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IMMUNOLOGY IN CROATIA

40th anniversary of the Croatian Immunological Society

Introduction

This special issue is dedicated to the 40th Anniversary of organized activities of the Croatian Immunological Society. On this occasion the Annual Meeting of Croatian Immunological Society will be organized in Šibenik, October 9-12 2008, where this supplement will be introduced and distributed.

The idea for such a collection of data was born (conceived) during my first presidency 10 years ago, when the first account of Croatian Immunological Society activities was published, in Croatian.

This time, we have tried to include not only the founding and activities of Croatian Immunological Society but also all research groups working in the field of immunology in Croatia during the past 40 years.

I wish to express my warmest thanks to all authors for their contributions. Especially for collecting all the relevant data, references and achievements of their research groups.

From the collected data it is evident that Immunology in Croatia developed very fast (rapidly), and achieved a prominent place among scientific disciplines. Both national and international collaboration resulted in the establishment of new groups, not only at universities where immunology first started, but also at several institutes and hospitals in Zagreb and Rijeka.

The number of Croatian Immunological Society members varies, being always more than one hundred and less than two hundred, but with a very good renewal rate of young members each year.

We hope that, with improved financing policy of basic science in Croatia, more novices will be attracted, and that immunologists will remain as active as they were throughout this past 40 years, despite some quite untoward conditions.

Sabina Rabatić
guest editor

EARLY BEGINNIGS

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Historical Roots of Immunology in Croatia

BISERKA BELICZA†

It is in the nature of man to pose questions on the beginning of the world, the origin and purpose of his own life and all that surrounds him, including the question of who, when, how and where first discovered the regularity of natural occurrences, the nature of their control or utilisation with the object of satisfying human needs and the realisation of human desires. Occasionally we ask why, how, when and where and who initiated the process of the creation and transfer of new knowledge and their application throughout the world, just as today, we ask where the early beginnings of immunology in Croatia lie, who were the people and what events marked its development and achievements on a national and world level.

Regardless of how we search for the answers to these questions we will inevitably come to the same conclusion. Namely, that man had a key role in this process, urged by the desire to control and avoid diseases which had for centuries determined the tragedy of his life, pervaded with death and invalidity of his nearest and dearest, or the premature loss of their or his own existential forces, the ability to see, hear, speak, understand, motoricity and many other blessings, of which in health we are frequently unaware. Time-honoured experience was carried from generation to generation in the knowledge that there are diseases of which man, after recovering from the disease, is protected for life, but also that there are diseases which provide such protection only for a short period or not at all. Such experience was applied in practice in many cultures and civilisations long before man had discovered the nature of the causes which lead to the occurrence of disease and resistance.

Thus, one can trace the roots of experience based immunology in the declaration issued on July 27, 1377 by Council of the Dubrovnik City Republic ordering precisely defined quarantine measures against various infectious diseases.

Unique measures were focused on prevention of possible outbreak of plaque epidemics, which could spread out by caravans or ships carrying merchandises from Ottoman Empire.

Latter on, Venetian republic and in particular Habsburg monarchy developed a Copley system of tightly controlled quarantine measures along Croatian Military Boarder with Ottoman Empire. Described system of preventive measures was mostly elaborated and at that time represented one of the greatest achievements of medicine.

The most impressive was that connected with variola. Today, aware of the historical sequence of events, we realise that it was in fact the discovery of Edward Jenner on the protective effect of cowpox that was a crucial moment, which in the prebacteriological era already established the foundation for the development of today's immunology. It is well known that his study, published in 1789 did not meet with the universal support of learned circles in the Royal Society in London. However, this did not prevent the introduction and promotion of vaccination with cowpox with the aim of preventing variola in London and soon after in other countries of the world, alarmed by the consequences of variola, which had intensified, while the plague had gradually receded. It was almost the only ray of hope at a time of medical scepticism and therapeutic nihilism, inhibited by all the problems of the range of medical theories, natural knowledge and technology at that time. As in the case of quarantine practice based on experience proved more effective than the relevant scientific doctrine at that time.

† The original text (in Croatian) was solicited from the late Professor Biserka Belitza, for the occasion of the 30th Anniversary of the Croatian Immunological Society, in 1998. To make this collection of texts a comprehensive account of the activities of immunologists in Croatia, we have translated Belitza's text into English and added it to the recently written collection of the Society activities from its beginnings.

We do not know exactly when the first news of Jenner's discovery reached these regions. However, we do know that as early as 1797 *Mihajlo Gellei*, a physician in Vukovar and later in Novi Sad, wrote articles on the usefulness of variolization, and that already in 1801 a report by the Italian physician *Constante Mudiano* was printed on vaccination against smallpox. In 1804 two books were printed in Zagreb with the support of the bishop, *Maksimilijan Vrhovac,* on the advisability of vaccination by *Michael Neustadter,* a protomedicus in Erdelj, translated into Croatian and »Illyrian«, and a third book printed in Rijeka, which was published with the support of the bishop of Senj-Modruš district, *Ivan Ježič*.

During those years *Luka Stulli*, a county and hospital physician in Dubrovnik, known as the casual poet, wrote a special elegy on vaccination by cowpox in honour of *Luigi Caren*, an Italian physician who lived in Vienna, where he dedicated a translation of Jenner's work on vaccination and propagation of vaccination. With the poem Stulli in fact announced Caren's book, which was printed in Dubrovnik in 1805 in his translation from French to Italian and Illyrian-Croatian.

It is interesting to note in these initial publications not only inevitable emphasis and praise for Jenner's work and success because of his method of vaccination against variola, but also that the reader is constantly dissuaded from doubt and fear that some other diseases can be spread by this method. In other words that the efficacy of vaccination weakens with the transfer of the vaccine from one child to the next. All the propagators of vaccination at that time advocated that vaccination should only be performed by experienced physicians or paramedics, and that they must procure the vaccine themselves, either in a dry or liquid form, so that in the case of greater needs they can continue to use puss from the pustules of children in whom the vaccine had been administered. Instructions were given to parents, fathers and mothers with dramatic messages and statistical data, in order to persuade them to vaccinate their children. Symptoms which accompanied vaccination were comprehensively and informatively described and directions given on how to feed and care for the vaccinated child. How to recognise that the vaccination had been effective, and when it was necessary to call for or consult a physician in the case of complications. In Caren's book there was a separate chapter and text on vaccination which had been carried out in London in 1801.

In 1807 *Vincenzo Dandolo*, an Italian physician, politician and high official of Dalmatia (from 1806 to 1810) during the time of the French government of Napoleon I, passed a decision on obligatory general vaccination in Dalmatia in the regulations of the work of physicians and surgeons in districts, cantons and counties, who were obliged to carry out vaccination in the region of their service. It was necessary to vaccinate all of the inhabitants of Dalmatia – not only children, primarily in order to protect Napoleon's soldiers in Dalmatia. This tradition in Dalmatia was continued later by the Austrian government, which in 1824 passed specific regulations on the vaccination of soldiers and civil inhabitants of the Croatian Military Border, while in the region of Croatian and Slavonia regulations from 1829 were valid, which were prepared by *Michael Lanhossek,* a Hungarian protomedicus. Obligatory vaccination was particularly insisted on at the time of the danger of an epidemic of cowpox in order to prevent further spreading. Consequently, we could anticipate that in time our physicians acquired their own experience on the effectiveness of vaccination and revaccination, their complications or contraindications and that they reported them not only to the appropriate organs but also to a wider medical public. However, it appears that the preparation of official reports on the results and observations completely satisfied or exhausted them, as only a few inaugural dissertations on vaccination can be found from that time. The oldest known dissertation which we found on the values of vaccination was printed in Vienna in 1825 by *Fran Folnegović*, later county physiologist in Zagreb. Another was written by *Antun Dragutin Laslović* in 1838 which was dedicated to the history of variola and discovery of vaccination against smallpox. Laslović later also worked as a county and town physiologist in Zagreb. They were graduate theses on the basis of which, after defending them and passing the examination, the candidate acquired the status of *doctor medicinae*. During the study of medicine great attention was paid to vaccination and in time a course on vaccination became one of the prerequisites for registration in the public medical health service. Historical materials in Croatian archives are full of reports by physicians on obligatory vaccination and revaccination, and also their repeated plea that they should be paid travel expenses and an appropriate fee.

Thanks to the investigations of a physician from Naples, Negrij, in 1840 technical progress was achieved which enabled easy, safe and abundant production of bovine vaccine. It was an opportunity to change to production of greater quantities of vaccine and its wider application. Around the year 1865 Negrij's method in some European countries prompted the development of the first successful institutes for the production of animal lymph. In the seventies of the 19th century Croatian physicians also took the initiative for affirmation of vaccination against smallpox, inauguration of vaccination with animal lymph, passing of new regulations and laws on obligatory vaccination, and also the start of the production of vaccine.

Already during the first year of its work the *Croatian and Slavonian Medical Association*, gave a lecture on vaccination against smallpox. In his lecture entitled *Collected works for evaluation of vaccination against smallpox Dr. Antun Schwarz* said: I felt it my duty to make these collected works known, and to show the numerous advantages of vaccination, so that, regardless of the unfounded quibbling of those who do not advocate vaccination against smallpox, the procedure was accepted, which, although such treatment does not always offer absolute immunity against the plague of smallpox, it does guarantee reduced disposition towards it, making the course of the disease less stressful and death the exception, a procedure which eventually cannot cause any harm.

In 1875 *Dr. Tomo Marek* proposed that in Croatia and Slavonia a *domestic institute should be founded for the production of pure bovine vaccine under the control of the Royal National Government.* His proposal was based on the following...*because vaccine is very difficult to obtain quickly, because of the very poor guarantee that it is actually good, because vaccine from private institutes varies considerably with regard to price and is generally too expensive.* In the Medical Association an ad hoc committee was constituted which considered his proposal and after requested amendments, concluded: *unable to consent to the proposal of Dr. Marek due to the fact that according to existing regulations only use of humanised vaccine is allowed.*

When in 1874 a law was passed on improvements of the medical service, it was emphasised, among other things, that one of the duties of the vice county physician was the *supervision and control of vaccination and revaccination against smallpox and the collection of excerpts from reports on yearly vaccinations performed.*

In 1876 the Royal Croatian Slavonic Government, Department of Internal Affairs, passed a special decree on implementation of the regulation for *Smallpox vaccination.* In the section *Procurement of vaccine* we read: & 29. *animal or humanised vaccine will be used for vaccination and revaccination....* In 1888 the following decree was passed on *Vaccination and revaccination of armed forces,* and in 1891 the *Law on vaccination and revaccination against smallpox* which in & 1 stresses: *Vaccination against smallpox is a national decree, and vaccination and revaccination will be performed only with animal vaccine.*

Thus the production of animal vaccine against variola was made possible in Croatia by the letter of the law. In 1890 *Dr. Izidor Schlick*, independently began production of animal lymph in Bjelovar. Thus with effect from 1891, when the law on vaccination with animal vaccine was passed, up until 1893 he was the main producer and supplier of vaccine for the use of Croatian physicians. On the proposal of protomedicus, Dr. Struppij, his institute was taken over by the national government in 1893 and was moved to Zagreb where production commenced in 1894. Already in the following years the government passed a decision on the building of new laboratories for the production of vaccine against smallpox at a location in Gundulić Street. On the 1st February 1896 the Institute moved into the new premises and acquired the official title: Royal National Institute for the production of animal vaccine in Zagreb**.** Dr. Julijo Rogina was Head of the Institute, and remained in this capacity until 1919. In 1926 the *Institute for the production of animal vaccine* became the *Department of the Institute of Hygiene with the Andrija Štampar School of Public Health* which took over and continued production of vaccine against variola.

It can be said that variola and vaccination provided the initial stimulus for the development of immunology. A new era commenced with the development of microbiology and natural sciences in the second half of the 19th century, which was characterised by increasing attempts to discover new therapeutic and preventive procedures in the control of disease. For example, in 1883 our Medical Gazette (Liječnički vjesnik) published a lecture by Prof. Liebermeister, which was held in Freiburg under the title *On the attempts of therapy in the newer era*, in which the author summarises that because of discoveries of specific causative agents in the new era, medicine experienced transformation from *pathological anatomical* to *etiological medicine,* which revived old hopes and belief in the possibility of specific therapy. To show that it was possible Liebermeister gave as an example **chinin – fever***, mercury-syphilis, salicylic acid-rheumatism and* **kalomel – at tyfa**.... and continued: Cannot we, in view of these facts, believe that we will some day find specific drugs/medicaments against smallpox, scarlet fever, diphtheria, cholera, dysentery and tuberculosis?

During the same year in 1883 the Medical Gazette (Liječnički vjesnik) published an article entitled *Aetiology of tuberculosis. A lecture held by Dr. Koch, national consultant for the Royal Office of Public Health, in the Physiological Society in Berlin, according to a report in the Berlin Clinic Wochenschrift in 1882, no. 15.* It is interesting to note that Prof. Liebermeister did not mention anthrax or rabies, and it was just Pasteur's discovery of vaccine against anthrax (1881) and later against rabies (1885) which was the first step forward after Jenner's vaccination in the development of therapy of diseases caused by microbes. The discovery of the causative agent of tuberculosis in 1882 was the first step which leads Robert Koch in 1890 to the discovery of tuberculin. Although there were sporadic cases of anthrax and rabies in Croatia far greater interest was aroused in this part of the world by Koch's discovery, most likely due to the fact that already in 1890 Dr. Antun Lobmajer had brought back from Berlin a small bottle of Koch's medicament. The same year the Medical Association passed a resolution, The method of treating tuberculosis according to Koch, and the national government issued a decree in December of 1890 *Treatment of tuberculosis according to Dr. Koch*, in which it was stressed that according to the opinion of the Royal National Medical Council this medicament was of great value in the diagnosis and treatment of tubercular patients, although in time it can cause serious, life threatening consequences in the organism, and for this reason rational use was necessary for the well-being of the patient. The decree stressed that the medicament should be obtained exclusively from the Institute approved by the Royal Prussian National District, and that the medicament can only be obtained by the Heads of institutes and scientific departments, and physicians with authorisation for practice. Treatment in private practice was only allowed on the condition that medical observation of the patient was ensured so that when necessary help could be provided. Outpatient treatment was forbidden. Physicians were obliged to record their observations and report everything to the appropriate organ, and each case of death of persons treated with Koch's tuberculin reported to the District administration.

The next stimulus for the development of immunology in Croatia was connected with diphtheria, a disease which our physicians had for centuries come up against and remained powerless before its intensity and consequences. Emil von Behring discovered a specific diphtheric antitoxin in 1890, and introduced it in practice in 1893. After the shaken hopes due to the failure with Koch's tuberculin, *Behring's lymph* aroused new enthusiasm and belief in the next age »aetiology of medicine, specific causative agents and specific therapy«.

Thus in 1894 our medical publications and medical legislation were greatly concerned with diphtheria and Behring's lymph, which the government very quickly procured and distributed for the use of physicians. In 1894 the National Government passed a decree *Treatment of diphtheria by blood lymph.* The Town representatives in Zagreb decided to use financial means, planned for a hygienist, amounting to 800 forints, to procure Behring's medicament for the poor inhabitants of Zagreb, regardless of their nationality, gratis, while those financially better off were required to pay for the medicament which they used. In a further decree in 1895 the Government stressed that for the *treatment of those suffering from diphtheria and immunisation of healthy children reserves have been ensured by the government: Behring's lymph and Schewring's Antitoxin, i.e. Arons' means....* In 1896 a decree was passed *Supply of patients with lymph in hospitals.*

According to published data from 1st August 1894 to 31st January 1895 Behring's blood serum was used in the following towns in Croatia and Slavonia: Zagreb, Varaždin, Osijek, Karlovac, Bakar, Bjelovar, Sisak, Brod, Mitrovica and Rijeka for 173 cases of diphtheria, of which 156 recovered and 15 children died. According to a medical report for the Austrian coastal area for the years 1893 and 1894, and in the area of Istria and the coastal area, diphtheria claimed the lives of its victims. However, it appears that only patients treated in the hospital in Trieste had the possibility of treatment with *Hochteterr – serum and Behring's antitoxin*. Therapy with the serum was also applied in Dalmatia, and according to a report for the years 1903, 1904 and 1905, we can conclude that in 1903 out of a total number of 872 patients, 95.3% were treated with serum, of which 88.09% recovered and 11.91% died, and only 4.76% were not treated with serum. In the comment we read »*Mortality of those treated with serum would have been less if in each case a physician had been at hand, and had been able to use serum at an opportune moment.....On quite a number of occasions in villages, much further away from the physician's headquarters, when he reaches the patient he finds him dead, or the disease had progressed so far that injecting serum can no longer be of help....*«

The first news with a detailed report of Roux's and Behring's investigations and application of Behring's serum in the treatment of diphtheria was published by the Medical Gazette (Liječnički vjesnik) in number 15 October 1894 in a presentation at the *VIII. International Congress on Hygiene and Demography*, which was held in Budapest at the beginning of September 1894. Immediately afterwards an article was published by *Dr. Julijo Heninger*, county physician in Vrbovac *Treatment of diphtheria of the pharynx (Angina membranacea)* in which Heninger wrote of his results in the treatment of diphtheria with *Liquor ferri sesquichlorati*, and thus the Editor considered it necessary to add a note in which he explained that the article had been written in July 1894, hence before Behring inaugurated his method in a lecture at a meeting of German natural scientists and physicians in Vienna. In order to also inform the reader about that meeting in the next edition the Editor included an article under the title *From the 66 Assembly of German natural scientists and physicians held in Vienna from 24th to 30th September 1894* in which the course of the meeting and Behring's lecture was reported with Erhlich's comments and notes of the Editor in the page margins: *We learnt that the Royal National Government distributed some of Behring's blood serum to the county physiologist for prophylactic vaccination. In Vienna collection of serum was started on the initiative of a private physician, in order to enable treatment of the poor. Under the supervision of Prof. Paltauf production started in the Institute for the treatment of animals with horse blood serum against diphtheria. Experiments with treatment were carried out by different hospitals, e.g. Rudolf in Vienna, St. Ana in Vienna, the hospital in Trieste etc.*

The first article on the results of our physicians with the application of *Berhing's medicament against diphtheria* was published in the Medical Gazette (Liječnički vjesnik) in the next number, as an excerpt from a report by *Dr. Isidor Schlick*, at that time county physiologist in Zagreb, submitted to the National Government and county of Zagreb. In the same number a supplement was published by *Dr. Julijo Heninger*, county physician in Vrbovec, who on this occasion wrote *Report on the use of Behring's serum* on the basis of personal experience and data from the literature.

In the same year in the book *Medical report for the year 1894. General and public Huttler-Kohlhoffer-Monspergove Foundation Hospital under national management in Osijek, Vatroslav Schwarz* emphasised... *The most important new drug/medicament introduced in therapy in 1894 was undoubtedly blood serum against infectious* **throat sore**....

Justifiably it was reported with pride that at the beginning of 1894 the director of the Osijek hospital organised a *bacteriological division, for which the instruments and apparatus were obtained from the firm Lautenschlager in Berlin*. He was clearly the only hospital director who could emphasise in his report on diphtheria for 1896: *1. In all cases true diphtheria was microscopically and bacteriologically unquestionably proved;* And on the basis of his own experience he concluded: *Hospital statistics on the treatment of diphtheria by blood serum are too scarce to enable final judgement on the true therapeutic value of this new drug/medicament: I believe that the whole question will soon be clarified by a greater number of accurately monitored and described cases in*

hospitals, in which a finding of diphtheria must be determined unquestionably, microscopically and bacteriologically, which can be easily performed, and by which Loffler's bacilli can be determined with no great technical difficulties. For all other physicians in Croatia such diagnostics, on the basis of a decree by the National Government in 1892 could only be performed by the biologist-botanist *A. Heinz* in the *Bacteriological Laboratory of the Botanical Institute University of Zagreb.*

In 1895 the Medical Gazette (Liječnički vjesnik) printed new articles on diphtheric and ante-diphtheria serum, among which was a contribution by *Dr. J. Havliček – Production of animal drug/medicament against diphtheria called curative serum, antitoxin or antidiphtherin.* He described in detail production of serum which was applied by Roux in Paris and the firm in Berlin according to Behring's method, revealing in the last part of the article his main motive – opinion on the need for the serum and the possibilities of its production in Croatia and Slavonia. With this purpose J. Havliček visited Prof. Palfauff in Vienna and Dr. Patrik in Budapest, whom he thanked for explaining to him and demonstrating the method of serum production. As we know production of diphtheric antitoxic serum commenced in Croatia by the same method in 1929.

The primary physician in the Osijek Hospital, Dr. Vatroslav Schwarz, also revealed to us that physicians in Croatia at that time followed with great attention and comment other events in the field of serotherapy. In the aforementioned report for the year 1895 in a chapter on *Scientific activity of institute physicians* we find his article under the title *New trends in serotherapy* which we present as an interesting and valuable document for the study of the development of immunology in Croatia, although it speaks of events in the world, mainly in Europe. Schwarz begins this article with the following words: »*More recently, particularly in this respect, two methods are prominent, and they are treatment with blood liquor (serotherapy) and treatment with animal organs (organotherapy); the first method has been successfully tested against diphtheria and the second in the treatment of struma/goitre. As the anticipated success of serotherapy in the treatment of diphtheria was justified, it was believed that this method was the only one, by which goals in the case of other diseases would be easily achieved, for which we have so far been more or less powerless with regard to therapy; and so we find in the literature the above serum against anthrax, pneumonia,* erysipelas*, tetanus, syphilis, and even against* measles…, *I suggest that of the diseases for which most success is anticipated by serotherapy in recent times, and which have been tirelessly studied for more than a decade, are cancer, pyemia, septicaemia and tuberculosis, in truth the most terrible disease of mankind.* A presentation followed on experiments and results of treatment by *blood serum against cancer* – from injection of pure virulent cultures of vrbacan-erysipelas in a tumour, injection of sheep serum surrounded with pure culture of *vrbančevih kokkah* – injected on the site of an operation and **Cancroin**. Schwarz commented in detail on the *serum against streptococci* and the results of its application for erysipelas and other diseases caused by streptococci,, acute diphtheria, streptococci inflamed neck in »scarlet fever« and finally *Maraglian's serum against tuberculosis*, reminding the reader that mass*, which Koch's tuberculin caused, also caused justified doubt in the medical world towards all new methods in the treatment of tuberculosis.* In each described attempt of *serotherapy* Schwarz gave his personal comment and opinion, insisting on scientific arguments and clinical proof. Today we would classify his work as a critical review article based on data from the literature. The journals which the primary physician from Osijek hospital, Dr. Vatroslav Schwarz, cited as his sources were: *Deutsche Chirurgie, 1880: The American Journal of the medical sciences, 1894; Deutsche med. Wochenschrift, 1895; Wiener klin. Rundschau 1895; Berliner klin. Wochenschrift 1895: Die Zeit. literarisch – socialpolitische Wochneschrift 1895.* This detail not only reveals the scientific interest and high expertise of V. Schwarz but also historically-scientifically clearly demonstrates how the process of the transfer of information and acceptance of new knowledge and methods in medicine evolved in our region.

Needless to say, apart from information on the transfer and acceptance of new achievements relevant institutional bases and appropriately educated experts were needed. In the case of serology, which in the 19th century took a step into the field of diagnostics it implied the need for a bacteriological and serological laboratory, and a specialist in microbiology. Although with regard to the medical circumstances in Croatia at the time, where acute and chronic diseases of microbiological aetiology dominated far in front of others, it was a case of essential institutions and in this respect private initiative was prominent in the realisation of such institutions. Although for years the need to found a bacteriological institute was discussed at the level of the National Government, it failed to go any further than discussion. Throughout the whole period from 1892 to 1907 Dr. Antun Heinz carried out all bacteriological tests for the requirements of the public health service in the area of Croatia*, in the Bacteriological Laboratory of the Botanical Institute University of Zagreb*. For this reason in 1907 *Dr. Ljudevit Gutchij* was prompted to found a private *bacteriological – hygiene institute at 120 Ilica street, opposite Primorska street.*

Ljudevit Gutschy (Sisak 1874 – Zagreb 1961) was our first physician specialist microbiologist and immunobiologist who had acquired specialisation in leading European centres at that time. In 1900 at the end of medical study in Graz he was accepted as assistant-volunteer in the Department of experimental pathology and bacteriology. At that time the National Government in Zagreb, prompted by the protomedicus**,** Dr. Ignjat Thaller, decided to found a bacteriological-hygiene institute, and on the recommendation of Prof. Klemensiewicz sent Gutschy to the Pasteur Institute in Paris for one year, where he had the opportunity of working with Roux, Bordet, Mečnikov and other experts in that institute. When after three years he returned to Zagreb he was confronted with the fact that a bacteriological-hygiene institute had not been founded. He went to

Vienna where he worked with Prof. Paltauf and later in veterinary surgery with Czukor, mainly engaged in zoonosis and the preparation of antirabies vaccine. From Vienna he went to the Koch Institute in Berlin, attending lectures by Wesserman and the pathologist Orth, and for two semesters worked in the Institute of Physiological Chemistry with Thierfelden. In 1905 he was promoted Assistant at the Medical Academy in Cologne where he became a coworker of Chaplewski. For a short time he worked with Calmett in Lubec. Returning again to Zagreb in 1907 he obtained permission to found a private *bacteriological-hygiene institute.* It was the first humanomedical – microbiological and chemical institute in Croatia, and thus with the extent and variety of business after five years in 1907 he offered to take over ownership of the Gutchy Institute. Apart from bacteriological research and examinations the newly founded *National Bacteriological and Hygiene Institute* was given the task of producing serum for diagnostic and therapeutic purposes, and was moved to No. 9 Kačić Street. The institute had a chemical-analytic and bacteriological department. Thus organised it worked up until 1918, headed by Dr. Lj. Gutschy as director, who had held the office from 1913. During the time of the typhus epidemic in Slavonia Gutschy carried out the first *antityphus vaccination* with his own vaccine in Croatia and achieved great success. During 1914 the Institute produced *vaccine against diphtheria*, and in 1915 against *typhus and cholera.* In 1916 Gutschy was engaged in solving the problem of rabies which prompted him to attempt to expand the institute with a serological-immunological and antirabies department. In the same year, in 1918, he received a ruling to immediately start production of *vaccine against rabies*, which on 1st January 1919 resulted in the founding of the first *Pasteur Institute* in Croatia, in which Gutschy, during the following five years, devoted all his knowledge and energy up to the moment of his unexpected retirement.

The Bacteriological Institute in Kačić Street continued work under the leadership of Dr. *Julijo Rogina*, and later for a short term under the leadership of Dr. *Vyšek*, until it was taken over by Dr. *Berislav Borčić.* In 1919, the Institute began production of *tetravaccine typhus-paratyphus-cholera*, mainly for the needs of the army, vaccine against *ozena*, various diagnostic serums and numerous autovaccines. In 1923, preparatory work was commenced for the production of diphtheric, and later also other, antitoxic serums and antibacterial preparations. At the same time preliminary work was carried out for the building of a new Institute of Hygiene at Zvijezda. In October 1927 the Bacteriological Institute moved from Klaić Street to a new location, where technical and organisational conditions were ensured for the work of the first department for biological products in Croatia and former Yugoslavia. Dr. *Josip Berlot was* Head of the *Department for the Production of Biological Preparations in the Institute of Hygiene in Zagreb* until the formation of a department in the new *Institute of Hygiene with the Andrija Štampar School of Public Health,* and the introduction on the market in 1929 of the first *diphtheric antitoxic serum* in Yugoslavia. This department later became the present Institute of Immunology in Zagreb. In 1928, an estate belonging to Vrapće Hospital in Kalinovica was purchased for the needs of the Institute of Hygiene, where in cooperation with the IV department immunisation of horses was carried out for the production of human serum. In time the assortment and amount of immunobiological human and veterinary preparations produced satisfied domestic requirements and in 1930 the import of these preparations from abroad was cancelled by a decree of the Ministry of Health. Increased attention was paid to the production of known serums, vaccines and biological preparations, as it was expected that during a possible New World war the use of poisonous gasses and bacteriological warfare could occur. On 1st December 1939, based on a decree on internal reorganisation of the Banovian Croatian Government, the *Banovian Institute for the Production of Medicines of Biological and Chemical Composition* was founded in Zagreb. As a separate institution, within the Department of Public Health, the *Institute for the Control of Medicines of Biological Origin* was founded with the purpose of testing the usability, sterility, harmlessness, potency, etc. of all medications/drugs of biological origin for human use, produced in the country or imported from abroad. This included all serums, vaccines, insulin as well as the toxicity and therapeutic value of chemotherapeutic domestic and foreign preparations. In 1941, PLIBAH changed its name to *Pliva*, which merged with *Kaštel*, a factory for the production of pharmaceutical preparations.

A search of bibliographic data on published papers in the field of immunology, serotherapy and immunoprophylaxis up until 1940 shows that Croatian physicians, apart from papers on vaccination for the prevention of variola, vaccination and treatment of diphtheria, typhus, paratyphus, rabies and cholera, also wrote about other serotherapeutical and prophylactic attempts.

Here are several examples: In 1902 an anonymous author wrote about *Moser's serum* in the treatment of scarlet fever; in 1906 J. Karinski gave a discussion *On treatment of dysentery by serum;* in 1908 M. Begić described a *Case of tetanus treated by »Hoechst« antitoxin;* in 1915 Beno Stein reported on the theme *Therapy of infectious* meningitis *by serum*, and in 1928 M. Sarvan published a *Contribution to the question of preventive vaccination against varicello*. A considerable number of papers concerned the problem of the preparation and production of serum and vaccines, while a relatively a small number of source papers and publications on the whole were concerned with theoretical questions, based on scientific research or the study of chemical and biological bases of the phenomenon of immunity. Interest in these problems gradually became evident in clinicians, particular paediatricians, internists and dermatovenereologists, who discussed these questions at their professional meetings.

After World War II, up until 1950, the production of human serums, vaccines, and antitoxins was still located within the *Pliva* factory. In 1950 production moved to the *Central Institute of Hygiene* in Zagreb, headed by Dr. *Josip Brodarac.* At that

time the problem arose of protection against certain viral diseases known about earlier, but which until then had not occurred on a large scale; for example poliomyelitis, and furthermore there was a need for the prevention of serious complications and consequences in morbilli, periostitis and rubeola.

The first compulsory vaccination of children against diphtheria in Croatia was probably carried out in 1947 or 1948. In 1950, Dr. Božidar Marković, apart from vaccination of children against diphtheria, also urged their vaccination against tetanus and pertussis. All this had an effect on the further development of the production of vaccines and serums, and consequently on the development of immunology in Croatia. In 1952 the *Division for the Control of Serums and Vaccines* was established within the Institute of Hygiene, with Dr. *Drago Ikić,* as head, while the *Division for the Production of Serums, Vaccines and Antitoxins,* was headed by Dr. *Vicko Krstulović*, until his retirement in 1956. In 1956, the *Institute for the Control and Testing of Immunobiological Preparations* was founded by a decree of the Parliament Executive Council. In the same year Parliament passed a decree that the *Central Institute of Hygiene* in Zagreb for the production of serums and vaccines and other biological preparations for human needs, continued work as a self-financing institution under the title *Serovaccination Institute*, with headquarters in Zagreb. From 1956 to 1958 Dr. Drago Ikić was Head of Serovaccinal Institute and from 1958 Dr. Drago Ikić became the head of the Institute for the Control and Testing of Immunobiological Preparations.

With the independence of the Serovaccinal Institute the production of bacterial vaccines increased. A new technique in the production of the diphtheric toxin was introduced due to the efforts of Prof. *Neboder Škarica; Dr. Neda Köller-Kubelka* developed the first production of vaccine on synthetic substrate against pertussis; Dr. *Josefina Rücker-Prpić* developed a new vaccine preparation against abdominal typhus; a preparation of an effective vaccine against the group Neisseria meningitides was developed by *Dagmar Sinković*, Mr., Dr. *Josip Petres* and Dr. *Dubravka Jušić,* while Dr. *Sonja Petrićević-Iveša* with co-workers, improved the production of toxin against tetanus, diagnostics of »legionary disease« and mycoplasma.

In 1961, the *Serovaccinal Institute* changed its name to the *Institute of Immunology,* which was registered as a *health institution with special assignments*, including scientific work, training and education of young scientists. The head of the Institute become Dr. Drago Ikić and he stayed at this position until his retirement in 1982. Thus, according to the writing of Dr. Berislav Pende, scientific-research work became a fundamental determinant for the future development of the Institute. In the same year work commenced on the construction of a building at 10 Rockefeller Street and the following year, in 1963, on the initiative of Dr. D. Ikić, publication started of the journal *Annals of the Institute of Immunology* on the production of viral vaccines and antitoxic serums. Dr. *Vladimir Lulić* initiated work in the field of poliomyelitis and Dr. *Neven Pasini* commenced initial experiments in the preparation of a live attenuated strain of influence. In those days biomedical investigations paved the way for new knowledge and methods in the production of vaccines, serums, antitoxins and similar biological preparations. For instance, the first possibilities for the prevention of morbilli were realised in 1954 when cultivation of a virus in a cell culture was successfully realised and laboratory procedures obtained an attenuated vaccine strain, known under the name Edmonston B. Primary and continuous cultures of human and animal cells had until then served as a model for the study of cellular biology and diagnostics of viruses. At the beginning of the 1960s, *D. Ikić* with co-workers *V. Lulić, I. Dedić, R. Asaj,* and Dr. *M. Juzbašić* introduced diploid cell cultures in the Immunological Institute, using them first in the production of viral vaccines such as against poliomyelitis, morbilli, influenza, variola. Virologists of the Immunological Institute, under the leadership of *D. Ikić, V. Lulić, M. Juzbašić,* succeeded in cultivating a strain of virus morbilli Edmonston – Zagreb, the most significant product of the Institute, which has been applied in the world in millions of doses. Success was achieved in the passage of the virus in a strictly controlled line in the cellular culture of human diploid cells HDC (Wi-38). It was the first attempt to use human diploid cells as a cellular substrate for attenuation of virus. In 1970, on the initiative of *D. Ikić,* preparation of leukocyte interferon was started. Apart from production the Immunological Institute also became known in the world for results in fundamental and applied research in the field of immunology, biochemistry, microbiology, transfusiology and vaccinology.

The development/progress of immunology in the world was reflected in pioneers in other disciplines. Prof. Nikša Allegretti (Brač 1920–Zagreb 1982) was a particularly prominent figure in the history of Croatian immunology, as the initiator of modern biomedical research in Croatia, a scientist who deserves credit for the development of physiology and immunology, one of the founders of modern experimental endocrinology, tumour and transplantation immunology. Within the framework of immunology he investigated radiation syndrome, anaphylactic autoimmunity reactions, tumour and transplantation immunology. He contributed to theoretic biology with his independent views on the origin of immunological phenomena. In the field of transplantation immunology mention should be made of Prof. *Berislav Nakić* (Omiš 1921–Zagreb 1982) who was the first in our regions to engage in fundamental investigations of homotransplantation of tissue and transplantation immunity on the basis of which he achieved world acclaim.

If we read the key bibliographic entries in the bibliographic processing of papers, published in the Medical Gazette (Liječnički vjesnik) and editions by the Croatian Medical Association, from 1874 to 1977, as indicators of the development of immunology in Croatia, we can see that in the publications from 1938, references are classified in the group *bacteriology-im-*

munology-serology and with particular diseases. In the bibliography from 1977, in successive chronology the oldest contributions are classified in entries: *immunity* /1908/; *immunochemistry* /1913/, *immunology* / 1924/; passive *immunity* /1933/: *immunisation* /1955/, *immunoflourescent technique* /1965/; *immunoglobulins* /1972/; *cellular immunity* /1973/; *interferon* /1973/; *immunosuppressive means* /1974/*; immunotherapy*/1975/. Other contributions were classified with entries of serum disease; serotherapy; serodiagnosis; vaccination – as vaccination; vaccines – exclusively connected with variola; vaccinotherapy; variola vaccine; morbilli vaccine; tissue culture, molecular biology; allergy; tumours; transfusion; transplantation, etc. One can hardly find an entry without at least one text with reference to immunology. In an atmosphere of such active professional and scientific-research work, the need arose for the organisation of scientific meetings and establishment of a professional society of immunologists, such as today's celebrated Croatian Society of Immunologists, which will be mentioned in later presentations.

It has been said that if historians, and thus historians of medicine, wish to be scientifically objective and neutral when writing about certain personalities, events and phenomena, they should keep a historic distance of at least fifty years. Therefore, this tribute to the people and events from the immunological past of Croatia, reaching back as far as the 1970s, will not be a historic contribution, but rather only a reminder of certain events, which should not be ignored by future investigators of the history of immunology in Croatia. There are still many living participants and witnesses of that time. It is up to them, if they so wish, to write their own perceptions of the period in which they worked, created and realised their objectives. Because behind each profession, each science and each association there is always man, who realises and defines them, both those who achieve recognisable success, and those who remain anonymous, unknown or forgotten.

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DEVELOPMENT OF CONTEMPORARY IMMUNOLOGY IN CROATIA

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INTRODUCTORY REMARKS

Organizers of the Annual meeting of the Croatian Immunological Society that is to take place this year in Šibenik asked us to give an overview of immunological research in the major Croatian institute for natural sciences, named after a prominent eighteenth century philospher, mathematician and constructor of Croatian origin, Ruđer Bošković, a learned member of the Jesuit order born in Dubrovnik who spent his fruitful life as scholar in several European cities.

In order to accomplish our task as objectively as possible, we asked our colleagues who considered themselves immunologists to describe their major acievements, offer pictures or grafs of historical value or scientific merit, and quote up to five references best representing their work. Original descriptions were observed as much as possible. A few colleagues who had worked in the Institute but continued careers elsewhere could not be reached and therefore their work was summarized on the basis of authors' recollections, publications retrieved from databases, and personal archives. With these limitations, the overview is fairly complete. Possible omissions can be amended and authors welcome suggestions to that end.

It should be noted that only the contributions to *immunology* or closely related fields have been recorded in this survey. Many colleagues have paralelly pursued other research topics or redirected their interests to hematology, oncology, molecular genetics etc., abandoning immunology. Those achievements, often of high scientific value, had to be disregarded for the purpose of restricting the presentation strictly to its subject, immunology.

All scientists represented in this overview have acquired their Doctor-of Science (PhD) degrees in the Institute, or exeptionally an MSc degree, and a majority (including all residents of the Institute) achieved positions of research associate or higher. In order to illustrate multidisciplinary character of immunological research in the Institute, however, the basic profession has been indicated only (MD, BSc Biol, BSc Med Biochem, DVM).

BACKGROUND

Ruđer Bošković Institute was founded in 1950 as a nuclear institute at the height of the cold war in Europe. The idea was to establish a scientific center devoted to research in nuclear physics, chemistry and biology, including the production of nuclear fuel. Biology department was to investigate protection from nuclear irradiation and fallout. Its organization and scientific leadership was entrusted in 1953 to a bacteriologist from the Military Medicine Academy Branko Miletić and to three propulsive scientists from the Zagreb Medical School – physiologist Nikša Allegretti, pharmacologist Zlatko Supek and hematologist Erik Hauptman.

FOUNDERS OF IMMUNOLOGICAL RESEARCH IN THE INSTITUTE

Nikša Allegretti (MD) (1920–1982) was at that time assistant professor of physiology and head of the Department of Physiology at the Medical School Zagreb. His original scientific preocupations were muscle and plasma proteins, food preferences, vitamin C, endocrinology, metabolism and diabetes *(e.g. 1, 2)*. Under the influence of his peer and colleague Borislav Nakić

Figure 1. **Nikša Allegretti** *(1920 – 1982). (photograph taken about 1980)*

(a surgeon who forsook clinical practice in order to pursue transplantation immunology at the Department of Physiology), Allegretti became also interested in immunology and transplantation. In 1954 he was appointed head of the Institute's Department of Biology and in view of its 'nuclear' commitment conceived and pursued research in radiobiology *(3, 4)*. Together with his young collaborators Stjepan Kečkeš, Miloje Matošić, Ljerka Hofman (all three BSc Biol) and Branko Vitale (MD) as well as with his colleague Veljko Stanković (DVM) and his collaborators Neda Šestan (BSc Med Biochem) and Šime Vlahović (MD) Allegretti explored effects of X-rays in guinea pigs and rats under a working hypothesis that radiation induced alteration of autoantigens and thus caused autoimmune reactions contributing to the radiation sickness *(5, 6, 7)*. Radiation chimeras were produced by transplanting allogeneic or semi-allogeneic bone marrow cells into irradiated recipients (*8*). With new group of young scientists joining his team – Vlatko Silobrčić (MD), Dragan Dekaris (MD), Mislav Jurin (MD) and Luka Milas (MD), Allegretti embarked on transplantation immunology and tumor immunology, with or without reference to radiobiology (see contributions of those colleagues).

It is worth mentioning that Allegretti payed particular attention to the experimental animal facilities. He imported from England several strains of inbred mice and rats required for studies in transplantation immunology and rigorously supervised the animal colony. During his affiliation with the Institute, which lasted until the seventies, Allegretti occupied important positions in the Institute as well as at the University and in governmental bodies concerned with science. He educated several immunologists in the Institute as well in his home institution, the Department of Physiology of the Medical Faculty Zagreb, and has been considered, together with Borislav Nakić, as one of the founders of Croatian immunology. Several of his co-workers acquired respectable scientific status in immunology and related fields and attained important positions in Croatian science as research leaders, scientific policy makers, founders of scientific societies and establishments, journal editors etc. Full account of Allegretti's work can be found elsewhere *(9)*.

Figure 2. **Veljko Stanković** *(1922 – 1982). (photograph taken about 1980)*

Veljko Stanković (DVM). Another founder of immunological research in the Institute was Veljko Stanković (1922–1982) who joined the Institute in 1957, coming from Sarajevo where he was assistant professor of pathophysiology at the School of Veterinary Medicine. Before that he was employed with Department of Pathophysiology of the School of Medicine in Zagreb. His research at that time was mainly concerned with metabolism, nutrition, proteins, essential aminoacids and scurvy *(10, 11)*. Conforming with priorities of the Ruđer Bošković Institute for radiobiological issues he redirected his research to that field and together with Neda Šestan (BSc Med Biochem) investigated effects of X-ray irradiation on insulin resistance in rats *(12)*. Under the influence of Nikša Allegretti Stanković entered the field of immunology as well (see their joint references) and together with his collaborator Šime Vlahović (MD) studied immunosuppressive effects of whole-body irradiation *(13)* and transfer of immune reactivity to irradiated animals by transfusion of bone marrow from sensitized donors *(14)*.

During a study visit to the Radiobiological Institute TNO in Rijswijk, The Netherlands, Stanković became acquainted with bone marrow transplantation in experimental animals and upon his return to Zagreb directed his young collaborators Milivoj Boranić (MD) and Ivo Hršak (MD) into that field of research. Radiation chimeras were eventually produced by transplantation of bone marrow from several donors into heterozygous mice *(15)*. With his third collaborator Milivoj Slijepčević (DVM) Stanković pursued studies of liver function in irradiated animals *(16)* and directed him towards studies of diabetes, resulting in successful treatment of alloxan-induced diabetes in rats by means of pancreatic islet transplantation *(17)*. (See description of their contributions.) Stanković also mentored several extramural disciples who continued research in immunology and related fields in faculties and other Croatian scientific institutions.

Veljko Stanković succeeded Nikša Allegretti as head of the Institute's Department of Biology (1966–1972) and until his sudden death at the age of sixty occupied important positions in the Institute as well as in governmental and international committees concerned with scientific research. Unlike majority of Croatian scientists who esteemed only the 'western' science, Stanković advocated collaboration with scientists from the 'eastern' block as well and established fruitful connections. More details about Stanković's work and personality may be found elsewhere *(18)*

As seen from a very abbreviated list of publications of the founders of immunology in the Institute, their work was of high quality and was published in prestigious international journals. That was so in spite of unfavourable circumstances and limited resources in Croatian science at those times.

THE FIRST GENERATION

Allegretti's disciples

Stjepan Kečkeš (BSc Biol, b. 1932) spent several years working with Nikša Allegretti since 1959 in the field of radiation biology and immunology *(19)*. In 1963 he was appointed head of the Institute's Center for Marine Biology in Rovinj and after 1976 continued a successful scientific career in United Nations Institute for Marine Protection in Monaco.

Branko Vitale (MD, b. 1932) spent his whole professional lifetime in the Institute (1959–2007). At the beginning, he investigated (together with Nikša Allegretti) the influence of BCG infection on the intensity of homograft reaction in rats *(20)* and the dynamics of immunological events in experimental allergic encephalomyelits in guinea pigs. After return from postdoctoral fellowship at the Mount Sinai Hospital in New York (1963–1968), Vitale's main interests included transplantation immunology (1966–1980) with emphasis on graft-versus-host reaction, followed by clinical immunology as a new discipline (1980–2005) *(21)*. To study the mechanisms of graft-versus-host reaction Vitale developed a model of acute allogeneic disease in lethally irradiated mice after inoculation of defined populations of allogenic lymph node cells. The model was exploited to ouline the dynamics and complexity of cellular processes and immunological reactivity of graft-versus-host reactive cells selectivelly retained in the spleen. Development of chronic graft-versus-host disease was shown to depend on the rectruitment of new T-cells derived from hematopoietic stem cells activated in the second week after transplantation. Splenomegaly in radiation chimeras is due to exuberant myelopoiesis stimulated by the activity of allogeneic T-cells *(22)*. As a member of the International Union of Immunological Societies' (IUIS) Committee on Clinical Immunology Branko Vitale actively participated in creating clinical immunology as a new discipline and in outlining the infrastructure needed for its implementation and development. His major contribution to that field included studies of chronic lymphocytic leukemia (CLL) that attained high international recognition. Vitale developed a new concept of CLL as a predominant consequence of a slowly developing homeostatic disequilibrium within the immune system due to age-related impairment of B-cell and T-cell comunication, accompanied by numerous cellular and humoral immune defects. The following aspects were studied: disbalance in homeostasis, total tumor mass as a new prognostic factor in CLL, use of nonlinear prediction methods, decision, tree approach, mathematical models of CLL, the role of thymus in CLL pathogenesis, the role of T-cell modulation of *in vitro* T-cell reactivity by alpha-1 thymosin, origin of leukemic B -1 cells, phenotyping, immunoglobulin production and multidrug resistence *(23, 24, 25)*. In 1988 Vitale organized the Eighth European Congress of Immunology in Zagreb. In addition to the scientific activities, Branko Vitale has lectured at graduate and postgraduate courses of immunology at the Universities of Zagreb and Osijek and mentored fifteen intramural and five extramural theses. His intramural PhD disciples were V. Burek, M. Kaštelan, V. Knapp-Tomažić, B. Kušić-Benković, J. Pavelić, Đ. Plavljanić, I. Vučenik, of whom B. Kušić and J. Pavelić remained in the Institute and the others continued careers in immunology or related fileds elsewhere. Extramular PhD disciple Branko Jakšić became professor of medicine and remained Vitale's collaborator for years (see publications). Branko Vitale occupied significant positions in the Institute such as director of the Department and president of Institute's bodies and committees, and completed construction of a new building for molecular biology and medicine. He developed productive collaboration with several clinical institutions and was vice-dean of Osijek Medical School. At present he is editor of Croatian scientific journal Periodicum Biologorum, indexed in SCI.

Miloje Matošić (BSc Biol, b. 1924) participated in research of bone marrow transplantation with Nikša Allegretti and later on with Branko Vitale. After employment in the Institute (1956–1976) he continued career as professor of biology and immunology at the University of Split.

Vlatko Silobrčić (MD, b. 1935) was associated with the Institute in 1956 when he accepted the Institute's fellowship offered by Allegretti. His employment with the Institute started in 1959 and lasted until 1970, with a three years interval for the Army service and a postdoctoral fellowship at the Baylor University and M.D. Anderson Hospital and Tumor Institute in Houston, Texas, USA (1963–1966). His main scientific interest, since the second year of his medical education, was transplantation immunology, in particular the graft-versus-host reaction and immunological tolerance to tissue transplants. With Borislav Nakić he coauthored a paper indicating that the graft-versus-host reaction was basis for the so-called »parabiotic disease« that develops in adult rats surgically joined to each other (parabionts), and accompanies their tolerance to mutual skin allografts *(26)*. That observation lead to his studies of chimerism in mice tolerating skin allografts and to the discovery that

cellular chimerism persisted during the life-long tolerance of the allografts *(27, 28)*. During his fellowship in Houston he also became interested in tumor immunology *(29)* as well as in drug-induced immunosuppression as a means of producing non-specific tolerance to tissue grafts. In 1969 he started teaching immunology at the Faculty of Natural Sciences and Mathematics in Zagreb, as the first assistant professor of immunology in former Yugoslavia. Together with Dragan Dekaris he paved way to transformation of the Croatian journal »Biološki glasnik« (Biological Herald), published by the Croatian Society of Natural Sciences, into an internationally recognized scientific journal »Periodicum Biologorum« and became its editor-in-chief. At that time he also initiated organization of the Yugoslav Immunological Society and became its first secretary. At the end of 1969 he left the Institute and continued a successful scientific career at the Institute of Immunology in Zagreb. His outstanding contributions to Croatian science have been rewarded by the election to full membership of the Croatian Academy of Sciences and Arts. During his employment with the Bošković Institute he mentored BSc theses of Mila Hršak and Lidija Šuman who took employment with the Institute, the MSc thesis of Ivan Bašić who became professor of immunology at the Faculty of Natural Sciences and Mathematics, and the PhD thesis of Jožef Mikuška who became director of the Kopački rit National park.

Figure 3. *Mouse tolerating skin graft from a semi-incompatible donor. Tolerance was induced by means of injection of donor-type spleen cells immediately after the birth. (V. Silobrčić, PhD thesis, 1963)*

Dragan Dekaris (MD, b. 1936) is full member of the Croatian Academy of Sciences and Arts and professor of immunology at the School of Medicine, University of Zagreb. Has been employed with the Institute from 1960 to 1971 working in the field of immunology with Nikša Allegretti. In that period he spent two years as a postdoctoral fellow in Pasteur Institute, Paris. His work in the Institute started with studies of the mechanism of anaphylactic shock in rats and of autoimmune processes in irradiated animals *(30)* as well as with effects of injecting foreign lymphoid cells into irradiated mice. Dekaris also developed an original method for detection of transplantation immunity in mouse skin (»direct« and »transfer« reaction) and at the same time organized and started red cell typing in Adriatic Sea sardine. During his interim fellowship at the Pasteur Institute Dekaris was isolating streptococcal antigens and studied hypersensitivity to group A streptococci in guinea pigs. Together with R. M. Fauve in 1968 he introduced a new method for *in vitro* detection of delayed hypersensitivity – the macrophage spreading inhibition *(31, 32, 33)* (see picture). On returning to Zagreb Dekaris' major interest was study of cellular immunity to bacterial, transplantation and tumour antigens. After leaving the Institute he continued a successful scientific career in the Institute of Immunology Zagreb, where he established a laboratory for clinical immunology and allergology. During his employment with the Ruđer Bošković Dekaris mentored MSc thesis of Branka Ugarković (MD) who continued career elswhere and the MSc and PhD theses of Blanka Veselić *(34)* who remained with the Institute in the group of Branko Vitale.

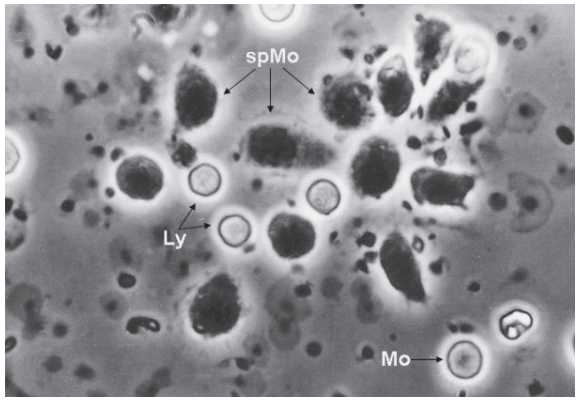

Figure 4. *Phase-contrast micrographs of mononuclear cells isolated from human peripheral blood and incubated with antigen. Monocyte (Mo), spread monocytes (spMo), lymphocytes (Ly), (D. Dekaris, personal archive)*

Mislav Jurin (MD, b. 1939) has worked in the Institute since 1964 till now and during the first twenty-five years was engaged in immunology. His initial research in transplantation medicine was performed using the model of parabiotic mice in order to study graft-versus-host and host-versus graft reactions *(35)* (see picture). During a fellowship in United States he studied modulation of immune reactivity by vitamin A *(36)* and developed interest in tumor immunology. By means of a tumor colony inhibition test the ability of lymphocytes from tumor bearing mice to kill tumor cells depended upon the tumor size and that the sera from tumor-bearing mice blocked lymphocyte action *(37, 38)*. The immune reactivity of tumor bearing mice against skin allografts and sheep red blood cells (SRBC) decreased as the tumor size increased, and recovered after extirpation or irradiation of the tumor. Spleen cells from tumor bearing mice immunized against skin allografts or SRBC transfered the immune memory to lethally irradiated mice *(39)*. His experience acquired in experimental models was implemented in clinical studies. A lymphocyte transformation test was used for monitoring the immune reactivity of patients having cervical, endometrial or ovarian carcinoma. The reactivity was suppressed prior to and during therapy but recovered later on. Patients whose immune reactivity did not recover were at risk of lethal outcome, and patients having the immune reactivity recovered but subsequently declined were at risk of tumor recurrence or metastasis. Similar studies were carried out in patients having multiple sclerosis. In vitro reactivity of their lymphocytes against specific antigens increased shortly prior to relapse of the disease, whereas reaction to a nonspecific mitogen phytohemagglutinin was decreased. Mislav Jurin mentored twelve PhD and three MSc theses. Two of his disciples (Neven Žarković, MD and Siniša Ivanković, DVM) have remained in the Institute pursuing research in experimental oncology or related fields and five of his extrramural disciples became university professors.

Figure 5. *Protection from lethal X-ray irradiation by means of parabiosis. Irradiated animal (left, grey) survived due to transfer of hematopoetic cells from the partner. (M. Jurin, personal archive)*

Luka Milas (MD, b. 1940) was employed with the institute for ten years (1963–72) and during that time spent three years as postdoctoral fellow in Houston, USA. His main interest was tumor immunology. Transplanted tumors stimulated granulocytopoiesis of their hosts *(40)* and on the other hand their skin grafts enjoyed prolonged survival in normal hosts *(41)*. Those effects were attibuted to a secretion of humoral factors subverting the immunohematopoietic system of the hosts. Delayed-type skin reactivity against tumor antigens was produced by means of sensitization with tumor antigens *(42)*. Splenectomy of the hosts increased the number of artificial lung 'metastases' produced by tumor cells injected intravenously *(43)*. Anti-tumor immune response could be improved by means of nonspecific immunization with *Corynebacterium parvum (44)*. Luka Milas continued a successful career in M. D. Anderson Cancer Center in Houston, USA.

Stanković's disciples

Neda Šestan (BSc Med Biochem) joined the Institute in 1956 and participated in early studies of radiation biology, metabolism and immunology with Nikša Allegretti and Veljko Stanković (see their contributions) until her tragic death in car accident in 1968.

Šime Vlahović (MD, b. 1932) worked in the Institute from 1958 until 1965, at first with N. Allegretti and then with V. Stanković studying the effects of irradiation on the immune response *(45)* (see also there). During a postdoctoral fellowship in Cooperstown, USA he became acquainted with bone marrow transplantation in mice *(46)*. Soon after the return from USA Vlahović left the Institute as to become head of the Department of Physiology at the newly founded Medical School in Rijeka. He continued fruitful research in immunology until his premature death in 1977. Several prominent immunologists emerged under Vlahović's leadership.

Ivo Hršak (MD, b. 1936) was employed with the Institute throughout his professional life (1961–2001) and pursued research in immunology for the first twenty–two years. At first he participated in Allegretti's research, using immunofluorescent microscopy in order to demonstrate altered antigenicity of irradiated tissues. Upon return from the military service Hršak joined Stanković's team and together with M. Boranić started bone marrow transplantation in mice *(47)* but subsequently

turned his interest to the influence of thymus on hematopoiesis (at that time a completely unexplored issue) and demonstrated a stimulating influence of thymus on the recovery of hematopoiesis heavily damaged by irradiation or by cytotoxic drugs *(48)*. A significant part of Hršak's research was devoted to immunosuppression accompanying neoplastic growth. In ascitic fluid of Ehrlich carcinoma in mice he discovered a very potent immunosuppressive agent responsible for the immunodeficiency of tumor-bearing animals *(49)*. Injection of a minimal quantity of ascitic fluid into normal mice caused strong immunosuppression. By stimulating the immune reactivity in tumor-bearing mice using injections of *Corynebacterium parvum* or by suppressing it using X-rays or drugs he was able to influence the growth rate of experimental tumors *(50)*. Together with Jelka Tomašić from the Institute's Department of Organic Chemistry and Biochemistry Ivo Hršak studied immunostimulatory properties of a water soluble nontoxic peptidogycan isolated from *Brevibacterium divaricatum (51)*. Parenteral application of that compound stimulated the immune reaction of experimental animals and suppressed the growth of transplantable tumors *(52)*. Pharmacokinetic studies elucidated distribution and metabolic pathways of peptidoglycan after intravenous administration. Much of Ivo Hršak's work was based on a simple but reliable experimental model for enumeration of antibody forming splenocytes, the Jerne's plaque-forming cell assay (PFC) that he introduced into his laboratory in 1968 and generously transfered to several other laboratories. Ivo Hršak mentored two intramural disciples, Krešimir Pavelić and Tanja Marotti who remained in the Institute and significantly contributed to immunology and attained leading positions in Croatian science (see descriptions of their work). Extramural disciples S. Pavičić, M. Šeremet, M. Bura and F. Žunter made successful clinical careers and H. Cena from Kosovo joined the faculty in Priština. Together with M. Boranić Hršak laid out plans for construction of a new building for molecular biology and medicine in the Institute in 1990.

Milivoj Boranić (MD, b. 1936) joined the Institute in 1961 and remained employed with it until retirement in 2001. Initially he was assigned by Veljko Stanković to master bone marrow transplantation in mice, produce bone marrow chimeras, and study the so-called secondary disease that develops in the chimeras due to an incompatibility between irradiated host and transplanted marrow. In 1963 radiation chimeras were obtained and pathology of secondary disease was studied. At that time a pioneer in human bone transplantation, Georges Mathé of Paris postulated that the reaction of the marrow graft against the host (the *graft-versus-host reaction*) might be able to eradicate leukemia cells remaining after whole-body irradiation of patients with leukemia. Boranić explored various possibilities of controling the reaction as to prevent irreparable damage to the host and during a two year fellowship at the Radiobiological Institute in Rijswijk eventually succeeded. The graft-versus-host reaction in mice with transplanted leukemia was mitigated by means of timely administration of immunosuppressive drug cyclophosphamide. Leukemia was eradicated without lethal damage to the host *(53)*. That experimental achievement was highly esteemed by the scientific community. Upon the return to Zagreb, Boranic continued research along those lines with Ivana Tonković (MD) who later became professor of surgery and with Marija Poljak-Blaži (BSc Biol) who continued her career in the Institute. Boranić's international connections and endeavours at home eventually resulted in 1982 in the establishment of a clinical bone marrow center at the University Hospital in Zagreb, the first one in south-east Europe. In the seventies Boranić devoted part of his research interest to tumor immunology *(54)*, together with extramural disciples Zlatko Pavelić (MD) and Božidar Vašarević (MD) and colleague Marko Radačić (DVM). With Jelka Gabrilovac (BSc Med Biochem) and Drago Batinić (MD) he investigated immunological features of mouse and human leukemias. In the eighties Boranić developed interest in neuroendocrine regulation of immunity at systemic level and together with neuropharmacologist Danka Peričić (MD) from the Institute studied effects of stress on immunity in mice and rats *(55)*. His collaborators Ljiljana Križanac-Bengez (MD), Lidija Šmejkal Jagar (MD) and Silvana Stanović Janda (MD) continued that work and investigated effects of various neurotransmitters on lymphoid *(56)* and hematopoietic cells *in vitro (57)*. In addition to scientific research, Boranić partici-

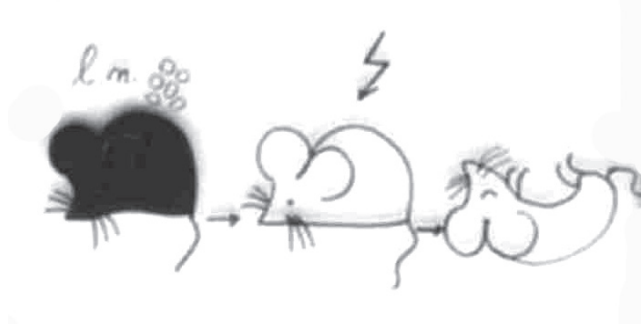

Figure 6. *Principle of lethal graft-versus-host reaction produced by injecting incompatible lymphoid cells into an irradiated host. Cartoon by Ivana Tonković, 1971 (M. Boranić, personal archive).*

pated at postgraduate teaching of immunological curricula. He was the first president of the Croatian Immunological Society (1971–1976), director of Institute's Department of Experimental Biology and Medicine (1973–1977), secretary of a governmental body responsible for construction of new Institute's facilities for molecular biology and medicine, and director of the Institute (2000–2001).

Milivoj Slijepčević (DVM, b. 1938) spent his professional lifetime (1963–2003) in the Institute. At first he participated in studies of bone marrow transplantation and thymus grafting (see contributions of M. Boranić and Ivo Hršak) but soon directed his interest to liver function and immunodeficiency accompanying thermal injuries and diabetes. Liver regeneration was studied in rats exposed to irradiation and bone marrow transplantation *(58)*. During a postdoctoral fellowship in Ulm, Germany, Slijepčević mastered technique of pancreas and pancreatic islet transplantation in rats rendered diabetic by means of pancreatectomy or treatment with streptozotocin *(59, 60, 61)*. That research was continued with his collaborator Mirko Hadžija and broadened to the problems of impaired immunity in diabetes. Diabetic condition was shown to facilitate growth of transplanted tumors *(62)*. Later on, in collaboration with colleagues from the Faculty of Pharmacy, Slijepčević investigated antidiabetic effects of various plant extracts, and with neurophysiologist Milica Bjegović from the Institute studied electrophysiological changes in brains of diabetic animals. Milivoj Slijepčević lectured pathophysiology at the Faculty of Pharmacy and mentored PhD thesis of Mirko Hadžija (see his contribution) and of two extramural disciples (Blanka Jamnicky, Dubravka Juretić).

Olga Carević (BSc Med Biochem) joined the Institute on invitation by Veljko Stanković in 1968 as an accomplished scientist. She came from Pliva Research Institute and before that had been associate professor at the Faculty of Pharmacy and Medical Biochemistry, Zagreb. Her primary interest were cellular lysosomes and in that context she studied immunosuppressive drugs *(63)*. In 1978 Olga Carević continued career in the Zagreb Institute for Health Protection. After retirement in 1989, particularly during the war in Croatia, she published a number of articles and four books advocating national reconciliation. Her disciple Višnja Šverko joined Slijepčević's group and after that, Tanja Marotti (see that contribution).

THE SECOND AND THE THIRD GENERATION

Silobrčić's disciple

Lidija Šuman (BSc Biol) has been employed with the Institute since 1972 and until 2000 pursued research in transplantation immunology. Her primary interest included the response of radiation chimeras to semiallogeneic skin grafts *(64)* and the reaction of female mice against minor H-Y transplantation antigens *(65, 66, 67, 68)*. Professional interests included laboratory animal genetics so that Lidija Šuman besides immunology parallely supervised the laboratory animal colony.

Dekaris's disciple

Blanka Burek (MD, maiden name Veselić) worked since 1969 with D. Dekaris investigating delayed hypersensitivity in rats by means of macrophage spreading inhibition test *in vitro (69, 70)*. Later on, until early retirement in 1998 she studied immunological features of mice with spontaneous leukemia *e.g.* metabolic activity of their lymphocytes *(71)* and immunomodulatory action of bacterial lipopolisaccharides *(72)*. After departure of D. Dekaris from the Institute she joined the group of B. Vitale and participated in studies of graft-versus-host disease and chronic lymphocytic leukemia (see there).

Vitale's disciples

Vesna Tomažič (BSc Biol, maiden name Knapp) started with N. Allegretti in 1964 but soon joined B. Vitale and participated in studies of graft-versus-host reaction *(73, 74)* and specific immunological unresponsiveness *(75, 76)*. She left the Institute in 1978 and continued career in USA in the field of laboratory animal care.

Mila-Kata Hršak (BSc Biol, maiden name Franceschi) spent her professional life in the Institute (1961–1995) working first with D. Dekaris and B. Veselić on delayed hypersensitivity *in vitro* (see their contributions) and later on participating in several studies of tumor immunology (*e.g.* 77, 78).

Mariastefania Antica (BSc Biol) has been employed with the Institute since 1981 and since 1994 has been head of the Laboratory for cellular and molecular immunology. She has had a long standing interest in stem cells and lymphocyte development and differentiation, starting with studies of the features and function of cells from leukemia patients and continuing

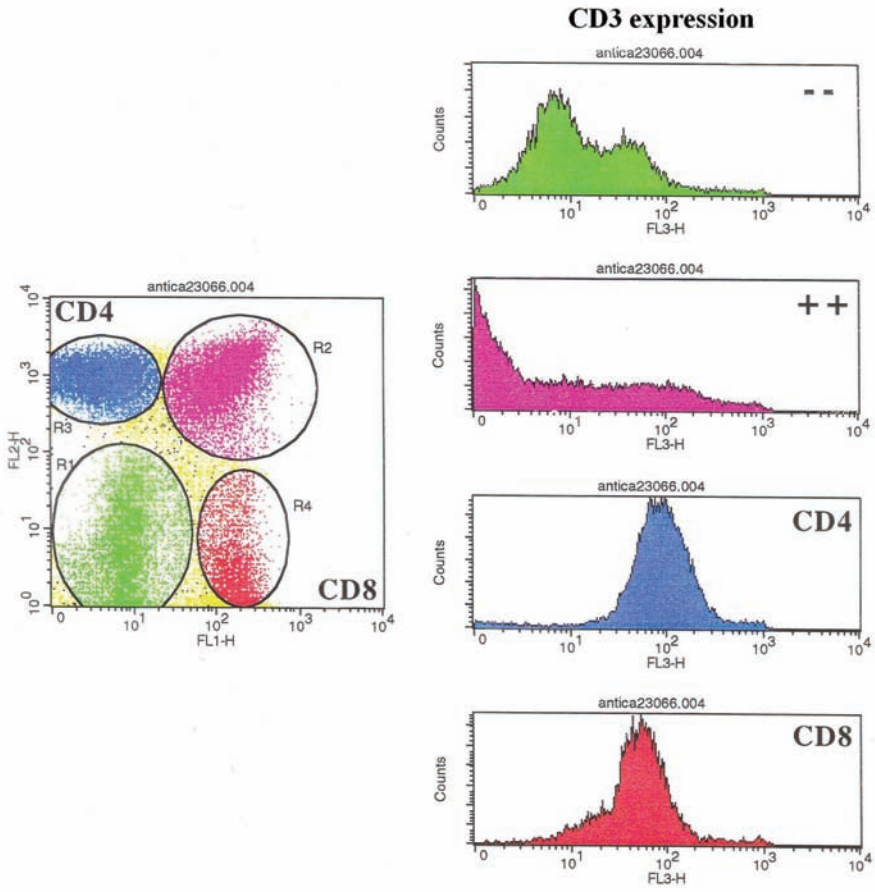

Figure 7. *Multiparametar flow cytometry of fetal organ thymic cultures labeled with antiCD4, antiCD8 and antiCD3 antibodies. Ruđer Bošković Institute, 1996 (M. Antica, personal archive).*

with basic and clinical studies of lymphoid stem cells, especially concerning the time and site of commitment of the lymphocyte lineage precursors. Antica discovered a new population of stem cells in fetal thymus and was the first to describe their activity and developmental potential (*79*). She also described a new population of stem cells in the bone marrow which, although very similar to the committed precursors, still preserved a potential to develop into other lineages besides T and B cells (*80*). With her collaborators Antica cloned murine U2 snRNP-A' gene differentially expressed during lymphocyte development in thymus. Those publications received high recognition and many citations as a significant contribution to immunology as they helped understand regulation of lymphocyte development from stem cells. Further Antica's work showed generation of mature single-positive T cells in lymph nodes, thus proving T lymphocyte development in extrathymic sites. Absence of T cell development in lymph nodes under normal conditions has been attributed to a failure in attracting T cell progenitors or in supporting some early event in T cell differentiation in the nodes (*81*). Antica's current work concerns transcription factors that regulate lymphocyte development and their impairment in lymphoma and leukemia (*82, 83*). A significant contribution of Mariastefania Antica to immunological research in the Institute has been the procurement and maintenance of flow cytometry as a scientific and technical support. In addition to research, Mariastefania Antica participates in undergraduate and postgraduate teaching at the Faculty od Science and Mathematics University of Zagreb and has mentored six extramural BSc theses related to immunology. One of her disciples is active in flow cytometry. Antica is member of several international societies, vicepresident of the Croatian Immunological Society and member of the editorial board of the Croatian Medical Journal and the Internet Hematological Journal.

Borka Kušić (BSc Biol, maiden name Benković) pursued research in immunology from 1977 to 2003. At first she tried to elucidate factors responsible for a great variability of mitogen induced DNA synthesis in lymphocytes *in vitro*. To that end she

studied dynamics of spontaneous and mitogen induced DNA synthesis in mouse lymphocytes harvested before or during rejection of skin allografts. The extent and differences in DNA synthesis reflected dynamics of cellular events induced by the allogenic transplantation, and were observed both in lymphocytes from ipsilateral and contrateral lymph nodes. Subsequent investigations were focused on the effect of insulin and oral antidiabetic drugs on immunological function of diabetic mice. Antidiabetic drugs were not able to restore the immunodeficiency and sometimes even deteriorated it. Turning to clinical immunology, Borka Kušić studied immunological responsiveness of patients with cervical carcinoma and found a suppressed state, particularly in patients with advanced disease. In some patients the suppression persisted long after the therapeutic procedures. After that, Kušić's major interest became characterization of T-lymphocytes and their subpopulations in chronic lymphocytic leukemia which is a clonal expansion of neoplastic B-cells. T cells were separated from B cells by means of rosetting with sheep erythrocytes and centrifugation over a gradient, yielding a 93 % pure T-cell population *(84)* (see picture). It contained immature $CD2^+CD5^+$ T cells, $CD21^+$ T-cells and double positive $CD4^+CD8^+$ cells. Stable phase of the disease was characterized by elevated percentage of $CD8^+$ T cells. Impaired functional reactivity in chronic lymphocytic leukemia was attributed to abnormalities of T-cell subsets (decreased CD4/CD8 index) as well as to abnormalities of B- and $CD8^+$ T-lymphocytes *(85)*. In collaboration with M. Antica, molecular methods were then applied for studies of malignant lymphomas and mucosa-associated lymphoid tissue tumors (MALT) *(86)*. A semi-nested PCR was used to detect clonal rearrangement of immunoglobulin heavy chain genes (IgH) in formalin-fixed paraffin-embedded lymphoid tissues in order to distinguish monoclonal B lymphocytic profiles (considered malignant) from the polyclonal ones. An asset of the method was the use of three primers only. It permitted accurate diagnosis in doubtful examples of lymphomas with apparently benign histological architecture, in gastric biopsies from patients infected with *Helicobacter pylor*i in which monoclonality of lymphoid infiltrates indicated appearance of MALT lymphoma, and in cases of splenic inflamatory pseudotumors *(87, 88)*.

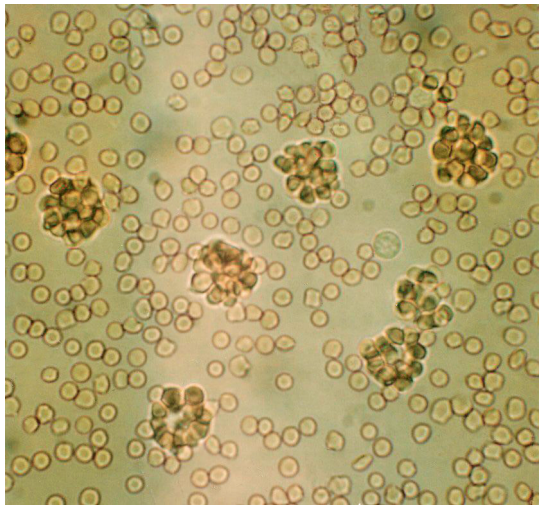

Figure 8. *Rosettes formed by T-lymphocytes from human peripheral blood after incubation with sheep erythrocytes. That technique permitted recognition and separation of T-lymphocytes long before the invention and use of monoclonal antibodies and flow cytometers. (B. Kušić, personal archive)*

Ljiljana Poljak (MD) was employed with the Institute for ten years (1988–98) and during that period spent six years as a postdoctoral fellow in DBMS/CEA, Grenoble, France and then in NIAID/NIH, Bethesda, USA. Starting with studies of chronic lymphocytic leukemia she showed that hypogammaglobulinemia resulted from clonal expansion of peripheral B lymphocytes *(89)*. In order to better understand molecular events underlying generation of leukemic cells she investigated mechanisms regulating the accumulation of $CD5^+$ B cells in blood and peripheral lymphoid organs. Functional heterogenity of leukemic B cells in spite of their clonal origin was found *(90)*. The heterogeneity was not related to the expression of $p53^{c\text{-}myc}$ protein, nevertheless a positive correlation was observed between c-myc gene activity and the ability of leukemic cells to activate apoptotic death. Further research of Ljiljana Poljak was focused on the role of NF-κB transcription factor in the development of follicular dendritic cells, a major cell class regulating fine microarchitecture of peripheral lymphoid organs (*91, 92*). Animals lacking some subunits of NF-κB were deprived of dendritic cells. It was concluded that due to an impaired development of dendritic cells the production of BLC chemokine, a major chemokine for B cells, is defective as well (*93*). These data helped develop a new field of investigation concerning the role of NF-κB transcription factor in the development of fine microarchitecture of peripheral lymphoid organs. Upon her return from abroad Ljiljana Poljak continued her scientific career at the Department of Physiology and Croatian Instiute for Brain Research at the Medical Faculty Zagreb.

Hršak's disciples

Tatjana Marotti (BSc Biol) has been affiliated with the Institute since 1973. At the beginning she investigated immunoregulatory properties of ascites fluid of various origin (mice and human) together with Ivo Hršak *(94)*. The main field of her subsequent and independent research has been dedicated to free radicals superoxide anion and nitric oxide as well as to antioxidative enzymes such as superoxide dismutase, catalase and glutathione peroxidase *(95)*. Their role in ageing as well as the gender differences in response to oxidative stress were explored. Tanja Marotti also studied influence of opioid peptides (mainly methionine- and leucine-enkephalin) on oxidative/antioxidative potential *(96)*. The main conclusion acquired from those studies was that opioid peptides were genuine immunomodulators, increasing moderate immunological responses and decreasing the enhanced ones. The response to oxidative stress was related to the age and gender, females being more resistant to stress *(97, 98)*. Those findings have been brought into connection with higher incidence of liver carcinogenesis in older male mice. Tanja Marotti is now head of the Laboratory for biological response modifiers and holds significant positions in the Institute as well as in several extramural committees and bodies. She participates in teaching immunology related curricula at the University of Zagreb and has mentored three intramural and four extramural PhD or MSc theses. Her disciples Tihomir Balog and Sandra Sobočanec have remained in the Institute and Helena Haberstok continued her career in the United States. All three have made valuable contributions to cellular and molecular immunology.

Krešimir Pavelić (MD) is correspondig member of Croatian Academy of Sciences and Arts and full member of European Molecular Biology Organization (EMBO). He joined the Institute in 1975 and has been employed with it until now. Currently he is director of the Institute's Division of Molecular Medicine and one of the most productive Croatian scientists. His early interests were focused on tumor immunology and endocrinology, and subsequently evolved to cancer genetics and molecular medicine. He spent two years as postdoctoral fellow in Buffalo and one year as a Fulbright visiting professor in Cincinnati and Rochester, USA. His work in the Institute started with studies of tumor immunology, particularly of the effects of immunosuppression and immunostimulation on tumor growth *(99)*. Later he described total recovery of immune system in diabetic mice after treatment with insulin *(100)*. Observed depression of immunological functions was attributed to impaired transport of glucose into immunocompetent cells. Insulin and glucagon suppressed growth of several tumors in animals, which could be attributed to maintenance of high immune reactivity and phagocytosis *(101)*. Together with S. Vuk-Pavlović and Ž. Bajzer he described self-incitement of tumor growth mediated by growth factors and an autocrine loop in tumor growth control by insulin-like growth factors *(102, 103)*. Later he introduced somatostatin in antitumor therapy *(104)*. Pavelić mentored 22 BSc theses and 20 PhD theses. Some of his disciples became directors of scientific institutes and clinics (R. Spaventi, V. Božikov, B. Pekić, S. Marušić, V. Baltić, S. Radić, Lj. Pavelić), university professors and heads of departments (D. Vrbanec, N. Pećina Šlaus, B. Krušlin, I. Zgradić, S. Levanat, S. Kapitanović). Pavelić payed special attention to rebuilding the laboratories and animal housing in the Institute. Modern molecular medicine facilities have been established, including a high-throughput unit for transcriptomics and proteomics coupled with DNA-chip technology and tandem mass spectoroscopy, which found useful application in clinical laboratory diagnostics. Pavelić has occupied leading positions in the Institute as well as in governmental bodies concerned with science. Presently he is vice-president of the European Molecular Biology Conference, president of the Croatian National Scientific Council and member of the standing committee of the European Medical Research Council, European Science Foundation.

Boranić's disciples

Ivana Tonković (MD) worked with Milivoj Boranić from 1970 till 1973 studying the antileukemic effect of graft-versus-host reaction in mice *(105)*. She left the Institute in order to became a surgeon (a rare commitment for ladies at that time) and made successful career in vascular surgery as professor at the Medical Faculty Zagreb.

Marija Poljak Blaži (BSc Biol) joined the Institute in early 1971 and explored separation of mouse spleen cells over a discontinuous gradient of Ficoll in order to obtain distinct subpopulations of lymphoid and hematopoietic cells. At that time cell separators and flow cytometry were still far ahead. Poljak Blaži succeeded in dissociating the cells into seven fractions: two lightest ones contained hematopoietic colony-forming units and repopulated mouse bone marrow damaged by irradiation and two heavy fractions contained immunocompetent cells mounting graft-versus-host reaction. The heaviest (bottom) fraction was immunology inactive *(106)* (see picture). If the light fraction was transplanted together with the heavy one the recipients' potential to generate immunocompetent cells producing antibodies against sheep red blood cells (PFC) was restored, provided the recipients were deprived of thymus. On the other hand the generation anti-host reactive cells was strongly suppressed in the absence of a thymus. These data showed a cooperation of T and B cells in humoral immune response *(107)*. In the eighties Poljak-Blaži joined M. Boranić in studies of the effects of stress on immunity (see there).

Poljak-Blaži explored in detail the mechanism of immune suppression elicited by means of administration of allogeneic red blood cells exposed to ultraviolet C (UVC) irradiation and found that the tolerogenic effect required function of the re-

Figure 9. *A discontinuous gradient of dextran loaded with mouse spleen cells and centrifuged. The cells have segregated into seven fractions seen as greyish rings at interfaces between layers of the gradient. (M. Poljak-Blaži, PhD thesis, 1971)*

cipient's T cells. Sera of mice treated with UV-irradiated blood showed low interleukin1 (IL-1) activity. Dilution of the sera, however, restored IL-1 activity, indicating the presence of an IL-1 inhibitor involved in the generation of immunological unresponsiveness *(108)*. In several other papers Poljak-Blaži studied immune reactivity of mice with leukaemia or melanoma in an attempt to explain the mechanism of that immunodeficiency. Her recent research has explored oxidative damage caused by reactive oxygen species (ROS) from activated neutrophils. In mice bearing B16-F10 melanoma that mechanism was responsible for tumour progression *(109)*. Neutrophils obtained from a papule elicited by means of a subcutaneous injection of Sephadex were cytotoxic for the B16-F10 cells *in vitro*, nevertheless Sephadex injection shortened the survival of tumor bearing mice. Sephadex-induced inflammation probably attracted neutrophils from the tumour site and thus allowed faster progression of the tumour *(110)*. Similar results were obtained in rats bearing Walker carcinosarcoma (W256). Those experiments stressed the contribution of natural immunity in tumor regression. Marija Poljak-Blaži mentored BSc theses of Mirko Hadžija and Morana Živković (both employed with the Institute), Berislav Bošnjak (now employed with a pharmaceutical company) and Andrea Kolesarić.

Jelka Gabrilovac (BSc Med Biochem) has been employed with the Institute since 1972. Her research interests included clinical immunology, neuroimmunology and molecular biology of inflammatory processses. She introduced clinical laboratory methods for the recognition of T- and B-lymphocytes and NK-cells. The rosette assay with sheep erythrocytes was used for studies of leukemia cells in experimental animals and patients *(111)*. Cytotoxic assay with ^{51}Cr labelled target cells was used *e.g.* for longitudinal study of NK-cell activity during normal human pregnancy and showed an initial increase in the first trimester followed by decrease in the second and third trimester. There was an inverse correlation between estrogen levels and NK-activity *(112)*. In children with acute lymphoid leukaemia, low NK-activity was associated with poor prognosis. Research in neuroimmunology showed endogenous opioid peptides to modulate immune responses either directly by their binding to opioid receptors on immunocytes or indirectly via opioid receptors on neuroendocrine cells *(113)*. Endogenous opioid peptides methionine enkephalin, leucine enkephalin and dynorphin-A modulated proliferative ability, NK-cell activity, cytokine secretion and NO production *in vitro* and *in vivo (114)*. Functionally active mu-opioid receptors associated with Ca^{++} as the second messenger was demonstrated on a human myeloid cell line. Recent studies of Jelka Gabrilovac explore the role of membrane peptidases in immune and inflammatory responses. Several membrane-bound peptidases (aminopeptidase-N/CD13, neutral endopeptidase/CD10 and dipeptidyl-peptidase IV/CD26) have been shown to interfere with growth, differentiation, activation and death of immunocytes by means of fine-tuning local concentrations of growth factors, hormones, chemokines and cytokines. Expression of membrane-bound peptidases, their regulation by cytokines and their roles in immune responses have been explored in various cell types. Expression of aminopeptidase N has been described on cultured human keratinocytes and shown to participate in regulation of their growth *(115)*. Neutral endopeptidase was also shown to participate in maturation of early B-cell precursors. Jelka Gabrilovac has been engaged in postgraduate courses at the University of Zagreb and mentored four PhD theses and three MSc thesis. Her intramural disciples Irena Martin Kleiner (BSc Med Biochem) and Barbara Čupić (BSc Mol Biol) have continued their research in the Instutute and Davorka Breljak (BSc Mol Biol) did so in the Institute for Medical Research. Extramural disciples Ljubica Raić (MD) and Marija Zekušić (BSc Biol) work elsewhere. Since 2000 Jelka Gabrilovac is head of the Laboratoty for experimental hematology, immunology and oncology.

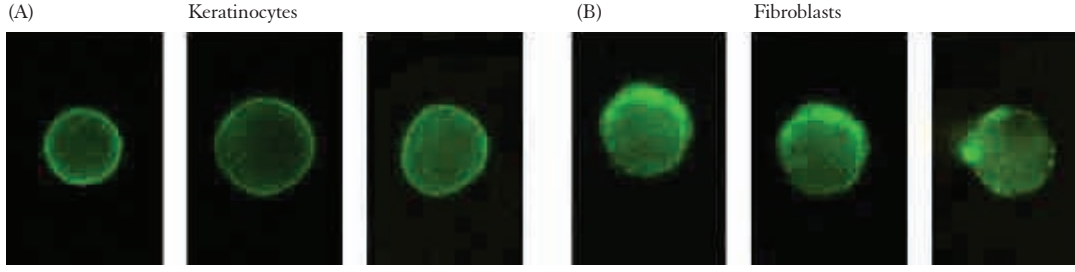

Figure 10. *Expression of CD13 on keratinocytes as compared to skin fibroblasts. Moderate, homogenous, ring-like CD13 distribution on keratinocytes vs. strong, patchy or polarized expression on fibroblasts (Gabrilovac J et al. 2004 Immunology Lett 91: 39–47)*

Marko Radačić (DVM) has been employed with the Institute since 1973. His interests have been tumor immunology and later on experimental oncology. Using three experimental mouse leukemias he investigated adherence, migratory ability and phagocytic activity of tumor cells *in vitro* and their distribution *in vivo* in order to see to which extent neoplastic cells retained functional features of their normal lymphoid and myeloid counterparts. The capability of tumor cells to adhere onto glass surface was tested in heparinized glass tubes *(116)*. Adherent cells were detached from the tubes and then incubated in petri dishes with *Escherichia coli* or latex particles. Tumor cells did ingest the particles *(117)*. Their migration from the tubes was limited *(118)*. Distribution *in vivo* was followed by means of labeling the cells with radioactive Cr^{51} before injection and found to resemble normal pattern *(119)*. Those experiments have shown that leukemia cells do retain some features of normal lymphoid and myeloid cells *(120)*. Marko Radačić also participated in studies of the effects of stress on immune response *(121)*. Later on his interest turned to experimental chemotherapy and in that field he developed fruitful collaboration with many scientists throughout Europe through the European Organization for Cancer Research (EORTC). Radačić participates in postgraduate teaching of laboratory animal care at the universities of Zagreb, Rijeka and Osijek.

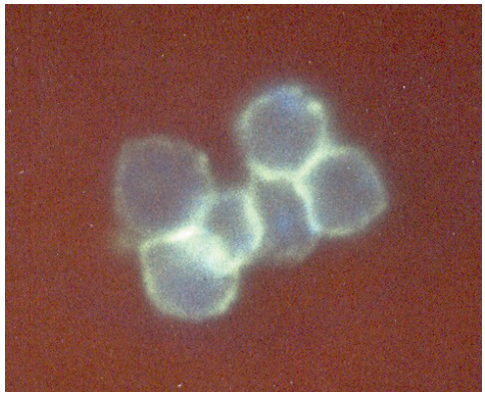

Figure 11. *Cells of lymphoid leukemia stained with FITC-conjugated anti-CD10 monoclonal antibodyFluorescent microscopy, 1985 (D. Batinić, personal archive)*

Drago Batinić (MD) was employed with the Institute for six years (1983–1988) and during that time pioneered application of monoclonal antibodies for the recognition of surface markers on normal human hematopoietic cells and leukemia cells. Flow cytometers were not available at that time and Batinić used fluorescent microscopy for his studies. In collaboration with clinicians he described the pattern of surface markers in childhood acute leukemia using a limited, but well-defined panel of monoclonal antibodies *(122, 123)*. That research was broadened by a wider antibody panel recognizing more differentiation antigens *(124)*. He also collaborated in studies defining the expression and specificity of an immature cell marker nowadays classified as CD34 *(125)*. After completion of his PhD thesis Batinić joined the Department of Clinical Laboratory Diagnosis at the Clinical Hospital Center Zagreb in order to coordinate a flow cytometry lab equipped with the first flow cytometer in Croatia *(126)*. He continued a successful career as a clinical laboratory immunologist and professor of immunology at the Zagreb Medical Faculty.

Ljiljana Križanac-Bengez (MD) was employed with the Institute from 1986 till 1998 and during that period spent five years as a postdoctoral fellow at the Fred Hutchinson Cancer Research Institute in Seattle, USA. She studied effects of opioid peptides on lymphatic and hematopoietic cells and pioneered establishment of clonal *(127)* and long-term *(128)* cultures of mouse or human bone marrow in the Institute.The effects depended on the circadian rhythm of the proliferative activity of harvested cells. In Seattle Ljiljana Križanac-Bengez investigated the features of pluripotent $CD34^+$ hematopoietic cells by means of flow cytometry. She contiunued her career in the States.

Lidija Šmejkal-Jagar (MD) spent in the Institute eight years (1988–1996) studying the immunomodulatory effects of agents affecting serotoninergic transmission *in vivo* and *in vitro*. The observed suppression was attributed to central actions of the drugs as well as to their direct effects on lymphoid cells expressing appropriate receptors *(129, 130, 131)*. Lidija Šmejkal-Jagar continued her career in pharmaceutical companies.

Silvana Stanović-Jagar (MD) continued the work of Lj. Križanac-Bengez and during employment with the Institute (1993–2001) studied effects of opioid peptide inhibitors in clonal and long-term cultures of human bone marrow *(132, 133, 134)*. She continued career in a trade company providing microbiological reagents for laboratories.

Slijepčević's disciple

Mirko Hadžija (BSc Biol) has been employed with the Institute since 1977 and since 2000 has been head of the Laboratory for Molecular Endocrinology and Transplantation. He has had a long standing interest in lymphocyte development and differentiation in diabetic condition. In the early period he investigated the effect of insulin and oral antidiabetic drugs on immunological function of diabetic mice. Antidiabetic drugs were not able to restore immunodeficiency accompanying the diabetic condition but recovery of diabetes as well as of the immunodeficiency was achieved by transplantation of isolated Langerhans islets *(135)*. Subsequently Hadžija extended experimental studies to clinical immunology of diabetes. With collaborator Ivana Djurinović-Bello he made significant contribution to understanding the function of the immune system

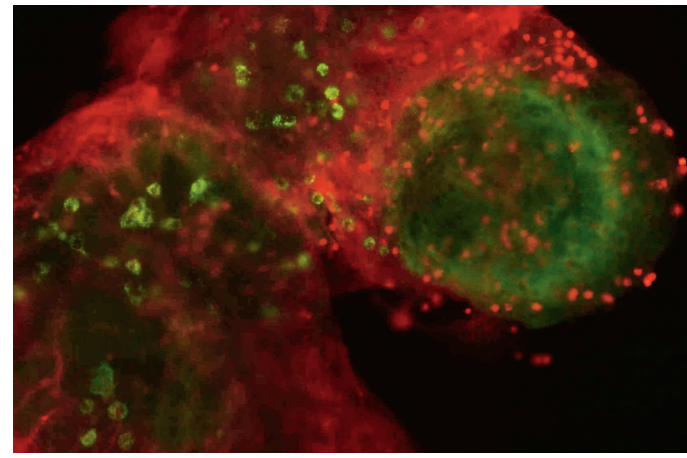

Figure 12. *Infiltration of CD4 T cells in Langerhans islet of a NOD-strain mouse with spontaneous diabetes. Fluorescent microscopy. (M. Hadžija, personal archive, 2005)*

in development of diabetes mellitus type I (*136*). Its pathogenesis could be attributed to failures in early stages of T-cell development resulting in an absence of T-cell progenitors or non-appearance of antibody-presenting cells (APC). Further work showed generation of »diabetogenic proteins« by APC and their role in maturation of $CD4^+$ T-lymphocytes in pancreatic lymph nodes (*137, 138*). During a postdoctoral fellowship in Canada Mirko Hadžija made significant contribution to immunopathology of diabetes by showing a possibility of reduction of inappropriate immune system function in the pathogenesis of diabetic neuropathy (*139*). In addition to research, Mirko Hadžija participates in undergraduate and postgraduate teaching at the Faculties of Pharmacy and of Science and Mathematics at the University of Zagreb and has mentored three BSc and one PhD theses related to immunology and diabetes.

Carević's disciple

Višnja Šverko (BSc Biol) has worked in the Institute since 1971, first with Olga Carević and later on with M. Slijepčević and T. Marotti. Her attention in immunology was focused on the recovery of the immune system and on changes of biochemical markers in stress *(140)*, diabetes *(141)*, lymphoid leukaemia *(142)* and malignant tumors in experimental animals and humans *(143)*. Improvement of immunological reactivity was assesed after application of biological response modifiers (peptidoglycans, enkephalins) or lysosomes, sialic acid, enzymes etc. *(144)*. Recent research has been directed toward the influence of biological response modifiers on oxidant/antioxidant status with respect to gender and age of experimental animals in physiological and pathological conditions. Biological response modifiers have been found to interfere with cell and lysosomal membranes and to modulate peroxidation of membrane lipids initiated by hydroxyl free radicals.

Marotti's disciple

Tihomir Balog (BSc Med Biochem) has been active in the Institute since 1992, mainly in three fields of immunology. First, the determination and definition of aminopeptidase (APN/CD13) and neutral endopeptidase (NEP/CD10) enzyme activities in human neutrophils. Donor-dependent APN and NEP enzyme activities were correlated with nonspecific immune response (release of superoxide anion from neutrophils) and with imunomodulatory actions of met-enkephalin *(145)*. Measurement of NEP/CD10 in neutrophils from patients with different types of adrenal gland tumors demonstrated that the enzyme activity could be used in diagnosis of pheochromocytoma *(146)*. Second field of Balog's scientific interest has been devoted to the activity of antioxidative enzymes (catalase, glutathion peroxidase and superoxid dismutase) in different tissues and cells after treatment with pharmacologically active compounds. The third part has been concerned with nitric oxides release from macrophages and human neutrophiles upon stimulation with opioid peptides and endomorphines, and with definition of immunomodulatory mechanisms of opioid peptide met-enkephalin *(147)*. Recent work is endomorphine regulation of inducible NO-synthase (iNOS) activity and iNOS protein expression in a macrophage cell line *(148)*. Amperometric detection of NO release, western blot analysis of iNOS protein and detection of proinflamatory cytokines TNF alpha and IL1 beta have been used as research tools. Those studies have been summarized in a monograph *(149)*. Tihomir Balog is currently mentoring a PhD student (Ana Šarić, BSc Biol).

Gabrilovac's disciples

Irena Martin-Kleiner (BSc Med Biochem) has been associated with the Institute since 1986 and was involved in studies of neuroimmunology and tumor immunology. She investigated *in vitro* the effects of opioid neuropeptide methionine enkepha-

lin on natural killer cell (NK) activitity and cAMP of peripheral blood lymphocytes. The enkephalin affected lymphocytes in a bimodal manner, both increasing and decreasing the NK-activity and cAMP level *(150)*. In leukemia and thymoma cell lines the enkephalin and its synthetic analogs decreased intracellular concentrations of calcium, the second messenger *(151)*. Synthetic delta, mu and kappa opioid peptides inhibited proliferation of a pre-B-leukemic cell line, showing participation of opioids in the control of lymphoid cell proliferation *(152)*. Methionine enkephalin increased the expresion of membrane marker CD10 in a pre-B-leukemic cell line. Irena Martin Kleiner also studied immunomodulator and antitumor effects of phorbol myristate acetate (PMA), diazenes and doxorubicin on proliferation and maturation of leukemic cell lines of T-, B- or myelomonocytic origin *(153)* and took part with J. Gabrilovac in investigations of NK-cell activity in childhood leukemia *(154)*.

Davorka Breljak (BSc Mol Biol) worked in the Institute from 1994 to 2004, at first with M. Boranić investigating the effects of opioid peptides and the expression of membrane peptidases in cell cultures. In clonal cultures of mouse bone marrow cells opioid peptides enkephalins inhibited CFU-GM generation depending on the time of marrow harvest and on the presence of accessory cells *(155)* showing the participation of neuropeptides in regulation of hematopoiesis *(156)*. Subsequently she joined Jelka Gabrilovac and together with her young collaborator Barbara Čupić investigated activity and expression of membrane enzymes aminopeptidase N (APN; CD13) and neutral endopeptidase (NEP; CD10) in human leukemia cell lines and in primary cultures of human fibroblasts and keratinocytes. In promyelocytic cell line HL-60 cytokine IFN-γ affected APN at the mRNA, CD13 and enzyme activity levels depending on duration of the exposure *(157)*. In B-lymphoid cell line NALM-6 dexamethasone at low concentrations affected expression and activity of NEP, suggesting a role glococorticoids in B-lymphocyte maturation under physiological conditions *(158)*. Davorka Breljak continued her scientific career in the Institute for Medical Research, Zagreb.

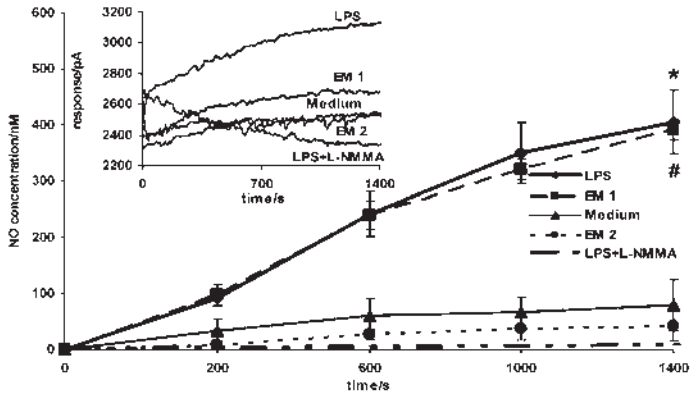

Figure 13. *Amperometric measurement of real time nitric oxide (NO) release from J774 macrophages incubated with endomorphines. From Šarić A, Balog T, Sobočanec S, Marotti T 2007 Neuroscience 144: 1454–1461*

Pavelić's disciple

Suzana Marušić (MD) joined the Institute as a graduate student in 1981 and worked with K. Pavelić until 1989, investigating dynamics of positive and negative selection of T-lymphocytes in thymus *(159)*, hormonal changes in immunized animals *(160)* and autocrine regulation of tumor growth *(161)*. During a study visit to Washingon University in Pullman, she mastered the technique for monoclonal antibody production *(162)*, implemented it in the Institute and disseminated to other scientific institutions. She continued career in USA.

CONCLUSION

As seen from this account of interests and achievements of scientists who spent whole professional life or at least a significant part of it in the Institute, immunological recearch in the major Croatian institute for natural sciences evolved from radiation biology and immunology in the fifties and sixties of the past century, over transplantation and tumor immunology in the sixties, seventies, and eighties, to molecular immunology in the nineties and today. At the beginning experimental models were rats, mice and guinea pigs, and later on, as the connections with clinical institutions were established and consolidated, cells and tissues from patients or healthy volunteers. Today, cell lines or primary cell cultures are increasingly used for the research, and modern methods of molecular biology and genetics have been been implemented.

Professional profiles of the scientists changed over time. The first echelon mainly consisted of biologists, followed by a propulsive group of physicians and veterinarians who consolidated, developed and ramified immunological research in the Institute and disseminated specific knowledge and techniques to many extramural sites. Newer generations are again dominated by biologists.

Genealogy of the »progeny« of the two founders of the Institute's immunology – Nikša Allegretti and Veljko Stanković, covering the first two generations should be presented in a future account about development and fate of the Institute's immunology.

From the very beginning immunologists of the Ruđer Bošković Institute have participated in postgraduate teaching at the University of Zagreb and at other Croatian universities and mentored dozens of scientists who continued their careers in immunology or related fields at universities, clinics, research institutions or pharmaceutical companies at home or abroad. In that manner the Institute played an important role in Croatian science as a breeding ground for young scientists.

Science is produced by dedicated individuals, but it also results from collective endeavour of research teams whose members complement each other with knowledge, skills, ideas and perseverance. In the course of fifty years of immunological research in the Institute many research teams have emerged, and flourished, then lost momentum and eventually disintegrated. Fortunately enough, scientific competition between the teams has never turned into factiousness. Institut's immunologists have preserved cohesion and friendship. And in spite of temporary financial restrictions, overused pieces of equipment, cramped space and limitations on import of chemicals and labware, they succeeded in keeping abreast with more fortunate colleagues working in well-equipped laboratories abroad.

Several immunologists who spent their professional lives in the Institute or continued career elsewhere have achieved high international recognition and/or occupied important positions as project leaders, scientific policy makers, book or journal editors, founders and presidents of scientific societies etc. Observing the paradigm set by the great Croatian scholar after whom the Institute was named, immunologists of the Ruđer Bošković Institute strived towards scientific excellence, international cooperation, and personal advancement during postdoctoral fellowships or study visits to leading scientific institutes abroad.

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INTRODUCTION

At the occasion of the 40th Anniversary of the Croatian Immunological Society, we would like to describe the activities of Croatian immunologists, members of the Society, working at the Institute of Immunology Inc., Zagreb (the Institute). Apart from Professor Drago Ikić (full member, Croatian Academy of Sciences and Arts, CASA), the founder of the Institute, whose activities date from 1961, the activities of other members include a time span of forty years. The period almost exactly overlaps with the formation of the Society.

We gratefully acknowledge the participation of the following colleagues in preparing this chronological report of the main topics of research and development at the Institute: Professor Vlatko Silobrčić full member, CASA; Professor Renata Mažuran; Ivna Svoboda Beusan, Ph.D.; Ante Sabioncello, Ph.D.; Alenka Gagro, M.D., Ph.D.; Branka Vranešić, Ph.D. and Professor Jelka Tomašić. The concepts of their written contributions were not changed. Our special gratitude goes to Drago Ikić, who helped us in selecting references pertinent to his research, and to Renata Mažuran for the brief account of his contribution.

ACTIVITIES OF DRAGO IKIĆ

Among the contributions of D. Ikić, the foundation of the Institute stands out as the single most important one. It was in 1961 when the Institute, as the successor to the Institute for preparation of immunobiologicals (founded in 1893), was established.

As the Director of the Institute for Control and Testing of Immunobiological preparations, he separated the Institute for Preparation of Sera and Vaccines from the Central Public Health Institute and founded the Institute of Immunology, remaining as its Director until retirement in 1982.

Internationally acclaimed production and sale of vaccines and his activities for the World Health Organization (WHO) are among other outstanding contributions of D. Ikić.

He initiated the development and production of bacterial *(1, 2)* and viral *(3–5)* vaccines, the human leukocyte interferon *(6–8),* and large-scale use of human diploid cells *(9)*.

WHO Expanded Programme on Immunization (EPI) was initiated at the 29th Assembly of the WHO (Geneva, 1976). The aim of EPI was to help national health services to extend their immunization programmes against 6 ubiquitous and potentially dangerous infections: diphtheria, pertussis, tetanus, poliomyelitis, measles and childhood tuberculosis. At that time, the following vaccines produced by the Institute in Zagreb had been licensed for massive (extensive) use (Table 1). Chronologically, production started in 1954 at the Serovaccinal Department of the Central Institute for Public Health (animal plasma products, bacterial and measles vaccines), was continued and expanded at the Institute including human plasma products, more viral vaccines and interferon.

TABLE 1

List of developed and licensed products at Institute of Immunology grouped by main product lines.

PRODUCT	FIRST REGISTRATION
Viral Vaccines	
Measles vaccine live, attenuated, Leningrad 16, CF	1967
Measles vaccine live, attenuated, Edmonston-Zagreb, HDC	1967
Rubella vaccine live, attenuated, HPV-77–Zagreb, HDC	1970
Rubella vaccine live, attenuated, RA27/3 HDC	1980
Mumps vaccine live, attenuated, L-Zagreb, CF	1972
Mumps vaccine live, attenuated, PZH-17 HDC	1980
Measles and rubella vaccine live, attenuated, Edmonston-Zagreb, RA27/3 HDC	1972
Measles and mumps vaccine live, attenuated, Edmonston-Zagreb, HDC and L-Zagreb, CF	1974
Measles, mumps and rubella vaccine live, attenuated, Edmonston-Zagreb HDC; RA27/3 and L-Zagreb, CF	1974
Influenza (type A) vaccine,live, attenuated	1968
Influenza (type A) vaccine, inactivated	1970
Influenza (type A + type B) vaccine, inactivated	1973
Inactivated polio vaccine	1959
Oral polio vaccine, live, attenuated, simian cells	1961
Oral polio vaccine, live, attenuated, human diploid cells (HDC)	1968
Small pox vaccine	1970
Bacterial vaccines (23,32*)	
Tetanus vaccine (adsorbed)	1954
Diphtheria and tetanus vaccine (adsorbed)	1955
Diphtheria and tetanus vaccine for adults and adolescents (adsorbed)	1962
Diphtheria, tetanus and pertussis vaccine (adsorbed)	1960
Diphtheria, tetanus, pertussis and parapertussis vaccine (adsorbed)	1965
Meningococcal polysaccharide (group A + C) vaccine, freeze-dried	1978
Typhoid vaccine, inactivated	1965
V.cholerae vaccine, inactivated	1960
S.Typhy, S.paratyphi A+B, V.cholerae (Inaba+Ogawa) vaccine inactivated	1960
Tetanus toksoid and *S.typhi* vaccine inactivated, adsorbed	1976
Human plasma products	
Albumin (human) 5% solution for infusion	1975
Albumin (human) 12% solution for infusion	1972
Albumin (human) 15% solution for infusion	1962
Albumin (human) 20% solution for infusion	1962
Coagulation factor VIII for iv. Use	
Human normal immunoglobulin for im. administration, 16% solution	1978
Human normal immunoglobulin for intravenous administration	1980
Human tetanus immunoglobublin	1970
Human rabies immunoglobulin	1985
Human hepatitis B immunoglobulin	1987
Human anti-Rh0 D erythrocytes immunoglobulin	1975
Human small-pox immunoglobulin	1970
Pertussis immunoglobulin	1964

Animal plasma products	
Tetanus antitoxin (equine)	1954
Diphtheria antitoxin (equine)	1954
Viper venom antiserum, European (equine)	1954
Lactrodectus tredecimguttatus antitoxin	1954
Gas-gangrene antiserum (equine)	1954
Antilymphocyte globulin (equine)	1972
Others (257,258,259)	
Human leukocyte interferon, freeze dryed for im. Injections	1972
Human leukocyte interferon, ointment	1972
Human leukocyte interferon, oil suspension	
Human leukocyte interferon (IFN alpha n3), vaginal preparation	1989
Allergen products	1959

Main accomplishments

Characterization of human diploid cells (HDC), and the development of viral vaccines on human diploid cell substrates

Successful use of human diploid cell substrates for the production of viral vaccines was demonstrated more than 40 years ago. The experience gained with oral poliomyelitis and other viral vaccines in immunizing millions of children in many countries clearly demonstrated the safety of vaccines produced on such substrates. The essential features of diploid cell lines of human (*e.g.* WI–38, MRC–5) or monkey origin are: they have a finite capacity for serial propagation, which ends in senescence; they are non-tumorigenic and display diploid cytogenetic characteristics with a low frequency of chromosomal abnormalities. The main advantage of diploid cell lines, in comparison to primary cells, is that they can be well characterized and standardized, and their use can be based on a cell bank system.

Development of the viral vaccine strains (Edmonston-Zagreb, L-Zagreb)

At the Institute new measles and mumps vaccine strains were developed. According to WHO, and in comparison to the other measles vaccine strains, Edmonston-Zagreb strain is safe and highly immunogenic with long lasting protection against measles.

Activities in collaboration with the World Health Organization (WHO)

Experts from the Institute served on the WHO Panel for Biological Standardization from 1957 to 1982. Also, many of them have been assigned as WHO consultants. The Institute organized WHO Interregional Courses for Biological Standardization in 1967, 1968, 1973 and 1976. Drago Ikić was the principal investigator for the Collaborating Laboratory for Scientific Research and Establishment of the Reference Preparations and Methods of the WHO. He also headed the WHO International Reference Centre for Bacterial Vaccines (1973–1981). This International Reference Centre was active until 1992, although additional collaborating studies (on pertussis vaccine and on diphtheria and tetanus toxoids) were performed even after this. In the period 1983–1993 another WHO Centre (Collaborating Centre for Research and Reference Service for Immunobiological and Biological Products) was active within the Institute. The Institute has organized eighteen International Meetings (mainly cosponsored by the Croatian, former Yugoslav, Academy of Sciences and Arts) resulting in 14 published Proceedings in English. The meetings and the published material cover the following fields: biological standardization (1957), immunology (1959), microbiological standardization (1960), characterization and use of human diploid cells (1963, 1970), biological standardization of cell cultures (1964), oncogenicity of viral vaccines (1968), smallpox eradication (1969), acute respiratory diseases (1969), influenza vaccine (live) (1971), bacterial vaccines (1971, 1977), combined vaccines (1972), field trials of vaccines (1973), production, standardization and clinical use of interferons (1975, 1977, 1979), effectiveness and stability of vaccines against measles, poliomyelitis and pertussis (1976).

Establishment of the Research Department at the Institute

While Director of the Institute, D. Ikić established the Research Department. He also initiated teaching of Immunology at the University of Zagreb including scientists from the Institute in the activities and teaching at the University.

Established by D. Ikić, the Department was chaired by Professor Dragan Dekaris, full member, CASA (1982 to 1987, and 1992 to 2001, when he retired). V. Silobrčić lead (was head of) the Department from 1987 to 1992, and Professor Sabina Rabatić took over the Department in 2001 and is presently its head.

THE ACTIVITIES OF THE RESEARCH AND DEVELOPMENT DEPARTMENT OF THE INSTITUTE

Transplantation Immunology

At the end of 1969, V. Silobrčić accepted the offer of a position at the Institute to organize a laboratory for transplantation and tumor immunology. These were the beginnings of investigations in those fields at the Institute.

The subject of the planned research at the Institute was defined by Silobrčić's previous interests in transplantation tolerance, immunosuppression and tumor immunology. Within these interests there was a practical goal connected with his participation in the initiation of kidney transplantation in Rijeka. Specifically, he organized the production of horse anti-human lymphocyte globulin (ALG), to be applied in patients with transplanted kidneys. Associated with the production of ALG, a test for the immunosuppressive potential of the product *in vitro* was developed, which showed a good correlation with the survival of skin allografts in monkeys *(10, 11)*.

With regard to transplantation tolerance interest was centered on the duration of cellular chimerism in life-long tolerance of allografts in mice *(12, 13)*.

In parallel with the mentioned investigations, tumor immunology occupied an important role in the investigations of the Unit. The question of detecting and following the immunological reaction of hosts to malignant tumors in experimental animals, and later on in humans, was intensively studied *(14–17)*.

Since infections and immunity were at the core of the activity of the Institute, a few papers were published on immunity in some diseases, particularly against mycoses (*18*). Since there was a need to stimulate the immune response in certain conditions, peptidoglycan monomer was studied as a possible immunostimulator *(19)*.

During Silobrčić's employment at the Institute, he spent two sabbatical years at the Department of Radiation Medicine, Massachusetts General Hospital and Harvard, Medical School, Boston, USA. On both occasions tumor immunology was the main subject of his research and a number of papers were published on the matter *(20, 21)*.

From 1992 to 1997 he was appointed General Director of the Institute. Besides successfully running the Institute (yearly profit was at the level of 10 % of the original capital) as a vaccine and sera producer with a global market, there were two events that should be mentioned as important: one was the celebration of the Hundredth Anniversary (Centenary) of the Institute, and the other was the transformation of the Institute into a shareholder company.

R. Mažuran lead the Transplantation Immunology Unit from 1989, after V. Silobrčić became Head of the Research and Development Department and later on the Director of the Institute. The research was focussed on innate immunity, i.e. the role of natural killer cells and cytokines (especially IFNs type I) in different pathological conditions (*22–29*). Modulation of innate immunity with different immunogenic molecules *in vitro* and *in vivo* was investigated (*30, 31*).The mechanisms of IFNs type I induction *in vitro* together with the characterization of secreted proteins was also studied *(32–34)*.

Cellular immunology

In 1971, when D. Dekaris joined the Institute, the Cellular Immunology Unit was organized. This meant broadening (extending) research to mechanisms of cellular immunity. The Unit was lead by D. Dekaris until 1996. S. Rabatić succeeded him, and in the year 2000 A. Gagro became head of the Unit.

Initial investigations were centered on experimental animal models, primarily using the *in vitro* correlates of cellular immunity, *e.g.* inhibition of macrophage migration and of macrophage spreading *(35)*. Very soon the investigations involved problems in clinical immunology. The accomplishments achieved during the period that D. Dekaris lead the Unit would not have been possible without the support and expertise of the staff (S. Rabatić and A. Sabioncello) and a number of young scientists (see References). In brief, the Unit investigated mechanisms of cellular immunity, macrophages, monocytes, phagocytosis, atopy, antibody dependent cellular cytotoxicity, cytokines, chemokines, local immunity, immunity to viral infections, chronoimmunology, psychoneuroimmunology, and the relation of stress to immunity.

However, the main research topics were allergology and the relationship between stress and immunity. In addition, the effect of supernates from thymic and splenic tumor cells on macrophage spreading *(36)* was studied, as well as the relationship of phagocytosis to macrophage spreading *(37)*. Correlation of *in vitro* inhibition of macrophages spreading by the antilymphocyte serum and prolongation of allograft survival were also studied *(38)*.

The following was also investigated: the effect of circadian rhythm on mouse leukocytes *(39),* the use of the test of human monocyte spreading inhibition for assessing the immunosuppressive potential of horse-anti-human lymphocyte globulin *(11),* the function of polymorphonuclear leukocytes and NK-cells in mature and immature newborns *(23),* local immunity *(40–42),* and the effect of ageing on the phagocytes of human peripheral blood *(43)*. In humans, the analysis of factors affecting the clinical applicability of the test of leukocyte migration inhibition *(44),* the cellular immunity in children with juvenile diabetes mellitus *(45, 46),* and cellular immunoreactions in patients with rheumatoid arthritis, ankylosant spondylitis and psoriatic arthritis were performed *(47, 48)*.Chronic lymphocytic leukemia was also investigated *(49–52)*.

Within the last ten years, investigations were directed to immune reactions to viruses, in particular hantaviruses initiated by Professor Alemka Markotić *(53–57)* and the respiratory sincytial virus *(58–61)*.

Allergic reactions

Laboratory clinical immunological investigations started with elaboration of the proper method (the test of the monocyte spreading inhibition) for *in vitro* detection of cellular immunity *(62),* and comparison of the method with the generally accepted test of the leukocyte migration inhibition *(63),* and continued with studies of varying allergic reactions.

Studies on delayed hypersensitivity by the test of macrophage inhibition *(64–67),* on the effects of supernates of antigen stimulated sensitized lymphocytes on macrophage spreading *(68)* and comparison of migration inhibition of peritoneal cells and peripheral blood leukocytes *in vitro* in tuberculin hypersensitivity in guinea-pigs were performed *(69)*.

An important new finding was that of cellular immunity to pollen antigens in patients with allergy to pollen *(70)*.

When the first flow cytometer for research purposes in Croatia was obtained (1990), the instrument was used to broaden the investigations.The research interest involved other aspects of allergic reactions, *e.g.* T-lymphocyte subgroups in children with allergic asthma, the role of low affinity receptors for E immunoglobulin (CD23) in allergic reactions, comparison of immune reactions in children with intrinsic and extrinsic asthma, immunological parameters for the purpose of evaluating the effectiveness of varying procedures of hyposensibilization, and the effects of new drugs for use in humans *(71–77)*.

An important contribution was the national standardization of the allergen from Dermatophagoides pteronyssinus, used for hyposensibilization *(78)*.

Recently research has been focussed on the role of regulatory T-lymphocytes in allergies *(79)*.

Psychoneuroimmunology

In the late 1980s our interest started to focus on interactions, well established by then, between immune and neuroendocrine systems.

Communication between all bodily systems is indispensable for integrity maintenance of an organism exposed to all kinds of internal and environmental signals. Fluctuating environmental conditions can overcome the relatively narrow homeostatic range, thus threatening the integrity. This threat (stressor) elicits physiological and behavioral responses through changes of boundaries of control that require extra energy to re-establish stability at a higher set-point (allostasis). Stress should be regarded as an essential element in the total adaptive system of the organism *(80)*.

Due to the outbreak of war we had an opportunity to thoroughly investigate the impact of chronic war-related stress on biological functions. The strain of prolonged elevated activity of physiologic system under challenge can predispose the body to disease. Consequences of war-related stress in severely traumatized victims may last for decades. This makes the understanding of mechanisms involved in the psychosomatic aftermath of trauma, including post-traumatic stress disorder (PTSD), of great importance.

At the onset of war we began to investigate the influence of trauma caused by forced expulsion from home in war-ravaged regions on psychological, hormonal, and immune responses in displaced persons. They revealed higher psychosomatic response, higher hormone (cortisol, prolactin, and endorphin) levels, an increase in activated phenotype of B, T, and NK cells, and enhanced proportion of proliferating lymphocytes in freshly isolated blood (*ex vivo*), but lower *in vitro*, mitogen-stimulated proliferative response *(81)*. Another study included civilian detainees just released from a prisoner of war camp after 4 to 7 months of imprisonment. They also had increased percentage and absolute number of activated T cells but diminished level of cortisol and prolactin in the peripheral blood. Regarding proinflammatory cytokines, function of NK cells, and phago-

cytes the level of TNF-α was increased, those of IFN-γ was decreased as well as NK and phagocytic (ingestion and digestion but not antibody dependent cellular cytotoxicity) activities *(82)*. Multiple interactions were established between psychological, endocrine and immune statuses in those groups *(83, 84)*.

In the first few years after the war our research efforts concentrated on biological consequences of posttraumatic stress disorder (PTSD) in soldiers (professional and enrolled). In general, fewer changes in immune and hormonal parameters were found in professional soldiers than in civilians or enrolled soldiers *(82, 85)*. Enrolled soldiers had an increased level of stress hormones, prolactin (unpublished) and cortisol but a decreased level of lymphocyte glucocorticoid receptors (GR). Although these results are opposite to those reported in literature for chronic PTSD (decrease of cortisol level and increase in GR expression) reduced GR expression still indicate a negative feedback response to increased level of cortisol *(86)*. In another study with enrolled soldiers, performed 10 years post trauma we found unchanged cortisol level but increased GR expression. NK activity was decreased despite of increased perforin content in NK cells *(87)*. With all these, seemingly inconsistent results in mind, we assumed that more time is needed for reversal of hormone and its receptor expression with consequent functional repercussions to take place due to prolonged hyperactivity of HPA-axis. Therefore, our further research efforts concentrated on follow-up with the veterans. We re-assessed the first group of veterans and control subjects after six years (8–13 years post trauma). At the second occasion only total lymphocyte count was still elevated in PTSD patients *(88)*. This confirmed our hypothesis that changes in immune and endocrine systems in PTSD are not static but depend on duration of allostatic load. A still longer follow-up period is needed to better understand the dynamics of changes in immune and endocrine systems and their interactions in people under prolonged stress.

By releasing chemokines and expressing activation surface molecules, platelets represent key mediators in leukocyte trafficking as well as in initiation and regulation of inflammation. We assumed that platelet activity might be elevated in PTSD patients and conducted a pilot study to determine baseline platelet function in war veterans diagnosed with combat-related chronic PTSD but no differences in measured parameters were observed *(89)*.

To determine the relation of psychological stress and the immune response to vaccination we measured serum antibody titre and frequency of $CD8^+$ influenza-specific T cells (HLA class I tetramer technology) before and 15 days, a month and 3 months after vaccination of healthy persons and combat-related PTSD patients. Although PTSD patients had a lower number of influenza-specific $CD8^+$ T cells before vaccination compared to healthy controls, they responded by a significant increase of antibody titre and antigen-specific citotoxic lymphocytes two weeks following vaccination *(90, 91)*.

Multidrug resistance

Half the human tumors are completely resistant, or respond to chemotherapy only temporarily, after which they can no longer be treated with commonly used drugs . Cells become resistant to a large number of drugs at the same time, so the phenomenon has been named multidrug resistance (MDR). Among other factors MDR is caused by hampered penetration and therefore low concentration of the drug in the cells, which happens due to membrane active ATP binding cassette transporters (ABCT), and the best known among them is ABC B1 P-glycoprotein (Pgp).

At the Institute, studies of MDR first started as clinical studies conducted together with the hematologists and Pgp expression was evaluated in patients with myeloid and lymphoid leukemia *(92)*. In 1998, I. Svoboda Beusan, Ph.D., having received a scholarship from the French Government, spent one year at the European MDR Center in Paris. Upon her return, testing of Pgp pump activity with vitro rhodamine Rh123 test was included in MDR research.

Until now MDR profile has been determined in subjects with multiple myeloma, gastric cells of patients with H. Pylori infection *(93),* in patients with chronic myeloid leukemia (CML) during treatment with the smart drug Imatinib mesylate (IM). Finally, comparison of Pgp/MDR activity and molecular level of response in CML patients treated with IM shows that the activity test is clinically valuable and that it correlates well with the outcome of treatment. Follow up of MDR status would enable planning and modification of therapy protocols (introduction of MDR-independent substrates or MDR modulation). In practice, this could improve efficiency, cost-effectiveness, safety and promptitude of treatment, and this would all significantly contribute to better survival of patients with malignant diseases.

Referral Center for Clinical Cellular Immunodiagnostics of the Republic of Croatia (RC)

Successful research in the field of laboratory clinical immunology resulted in the foundation of the RC in 1990, under the leadership of D. Dekaris *(94)*.

One of the first activities of the RC was standardisation in laboratory clinical immunology.

»The problem of insufficient reproducibility of the methods **in laboratory clinical immunology** was immediately recognized. It was obvious that a standard regarding the performance of the test and ways of interpreting their results was required.

In 1975, we organized the first meeting on the problems of standardization of the most widely used test for *in vitro* detection of cellular immunity – the inhibition of macrophage/granulocyte migration – in the Croatian Medical Academy.

Our group at the Institute has actively participated in the development and application of tests for cellular immune reactions from the very beginning. We soon realized that it was necessary to establish a center as a focal point for standardization and development of the methodology for monitoring of cellular immune reactions.

In order to make the center fulfill its task efficiently, we had to meet two prerequisites: 1) to follow scientifically the developments of immunology in the world; 2) to create the legal basis for the establishment of a referral center for standardization and development of methods for clinical diagnostic of cellular immune reactions« *(94)*.

The programme of RC was:

1. Coordination and quality control of Croatian laboratories dealing with cellular immunodiagnostic;
2. Assistance to the Croatian health authorities in establishing the standards for laboratory procedures used in clinical cellular immunodiagnostic;
3. Providing expert opinion on the indications and usefulness of given procedures (upon the request of the health fund);
4. Developing new procedures for the detection of immunological phenomena that cannot be detected by the existing tests, as well as those procedures that are significantly more selective than the existing ones;
5. Improving the existing procedures with the aim to find a quicker, cheaper, simpler and more reproducible variant;
6 . Establishing the procedures by which a certain immunological phenomenon is most adequately followed and the analysis of the applicability of a given assay in diagnosis and monitoring of immunological diseases.

Over the years, the RC has, in compliance with the suggestions of the World Health Organization and the International Union of Immunological Societies, as well as the needs of health service of Croatia, widened the programme of its operation to the following:

7. Education and other forms of dissemination of knowledge in the field of clinical immunology;
8. Assistance in creating programmes for the specialist's training in clinical immunology;
9. Publishing textbooks in the field of allergology and clinical immunology;
10. Organizing scientific meetings in the field of clinical immunology within Croatia, former Yugoslavia and Alps-Adria Working Community;
11. Associating with international societies of clinical immunologists;
12. Establishing a Croatian register for primary immunodeficiency;
13. Setting up documentation on changes of immune reactivity of war-victims in Croatia« (*94*).

In conclusion, the activity of the group of scientists in the Unit for cellular immunology (head D. Dekaris, and later S. Rabatić, and including A. Sabioncello, A. Gagro and a number of their young collaborators) together with V. Silobrčić, R. Mažuran, I. Svoboda Beusan in the Unit for transplantation immunlogy, provided an important impetus to the development of Clinical immunology, in particular of Laboratory clinical immunology in Croatia.This was evidenced by the foundation of the above mentioned Referral Center of Croatia.

Collaborations

The success in promoting Clinical immunology was assured by a continuous collaboration with these clinical departments, hospitals and institutes:

University Hospital »Dr. M. Stojanović« Zagreb, Croatia; University Hospital »Rebro«, Zagreb, Croatia; University Hospital for Infectious Disease »Dr. F. Mihaljević«, Zagreb, Croatia; Clinical Hospital »Dr. O. Novosel«, Zagreb, Croatia; Clinical Hospital »Dr. J. Kajfeš«, Zagreb, Croatia; Children's Hospital for Tuberculosis and Pulmonary disease, Zagreb, Croatia; University Children's Hospital, Zagreb, Croatia; Dubrava Clinical Hospital (University Hospital Dubrava), Zagreb, Croatia; Vrapče Psychiatric Hospital, Zagreb, Croatia; Institute »Ruđer Bošković«, Zagreb, Croatia; Croatian National Institute of Public Health, Zagreb, Croatia; »A. Štampar« School of Public Health, University of Zagreb, Zagreb, Croatia; »J. Benčević« General Hospital, Slavonski Brod, Croatia; Varaždin General Hospital, Varaždin, Croatia; School of Medicine, University of Ljubljana, Ljubljana, Slovenia; School of Medicine, J.J. Strossmayer University, Osijek, Croatia; Institute for Virology, University of Vienna, Austria; Clinic of the Pfilipps University, Marburg, Germany; CDC, Atlanta, GA, USA; University of Pecs, Faculty of Medicine, Pecs, Hungary; MRC Center for Immune Regulation, The Medical School, University of Birmingham, UK

Financial support

Beside the continuous financial support by the Ministry of Science, Republic of Croatia through financing scientific projects and programs, several successful technological projects guided by investigators from the Unit were financed (TP-04/021–08, principal investigator S. Rabatić; TP-01/021–05, principal investigator A. Gagro; and TP-01/021–06,principal investigator A. Markotić). The investigations were partly supported also by scientific projects financed by international foundations: The Wellcome Trust, UK (principal investigator A. Garo); MSD, USA (principal investigator A. Gagro), Soroš foundation (principal investigator D. Dekaris).

Radioimmunology and Chemistry

Twentyfive years ago, in the summer of 1983, a group of already established scientists in the field of biochemistry and immunochemistry joined the Research and Development Department at the Institute. Under the leadership of Jelka Tomašić a new Radioimmunology and chemistry unit was formed and a new era from the immunochemical point of view started. Due to the urgent need for the kits for determination of T_3, T_4 and cortisol on the Croatian market the production of respective radioimmunoassay kits was started at the Institute at the newly established unit within R & D department. Development and assembly of a radioimmunoassay kit for thyroid hormones and cortisol determination meant the preparation of immunogene *e.g.* conjugation of the hormone to a protein carrier, immunisation of experimental animals with the obtained conjugate, retrieval of the polyclonal antisera and determination of their quality. In parallel, labelling of a hormone was performed and subsequently the radioimmunoassay kits were assembled. For almost ten years this small production was the only source of the mentioned RIA – kits for the Croatian hospital laboratories.

In parallel the PRIST and RAST kits for determination of total and specific IgE's were developed and have been used for couple of years in several laboratories in Zagreb for allergy testing. Establishment of the PRIST and RAST for determination of IgG's led to characterisation of the Croatian national standard for *Dermatophagoides pteronissinus* allergen extract *(78)*.

Furthermore, in the field of radioimmuno assays, the in house IRMA kit for determination of HBsAg was prepared and standardized, to be used for testing plasma donors at the Institute.

The method for the determination of fetal calf serum in vaccine preparations by immunoradiometric and enzyme-linked immunosorbent assay (ELISA) was worked out *(95),* and has been used, among other tests, for quality control of the viral vaccines produced at the Institute.

Along the same lines a competitive radioimmunoassay for peptidoglycan monomer determination *(96)* and novel ELISA's for determination of polysaccharide specific immunoglobulins and for T-B-epitope of measles virus specific immunoglobulins were set up *(97, 98)*.

In parallel to the described work in the field of immuno-tests intensive scientific activity was taking place in the area of the study of immunostimulating substances of natural and synthetic origin. The majority of the research has been performed on the peptidoglycan monomer (PGM) derived from *Brevibacterium divaricatum (99–102),* its synthetically modified derivatives *(103, 104)* and newly synthesized adamantly tripeptides *(105–107)* comprising the peptide portion characteristic for PGM.

Humoral immune response to different immunogenes *(98, 108, 109)* in various experimental models was investigated and within years a reliable model for studying potential immuno stimulators in different application systems (liposomes, oil emulsions) was established *(110–112)*.

The Ministry of Science and Technology, as well as the EU Commission for Science, Research and Development, have recognized the potential in the work involved in immunomodulation with the emphasis on immunostimulation, obtained by the novel substances, by assigning financial support for several projects led by Jelka Tomašić from 1984 to today. In addition to financing the scientific projects, two techonological projects (TP-01/021–04, principal investigator J. Tomašić; TP-05/021–01, principal investigator B. Halassy) were supported as well.

Molecular Biomedicine

The development of research in molecular biomedicine at the Institute began in 1992 when Renata Mažuran equipped the former Transplantation Immunology Unit with necessary instruments for recombinant DNA technology methods. It was not until 1996 that the laboratories acquired their proper shape (form): the automated DNA sequencer came just in time to finalize in-house RT-PCR method for detection of HCV RNA in biological samples *(113, 114)*. The method is exploiting for follow-up study of human plasma and leukocytes used in biotechnological processes *(115–118)*.

In recent years (1995–2008), we have focused our efforts on molecular biology of RNA viruses, by exploring their genomes as well as the relationships between viral and cellular components, of diverse enveloped and non-enveloped, positive and negative single-stranded RNA viruses including Flaviviridae (hepatitis C virus), Orthomyxoviridae (influenza virus), Togaviridae (rubella virus) and, most actively, Paramyxoviridae (measles, mumps). The Ministry of Science, Education and Sports support(ed) several research projects with Dubravko Forčić Ph.D. and Maja Šantak Ph.D. as principal investigators.

In the frame (field) of measles eradication activities, sera from 1205 Croatian citizens were tested by ELISA for anti-measles IgG and results indicated that vaccination coverage higher than the reported 90–94% should be attained to eradicate measles *(119)*.

To confirm the genetic stability of the Edmonston-Zagreb (EZ) measles vaccine strain, we determined and compared the nucleotide sequences of genuine EZ seeds *(120)*. Sequence analysis and comparison to the reference strains revealed differences at the molecular level between EZ from different sources which required periodical sequence analysis of the same strain in the hands of different vaccine manufactures.

Regarding pathology of measles virus (MV) infection, two cases of subacute sclerosing panencephalitis (SSPE), diagnosed in Croatia in 2002, were investigated *(121)*. Additionally, possible influence of mutations in untranslated regions (UTRs) of MV on establishment and maintenance of chronic progressive CNS disease caused by MV persistence was investigated *(122)*.

Determination of inter- and intragenotype stability and variability are the basic tools for the molecular epidemiology and evolutionary investigation of MV. We detect the presence of unique residues on the level of the entire genome as a new important parameter in the investigation of molecular evolution of MVs *(123)*.

Viral epidemiology is determined by the movement of infected people within and between geographical areas. The genetic characterization of wild-type isolates combined with standard epidemiological methods may enable the identification of the source and transmission pathways and permit differentiation between indigenous and imported viruses. We investigated the genetic characteristics of the wt MVs isolated in Croatia during 2003–2004 outbreaks *(124)* and we confirmed the presence of the D4 measles virus genotype in Europe.

Eleven mumps vaccine strains, all containing live attenuated virus, have been used throughout the world. Although L-Zagreb mumps vaccine has been licensed since 1972 (Institute of Immunology Inc., Zagreb, Croatia), only its partial nucleotide sequence was previously determined. Therefore, we sequenced the entire genome of L-Zagreb vaccine strain *(125)*. The molecular characterization of a historical mumps isolate *(126)* was also performed. With funding from the Ministry of Science, Education and Sports the obtained biological material was used for development of new mumps vaccine strain(s) attenuated on human diploid cells (technological project TP-01/0021–01; Principal investigator R. Mažuran). Process of development resulted in an adequate volume of cGMP materials for production of Clinical materials. Entry into the next stage of clinical trials (Phase II and Phase III) requires the completion of Phase I (safety) by testing neurovirulence in monkeys or by alternative testing in newborn rats Implementation and validation of the test is in process (technological project TP-01/0021–02; Principal investigator R. Mažuran).

More recently, two wt mumps virus strains and were isolated in two locations in Croatia in 1998 and 2005 *(127)*.

Often, for our investigations we need concentrated biological material, free of impurities. Chromatographic purification, especially the introduction of ion exchange chromatography and affinity chromatography has enabled the production of highly purified macromolecules. Monoliths are considered to be a novel generation of stationary phases introduced in the past 15 years. Mass transfer in monoliths is mainly based on convection and that is the basis for naming one particular type of these supports as Convective Interaction Media (CIM). Our previous research on the purification of plasmid DNA *(128),* prokaryotic, eukaryotic genomic DNA (129–131), viral RNA *(132)* and satellite replicative dsRNA *(133)* has shown that CIM® DEAE disks are excellent chromatographic support for nucleic acids in preparative and analytical work. Based on these results and on the fact that the pores in monolithic columns are large enough to harbor nanoparticles, we are developing a new approach in the separation and analysis of different biological macromolecules.

Teaching

Teaching Immunology started by D. Ikić at the Faculty of Natural Sciences and Mathematics, University of Zagreb. In 1971 V. Silobrčić was duly appointed Assistant Professor of Immunology at the same Faculty, as the first habilitated professor in former Yugoslavia. Soon after (1972), D. Dekaris and Professor Branko Vitale (Institute »Ruđer Bošković«, Zagreb) started teaching Immunology (graduate level) at the Medical Faculty, University of Zagreb. Teaching of Immunology involved a great deal of practical work at the modern facilities of the Institute. Many staff members of the Institute participated in the teaching with their expertise.

Throughout the years we have taught various aspects of Immunology at graduate and postgraduate level at the Faculties:

Faculty of Natural Sciences and Mathematics, University of Zagreb, Postgraduate studies
(Vlatko Silobrčić, Dragan Dekaris, Renata Mažuran, Ante Sabioncello, Sabina Rabatić, Ivna Svoboda-Beusan, Alenka Gagro, Branka Vranešić, Jelka Tomašić)

School of Medicine, University of Zagreb, Postgraduate studies
(Dragan Dekaris, Vlatko Silobrčić, Renata Mažuran, Ante Sabioncello, Sabina Rabatić, Alenka Gagro, Jelka Tomašić)

School of Medicine, University of Rijeka, Postgraduate study
(Dragan Dekaris, Vlatko Silobrčić, Renata Mažuran, Ante Sabioncello, Sabina Rabatić, Alenka Gagro, Alemka Markotić)

School of Medicine, University of Osijek, Postgraduate study
(Alenka Gagro, Alemka Markotić, Ante Sabioncello)

School of Medicine, University of Sarajevo, Postgraduate study
Dragan Dekaris

School of Medicine, University of Ljubljana, Postgraduate study
Dragan Dekaris, Vlatko Silobrčić

School of Medicine, University of Priština, Postgraduate study
Dragan Dekaris, Vlatko Silobrčić

School of Veterinary Medicine, University of Zagreb, Postgraduate study
Dragan Dekaris

Institute Pasteur (Paris), Postgraduate study
Dragan Dekaris

Textbooks, dictionaries, books and encyclopedias

To help the education in Immunology, D. Dekaris and V. Silobrčić translated from English the book I. Roitt: Essential Immunology. The first edition appeared in 1974, the second in 1979 *(134, 135)*. The second edition contained also a Glossary of immunological terminology in Croatian, *(136)* written by D. Dekaris, V. Silobrčić and Josip Silić (Croatian Department, Faculty of Philosophy, University of Zagreb).

Scientists from the Institute took part in translating the first textbook on Clinical Immunology *(137)*. D. Dekaris published the first book on basic allergology *(138)*.

A number of scientists from the Institute wrote chapters in textbooks of Pathology, Pathophysiology, Oncology, Pediatrics, Hematology and Transfuziology, Internal medicine and Molecular biology.

D. Dekaris with the work on immunological terminology contributed in publishing the Encyclopedic Dictionary of Human and Veterinary Terminology *(in Croatian, 139)*. D. Dekaris and Professor Filip Čulo (Medical Faculty, University of Zagreb) edited a multiauthored book on clinical immunology in Yugoslavia (*140*).

V. Silobrčić was the first to introduce the subject of scientific writing at postgraduate level, and wrote a book on it *(in Croatian, 141)*

TABLE 2
Mayor awards.

Year	Awardees	Award
1972	Drago Ikić	»Ruđer Bošković« annual award of Republic of Croatia for outstanding scientific achievement
1973	Dragan Dekaris	»Ruđer Bošković« annual award of Republic of Croatia for outstanding scientific achievement
1974	Dragan Dekaris	»Pavao Ćulumović« award for medical scientific research, Academy of Medical Sciences of Croatia
	Vlatko Silobrčić	»Ruđer Bošković« annual award of Republic of Croatia for outstanding scientific achievement »Pavao Ćulumović« award for medical scientific research, Academy of Medical Sciences of Croatia
1980	Drago Ikić	National science lifetime achievement award

1981	Vlatko Silobrčić	Annual Award of the City of Zagreb for achievements with the scientific journal **Periodicum biologorum**
1987	Vlatko Silobrčić	Medal of the Massachusetts General Hospital, Boston, MA, USA
1991	Vlatko Silobrčić	»Fran Tućan« annual award of Republic of Croatia for popularization and advancement of science and scientific culture
1994	Dragan Dekaris	»Ante Šercer« annual award for the most valuable medical publication, Academy of Medical Sciences of Croatia
1995	Sabina Rabatić and Alenka Gagro	Croatian Academy of Sciences and Arts annual award in the field of medical sciences
1996	Alenka Gagro	»Ante Šercer« annual award for the most valuable medical publication, Academy of Medical Sciences of Croatia
	Alemka Markotić	National science annual award for junior researchers in biomedicine
1999	Sabina Rabatić	National science annual award for scientific achievement in biomedicine
	Vlatko Silobrčić	National science lifetime achievement award in biomedicine
2000	Alemka Markotić	USAMRIID Coin, U.S. Army Medical Research Institute for Infectious Diseases, Frederick, Maryland, USA for outstanding contribution to USAMRIID
2001	Dragan Dekaris	National science lifetime achievement award in biomedicine
		»Ladislav Rakovac« award, Croatian Medical Association
2002	Alemka Markotić	Croatian Academy of Sciences and Arts annual award in the field of medical sciences
2003	Vlatko Silobrčić	Gold Medal of the Gilbert Fletcher Society, Houston, Texas, USA
2005	Renata Mažuran	National science annual award for scientific achievement in biomedicine
2006	Beata Halassy	»Vera Johanides« award for scientific achievement in five years in biotechnical sciences, Academy of Technical Sciences of Croatia

Activities within the Croatian Immunological Society

V. Silobrćić, prompted by a letter from Professor B. Cinader, started preparations for the organization of the Yugoslav Immunological Society, and served as its first Secretary General, and subsequently as its Vice-president and President (1970 to 1976). As the Yugoslav Immunological Society was among the first national immunological societies founded, the Society became the Founding member of the International Union of Immunological Societies. As Vice-president of the Commission for Europe and a member of the Symposium Committee of the Union, V. Silobrčić organized its First international symposium, held in Rovinj (1970).

V. Silobrčić and D. Dekaris transformed the local journal »Biološki glasnik« into an international scientific journal »*Periodicum biologorum*«, which shortly became indexed in Current Contents and Science Citation Index of the Institute for Scientific Information, Philadelphia, USA. V. Silobrčić was its Editor-in-Chief from 1974 to 1993, and the Journal remains the official Journal of the Croatian Immunological Society.

After Croatia's independence, from 1995 the Society continued its activities as the Croatian Immunological Society. Scientists from the Institute were very active as members of governing bodies of the society; R. Mažuran and S. Rabatić served as its Presidents.

Since 1996, one of the main activities of the Society was the organization of 12 regular annual meetings, held in English and with participation of eminent foreign scientists. R. Mažuran, S. Rabatić, A. Gagro and A. Markotić were continually involved in organizing these meetings.

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Immunological investigations in the Department of Physiology at the School of Medicine started at the beginning of the 1960s , when the Head of the Department, the late academician Nikša Allegretti, began to study the influence of ionizing irradiation on immunological processes in the organism, mechanisms of autoimmunological diseases and transplantation reaction. Proceeding to these investigations was acquisition of inbred strains of mice which Professor Allegretti personally brought from the Medical Research Council, Harwell, England. His original idea was that irradiation can induce somatic mutation of immunologically competent cells, so that the normal tissue antigens appear foreign *(1, 2, 3)*. This hypothesis he checked with collaborators in several experimental models.

It was found that irradiated animals succumb to experimental allergic encephalomyelitis (EAE) more frequently than normal animals and that EAE, together with lung lesions, can appear in animals which were immunized with lung tissue. These investigations were published in one of the most prestigious biomedical journals – Nature *(4, 5)*. For induction and development of EEA immunocompetent host cells (T-lymphocytes) are necessary – incidence of EAE is significantly reduced in mice in whom the thymus was removed before the irradiation and transfer of syngeneic bone marrow cells (T-deficient mice) *(6)*.

At the same time, he studied the mechanisms of transplantation reaction, both that caused by dominant immunoreactions of host to allogeneic graft as well as by graft versus host in the case of transfer of foreign lymphoid (immunocompetent) cells. To ensure that in the latter case the reaction is unidirectional, the irradiated (immunosuppressed) hosts or transferred lymphoid cells F1 hybrid of inbred mice strain were used (which cannot react to cells of parental strains). At the same time, he follows the cytodynamics and expression of chimerism in recipients which have received immunocompetent donor cells. On the basis of observed late mortality in midlethally irradiated hosts which have received F1 hybrid lymphoid cells, which lead him to the hypothesis of »allergic death« of immunocompetent cells. Allogeneic disease would ensue as a result of decay of the immunological system because of mutual attack and exhaustion of immunocompetent cells *(7–13)*. The death of most allogeneic partners joined in parabiosis is maybe the best proof of immunological exhaustion and »allergic death« of the immunocompetent recipient *(14)*. The transplantation reaction can be manifested and demonstrated as skin reaction of delayed type *(15)*. For its expression the presence of host T lymphocytes is needed *(16)*.With associates he has shown that nonspecific stimulation of the immunological system, with BCG bacteria, enhances the rejection of allogeneic skin transplant *(17)*. This paper, which is widely cited in the literature, has paved the way for nonspecific immunotherapy of tumors, which was the main preoccupation in investigations of the academician Allegretti until his premature death.

With his collaborators he investigated various aspects of immunological reaction to tumor. It was shown that adherent peritoneal exudates or spleen cells or (especially macrophages) play a role in defense, take and growth of transplantable tumor in rodents *(18–23)* and that midlethally irradiation of the host deceases the resistance of mice to transplantable tumor *(24),* and that immunosuppression with cyclophosphamide increases the number of artificially induced metastases in mice *(25)*. Specific antitumor immunity can be induced by immunization of mice with dead tumor cells given in a complete Freund adjuvant *(26)*. For generation of antitumor immunity to xenogeneic tumors the presence of functional T lymphocytes – in animals

deficient on T lymphocytes xenogeneic tumors can grow *(27, 28)*. For rejection of these tumors the cooperation of lymphocytes T and B is needed *(29)*. In resistance to transplantable ascites tumor in mice, besides cellular immunity, a role is also played by humoral immunity *(30)*. Mice which finally succumb to tumor, express concomitant immunity to the same tumor in the early phase of growth of the tumor *(31)*.

With his permanent collaborators in the Department of Physiology, Professors ndries, Čulo, Marušić and Taradi, he investigated various other aspects of immunological host tumor relations. By using mice with absence of thymus (thymectomized, lethally irradiated mice to whom syngeneic bone marrow cells were transferred) it was shown that T lymphocytes do not have an essential role in surveillance of the emergence of solid tumors induced with chemical carcinogens – appearance of tumors induced with methylcholantrene was not higher in T-deficient than in normal mice *(32)*. By combined application of cyclophosphamide and passive transfer of syngeneic spleen cells from anti-tumor immunized mice it was possible to cure and generate specific immunity in mice with an established tumor *(33, 34)*. Exogenous application of intracellular proteases – cathepsins can inhibit growth or cause regression of already established transplantable tumor in rats *(35, 36)*. For cyclophosphamide it was shown that, besides direct cytostatic antitumor action, it acts as an immunomodulator. If given 2–4 days after implantation of ascitic tumor it acts immunosuppressively, primarily on proliferating anti-tumor lymphocyte clone, causing its deletion, and if given after that overwhelms its cytostatic effect if given alone or in a model of combined chemotherapy and adoptive immunotherapy, i.e., the effect is stronger when the therapy is applied after implantation *(37–41)*.

During his investigations of host tumor relationships, academician N. Allegretti came across the phenomena of immunological enhancement – facilitation of tumor growth after attempts of modulation of immunological reaction. First he found that the appearance and growth of liver carcinoma can be enhanced after immunization of rats in precancerous state with homogenized tumor tissue in Freund' adjuvant *(42)*. The phenomena of immunological enhancement of tumor growth prompted him to perceive (examine) the mechanism of facilitation (then actual area of investigations in transplantation immunology) as a general mechanism of coexistence of cells of different antigenog structure *(43)*. According to this perception, in a simplified view, facilitation of immunological reaction of the host, especially its humoral part – antibodies, would actually protect tumor cells from the attack of cellular immunological effectors. He published with collaborators several papers in which he obtained some antitumor effect by giving xenogeneic antibodies against immunoglobulin of tumor host *(44–49)*. Unfortunately, the premature death of academician Allegretti interrupted these investigations and definite confirmation of this hypothesis.

In parallel with investigations, and especially after the death of Professor Allegretti (1982), his collaborators from the Department of Physiology carried out some investigations in which they were supervisors, in which other colleagues at the Department frequently collaborated. The researchers who collaborated with Professor Allegretti in immunological investigations from the beginning of their employment at the Department of Physiology were: I. Andreis, F. Čulo, M. Marušić and M. Taradi. Latter, this group was joined by Prof. S. Kukolja-Taradi. Besides working and collaborating in immunology research all the members of this group were co-authors of texts and editors of the textbook »Imunologija« (Immunology). This was the first textbook on immunology in the Croatian language for graduate students of medicine. The first edition of the book was issued 1984, and presently the sixth edition of the book is in progress.

I. Andreis was included in immunology research during the time he spent at the Department of Physiology (1965. till retirement in 2002.). He was interested in many fields of immunology, but his attention mainly focussed on the immunological approach to tumor diseases.

In the first period I. Andreis was interested in the role of the specific antibodies in accelerated tumor growth (facilitation). He attempted to eliminate the unwanted effect of facilitation with antiglobulin antiserum and proteolytic enzymes and the results of the investigations supported these ideas *(35, 36, 44, 48, 50, 51)*.

The other part of research was concerned with the introduction of some new immunologic diagnostic methods. One part of the study in this area focussing on different autoantibodies in autoimmune diseases, was performed in the Central Hematology and Immunology Laboratory, St.Antoine Hospital, Paris. The paper describing the antimitochondrial antibodies in hepatitis patients was the very first concerning this subject *(52)*. The other part of work is related to the introduction of a specific rosette test in animals with malignant tumor. Briefly, isolated tumor antigens was (were) coupled by using glutaraldehyde to immunoglobulins against sheep red blood cells (SRBC) and SRBC were sensitized with this complex. Such sensitized SRBC were incubated with the splenocytes of immune or normal animals and later the frequency of splenocytes which form rosettes was determined. The experiments indicate the possibility of using the rosette test in the evaluation of specific reactivity of a host to tumor *(54–59)*.

The major part of papers is related to concomitant immunity (CI) phenomenon. CI is resistance to secondarily introduced tumor cells in animals already bearing the same tumor. Depending on the model used, CI first reaches its maximum, and thereafter subsides or disappears completely. The results indicate that the weakening of CI may be due to the participation of suppressor cells *(31, 60–64)*.

Another part of the papers is concerned with the role of macrophages on tumor growth and on experimental allergic encephalomyelitis development. On the basis of the obtained results it appears justifiable to conclude that normal, non-activated macrophages can prevent nidation of tumor cells by the local non-specific destruction of these cells. The data support the hypothesis that macrophages can serve as effectors of immune surveillance. On the other hand, it can be concluded that macrophages are important for EAE induction in rats: without functionally capable macrophages, the induction generally fails *(19, 22, 65–71)*.

Beside scientific papers, I. Andreis published several other texts related to immunology. He is the author of several chapters in the textbook »Imunologija« (he was also the editor of the 6th edition of this textbook) and of numerous articles in the Croatian Encyclopedia and Medical Lexicon (Leksikografski zavod »Miroslav Krleža«, Zagreb).

Filip Čulo, was on leave of absence (on postdoctoral fellowship) from 1977 to 1979 at the Cancer Research and Treatment Center, Albuquerque, University of New Mexico, USA, were he studied the action of anti-tumor effectors *in vitro*, in a model multicellular tumor spheroids, which display many characteristics of tumor growth *in vivo (72)*. Latter he investigated the mechanism of action of radioprotective agent WR-2721, which showed an unusual important feature, that is, that it protects normal tissues, but not the tumor cells from toxic action of X-irradiation and alkylation chemotherapeutics (this drug was approved for clinical use, under the name aminofostin, trade name Ethyol). He published 5 papers *(73–77)* in that field, of which two have been quoted in the world literature more than 200 times each.

After his return, he investigated various factors that influence the effect of adoptive immunochemotherapy of tumor. He has shown that the adoptive transfer of immunity with the spleen cells from immunized syngeneic donors is much less successful if the recipient was previously irradiated with a mid-lethal dose of dose of X-rays, which was explained by lack of some radiosensitive component of the host needed for action of transferred cells *(78, unpublished results)*. On the other hand, pretreatment of recipients with cyclophosphamide enhances their efficacy *(79)*. It seems that for efficient activity of transferred cells their homing in lymphoid tissues of recipients is needed *(79)*. He called special attention to investigation of positive action of cyclophosphamide in a model of adoptive immunochemotherapy malignant tumors. With collaborators he showed that tumor cells which escape the direct killing with a sublethal dose of cyclophosphamide are, for about three weeks, more sensitive to an immunological attack of immunological cells. When these cells are transferred into a normal recipient, their translatability is decreased and sensitivity to lytic action of immune cells increased, without change in their immunogenicity *(80, 81)*. Cyclophosphamide cancels the action of suppressive factors in mice with advanced tumors. Mice with advanced tumors (induced with methylcholanthrene), in contrast to mice with early tumors, does not manifest the signs of concomitant immunity, i.e., to be able to reject the cells of the same tumors. Even more, lymphoid cells from mice with advanced tumors inhibit (suppress) the anti-tumor action of lymphoid cells from immunized donors in Winn's neutralization test. Application of a single sublethal dose of cyclophosphamide induces after 7 to 15 days reappearance of concomitant immunity in mice with advanced tumors, and cells from such treated mice only partially suppress the anti-tumor action of cells from immunized donors *(82–84)*. In other experiments, the action of suppressor (regulator) cells was demonstrated in a model of adoptive immunotherapy of malignant tumors and their characteristics described *(85–87)*.

Later, F. Čulo investigated the role of prostaglandins, especially PGE2, in growth malignant tumors, particularly squamous carcinomas of the head and neck in humans. He showed that the amount of PGE2 production is commonly a bad prognostic predictor for survival of the patient or reappearance of the tumor, which may partially be due to the result of the general immunosuppressive action of PGE2 on reactions of cell-mediated immunity *(88–91)*. Experiments with different mouse tumors have shown that the anti-tumor effect of nonspecific inhibitor of prostaglandin synthetase, indomethacin, depends on the amount of total prostaglandin production or production of PGE2, and (that for its action is presence of host T lymphocytes?) *(92)*

In parallel with these investigations, F. Čulo studied the mechanisms of acute injury of the liver by xenobiotic and its *protection*. Acute liver damage in laboratory mice was induced by administration of a lethal or sublethal dose of acetaminophen (Paracetamol, APAP). Toxic effect of APAP was followed based on survival of mice, concentration of serum aminotransferases (ALT, AST) and histopathological changes in liver tissue. It was shown that PGE2 or its stabile analogue – dibutyryl-PGE2) has a protective effect on APAP toxicity when given several hours before administration of APAP or up to two hours after it (when the conversion of APAP into toxic metabolite is already done) *(93)*. Later it was shown that IL-1α, IL-1β and IL-6 have a protective effect, but only if applied before intoxication of mice with APAP. Protective effect of IL-1α can be partially cancelled by administration of anti-PGE2 or anti-IL-6 antibodies *(94, 95)*. Ketoconazole, an antifungal agent, and dipyridamole, which influence the activity of prostaglandin synthetase havea protective effect on toxicity of APAP, increase the synthesis of PGE2 and PGI2 and decrease synthesis of TXA2 in liver tissues (96 and unpublished results). It was shown that APAP alone induces the synthesis of some of these mediators (IL-1α, IL-1β and IL-6), as a part of the host defensive mechanism, which however is not sufficient to complete protection against xenobiotic toxicity *(97)*. These results show that these mediators act

in a cascade (that they, perhaps,) through a common protective pathway. Some preliminary results show that a common protective pathway starts with synthesis of cAMP in liver cells *(98)*. Radioprotective drug (WR-2127) decreases the toxicity of APAP *(99)*.

In a smaller part of his investigations he studied the regulation of secretion and action of cytokines in various physiological and pathological states *(100–103)*. He showed that nitrogen oxide (NO) is excreted in a smaller quantity in patients with periodontitis than in normal persons *(104, 105)*. Recently, he has been included in investigations of immunological mechanisms in pathogenesis of rheumatoid arthritis *(106)*.

He is co-author of the Catalogue of Knowledge in Immunology of the Association of Societies of Immunology of Yugoslavia *(107)*. He is co-author is of an article and one of two editors of the monography »Klinička imunologija u nas« *(108)*. He was the president of the Croatian Immunological Society in the period from 1999 to 2003, in which he initiated activities for separation of the Croatian Society for Immunology from the Association of Societies of Immunology of Yugoslavia and carried out preparaton work for its acceptance in EFIS and IUIS, which was realised about one year after termination of his mandate. At that time, he was one of the initiators and co-authors for composition of the Catalogue of knowledge and skills for specialization in the field of basic immunology, which, unfortunately, was never realised.

Matko Marušić joined the Department of Physiology in 1968, as a student of medicine, and worked with F. Čulo until he graduated. This cooperation continued after 1971, when M. Marušić formally became a member of the Faculty. Čulo, Marušić and coworkers worked together in the field of tumor immunology and immunotherapy *(30, 39–41, 79, 91,109–114),* but also developed their own research projects. Some degree of cooperation, however, was maintained until retirement, and later mostly in their teaching and scholarly activities.

In his independent research, M. Marušić studied the relationship of the immune system and carcinogenesis where he was the first to demonstrate that the frequency of chemically induced tumors in immunosuppressed mice was not different from that in normal mice, a finding that seriously challenged a crucial part of the theory of immune surveillance *(32)*. However, in concordance with the immune surveillance theory, M. Marušić and coworkers found that immune deprivation enhances tumor growth *(115)*. The phenomenon was much stronger in female mice *(116, 117)*.

The aim of the team at the Department of Physiology was to study immune reaction to tumor by using T-cell deprived mice, hoping that in severely immunodeprived mice it would be possible to grow and study reaction to human tumors. Thus Marušić was assigned the task of producing T-cell deprived mice by adult thymectomy, lethal irradiation and transplantation of syngeneic bone marrow cells (TIR mice). However, it was soon discovered that the transfer of bone marrow cells under certain conditions might also bring about a transfer of immunological memory *(28)*. Soon, it became obvious that the memory transfer was due to transfer of very small numbers of T lymphocytes contaminating the bone marrow transplant *(118, 119)*. The number of T cells necessary to transfer the full second-type immune response to thymectomized, irradiated mice was determined to 10^4 *(120)*. The model also revealed that T cells have different effectiveness against established versus nonestablished allogeneic grafts *(121)*.

In his studies of the immune reaction to tumor, M. Marušić discovered that thymectomized, lethally irradiated mice reconstituted with bone marrow (TIR) are unable to reject xenogeneic (rat) tumor and that the rejection of the tumor could be achieved by addition (IV injection) of syngeneic T lymphocytes *(16, 122)* which M. Marušić recognized and developed into a model for study of T-B cell cooperation *(123)*. After it was shown that in this model rejection of the xenoantigens can only be achieved by syngeneic, and not allogeneic T cells, M. Marušić completely switched to the study of genetic restrictions of T-B lymphocyte collaboration. This area of research was enhanced by his postdoctoral training in Oak Ridge national Laboratory, Oak Ridge, TN, USA 1976-1978 (124–127). He developed a model of genetic requirements for T-B cell cooperation *(128)* although the scheme was disproved by the studies of R. Zinkernagel and P. Doherty, who in 1996 were awarded the Nobel Prize for clarification of the physiological mechanisms of immune cell interactions.

In his efforts to elucidate the genetic restrictions of T-B cell cooperation, M. Marušić also encroached into immunogenetics, mainly through cooperation with the renowned immunogeneticist, Dr Jan Klein of Tuebingen, Germany. During 1991–1994, Marušić spent one year of research in Tuebingen *(129, 130, 131)*. His most significant finding was that common environmental antigens are recognized through both class II antigens of the major histocompatibility complex *(132)*.

One of his relatively limited, but possibly most important, contributions is his study of the function of aging human thymus *(133)*. With his collaborators, he was the first to prove that human thymus retains its function of T-cell maturation throughout life *(134)*.

In 1986, M. Marušić was invited to lead (head) the Clinical Immunology Laboratory at the Zagreb University Hospital Center (Rebro), primarily to boost the bone marrow transplantation program by his immunological laboratory expertise. He worked there until the end of 1995. A number of publications resulted from the clinical work of the Bone Marrow Transplantation Team *(135–137)*. The most significant finding that ensued from this work is the demonstration that the bone marrow

to be harvested from a human donor mathematically has zero volume, and is sucked (suspended) in the peripheral blood, i.e., that the volume of the peripheral blood in the bone marrow transplant determines the amount of T lymphocytes (abundant in the peripheral blood) in the transplant *(138, 139)*. This research field was also extended to limited efforts to study details of bone marrow transplantation in animal models (140, 141), with the general idea of separating the transplant's graft-versus leukemia from graft versus host effects *(142)*.

Working in the clinical laboratory, primarily aimed at supporting the bone marrow transplantation program, M. Marušić and his coworkers concentrated on the introduction of flow cytometry cell typing method and the respective analysis of hematological malignancies (143–154)

However, Marušić's laboratory also supported other areas of clinical immunology research, such as neurology *(155, 156),* pediatrics *(157),* anesthesiology *(158, 159),* and oncology *(160–166)*. A good portion of Marušić's activities in clinical immunology was devoted to studies of autoimmune diseases (167–172). Some of his work, especially important for the development of clinical immunology at the new Medical School in the city of Split, was in allergology *(173–179)*.

Milan Taradi was already involved during his medical study in scientific research in the field of the effects of prenatal stress on corticosteron and in the field of autoimmune thyroid diseases.He started his scientific and teaching career in the Department of Physiology at the School of Medicine, where he is working to this day. He was involved in the research team of Prof. N. Allegretti, which initially dealt with tumour immunology, especially tumour-host relations in early stages of carcinogenesis *(18)*. Later, he studied the tolerance and prevention of tumour growth in immune deficient mice *(180)*. and the microenvironmental conditions during tumour growth, particularly the impact of selective pressure from the tissue microenvironment on tumour growth and its clonal nature in a model of Yoshida ascites sarcoma in rats *(19, 181)* The process of tumour integration into multicellular organism consists of two phases: the nidation of tumor cells and the immunological reaction. In our laboratory we developed a model that enables the study of both phases separately. In the first phase the dominant role is played by macrophages, which are able to destroy tumour cells by nonspecific action without general immunological reaction *(66, 67)*. M. Taradi has developed a method of observing tumour nidation by autoradiography. He researched the modification of macrophage cytostatic antitumor activity by phorbol esters and calcium ionophores He also studied the proton transport mechanisms in rat peritoneal macrophages and particularly the role of the Na-H exchanger *(182)*

The second field of his scientific interest is the research of experimental allergic encephalomyelitis (EAE) in rats, a model for the study of multiple sclerosis in humans. He found an important role of macrophages in induction of EAE. The EAE induction, along with myelin basic protein (MBP) also requires functionally competent macrophages *(183)*. Also, specific immunological tolerance and prevention of EAE can be induced by administration of MBP before EAE induction *(184)* He also studied immuno-regulatory cells involved in weakening of concomitant immunity *(64)*.

During his post-doctoral fellowship in 1988/89, first in the Department of Radiation Medicine at the Massachusetts General Hospital, Boston (USA), and later in the Department of Radiation Medicine, University of Kentucky (USA) he focused his research on biological response modifiers that potentate the immune response with the aim of improving cancer therapy.

Sunčana Kukolja Taradi demonstrated that the local administration of OK-432 (Picibanil) enhanced the response of murine tumour and normal tissue to elevated temperature *(185)*. This OK-432 enhanced thermal response was mediated by the potentiating of NK cell activity *(186)*. This treatment has been shown to strongly inhibit not only local tumour growth, but also reduce the development of spontaneous lung metastases in mice *(187)*

Recently, M. Taradi and S. Kukolja Taradi have been particularly focussed on the use of information and communication technology (ICT) in research and presentation of knowledge in the field of immunology (physiology and other medical fields *(188, 189, 190)*

This review shows the work of a group (team) of researchers at the Department of Physiology who started their research in collaboration with the late Prof. Nikša Allegretti. About 15 years ago, other young researchers at the Department, heade by Assistant Professor D. Grieve, started investigation of the immunological processes connected with mechanisms of bone formation in collaboration with Prof. Ana Marušić from Department of Anatomy School of Medicine in Zagreb. Their contribution is reviewed in the appending review.

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OSTEOIMMUNOLOGY AS A NOVEL RESEARCH FIELD

The interdisciplinary field of osteoimmunology, focusing on the close relationship between the immune and skeletal systems, has recently attracted much attention due to the observations that bone metabolism is often disturbed by an abnormal response of the immune system and that mice lacking immunomodulatory molecules often exhibit an unexpected bone phenotype *(1–3)*. Within the bone marrow, the bone and the immune system are anatomically, developmentally and functionally related. Bone and immune cells share the same progenitors, their differentiation may be driven by the same supporting cells, and the same regulatory molecules play a crucial role in the regulation of both bone and immune cell differentiation and activity *(3)*. Therefore, the role of any regulatory molecule within the bone marrow microenvironment should always be considered in studying both the immune and bone cells.

In addition to the investigation in the field of transplantation immunology and hematology *(4–9),* our research group opened the new and intriguing field of osteoimmunology some 13 years ago, searching for both bone and immune effects of the same regulatory factor. Interestingly, one of our first studies showed that the complexity of the genetic regulation of cell functions was visible not only as a variation in the immune response among different inbred mouse strains or differences in the peak bone mass, but also in the ability to form new bone after an osteoinductive stimulus *(10)*.

Our group developed two experimental models of osteoinduction in the mouse, which replicate endochondral or membranous bone formation during fetal development. We were the first to show that blood can serve as a carrier for osteoinductive bone morphogenetic proteins (BMP) instead of collagen or calcified matrices, which led to the development of an *in vivo* model of bone induction by recombinant human (rh)BMP-2 administered in a blood clot *(11)*. We also adapted to the mouse model the method of stimulating membranous bone induction by mechanical ablation of the tibial bone marrow *(12, 13)*.

Studying interactions between cells of the immune system and the bone

Both major lymphocyte populations are involved in the regulation of bone metabolism. Our group studied the role of B-lymphocytes in the regulation of new bone induction and regeneration, using mice produced by targeted disruption of the μ-chain (μMT mutation), in which the development of B-lymphocytes is arrested at the pre-B-cell maturation stage *(12)*. Using the model of new bone induction by implantation of rhBMP-2 in a syngeneic blood clot *(11),* we showed that the lack of mature B-lymphocytes in these mice did not directly affect bone formation, but rather enhanced recruitment and proliferation of osteoprogenitor cells *(12)*. In addition, to be involved in the regulation of bone cells differentiation, B-lineage cell population contains progenitors that can differentiate into osteoclasts *in vitro*. Based on these findings, we investigated whether estrogen withdrawal by ovariectomy affects the ability of receptor activator of NF-κB ligand (RANKL) and macrophage colony-stimulating factor (M-CSF) to stimulate formation of osteoclasts in unfractioned and B-cell lineage marker CD45R-positive murine bone marrow cell culture, and showed that CD45R expression identified a subset of murine bone marrow cells whose ability to form osteoclasts *in viv*o is regulated by estrogen *(14)*.

By investigating the role of T-lymphocytes in the regulation of bone metabolism, we first showed that T-lymphocyte depletion by neonatal thymectomy in rats primed the osteoinductive sequences of endochondral new bone formation *(15)*. Then we showed that bone turnover in mice deficient for β_2-microglobulin, which is a constituent of the class I major histocompat-

ibility complex molecule and is crucial for its normal function in cell recognition by $CD8^+$ subpopulation of T-lymphocytes, was adequate for the stage of their skeletal maturation and similar to that in their wild-type littermates *(16)*. Finally, we assessed the role of T-lymphocytes in endochondral new bone formation in the model of *in vivo* rhBMP-2 implantation in $CD4^+$ and $CD8^+$ T-lymphocyte depleted mice *(3)*. By demonstrating increased cartilage formation in mice depleted of $CD4^+$ and $CD8^+$ T-lymphocyte subsets *in vivo*, we confirmed that T-lymphocytes play an important role in the endochondral sequence of new cartilage and bone formation.

Our group also provided a body of evidence that T-lymphocytes play an important role in the regulation of osteoclast differentiation and function. To investigate the role of T-lymphocytes in osteoclast differentiation, we selectively depleted $CD4^+$ and $CD8^+$ T- lymphocyte subsets *in vivo* and analyzed osteoclastogenic potential of the murine bone marrow and splenic progenitors after stimulation with 1,25-$(OH)_2$ vitamin D_3 or RANKL and M-CSF *(17)*. T-lymphocyte depletion did not affect osteoclast formation from bone marrow and spleen cells treated with RANKL and M-CSF, but it significantly stimulated osteoclastogenesis in bone marrow cultures stimulated with 1,25-$(OH)_2$ vitamin D_3. This effect was mediated by enhanced prostaglandin production and subsequent upregulation of RANKL and downregulation of osteoprotegerin (OPG) expression. In order to further test the effect of activated T-lymphocyte on bone remodeling we used several models of T-lymphocyte activation – mitogen-pulse, anti-CD3/CD28 stimulation, and *in vivo* and *in vitro* alloactivation *(18)*. Each method of activation had an inhibitory effect on *ex vivo* osteoclastogenesis stimulated by RANKL and M-CSF from murine hematopoietic progenitors and showed that activated T-lymphocytes inhibit osteoclast differentiation by diverting early hematopoietic progenitors towards dendritic cell differentiation through down-regulation of RANK and c-Fos.

Studying cytokine microenvironment of the bone and bone marrow

Bone metabolism and differentiation of bone cells are influenced by different cytokines and growth factors, including members of the TNF-superfamily. To estimate the role of a proinflammatory cytokine TNF-α and its receptor TNFR1 in new bone formation, we used two models of new bone induction (intramembranous ossification following mechanical tibial marrow ablation and endochondral ossification induced by rhBMP-2 implantation) in TNFR1-deficient (TNFR1 –/–) and wild-type control mice. Intramembranous osteogenesis was not altered in TNFR1 –/– mice but these mice formed more cartilage and bone during endochondral osteogenesis *(19)*. We concluded that the TNFR1-signaling pathway is involved in the restriction of endochondral but not in the intramembranous osteogenesis by down-regulating BMPs and the RANKL/RANK cytokine system.

Another cytokine from the TNF-superfamily extensively studied by our group is the Fas ligand (FasL). To assess the role of Fas/FasL system in the regulation of bone metabolism, we first studied the bone phenotype in mice with the point mutation in FasL gene – *gld* mice. We showed that *gld* mice had greater total body bone mineral density and increased trabecular bone volume and did not lose bone after ovariectomy *(13)*. They formed more trabecular bone after the model of intramembranous osteogenesis, having fewer osteoclasts on bone surfaces and less apoptotic osteoblasts. Osteoclastogenesis from the bone marrow progenitors in *gld* mice was similar to that in wild-type mice whereas osteoblastogenesis was significantly increased. Bone and bone marrow from *gld* mice expressed more OPG than that from wild-type mice.

Our group also studied the effect of FasL absence on endochondral ossification induced by rhBMP-2 implantation in *gld* mice *(20)*. This study showed that FasL deficiency resulted in the increased formation of cartilage and mesenchyme early in the osteoinduction sequence whereas there was no difference in new bone formation between *gld* and wild-type mice at later stages of osteoinduction sequence. We concluded that Fas/FasL interaction was important for early endochondral bone formation, whereas at later stages its effect was probably bypassed by the effect of other cytokines and growth factors present at the site of induction.

To investigate the underlying mechanism of the development of the bone phenotype in *gld* mice, we established a model of parabiosis between *gld* and wild-type B6 mice *(21)*. We hypothesized that the transfer of blood-borne cells could lead to the alteration of bone phenotype as well. Parabiosis resulted in the appearance of double-negative ($CD3^+CD4^-CD8^-B220^+$) T lymphocytes in peripheral blood, bone marrow, spleen and lymph nodes in wild-type mice of the B6-*gld* parabiotic pairs, and their decrease in *gld* mice. During parabiosis of B6-*gld* parabiotic pairs, osteoclastogenesis decreased in wild-type parabiotic mice similarly to the osteoclastogenesis in their *gld* counterparts, whereas osteoblastogenesis increased in wild-type parabiotic mice, resembling the osteoblastogenesis in *gld* mice. Our study showed that the circulation of cells from the *gld* to the wild-type mice resulted in the transfer of the *gld* bone phenotype to the wild-type parabiosis pair counterpart.

To clarify the cellular and molecular mechanisms responsible for increased bone formation in the absence of Fas in *gld* mice, first we investigated the expression of Fas and FasL during *in vitro* osteoblastogenesis and osteoclastogenesis and the effect of FasL on bone cell apoptosis and differentiation *(22)*. We showed that FasL added *in vitro* was a weak inducer of apoptosis in both osteoclastogenic and osteoblastogenic cultures. However, FasL specifically inhibited osteoblastic differentiation *in vitro* by the mechanism involving caspase 8 activation. The absence of Fas or FasL *in vivo* resulted in a significantly increased osteoblastogenesis, confirming that Fas/FasL system primarily controls osteoblastogenesis by inhibiting progenitor differentiation rather than by inducing apoptosis.

Future research directions

Osteoimmunology is certainly a scientific field that has dramatically expanded during the last ten years. Our group currently investigates functional relations between the bone and immune systems, with an aim to detect changes in the bone microenvironment as a result of disturbances within the immune system, including inflammation, autoimmune reaction and malignant transformation, and to further investigate specific signaling pathways involved in the differentiation of bone cells. New findings in the field of osteoimmunology will contribute to better understanding of several pathological conditions that develop in bone microenvironment and affect bone metabolism, as well as help identifying potential osteoprotective therapeutic targets.

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INTRODUCTION

In this paper we describe the period, starting from the middle sixties of the last century, as a very important time in the history of the development of immunological research and teaching of immunology at the Department of Animal Physiology (DAP), Faculty of Science, University of Zagreb, when the late Professor Borislav Nakić took a leading part in both research and teaching of immunology in Croatia (CIS), as well as in the organization of the Croatian Immunological Society. The past and present time has been presented and the most important scientific work of some members of the Society from DAP was specified. In addition, we describe present scientific achievement of the researchers presently working in DAP in the field of tumour chemoprevention and the new approach to chemoimmunotherapy and radioprotection.

History

Founder of the Department of Animal Physiology (DAP) at the Faculty of Science, University of Zagreb, was Borislav Nakić, M.D., Ph.D., who in 1963 became Professor and Head of DAP. Professor B. Nakić introduced the course of Immunology for undergraduate students of Biology (1965) and graduate students of Biomedicine; years later Immunology courses for undergraduates were taught at the Faculty of Medicine and the Faculty of Veterinary Medicine. Classes of Immunology for undergraduates were provided by staff members of DAP headed by Professor B. Nakić and Professor D. Ikić (Institute of Immunology, Zagreb) and were continued by Professor V. Silobrčić and D. Dekaris until 1987 when Professor I Bašić took over the Immunology classes. During the last 5 years Professors N. Oršolić, B. Malenica and Z. Tadić have also lectured the classes of »Immunology and Immunogenetics«, »Immunoecology«, »Comparative Immunology« and »Tumour Immunology« as well. Professors from other institutions in Zagreb and the Faculty of Medicine in Rijeka have been very much involved in courses on different subjects in immunology for graduates at the Department of Biology at the Faculty of Science, Zagreb (Professors S. Rabatić, R. Mažuran, A. Sabioncello, I. Svoboda-Beusan, B. Vranešić and A. Gagro – Institute of Immunology, Zagreb; M. Jurin, M. Boranić and T. Marotti – Institute Rudjer Bošković, Zagreb; D. Rukavina, S. Jonić and P. Lučin – the Faculty of Medicine, Rijeka).

Borislav Nakić's activity

Regarding the founder of DAP, the late Professor B. Nakić, we all remember him as a great teacher, he was a generous teacher, always ready to help out a student in the scientific methods. He dedicated much time to teaching young scientists, working directly with them, and enabling them to receive further training at some of the world's most prestigious institutions. He truly was an example of a scientist who passed his knowledge and experience onto his associates and students. He was also an excellent lecturer, speaking concisely, clearly and giving many examples. He made every effort to impart knowledge

to his students, but sought a great deal in return. He was strict but just, and his students respected him, just as he respected them. On one occasion (in 1970), he told his students, »*I believe that students are adults, aware and responsible within their own knowledge and experience. But sometimes it's difficult to speak to young people. I was like that. But a man learns some things only when he's over 40. You should try to be more flexible. Sometimes I fear you are going off to extremes. You compare young and old, and forget that young and old are the same, because the old were once young, just as you are, and that you will be old in just a few years' time.*«

In 1968, B. Nakić was appointed full Professor at the Faculty of Science, University of Zagreb. One year later, he received national recognition with the Ruđer Bošković Award for scientific achievement. His scientific contribution to world science enabled him to become a member of the Royal Society of Medicine (London), Transplantation Society and European Society of Radiobiologists. He published 31 scientific papers which stood out primarily for the quality of ideas and precision. This is best seen in the fact that even today Nakić's papers are often cited by scientists worldwide *(1–9)*. It should be mentioned that the 1960s was a period of intensive study of the basics of transplantation immunology and transplantation rejection reactions in incompatible recipients. The public was informed on the issue of transplantation rejection after the sensational first heart transplant conducted by South African cardiosurgeon Christian Barnard in 1967. Though the first kidney transplants with unrelated donors were already successfully carried out in 1959 with the application of strong immunosuppressive drugs, these successes failed to arise great interest in general publications. Owing to B. Nakić, the scientific researches conducted in Croatia were world class. Nakić launched the initiative to establish the Laboratory for Tissue Typisation within the Department of Animal Physiology. However, it was only after Nakić's death that the Centre for Tissue Typisation was set up at the Urology Ward of Rebro Clinical Hospital in Zagreb, as a necessary centre for establishing compatibility between kidney transplant donors and recipients.

Tissue Typisation

From the time of its establishment, the Centre was run by Professor Andrija Kaštelan, who worked at the DAP as Head of Department after Nakić's death in 1970.

With kidney transplantation that started in Ljubljana in 1969, preparations were carried out in Zagreb and Rijeka, and the issue arose concerning ethics of tissue and organ transplantation, as well as the preparation of legal guidelines for transplanting organs from the deceased. Nakić was aware of the danger involved in transplanting organs and wrote the following on that topic: »*There is an important circumstance that forces us not to condemn the transplantation of organs as an act that is not in line with our principles of ethics. At this time, organ transplant is the only way to ease the difficulties and extend the life of a patient with severe or irreversible damage of both kidneys, even returning the patient to an active life. From the ethics standpoint, to a patient who is doomed to imminent death we cannot deny the help that today's science can offer…However, it is necessary to respect the principles of human rights and to create the legal mechanisms that will protect donors from possible abuses…What are the reasonable boundaries we can go to in replacing diseased organs and tissues, without threatening the integrity of the human character? This perhaps relates more to the transplantation of living organs, and in particular the possibility of transplanting brains in humans. Fortunately, such an operation is such a difficult biological and technical issue that it is unlikely we will be able to resolve it in the near future.*«

As mentioned above, Professor A. Kaštelan succeeded Professor B. Nakić after his death and headed DAP up to 1986 when he moved to the Faculty of Medicine, Zagreb. From that time on he headed the Department of Tissue Transplantation at the Clinical Hospital Centre Rebro, Zagreb, until recently; his scientific activities were mostly devoted to the field of tissue transplantation (see article by V. Kerhin in this issue). Professor I. Bašić headed the DAP from 1986 until 2007.

Immunology research

Research in immunology during the last 20 years mostly covered experimental haematology chimerism after transplantation of hematopoietic tissue (Professor D. Volf, *9, 10, 11, 12*); thymus gland and its products (Professor O. Springer, *7–9, 13*); the field of Veterinary Immunology (I. Bašić's work described in the article by I. Valpotić in this issue); tumour immunology and biotherapy *(14, 15, 16);* chemoprevention of tumour development by honey bee products and their polyphenolic compounds as well as combined therapy of tumours by these products and chemotherapeutics, and the radioprotection by them conducted by N. Oršolić, appointed Professor and Head of DAP, and by I. Bašić.

Further in this publication we report on our work done within the DAP during the last ten years in which the particular work in chemoprevention, combined treatments and radioprotection by honey bee products has been described.

Chemoprevention by honey bee products; their influence on the immune system, combined treatments with chemotherapeutics and radioprotection

The immune system plays an important role in maintenance of the body homeostasis by eliminating endogenously formed mutated cells such as virus-infected or tumour cells as well as exogenous cells by invading microbial organisms. Many immunological modulators capable of improving immune responses have been developed so far, some being widely used in clinics such as lentinan, cyclosporine and ascorbate derivatives. One might expect the advent of novel immunostimulators, because safe and effective drugs are of critical importance in the control of infectious diseases, tumour development, and various types of allergic diseases. Anti-infectious drugs such as antibiotics or antivirals are beginning to exhaust their therapeutic capabilities. The occurrence of dysfunction of the immune system requires new approaches. Immunomodulation through natural or synthetic substances may be considered as an alternative for the prevention and cure of infectious diseases and of neoplastic diseases *(17–33),* respectively.

The target of much research has been connected to the discovery of natural and synthetic compounds that can be used in prevention and/or in treatment of cancer. Many plants have shown to possess various biological activities like immunopotentiating and antitumor efficiency. Honey bee products and their flavonoid components are the most promising as antitumor *(17–33),* immunomodulatory *(18, 20, 22–24, 27–32)* and radioprotective *(34–39)* agents.

There has been a revival of interest in medicinal properties of honey bee products because they are thought to exhibit a broad spectrum of activities including antibacterial, antifungal, antioxidant, cytostatic, wound healing and antitumor effects together with anti-inflammatory properties *(40–46)*. Apitherapy is the medical use of honey bee products to promote health and healing. This can include the use of honey, pollen, propolis, royal jelly, and bee venom.

Since the target of much research has been aimed at the discovery of natural and synthetic compounds that can be used in prevention and/or in treatment of cancer, our interest was investigation of honey bee products and their effect on tumour growth and metastases formation as well as the search for the mechanism(s) of their antitumor action. Our first paper in the field of apitherapy was published in 1996 *(20)* entitled »A royal jelly – a new potential immunomodulator in rats and mice« and described the use of royal jelly, which serves as food for all young larvae and as the only food for larvae that are developing into queen bees. Royal jelly contains free amino acids, proteins, sugar, fatty acids (mainly 10-hydroxy-2-decenoic acid), minerals (mainly iron and calcium) and vitamins (thiamine, niacin, riboflavin). Our findings demonstrated that royal jelly given *ip* or *sc* before or after tumour cell inoculation had no effect on metastasis formation *(25, 33)*. However, royal jelly administered to mice *iv* synchronously with tumour cells exerted significant effect on metastases formation of these cells in the lungs. It is likely that 10-hydroxy-2-decenoic acid and saturated fatty acid present in royal jelly directly affect metastatic ability of injected tumour cells *(25, 33)*. In addition, our data indicated that royal jelly exhibits immunomodulatory properties by stimulating antibody production and immunocompetent cell proliferation in mice or through the depression of humoral immune function in rats *(20)*. Both phenomena could probably be reversed by changing the dose or the route of royal jelly application.

The investigation of immunomodulatory and antitumor properties of bee venom, propolis, and its polyphenolic compounds, were part of the studies described in Ph.D. thesis entitled »Antitumor activity of bee venom and propolis« by Nada Oršolić under the mentorship of I. Bašić; results concerning bee venom published in *(22, 25, 33)* described antitumor effects of bee venom. Bee venom contains major components including histamine, catecholamines, polyamines, melittin, and phospholipase A_2 *(25, 33)*. Melittin, present in bee venom about 50–70%, is antimicrobial and antitumoral peptid of bee venom. Melittin has been shown to revert the transformed phenotype of H-ras transformed cells *(described in reference 22)*. It was demonstrated that melittin is one of the most potent inhibitors of calmodulin activity, and as such is also a potent inhibitor of cell growth and clonogenicity *(22)*. Calmodulin inhibitors are cytotoxic to malignant cells both *in vitro (22)* and *in vivo (25, 33)*. Mechanisms of antitumor action were described as apoptosis, necrosis and lyses of tumour cells *(22)*. We demonstrated direct antitumor effect of bee venom given intratumourally at different times after tumour cell inoculation in mice and cytotoxic effect of bee venom on HeLa cells and on primary culture of MCa cells. In our study *(22)* we demonstrated that antitumor and antimetastatic effects of bee venom could be highly dependent on the route of injection and on close contact between bee venom and tumour cells. When bee venom was injected intratumourally, tumours decreased in size; some sort of shrinkage of tumours occurred and the delay of tumour growth was evident. Survival of bee venom treated mice was prolonged as compared to control mice. Bee venom significantly inhibited tumour growth; tumour inhibition effect of bee venom was dose- and time dependent *(25)*. We have recently shown that inhibited tumour growth by bee venom was the consequence of apoptosis and/or necrosis of tumour cells or it was the result of the activation of immune system by bee venom *(22)*. It was also shown that less differentiated cells (leukemic cells, tumour cells) were 2–4 times more sensitive to the lytic effects of bee venom than normal splenocytes or bone marrow cells *(22)*; the reason for this is probably the loss of amino/carbohydrate binding structures which led to destruction of tumour cells that were in close contact with bee venom.

Requirements for close contact between mammary carcinoma (MCa) and bee venom for *in vivo* effect was also shown in *in vitro* studies *(22)*. The degree of growth inhibition of MCa cells in the presence of bee venom was dose-dependent up to 24 hours. It is likely that bee venom has a short half-life for the effect on thymidine incorporation since its active ingredients could be unstable in tissue culture medium. This may be explained by inhibition of calmodulin or by the effect of bee venom on induction of apoptosis and necrosis of tumour cells described in our *(22)* studies. 48-hour incubation of MCa is important for the appearance of tumour cells resistant to bee venom (dose 1.43). Our *in vitro* results with other cells (HeLa and V79) showed that the increase of the glutathione levels might be involved in their resistance to bee venom.

The responses of regional lymph node cells were increased in animals treated with bee venom *(22)*. The results related to the lytic activity of popliteal lymph node cells on MCa cells *(22)* indicate that bee venom is a strong activator of antitumor lytic activity in lymphoid cells deriving from the regional lymph node. Inactivity of spleen cells in this respect indicates that concentration of bee venom is an important factor for activation of antitumor lytic activity in mice. The possible mechanism(s) of antitumor lytic activity may include factors related to activation of cytotoxic T lymphocytes. Local treatment with bee venom increased the $CD8^+$-T cell subset and led to progressive reduction of the immune index ($CD4^+/CD8^+$ ratio) in favour of $CD8^+$ cells. A low $CD4^+/CD8^+$ ratio in lymph node of 0.75 is observed in the treated group compared with the control group (1.43). The $CD4^+/CD8^+$ ratio in spleen between the control and treated group was 1.45 and 1.35, respectively. This implies that the phenotype of $CD8^+$ cells increased by local stimulation of regional lymph nodes by bee venom may have an important role in tumour cytotoxicity. Moreover, *iv* treatment with bee venom increased the response of spleen cells to polyclonal mitogens *(22)*. Thus, bee venom might have a direct and an indirect action on tumour cells by stimulating the host cells, mainly macrophages and cytotoxic T lymphocytes.

Our investigations with propolis and its polyphenolic/flavonoid compounds revealed that macrophage activation was likely to be the most important effectors mechanism of antitumor activity of test compounds *in vivo (21, 25–28, 32)*. These findings suggested that propolis and some of its components stimulated macrophages and reduced the number of mammary carcinoma metastases in CBA mice *(23, 32)*. Results also demonstrated a reduction of tumour volume and increase in life span (ILS) when test compounds were given before tumour cell inoculation *(25)*.

Since immunomodulation is known to be of importance in controling tumour growth and its spread, we studied the effect of WSDP and its polyphenolic components on haematological and immunological parameters in mice. Findings demonstrated that the mitogenic effect of supernatant of macrophages from mice treated with WSDP exerted strong activity on production of lymphocyte activation factor (LAF) that influenced incorporation of ^{3}H- thymidine in primary culture of syngeneic mouse thymocytes (21). Increased level of LAF activity produced by WSDP activated macrophages correlated directly with the reduction of metastases in the lungs of treated mice and with tumour cytotoxicity *in vitro (21)*. These findings suggested that WSDP possesses the ability to activate macrophage to produce factors capable to regulate the function of B- and T-cells, respectively. The elevation of both $CD4^+$ and $CD8^+$ T-cell subsets in tumour-bearing mice, after treatment with WSDP, showed a dose-dependent effect of WSDP that leads to progressive reduction of the $CD4^+/CD8^+$ ratio in favour of $CD8^+$ cells *(30)*. It thus appears that, the antimetastatic activity of WSDP was, at least in part, the consequence of immunomodulation of the host's immune system.

Since interferon gamma (INF-γ), TNF-α and IL-2, produced by Th-1 lymphocytes as promoters of host defence, induce the synthesis of NO by macrophages, it was in our interest to check whether WSDP and related polyphenolic compounds (CAPE, CA) influence the synthesis of NO. Studies revealed that peritoneal macrophages from mice treated with 50 mg/kg of WSDP or CAPE when cocultured with HeLa cells produced significantly higher amount of NO than the control cells *(32)*. At the same time, the percentage of ^{3}H-TdR incorporation into tumour cells was lower than in control cells. In contrast, peritoneal macrophage from mice treated with CA expressed very strong cytotoxicity to HeLa cells as compared to control ones, suggesting that (an) other mechanism(s) different from that of WSDP and CAPE should be taken into consideration. Activated macrophages produce increased level of reactive oxygen species, including H_2O_2, which are known to modulate cellular functions including those of lymphocytes *(26)*. Our results supported these findings since the response of spleen cells to polyclonal mitogens was suppressed in mice treated with CA *(26)* while WSDP exerted an opposite effect. These pro-proliferative effects may predominate since WSDP increased LAF *(21)* activity that might be associated with enhanced T and B cell proliferation.

These findings, however, confirmed that the dose of WSDP was an important factor for activation of the mechanisms involved in antibody production, as well as the time intervals between antigen introduction and treatment with WSDP. Thus, findings suggested that a continuous presence of WSDP is necessary to assure activation of mechanisms involved in antibody production, such as macrophage activation and production of factors regulating the functions of B- and T- lymphocytes *(21)*. Concerning the plaque formation, the number of IgM PFC of spleen in our studies was significantly increased in WSDP treated mice when SRBC were given to mice at the time of injection of WSDP and 24 or 48 hours before WSDP respectively;

the most pronounced effect was achieved in a group of mice receiving SRBC 24 hours before WSDP treatment *(26)*. These findings suggested an adjuvant effect of WSDP. Although PFC is an endpoint to evaluate the humoral immune response, the response to SRBC requires the cooperation of a number of cell populations, including B cells, T helper cells, and macrophages. Findings from these experiments confirmed that WSDP could strongly activate the processes included in production of antibodies. These findings, however, confirmed that the dose of WSDP was an important factor for the activation of mechanisms involved in antibody production *(26)* as well as the time intervals between antigen introduction and treatment with WSDP. WSDP also increased, in a time-dependent manner, the levels of γ-globulins in treated mice when mice were given WSDP before immunization with antigen; γ-globulin levels were higher when the time between WSDP and antigen injection was longer *(26)*. Treatment with WSDP at the time of antigen injection resulted in the lowest level of γ-globulins.

Our findings also suggested that WSDP possesses the property to activate macrophage to produce factors regulating the function of B- and T-cells, respectively *(21)*. Macrophage spreading revealed that the treatment with test components affected the functional state of macrophages. Results showed that macrophage spreading involved an increase in size and content of large cytoplasmic vacuoles *(27, 28, 43)*. The highest macrophage spreading was achieved with preparations of WSDP (Croatian and Brazilian) as compared by a single flavonoid *(32)*. It is likely that the antitumor activity of WSDP was the result of synergistic activities of its polyphenolic compounds *(28)*. It should also be pointed out that, for the tumour-bearing mice treated with test components as compared to control, there was an increase in the median values of percentage spreading accompanied with an increase in cytotoxicity on EAT cells and in their effect on induction of apoptosis in tumour cells. Treatment with WSDP and polyphenolic components also yielded an increase in the percentage of PMN cells when compared with control group *(28)*. However, in spite of a quantitative increase of PMN cells in test component treated mice, a marked decrease in the percentage of tumour cells was not seen in our studies. This suggests that PMN cells alone were not capable of inhibiting tumour growth. It is well known that MN cells, mainly macrophages, are the major cells involved in tumour rejection. Namely, activated macrophages have higher capacity to survive in acid environment, since the oxidative burst is a sequential step after the processes, such as phagocytosis, enzyme liberation, free radical generation, as well as production of mediators of inflammatory processes. Our findings *(27, 28, 43)* suggested that the immunostimulatory and antitumor activity of propolis may be associated with macrophage activation and enhancement of macrophage phagocytic capacity *(27, 28, 43)*.

In 2005 we used propolis or its corresponding flavonoids in combined therapy with chemotherapeutic drugs in treatments of murine tumours. The combined treatment with WSDP and Epirubicin profoundly inhibited metastasis formation as shown in *(31)*; this synergistic effect is maximal when Epirubicin and WSDP were administered after tumour cell inoculation. In preventive treatment the combination of chemotherapy and WSDP was ineffective. Furthermore, in curative treatment it is likely that more expressed antitumor effect of Epirubicin is elevated by the flavonoids present in WSDP which, through their property to inhibit different kinases and topoisomerase II activities, reduced the growth of tumour cells. In addition, we demonstrated that WSDP prevent the Epirubicin induced haematological toxicity in mice bearing metastases of the mammary carcinoma. In mice treated with WSDP and Epirubicin number of leucocytes and erythrocytes was higher as compared to untreated and mice treated with Epirubicin alone.

Our results in *(18)* provide the first demonstration for the possible mechanism based on inactivation of mice CYP1A2 by WSDP; CYP1A2 is expressed principally in the liver, where it is responsible for metabolism and biotransformation of carcinogens and chemotherapeutics. Results showed that WSDP inhibited CYP1A2 responsible for transformation of 3-methylcholanthrene (MC) in hepatocytes of mice. WSDP was potential inhibitor of CYP activity prolonging chemotherapeutic action in tumour cells, thus increasing their antimetastatic effects. Collectively, these results suggested that the antimetastatic effect of WSDP reflects its multiple mechanisms of action that include antioxidant potential, immunomodulation and effects on xenobiotic metabolizing system.

The combination of WSDP with chemotherapeutic Epirubicin influenced the proliferation of leukocyte populations in peripheral blood *(31)* that was inhibited by chemotherapeutic agents. Thus results in our paper *(18, 31, 43)* suggest that flavonoids from WSDP possess hemostimulative, antioxidative, protective and regenerative properties.

Moreover, our work described in *(36)* has shown that WSDP, EEP, naringin and quercetin given in combination with chemotherapeutic agent Irinotecan delayed Ehrlich ascites tumour (EAT) growth and increased the life span of EAT-bearing mice. EEP and WSDP in combined treatment with Irinotecan increased median survival time compared to the control group or the group treated with Irinotecan alone. In this study, the analysis of the total number of cells present in the peritoneal cavity of mice revealed that all the experimental groups inoculated with tumour cells in the presence of WSDP or polyphenolic compounds of propolis exhibited significantly lower number of cells in peritoneal cavity as compared to control. Combined treatment of test components with Irinotecan showed strong antitumor activity; total number of cells in peritoneal cavity of mice treated with Irinotecan in combination with quercetin or naringin was reduced as compared to control and with Irinotecan alone, respectively *(36, 43, 44)*. Some of the possibilities include: a) maintaining high circulating levels of Irinotecan by WSDP or related

flavonoids due to P-glycoprotein pump efflux activity as demonstrated in paper *(44)*, b) test component may act as an efficient vehicle for selective delivery of chemotherapeutic agents to tumour cells, rather than acting on tumour cells *(19)*, c) test components may act on tumour cells through enhanced immunity and direct DNA damage induced by apoptotic processing *(18)*, d) test components may have a potential to alter metabolic activation of therapeutically administered drug *(18)*, e) synergistic action of flavonoids and chemotherapeutic on topoisomerase I and II *(36)*. We determined that the efficacy profile of propolis and related polyphenolic compounds treatment alone, or in combination with chemotherapeutic Irinotecan, enhanced the activity of immunological effector cells and haematopoiesis in mice bearing tumour. In addition, this study showed that propolis and its polyphenolic compounds may reduce the toxicity of Irinotecan to normal cells (liver, kidney and blood) *(43, 44)*. The major new findings are that the pre-treatment of mice-bearing tumour with propolis and propolis related compound such as naringin or quercetin in combination with Irinotecan resulted in (i) decreased number of total cells in peritoneal cavity, (ii) increased number of WBC, (iii) enhanced macrophage and PMN activity, (iv) protection of liver and kidney cells against Irinotecan-induced toxicity, (v) decrease in the number of micronucleated cells in peripheral blood. According to this observation, it is likely that activation of the immune system is involved in *in vivo* tumour regression.

Usually, in cancer chemotherapy, the major problems encountered are of myelosuppression and anaemia *(31)*. So, it is known that the major toxicities of Irinotecan in clinical use are myelosuppression and diarrhea. Our results have clearly shown that propolis and related flavonoids (100 mg kg^{-1}) in combined treatment with cytostatic may protect WBC, but have not effect on the haemoglobin content and red blood cells (RBC) *(45)*. Moreover, propolis and related flavonoids may guard RBC in the peripheral blood from Irinotecan–induced toxicity as shown by micronucleus assay in *(43)*. In this experiment and our studies performed previously *(36)* we did not observed side effect such as diarrhea and a loss of body weight in combined treatment.

Moreover, propolis and related flavonoids reduced Irinotecan-induced DNA damage of kidney, liver, and leucocytes *(43)* as well as chromosomal breakage *(43)* in groups of mice treated with test compounds without tumour and in all groups with tumour except in a group of mice treated with quercetin combined with Irinotecan. In micronucleus assay quercetin in combination with Irinotecan increased number of micronucleated cells indicating again prooxidative effect of quercetin.

Combination of flavonoids and chemotherapy with Irinotecan produced a clear reduction of drug toxicities *(43, 44)* while Irinotecan alone increased activities of ALT and AST indicating organ dysfunction and cellular injury. Administration of propolis and its flavonoids with cytostatic caused the activities of these enzymes to return to normal levels; protective effect against organ dysfunction and cellular injury of liver or kidney was more expressed in preventive than in curative treatment with test components. This may be due to antineoplastic property of the test components and drug indicating the protective role on tissue damage. Propolis and related flavonoid administered alone to mice showed no alterations in the specific activities of AST and ALT or LDH, suggesting that it did not affect the pancreas and liver, respectively *(43)*. In this work, the evidence that propolis did not induce kidney damage *(43)* came from the findings indicating urea and creatinin levels.

We also showed that propolis and its flavonoids expressed strong radioprotective effect. Our data suggest that propolis preparation (water or ethanolic extract of propolis; WSDP or EEP) and propolis polyphenolic compounds (caffeic acid, naringin, chrysin, or quercetin) posses promising radioprotective effects, comparable to well-established chemical radioprotector aminoethyl isothiourea (AET). Synthetic protector AET in a dose of 1 mM kg^{-1} was used as a positive control in our studies. Propolis and its flavonoids given to mice before or after whole body γ-irradiation (WBI) (9 Gy) may protect mice on molecular *(34, 37, 39),* and on the organism level *(34, 35)*.

Moreover, our studies indicated statistically significant differences in the survival times of WBI mice pretreated with test compounds as compared to control (solvent:H_2O or ethanol). The most effective compound regarding survival of mice was QU, showing protection similar to that achieved by the AET; such a huge protective effect of QU could result from its chemical structure, which consists of the most suitable structural form for scavenging free radicals *(34, 37, 39)*. Treatment with test components after irradiation was ineffective. All other polyphenolic compounds used were also effective in protection against radiation induced damage as well as propolis preparations (EEP and WSDP); the effectivity in radiation protection between WSDP and EEP could be explained by higher contents of polyphenols present in both WSDP and EEP preparations, as presented in (34). The Kaplan-Meier method and the log-rank test have revealed that the surviving time of mice was as follows: quercetin, WSDP, naringin, caffeic acid, chrysin and EEP.

Our recent observations *(31)* proved the protective effect of propolis on bone marrow and lymphoid tissues of mice from cytotoxic drugs and radiation. Augmented immunological activity as seen in increased activity of macrophages, cytotoxic T cells, B cells and NK cells by propolis and related compounds *(31, 34, 37–39),* seems to play a central role in preventing secondary infections associated with irradiation, contributing to further acceleration of haemopoietic regeneration and increasing survival following radiation-induced lympho- and myelo-suppression.

Our results *(31)* show that WSDP protected more of those cells, possibly due to the elevation of glutathione synthesis in their bone marrow which was not compromised by either 3 Gy or 6 Gy WBI. It is likely that treatment with WSDP increases

the ability of haematopoietic tissue to synthesize glutathione in bone marrow compartment, making treated mice more resistant to the harmful damages of irradiation.

More CFUs in haematopoietic tissues of mice as shown by exogenous spleen CFU assay indicate that WSDP used *po* for long period of time exercised stimulative effect on haematopoiesis. The stimulative effect of WSDP on haematopoiesis may be due to IL-1 production and its action to stem cells *(31)* or through its influence on glutathione level. It is likely that the antioxidative effect of flavonoids in biological system is related to a great deal of events including: (i) their ability to scavenge ROS including $^1O_2$, $OH^\bullet$, H_2O_2, $O_2^{\bullet -}$, $HO_2^\bullet$, lipid radical ($LO^\bullet$) and lipid peroxy radical ($LOO^\bullet$; (ii) ability to scavenge nitric reactive radical (HOONO, NO, NO3 i dr.); (iii) inhibition of oxidative enzymes; (iv) metal ion chelation (Cu^{2+}, Fe^{2+}, Zn^{2+} i Mg^{2+}); (v) increase the activity of antyoxidant enzymes and their protection *(34, 35, 39, 45)*.

The development of drugs that radiosensitize the malignant cell and radioprotect the normal tissues remains a challenge for oncologists and radiobiologists.

Whole body exposure of tumour-bearing animals to γ-radiation (4 Gy) resulted in an increase in the comet parameters (such as tail length, %DNA in tail, tail moment) of blood lymphocytes, as well as in tumour cells, as a result of cellular DNA damage. Using the comet assay, we clearly demonstrated that propolis and related flavonoids applied to mice-bearing tumour before or after irradiation have different effect on normal and tumour cells; a significant decrease was shown in comet parameters of blood lymphocytes but not in the tumour cells of irradiated animals *(35)*. Moreover, the number of tumour cells in peritoneal cavity of mice treated preventively with test component was significantly decreased as compared to control *(35)*. It is possible that synergism between radiotherapy and polyphenolic compounds, due to selectively induced apoptosis in cancer cells, was the base for these findings. We proposed that variability in antioxidant defence or DNA repair capability, induction of apoptosis as well as above mentioned difference between cell types and growth state, could be important in determining the susceptibility of the cells to genetic destabilization, cell death or mutation.

To conclude, the DNA damage caused by test components and radiation in our study *(35)* can be based on the experimental evidence of radiation and different mode of action of the test component on tumour as compared to normal cells. The mode of action may include: (i) inhibition of various enzymes involved in DNA repair; (ii) induction of reactive oxygen species (ROS) capable of inflicting DNA damage, (iii) the inability of tumour cells to use extra antioxidants in a repair capacity, (iv) the difference in cellular biochemistry or a lack of sufficient concentration of the propolis and polyphenolic compounds in tumour tissue to elicit radioprotection, (v) the biodistribution of this compound in tumour and normal tissues, the hypoxic environment of the tumour and the poor vasculature in the tumour, (vi) the variations in the physiological and biochemical status of the cells of the tumour compared to normal cells at the time of irradiation and (vii) a selective protection of normal tissues from damage induced by irradiation and cytotoxicity to tumour cells.

A small part of our investigation was based on antitumor effect of honey and pollen on metastases formation. Honey is the foodstuff made by honey bees from the nectar of flowers or secretions from other parts of the plants, which they gather, transform together with their own specific materials, and store in a honeycomb. It is a supersaturated solution of sugars, mainly fructose, glucose, and maltose-like sugar, with traces of sucrose- and glucose- oxidase, hydrogen peroxide, phenolic acid, flavonoids, terpenes, etc. It is shown *(25, 33)* that honey significantly affected the formation of lung metastasis when applied before tumour cell inoculation; however, given to animals after tumour cell inoculation honey enhanced metastasis formation in the lung. The latter suggests that the antitumor effect of honey mostly depended on the time of application; it is likely that polyphenolic components present in honey stimulate host antitumor defence, while in the presence of tumour nutritive constituents of honey prevail the effect of the former. It is possible that honey in the presence of tumour alleviates tumour growth since it contains a mixture of vitamins, minerals and amino acids as those present in plasma, as well as large amounts of glucose. In addition, its high osmolarity induces an outflow of lymph which enhances nutrification and oxygenation, and its acidity favours release of oxygen from haemoglobin in the capillaries of adjacent tissues. In studies, the combination of honey with chemotherapeutics influenced the proliferation of leukocyte populations in the peripheral blood, that was inhibited by chemotherapeutic agents. These results are in line with previous results from this laboratory *(25, 33, 45)* suggesting that flavonoids from honey possess hemostimulative, antioxidative, protective and regenerative properties.

»Bee pollen« is actually pollen from flowers that is collected by bees. Pollens are the male reproductive cells of flowers. Flower pollens are bees' primary food source, and they contain concentrations of phytochemicals and nutritients and are rich in carotenoids, flavonoids and phytosterols. In addition to most vitamins and minerals, bee pollen also provides amino acids, enzymes and coenzymes, fatty acids, carbohydrates and 25% protein by weight. Our studies concerning antimetastatic capacity of pollen reveal that some pollens such as *Castanea* and *Raphanus* given to mice orally before tumour cell inoculation manifested significant antimetastatic effect while the treatment of mice with pollen from *Cistus, Salix,* and *Papaver* was ineffective *(46)*. These results suggest that different antimetastatic effects of pollens from different plant species may be prescribed to their constituents and probable polyphenolic compounds of pollens *(46)*. According to literature presented data are the first ones describing antimetastatic effect of pollen in animal model of metastases formation.

In conclusion, our results confirm that pre-treatment with natural antioxidant such as propolis, honey, pollen and their flavonoids can reduce tumour size and metastases formation as well as reduce the adverse effects of same chemotherapeutic agents on normal cells with equal or increased efficacy on tumour cells. In addition, the results of the present study in mice have given us the hope that propolis and flavonoids can act in the prevention or the reduction of the side effects due to chemotherapeutic agents, and in the future may be used in humans as an adjunct to standard cancer therapy. The haematological, liver and kidney toxicity due to common drugs such as Irinotecan or other chemotherapeutics may be avoided by the use of propolis and related flavonoids.

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Development of immunology in Rijeka is entirely connected with the development of the Medical Faculty in Rijeka which was established 52 year ago (in 1955). This was first higher education institution in Croatia that was founded outside Zagreb. Today, it is the largest scientific and teaching higher education institution outside Zagreb incorporated into the University of Rijeka since 1973. It has more than 450 teaching staff, several undergraduate, graduate and postgraduate training programs, and distinguished international scientific reputation. Immunology, as one of first scientific disciplines developed at the new Medial Faculty, contributed substantially to this reputation.

In this paper we will give a brief overview of the last 45 years of development of immunology in Rijeka that started with the establishment of the Department of Physiology and disseminated towards a number of Medical Faculty departments and health system institutions in the city of Rijeka. It will not be a strict historical listing of major events, but rather description of dissemination of immunology with development of major actors, researchers and physicians, and their achievements over four decades. At the end of the article, we believe that the reader will recognise the power of Rijeka's immunological community and huge immunological expertise that lays in this part of Croatia.

DEPARTMENT OF PHYSIOLOGY AND IMMUNOLOGY

The foundation of the Medical Faculty in Rijeka provided the conditions for the development of basic medical sciences and for scientific and highly professional enrichment of the overall medical activities in Rijeka. However, the origins of immunology in Rijeka date back to 1964, when Professor Nikša Allegretti was for a one year period elected acting head of the Department of Physiology. Professor Daniel Rukavina (since February 1962) and Professor Predrag Eberhardt (since September 1963) had already been his collaborators at the Department.

Subsequently, thanks to N. Allegretti, in September 1965, head of the Department of Physiology became Šime Vlahović (Figure 1). Šime Vlahović had acquired his basic scientific education at the Ruđer Bošković Institute and then stayed in the USA for three years. He worked in Cooperstown (1963–1965) and in Boston (1965) on the problems of bone marrow transplantation to irradiated adult recipients (mice and dogs) exposed to lethal and sublethal irradiation doses, and on selection of the most suitable transplant donor. It later turned out that his research interest and education were of extraordinary significance for the development of immunology and clinical transplantation in Rijeka.

Figure 1. ***Dr. Šime Vlahović.*** *First head of the Department of Physiology and Immunology (1965–1977).*

At that time, several groups of excellent surgeons worked at the Medical Faculty and the Clinical Hospital, of which the group led by Professor Vinko Frančišković was exceptionally interested in new approaches to therapy of irrecoverably lost kidney function (chronic dialysis and transplantation). With the arrival of Š. Vlahović at the position of the Department head, research interest was focused in several areas: (1) study of conditions to prolong the life of tissue transplants, (2) immunology of the mother/foetus relationship, and (3) the role of lymphatic system in control of the rapid growth of normal tissue (compensatory growth after unilateral nephrectomy and partial hepatectomy). Soon afterwards, in October 1966, Biserka Radošević-Stašić became an employee of the Department and took active part in dealing with this problem area. After some time she also established her own research group that was later joined by Mira Ćuk. A great contribution in the field of immunoregulation gave also Vlasta Linić-Vlahović, who jointed to the Department. In 1972 D. Rukavina obtained a one year Fulbright postdoctoral fellowship to work at the University of Texas, South-Western Medical School in the group of Professor Rupert Billingham, one of leading scientists in the field of transplantation at that time. He was granted also by special travel grant to visit ten departments of his preference in the field of immunology and transplantation which helped him to establish professional and personal contacts with many leading scientists in the field.

After a too early demise of Š. Vlahović (March 9, 1977, in his 45th year), D. Rukavina became head of the Department. In the same year, the Department underwent the first substantial functional and architectural reconstruction and three laboratories for research in the field of cellular immunology were established. Thus, lymphocyte subpopulations have been determined since 1977 in Rijeka using the techniques of forming rosettes with xenogeneic red blood cells, serum immunoglobulins have been determined in serum and secretions by immunochemical methods, and functions of lymphocyte reactivity on the panel of polyclonal mitogens, specific antigens and alloantigens were investigated. Determination of alloreactivity in cultured mixed lymphocyte reaction (MLR) was of particular interest also because of its application in clinical transplantation.

In 1979, the Department of Physiology and Immunology became the teaching base also for the course in Pathologic Physiology. B. Radošević-Stašić took up the leadership of this course. All these events in the 1978–1980 period allowed employment of a large group of young collaborators who distinguished themselves during studies as best students, worked at the Department as undergraduate assistants and took part in research activities. An atmosphere filled with extraordinary enthusiasm and the wish to perform new breakthroughs in science was established. We can especially point out Miljenko Dorić, Stipan Jonjić, Miro Morović and Miljenko Kapović in that group (Figure 3).

Figure 2. ***Teaching staff at the Department of Physiology and Immunology in 1977.*** *From the left: Miro Morović, Predrag Eberhardt, Daniel Rukavina, Biserka Radošević-Stašić and Mira Ćuk.*

In 1986, a major reconstruction was carried out at the Department of Physiology and Immunology including very modern furnishing and equipment of research laboratories, rooms for experimental animals, and rooms for classes. In the following ten years a number of cellular and molecular immunology techniques was introduced and expanded, including the cell culture, hybridoma technology and maintenance of cell lines. This was followed by extensive production, purification, characterization and usage of monoclonal antibodies, particularly through flow cytometry, various enzyme linked immunoassays, Western blotting and immunoprecipitation. In the decade 1995–2005, a number of modern molecular biology techniques were introduces.

During two decades of intensive growth (1977–1998), more than ten young collaborators were employed at the Department during one to eight years who, after acquiring academic degrees and basic scientific education under the supervision of D. Rukavina and B. Radošević-Stašić, transferred to many other departments of the Medical Faculty. Today, 22 researchers are employed at the Department, of which 12 have scientific-teaching position and 10 assistants.

Figure 3. ***Members of the Department of Physiology and Immunology in 1981.*** *Sitting from the left: Biserka Radošević-Stašić, Vlasta Linić-Vlahović, Antonija Peršić, Daniel Rukavina, Dragica Kovačević, and Predrag Eberhardt. Standing from the left: Stipan Jonjić, Mira Ćuk, Miljenko Kapović, Marija Kaštela, Jelena Đirlić, Davorka Perčinić, Nadija Peraić, Miljenko Dorić, and Vjera Vučenov.*

Through organized choice of collaborators and research problem areas and through education of collaborators in top-level European laboratories, an atmosphere for further strengthening of immunology in Rijeka was created. Thus M. Dorić stayed on several occasions in Paris, S. Jonjić in Tübingen and Ulm, M. Kapović in Paris, P. Lučin in Ulm and Heidelberg, Vesna Barac-Latas in Wienna, Damir Muhvić in Borstel-Lübeck and Zlatko Trobonjača in Ulm. Researchers and collaborators of the Department have published more than 250 papers in international journals indexed in the Current Contents database. They maintain close collaboration and contacts with a number of European and American research groups.

The expansion of research activities at the Department dates back to 1977, when first projects of the former Republic Scientific Fund were allocated to D. Rukavina. After that, the number of organized research activities was constantly increasing, including national and international grants. This resulted with the inclusion of the group of researchers from Department, leading by D. Rukavina into the Framework Programme 6 network of excellence EMBIC (Embryo Implantation Control – Understanding the molecular tuning of the beginning of life).This network is the first large-scale project funded by the European Commission within the FP6 to reinforce European research on female infertility. It concentrates research potential of more than 200 scientists and clinicians from 19 leading European institutions and 2 private companies from 11 countries**.** D. Rukavina was one of the initiators of the network and the first meeting of the initial group of nine laboratories to define the project proposal was held in March 2002 in Rijeka *(1)*.

During last four decades, the work in laboratories of the Department resulted with more than 90 Doctoral and Master of Science theses. This means that a number of researchers and teachers of the Medical Faculty and Clinical Hospital Centre of Rijeka acquired their basic scientific competences at the Department.

In addition to their research and teaching activities and achievements, the entrepreneurial and free academic spirit at the Department contributed also to the development of leadership and management competencies of teaching staff of the Department. These competences significantly contributed to the development of the Medical Faculty, University of Rijeka and national policies. D. Rukavina, S. Jonjić and M. Kapović were elected Deans of the Medical Faculty, and D. Rukavina, B. Radošević-Stašić, P. Lučin and V. Barac-Latas Vice-Deans in several mandates. Since 2000, D. Rukavina is the Rector and P. Lučin Vice-Rector of the University of Rijeka. P. Lučin is the first President of the National Foundation for Science, Higher Education and Technological Development of the Republic of Croatia, and Croatian accession negotiator with the EU for chapters Education, Culture and Science. In addition to that, members of the Department chaired a number of committees at the institutional, university and national level (M. Ćuk, Z. Trobonjača, J. Ravlić-Gulan, G. Laškarin). S. Jonjić was the president and P. Lučin vice-president of the Croatian Immunological Society. D. Rukavina was the first president of the re-established Croatian Physiological Society (1999), the first president of the European Federation of Immunology and Reproduction (EFIR), and president of world organization of reproductive immunologists (International Society for Immunology of Reproduction) in the period 2004–2007.

BROADENING OF THE COLLABORATION TO OTHER DEPARTMENTS, CLINICS AND FOREIGN INSTITUTIONS

Such development of research potential at the Department of Physiology and Immunology was exceptionally stimulating for the advancement of general research atmosphere at the Medical Faculty and for the establishment of research cores also on other departments and clinics. Medical Faculty in Rijeka resolved many personnel problems by selecting collaborators who attained basic scientific education at the Department of Physiology and Immunology. Thus, M. Dorić became head of the Department of Microbiology and Parasitology in 1987, M. Kapović of the Department in Biology in 1991, S. Jonjić of the Department of Histology and Embryology in 1996, and M. Morović led the Clinics of Infective Diseases. Immunological studies developed then in these basic departments, as well as in the Department of Pathology where Nives Jonjić was elected head in 1993, while Marija Petković became head of the Department of Oncology. A significant number of clinicians who got their basic scientific education and acquired academic degrees at the Department of Physiology and Immunology were in the past period or are presently heads of departments or division heads in clinics: Nikola Matejčić, Herman Haller, Oleg Petrović, Darko Manestar, Darko Ledić, Luka Zaputović, Ksenija Vujaklija-Stipanović, Sanja Balen-Marunić, Anđelka Radojčić-Badovinac, Jovan Tofoski, and Vladimir Vincek.

Collaboration with numerous institutions and eminent researchers from abroad is very extensive and has yielded significant results, which was evident in continuous education of young scientists in foreign institutions, in numerous publications in famous world journals and abundant help in material and equipment. Thus, collaboration was established with laboratories in Paris (G. A. Voisin and G. Chaouat), Chicago (A. E. Beer and K. Beaman), Pittsburgh (T. J. Gill III), Miami (E. R. Podack), Ulm and Tübingen (U. Koszinowski, T. Mertens, J. Thiele, M. J. Reddehase), Borstel-Lübeck (H. D. Flad), Vienna (H. Lassmann), Stockholm (S. Efendić), Pecs (J. Szekeres-Bartho), Liverpool (P. Johnson and S. Christmas), and Milano (A. Mantovani).

DISSEMINATION OF IMMUNOLOGY TO THE DEPARTMENT OF HISTOLOGY AND EMBRYOLOGY

The group of immunologists currently affiliated at the Department of Histology and Embryology originally started their research at the Department of Physiology and Immunology. In 1996 S. Jonjić and part of his group moved to the Department of Histology and Embryology and continued their research work in immunology and experimental pathology. In addition to Bojan Polić and Astrid Krmpotić, several PhD students also joined them and the group continued their research without interruption. The Department was completely renewed and several fully equipped new laboratories were established. Two scientists already affiliated at the Department also joined this group – Ester Pernjak-Pugel and Jelena Tomac.

The group was growing from year to year and through several international and national research projects very high scientific standard was reached, with several publications in leading journals in the field of immunology and virology. Thanks to this, B. Polić was awarded Humboldt Fellowship (1997–1999) and spent his postdoctoral studies in the group of Klaus Rajewsky in Cologne. After finishing her PhD Milena Hasan received EMBO Fellowship and did her postdoctoral studies in Pasteur Institute in Paris and Luka Čičin-Šain spent several years in Germany working in the group of Ulrich Koszinowski. Both of them later on decided to continue their careers abroad. In 2006, A. Krmpotić was awarded Howard Hughes Medical Institute International Research Scholar Grant to perform the research in her home laboratory.

Throughout these years research groups at the Department developed collaborative research activities with many laboratories abroad including Ulrich Koszinowski (Max von Pettenkofer-Institute, München), William J. Britt (UAB, Birmingham), Mathias Müller, (Veterinary University of Vienna), Hartmut Hengel, (Heinrich-Heine-University Düsseldorf), Martin Messerle (University of Halle-Wittenberg), Wayne M. Yokoyama (Washington University Medical Centre, St. Louis), Joanne Trgovcich (Ohio State University, Columbus), and Silvia Vidal (McGill University, Montreal).

IMMUNOLOGICAL RESEARCH AT THE DEPARTMENT OF MICROBIOLOGY AND PARASITOLOGY

Immunological research diffused to the Department of Microbiology and Parasitology when Miljenko Dorić moved to this department in 1987. He has a long standing interest in scientific research on the pathogenesis of bacterial infections. At the beginning the research was primarily focused in new approaches of bacterial identification by the assistance of the numerical-computer system (M. Dorić and Janko Makiš) and in 1997 Dorić introduced a model of experimental murine congenital listeriosis. Almost simultaneously, Maja Abram established a murine model of pregnancy-associated listeriosis, Darinka Vučković *in vivo* model of *Campylobacter jejuni* infection, Tomislav Rukavina started model of *Klebsiella* infection and Brigita Tićac model of *Legionella pneumophila*. The Legionella team was extended in the period by two new researchers, Marina Šantić (arrived in 1998) and Ivana Gobin (2001), while Marina Bubonja who came in 2000 joined the research concerning listeriosis.

In the period 2002–2006 scientific research at Department was extended, as two researchers (M. Abram and T. Rukavina) became independent, leading their own projects. Subsequently in 2006, three other researchers were able to submit their projects and establish their own research teams. In the new project period started in 2007, M. Dorić is coordinating the research program composed of 6 research projects, which is focused on different pathogens that are associated with infectious diseases of significant public health impact: legionellosis, listeriosis, tularemia, campylobacteriosis and gram-negative sepsis. Besides studying the properties of pathogens, the research groups also analyze the host's susceptibility to infection and the ability of the host immune system to control and eliminate the microorganisms. The program is realized in collaboration with researchers from Ljubljana (B. Wraber, S. Smole-Možina, B. Jeršek), Louisville (Y. Abu Kwaik), Cologne (M. Deckert), and Stuttgart (M. Šuša).

IMMUNOLOGY AT THE DEPARTMENT OF PATHOLOGY

Although a majority of research focuses of the Department of Pathology do not fit into the field of immunology, it is important to note that immunological techniques are in the basis of the routine diagnostic performed by the Department. These techniques were introduced since 1993, when Nives Jonjić became head of the Department. In the first period the imunnohistochemistry was performed on frozen sections and couple of years later extended to termostabile epitopes on paraffin embedded tissue sections. Today, the Department is characterizing a panel of more than 200 tissue antigens in phenotyping of tissue histogenesis, particularly in tumour diagnostics. Lymphoma phenotyping was introduced in 1996 and since then the pathologists from the Department are following trends in modern diagnostics of lymphoma, the revised classification from 1997 and the WHO classification from 2000. The determination of tumour histogenesis, particularly predictive diagnostics of tumour markers and receptors is exponentially growing, including also recognition and competencies of Rijeka's pathologists.

In addition to immunihistochemistry, immunofluorescent techniques in diagnostics of autoimmune diseases and transplant rejection were introduced since 1993. It can be expected that also immunogold electron microscopy will be introduced in the close future, given that the complete infrastructure was established in the last five years.

CLINICAL IMMUNOLOGY AND TRANSPLANTATION IN RIJEKA

Development of basic immunologic research at the Department of Physiology by Š. Vlahović in 1965 could not pass unnoticed so that clinicians expressed their interest in collaboration. The core transplantation group was soon set up consisting of clinicians headed by V. Frančišković and scientists from basic research institutes led by Š. Vlahović (D. Rukavina, V. Linić-Vlahović, P. Eberhardt, and B. Radošević-Stašić). Regular meetings were held (27 meetings in total) where current aspects of immunology and transplantation and clinical experiences were discussed. Young clinicians went to other centres to acquire education (Petar Orlić, Ksenija Vujaklija). All that resulted in quality preparations for the first successful kidney transplantation in 1971, not only in Croatia but also in the former state and even in southern Europe (Figure 4). It was an extraordinary impetus to development of clinical medicine in Rijeka, as well as to progression of immunological studies.

Figure 4. ***First transplantation team in Rijeka (1971).*** *Gianpaolo Velčić, Miomir Zelić, Damir Dimec, Nikola Gržaja, Daniel Rukavina, Anton Šepić, Alemka Suzanić, Vjerislav Peterković, Duje Vukas, Marija Rakidžija, Branimir Budisavljević, Andrej Gudović, Petar Orlić, Ksenija Vujaklija-Stipanović, Šime Vlahović, Vinko Frančišković, Tomislav Tićac and Jerko Zec.*

This stage of development could be considered extremely significant for development of immunology in Rijeka. Although we cannot speak of top-level scientific results, still an atmosphere was created of enthusiasm and general support to the development of immunology in Rijeka. Kidney transplantation has become a routine method in the treatment of patients on dialysis so that over 650 transplantations have been performed so far, and Rijeka has remained the leading centre in kidney transplantation. Regrettably, the development of clinical transplantation did not proceed at the pace that it could take so that the transplantation of pancreas took place not before 1994 (P. Orlić, M. Zelić, K. Vujaklija), and the liver in 2006 (M. Uravić).

TRANSFUSION MEDICINE IN RIJEKA

Development of transfusion medicine was closely linked to the development of immunology and transplantation medicine in Rijeka. In 1956 routine determination of blood groups and Rh factor and in 1964 the indirect Coombs test was introduced. K. Vujaklija established transfusion Centre in 1968 that was transformed into Laboratory for tissue typing in 1971, alongside with the development of clinical transplantation. In 1987, the laboratory was transformed into Department of Transfusiology of the Clinical Hospital Centre (K. Vujaklija-Stipanović, Chairman). Department now employs 10 physicians, 9 laboratory engineers, 29 technicians and 10 accessory staff. Head of Department is Sanja Balen who obtained basic immunology education and PhD at the Department of Physiology and Immunology.

CENTER FOR PROTEOMICS

The Center for Proteomics was established at the Medical Faculty, University of Rijeka in 2006 with focus on monoclonal antibody (mAb) development for cutting-edge applications including proteome analysis. The Medical Faculty made major investments to bring the Center for Proteomics into life. The University allocated financial resources for the building construction, which was completed in summer 2003. The necessary equipment and laboratory furniture was purchased through the Jezgra 17 Grant, awarded by the Croatian Ministry of Science, Education and Sports. Since its start, the basic technology for protein expression and high-throughput mAb production at the Center was organized and supported by the EU FP6 SSA3 Grant. As a first project, generation of mAbs against the entire proteome of the Varicella Zoster Virus (VZV), which consists of 69 distinct proteins, has been performed. Up to now, mAbs have been generated to the vast majority of the VZV proteins. Apart from that, using this comprehensive pool of mAbs as well as other mAbs produced within the Center, the platform for a variety of mAb-based tools for protein analysis will be developed, which should increase the throughput and reliability of proteomic discovery.

In addition to the project mentioned above, the Center for Proteomics is already well integrated into international scientific community. These collaborations are mainly based on the production and characterization of mAbs for research purposes *(2)*. On top of that, the Center also collaborates with several biotech enterprises on the protein expression and the production of mAbs. The Center is taking part in the EU FP6 consortium on the production of various binders to human proteins (Bojan Polic, co- PI).

In collaboration with Dr. Joanne Trgovcich, Ohio State University, Columbus in frame of project supported by Unity through Knowledge Fund the mouse model is used to characterize the entire CMV transcriptome. Altogether, transcriptome research will expand the technological capacity of the Center for upgrading a comprehensive research facility. In the project approved by the National Foundation for Science, Higher Education and Technological Development of the Republic of Croatia a recombinant fusion protein composed of the ligand for the NKG2D receptor and the variable region of the immunoglobulins specific for the viral protein expressed on the surface of infected cells will be generated. It is predicted that the recombinant protein should have the ability to bypass the components of the innate (NK cells) and specific (antibodies) immune responses. If the results are as expected, this will be the first use of mAb-based experimental immunotherapeutic approach designed at the Center.

The Center is engaged in several other ongoing projects, *e.g.* generation of mAbs to ribosomal proteins and several proteins expressed on human NK cells, viral MHC I like proteins for studies in structural biology, potential markers on pancreatic tumour as well as neuro-stem cells and others.

ANIMAL FACILITIES

The greatest obstacle to further progress of not only immunological but biomedical research in Rijeka was the lack of adequate space for cultivation of a laboratory with rodents. Therefore it was necessary to tackle the construction of a special building of vivarium on the grounds of the Medical Faculty. D. Rukavina was active in the realization of this idea and the concept of a modern vivarium in the period 1987–1991, with the support of the Medical Faculty management. Funds for this investment were partly provided by the Ministry of Science, and the remaining funds were made available by the Medical Faculty.

S. Jonjić has taken up the management of the Vivarium since its opening. In time, more than 50 conventional and transgenic mouse strains have been collected and bred in the facility and thus, it became the biggest resource of laboratory mice in Croatia. However, considering the scientific development at the Medical faculty, particularly at the end of 90ties, and new standards in breeding and maintenance of laboratory mice, reconstruction of the facility became a necessity which was recognized by S. Jonjić, who was at the time dean of the Medical School (1999–2003). Therefore, he and B. Polić have designed and reconstructed the Vivarium (2001–2003) in the most modern manner and according to the accepted international SPF standards including strict barrier conditions, IVC systems and quarterly health monitoring. This reconstruction was largely supported by the University of Rijeka and Ministry of Science.

The new Vivarium also contains Laboratory for microinjection and embryo transfer, which is a part of mouse gene targeting program. In 2003 the name of the Vivarum was changed in the Laboratory Mouse Breeding and Engineering Centre Rijeka (LAMRI) and, since than, the management of LAMRI has been taken up by B. Polić. LAMRI is today the most modern laboratory mouse breeding facility in Croatia and represents an important foothold for long-term development of basic biomedical sciences studies in Rijeka and in Croatia.

TEACHING IMMUNOLOGY AT THE MEDICAL FACULTY IN RIJEKA

Teaching of Physiology at the Medial Faculty of Rijeka, led by Professor of the Zagreb Medical Faculty Ljubomir Božović started in the academic year 1957/58. Due to endeavours of Š. Vlahović, the contents of modern immunology were from 1965 gradually included in the teaching of Physiology, and in 1976 a catalogue of competences to be acquired by every student was defined for the first time. V. Linić-Vlahović together with Š. Vlahović was the author of our first textbook in immunology (Bases of Medical Immunology, Otokar Keršovani Publisher, Rijeka 1977). Since 1979, immunology has become the constituent of the course in Physiology (Physiology and Immunology), and the Department of Physiology was in the same year renamed into Department of Physiology and Immunology, with the Chair of Physiology, Immunology and Pathological Physiology active within it.

In the academic year 1992/1993, Immunology disassociated itself as a distinct course and the exam in this course is taken apart from that in Physiology. Comprehensive two-year scientific postgraduate study in Clinical Immunology was founded at the Medical Faculty in 1984 (led by D. Rukavina), with enrolment of a new generation of students every second year. In the 1984, under the supervision of the Dean (D. Rukavina), the first reform of postgraduate studies was performed. Postgraduate

studies in Clinical Pathophysiology with eight different programs were introduced. Comprehensive two year scientific program in Clinical Immunology and Transplantation was established (led by D. Rukavina) with two modules: Clinical Immunology and Experimental and Clinical Transplantation. Establishment of postgraduate studies with lecturers from Rijeka, Zagreb and a substantial number from abroad represented logically rounded off comprehensive activities to ensure a broad personnel basis that was in the near future to even more strengthen the prestige of immunology in Rijeka.

In the 1994/95, postgraduate studies at the Medical Faculty of Rijeka underwent the comprehensive reform, a project led by P. Lučin and S. Jonjić, who was Vice-Rector of the University of Rijeka for science at that time *(3)*. Flexible postgraduate study program Biomedicine, managed for five years by P. Lučin, was created with a number of elective courses in immunology. Former postgraduate Master of Science study program in Clinical Immunology was incorporated in the program of Biomedicine as an elective package of courses led by D. Rukavina since 2000. Seven generations of students were enrolled into postgraduate clinical immunology training during 15 years.

Postgraduate study of Biomedicine was renewed again in 2001 and 2005, and the Clinical Immunology as elective course disappeared from the program. In addition, a number of specialized immunology courses are organized within the new postgraduate program Medical Chemistry, established in 2008 at the level of the University of Rijeka.

ORGANIZATION OF IMMUNOLOGICAL MEETINGS

Numerous national and international meetings were organized by immunologists from Rijeka and they contributed to recognition of Rijeka and Croatian immunology, bringing hundreds of top-level scientists worldwide to Croatia. These meetings allowed and facilitated the establishment of close personal contacts and collaboration and helped in finding bursaries and support for education of young researchers abroad.

First conference of immunologists from the former state with 650 participants was organized in 1985 (Opatija) by immunologists from Rijeka and D. Rukavina was the President of the Organizing Committee.

Dragan Dekaris and D. Rukavina in collaboration with J. Sepčić and M. Dorić and with a support of Croatian Immunological Society organized First Alps Adria Immunology and Allergology Meeting (Opatija, October 1990). The meeting was held under the auspices of Dr. Franjo Tuđman, President of the Republic of Croatia. The meeting was attended by remarkable number of leading immunologists (350 participants) from Alps Adria region and other parts of Europe and USA *(4)*.

The war against Croatia prevented the organization of the second meeting which was planned for 1992. In the meantime the profile of the meeting was changed and the official names of the meetings were »Mechanisms in Local Immunity« that were held in 1994, 1996 and 1998. The organizers of these meetings were D. Rukavina and Thomas J. Gill. Presentations of invited speakers from these conferences were published in special issues of distinguished journals: Regional Immunology, American Journal of Reproductive Immunology and Periodicum Biologorum. During the Meeting which was held in 1994 the Alps Adria Society for Immunology of Reproduction (AASIR) was founded as an affiliated society to the International Society for Immunology of Reproduction (ISIR). D. Rukavina was elected as a first president (2004–2007) and in the next ten years AASIR was the most active European group in reproductive immunology.

The outstanding reputation of AASIR meetings in the International Society for Immunology of Reproduction (ISIR) resulted with the organization of 8th and 10th International Congresses of Reproductive Immunology, which were held in Opatija in 2001 and 2007, respectively. In the period 2004–2007, D. Rukavina acted as the president of the ISIR.

NATIONAL AND INTERNATIONAL RECOGNITION OF IMMUNOLOGISTS' FROM RIJEKA

Scientific and public activities of Rijeka's immunologists was recognised and awarded nationally and internationally. D. Rukavina became in 2000 full member of the Croatian Academy of Sciences and Arts in the Department of Medical Sciences and was awarded in 2003 by the Republic of Croatia for lifetime achievements. Croatian annual National Prize for Science »Ruđer Bošković« was awarded to Š. Vlahović (1972), D. Rukavina (1985), S. Jonjić (1993), P. Lučin (1998), B. Polić (2002) and A. Krmpotić (2002), and for Young Researchers to G. Laškarin (2000). The Annual Award of the Croatian Academy of Sciences and Arts for Scientific Achievements was given to P. Lučin (1996), D. Rukavina (1998) and S. Jonjić (2003). The Annual Award of the City of Rijeka was given to the Department of Physiology and Immunology, Š. Vlahović and V. Frančišković (1971), to D. Rukavina (1987), S. Jonjić (1991), K. Vujaklija-Stipanović (1972 and 1994), P. Orlić (1994), and B. Radošević-Stašić (2000).

S. Jonjić was awarded in 1994 with the Günther Weitzel Science Award by the League for the development of molecular biology and biotechnology, (Tübingen, Germany) and the Raine Foundation Visiting Professorship at the University of Western Australia (2008). D. Rukavina was awarded also by the Academy of Medical Sciences of Croatia (2000), Award for scientific contribution of the government of Carinthia (1998), Award of Japanese Society for Reproductive Immunology (2000),

American Society for Reproductive Immunology (2005) and International Society for Immunology of Reproduction (2007), in which he served as president. In 2008 D. Rukavina was awarded by Blackwell Munksgaard Award, the highest award of American Society for Reproductive Immunology for outstanding contribution to the field of reproductive immunology.

RESEARCH FOCUSES AND ACHIEVEMENTS RIJEKA'S IMMUNOLOGISTS

Clinical immunology and transplantation

The group led by D. Rukavina is developing research in the field of clinical immunology and transplantation for more than three decades. This research was supported by a number of national and international research grants (UK grant ALIS in collaboration with P.M. Johnson and NIH grant in collaboration with E.R. Podack). In the period 1986–1990, D. Rukavina was coordinator of the national programme Transplantation and Clinical Immunology. Through these programs, he developed a fruitful cooperation with a number of clinics and more than 60 young researchers achieved either Master of Science of Doctoral degrees under his supervision. More than 30 are today teachers at clinics or departments of the Medical Faculty of the University of Rijeka or abroad.

Basic and clinical research programs in the field of neuroimmunology were developed in collaboration with J. Sepčić, Chairman of the Neurology Clinics. Multiple sclerosis (MS) as a clinical entity and chronic relapsing form of experimental allergic encephalomyelitis (CR-EAE) were used by M. Morović, H. Haller, D. Ledić and L. Zaputović as research models. CR-EAE as an experimental model for MS was induced in rats *(5, 6)*. The distribution of tissue compatibility antigens (HLA) was investigated in MS patients from cluster Gorski Kotar, known as an area of high prevalence for disease *(7)*.

The investigation of lymphocyte subpopulations dynamics (active T lymphocyte) and cytolytic mechanisms and cytolytic molecules expression (perforin) in the peripheral blood and cerebrospinal fluid confirmed the potential role of these mechanisms in the clinical course of disease, particularly in the phase of exacerbation. It has been suggested that $CD4^+P^+$ cytotoxic cells may play a role in the pathogenetic mechanisms of MS. These cells are upregulated in active disease in cell number, in the level of P expression per cell and in the level of cell activation *(8, 9)*.

Collaboration of the Department of Physiology and Immunology with Transplantation center at Clinical Hospital in Rijeka, which was established before the first successful kidney transplantation (1968) is lasting for four decades now *(10)*. Successful collaboration in research and clinical programs was established with clinicians from the transplantation team (P. Orlić) and Department of Transfusion and Tissue typing (K. Vujaklija-Stipanović and S. Balen-Marunić). Investigation and collaboration was centered to programs of donor selection, prediction of immunological rejection crisis, detection of immunological competence of transplanted patients, the level of immunosuppresion, effects of donor specific transfusion pre-treatment etc. It has been shown also that the mechanisms of lymphocyte cytotoxicity mediated by cytolytic molecule perforin are potentially important in allogeneic kidney rejection *(11, 12)*.

The research interest of Gordan Gulan and Jagoda Ravlić-Gulan in the field of human rheumatoid arthritis is based on intensive collaborative work among Clinic of Orthophaedic Surgery of Lovran, Clinical Hospital Centre of Rijeka and Department of Physiology and Immunology. They investigated the role of the cytolytic action mediated by perforin in the course of rheumatoid arthritis (RA) at systemic (peripheral blood) and local level (synovial fluid and synovial membrane) in patients during the acute or chronic phase of RA. In acute RA highly significant changes in P expression were found in all compartments with strong increase of P^+ cells, $CD8^+P^+$ and $CD56^+P^+$ cells as well as the content of P/cell. Strong evidence was obtained that P mediated cytotoxicity can participate in the acute phase of RA by maintaining and perpetuating the inflammation and contributing to tissue destruction *(13, 14)*.

Very interesting results were obtained by D. Rukavina and his coworkers in investigations of the role(s) of lymphocyte cytotoxicity mediated by perforin in various physiological conditions. Proportions of perforin (P) positive lymphocytes showed age-related changes with strong decline after the age of 70 years for T cells and $CD16^+$ and $CD56^+$NK cells as well as a highly significant reduction in mean levels of P per cell. All these changes resulted in the deficiency of cytotoxic potential in old age which may have implications for antiviral and antitumour immunity in elderly persons *(15)*.

In investigations of chronic hepatitis C virus infection, in collaboration with immunologists and clinicians from Pecs University (Hungary), a decreased percentage of $CD3^-CD8^+$, $V\gamma9/V\delta2TCR^+$ and perforin-posititve T cells was found with decreased peripheral NK activity which may contribute to the impaired cellular immune response and the chronicity of the disease *(16)*.

Larisa Prpić as a PhD student investigated the role played by cell–mediates cytotoxicity in the course of psoriasis and *lichen planus* at both systemic and local level (affected skin lesions). Significant accumulation of T cells was found in both epidermis and dermis of *lichen planus* lesions suggesting a potential role of perforin in the apoptosis of basal keratinocytes. Strong infiltration of T lymphocytes and accumulation of perforin was found in the epidermis of psoriatic lesions, suggesting the potential role for perforin in the creation of the psoriatic plaque and in the disease severity *(17, 18)*.

Immunology of reproduction

Immunology of reproduction and immunological relations between mother and foetus, defined by the Nobel Prize winner Peter Medawar (1953) and Rupert Billingham (1964), as a most intriguing puzzle of the transplantation immunology attracted very early research interest of D. Rukavina and later many of his coworkers. In master thesis (1968) and in doctoral thesis (1972) D. Rukavina investigated immunological aspects of the maternal-foetal relationship and found the interesting effect of maternal transplantation immunity on the specific reactivity of the offspring to the same transplantation stimuli to which the mother had been exposed. This approach was the focus of his research interest in Dr. Billingham's laboratory (Dallas, 1972/73) where he worked as a Fulbright fellow. Upon his return to Rijeka, D. Rukavina organized his own research group (M. Dorić, S. Jonjić, M. Kapović) at the Department and established close collaboration with clinicians from Department of Obstetrics and Gynaecology (N. Matejčić and J. Stašić). The research was centered to the investigation of the consequences of maternal systemic and intrauterine allogeneic sensitization to the outcome of pregnancy and the offspring reactivity to these specific stimuli. The animal models were mice and rats with haemochorial placenta *(19, 20)* and sheep with syndesnochorial placenta, which is thought as an impermeable barrier *(21)*.

Interesting results were obtained showing that pregnancies of sheep sensitized to parental partner transplantation antigens during pregnancy rejected paternal grafts as second set grafts.

In the next period the research interest was focused on the mechanisms that control embryonic implantation. Many PhD students and young clinicians were involved in the investigation of bone-marrow derived immunocompetent cells infiltrating cyclic endometrium and decidua of human early pregnancy. H. Haller and O. Petrović showed accumulation of $CD56^+$ cells in the first trimester decidua, five fold increase of CD56:CD3 cell ratio and analyzed the consequences of decidua-trophoblast interactions on the phenotype, spontaneous and induced proliferation and immunoregulatory potential of decidual leucocytes in normal and pathological pregnancies *(22, 23)*.

This group was the first showing that decidual NK cells, the predominant population of decidua infiltrating lymphocytes, are $CD56^+CD16^-$ and full of perforin (perforin bright$^+$). The cytolytic molecule perforin is expressed in first trimester pregnancy decidua in quantities higher than in any other pathological condition (tumours, inflammation) *(24)*. The investigations of the phenotype and distribution of decidua infiltrating leucocytes, expression of cytokines and cytolytic molecules, functional activity, secreted molecules such as cytokines, gene expression and protein profiles were the themes of Master and PhD theses of G. Rubeša, L. Gudelj, G. Gulan, G. Laškarin, I. Bedenicki, N. Štrbo, V. Sotošek, K. Ćupurdija, T. Bogović Crnčić and K. Juretić Franković *(25–29)*.

G. Laškarin is now leading her own research project on immunoregulatory functions of antigen presenting cells in early pregnancy. Interesting results were obtained in investigation of the role of mannose receptor (MR) in the initiation of the immune response and regulation of homeostasis during inflammation and tissue remodelling at the maternal-foetal interface. Decidual MR^+ macrophages, surrounding early decidual glands are able to internalize ligands for carbohydrate recognition domain of the receptor, including decidual secretory phase mucin TAG-72. TAG-72 efficiently downregulate Th_{12} oriented cytokine/chemokine production, whereas MUC I up-regulated pro-inflammatory decoy receptor expression *(29)*.

N. Štrbo and V. Sotošek in collaboration with E. R. Podack (Miami, USA) demonstrated an essential role for perforin-mediated functions in the activation of innate and adaptive immunity by heat shock protein gp96- peptide complexes. Cooperation between NK and dendritic cells was necessary for both NK activation and clonal CTL expansion *(30, 31)*.

In the frame of EMBIC network we examined the relative contribution to the cytotoxic function of different NK activating receptors. Specific engagement of NKp46 induced intracellular calcium mobilization, perforin polarization, granule exocytosis, and target cell lysis. This was dramatically blocked by NKG2A co-engagement. From the other side the engagement of NKp30 triggered the production of proinflammatory molecules (IFN-gamma, TNFalpha etc.) *(32)*.

In collaboration with A. Mantovani (Milano) as a part of EMBIC project, G. Laškarin and J. Dupor, PhD student participated in the investigation of decoy receptors, an emerging family of negative regulators of different classes of immune mediators. D6 molecule is the best defined chemokine decoy receptor with scavenger activity and is strongly expressed on the syncytiotrophoblast cells. D6 is strategically located as a protective barrier at maternal-fetal interface to tune inflammation by means of chemokine scavenging. Exposure of $D6^{-/-}$ pregnant mice to LPS or antiphospholipid autoantibodies results in increase in foetal loss which is prevented by blocking inflammatory chemokines *(33, 34)*.

Immune control of organ growth

Among the first projects financially supported by Republic funds (1965–1975) were the investigations of radiation biology, transplantation immunology and compensatory renal growth, led by Š. Vlahović. Subsequently a coordinator of the projects investigating the immune aspects of organ growth and neuro-endocrine influences on lymphatic tissue became B. Radošević-Stašić, with the co-workers M. Ćuk, M. Petković, L. Polić, D. Muhvić, Z. Trobonjača, J. Ravlić-Gulan and I. Mrakovčić-Šutić.

She established a very close collaboration with the researchers on Department of Chemistry and Biochemistry led by Mladena Kirigin and than by Čedomila Milin, enlarging the investigations to metabolic aspect of organ growth and immune reaction. Their early data have shown that disturbance of morphostasis results in activation of lymphatic cells with morphogenetic properties *(35, 36)*. Later, they found that the main regulators are autoreactive NKT and regulatory T cells (*37, 38*), which might be found particularly in the liver, and that these events were significantly affected by metals, particularly zinc *(39)*. Working on immunoregulatory effects of peptidoglycan-monomer linked with zinc (PGM-.Zn) and on projects supported by Pliva, they also found that PGM-Zn has immunocorrective and hepatocorrective properties in conditions of immunosuppression *(40, 41)*. These data were in 1992 protected by Pliva by the patent P920488A.

Previous members of this group are now leading their own projects, working on tumor immunology (M. Petković and I. Mrakovčić Šutić), autoimmune diseases (J. Ravlić-Gulan) and cytomegalovirus infection (Z. Trobonjača).

The new research fellows from the group of B. Radošević-Stašić, H. Jakovac and D. Grebić, subsequently showed by immunocytochemistry and PCR that activation of lymphatic cells during disturbance of morphostasis depends on damage-associated signals, and particularly on metallothioneins and endoplasmic reticulum resident heat shock protein gp96, which might be found in regenerating tissue, liver, thymus, at fetoplacental unit and in CNS during experimental allergic encephalomyelitis (EAE) *(42, 43)*. Moreover, in a current scientific project with Vladimir Mićović from Teaching Institute of Public Health, Primorsko-goranska County they demonstrated that expression of metallothioneins and heat shock proteins in the marine shells are in high correlation with environmental pollution in Kvarnerian bay.

Neuroimmunomodulation

Very early the group of B. Radošević-Stašić in coordination with Suad Efendić (Stockholm) started to investigate also the effects of somatostatin (ST) on compensatory renal growth and on the lymphatic tissue. Among the first in the world (in 1983) they reported about the immunosuppressive and growth regulatory properties of ST and its ability to modulate the graft-versus-host reaction, EAE and the processes of differentiation and proliferation in the thymus *(44–48)*.

Besides in coordination with Walter Pierpaoli and George Maestroni the group of B. Radošević-Stašić showed that pineal gland and melatonin are directly involved in the control of organ growth and immune functions, pointing to its importance for chronobiology. The group of B. Radošević-Stašić also contributed to the elucidation of the effects of cholinergic influences on immune response, showing that lesions of nucleus basalis and damage of projections to neocortex markedly affect the immune response *(49, 50)*.

Immunosurveillance of cytomegalovirus infection

As a continuation of the work that S. Jonjić performed while he was with Ulrich Koszinowski in Germany, later on, at the Department of Physiology and Immunology, he and his group were involved in studies of immunosurveillance of cytomegalovirus (CMV) infection *(51)*. Interestingly, although it has been clearly shown that $CD8^+$ T cells play a dominant role in control of CMV infection, they provided new evidence that this does not apply to all tissues. Namely, in absence of $CD4^+$ T cells, CMV infected mice develop persistent infection in salivary glands, showing for the first time the importance of $CD4^+$ T cells for the prevention of horizontal virus spread *(51)*. In subsequent series of studies it was shown that $CD4^+$ T cells mediate their effect via IFN-gamma, which is necessary for preventing the horizontal transmission of MCMV infection *(52)*. Furthermore, although $CD8^+$ T cells are the major protective subset in the control of CMV infection, mice lacking $CD8^+$ T cells eliminated virus via the compensatory response mediated by $CD4^+$ T cells *(53)*. To our knowledge this was the first example of physiological compensation in immune response. This finding was additionally confirmed by experiments in mice lacking MHC class I complexes *(54)*. It was shown that these mice can control primary CMV infection almost with the same kinetics as normal mice. Series of studies by P. Lučin, I. Pavić, B. Polić and others provided evidence that cytokines such as IFN-gamma but also TNF-alpha are important in virus control *(55, 56)*.

The group of S. Jonjić also contributed to the understanding of immunosurveillance of chronic and latent CMV infection and conditions that lead to viral reactivation from latency *(57)*. By use of B-cell deficient mice, a model to study the immunosurveillance of CMV infection in the absence of antibodies was established *(58)*. They were the first to show that antiviral antibodies are not required for the resolution of primary infection and the establishment and maintenance of CMV latency. B cell deficient mice were able to control infection and establish latency with similar kinetics as normal mice. However, the results confirmed that antiviral antibodies play a key role in limiting recurrent infection *(58)*. The results clearly demonstrated that immunosurveillance of latent CMV infection is organized in a hierarchical and redundant fashion. Namely, not only $CD8^+$ T cells but also $CD4^+$ T cells and NK cells contribute to control of MCMV latency and prevention of recurrent infection *(59)*.

An important aspect of the research was the characterization of the role of viral immunoevasins of $CD8^+$ T cells by down modulation of MHC class I molecules *(60)*. Together with Koszinowski's laboratory they provided evidence that MCMV in-

hibitors of MHC class I molecule play a role in virus control by $CD8^+$ T cells *in vivo (61)*. The deletion of the MCMV m152 gene results in virus attenuation with an impaired virulence and replication *in vivo* due to a more stringent immune control by $CD8^+$ T lymphocytes.

Viral regulation of NK cells

Guided by the observation that during the first days post infection most of laboratory mouse strains develop no significant NK-cell dependent virus control immunologists at the Department of Histology and Embryology postulated that this could be the consequence of viral immunoevasion of NK cells. As a result, over the past several years they have characterized several MCMV genes encoding proteins involved in the evasion of NK cells. Namely, it was shown that MCMV prevents NK cell activation by down-modulating cellular ligands for the activating NK cell receptor NKG2D. This group was first to describe MCMV protein involved in this function *(62)*. Apart from the downregulation of MHC class I molecules, m152 prevents expression of NKG2D ligand RAE-1 from the cell surface. Importantly, the mutant virus lacking m152 was attenuated on day 3 post-infection and the attenuation could be abolished by the depletion of NK cells *(62)*. Of note is that in the same issue of the Nature Immunology Klas Karre wrote News & Views article emphasizing the significance of this finding. In subsequent years in this laboratory three additional MCMV genes involved in downmodulation of NKG2D ligands have been characterized *(63–65)*. Whereas m152 is involved in down-regulation of RAE-1 isoforms, the m155 down-modulates H60 and m145 negatively regulates the expression of MULT-1. Interestingly, the product of *m138* gene, originally characterized as viral receptor for Fc fragment of immunoglobulins, is involved in down-regulation of at least two NKG2D ligands – H60 and MULT-1. It was previously shown that the virus lacking *m138* is strongly attenuated during the early days post infection even in immunoglobulin-deficient animals suggesting that Fc binding is not the only immunoevasion property of this protein (66). In addition to molecular and functional characterization of viral NKG2D immunoevasins it was shown that they play a role *in vivo (67, 68)*.

Currently, in frame of HHMI research fellowship, A. Krmpotić and colleagues are studying the significance of viral immunoevasins of innate immunity for the long-term control of CMV infection.

Apart from work on the characterization of viral inhibitors of NKG2D, the Jonjić's group took part in the characterization of MCMV proteins involved in activation of NK cells. Others have shown that MCMV m157 protein serves as a ligand for activating Ly49H receptor C57BL/6 mice. By deleting m157 gene, the group from Rijeka together with Koszinowski and colleagues have shown that the entire NK cell control of the virus was abolished, suggesting that no other MCMV protein is able to engage Ly49H *(69)*. Of note is that under selective pressure by Ly49H the m157 gene is subject for mutation leading to mutant strains able to escape NK cell control *(70)*. Apart from Ly49H, in a very recent study, this group took part in the collaborative research with Silvia Vidal aimed at characterization of another viral protein involved in NK cell activation via Ly49P receptor (*J Exp Med* submitted). It was shown that MCMV m04 is essential for the recognition of infected cells by Ly49P in MA/My mice. Consequently, the deletion of m04 resulted in loss of virus susceptibility to NK cells. Interestingly, unlike Ly49H, for the activation of Ly49P in addition to viral protein the specific MHC class I molecule is required. The results in PWK mouse strain also indicate that NK cell dependent resistance of this strain to MCMV also includes recognition of so far unknown viral protein (*71*).

Congenital CMV infection of developing CNS

More recently, the mouse CMV as a model to study human CMV infection in the developing CNS was established through collaboration between Jonjić's group and Bill Britt at the University of Alabama, Birmingham and supported by NIH RO1 Grant *(72)*. Congenital HCMV infection of CNS represents one of the major viral causes of congenital abnormalities in CNS. Inoculation of newborn mice with MCMV resulted in virus spread to the brain and the infection was associated with the induction of inflammatory response (focal encephalitis) and defects in cerebellar development. Specific defects included decreased granular neuron proliferation and migration and the activation of neurotrophil receptors. Ongoing studies in this laboratory and collaborators have already proven that this model is going to play an important role in understanding the immunobiology of congenital CMV infection *(72)*.

The role of NKG2D and other immunoreceptors on the development, homeostasis and effector functions of the immune system

B. Polić, upon his return from the postdoctoral education in Germany, has established mouse gene targeting program at the Department of Histology and Embryology (2002), which was a pioneering endeavour in Croatia. This program has been giving to scientists a possibility to genetically create new mouse models to answer fundamental biological questions, and thus,

an additional flexibility and quality to the present research at the Medical Faculty. He and his co-workers have produced, among the others, the conventional and conditional mouse mutants for gene encoding NKG2D receptor, which are now under the scope of investigation. NKG2D, as an activating receptor, is present on various immune cell populations (NK, NKT, and T) and, according to the present literature; it is implicated in their effector functions. The analysis of NKG2D knock out mice has revealed impaired NK cell development as well as homeostasis and effector functions of NK cells, which is going to be reported soon. Interest of this group is to further investigate the role and mechanisms of action of this and other immunoreceptors in innate and adaptive immunity (Figure 5).

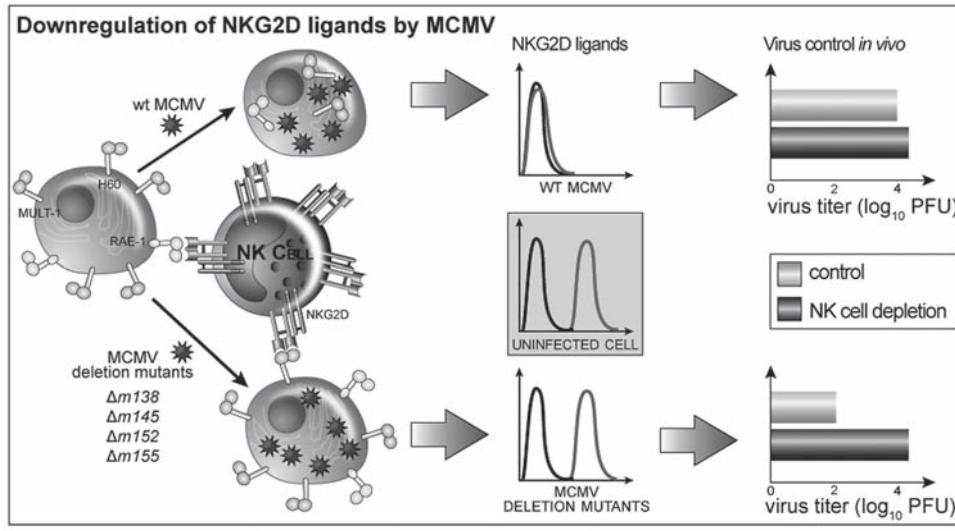

Figure 5. ***MCMV inhibitors of NKG2D ligands*** *(Jonjić et al., Current Opinion in Immunology, 2008).*

Cell biology of murine cytomegalovirus infection

Cell biology of murine cytomegalovirus infection was for a decade in the background of immunological research. In the 1994, P. Lučin described synergistic inhibitory effect of interferon-gamma and tumour necrosis factor on the late phase of murine cytomegalovirus replication *(56)*. Their effect was also extended on restoration of defective antigen presentation *(55)*. Natalia Kučić demonstrated that MCMV replication requires activity of protein kinases C in the early phase *(73)*.

Murine cytomegalovirus interference with MHC class I molecules

Immune evasion mechanism of murine cytomegalovirus has been studied from several aspects. The initial observations were made within research project of S. Jonjić in collaboration with Ulrich H. Koszinowski on presentation of MCMV antigens to cytotoxic lymphocytes. In the 1992, it was published that cytomegalovirus prevents antigen presentation by blocking the transport of peptide-loaded major histocompatibility complex class I molecules into the medial-Golgi compartment *(74)*. Pero Lučin continued research in Ulm and Heidelberg (1992–1993) and upon return in Rijeka established independent group that was joined by N. Kučić in 1995, and Hana Mahmutefendić in 1999. First attempt to identify the MCMV gene responsible for the antigen presentation block vas unsuccessful, but led to the discovery of a MCMV gene with Fc receptor function *(75)*. The MCMV genomic region affecting MHC class I molecule transport *(76)* was first identified and then MCMV glycoprotein m152 that retains MHC class I complexes in the ERGIC/cis-Golgi compartments *(77)*. In addition to this protein, two MCMV proteins that affect MHC-I transport were identified: gp48, that reroutes MHC class I complexes to lysosomes for degradation *(78)*, and gp34 that forms a complex with folded class I MHC molecules in the ER which is not retained but transported to the cell surface *(79, 80, 81)*.

Initial observation that MCMV also affects MHC-I molecules at the cell surface and cause their backsorting was published in 1994 *(81)*, but attempts to characterize mechanism were unsuccessful. At that time, the overall expertise in the endocytic system were poor in Rijeka and Lučin's group shifted research focus towards endocytic trafficking of MHC-I molecules.

Endocytic trafficking of MHC class I molecules

Studies of endosomal trafficking of MHC class I molecules were initiated in 2000 when H. Mahmutefendić joined the group of P. Lučin. Early research indicated that MHC-I sorting depends on their conformation *(82),* and spontaneous internalization as a consequence of the bulk cellular membrane flow demonstrated that conformed and nonconformed MHC-I molecules use different internalization and trafficking route *(83)*. In 2003, the group was joined by Gordana Blagojević, who established model of cholera toxin endocytic trafficking *(84),* and studying trafficking of human MHC-I molecules in transfected cells. In the 2005, Maja Ilić Tomaš joined the group and continued research of endosomal sorting of MHC-I molecules in MCMV infected cells.

Immunopathogenesis of bacterial infections

The group led by M. Dorić has a long standing interest in scientific research on the pathogenesis of bacterial infections. In the period 1988–1991, an original computer-assisted data base system for identification of gram negative nonfermentative and fermentative bacteria was constructed, followed by development of 11 computer-based programs for bacterial and yeasts identification in the period 1991–1996. At that time antibacterial effectiveness was correlated with the effect of antibiotics on the host defence system (lymphocyte subpopulations, antibody secretion, macrophages and neutrophils) which resulted with four Master Thesis mentored by M. Dorić and two original scientific papers *(85, 86)*. In the 1997, as a continuation in the field of bacterial pathogenesis and immunology of reproduction, M. Dorić started research on pathogenesis of experimental murine congenital listeriosis. Murine model of pregnancy-associated listeriosis and the host immune response to *L. monocytogenes* infection was established by M. Abram *(87),* and *in vivo* model of *Campylobacter jejuni* infection by, D. Vučković. Proinflammatory cytokine response to these two pathogens indicated that IFN-γ and TNF-α production correlate with bacterial clearance from the liver *(88)* and that, in contrast to *L. monocytogenes*, *C. jejuni* does not promote induction of IL-6 *(89)*.

T. Rukavina produced monoclonal antibodies against *Klebsiella* capsular polysaccharides *(90)* and showed their neutralizing and protective effect *(91, 92)* in the murine model of lung and systemic *Klebsiella* infection *(93)*. B. Tićac showed that the ability of *Legionella pneumophila* to cause pneumonia depended on its capacity to invade and replicate within alveolar macrophages, monocytes, and potentially alveolar epithelial cells *(94)*. Vanja Vasiljev-Marchesi joined T. Rukavina group in 2002 and demonstrated that reduced cytokine production was involved in the survival of animals protected by antilipopolysaccharide antibodies *(95)*. The ratios between IL-10 and proinflammatory cytokines confirmed the suppressed pro-inflammatory response in protected animals, especially 24 hours postinfection *(96)*.

Marina Šantić, a member of the M. Dorić group, continued with *Legionella* research and showed that the rsmA, htrA and ligA genes are essential for the replication of *L. pneumophila* and for the expression of mRNA for IL-1α and IL-18 *(97)*. Ivana Gobin, who came in 2001, compared the pathogenic potential of *L. pneumophila* and *L. longbeache*, showing that there are significant differences in the infective doses, permissiveness of experimental animals and pathohistological changes in the lung tissue This group also showed that Dot/Icm type IV secretion system is essential not only for modulation of phagosome biogenesis but also for the activation of caspase-3 *(98, 99)*. When comparing intracellular survival of different bacterial species, the research was enlarged to pathogenesis of *Francisella tularensis subsp. novicida (100, 101)*.

The M. Abram's group focused on cellular and molecular mechanisms in the pathogenesis of congenital listeriosis and demonstrated that listeria could traverse the placenta and cause serious foetal damage by immunopathological mechanisms *(102, 103)*. M. Bubonja, a member of the group, established a mouse model that mimic the natural route of *L. monocytogenes* infection, showing that intragastric administration of listeria for three consecutive days led to the development of severe systemic illness with involvement of brain tissue in all infected mice. Comparison with campylobacteriosis revealed that *C. jejuni* is not typical extracellular bacterium *(104)*.

Experimental autoimmune models

The main animal model for investigation of pathogenesis of multiple sclerosis was experimental allergic encephalomyelitis (EAE), introduced at the Department of Physiology and Immunology by D. Rukavina and coworkers in this field – V. Barac-Latas, M. Morović and D. Muhvić. It was induced in its relapsing and monophasic form in AO (resistant) and DA (susceptible) strain of rats, forming a basis for investigations of mechanisms that induce the chronic inflammatory demyelinating disease. Working in the group of H. Lassmann in Vienna, Vesna Barac-Latas studied also the patterns of oligodendrocyte pathology in coronavirus-induced subacute demyelinating encephalomyelitis in the Lewis rat, showing that infected oligodendrocytes were destroyed by necrosis, whereas oligodendrocytes that did not contain detectable virus antigen or RNA were dying by apoptosis *(105)*. D. Muhvić later showed that some of early manifestations might be changed by peripheral or intracerebroventricular application of somatostatin *(46, 47)*.

Using the streptozotocin-induced autoimmune diabetes as a model for diabetes mellitus type I, I. Mrakovčić-Šutić showed that it involves high accumulation of NKT cells in the liver with increased cytotoxicity against syngeneic thymocytes *(38)*.

Studies on ethiopathogenesis of the inflammatory bowel disease were introduced by Z. Trobonjača when he moved to Ulm, Germany and joined group of Joerg Reimann. They published interesting observation that small population of spleen $CD4^+$ class I-restrictive cells can induce extremely aggressive and lethal colitis in immunodefficient RAG KO host mice *(106)*. Simultaneously to colitis investigation this group carried out experiments on liver antiviral immunity that were partially completed in Rijeka. They showed importance of dendritic cells in the activation of hepatic NKT cells *(107, 108)* and a mechanism of crosstalk between liver NK, NKT and DCs that is multiplying hepatic IFN-gamma production *(109, 110)*.

CONCLUDING REMARKS

Four decades of investment in people and their training at the Department of Physiology and Immunology created the group of immunologists in Rijeka that have international scientific reputation and significant impact on development of biomedicine in Croatia. As an open training centre with creative and productive spirit, Department of Physiology and Immunology substantially contributed to the development of the Medical Faculty of Rijeka. Dissemination of early doctorants to the chairs of a number of departments and clinics in Rijeka, as well as collaboration with numerous scientists outside department helped in profiling the scientific mindset in the Rijeka's academic community. Group at the Department of Histology is nowdays one of the most productive groups in Croatia, but similar pattern of scientific behaviour is growing also at microbiology, biology, biochemistry and many other departments. This paper, made on the occasion of the 40 years of Croatian Immunological Society, is and opportunity to thank to the founders of immunology in Rijeka: to Šime Vlahović, Vinko Frančišković and Daniel Rukavina. Their vision, creative spirit and entrepreneurial energy made an adamant base for the competitive research environment of the University of Rijeka.

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CHRONOLOGY OF IMPORTANT DATES

1972 Foundation of the Tissue Typing Centre headed by Andrija Kaštelan (1970–2001) and Vesna Brkljačić-Kerhin (2001–now).

From 1970–2007 the center was part of the Urology Clinic of the Clinical Hospital Center Zagreb and Zagreb University School of Medicine.

In 2007 the center became part of the Clinical Institute of Laboratory Diagnosis and changed its original name to Department for Tissue Typing.

1977 Foundation of the Laboratory for Cellular Immunology headed by Maja Kaštelan (1977–1981). From 1977–1981 the laboratory was part of the Oncology and Radiotherapy Clinic, Clinical Hospital Center Zagreb.

From 1981–1985 the laboratory was part of the Division of Immunology within the Department for Clinical Laboratory Diagnostic of the Clinical Hospital Center Zagreb.

1978 Foundation of the Division of Clinical Immunology and Inflammatory Rheumatic Diseases as a part of the Clinic for Internal Medicine, Clinical Hospital Center Zagreb and Zagreb University School of Medicine; the division was headed by Zvonimir Horvat (1978–1990).

Foundation of the Laboratory for Serologic Immunodiagnostics as a part of the Division of Clinical Immunology and Inflammatory Rheumatic Diseases, also headed by Zvonimir Horvat (1978–1981).

1981 Foundation of the Department for Clinical Laboratory Diagnostic (so called »central laboratory«) of the Clinical Hospital Center Zagreb headed by Ana Stavljenić Rukavina (1981–1985).

Laboratory for Cellular Immunology and Laboratory for Serologic Immunodiagnostics were united and became a new organizational unit – Division of Immunology – within the Department for Clinical Laboratory Diagnostics, Clinical Hospital Center Zagreb. The head of this new division was Maja Kaštelan.

1985 Department for Clinical Laboratory Diagnostics of the Clinical Hospital Center changed its name to the Department for Clinical Laboratory Diagnostics of the Zagreb University School of Medicine (headed by Ana Stavljenić Rukavina (1985.–1992.)

1986. Matko Marušić, a professor of physiology and immunology from the Zagreb University School of Medicine became head of the Division of Immunology of the Department for Clinical Laboratory Diagnostics.

1990 Division of Clinical Immunology and Inflammatory Rheumatic Diseases changed its name to the Department of Clinical Immunology and Rheumatology of the Internal Clinic, Zagreb University School of Medicine, headed by Nada Čikeš (1990–until now).

1990 Department of Clinical Laboratory Diagnostics changed its name to the Clinical Institute of Laboratory Diagnosis of the Clinical Hospital Center Zagreb and the Zagreb University School of Medicine headed by Ana Stavljenić Rukavina (1992–2005) and Jadranka Sertić (2005–until now).

Division of Immunology changed its name to the Department of Immunology as part of the Clinical Institute of Laboratory Diagnosis. In the period 1992–1997 the Department was headed by Matko Marušić 1992–1997 and Drago Batinić (1997–until now).

1998 Department of Immunology became the Referral Center of the Croatian Ministry of Health and Welfare for Clinical Laboratory Immunodiagnostics of Hematological and Immunological Diseases.

Department for Tissue Typing obtained the status of the Tissue Typing Referral Center for the Ministry of Health and Welfare of the Republic of Croatia.

2007 Department of Tissue Typing obtained accreditation from the European Federation for Immunogenetics (EFI).

DEPARTMENT OF CLINICAL IMMUNOLOGY AND RHEUMATOLOGY

Staff members (2008): Nada Čikeš, *head*, Branimir Anić, Dubravka Bosnić, Jasenka Markeljević, Miroslav Mayer and Mirna Sentić.

Former staff member: Zvonimir Horvat *(a founder and former head of the department)*.

Patients' management and treatment

Since its foundation the Department of Clinical Immunology and Rheumatology has been involved in the complex diagnostic procedures and treatment of patients with systemic connective tissue diseases, systemic vasculitis and primary and secondary immunodeficiency. As will be discussed in more detail, the Department of Clinical Immunology initiated and actually(in fact) founded the first laboratory for specific immunodiagnostic procedures (*see below*). Concerning the specific treatment of autoimmune patients, clinical investigation protocols included patients with systemic lupus erythematosus, rheumatoid arthritis and systemic vasculitis *(1–8)*. Application of intravenous cytotoxic drugs and immunoglobulins as a part of the treatment procedures and follow-up of patients is organized in an out-patient fashion (way). The Department is continuously introducing new treatment modalities in routine clinical practice. Corticosteroids were first introduced in the treatment of patients with systemic autoimmune diseases in 1951, specific cytototoxic and immunosuppressive drugs in 1960, whereas biologic therapy with specific monoclonal antibodies was introduced in 1999. The Department is a training center for subspecialty of clinical immunology and allergology as well as subspecialty in rheumatology.

Scientific activity

Four research projects supported by the Croatian Ministry of Science and Technology
(project leaders: N. Čikeš, J. Markeljević)

International collaboration activity:

EULAR: The European League against Rheumatism

1. EULAR Standing Committee for Education in Rheumatology – ESCET (*N. Čikeš*)
2. EULAR Standing Committee for International Clinical Studies including Therapeutic Trials-ESCISCIT (*Branimir Anić)*
3. EULAR Standing Committee for young specialists-EURORITS (*Miroslav Mayer*);
4. Department of Immunology, Mayo Clinic, Rochester, Minnesota, USA. Project: Unified musculoskeletal undergraduate curriculum.

Educational activity

Graduate and Postgraduate courses:

1. Zagreb University School of Medicine (N.Čikeš, B.Anić, J. Markeljević)

Specialistic education

Training center for clinical specialties in internal medicine, physical medicine and rehabilitation, infectious diseases, subspecialty in clinical immunology and allergology as well as in rheumatology. Teaching clinical immunology and rheumatology for various professional postgraduate medical courses, including dermatovenerology, neurology, ophthalmology, orthorhinolaryngology, etc.

DEPARTMENT OF LABORATORY IMMUNOLOGY

Staff members (2008): Drago Batinić (*head),* Klara Dubravčić, Ivana Franić-Šimić, Ana Kozmar, Branko Malenica, Sanja Mrsić, Marija Rudolf

Former staff members: Mila Hršak, Antonio Juretić, Maja Kaštelan, Ljerka Krajina, Matko Marušić, Mladen Petrovečki, Branka Užarević

Immunodiagnostic activity

Immunological tests have been used for the correct diagnosis and management of patients with several categories of diseases, such as allergic diseases, autoimmune diseases, neoplastic diseases, immunodeficiency diseases, as well as for histocompatibility testing and transplantation. During the last three decades, there have been significant changes in methodologies and techniques used for immunodiagnostic purposes: from immunoelectrophoresis, immunodiffusion and radioimmunoassay to nephelometry, turbidimetry, ELISA, microchip and microsphere technology, from UV-microscopy to flow cytometry, from radioactive lymphocytes functional assay to various flow cytometric assays, from standard ELISA and microcytotoxic assays to microsphere technology and molecular biology.

Immunodiagnostics of systemic autoimmune diseases

Classical immunochemical methodologies such as electrophoresis and immunoelectrophoresis were introduced in 1960 in the routine work of the Laboratory for Protein Biochemistry and Immunochemistry at the Internal Clinic *(9)*. More intensive immunodiagnostics of systemic autoimmune diseases began approximately three decades ago (1978) in the Laboratory for Serologic Immunodiagnostic of the Division of Clinical Immunology and Inflammatory Rheumatic Diseases (Internal Clinic Rebro). During this early period, the most common tests were LE-cells, anti-nuclear antibodies (ANA) using immunofluorescence on tissue imprints and rat liver sections, anti-dsDNA on *C. Lucilliae*, Waaler-Rose and latex-test for rheumatoid factor *(10–14),* complement assays (CH50, C3 and C4) and anti-streptolysin titer (AST). Later , several »*in house*« ELISA formats for determination of anti-dsDNA and anti-cardiolipin autoantibodies were standardized for routine diagnostics *(15)*. Over time, the number of tests in the panel have increased and today include ANA, anti-phospholipid antibody (aPL), anti-gamma globulins antibody (RF; rheumatoid factors), anti-streptolysin antibody (AST) and anti-cyclic citrullinated peptides antibody (CCP). In addition, CH50, complement components C3 and C4 and C1-inhibitor are performed routinely for monitoring of the complement system. All suspected sera for ANA-ENA (extractable nuclear antigens) are screened on Hep-2 cells. The target diagnostic autoantigens in positive are further analyzed by a multiplexed fluorescence microsphere assay (AtheNA-Multi-lyte ANA system – Luminex™) *(16)* This system allows simultaneous detection of autoantibodies specific for double stranded DNA, histones, SS-A, SS-B, Sm, RNP, DNA topo I, Scl-70, centromere B (CENP B) and Jo-1. These autoantigens are helpful in differential diagnosis of various forms of systemic autoimmune diseases.

Besides immunodiagnostic work, a number of studies using flow cytometry have been performed to analyze various leukocyte subsets in patients with autoimmune diseases at diagnosis and during treatment *(1, 2, 4, 17)*. During the last two years, research interest has been focused on regulatory T-cells enumeration in patients with systemic autoimmune diseases, and the peripheral blood cytokine profile in various conditions, including autoimmune disease and patients on hemodialysis *(18)*. The latter has been done by a novel microchip technology that simultaneously detects a series of cytokines *(19)*.

Immunodiagnostic of systemic vasculitis

Anti–neutrophil cytoplasmic antibodies (ANCA) characterize systemic small vessel vasculitis. All suspected sera are screened using »in house« ethanol fixed cytospin preparation of human granulocytes. The target diagnostic autoantigens (proteinase 3 – PR3, and myeloperoxidase – MPO) in positive sera are further determined by specific ELISA or immunoblotting. These assays are very sensitive, specific and helpful in the diagnosis of different types of small vessel vasculitis such as Wegener's granulomatosis, microscopic poliangiitis, idiopathic glomerulonephritis and Churg-Strauss syndrome *(20, 21)*. The antibodies against glomerular basement membrane (GBM) are also helpful in this respect.

Immunodiagnostics of autoimmune liver diseases

Autoantibodies against mitochondria (AMA), cell nucleus (ANA), smooth muscle (SMA), liver-kidney microsome type-1 antigen (LKM-1), cytosolic liver antigen type 1 (LC-1) and soluble liver antigen/liver pancreas antigen (SLA/LP) are useful diagnostic tools for the management of patients with suspected primary biliary cirrhosis (PBC), autoimmune hepatitis (AIH) and primary sclerosing cholangitis (PSC). The analysis of AMA and SMA using indirect immunofluorescence (IIF) was introduced in the lab routine three decades ago. During the last decade, the spectrum of autoantigens related to liver diseases has been broadened. All suspected sera are first screened on the appropriate tissue preparations (liver, kidney or stomach) and HEp-2 cells. The target diagnostic autoantigens in positive sera are further determined by antigen-specific ELISA or immunoblotting. Immunoblotting allows simultaneous detection of autoantibodies specific for AMA-M2 (pyruvate-dehydrogenase complex), Sp100 (nuclear granular protein, nuclear dots), gp210 (integral protein of the nuclear membrane, nuclear pore complex), LKM-1 (cytochrome P450 II D6), LC-1 (forminotransferase-cyclodeaminase) and SLA/LP (UGA suppressor tRNA-associated protein). The detection of AMA is of great importance in the diagnosis of PBC. In accordance with the autoantibody status it is possible to differentiate between the three types of AIH: type I (ANA, SMA), type II (LKM-1, LC-1) and type III (controversial; SLA/LP).

In collaboration with the Department of Gastroenterology (*head Boris Vucelić*) and Referral Center for Chronic Hepatic Diseases (*head Rajko Ostojić*) of the Clinical Hospital Center Zagreb, there has been a fruitful study on the diagnostic accuracy of an atypical perinuclear anti-neutrophil cytoplasmic antibody (a/P-ANCA) in patients with AIH. The results show that a/P-ANCAs have importance in the diagnosis in AIH type I *(22)* and may also be helpful in the diagnosis of PSC.

Immunodiagnostics of inflammatory bowel diseases

The test for antibodies against parietal cells (PCA), neutrophil cytoplasmic antigens (ANCA), Saccharomyces cerevisiae (ASCA) and endomysium (EMA) have been used as diagnostic tools for the proper (correct) management of patients with suspected inflammatory bowel diseases, chronic atrophic gastritis, pernicious anemia and celiac disease. All suspected sera are first screened by immunofluorescence on the appropriate tissue preparations (stomach and esophagus) and smear of the *Saccharomyces cerevisiae*. The diagnostic target autoantigens in positive sera are determined with antigen-specific ELISAs. The determination of the tissue transglutaminase (tTg) in EMA positive sera is of great importance in the diagnosis of celiac disease. Autoantigens H+/K+ ATPase and intrinsic factor are helpful in the diagnosis of autoimmune gastritis and pernicious anemia.

During the last decade research interest has been focused on the diagnostic accuracy of the simultaneous determination of perinuclear anti-neutrophil cytoplasmic antibodies (a/P-ANCA) and ASCA in patients with inflammatory bowel diseases. The results of this study show that the simultaneous determination of these antibodies may be important in differential diagnosis of ulcerative colitis (UC) and Crohn's disease (CD) *(23, 24)*.

Immunodiagnostic of autoimmune neurological diseases

Autoantibodies against various gangliosides, striated skeletal muscle, myelin and neurons have been used as laboratory tools in the proper (correct) management of patients with suspected autoimmune neurological diseases and especially for directing the search for underlying tumors. The laboratory determination of antibodies against myelin oligodendrocyte glycoprotein (MOG) and against myelin basic protein (MBP) may be helpful in the diagnosis of multiple sclerosis (MS). On the other hand, antibodies against gangliosides asialo-GM1, GM1, GM2, GD1a, GD1b and GQ1b are helpful in distinguishing acute neuropathies such as Guillan-Barré syndrome and its subtypes. Antibodies to myelin–associated glycoprotein (MAG) are associated with chronic inflammatory demyelinating polyradiculoneuropathy. The determination of the autoantibodies against acetylcholine receptor (AChR) and muscle-specific kinase (MuSK) is a sensitive and specific laboratory test in the diagnosis of myastenia gravis. The presence of these autoantibodies is routinely checked in the Laboratory for neurochemistry (*head Milica Trbojević-Čepe*) as a part of the Department of Special and Molecular Biochemistry (*head Dubravka Čvorišćec*). Antineuronal antibodies characterize paraneoplastic neurological diseases (PND). All suspected sera are first screened on the appropriate tissue preparations (primate cerebellum, intestinal tissue and peripheral nerve). The diagnostic target autoantigens in positive sera (cut off dilution 1:20) are detected by immunoblotting (IB). IB allows for simultaneous determination of autoantibodies specific for Hu (ANNA-1), Yo (PCA-1), Ri (ANNA-2), amphiphysin, Tr, CV2 and Ta antigens. Hu antigens are associated with small cell lung carcinoma (SCLC) and paraneoplastic encephalomyelitis/paraneoplastic sensory neuropathy (PEM/PSN), Hu and Ta with SCLC and testicular tumors and paraneoplastic encephalitis (PLE), Yo with ovarian, breast and uterus tumors and paraneoplastic cerebellar degeneration (PCD), Ri with breast and SCLC and opsoclonus/myoclonus syndrome (OMS) and amphiphysin with breast and paraneoplastic Stiff-Person syndrome (SPS).

Immunodiagnostics of autoimmune skin diseases and hypersensitivity

Immunodiagnostics of autoimmune skin diseases (by direct and indirect immunofluorescence) and skin testing with various allergens are performed by specialists in the Dermatovenerology Clinic (*head Jasna Lipozenčić*) of the Clinical Hospital Center Zagreb. The laboratory testing for skin allergy includes total and specific IgE to »classical« allergens. Hypersensitive reactions to various drugs are determined by the lymphocyte transformation test (LTT) and cellular antigen stimulation test (CAST) *in vitro (25–26)*.

The immune status and diagnostics of primary and secondary immunodeficiencies

Diagnostic activities of the Department of Immunology have been intensively focused on primary and secondary immunodeficiencies. The pioneering work in that sense have been studies on the immune status in various pathologic conditions, using at the time standard methods for lymphocyte enumeration (E-rosettes and immunofluorescent surface Ig) and functional lymphocyte proliferation assay, respectively *(27–29)*.

The immune status in various pathologic conditions and the functioning of the immune cells was a continuous subject of the research of staff members *(1, 2, 4, 30, 31)*.

During the 1990s , activities were focused on primary immune deficiency (PID) diseases which sometimes represent a real diagnostic challenge. Thanks to fruitful collaboration with pediatricians within the Clinical Hospital Center Zagreb and other pediatric units in Croatia, a number of cases with various forms of immunodeficiencies have been determined and successfully treated by substitution therapy or bone marrow transplantation (BMT). Among them are children with various forms of severe combined immunodeficiency (SCID), Brutton's agammaglobulinemia, hyper-IgM syndrome and chronic granulomatous disease (CGD). In addition to standard flow cytometric (FC) lymphocyte enumeration, a number of complex and time-consuming cellular assays based on FC have been developed and introduced, including lymphocyte proliferation assay, CD40-ligand assay, phagocytosis and respiratory burst.

As previously mentioned, the early 1990s witnessed significant work on the analysis of specific lymphocyte subsets in patients with systemic autoimmune diseases *(1, 2, 4)*. Recently, the laboratory introduced several new FC assays, including those for enumeration of regulatory T-cells (Tregs) and allergen-activated peripheral blood.

Immunophenotyping of leukemia, lymphoma and other hematological diseases

Immunological classification of leukemia and lymphoma began during the late 1970s at the Clinical Hospital Center Zagreb *(32, 33)* and soon after at the Ruđer Bošković Institute *(34, 35)*.

The older methods (such as E-rosettes) were soon replaced by monoclonal antibodies *(33–36)* and flow cytometry *(37–40)*. Namely, the Division of Immunology of the Department of Clinical Laboratory Diagnostics was the first institution in this part of Europe (Middle and South-East Europe) to purchase and install fluorescence activated cell sorter (flow cytometer, FC). The Coulter's cell sorter EPICS was installed in 1986 and since then this technology has been engaged (used) in routine diagnostics of immunological and hematological diseases, as well as in scientific and educational activities within Republic of Croatia and at international level.

From the very beginning, the machine (apparatus?) was used for lymphocyte enumeration in various clinical conditions *(1, 2, 4, 17, 31, 41),* but was especially valuable for the immunophenotyping of leukemia and lymphoma *(39, 42)* and DNA-analysis of tumors *(43)*. At that time, novel approaches to the immunodiagnosis of leukemia/lymphoma were tested, such as computer assisted method for recognition of leukemia/lymphoma phenotype pattern *(44)*.

Although for many years it served as an unofficial referral center for immunophenotyping of leukemia and lymphoma, in 1998 the Department of Immunology finally became the official National Referral Center for Immunodiagnostics of Hematological and Immunological Diseases for the Croatian Ministry of Health and Welfare.

Besides routine immunodiagnostic and scientific activities, laboratory members have been engaged in several international studies, especially those dealing with treatment of adult acute leukemia (EORTC Leukemia Group) and pediatric acute leukemia (BFM; ALL-IC group) *(46)*. Recently, a significant amount of work has been devoted to the problem of minimal residual disease (MRD) in acute leukemia, again in the context of international collaborative studies, especially BFM-ALL-IC for pediatric leukemia *(46)*.

In addition to hematological neoplasms, the laboratory has gradually developed and introduced many valuable flow cytometric assays, including CD34+ enumeration, assay for paroxysmal nocturnal hemoglobinuria (PNH), analysis of platelet glycoproteins, leukocyte functional assays such as respiratory burst, etc. *(47)*.

Hematopoietic stem cell transplantation

From the very beginning, the Department of Immunology was actively involved in the field of hematopoietic stem cell transplantation (HSCT). (In that sense) Consequently, flow cytometry *(41)* and short term bone marrow culture for the detection of colony forming units (CFU) *(48)* were important for the development of a clinical stem cell program and corresponding scientific activities carried out in collaboration with the Department of Hematology. Besides routine diagnostics in patients undergoing HSCT, the laboratory was actively engaged in the immunological follow-up of patients after HSCT and research work dealing with cellular composition of bone marrow transplant *(41)*.

The Immunology Laboratory started to play an even more critical role during the early 1990s , when autologous HSCT came to light: enumeration of hematopoietic CD34+ stem/progenitor cells in peripheral blood and leukapheresis product (i.e. transplant) by FC, as well as short term bone marrow culture, became essential laboratory tools for planning and performing auto-HSCT *(49)*. Although modified over time, these two methods have preserved their importance for auto-HSCT until today. In parallel with diagnostic CD34+ enumeration, the Department of Immunology was actively involved in the first clinical program of CD34+ selection for auto-HSCT. Namely, in 1994 the laboratory staff performed the first CD34+ positive selection from leukapheresis product, using an affinity cell-chromatography-based device (CellPro™) *(49)*.

During next two years, a total of 40 CD34+ selections for auto-HSCT and two combined procedures (CD34+ selection followed by T-cell depletion) for haploidentical allo-HSCT were performed. By the end of the 1990s , the chromatography-based CD34+ selection was replaced by an immunomagnetic one routinely performed by specialist transfusiologists.

It should be mentioned that the Clinical Hospital Center (quickly) recognized the value of umbilical cord blood as a potential source of hematopoietic stem/progenitor cells for transplantation *(50)*. Soon after, a study of cord blood was performed including lymphocyte subsets enumeration, CD34+ content and CFU-content *(V. Šneler, unpublished*). Fifteen years after those pioneering steps, the Clinical Hospital Center established the Cord Blood Bank »Ana Rukavina« to serve public and private needs for hematopoietic cells *(51)*.

Immunocytogenetic analysis of hematological and solid malignant tumors

The Laboratory of Immunocytognetics was formed (established) during the late 1990s to serve increasing demands for accurate cytogentic and molecular diagnosis of various hematological neoplastic diseases as well as solid tumors. In addition to conventional cytogenetics, the laboratory soon introduced sophisticated molecular methodologies, including M-FISH and I-FISH *(45, 52)*. FISH technology has been used in several collaborative research sudies, especially a study investigating telomere dynamics in human tumor cell line *(53)*.

Scientific activity

Six research projects supported by the Croatian Ministry of Science and Technology
(project leaders: M. Marušić, D. Batinić, B. Malenica)

Five collaborative projects supported by the Croatian Ministry of Science and Technology
(project leaders: B. Labar, H. Banfić, B. Vucelić)

International collaboration activity

1. UEMS, Union European of Medical Specialty, Section of Medical Biopathology-Immunology Division (B. Malenica)
2. EORTC, European Organization for Research and Treatment of Cancer (D. Batinić)
3. BFM, International cooperative study on diagnosis and treatment of childhood acute lymphoblastic leukemia (ALL-IC) (D. Batinić, Klara Dubravčić*)(46)*.

Educational activity

Graduate and Postgraduate courses:

1. Zagreb University School of Medicine (D. Batinić, B. Malenica)
2. Mostar University School of Medicine, BIH (D. Batinić)
3. Zagreb University Faculty of Natural Sciences(B. Malenica)
4. Zagreb University Faculty of Pharmacy and Biochemistry (B. Malenica)
5. Immunology course, High School for Laboratory Medicine (B. Malenica)

Specialistic education

Teaching clinical and laboratory immunology as a part of education in various medical specialties and subspecialties: clinical immunology and allergology, rheumatology, hematology, transfusiology, dermatology and medical biochemistry.

DEPARTMENT OF TISSUE TYPING

Staff members (2008): Vesna Brkljačić-Kerhin, h*ead,* Vesna Balog, Esma Čečuk, Zorana Grubić, Ines Humar, Željko Kovač, Natalija Martinez, Biserka Palfi, Renata Žunec

Former staff members: Ljerka Brkljačić Šurkalović, Ivana Đurinović Bello, Marijan Gerenčer, Andrija Kaštelan, Vesna Mezulić, Marija Tomašković.

Transplantation

Since its foundation in 1972, the role of the Tissue Typing Center (TTC) was to support the kidney transplantation program in Zagreb, which started the same year with the first cadaveric kidney transplantation in the Clinical Hospital Center Zagreb. From that date, a national kidney transplantation waiting list has been kept in the TTC. The very first software for donor-recipient matching in cadaveric kidney transplantation was developed in TTC and for many years it served as a national standard program. In the following years the TTC has also taken a role in improving the outcome of other organ transplantation programs by performing tests prior to transplantation (HLA typing, screening, cross-match) as well as post transplantation follow-up.

In 1983 the TTC started to support the National Bone Marrow Transplantation Program based on the selection of the HLA-identical sibling donor which was initiated at the Department of Hematology of the Clinical Hospital Centre Zagreb.

Diagnostics

During the 1970s , the TTC established HLA typing for diagnostics, firstly for HLA-B27 as an indicator of many rheumatic diseases but also for HLA class II typing as tests for autoimmune diseases associated with HLA genes. In the same period a mixed lymphocyte culture (MLC) as well as other tests based on cultured cells *in vitro* were introduced into the routine work of the TTC. Prenatal diagnostics of congenital adrenal hyperplasia (CAH) based on HLA typing was established in 1984.

At the beginning of the 1990s , theTTC introduced molecular typing of HLA class II genes (DRB1, DQA1, DQB1, DPB1) for the transplantation program in Croatia, as well as for routine diagnostics. A few years later molecular typing of HLA class I genes (A, B and Cw) for the transplantation program was established.

Population and disease HLA association studies

The first study on HLA polymorphism in the Croatian population was launched in the early 1970s . The results of that research were published in Tissue Antigens in 1974 *(55)*. For the first time this study provided data about HLA class I antigen characteristics among Croatians. Since 1977 the TTC has participated in research projects of the International Histocompatibility Workshops (7th IHW – Oxford, 1977; 8th IHW – Los Angeles, 1980; 9th – Munich Wien, 1984; 10th IHW – New York, 1987; 11th IHW – Yokohama, 1991; 12th IHW – Paris, 1996, 13th IHW Seattle, 2002; 14th IHW – Melbourne, 2005; 15th IHW – Rio de Janeiro, 2008) *(56–61)*. The aim of these projects was not only to investigate population characteristics of the Croatian population in general and in isolated island populations, but also to explore (investigate) the association (correlation) of different diseases with HLA genetic background. All these studies have succeeded in contributing to the resolution of the pathogenesis of the studied diseases.

The following research projects dealt with HLA and disease associations (correlations) (Ankylosing Spondylitis, Diabetes type 1, CAH, Psoriatic Arthritis) and the role of HLA in human reproduction *(61–64)*.

During the 1990s the TTC research projects were based on the investigation of HLA region on DNA level and have provided help in obtaining a more precise picture of HLA polymorphism in the Croatian population, and also elucidation of the role of HLA in association with diseases as well as transplantation *(65, 66)*.

Scientific activity:

Five research projects supported by the Croatian Ministry of Science and Technology
(project leaders: A. Kaštelan, R. Žunec, Z. Grubić)

International collaboration activity:

1. Terasaki Quality Control Program (chairperson: Terasaki PI)
2. Collaborative Transplant Study (chairperson: Opeltz G)
3. Central Europe Typing (chairperson Fischer G)
4. Eurotransplant (chairperson Doxiadis I)

Educational activity

Graduate and Postgraduate courses:

1. Zagreb University of Natural Sciences (V. Brkljačić-Kerhin, Z. Grubić)
2. Zagreb University School of Medicine (V. Brkljačić-Kerhin, Z. Grubić)
3. Osijek University JJ Strossmayer (Z. Grubić)

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INTRODUCTION

The well known evolutionary biologist Theodosius Dobzhansky once stated that nothing in biology makes sense except in the light of evolution. As immunology is not exempt from this truism, the distinguished immunologist Jan Klein, in 1995 paraphrased Dobzhansky *(1),* claiming that if one wants to make sense of an immunological phenomenon, a comparative study is a requirement. The recent development of modern immunology is not restricted to the study of mouse and human immune systems. Extension of the scope of immunology to the study of other species of vertebrates and some invertebrates is turning the discipline away from a story of »Mice and Men« (citation of John Steinbeck's title) and make it a biological rather than a medical discipline. The former is what it should be, because self-non-self discrimination is evoulutionary biology and not a specifically medical characteristic.

Veterinary Immunology Mission

Veterinary immunology is dedicated to the improvement of animal health. The field of veterinary immunology is expanding rapidly due to an immense number of species and topics to be covered. An understanding of the immune system of animals other than mice and primates has several essential aspects: (a) in domestic animals it has obviously important applications in veterinary medicine (diagnosis, vaccination, nonspecific immunomodulation, immune-mediated diseases, immunogenetics or immunodeficiencies); (b) animal health/welfare and animal products hygiene/quality, which benefit from immunology, are now of primary importance for human health; (c) particularly significant for the health of food animal species for the World's economy and public health; (d) several animal species (swine, sheep, rabbit, horse, dog, and poultry) also represent unique models for medical studies and greatly conbtribute to the development of immunology; (e) evolutionary or comparative immunology research constitutes a crucial way of increasing our knowledge with both basic and practical information of the immune system; (f) the growing interest in xenografts clearly implies a thorough immunological description of the animal tissues to be grafted. The authors of this article supplemented some of these aspects summarized in 1998 by Bernard Charley *(2)* a past chairman of the Veterinary Immunology Committee(VIC) of the International Union of Immunological Societies (IUIS).

History

In 1986, the 1st International Veterinary Immunology Symposium (IVIS) was convened at the University of Guelph in Canada, as a satellite meeting of the 6th International Congress of Immunology held in Toronto (Canada). The goal of the

IVIS was to bring together scientists interested in the immune system of species other than mouse and man, and represented a significant milestone in the development of comparative and veterinary immunology. Since then veterinary immunology has been defined as a part of immunology that considers the animal the aim and not the tool of immunological investigations. The first meeting was so successful that funds were transferred to the VIC of the IUIS to seed future conferences. Thus, the tradition continued and symposia have been held in Germany (Hannover, 1989), Hungary (Budapest, 1992), USA (Davis, CA, 1995), India (Ludhiana, Punjab, 1998), Sweden (Uppsala, 2001), Canada (Quebec City, 2004) and Brasil (Ouro Preto, 2007). The next meeting in 2010 will be in Tokyo, Japan.

Since 1995 meeting, the Distinguished Veterinary Immunologist (DVI) Award is presented at the IVIS, during which the recipient delivers the Award lecture. The award was presented by the VIC of the IUIS and generously sponsored by the Animal Health Biological Discovery Department of Pfizer Inc. Recipients are selected by a committee comprising members of the VIC, on the basis of their academic excellence and their internationally recognized scientific stature/career or authorities and leaders in the field. Dr. John Butler from the University of Iowa, Ames, IA, USA was chosen as the DVI for 2007.

Besides organizing the IVIS, the VIC of the IUIS facilitates international and interdisciplinary research contacts by sponsoring/organising subcommittees /workshops such as:

– Swine Immunology Subcommittee
– Ruminant Immunology Subcommittee
– Equine Immunology Subcommittee
– Avian Immunology Subcommittee
– Human Leucocyte Differentiation Antigens (HLDA8) Animal Homologues Workshop
– Comparative Immunology Workshop
– Other species Workshops (planned on (companion animals?) and fish)
– Comparative MHC Workshop (bovine, canine and porcine sequences are (underway to submit?))
– Immune Toolkit Workshop (development and distribution of new immune reagents)
– Electronic Workshops (on topics specific for the veterinary immunology community)
– Education (web linked veterinary immunology courses and curricula plans)

Today several countries have VIC within their national immunological societies and/or separate organizations, *e.g.* Germany, India, UK and USA. An example of the latter is the American Association of Veterinary Immunologists (AAVI), formed in 1979 by a group of veterinary immunologists assembled at the annual Conference of Research Workers in Animal Diseases. The AAVI is open to all veterinary immunologists internationally and with 463 members is the biggest (largest) society of veterinary immunologists.

By the end of the 1970s, the constant awareness of the need of further development, promotion, and dissemination of knowledge in veterinary immunology resulted in the foundation of an international journal dealing with fundamental and applied immunology of animal species of veterinary importance. Such a journal, entitled Veterinary Immunology and Immunopathology (VII), commenced publication in 1980, is devoted to comparative immunology and deals with the study of veterinary immunology and immunopathology as applied to animals, particularly agricultural and companion animals as well as wildlife. Today the VII is the most prominent journal (ranked 8th in the Veterinary Sciences ISI category with an impact factor of 1.994) dealing with issues of veterinary immunology.

Recent Perspectives

New veterinary immunology contacts are being extended as contacts are suggested by the country's immunological society or requested by the IUIS–VIC Chair-Elect Jan Naessens, Nairobi, Kenya. These efforts are expanding as veterinary immunologists further develop their efforts to have the VIC with their regional federations. Thus, in 2001 the European Veterinary Immunology Group (EVIG) was founded under the auspices of the European Federation of Immunologic Societies (EFIS). The first meeting/workshop of the EVIG, the newly established VIC in EFIS, was held in 2003 in Berlin *(3)*. The next meeting was held in late summer of 2006 in Paris.

The term »veterinary immunology« is now traditionally used to refer to the immunology of domestic animals, including companion and food-producing species, and wild animals having economical or sentimental value to man, and provides both practical knowledge that is useful to animal husbandry, and new insights into fundamental immunology. Scientists working on the immune system of domestic animals obviously pursue both applied and basic objectives. The applied aims are directly linked to veterinary medicine and include studies on the immunological improvement of health of domestic animals (by nutritional and immune modulation) and the immune mechanisms involved in the pathogenesis of animal diseases (such as autoimmunity and hypersensitive reactions). Defining new immunologically based diagnostic tools (monoclonal antibodies,

ELISA procedures), vaccination strategies and products (vectors, immunoadjuvants, antigen delivery systems), genetic selection of resistant animals based on immune parameters, and immunotherapy or gene therapy against animal diseases, obviously still represents important applied objectives *(4)*. Simultaneously, »veterinary immunology« is a part of an immunological continuum, in which data generated from a wide variety of animal species have a crucial role in illuminating our understandsing of general immune mechanisms. New contributions to basic and human immunology arise from so- called »immunological opportunities in farm animals« which may well rest upon their *»in vivo* increased relevance«. In this respect, immunological studies on well controlled experimental models using domestic animals, as well as on spontaneously occurring animal diseases, afford valuable means of manipulating and analyzing the immune system *in vivo* and of evaluating immunoprophylactic/immunotherapeutic approaches, which would be either impossible or unethical to carry out in other species *(5)*. Additional advantages of domestic animals for fundamental research include impermeability of the placenta of ungulates to large molecules which simplifies studies on the ontogenesis and development of the immune system and immunocompetence respectively, or on the maternal immunity in the absence of any external antigenic/antibody influence. Also, increasing knowledge of the immune system of domestic animals contributes to the field of transplantation immunology as illustrated by extensive studies on the potential use of xenografts, mainly swine tissues/organs, in humans. Further need for immunological improvement of the health of food animals will be strengthened by the increasing economical importance of animal products for food supply. For instance, novel immunization strategies using DNA plasmid vaccines, targeted to the mucosal sites, which offer stablity, safety and effectiveness, will be fully evaluated. The interactions between neuroendocrine and immune systems will lead to applications such as immune-mediated castration or immune control/improvement of fertility. The assessment of immune parameters of animal welfare and responses to environmental agents will also be a major goal for veterinary immunologists. Finally, improving interactions between veterinary immunologists and veterinary clinicians which are likely to grow (increase) in the future will include the use of spontaneously/genetically occurring animal diseases (autoimmune diseases, acquired immunodeficiency syndrome and cancer) as models for human diseases *(6)*. Thus, evaluation of immune manipulations (immunotherapies and gene therapies) against specific human pathological entities, including tumors, should benefit from studies carried out in animals, particularly small animals and swine.

The purpose of this article is to gather and summarize in a comprehensive way all available data on veterinary immunology from the scientific community in Croatia and Croatian immunologists working in scientific institutions on domestic or wild animals in the manner that they consider these animals the aim and not the tool for their immunological studies or trials. Due to its limited volume this article will rather focus on research achievements in veterinary immunology over the period of the last 20 years (between 1985 and 2006), which coincides with the revival of the discipline by a group of Croatian immunologists who presented their papers at the 1st Congress of theYugoslavian Immunological Society held in Opatija, Croatia in 1985 within the framework of the newly established section termed »Veterinary immunology«, on the »early days« of the immunological investigations that were aimed at solving actual animal disease problems. However, the pioneers of veterinary immunology in Croatia and their research efforts must not be forgotten.

Veterinary immunology in Croatia

Although the number of scientists and groups working on veterinary immunology in Croatia is relatively small, there are still several scientific institutions where researchers deal with immunology of domestic and wild animals such as:

– Veterinary Faculty (VF), University of Zagreb
– Croatian Veterinary Institute (CVI), Zagreb
– Department of Animal Physiology (DAP), Division of Biology, Faculty of Science, University of Zagreb
– Animal Health »Veterina«, Pharmaceutical Company Pliva, Zagreb

However, the research results on the veterinary immunology issues attained (achieved) in the firm »Veterina« are not included in this article since its aim was to describe the state of veterinary immunology research achievements in scientific institutions in Croatia. Recently, a nucleus of veterinary immunology research was established at the Faculty of Agriculture (FA) University of Osijek.

The concept of this article was to keep it as short as possible but sufficiently documentative to provide essential information about two major institutions working in the field of veterinary science, *i.e.* VF and/or CVI. The work of veterinary immunologists from the two above mentioned institutions (excluding those from Pliva) will be mentioned briefly within the scope of collaborative research with researchers from VF and CVI.

The history of research at VF and CVI dedicated to veterinary immunology can be divided essentially into two periods. The first (1902-1984) was characterized by a wide range of interests in immunology of infectious diseases, aimed at solving animal disease problems (foot and mouth disease, swine plague, brucellosis, leptospirosis, viral pneumonia, viral and bacterial diarrhea, fowl cholera and Aujeszky's disease; AD) on the new large-scale farms, by introducing serological diagnostic

techniques and/or developing vaccines. This pioneer epoch or »early days« of veterinary immunology was essentially a constant struggle for existence and produced a relatively large number of publications, prevalently (mainly) in the Croatian language. In the second period (1985-2006) the focal points were immunity to infections and development of effective vaccines against infectious diseases, particularly of viral etiology. Such diseases jeopardize animal health/welfare and the agricultural economy in both developed and developing countries. The situation is exacerbated by the fact that some diseases (such as brucellosis, classical swine fever; CSF, trichinellosis, paratuberculosis, distemper and rabies) can be readily transmitted between domestic and wildlife species *(7)*. Moreover, many animal diseases pose a potential threat to human health. Among these, according to the number of cases per year the most frequent zoonoses in 2004 in Croatia were: leptospirosis, trichinellosis, Q-fever and toxoplasmosis. Recently, worldwide attention has been focused on efforts to control newly emerging infectious diseases, for example, porcine reproductive and respiratoty syndrome (PRRS), bovine spongioform encephalopathy (BSE), porcine circovirus associated diseases (PCAD) and highly pathogenic avian influenza (HPAI) in farm animals. However, the second period was also characterized by focus on more basic research in immunobiology of livestock, companion animal species and wildlife, including birds and aquatic animals, increased application of modern cellular and molecular immunological methods and more publications in internationally recognized scientific journals.

Parallel with the definition of »veterinary immunology« in the mid-1980s , the group of immunologists in Croatia who had already accepted such a concept contributed to its realization with five congress papers assigned to the section termed »Veterinary immunology« presented at the 1st Congress of the Yugoslavian Immunologists held in Opatija, Croatia in the autumn of 1985. These papers were published in this journal in 1986 (Period biol 88, Supp 1/A: 445–456). This episode in the history of veterinary immunology in Croatia is interesting and of sentimental value for those who participated at the Congress as authors of these papers. The discipline which gathered together scientists interested in the immune system of animals and improvement of their health (such as Slavko Cvetnić, Ivan Bašić, Branko Lugović, Zvonko Modrić, Damir Rapić and Ivica Valpotić) was defined and recognized, and also revived. Namely, we can trace very far back in the history of veterinary science in Croatia the approach that complies well with what today is the scope of veterinary immunology.

Early days

In Croatia, like in other countries, veterinary immunology is dedicated to the improvement of animal health and, thus, immunity to infectious diseases and vaccine development were focal points of pioneer research. A brief hisoric overview of these »early days« is given below.

According to Topolnik *(8)* in 1901, the Croatian Government authorized the first head of Veterinary Services in Croatia, Radoslav Krištof, to establish a Department for Veterinary Bacteriology (DVB) in Križevci. The founding head of the DVB, Ferdo Kern, started work with a staff consisting of five assistants (N. Ritzoffy, E. Kolibaš, F. Gabrek, F. Švrljuga and J. Haramin̆čić), and between 1902 and 1904, they initiated the production of vaccine against blackleg. The proposition of Kern to establish a department for production of vaccines against infectious diseases of domestic animals was declined by the Government in 1912. In 1916, Kern and Bogoslav Ljevačić, chief veterinary inspector, went to Budapest, Hungary for training at the State Department for Production of Vaccines. Kern was, however, did not succeed in establishing a serum department in Croatia until 1918, when the assembly of the newly established Department for Production of Vaccines (DPV) in Križevci approved the production of vaccines against antrax, swine erysipelas and blackleg as well as immune sera against anthrax and swine erysipelas. Major progress was achieved in 1920, when 737 liters of immune serum against swine erysipelas and over 52 thousand doses of vaccine against anthrax were produced. When Kern retired in 1922 Andrija Hupbauer, a former assistant (since 1914) bacame head of the DPV. He organized a division for production of vaccines against infections of livestock within the DPV. By the end of that year the Veterinary Administration in Zagreb passed a resolution on the production of vaccines and changed the name of the DPV into the Department for Bacteriology and Serology (DBS). At the same time in Zagreb the Yugoslavian Serum Department was active (established in 1920 by Aladar Lukacz who had similar departments in Budapest, Frankfurt and Madrid) producing vaccines against anthrax, blackleg, swine plague, swine erysipelas, fowl cholera, strangles, salmonellosis, brucellosis, distemper, glanders, hyperimmune sera using horses as donors as well as alergens/immunogens such as mallein/tuberculin. Parallel with the serological diagnostics performed at the DBS in Križevci, animal infectious diseases were diagnosed (since 1922) at the Department for Infectious Diseases (DID) and the Department of Veterinary Hygiene and Microbiology of the Veterinary High School, since 1924 of the VF University of Zagreb. The first head of these two departments was Stjepan Plasaj who in his early research (since 1921) studied the feasibility of immunization against foot and mouth disease and control of this disease by immunotherapy. He also conducted vaccination against sheep pox and bovine tuberculosis by Calmette-Guerin's vaccine, validated serological methods and malleinization alergic reaction for diagnosis of glanders, and applied peroral immunization against swine plague and fowl cholera *(9)*.

In the second half of the1930s Josip Ježić, Božidar, Tunk and Marko Zeljko at the CVI conducted investigations on the immunogenicity of vaccines against swine plague, ND and swine erysipelas.

In 1940, Hupbauer left his position at the DBS in Križevci and devoted his further career to academic research activity at the DID of VF in Zagreb. Comprehensive research on vaccines against anthrax (glucoside vaccine), blackleg (bivalent vaccine), swine plague (crystalviolet vaccine), foot and mouth disease, atypical poultry plague and swine erysipelas (adsorbate vaccine) had outstanding theoretical and practical importance. Within the framework of immunological studies, he wrote about serum sickness and anaphylaxis in veterinary practice, enhancement of antibody production by nonspecific stimuli and adjuvanticity of saponins with vaccine against swine plague *(10)*.

At the DID in the early 1950s Zvonimir Brudnjak developed an agglutination test for serological diagnosis of swine erysipelas and analyzed the influence of the route of antigen application upon the formation of antibodies. At that time veterinary immunology teaching dealt with two main issues: (i) the struggle to gain recognition for immunology as a distinct discipline at the VF, and (ii) the need to identify the essential immunologic concepts that all veterinary students should learn. In an effort to support these issues a group of authors from the DID, lead by Franjo Mlinac, prepared and issued in 1963 the first teaching text entitled »Lessons in microbiology and immunology«, with described serological methods for the diagnosis of infectious diseases.

A serological survey on leptospirosis in animals and humans was performed by Ivan Zaharija, who organized a Laboratory for leptospires at the VF in 1950. He collaborated through the Center of the Yugoslavian Academy of Science and Arts (YASA) with numerous human and veterinarian infectologists/immunologists. His finding of equine leptospirosis was the second description of a case of the disease in horses in the World.

In the late 1950s at the CVI, Čedomir Pauković developed serological diagnostics of some viral diseases (Newcastle disease; ND, bovine viral diarrhea; BVD, fowl pox and Rift Valley fever). In the mid 1960s Hrvoje Kovačić performed studies on immunobiology of bacterial zoonoses (leptospirosis, brucellosis, bovine tuberculosis and Q-fever).

In the 1970s, Slavko Petričević worked on immunoprophylaxis of swine erysipelas, porcine Clostridial infections, bovine trichophytosis and tuberculosis. Later on, he and Ivan Udovičić (whose main interest was the immunoprophylaxis of swine necrotic enteritis and examination of antibody responses following specific immunization) performed comprehensive expertise on development/evaluation and registration of bacterial vaccines.

Research at the VF on veterinary immunology issues in 1950s can also be ascribed to the investigations of Milan Kralj who worked on serological diagnostics of fowl typhoid and pullorum disease. However, before he started exclusively with research on avian diseases in the late 1960s, his work was also oriented towards:

– validation of the agglutination test in diagnosis of swine erysipelas and bovine brucellosis,
– detection of heminhibiting serum antibodies to equine influenza virus A1 (Prague) 56 and parainfluenza 3 virus,
– evaluation of the significance of specific neutralizing antibodies in the serodiagnostics of infectious bovine rhinotracheitis (IBR).

Since the 1970s, a well known immunological tool for detection of specific antibodies against equine infectious anemia (EIA) virus, *i.e.* gel diffusion precipitation (GDP) test was introduced in 1970 by Davor Petrović and his research group at the VF immediately following its first description by L. Coggins and N. L. Norcross in the USA *(11)*. This was probably the first utilization of the GDP test in Europe.

Research on the immunology of poikilotherm vertebrates was conducted at the VF on a carp between 1964 and 1977 by Nikola Fijan and Đuro Sulimanović who focused their work on:

– Detection of the humoral immune reactivity of carp depending on the environmental conditions by the method of hemolytic plaques and serum neutralization test.
– Immunization and hyperimmunization of the carp against spring viremia with a live virus vaccine by different routes of application.
– Determination of the antibody response of carp to *Rhabdovirus carpio* by indirect hemagglutination.

In 1980, Eugen Toplnik and collaborators at the VF issued the textbook »General Microbiology and Immunology«, intended as another textbook for veterinary students. However, the major impact in the recognition of veterinary immunology occurred between 1937 and 1980, due to his research work on:

– Immunoprophylaxis of ND of poultry.
– Immunobiological relationship between equine viral abortion and equine influenza.
– Development of serological methods for diagnosis of infectious diseases, such as equine viral abortion, EIA, bovine parainfluenza 3, IBR, BVD and bovine adenovirus type 1.
– Evaluation of the immunogenicity of attenuated vaccines against bovine parainfluenza and IBR.

As an outstanding research personality, his scientific curiosity prompted him to publish reviews on interferon and the role of viruses in oncogenesis in 1962 and 1967, respectively.

State of art (1985-2006)

More recent history of veterinary immunology in Croatia has been written by researchers at six departments and one clinic of the VF, but also at the CVI and DAP as well as by several Ph.D. students currently working elsewhere.

At the Department of Biology (DB) of the VF the research topics in veterinary immunology were initiated in the early 1980s by Terezija Hrženjak who dealt with:

– Iimmunogenicity of fluke (*Fasciola hepatica*) cytolipin-P.
– Molecular similarity of parasitic larval antigens (isolated from *Echinococcus granulosus*) and human tumor antigens.
– Mitogenicity of lectins isolated from trout (*Salmo trutta fario)* mature spawn. In the mid 1980s research of Ivica Valpotić had valuable importance for theprompt recognition of veterinary immunology in Croatia as a separate discipline within general immunology. His work had outstanding theoretical and practical importance, particularly regarding the mentorship of numerous M.S./Ph.D. students (3/11) and the leadership of efficient projects (8 domestic and 5 international) focussing on the improvement of animal health by immunological approaches. The key issues/outcomes with which he and his collaborators (names given in parentheses), including M.S./Ph.D.students, (whose names are given in italics in parentheses) have been engaged or have achieved between 1981 and today are:
– Humoral and cellular immunity in horses naturally or experimentally infected with the EIA virus; an original finding of differentiate lysis of autologous virus infected cells by direct cytotoxicity and antibody-dependent cell-mediated cytotoxicity in the lentiviral infection.
– Unique features of lymphocyte reactivity to nonspecific/specific stimuli in food (pig, cattle, sheep, goat) and companion (dog, cat, horse) animals.
– Passive transfer of immunity in swine and immunoprophylactic potential of allogeneic immunoglobulin preparation in the prevention of diarrheal disease in suckling pigs.
– *In vitro* and *in vivo* studies of immunomodulatory effects of egzogenous and endogenous immune response modifiers (IRMs) in species of veterinary importance, particulatly in young pigs.
– Age-dependent responsiveness of porcine peripheral blood lymphocytes to common mitogens, specific antigens or allogeneic cells.
– Porcine leukocyte cluster of differentiation (CD) antigens and their distribution on lymphoid/myeloid cell subsets in lymphoid tissues/organs; participation as research leader of the laboratory authorized for testing of mAbs reactive with swine lymphoid CD antigens for three International Swine CD Workshops held between 1992–1998.
– Development of an enzyme immunoassay for detection of porcine enterocyte receptor for adhesion of the F4ac fimbrial antigen of enterotoxigenic *Escherichia coli* (ETEC).
– Screening of porcine peripheral blood and Peyer's patch lymphocytes for the presence of the F4ac adhesion receptor homologue.
– Demonstration of specificity and function of porcine immune cells in defense of the intestinal mucosal surfaces against enteric infections of bacterial etiology.
– Modulation of porcine intestinal mucosal immunity with $F4^+$ and $F18^+$ nonenterotoxigenic *E. coli* (non-ETEC) vaccine candidate strains, IRMs/mucosal adjuvants and/or prebiotics/probiotics in order to control/prevent postweaning colidiarrhea/colienterotoxemia (Ivica Valpotić).
– Immunogenic and molecular characteristics of the isolate New scotia-1 of nematode subspecies *Trichinella spiralis nativa* (*Albert Marinculić*).
– Analyses of cellular immune response parameters in the gut-associated lymphoid tissues (GALT) of pigs experimentally infected with ETEC or non-ETEC strains of *E. coli* (*Nada Vijtiuk*).
– Humoral and cellular immune responses of horses hyperimmunized with tetanus toxoid (*Nikica Petrinec)*.
– Phenotypic expression and tissue distribution patterns of porcine CD^+ immune cells *(Marija Tomašković)*.
– Levamisole synergizes immunostimulatory effect of a live oral vaccine against postweaning colibacillosis*(Frane Božić)*.
– Humoral and cellular immunity of pigs following immunization with either attenuated China strain of the CSF virus or with glycoprotein E2 of the virus *(Svjetlana Terzić)*.
– Immunophenotypic and functional characteristics of T and B cell subsets in the GALT of pigs experimentally vaccinated with an adherent nontoxigenic *E.coli* strain.

– Modulation of the intestinal mucosal immunity in weaned pigs with fimbrial antigens of *E. coli* and/or IRMs *(Lidija Šver)*.
– Stimulation of nonspecific immunity by inactivated *Parapox virusovis* preparation decreases mortality in young pigs *(Marcela Šperanda)*.
– Reproduction characteristics of breeding bulls of the simmental breed can be improved by immunomodulator Baypamun.
– Immunostimulatory effectiveness of levamisole can improve the sperm quality in boars while homeopathic preparation traumeel was shown to be ineffective *(Franjo Marković)*.
– Cellular immune responses of pigs with postweaning multisystemic syndrome are-related to the magnitude of changes in their lymphoreticular tissues (Željko Mihaljević).
– Immunophenotypic and morphometric characteristics of the small intestinal immune cells of weaned pigs immunized with a live oral vaccine against colienterotoxemia.
– Analyses of porcine intestinal immune system during perinatal ontogenesis and following egzogenous immunomodulation by immunohistological and morphometric techniques *(Ana Kovšca Janjatović)*.
– Functional *in vitro* analyses of cellular immunity in EIA virus-infected horses show homology with the anti-viral responsiveness in AIDS.
– Immunosuppression of *in vivo* and *in vitro* porcine lymphocyte responses induced by *T. spiralis* or excretory-secretory (ES) antigens of the parasite (Marijan Gerenčer).
– Identification and distribution of CD^+ leukocyte subsets in the porcine GALT compartments by immunofluorescence or immunoperoxidase methods.
– Differentiating cytolytic and suppressor T cells in the small intestine of pigs by S-100 protein marker (Gordana Lacković).
– Immunological characterization of the immunoglobulin-like adhesins isolated from earthworm *Eisenia foetida*.
– Analyses of postnatal development/maturation of cellular immunity in dog, cat, cattle, horse, goat, sheep, swine, rabbit and chicken by expression of leukocyte/lymphocyte CD antigens using flow cytometry.
– Demonstration of the anti-stress effect of immunomodulator Baypamun by decreasing the level of plasma cortisol after regrouping of pregnant gilts.
– Cytometric detection of qualitative/quantitative changes in patterns of the immune cells from roosters following coponization and in fattening chickens immunized with live or inactivated vaccines against ND.
– Assessment of changes in the expression of leukocyte cell surface CD molecules in cows naturally infected with enzootic bovine leukosis (EBL) virus (Maja Popović).

I. Valpotić has been a member of the AAVI (since 1989), past member of the VIC of the IUIS (1993- 1996) and was awarded (in 2004) with the Annual Croatian Award for significant scientific achievement in elucidating the mechanisms of modulation of porcine intestinal mucosal immunity by bacterial antigens and IRMs.

At the Department for Biology ond Pathology of Fish and Bees investigations on fish immunology were aimed at:

– Demonstration of the antigenic characteristic of *Rhabdovirus carpio* causative-agent of spring viremia of the carp (Zdravko Petrinec).
– Immunoprophylaxis of vibriosis (*Vibrio anguillarum*) and yersiniosis (Yersinia *ruckeri*) in the californian trout *Oncorhynchus mykiss* (Dražen Oraić).
– Vaccination of carp against spring viremia by an inactivated virus vaccine.
– Determination of the role of rodlet cells in the cellular immunity of carp (Željka Matašin).

At the Clinic for Internal Diseases (CID) investigations within the framework of veterinary immunology was started recently by Vladimir Mrljak, with a staff consisting of three researchers (Vesna Matijatko, Renata Barić Rafaj and Nada Kučer). Their research objectives were to define early diagnosis of protozoan infection of dogs, *i.e.* canine babesiosis, by screening of the plasmatic systems activated during the course of the disease for:

– Acute phase proteins such as C-reactive protein and haptoglobin.
– Neopterins.
– Arachidonic acid metabolites. which indicate that an infection/inflammation is occurring in the diseased animal.

The Department of Microbiology and Infectious Diseases (DMID) with the Clinic is considered the unit of the VF where veterinary immunology was »born« in the early 1920s as an academic research discipline of immunological continuum. Major progress in veterinary immunology issues (between the 1960s and mid 1990s), particularly in vaccinology was achieved by the outstanding contribution of Slavko Cvetnić, who dealt with:

– Development of diagnostic tools and immunoprophylaxis of equine influenza with an inactivated vaccine following determination of the virus A_2–equi (H3N8).
– Demonstration of the specific antibodies against human influenza virus in horse and swine.
– Development of the indirect hemagglutination test in diagnostic of IBR and the attenuated virus vaccine against bovine herpes virus 1.
– Evaluation of the effectivenes of immunoprophylaxis of bovine respiratory diseases with the attenuated bivalent vaccine comprising bovine herpes virus 1 and parainfluenza 3 virus.
– Serological detection of antibodies against adenovirus 1, causative agent of BVD and bovine immunodefficiency virus.
– Dose-dependent validation of inactivated or attenuated vaccines in the immunoprophylaxis of AD and potential use of the marker vaccines.
– Research on immunogenicity of the F strain of ND virus and a subunit vaccine against ND prepared from La Sota strain of the virus.
– Determination of vaccine potential against epidemic tremor of poultry (Slavko Cvetnić).

Within the framework of more recent veterinary immunology issues (between the mid 1960s and today), investigations of the DMID (respective names are in parentheses) have been:

– Investigation of immunogenicity of F strain of ND virus multiplied on the FL cell culture and epizootiological justification of the vaccination of breeding poultry against infectious bronchitis.
– Determination of the immunity of calves following vaccination with attenuated parainfluenza 3 virus.
– Study of the effectiveness of attenuated gE^- marker vaccine against AD in pigs.
– Demonstratation of specific antibodies for canine/porcine parvovirus, bovine adenovirus, equine influenze virus in humans, parainfluenza 3 virus in sheeps/goats, human influenza virus in birds, sheeps/goats and wild boars, CSF virus, PRRS and BVD in wild boars (Željko Župančić).
– Continuation of the work of I. Zaharija and demonstration by microscopic agglutination test antibodies specific for 10 serological groups of leptospires in Croatia.
– Systematically monitoring the seroprevalence of leptosirosis in animals and humans and firstly discovered this disease in the cat and hare in Europe (Zvonko Modrić).
– Continuation of the work of D. Petrović on EIA, and extending it by serological research of distribution of this retroviral disease in Croatia, Slovenia and Bosnia and Herzegovina.
– Development of an original technique of viral antigen production from the spleen of EIA infected horses.
– Study of the connection between influenza of domestic and wild animals with that of humans using different serological methods for detection of specific antibodies (Berislav Jukić).
– Testing of vaccine potentials in immunoprophylaxis/immunotherapy of bovine dermatophytoses and porcine necrotic enteritis.
– Investigating serologically the infection of horses with equine influenza virus A_1-equi, *Salmonella abortusequi* and *Actinobacillus pleuropneumoniae*.
– Serodiagnosis of the prsence of *Mycoplasma equigenitalium* in the sexual organs of horses and cattle; this was the first finding of this bacterium in a stallion in the World.
– Determination of the antigenic and genomic characteristics of *M. mycoides* subsp. *mycoides* and *M. conjunctivae* isolates from kids and sheep.
– Participation in the introduction of collegium Veterinary immunology in the programs of undergraduate/postgraduate studies of veterinary medicine at the VF in academic year 1991/1992.
– Coauthor of the texbook Veterinary Immunology, issued in 1993 as the first book in the Croatian language that offers comprehensive and fresh insight into basic and clinical immunology of species of veterinary importance (Tomo Naglić).
– Development of the toxoid vaccine against ovine gangrenous mastitis (bluebag) for the immunoprophylaxis of this disease in Croatia.
– Participation in the introduction of collegium Veterinary immunology in the program of undergraduate/postgraduate studies of veterinary medicine at the VF in academic year 1991/1992.
– Coauthor of the texbook Veterinary Immunology, issued in 1993 as the first book in the Croatian language that offers comprehensive and fresh insight into the immunology of species of veterinary importance (Danko Hajsig).
– Investigation of the vaccine potentials of inactivated and dissociated vaccines against equine influenza and introduction of a one-way radial hemolysis for its diagnosis.

– Application of immunoprophylaxis of AD and IBR in pigs and cattle, respectively.
– Study of immunobiology of IBR (antibody isotypes in serum and mucosal secretions) in experimentally infected calves following inoculation, reinfection and restitution of viral activity by different procedures of the immunoenzyme assay.
– Description of the immunogenic characteristics of herpes virus 1 strain Zagreb (deletion mutant for gen encoding gE); this was the first finding of such a natural mutant in the World which changed strategies of the control and prevention of IBR worldwide.
– Testing of the effectiveness of the vaccines against canine and porcine parvovirosis comprising the precipitated immune complexes.
– Demonstration of the specific neutralizing antibodies against West Nile virus in horses in Croatia.
– Serological/epizootiological investigation of the distribution of EBL virus, bovine immunodeficiency virus and feline leukaemia/immunodefficiency viruses (Josip Madić).
– Study of the level and kinetic of the humoral immune responses of cats/dogs naturally or experimentally infected with dermatophytes caused by fungal species such as *Microsporum canis*, *Microsporum gypseum* and *Trichophyton mentagrophytes.*
– Characterization of the cytoplasmic antigens of *M. canis* strains isolated from dogs and humans by SDS-PAGE technique and preparation of mAbs specific for these antigens.
– Clinical investigation of canine/feline allergic diseases (particularly atopic dermatitis of dogs caused by allergen from fungi *Malassezia pachydermatis)* based on originally developed indirect IF diagnostic method (Ljiljana Pinter).
– Serological investigation of epzootiology and epidemiology of infectious bacterial diseases, particularly zoonoses such as leptospirosis and Lyme borreliosis.
– Diagnosis of leptospires in domestic/wild animals and humans by serological and molecular methods.
– Development of an immunoenzyme assay for diagnosis of canine Lyme borreliosis.
– Screening of serum antibodies against human influenza virus in wild boars and birds.
– Clinical survey of the effectiveness of autovaccine against bovine papillomatosis (Nenad Turk).
– Investigation of seroprevalence of EIA in Croatia using originally isolated viral antigen on equine derma cell (fibroblasts) line culture in the GDP test (Vili Starešina).
– Investigation of nonspecific anti-virus/anti-stress effects of immunomodulator Baypamun on the transmission of AD virus and cortisol levels following transport of pigs (Nevenka Biuk Rudan).

At the Department of Parasitology and Invasive Diseases research on the immunity to parasites was initiated by Damir Rapić (and supported by his collaborators from the DB) and in the mid 1980s (who) he elucidated the immunosuppressive effects of nematode *T. spiralis* and the ES antigens of the parasite on *in vivo* and *in vitro* lymphocyte responses of either experimentally invaded swine or their ES-pretreated cells, respectively. However, he left the VF soon afterwards and his work was continued by Albert Marinculić who performed comprehensive research on:

– Development of postmortal diagnosis of swine trichinellosis by ELISA using originally produced ES antigen from the mucle larve of *T. spiralis.*
– Seroprevalence of trichinellosis as determined by the presence of specific antibodies in swines from farms in Germany and Croatia; these antibodies can only be detected in animals with more than 0.38 larve per gram of the musculature.
– Expulsion of adults of *T. spiralis* following inflammatory responses and significant increase of $\alpha/\beta TCR^+$ and $CD8^+$ intestinal intraepithelial (i-IEL) T cells in mice pretreated with dexamethasone.
– Immunogenicity and vaccine potential of an arctic species of the parasite, *i.e. T. nativa,* which were assessed in swine by sIgA antibody presence in the immunoblotting assay and by challenge infection with *T. spiralis.*
– Standardization of the antigen preparations (by SDS-PAGE and immunoblot techniques) secreted/excreted from different developmental stages of *T. spiralis* for serodiagnosis of trichinellosis in swine and humans; the highest specificity was obtained with the antigen from stichocytes.
– Seroprevalence of zoonosis, canine leishmaniosis, in an endemic area of Croatia (Dalmatia) using the DOT ELISA; up to 43% of tested dogs were found to be seropositive.
– Alternative diagnostic antigen isolated from simbiontic flagellate of the insect *Crithidia luciliae,* to commonly used antigen in the DOT ELISA produced *in vitro* from promastigotes which are potentially infectious for humans.

At the Department of Pharmacology and Toxicology, by the end of the 1990s, Frane Božić dealt with immunity to parasites and more recently (since 2000) with the immunopharmacology using a model of exogenous immunomodulation in swine. His research efforts were focused on:

– Analysis of the γδTCR$^+$ i-IEL in the local immunity of mice/swine against experimental invasion with nematode *T. spiralis* and *T. pseudospiralis;* it was the first phenotypic characterization of the IEL in murine small intestine.
– Dose-dependent early recruitment of i-IEL following experimental *T. spiralis* gut infection and their role in resistance to the parasite by accelerating worm expulsion.
– Modulating effect of the immunosuppressive drugs such as dexamethasone on jejunal goblet cells hyperplasia during *T. spiralis* gut infection of mice.
– Immune protection of swine against trichinellosis induced by a vaccine candidate species *T. nativa*.
– Recruitment of intestinal CD45RA$^+$ and CD45RC$^+$ cells induced in weaned pigs by a candidate oral F4 ac$^+$ non-ETEC vaccine against porcine colibacillosis.
– Levamisole as a mucosal adjuvant in synergizing the immunogenicity of a live attenuated F4 ac$^+$ non-ETEC vaccine candidate strain against postweaning diarrheal disease of swine by activating intestinal T cells.

At the 9th Internatinal Congress of the European Association for Veterinary Pharmacology and Toxicology in Lisabon, Portugal in 2003 he was awarded for the best oral presentation with a paper entitled »Levamisole stimulates intestinal T cell-mediated immune responses of weaned pigs to vaccination against colibacillosis«.

At the Department for Poultry Diseases with Clinic research on veterinary immunology issues coincided with its foundation in 1968 by M. Kralj who was already experienced in such issues. In the newly founded Department, he started work with a group of young researchers, among which the most prominent were Hrvoje Mazija and Zdenko Biđin. Their research activities included:

– Specific immunoprophylaxis of ND in hens and evaluation of the immunogenicyty of vaccine candidate strains of ND virus multiplied on FL cell culture.
– Improvement of specific hemagglutination of ND virus by removal of nonspecific inhibitors from pheasant serum.
– Potentiation of innate immunity against ND in pheasant chickens by vaccinating their parents.
– Detection of specific antibodies against numerous avian viroses (such as chicken embryo lethal orphan; CELO-virosis, rheoviral arthritis, infectious bronchitis/laryngotracheitis and avian encephalomyelitis). This was in fact the first finding of the humoral immune response of poultry to these viral infections.
– Development of a novel vaccine against CELO-virosis and improvement of effectiveness of vaccine against ND, infectious bursal disease (IBD) and infectious bronchitis.
– Serological detection of specific antibodies against the virus of egg drop syndrome (EDS′76).

When M. Kralj retired, H. Mazija, Z. Biđin and Estella Prukner Radovčić (complemented by Ph.D. students Ivana Lojkić and Irena Ciglar as well as two young researchers) targeted their research on:

– Immunosuppression of specific immune responses to homologous/heterologous antigens of IBD virus and genetic characterization of the virus.
– Immunosuppressive diseases of turkeys such as astrovirosis and rheovirosis.
– Standardization of the specific protection of some polutry vaccines (particularly ND vaccine) applied by ultrasound aerosolization in laboratory and field conditions.
– Diminishing lentiviral contamination of some live poultry vaccines.
– Serological diagnosis of turkey rhinotracheitis was the first finding of this disease in Croatia.
– Development of serological diagnosis of chlamidophylosis and chlamidiosis in domestic fowl and free-living (wild) birds.
– Novel approach to vaccinology against viral diseases of poultry and wild birds by aerosol vaccination at the hatchery; such an originally developed route of vaccine application has both scientific importance for veterinary immunology (delivery of microparticles of vaccinal immunogen to the respiratory system induces immune protection) and practical value for poultry production.

At the CVI a great deal of scientific research was oriented (directed) to veterinary immunology aspects, particularly those regarding diagnosis, control and prevention of infectious diseases in animals of veterinary importance. With regard to viral vaccines, Mirko Lojkić performed (between the late 1970s and 1990s) intensive research on the preparation and validation of vaccines against porcine (transmissive gastroenteritis, AD, parvovirosis and rotavirosis) and bovine diseases (IBR/infectious pustular vulvovaginitis).

In the past decade, several members of the CVI have dealt with veterinary immunology issues as follows:

– Systematical monitoring of epizootiology and epidemiology of bacterial diseases (Željko Cvetnić), particularly zoonoses, of domestic and wild animals such as brucellosis, leptospirosis, porcine/bovine tuberculosis and Q-fever as well as viral diseases such as BVD, border disease, PCAD and CSF (Lorena Jemeršić) by serological and molecular methods.

– Characterization of phenotypical/functional patterns of cellular immunity and detection of antibody responses in pigs following vaccination with a subunit or attenuated vaccines against CSF were studied by either flow cytometry/lymphocyte stimulation test (LST) or by ELISA (Svjetlana Terzić).
– Evaluation of immunogenicity of inactivated subunit vaccine against parvovirosis of swine (Besi Roić).
– Qualitative/quantitative analyses of porcine CD^+/SWC^+ immune cell subsets in weaned pigs affected by PCAD, and comparison of their cellular immune status with the virus-induced changes in lymphoreticular tissues (Željko Mihaljević).
– Cytometric analyses of bovine leukocyte subsets in secretions from the udder of cows with mastitis (Miroslav Benić).
– Development of immunoenzyme test for detection of antibodies against *Trichinella spp.* in swine (Sanja Bosnić).
– Immunomodulation of humoral immunity in boars by levamisole and potential use of the RIA for detection of the changes in serum IgG levels (Nina Bilandžić).
– Development of serological diagnostic methods, such as inhibition of hemagglutination test for detection of antibodies against ND, EDS'76 and infectious viral bronchitis (IVB; strains M-41, 793/B, D-1466 i D-276), ELISA test for detection of antibodies against IBD, IVB, avian inflenza (in domestic and wild birds), avian encephalomyelitis, turkey rhinotracheitis, swollen head syndrome, rheovirosis, Marek's disease, coccidiosis and infection wirh *Salmonella typhymurium* in order to assess the immune status of the flock.
– Investigations of interferons (IFN-) α and γ in hens exposed to natural or experimental infections with virus of infectious anemia and IBD by mRNA hybridization method (Vladimir Savić).
– Screening of postvaccinal immunity against ND and analysis of immunosuppressive effect of the virus by studies of expression of mRNA for IFN-α, IFN-γ i interleukin (IL)-2 using real-time RT-PCR method as well as a flow cytometric demonstration of phenotypes of lukocyte subsets in whole blood (Mirta Balenović).
– Influence of acute heat stressors on the humoral immune responses of fattening chickens and laying hens of light breeds (Milivoj Mikec).
– Serodiagnosis of infections by bacterial species (*Ornithobacterium rhinotracheale*, *Mycoplasma gallisepticum* i *Mycoplasma synoviae*) or by avian leukosis virus and rheovirus using the ELISA, serum agglutination test and gel immunodiffusion test (Tajana Amšel Zelenika and Irena Lukač Novak).

At the DAP only a part of the research activities of Ivan Bašić *et al.* were oriented towards veterinary immunology issues, but they had outstanding theoretical and practical importance, paticularly in the mentorship and leadership of the pioneer (in the early 1980s) projects dealing with comparative immunology and the improvement of animal health. His contributions to the contemporary knowledge of the immunobiology of species of veterinary importance are as follows:

– Phenotypical and functional characteristics of canine, equine and porcine lymphocytes determined by LST and rosette techniques.
– Diminishing porcine perinatal losses by immunotherapy with allogeneic-immunoglobulins extracted from swine plasma by polyethylene glycol (PEG) sedimentation technique.
– Influence of perorally administered allogeneic immunoglobulins on cellular and humoral immunity of neonatal piglets (in collaboration with I. Valpotić).
– Protection of neonatal piglets against infection with *Leptospira interrogans* sergroup *pomona* by orally administered allogeneic immunoglobulins.
– Uptake and transport of allogeneic immunoglobulins by the small intestine of neonatal piglets as measured with FITC/^{131}I –labelling (in collaboration with Stipica Ćurić).

Full details on the references related to the aforementioned veterinary immunology issues published since 1997 can be found at http://bib.irb.hr searching either by the name of author(s) or by the number of the institution holder of the project. Older references can be provided by the National University Library or by the authors of this article on request.

Future prospectives

Veterinary immunology continues to meet the challenges of the Croatian scientific community in comparative and clinical immunology as well as those of the »rest of the World«, particularly of the EU countries and strict requirements of the Federation of Veterinarians of Europe (FVE). However, only a part of these challenges, which is rather modest although proportional to the relatively small numer of researchers dealing with veterinary immunology, will be met. This should be attained through 14 scientific projects *appertaining to the discipline of veterinary immunology funded by the MSES of Croatia in the period from 2007 to 2011 (project leaders:* I. Bašić; Lj.Bedrica; Z.Biđin; F.Božić; Ž.Cvetnić; J. Madić; A. Marinculić, H. Mazija; V. Mrljak; V. Savić; M. Šperanda; S. Terzić; N. Turk; I. Valpotić)

– Summarizing all available data on these 14 projects (after almost one and half years of their duration) we can say that our future work will continue to focus on:
– Improvement of the control and prevention of infectious animal diseases by performing fundamental and applied immunological research for better understanding of innate or acquired immunity to selected important pathogens (such as avian enteric/respiratory viruses, bovine lymphocytotropic virus, canine protozoans, porcine enteric bacteria and zoonotic bacteria, including leptospires) in order to develop safe and effective means of specific/nonspecific immunization against these organisms and their products, and by addressing the challenges posed by new (PRRS, BSE, PCAD and HPAI) and re-emerging diseases (avian influenza, Lyme borreliosis and leptospirosis) , among which zoonoses are prevalent;

– Improvement of food safety control/prevention measures by developing vaccines against foodborn parasitic nematods and by monitoring their effects as well as the effects of newly introduced drugs (with special regard to their transformation in food-producing animals) on immune parameters in animals;
– Immunostimulation of immune responses to the above mentioned causative agents and, hence, enhancement of resistance to the infectious diseases which they induce, through the use of specific immunoprophylaxis, including vaccine development and delivery, nonspecific immunomodulation by natural and synthetic immune response modifiers (IRMs), adjuvants or nutraceuticals, and immunoresoration with antioxidants.

Regulations on drug usage, particularly the nonclinical use of antibiotics in animals for consumption, have resulted in increased investment in veterinary immunology investigations of basic veterinary species immunology to substantially improve disease prevention and (promote/assist) development of biological products for immunization (such as DNA, edible and mucosal vaccines, natural IRMs and nutraceuticals), primarily, for the benefit of animals but also for humans, in terms of safer foodstuff and advanced biomedical models. A good slogan, »from DNA to the dinner plate«, created by Babiuk *et al. (12)* is apparently summarises the former, and is worth repeating because it is so true. This slogan has by now become an official statement of the FVE. It also stimulates veterinary immunologists for further research in those species and areas of immunology about which little is presently known.

Concluding remarks

Veterinary immunologists need not be reminded that our field is the most important basis of knowledge for the development of technologies to promote healthier animals, safer products and international trade without sanitary barriers. Increased investment within the pharmaceutical industry in new drugs and vaccines is required to provide innovations and to facilitate advances in animal health (including livestock and poultry, companion animals, fish and marine mammals), and the diagnosis and prevention of their diseases, in terms of seafer animal foodstuff and public health. The optimal fulfillment of the objectives of veterinary immunology requires its full integration with basic and human immunology, as well as the flow of information and communication among veterinary immunologists and the rest of the immunological community, irrespective of the species on which they are working.

Acknowledgements: *Gathering this amount of information would not have been possible without the commitment and dedication of a number of contributors from the VF (Frane Božić, Zdenko Biđin, Albert Marinculić, Vladimir Mrljak, Željka Matašin and Maja Popović) and from the CVI (Želko Cvetnić, Vladimir Savić and Svjetlana Terzić). We are greatly indebted to them for their work and willingness to set aside other priorities for this compilation.*

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DEVELOPMENT OF ALLERGOLOGY AND CLINICAL IMMUNOLOGY IN CROATIA

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Historical overview

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Already in 1909 and later with his collaborators in the journal Liječnički vjesnik in 1928, Ernst Mayerhofer brought attention to the importance of allergies in childhood. The presented information pointed that antibodies caused by cow milk allergen were found in 70% of artificially fed infants, and that the so-called toxic exanthema in newborns are of allergical origin and accompanied by leukopenia and eosinophilia.

Ivan Hugo Botteri was among the first physicians worldwide to apply skin test for diagnosing echinococcosis on the basis of allergic skin reaction to the allergen of this parasite.

We should also mention studies by Fran Kogoj who made Croatian dermatovenerology famous in the world where it earned its place in allergology and clinical immunology. His publistic article »On Allergology«, »L'allergie, c'est la vie«, was started in 1926, with significant contributions in the fields of dermamycosis, epidemiology and serology of syphilis, urticaria, strophulus, pruritus, »diagnostics of allergic diseases«, vasculitis, drug allergy, allergodermias, detection of Mljet disease in 1937 and numerous other contributions.

As an internist, Vinko Vuletić indebted Croatian allergology and scientific community by his early studies of diagnostics and therapy of bronchial asthma and pollinosis and by research of breathing in asthmatics, published in the distant 1931–1934 period in the journal Liječnički vjesnik.

Academician F. Kogoj was a competitive organizer and initiator. In 1948, he initiated the establishment of Allergologic Station as part of the Dermatology Department, of an Outpatient Clinic for Occupational Skin Diseases in Zagreb in 1956, of Allergologic Laboratory in Rijeka (hospital »Braća Sobol«) in 1957, and of an Allergologic Ward in Rijeka (hospital »Dr. Kučić«). On academician Kogoj's initiative in 1951, the Yugoslav Academy of Sciences and Arts the Academy organized »Symposium on Allergy« and published the proceedings in 1952. The Symposium was specific by its covering of a number of fields in medicine together with allergology. In addition to F. Kogoj (dermatovenerology), the experimental background of allergology was edited by Petar Sokolić, the endocrine system by Vuk Vrhovac, bronchial asthma by Vinko Vuletić, allergy and digestive tract by Arpad Hahn, tuberculosis by Stanko Ibler, allergy in neurology by Radovan Lopašić, otorhinolaryngology by Srećko Podvinec, stomatology by Ivo Čupar, while Branko Kesić was editor for occupational allergic diseases.

In 1952, again on the initiative by F. Kogoj, Allergological Section of the Croatian Medical Association was founded which gathered several dozen members. F. Kogoj was its first president, and Štefanija Puretić secretary. They successfully managed the Section for a number of years, and F. Kogoj was later elected lifetime honorary president.

A momentum in the development of Croatian allergology was made possible by two important factors. The first one is the fact that production of allergens for testing and desensibilization started in 1956 in the then Serovaccinal Institute (later Institute of Immunology); Neda Keler-Kubelka and Miroslav-Miro Mimica were particularly involved in this process. In May 1959, the so-called »Minor Meeting of the European Academy for Allergology« was held in Belgrade where studies with nationally produced allergens were presented, as well as initial experiences in that field. The second factor of importance for Croatian allergology is the research of airborne pollen, with a pioneering study in that field by Ivo Volarić-Mršić. The investigated area in Croatia was Zagreb municipal area and the island of Hvar, and first results were presented in 1959 at the abovementioned meeting of the European Academy. These were also the most important contributions from Croatia at that meet-

ing. The movement of airborne pollen is monitored today in every modern country, with media-released information to patients. Results of a study in Croatia were published in 1960 in the journal Acta Allergologica and in the European pollen calender. These investigations have lately been performed by Željka Eberhardt-Lovašen with support of several working groups of allergists in Croatia, but such research is based exclusively on enthusiasm of individuals (Nikša Sindik, Karmen Kavuric-Hafner, Branko Čvorišćec and others). Funds are never available to set up such a service, and a blind eye is turned to 50-fold increase in ragweed pollen in the Zagreb area during the 1970–1982 period.

Considering excellent weather conditions, Croatian coast has, like in hardly any other country, very good conditions for asthma treatment with the aid of climatic factors. These favorable conditions were observed long ago so that the 10th anniversary of the Hospital for Children's Diseases was celebrated in 1988 n Veli Lošinj. On May 5–9, 1964, International Symposium on Allergology (Zagreb, Split, Hvar) was organized by the Academy and Allergological Section of the Croatian Medical Association, with the main topic »Climate and Allergy« and participation of the then most famous names in European allergology. At the initiative by F. Kogoj and the Academy president Grga Novak, European Climate Center for Treatment of Allergic Diseases was founded in the island of Hvar, with W.I.O. van Ufford from the Netherlands as one of the chairpersons of the Founding Committee. Other collaborators were R. Surinyach from Barcelona and Mladen Debelić who was later the president of the European Academy of Allergology and Clinical Immunology and who has until the present day done many useful steps for the development of allergology in Croatia. Similar centers were also established in Dubrovnik, Baška Voda, Children's Village near Makarska, in Rab, and in Crikvenica. On October 16–18, 1961, members of Allergological Section organized the »1st Yugoslav Congress of Allergists« in Zagreb. The president of the Organizing Committee was Fran Kogoj, and the Section compiled data for allergological bibliography for the 1913–1961 period with more than 300 titles. Considering its organization and professional level, the congress was very successful. In 1962, the Section organized the »First Professional Meeting of Yugoslav Allergists« in Dubrovnik on the occasion of its 10th anniversary, and Hospital for Allergic Diseases of Respiratory Organs in Lapad participated in organization of the meeting. As physicians in Croatia were showing increasing interest in allergology, a need was present for organized professional education in this field. As part of the 3rd Congress of Croatian Physicians, Miro Mimica organized in 1964 a seminar on »Bases of Clinical Allergology« in »Dr. Josip Kajfeš« hospital and at the Dermatovenerology Clinic in Zagreb. In 1965, a new management board of the Section was elected with Zvonimir Krajina as president. During his absence in 1968–1969, this duty was performed by Sead Midžić, while Neda Keler-Kubelka was elected secretary.

In February 1966, the Section organized a panel discussion entitled »Allergy for a Practitioner« together with the Zagreb subsidiary of the Croatian Medical Association (CMA). In March 1967, the CMA Allergological Section organized the »First Republic Symposium of Allergists of Croatia«.

On the occasion of the 100th anniversary of the Academy, a »Symposium on Respiratory Tract Allergoses« was held in May 1967 in Hvar where Allergological Section members participated and cooperated; they participated with their presentations in all congresses of allergists of Yugoslavia in 1965 and in the 2nd Congress held in 1968 in Sarajevo.

A new Management Committee was elected in 1970. Miro Mimica was elected president, and Ivan Paleček was elected secretary. I. Paleček took up the duty of a vice-president in 1972, and Božica Kanceljak-Macan was elected secretary. The Section was very active at that time. It associated with the Croatian Immunological Society and numerous meetings have been organized together since 1970 even more frequently than once a month. It was the time when association of practitioners and basic researchers was becoming a necessity. New *in vitro* tests were developed whose validity was to be evaluated in practice. Quantitative immunoglobulin determinations were carried out as well as tests of degranulation of basophilic leukocytes and rat mastocytes, lymphoblastic transformation, leukocyte migration inhibition, etc. This collaboration was evident at the 4th Congress of Allergists of Yugoslavia, held in 1972 in Ljubljana, where many Immunological Society members participated.

Allergological Section became a member of the European Academy of Allergology (later) and Clinical Immunology (EAACI) through Yugoslav Association of Allergists, and later also of the International Allergological Society. In 1978, this membership was renewed and in 1996, when Croatian Society of Allergology and Clinical Immunology (CSACI) gained international recognition, the Society became a member of the European Academy of Allergology and Clinical Immunology.

Along with publications at congresses and scientific meetings, a Bulletin of the the Academy Allergological Center was periodically published in Hvar and its editors and active collaborators were mostly members of Allergological Section. In 1973, the Section had 68 members.

It was decided at the major annual meeting held on September 23, 1972 that the headquarters of the Association of Allergists of Yugoslavia was to be in Zagreb. Zvonimir Krajina was elected president, Željko Poljak secretary, Ivan Paleček was elected second secretary, while majority of the management board were members of the Section. In 1974, the 5th scientific meeting of allergists of Yugoslavia was held in Hvar and featured two major topics: Evaluation of allergological tests in vivo and *in vitro*, and: Therapy of allergic diseases of respiratory pathways in children and adults.

In May 1976, the Section organized the 5th Congress of Allergists of Yugoslavia near Šibenik. In line with contemporary trends, it was agreed that the Association and the Section change their names. Since then, the name of the Section was: Section for Allergology and Clinical Immunology (SACI), Croatian Medical Association (CMA).

As interest in allergology was undoubtedly growing, there was a need for organized education in the field. Toward the end of 1972, CMA invited all sections to propose a program of specialist and subspecialist training. Allergological Section of CMA put forward its proposal on February 20, 1973 for subspecialist training in allergology as an interdisciplinary narrow specialty for physicians in internal medicine, dermatovenerology, otorhinolaryngology, opthalmology, pneumophysiology, and occupational medicine, in the duration of one year. As in the former state this issue was not finally resolved based on preliminary programs which have changed in the meantime, SACI gave a considerable contribution to the development of postgraduate study in allergology and clinical immunology at the School of Medicine in Zagreb. The organization of the postgraduate studies was initiated by M. Mimica, with the assistance of I. Paleček, and continued by Vladimir Kolbas. As a faculty member of the Medical School, Željko Poljak managed in 1979 to implement postgraduate teaching in practice. There was a keen interest and 40 candidates applied already in the first year. Postgraduate teaching has continued to the present, and the second year was completed and the title of a master of medical sciences was acquired by 71 postgraduate students.

Electoral meeting of SACI took place in 1974. Vladimir Kolbas was elected president and Renata Lokar secretary. The term of office of the new board lasted until June 1978. At a meeting of the SACI management board held on April, 1976, it was proposed to request at the then forthcoming 5th Congress of Allergists in Šibenik the recognition of the status of a specialist in allergology from the Federal Secretariat for Health and the Confederation of Medical Associations. The Section proposed criteria for such title: minimum 10 years of practice mostly in allergology, at least five scientific papers in the field, and participation in scientific meetings, along with organizational and educational activities. This proposal was accepted at the annual meeting of the Society in Šibenik in 1976, so that the assembly of the Association of Allergists awarded certificates with a title: allergist and clinical immunologist, to twenty colleagues from Croatia at two congresses that followed (in Struga in 1980 and in Igalo in 1984). These colleagues were Croatian experts, not only clinicians but also those from basic professions, and Federal Health Secretariat was not included in preparation of the certificates. Allergology has been rarely left out at congresses of Croatian physicians. The 6th congress of Croatian physicians, held in 1974 on the occasion of the 100th anniversary of CMA, was of particular importance for promotion of interest in allergology and clinical immunology. As part of the Congress, the Section organized a symposium entitled: *In vitro* tests in allergy diagnostics.

A new Section board was elected in June 1978, with Željko Poljak as a president and Drago Buneta as a secretary. During their term of office, SACI organized an important meeting: Seminar on Respiratory Allergoses, in September 1979. Also, during the term of office of this board, six Section meetings with ten topics were organized by the end of November 1980, including also another four organized together with immunological societies and other sections and associations. Thus on March 6, 1979, as many as six sections and societies participated in organizing a lecture by Nikša Allegretti entitled »Information, recognition, natural selection«.

In November 1980, a new board was elected; Branimir Čvorišćec was elected president and Ivan Paleček secretary. The newly elected president was during the past ten years active vice-president and a member of the Section Board, taking the major credit for the organization of Allergologic Service in Croatia according to a program published in the Bulletin of the CMA Medical Academy (12; 34) in 1979. The Section was very active during that period. During the first term of office of the new board, a total of 18 meetings were held until 1984. Among intersectional meetings, we should point out a round-table discussion on malignant melanoma, and discussion on diagnostic procedures in clinical immunology.

Particular attention should be dedicated to the symposium: Standardization of Diagnostic Procedures in Allergology and Clinical Immunology, that was held in October 1983. The symposium gathered more than a hundred participants, and covered the following topics: standardization of nomenclature in immunology, standardization of survey questionnaires in allergology (standardized medical history), standardization of allergic preparations for testing, and standardization of procedures during performance of skin and other tests and tests in vitro. Symposium proceedings were published, edited by B. Čvorišćec, D. Dekaris and I. Paleček. Professors Einarson and Dreborg from Sweden also participated in the symposium.

In the meantime, the activity of Yugoslav Association of Allergists and Clinical Immunologists was improved, and the Section's president and secretary were delegated to the Association management board. Discussions were held on a new act on specialist trainings and organization of scientific meetings and congresses. Presenters at the symposium »New Aspects in Asthma Therapy«, held in 1982 in Herceg Novi, were Zdenko Tudman, Tihomil Beritić and Ivo Bakran. 7th scientific meeting of allergists and clinical immunologists of Yugoslavia was also held in Herceg Novi in September, 1982, with substantial participation of Section members. Members of the Section participated with nine presentations in the congress of pneumophysiologists in Portorose in June, 1983. The 7th congress of allergists and clinical immunologists of Yugoslavia took place in Igalo in October 1984. The Section's task was to organize a round-table discussion on drug allergy, with I. Paleček as a chair-

person. Section members also participated in round-table discussions on occupational diseases and nutritive allergies. It was evident that the needs for allergologic service had increased, and that related diseases were topical. Thus, the number of Section members at the end of 1984 rose to 135. At electoral meeting in December 1984, it was proposed that the secretary's report be published in the journal Liječnički vjesnik, which was subsequently done (1985;107:409). The term of office of the then-governing board was prolonged with minor changes. Ž. Poljak continued to be a delegate in the editorial board of the journal Liječnički vjesnik, and D. Dekaris in the Croatian Immunological Society. The 1st Yugoslav Congress of Immunology was held in Opatija in 1985 where Section members participated as organizers and presenters. Among numerous reports, biological standardization of pollen of the grass Dactylis glomerata using the method of RAST inhibition was for the first time presented at that congress; the credit for that is due to Asja Stipić-Marković, Neva Sket-Janković and others. This was the first step in forming the national standard for biological potential of a certain allergen. As a result of collaboration with the Institute of Immunology, such standardization has hitherto been carried out also for the allergen of the itch mite Dermatophagoides pteronissynus and for pollen of the ragweed Ambrosia artemisiifolia. The ragweed represents a significant medical and epidemiological-ecological issue particularly in new Zagreb suburbs as an example of lack of concern for environment. From October 1984, the headquarters of Yugoslav Association of Allergists and Clinical Immunologists was in Vojvodina. Its 7th scientific meeting took place in Sremska Kamenica in 1986, the main topic was: Therapy of Allergic Conditions, and the speakers were noted for their well-made contemporary reports. The next 8th congress was held in Novi Sad in 1988, and our members were moderators in the following main topics and round-table discussions: D. Dekaris: Allergological – immunological diagnostics, B. Čvorišćec: Standardization of allergens, I. Paleček: Pseudoallergic reactions. During the board's continued term of office, permanent collaboration was established with the Austrian Society of Allergology and Immunology. Annual meeting of SACI was held on December 21, 1988; Drago Buneta was elected president and Jasna Lipozenčić secretary.

A symposium »Clinical Colloquium« was organized as a novelty in SACI activities. A hundred and twenty Society members took part in the Colloquium and accompanying social events, and professor Mladen Debelić, a Croatian allergist from Germany was a guest scientist at the meeting.

In addition to the colloquium, SACI organized in October 1992 a celebration of the 40th anniversary of Allergological Section »Allergologia croatica 1952–1992«. Plenary lectures on leading figures in allergology paid on that occasion full tribute to academician Kogoj and professor Mayerhofer, and also reported on the following: current state of scientific and professional activities in allergology and clinical immunology, modern basic immunological studies in Croatia, reviews of the most recent investigations of some important hypotheses of immunology and related professions worldwide. A number of reports from that meeting were published in Proceedings of the Croatian Academy of Sciences and Arts, Medical science, vol. XXVII, Zagreb, 1944:466.

A symposium »Clinical Immunology in Croatia« was held under the patronage of the Croatian Academy of Sciences and Arts and in collaboration and with Croatian Immunological Society and SACI on February 21–22, 1989, and a monograph was published in 1990 (D. Dekaris and F. Čulo, editors) with comments on the state-of-the-art of the field in Croatia and the world. A hundred and twenty authors participated.

At electoral meeting of the Croatian Society of Allergology and Clinical Immunology (CSACI) in February 1993, Ivo Bakran was elected president and Mirna Sentić secretary by 132 members. Twelve professional meetings were held during their term of office, as well as a one-day professional conference »First Spring Meeting« on April 1994, and they included the following topics: pathophysiological mechanisms of immunotherapy, assessment of immunotherapy, allergic preparations, methods of hyposensibilization, indications and contraindications for specific immunotherapy, monitoring immunotherapy effects, cost-benefit of immunotherapy, rush-immunotherapy of contact allergy, decline in CD23+B cells following »rush-immunotherapy«, specific immunotherapy of hay fever in children and others. Upon request by the Ministry of Health, diagnostic and therapeutic algorithms were prepared, as well as the proposal of subspecialist training in clinical immunology (Nada Čikeš, B. Čvorišćec, D. Dekaris). A young investigator award was established in 1993.

In February 1994, CSACI became member of the European Academy of Allergology and Clinical Immunology (EAACI). During that year, CSACI members participated in meetings in Rotterdam, Stockholm, Nice, Graz and Bled.

At the CSACI electoral meeting in February 1995, Jasna Lipozenčić was elected president, Asja Stipić-Marković secretary, B. Čvorišćec was elected EAACI delegate, and Nada Čikeš a delegate in the editorial board of the journal Liječnički vjesnik. Professional activity was marked by participation of foreign guests from the USA J. G. Brooks and R. Klein, then Kapsenberg (Holland), Drachenberg (Germany), Wheeler (England), M. Debelić (Germany), J. Streilen (Boston), J.E. Stein-Streilen (Boston), and Jacobsen (Denmark). The program of CSACI activities included International Clinical Colloquium »Topical issues in clinical and experimental allergology« under the auspices of CASA, in May 1996 with a total of 45 authors with reports on the following topics: Epidemiology and prevention of allergic diseases, Allergic skin diseases, Therapy of allergic diseases. All abstracts were published in the Colloquium proceedings. In the 1995–1997 period preparatory activities were carried out and

documentation was submitted for membership in the International Association of Allergology and Clinical Immunology (IAACI) which was realized in July 1997. For the first time in the history of the Society, eight issues of Vjesnik, an informative CSACI bulletin, were published during that period.

Electoral meeting of CSACI in February 1997 resulted in election of Nada Čikeš for president, Jasna Lipozenčić for vice-president, and Mirna Sentić for secretary, with the same management board as in the previous term of office. With regard to further activities, CSACI succeeded in recognition of allergists and clinical immunologists by Croatian Ministry of Health, and was actively involved in postgraduate studies in allergology and clinical immunology. New CSACI leadership was elected in 2004 at electoral meeting: Asja Stipić Marković, was elected president, and Mirna Sentić was elected vice-president.

Under the auspices of the Zagreb Municipal Office for Health, Labor and Social Welfare and CMA, annual meeting of CSACI was held on September 25, 2004. Also, a pollen measurement station was established on the roof of the Dubrovnik general hospital.

In the same year, we hosted the GA2LEN/EAACI Summer School. In the opinion of Professor Cauwenberg, this summer school was to serve as a standard for all future EAACI schools, which can be read in a letter recently forwarded to all our participants together with a CD containing snapshots of our activities. EAACI Newsletter featured a report on our School.

From other activities organized under the auspices of the Society during past period, we had a symposium on application of intravenous immunoglobulins. Annual CSACI meeting was held in September 2005. In the same year, a continuous medical education course of the 1st category was held under CSACI auspices: »Determination of ventilation disorders by portable spyrometer«.

In 2007, Croatian Society of Allergology and Clinical Immunology in collaboration with Zagreb University Medical School, Ministry of Health of the Republic of Croatia and Croatian Medical Chamber developed specific core curriculum, educational and training programs for allergy specialists, according to recomendations of the Section of Allergology and Clinical Immunology of the Union of European Medical Specialists (UEMS). Treatment of allergic diseases in Croatia was until nowadays in competence of various medical professionals, like specialists in internal medicine, pediatricians, ear, nose and throat (ENT) specialists, pulmologists and dermatologists who collaborated through clinical praxis, scientific meetings and educational activities. Organ-based specialists will continue to play an important role in the medical care of allergic patients but a core service of care will be provided by allergists with training based on internal medicine and/or pediatrics. Logbook for evaluation of competence and progression in the learning process will be required to qualify as an allergy specialist.

Croatian Society of Allergology and Clinical Immunology and Institute for Medical Research and Occupational Health organized a scientific conference »Immunological Reactions to Drugs« in April 2006. Eleven speakers covered many topics important in continuing education of practising allergists.

On December 2007, Croatian Society of Allergology and Clinical Immunology organized scientific symposium »IgE-mediated reactions between inhalant allergens and their homologs in food«. The topics covered were: profile of sensibilization in different European regions, pathophysiological mechanisms of gastrointestinal system sensibilization, role of T-like receptor in inflammatory bowel disease, inhibition of IgE binding by homolog allergens, homology of allergens from inhalant and nutritive sources. A book of symposium abstracts was published.

In May 2008, Croatian Society of Allergology and Clinical Immunology in collaboration with Croatian Immunological Society organized a scientific symposium »New facts in pathopysiology of allergic diseases« in Zagreb. The meeting was realization of the long-standing tendency to unify the professional work of basic and practising immunologists. The topics covered by fourteen speakers included: role of T-reg cells in allergic diseases, interaction of virus infection and allergic disease, basophil activation test, recombinant allergens, bio-chip in allergy diagnostics, seasonal appearance of Cladosporium, Penicillium and Alternaria in the city of Zagreb and Medvednica mountain, new facts in pathophysiology of asthma and COPD, news in atopic eczema, rationale for preventive strategies, diagnostic possibilities in drug allergy, comparison between sublingual and conventional immunotherapy, new clinical data about antihistamine pharmacology and the role of biological drugs in immune diseases. Extended abstracts were published in the book of abstracts.

CROATIAN IMMUNOLOGICAL SOCIETY

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Foundation

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All my endeavors to find original Cinader's letter in the archives of the Croatian Immunological Society have been unsuccessful. Therefore at least part of this text will depend on my memories which are certainly not an unquestionable category. Thus, according to my recollection, I received a letter in the distant year 1967 from B. Cinader with a request to inquire into a possibility of founding an immunological society in the then Yugoslavia. Further intention was that several national societies unite into International Union of Immunological Societies (IUIS). With this letter, I went to talk to Dr. Boris Nakić. We immediately agreed that founding such a society would be excellent for promoting immunology and international connections. We thought that we should offer Dr. B. Janković in Belgrade who, as we knew, was engaged in immunological studies, to found Yugoslav Immunological Society together with him and his associates. Nakić made contact with Janković and in a few following days we agreed on a meeting in Belgrade where we we would discuss it and found a society.

Our delegation went to Belgrade (since Janković was engaged in immunology longer than us) for the final agreement. I don't remember if there was anybody else from Zagreb apart from Nakić and me. The agreement was reached and the society was founded on December 17, 1968, with its seat in Zagreb. The first president was B. Nakić, B. Janković was vice-president, and I was elected secretary. The society was entitled Yugoslav Immunological Society (YIS).

We also agreed that the head office was to shift every two years. According to what I remember, the meeting was held in a pleasant and constructive atmosphere.

Accordingly, YIS head office transferred to Belgrade after two years. The following president was B. Janković, I was elected vice-president, and the secretary was M. Simić (he was after some time replaced by K. Isaković).

The headquarters of YIS in the third term of office were again in Zagreb. I became a president, and M. Jurin was a secretary. Our term of office lasted somewhat longer than two years since at its end it was necessary to implement the transformation of YIS into Federation of Yugoslav Immunological Societies (FYIS). In agreement with colleagues from Belgrade and Ljubljana, we carried out all preparations, but FYIS was not founded until 1975. I was then already gone to a one-year stay in Boston, USA. Decision to found FYIS was made in due time by ballots but adjusting the text of the Statute took very long time.

Our relatively fast response in founding YIS was rewarded: YIS was one of the 10 founders of the International Union of Immunological Societies (IUIS); these were: American Association of Immunologists, British Society of Immunology, Canadian Society for Immunology, Dutch Society for Immunology, Gesellschaft fur Immunologie, Israel Immunological Society, Polish Society of Immunology, Scandinavian Society of Immunology, Societe Française d' Immunologie, Yugoslav Immunological Society. The Union was founded on May 5, 1969 at a meeting in Brugge (Belgium). An Interim Council was elected but I don't remember who was on it (whether B. Nakić or myself). The Statute was confirmed at the first Annual Assembly in Washington on June 30, 1972. The first Council was then also elected according to the Statute for the 1971–1974 period. B. Janković represented YIS in the Council. B. Cinader was elected president of IUIS, J. Humphrey was vice-president, and A. de Weck a secretary. I was elected a member of the Symposium Committee because the First International IUIS Symposium was held in Rovinj in May 1971. IUIS objectives were: 1) organize international cooperation between immunologists and promote collaboration between different immunological and related disciplines; 2) encourage cooperation of all societies within a country that represent interests of immunology; 3) contribute to the advancement of immunology in general. According to assessment at that time, there were about 7,000 immunologists worldwide.

Soon after the foundation of IUIS, European immunologists carried out a sort of a coup in this Union (the Frenchman A. Bussard was particularly active in it) because they wanted to establish a federation of European immunological societies. IUIS Commission for Europe was established with this aim. The chairman was J. Humphrey, and I was elected one of the three vice-chairmen. As you know, the Federation was eventually founded (European Federation of Immunological Societies, EFIS). Three events marked my participation in the activity of YIS and my presidential term of office: a symposium in Rovinj positively, and negatively a conflict with B. Janković about election of YIS scholarsip holder and that with M. Simić on election of YIS vice-president.

The symposium in Rovinj was the first one organized by the IUIS Symposium Committee. Actually, it was organized by D. Rukavina, O. Springer and me, including also Saša (I don't recall his last name) from Generalturist Travel Agency. It was a real adventure. One of the most dangerous parts was transport by hydrofoil boat from Venice when a number of Israeli participants did not have the entry visa. We literally smuggled them under the cover of the dark. They had problems when leaving the country but it all ended well. There were a number of things that particularly colored my life as the principal organizer: everyday trifles in a newly opened Eden Hotel as, *e.g.*, the fact that the staff did not manage to wake up a lecturer who arrived from the USA. The man in question simply overslept and missed his lecture. Invariable trifle was also the fact that electricity was out every day around 10 o'clock. There were also some funny things: for instance, when van Rood came running barefoot to have his lecture, holding sandals and still wet swimming trunks in his hands.

A hundred and fifty participants from 22 countries took part in the Symposium. Participants from our Society and the English one were most numerous. I'll state here some of the most famous names, without any particular order: E. Diener, M. Simonsen, G. Biozzi, G. Voisin, K. Rajewsky, B. Askonas, J. Batchelor, D. Dresser, H, Festenstein, J. G. Howard, G.E. Roelants, I.M. Roitt, J. J. van Rood, M. Sela, G. Petranyi, M. Colnaghi, F. Spreafico, E. Thorsby, A. A. Coutinho, E. Moller, H. Wigzell, J. Dausset, K.T. Brunner, J-C. Cerrottini, F. Bach, G. Houghton, H.O. McDevitt, H.O. Sjogren, R.L. Walford, and about 23 members of our Society. According to everything we know, it was a successful meeting, which was also confirmed by Brigite Askonas, the president of the IUIS Symposium Committee; she wrote to me: »I should like to thank you for making the first meeting of the International Union Symposium Committee such a success...«. The conflict with the late B. Janković broke out because he insisted that the list of 10 potential Yugoslav grant holders (I think that grants for participation in the World Congress of Immunology were in question) be made in the manner that five of them were from Serbia and five from Croatia. Seemingly, according to a fair principle. However, it should be clear that at that time (the beginning of 1974) Croatia had better candidates. Thus, I remember, for example, that Blanka Veselić, who achieved PhD and had several papers in journals worldwide, had the same rank in the list as D. Marković who was a novice in immunology (but was a relative of D. Marković, a leading Serbian politician). After exchanging a few sharp-worded letters with Janković, the things settled down. I don't remember if any of our candidates eventually obtained the grant. Luckily, I managed to avoid growing a gastric ulcer. Besides myself, Mislav Jurin (secretary) and Oskar Springer (member of the YIS Board) were also subjected to hardships due to this conflict.

The conflict with Simić occurred because I proposed Katarina Isaković for vice-president rather than him. Simić was especially hurt by this proposal as I was at that time in continuous conflict with a group led by Janković. The main reason for my proposal which was, of course, put forward after consultations, was that Mirko Simić, when he was YIS secretary, was not very successful (as stated by Janković!) and was eventually replaced by K. Isaković who was YIS treasurer. Indeed, I received an open letter and answered. All this was also soon forgotten, but it cost me nerves. The entire conflict was later forgotten and M. Simić and I had good collaboration.

Everything else was rather routine during my term of office, if a normal activity of a society which was active in the area of the entire former state could be described by this category. This routine included organization of our regular YIS symposia. After my term of office as a president, the Yugoslav Immunological Society ceased to exist, and histories of independent societies in Croatia, Slovenia and Serbia began. Although we were at the time content with such developments, we were of course not in our dreams able to assume that Yugoslavia was finally to break apart and that we were to break the otherwise fragile cooperation with Belgrade immunologists, protesting against their silence during aggression on Croatia.

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Past presidents personal recollections

MILIVOJ BORANIĆ, ANDRIJA KAŠTELAN, IVAN BAŠIĆ, BRANKO VITALE, FILIP ČULO, RENATA MAŽURAN, SABINA RABATIĆ, TANJA MAROTTI, STIPAN JONJIĆ and SABINA RABATIĆ

1971–1976 Milivoj Boranić

Croatian Immunological Society had been a section of the federal Yugoslav Immunological Society until April 11, 1974 when it registered as a republic society under the Federation of Yugoslav Immunological Societies. Actually, political developments after dramatic year 1971 ended in 1974 with the enactment of a confederate constitution of the former joint state, so that professional societies were also subjected to confederate transformation. Thus, it happened that I began my term of office in 1971 as a president of the Section and ended it in 1974 as a president of the Society. Thus, I was discharging this duty for five years, i.e. two years in Section and two in the Society, including also a one-year interregnum period of administrative re-registration.

To refresh the memories of older Society members and evoke to some extent the spirit of the time to younger members, I will indicate basic determinants of the sociopolitical and economic environment in which we lived and worked. After the breakdown of the Croatian national movement (during which, fortunately, none of the prominent immunologists fell victim, although some exposed themselves), radical social changes took place. They ushered a period of self-management in the middle of the 1970s. The then social structures (enterprises, institutions, associations) were split into basic organizations of associated labor which were, as elementary cells, entrusted with management of social assets. All business and professional problems were discussed on Worker's Councils and Workers' Meetings. Self-management was also present in science, culture and art. All employees, that is, not only researchers but also technical and ancillary staff discussed personally and through their delegates in Workers' Councils and decided on all activities of the collective, including work plans, equipment provision, employment policy, participation in scientific meetings... Scientific activities were in a such social framework financed and directed through Self-management Interest Communities which were composed of delegates from research basic organizations of associated labor and organizations involved in material production or service sector. From today's perspective, this period appears to be unusual (some politicians call it unnatural), but this was the environment where we lived, worked and created. It was also the golden period of the Croatian immunology – but we'll talk about it later. We may add at this point that the overall economic power of the country increased in the 1970s, which was also evident in science. The country's non-alignment policy and improvement of relations with Western developed countries allowed easier and freer than before travel abroad to scientific meetings and professional education. The gap between developed countries in personal and professional standards decreased. In short, life was somewhat better and laboratories were better equipped in the 1970s than in the 1960s.

With regard to political events after stormy year 1971, it is interesting to note that there was much discussion during registration of the society about whether it should be called Croatian Immunological Society or Society of Immunologists of Croatia. In fact, after the breakdown of the Croatian national movement, general political climate was such that names with national adjective bore negative connotation and were not benevolently regarded by official and administrative institutions. Therefore, the latter name would be more appropriate to the time and circumstances. In addition, colleagues from Slovenia (who were not numerous enough to found their own society) were also active within the then Croatian Section of the Federal Society of Immunology, so it wasn't quite clear how they would stand if the title of the society emphasized that it was Croatian. Nevertheless, we decided for the former, inopportune variant of the name. We explained our attitude (for official, i.e. administrative use) by the statement that foundation of national immunological societies – Croatian, Serbian and predictably of others, provides the

basis for federal society which was to be a supernational association of legally equal subjects. As it is currently desirable to emphasize national awareness at the time when it was extirpated, we could have a reason for complacency in this regard.

At the beginning of the 1970s, i.e. at the time when I was conferred the honorable duty of the first president of the Society, scientific and professional activities of its members were at an enviable level. Among us, there were still the founders of the Croatian immunology Borislav Nakić and Nikša Allegretti, and their peer Veljko Stanković. Prominent names of Croatian immunology: academicians Dragan Dekaris, Andrija Kaštelan and Vlatko Silobrčić, and the late Šime Vlahović, were agile 35- or 40-year-old scientists creative at their best and acquiring scientific and teaching degrees, and building their reputation in national and international circles. The similar was also true of other members of that generation of immunologists (I. Hršak, M. Jurin, L. Milas, M. Slijepčević). Republic awards for scientific achievements were at that period granted to Šime Vlahović (1972), Dragan Dekaris (1973), Vlatko Silobrčić (1974), Ivo Hršak (1975) and Andrija Kaštelan (1976). There were other awards as well, for example the Zagreb Municipal Award was in 1973 conferred to Milivoj Boranić and Andrija Kaštelan and collaborators.

Many Society members received invitations and support to participate in eminent international scientific meetings like the 1st International Congress of Immunology in Washington (1971), 4th International Congress of Transplantation Society in Washington (1972), and 4th Congress of the International Society for Experimental Hematology in Paris (1974). They were also on short or prolonged study stays abroad.

The second generation of Croatian immunologists at that period was completing their postgraduate studies and acquiring master's and PhD degrees. I will name only those where I was a member of Thesis Evaluation- or Defense Committees: I. Andreis, F. Čulo, J. Gabrilovac, M. Poljak-Blaži, M. Radačić, D. Volf. I apologize but I don't have exact information on others.

The journal *Periodicum biologorum* was accepted as the official paper of the Society. Valuable manuscripts in immunology and related fields were published in this journal, which certainly contributed to its inclusion in the Current Contents database. At the beginning of the 1970s the Society regularly (once or twice a month) held scientific meetings where members presented most recent results, often using only a blackboard and a chalk. Discussions were lively and argumented. Immunological terminology was being harmonized, and many terms adopted that are presently used as common good in the language. Joint meetings with Allergological Section, Croatian Medical Association, were organized, and occasionally also with other sections of this Association. In this manner, professional collaboration was fostered with colleagues who dealt with practical aspects of immunology.

To illustrate this, I will outline, based on my notes, a discussion at the annual assembly of the Society in July 1971. The discussion was about collaboration with clinicians, teaching activity, organization of professional meetings and criteria for admission of new Society members. It was proposed that the Society recommended establishment of a unique hemodialysis-transplantation center for entire Croatia, and the need was emphasized for the development of laboratory diagnostics of allergic and autoimmune diseases. A necessity for a modern textbook of immunology was observed. It was agreed that joint meetings of Zagreb and Rijeka immunologists were to be held from time to time.

The Society organized three meetings of Yugoslav immunologists: the 4th Symposium on Immunology and Transplantation as part of the 8th Congress of the Yugoslav Physiological Society in 1973 in Opatija (Š. Vlahović), immunology section during the 9th Congress of Yugoslav Physiological Society in Portorož in 1975 (M. Jurin, V. Silobrčić, S. Banič), and the 5th Symposium of Immunologists from the Socialist Federative Republic of Yugoslavia in Stubičke Toplice in 1976. (M. Jurin). In 1975, the 5th Congress of the International Society of Experimental Hematology was held in Trogir (M. Boranić). This international meeting remained in memory of many participants.

It is evident that I was fortunate to be the president of the Croatian Section of Immunological Society of the former Yugoslavia and the first president of the Croatian Immunological Society during a very dynamic and vital period of Croatian immunology. It was at the same time a rich period in my life. At the age of thirty-four, I was appointed in 1970 the head of the then Division of Biology, Ruđer Bošković Institute, and soon elected a president of the Institute's Managing Board. Thus, I found myself in the middle of turbulent developments related to self-management transformation of the Institute. Biology Division changed its name into Division, and then into Basic organization of associated labor for experimental biology and medicine, and I was elected the first director. I exercised this function until 1977. I may say without hesitation that biomedical studies acquired their status at the Institute, which was (also) expressed in the changed official name of the Division, and one of the major directions of scientific research was immunology. Although immunologic studies at the Institute later lost its momentum, some others were initiated and replaced them as, for instance, molecular biology, so that biomedicine is one of the cornerstones of scientific research at this largest Croatian scientific institution. Apart from administrative duties, I was a coordinator of scientific projects, promoter of collaboration with foreign scientific institutions – US National Cancer Institute and European Organization for Cancer Research, leader of collaboration with clinical institutions in Zagreb, a supervisor of

several young collaborators, a member of the editorial board of three international and one national journal, organizer of the 25th anniversary of the Institute and occasional exhibition » A Meeting of Science and Arts«, organizer of the congress of International Society for Experimental Hematology, sub-editor and translator of a major textbook in physiology... In addition, I published a large number of scientific and professional papers and reviews, including also texts in the media on social role and position of scientific research. In brief, my scientific and professional activities were not confined (only) to the field of immunology. Actually, many other members of the Society were also not engaged exclusively in immunology but also in related fields of biomedicine.

Through the written word, social engagement and professional activity, I attempted to assert the attitude that scientific and research work should not be solely fundamental, but that it should be rather desirable to see the possible application of results in closer or more distant perspective. I also worked hard for recognition of the dignity of scientific and professional papers that were published in Croatian language in national professional journals. Such views were (and it seems that they still are) rather at variance with the attitudes of the then scientific establishment, including also immunological circles. In my opinion, the developments in science which we have witnessed still support the principles which I advocated. Thus, for example, our work on experimental problems and model systems in immunology has paved the way for acceptance of new findings and employment of new methods and skills in clinical and laboratory practice regardless of the principles and motives that were proclaimed and promoted.

1976–1978 Andrija Kaštelan

I took over the term of office of the Croatian Immunological Society president from Dr M. Boranić on June 16, 1976 and was succeeded by Dr I. Bašić since November 15, November 1978. Due to the then general confederalist spirit (Croatian national movement in 1971 and Constitution from 1974) and to some extent related to the mandate, Federation of Yugoslav Immunological Societies was founded only a short time before in Stubičke Toplice (February 1976); at that time the Federation consisted of two republic societies, i.e. the Croatian and the Serbian (they were subsequently joined by the Slovenian Immunological Society). On insistance of the Croatian delegation, which I headed, the principle »one society, one vote« was introduced in the Federation Statute with the intention of ensuring acceptable autonomy of the Croatian Society. The Federation was founded on the occasion of the 5th Symposium of the then Yugoslav Immunological Society where Croatian representatives presented 75 of the total of 106 abstracts. It may be interesting to mention the areas of research that were topical at that period and which have not essentially change in the following terms of offices. These were as follows: mechanisms of immunological reaction, autoimmunity, allergic reactions, histocompatibility and organ transplantation, immunologic functions of T-lymphocytes, lymphocyte membrane markers, immunology and immunotherapy of tumor, immunostimulation and immunosuppression, HLA and diseases, immunology of reproduction, etc. One of the characteristics of that period was a strong surge of experimental immunology into clinical professions and lively collaboration with fellow clinicians. This is, among other things, evident also from congress and symposium abstracts from that time. I will mention some meetings that will depict the clinical professions with which we collaborated at the time: Meeting of Yugoslav Allergologists and Immunologists, Ohrid, 1978 (allergology); Symposium on Kidney Dialysis and Transplantation, Bled, 1978 (dialysis and transplantation); 3rd Congress of Yugoslav Hematologists and Transfusiologists, Sarajevo, 1978 (hematology and transfusiology); 1st Yugostransplant Congress, Opatija, 1978 (transplantation organization issues); Symposium on Ankyloses and Kyphoses, Zagreb, 1977 (orthopedics); 1st Scientific Meeting of Yugoslav Nephrologists, Struga, 1977 (nephrology); 9th Meeting of Croatian Pediatricians, Stubičke Toplice, 1978 (pediatrics), etc. Two international meetings were also held at that time: 4th European Immunology Meeting, Budapest, 1978, where Croatian immunologists presented 20 abstracts, and International Intertransplant Workshop, Berlin, 1977.

Some events from that period seem to be of particular relevance, at least from my point of view, so I am going to sketch them briefly below.

In 1977, Rebro Tissue Typing Center (founded in 1970) met all stringent criteria and for the first time participated, together with 150 laboratories worldwide, in a highly controlled experiment which eventually resulted in detection of HLA-DR locus. It may be interesting to mention that these 150 laboratories were classified into 22 groups. The Zagreb laboratory was active in group France 1 (rather than in group Eastern Europe). During two years (1976/1977), all laboratories worked together and used the same techniques and the same typing sera. A total of 13,000 subjects were typed with over 400 anti HLA sera, while cellular techniques were used on 2,000 subjects. In addition to 7 new antigens at DR locus (DRW1-DRW7), another twenty new HLA specificities were detected at previously known loci, i.e. HLA – A, B and C. Also, 28 different diseases were investigated in terms of defining the role of HLA antigen in their etiopathogenesis. Comprehensive results of the

7th International Workshop (Oxford, 1977) were impressive and provided the basis for further studies in the field. Since then, the Zagreb Tissue Typing Center took part in all histocompatibility workshops (there were five more of them), which provided it with international and national reference in the fields of histocompatibility and clinical transplantation of organs and bone marrow. Regarding the Registry, we have had a continuous support of many laboratories and institutions worldwide, particularly of Francetransplant and J. Dausset, and Eurotransplant and J.J. van Rood. We made requital, to some extent, to J. Dausset by proclaiming him a member (1978) of the then Yugoslav, and presently Croatian Academy of Sciences and Arts, and by awarding him a honorary PhD degree of the University of Zagreb (in year 1986). We are still bound to make recompense to J. J. van Rood.

In October 1977, a Symposium on the need and possibilities of bone marrow transplantation was held in Stubičke Toplice. Among 19 participants, there were four immunologists: M. Boranić, A, Kaštelan, V. Silobrčić and B. Vitale. The meeting was supposed to provide an answer to the following questions: a) Is there a need in Croatia for clinical transplantation of bone marrow? and b) Are there experts and technical possibilities necessary to carry out such a complex task? Nineteen presentations were held. (see: Liječ Vjesn 1978;100:376–96). After two-day discussion that encompassed all aspects of the problem area – from epidemiological, tissue typing (donor selection), immunological, clinical-diagnostic-therapeutical, economic-material, to ethical and legal aspects, a conclusion was reached stating that all preconditions have been met in Croatia to organize such a program, but also that additional funding is necessary, particularly to establish and equip a transplantation center and some other laboratories. A commission for implementation of the bone marrow transplantation program was established during 1978 in the Zagreb Clinical Hospital Center where Tissue Typing Center had already been established and furnished up-to-date (the selection of a histocompatible donor is the basic requirement for successful bone marrow transplantation), with already several years of successful kidney transplantation program (experience in immunosuppression). Expert support (education of hematologists) was provided by Paris Transplantation Center (J. Dausset), and financial support was ensured by Croatian electric power industry as part of the project on prevention of lethal irradiation at Krško nuclear power plant. Preparations lasted until 1983 when the first bone marrow transplantation was performed in Croatia; so far 350 transplantations have been performed, 338 from related donors and 12 from unrelated donors from international registries.

In 1977, five or six years had already passed from the first kidney transplantation (from a live relative) (Rijeka, 1971, Zagreb, 1973). Yugotransplant had been founded before this event (in year 1974) for the purpose of organ exchange and transplantation from deceased persons according to the principles of histocompatibility. Zagreb Tissue Typing Center became the Referral Center for Yugotransplant. With support from the Croatian Immunological Society, this Center organized the 1st Workshop on Histocompatibility (February-March 1975) with the aim to establish a battery of typing sera for Yugotransplant. Based on this battery, the first Yugotransplant Waiting List for cadaveric kidney transplantation was announced. The List included 218 patients from 13 dialytic centers in the former state (7 centers were from Croatia). The List was set up in the same manner as the battery of tissue typing sera, i.e. it was based on our own applications on the then electronic computer of the University Computing Center (UCC), and the terminal in the Referral Center which was at that time the only terminal dislocated from the principal UCC computer. I remember that two patients from that List obtained their kidneys from abroad, one from Chicago (delivered on perfusion instrument) and another from Manchester (also on perfusion instrument). The List was, more or less regularly, being announced until 1985 when it was reduced to the Patient Waiting List only for patients from Croatian dialytic centers (there were 18 such centers at that time with a total of 847 patients on dialysis, and with 309 of them on the Waiting List for transplantation). Currently the List includes approximately 1,000 patients, and the number of transplanted kidneys has, on average, been 50 per year. However, let us return to year 1977. Despite comprehensive organizational and other enterprises (Yugotransplant, Waiting List, etc.), the program of cadaveric kidney transplantation was at the time making poor progress, that is, the number of transplanted organs was much lower than required. In professional circles, attempts were made to account for such state by unresolved ethical and legal questions, or by absence of a national transplantation law which should define cerebral death and regulate the collection and transplantation of an organ from a deceased individual. Therefore, the then Republic Health Secretariat set up (in 1977) a Commission that was to prepare a proposal of an act on transplantation of human body parts for therapy purposes (an immunologist was also a member of this Commission). After almost two year activity of the Commission, the Croatian parliament was finally, in year 1980, to enact a Law on collection and transplantation of human body parts for therapeutic purposes (Official Gazette of the Republic of Croatia, No. 31/1980, and No. 40/1988) which is currently still valid and follows the most exact laws on organ transplantation.

Finally, to sum up all that was said above but also much of what was not said but refers to my term of office during that time, I think that the 1980s were enthusiastic years of the Croatian Immunological Society.

1978–1981 Ivan Bašić

Two fundamental activities of the Croatian Immunological Society marked the terms of office of this Management Board:

a) Celebration of the 60th anniversary of academician Nikša Allegretti

b) Symposium on the occasion of the 25th anniversary of Transplantation Immunology in Croatia, dedicated to the memory of the pioneer of transplantation immunology in Croatia Professor Borislav Nakić, PhD.

Both of these activities were registered in publications in Periodicum biologorum (1980;82(2):51–217) and in Liječnički vjesnik (1982;104(3–4):133–161), and I enclose excerpts from these journals:

a) An excerpt from the preface to scientific papers published in Periodicum biologorum, Vol. 82, No.2, which was written by the editor-in-chief, academician Vlatko Silobrčić:

Nikša Allegretti – on the occasion of his 60th birthday

This issue of Poeriodicum biologorum is devoted to NIKŠA ALLEGRETTI, on the occasion of his 60th birthday. In preparation of the issue, we have collaborated with the Croatian Immunological Society and the Croatian Physiological Society. Together we invited several friends, colleagues and students of N. Allegretti, from Zagreb and from S.R. Croatia, to contribute to this issue a paper on the subject of their choice. In answer to our letters, we received 34 papers. The papers have been reviewed by N. Allegretti and myself. They all have one thing in common: they are sincere attempts on the part of their authors to show appreciation for Academician Allegretti and his activities.

V. Silobrčić Comment: Twenty manuscripts were in this issue of Periodicum biologorum (CC indexed journal) submitted by the Society members. Other papers were contributions by members of the Croatian Physiological Society. With regard to this celebration, a formal session of Croatian Immunological Society was held where a plaque of the Society was awarded to Academician N. Allegretti.

b) An excerpt from Liječnički vjesnik (1982;104(3–4):133–147) on the occasion of organizing a symposium dedicated to Professor Borislav Nakić.

Symposium on the occasion of the 25th Anniversary of Transplantation Immunology in Croatia – Beginnings of Transplantation Immunology in Yugoslavia dedicated to Borislav Nakić, held in Zagreb on December 18, 1980. Organizer: CROATIAN IMMUNOLOGICAL SOCIETY

This supplement of Liječnički vjesnik contains presentations from the Symposium held on the occasion of the 25th Anniversary of the development of transplantation immunology in Croatia which was organized by the Croatian Immunological Society and dedicated to the pioneer of this scientific discipline in Croatia, the late Borislav Nakić. In terms of contents, the Symposium consisted of two parts: the first was dedicated to the development and significance of transplantation immunology worldwide and in Croatia, including the participation of Boris Nakić and his contribution to foundation of transplantation immunology in Croatia. The second part was about clinical bone marrow transplantation and the possibilities for its implementation in Croatia. Presentations at the Symposium aroused considerable interest, and ensuing discussions and conclusions elucidated many of the difficulties occurring in investigations in basic immunological science and its clinical application.

The Symposium introduction was given by Ivo Bašić who began with the following citation:

»The (immune) system must always be on a 24 hour alert to combat foreign instructions as well as disloyalty from within... One of the chief functions of the system is to maintain a series of checks and counterchecks against inappropriate action by its own agents.« (F.M. Burnett).

The Croatian Immunological Society dedicates this silver anniversary of transplantation immunology to the memory of Professor Borislav Nakić who made a step 25 years ago in a new path whose route began to loom in the 1950s. Today, this route is a modern way of transplantation immunology with lanes full of vehicles loaded with knowledge and information on tasks and functions of immunological system. It does not only help in better understanding of epidemiology and pathogenesis of diseases caused by bacteria, viruses or other microorganisms, but also in understanding of certain relations between cells, tissues and the system, reactions to what is our own and that which is foreign, and genetic aspects of control within an individual.

The basis of investigation in transplantation immunology, or better of modern immunology, is the immune system; a system which is anatomically very simple, and one of the most complicated according to its function and ways of action. Besides duties shared by other systems in an individual's body and occasional or continued carrying out of tasks with which they are entrusted, this system, to quote the introductory motto »must always be on alert to combat foreign intrusions and disloyalties from within...«.

1981–1988 Branko Vitale

Experimental immunology was one of the scientific disciplines that were marked by particularly fast progress in Croatia after the Second World War. It was, actually, a continuation of the long tradition of classical immunological studies in Croatia at the end of the 19th and the beginning of the 20th century. In the period from the late 1950s and the middle of the 1970s, numerous research groups were established at the School of Medicine in Zagreb and in Rijeka, at the Faculty of Science and Mathematics in Zagreb, and at the Institute of Immunology and Ruđer Bošković Institute in Zagreb. In relation to the size of Croatia and the level of development of other natural sciences, the contribution of Croatian experimental immunologists in that period and later to the world science can be rated as very significant.

Such a rapid development of experimental immunology also imposed the need for a professional organization supposed to ensure continuous communication and exchange of experience between our scientists and provide them with the possibility of organized international cooperation. This communication was first realized in the Croatian Immunological Section of the Yugoslav Immunological Society and, since 1968, in the independent Croatian Immunological Society and the Federation of Yugoslav Immunological Societies. In these organizations, symposia of immunologists were regularly held every three years at the level of the former state, with monthly professional meetings of the Society. Thus, the first symposium of immunologists was held in 1966 in Zagreb (organizer: N. Allegretti; 23 presentations), the 2nd symposium took place in Ohrid in 1969 (organizer: B. Nakić; 58 presentations), the 3rd in Belgrade (organizer: B.D. Janković; 74 presentations), the 4th symposium was held in 1974 in Opatija (organizer: Š. Vlahović; 83 presentations), the 5th in Stubičke Toplice in 1976 (organizer: M. Jurin; 104 presentations); the 6th in Kaluđerske bare in 1979 (organizer: M. Simić; 178 presentations), while the last symposium took place in 1982 in Radenci (organizer: S. Banić; 194 presentations).

In addition to organization of professional meetings, Croatian Immunological Society was actively involved in activities related to introduction of an autonomous immunology course at the Faculty of Science and Mathematics in Zagreb (1964), at the School of Medicine in Zagreb (1972) and in Rijeka (1975), and postgraduate studies in Zagreb (1980) and in Rijeka (1985), and played an important role in the development of clinical immunology.

1981 At the 3rd regular electoral meeting of the Society held on March 12, 1981, I was elected president and took the leadership of the Society together with the Management Board that consisted of M. Dorić, R. Mažuran and S. Rabatić. As the above-mentioned forms of professional activities of the Society had already been defined, they had to be maintained and possibly followed by the development of new ones that were imposed by rapid development of immunology.

During 1981, we consolidated professional activities of the Society and, together with the Allergological Section of the Croatian Medical Association, we organized 13 professional meetings with five foreign and eight national lecturers. Also, we renewed and tightened our professional and friendly relations with the Society members from Rijeka.

During a joint meeting of the members of the Board and the Society members from Rijeka held on April 22 in Rijeka, we discussed possible organization of a section within Croatian Immunological Society, organization of a European immunology congress, holding of joint meetings, and the development and organization of clinical immunology in Croatia. As a result of this initiative, a very successful joint professional meeting of Zagreb and Rijeka Society members was held on June 19 in Rijeka, which resulted in numerous professional and friendly contacts.

At international level, we began with activities related to the candidacy of our Society for organization of a European immunology congress in Zagreb; we started with a letter of intention addressed on May 28, 1981 to the then EFIS president Professor J. Gergely, PhD, Budapest, Hungary.

In the fall of the same year, we started to publish our Bulletin thanks to great efforts exerted by R. Mažuran and S. Rabatić.

1982 Regular monthly professional lectures were continued during 1982. Of the total number of the 17 lectures held, seven were by foreign lecturers, seven from other Yugoslav republics, and five by Croatian lecturers.

A discussion on diagnostic procedure in clinical immunology was organized in collaboration with the Croatian Medical Association already in April. Besides, the Society had the pleasure to propose its member Professor Matko Marušić, PhD, for the Zagreb Municipal Award for his book »Immunologic Recognition«.

In May, Dr Čulo started an initiative to set up a joint bank and register of experimental animal tumors in Croatia.

In year 1982, the Croatian Immunology Society was stricken by two sudden and irrecoverable losses: the death of Professor Veljko Stanković (16th April) and of Professor Nikša Allegretti (29th October). Thus we lost with them our Society founders, our teachers, collaborators, friends and, above all, great humanists.

1983 Croatian Immunological Society activities related to its candidacy for organization of the Euroepan immunology congress were continued during 1983, as well as its regular professional meetings. Throughout the year, six such meetings were held with five foreign and six Croatian lecturers.

In April (April 5th), we organized a memorial symposium on experimental allergic encephalomyelitis dedicated to Academician N. Allegretti with presenters from Belgrade, Rijeka and Zagreb.

In June, we had an interrepublic meeting with Serbian immunologists in Vinkovci where introduction of immunology in university schools of veterinary medicine was discussed.

Finally, the 7th Immunology Symposium was held in Radenci in November, with a high number of participants from Croatia. One of the sessions was dedicated to the memory of the Academician Allegretti where B. Vitale spoke of this deceased member of our Society.

At the end of November (November21st), the 4th regular Croatian Immunological Society Electoral Meeting was held where a new Management Board was elected consisting of Vitale, Jonjić, Jurin, Benković.

1984 Only three professional meetings were organized during 1984, with one foreign and two Croatian lecturers. The Society seconded a motion by Prof Rukavina that the 8th Immunology Symposium take place in Opatija on the occasion of the 30th Anniversary of the Rijeka School of Medicine. Also, a proposal by Professor M. Simić from Belgrade that immunology symposia grow into congresses was accepted. Therefore the meeting in 1985 was renamed into the 1st Yugoslav Congress of Immunologists.

In May, Professor V. Frančisković, the founder of clinical transplantation in Croatia, passed away in Rijeka. Professor Frančisković had successfully led a kidney transplantation team in Rijeka during a ten-year period.

The members of the Society Management Board located in Zagreb took up the organization of the scientific segment of the 1st Congress of Immunologists, and Board members from Rijeka took over the local organization of the Congress in Opatija together with other colleagues from the Rijeka School of Medicine.

After unsuccessful candidacy of the Society for the organization of the 6th European Immunology Congress in 1984, the new Management Board renewed our candidacy in EFIS at the assembly held in Interlaken during the European Immunology Congress. Our candidacy received general support at this Assembly and it was decided that the 8th European Congress of Immunology was to be held in 1987 in Zagreb. In the preparatory period of the Congress, we announced and communicated information on the course of preparations at EFIS immunological congresses and assemblies in Jerusalem 1985 and Toronto 1986.

1985 During 1985, the main activities of the Society were focused on the organization of the 1st Congress of Immunologists in Opatija which was held on October 21–25. A total of 330 papers were presented at the Congress which had about 450 participants. Exhibition of scientific equipment and biological reagents was organized during the Congress. Furthermore, establishment of a Committee for Veterinary Immunology was proposed at the Congress, and initiative was launched to introduce immunology teaching at university schools of veterinary medicine according to IUIS suggestions. The general feeling was that the Congress was well organized and provided proper insight into the section of immunological research in the country. In the same year, a group of Croatian Immunological Society members (Vitale as a coordinator, B. Čvorišćec, D. Dekaris, A. Kaštelan, V. Silobrčić) presented a proposal of organization of clinical immunology for Zagreb city districts. Despite having prepared a regional program for a part of Croatia, the authors presented an integral proposal of the scope of activity for a new clinical discipline, its gradual organization within the framework of health care service for entire Croatia, and the proposal of specialist training in clinical immunology. The aim of the proposal was an attempt at organized development of this important clinical discipline in Croatia through a synthesis of knowledge and experience worldwide. At the end of the year (December 18, 1985), Scientific and Organizing Committees of the 8th European Congress of Immunology in Zagreb were elected. President of Croatian Immunological Society, Professor B. Vitale, was elected the chairman of both Committees.

1986 Besides professional meetings during 1986 where one foreign and four Croatian lecturers participated, the major event at the professional level was the conference entitled: Chronic Lymphocytic Leukemia as a Model for Studying Lymphoproliferative Diseases which took place in Dubrovnik on May 21–23, 1986.

The organizers were the members of the Society, B. Vitale and B. Jakšić. A special issue of the journal Blood Cells was dedicated to this Conference, and B. Jakšić and B. Vitale were guest editors. About a hundred eminent scientists, both national and from around the world, participated in the Conference.

During preparations for the 8th European Congress of Immunology to be held in March 1986, a meeting of the EFIS Management Board was held in Zagreb which on that occasion coordinated the Congress framework together with the Croatian Immunological Society Management Board. The EFIS Management Board completely accepted the Congress's conception we had proposed. On that occasion, EFIS allocated a financial support to the organizers in the amount of 10.000 dollars. Scientific Committee met once more on October 31, 1986 to determine the final program of the Congress.

1987 During 1987, all Croatian Immunological Society activities were focused on the organization of the 8th European Congress of Immunology in Zagreb which took place on August 30 – September 5, 1987.

The Congress was held under the auspices of the Presidency of the then Socialist Republic of Croatia (A. Marković, BSc) and the then Zagreb City Council (Dr M. Mikić), then of IUIS (Professor G. Nossal), and of EFIS (Professor M. Hess). It lasted five days: four active days, and one rest day which participants spent at the Plitvice Lakes. Overall, approximately 1,200 participants were present from all continents, and 850 congress papers were presented. On the occasion of the Congress, an article written by B. Vitale and entitled »Immunology in Yugoslavia« was published in the journal *Immunology Today* (Volume 8, No. 6, 163–190, 1987). After the Congress, a special double issue of *Immunology Letters*, the official EFIS paper, was published that was dedicated to the Congress and contained 28 plenary and symposium lectures. The preface to this issue was written by B. Vitale and B. Jurin as guest editors who outlined, among other information, the conception of the Congress in Zagreb. A portion of text from the preface is singled out here which refers to the Congress's conception:

...Immunology has become one of the most important life sciences; its achievements have had a profound beneficial effect on the quality of human life in the past and promise to have it in the future. The horizons of contemporary immunology are continously widening, both conceptually and technologically. Nowadays a profound impact is being made by molecular biology and neuroimmunology, which contribute to better understanding of the cellular and molecular mechanisms underlying immunological events and mutual influences operant between neural, endocrine and immune systems in enabling an organism to be a functional unity. In addition, research in experimental and clinical immunology, closely connected and complementing each other, is further broadening our understanding. The greatly enlarged scope of immunology today requires, for such a meeting as this, the definition and selection of a common core of topics in order to facilitate and promote understanding and collaboration between immunologists working in different fields of this science. With this in mind, an attempt was made to give participants of this meeting an overview of research and progress both in mainstream and specialist areas of experimental and clinical immunology...

According to general ratings of the participants, that congress was, until then, one of the best organized international immunological congresses. In addition to extensive participation of our scientists and establishment of numerous contacts with scientists from around the world, it should be particularly pointed out that the city of Zagreb, with all its cultural potentials, presented itself to participants and guests in indeed a grandiose light.

An understandable relaxation ensued after the Congress, with flagging Croatian Immunological Society activities and fatigue of the the then Management Board. What the Society needed was a new Management Board. Our term of office ended at the Electoral Meeting held on May 26, 1988, and we wished success to the newly elected Management Board in leading and further advancement of activities of the Society.

1988 – 1993 Filip Čulo

Professor Filip Čulo, Zagreb University School of Medicine, took the helm of the Croatian Immunological Society in 1988 from Professor Branko Vitale. At that time, there were Immunological Society of Croatia, Immunological Society of Serbia, and Slovenian Immunological Society (Croatian Immunological Society is the name to be adopted not before 1993!), all united in the Yugoslav Federation of Immunological Societies (YFIS). In May 1989, we participated in the last (2nd) Congress of Yugoslav Immunologists in Vrnjačka Banja where an initiative was started to establish new republic societies. As a consequence of this initiative, Professor Momir Macanović announced in June 1990 the founding assembly of the Immunological Society of the Republic of Bosnia and Herzegovina. However, we were together in Federation for not longer than a year! The last meeting of the members of the Executive Committee of the Federation of Immunological Societies was held during Alps-Adria Immunology Meeting in Opatija in October 1990. The 3rd Congress of Yugoslav Immunologists was agreed on at that meeting for 1992 in the organization of the Slovenian Immunological Society. At the time of anticipated meeting of the Scientific Council of the Congress, i.e. March 1991, the event at Plitvice happened and we separated forever. This was officially confirmed by letters with the same content addressed on October 7, 1991, by Professor Filip Čulo, the president of the Croatian Immunological Society, to the following addresses:

– Asst Professor V. Kotnik (president of the Slovenian Immunological Society)

– Professor M. Macanović (president of the Society of Immunologists of Bosnia and Herzegovina)

– Professor V. D. Miletić (president of the Serbian Immunological Society)

– Professor M. Simić (president of the Federation of Yugoslav Immunological Societies), with the following content:

»We hereby inform you that the Croatian Immunological Society has resigned from the Federation of Yugoslav Immunological Societies. The reasons for our withdrawal are explained in a letter to the Federation of Yugoslav Immunological Societies, the copy of which is attached.«

On the same day, the president of EFIS (European Federation of Immunological Societies), Professor Max Hess, was informed on the withdrawal of the members (69 of us) of the Croatian Immunological Society from the Federation of Yugoslav Immunological Societies. The intention that the Croatian Immunological Society become an EFIS member was expressed in that same letter. The path of realizing this intention was to show to be very toilsome and the stuggle to achieve prolonged to the following term of office when Dr. Renata Mažuran and her enterprising female team (Dr. Sabina Rabatić and Dr. Alenka Gagro) were heartily fighting for it and succeeded.

In the meantime, EFIS president changed and we again informed in March 1992, but this time Professor A. Capron, on the separation of our Society from YFIS and we again expressed our wish to become an EFIS member. In May of the same year we forwarded a letter to the president of International Union of Immunological Societies Professor J. B. Natvig with a request for membership in this umbrella organization. Later, the presidents of both societies changed and we kept on writing. Thus in October, we again forwarded a request for membership in both societies to a new EFIS president Professor K. Eichman and a new IUIS president Professor H. Metzger. As a vice-president of the Croatian Immunological Society, Professor Stipan Jonjić attended the EFIS Meeting in Budapest in 1992 and learned that EFIS was to reach its decision after a decision on our membership was made by IUIS, which was also confirmed in a letter by Professor Eichman from November 1992: »The EFIS board has resolved that the Croatian Immunological Society will become a full member of EFIS as soon as it has become a full member of IUIS«. EFIS showed its good will by inviting us as observers to its meetings and providing us with all EFIS information. The answer from Prof Metzger did not arrive so we reminded him again in March 1993 (How time flies!) of our wish to become an IUIS member. We also addressed again the EFIS president Prof Eichman expressing our hope that our membership would be confirmed before the meeting in Leipzig in the fall of 1993. In May of that year, our team left the »battlefield« and left over the struggle to a courageous and combative team of Dr. Renata Mažuran. What else can be said today of the period when all of us, aside from our obligations at university schools, clinics or institutes, worried for our family and interrupted experiments due to wartime alerts? Society meetings were not as regular as today, although we tried. We received several new members to the Society at that time but it seems to me that perhaps the best indicator of our invincible spirit was the fact that we published as many papers in that period as during the pre-war time. After that, the Society was led by two excellent teams whose, I hope, contagious enthusiam and optimism will be transmitted to new and young Society members.

1993 – 1995 Renata Mažuran

HID has the heart[1] Why I accepted the honour, but also the duty of the president of the Croatian Immunological Society (thesis argumentation)

I encountered immunology as a biology student in 1969 in the laboratory of Borislav Nakić[2] where I was learning the basics of experimental work on chimerism and tissue transplantation. At that time, graduate students encountered immunology only at the Faculty of Sciences and Mathematics, University of Zagreb, where immunology course was introduced in 1968 as part of the study of experimental biology. Suggestiveness of Borislav Nakić, his way of conveying knowledge and of handling scientific facts sealed up forever my professional choice. I decided to become an immunologist. His too early, tragic decease redirected me to another location[3], to the laboratory of his student and colleague Vlatko Silobrčić. I began my scientific life in 1971 – the year when our first lymphokine was characterized (macrophage migration inhibitory factor). A year later (1972), a course in immunology was introduced in graduate medical studies at the Zagreb University School of Medicine. Looking back, I am amazed at the ease with which a small team of enthusiasts in one of a few top quality institutions in which immunology in Croatia was developed, reached subtle solutions that led to original scientific results, always at the level or just a small step behind major immunological developments worldwide. I can define my personal scientific interest with three key concepts: immunoreaction mechanisms, innate immunity and immunomodulation. All my friends from that time are still here[4], still productive and still enjoying (although not infinitely any more) their work: Vlatko Silobrčić, Dragan Dekaris, Ante Sabioncello, Rabatić i Ivna Svoboda-Beusan. For a shorter or longer period, about 50 researchers were part of this team, and many of them left an indelible imprint on Croatian biomedical sciences. The result of lively activity is ten international projects of various duration and virtually continuous projects financed by the Ministry of Science or other government institutions.

At the beginning of our collaboration, our objectives were correlates of specific cellular immunoreaction *in vitro*. Leukocyte migration inhibition test and monocyte spreading inhibition test were introduced in 1973 and their application started in

[1] A paraphrase of the title of a book by Shel Silverstein: The giving tree (Ilyricum, 1992), in Croatian translation, literally: The tree has a heart. On this occasion, I warmly recommend this book.

[2] Department of Physiology, Faculty of Sciences and Mathematics, University of Zagreb

[3] Division of Transplantation Immunology, Institute of Immunology, Zagreb

[4] School of Medicine, University of Zagreb, Ruđer Bošković Institute, Faculty of Science and Mathematics, University of Zagreb, Institute of Immunology, and School of Medicine, University of Rijeka

basic (animal model for immunosuppression efficacy) and applied immunology (pollen allergy). We began to investigate pleiotropicity of cytokines during the same year by studying lymphokine activities in human leukocyte interferon.

Translation of the first textbook in immunology[5] was done in 1974. The discussions occurring during translation have permanently established our loyalty to terminology and standards in immunology.

During this same year we began to differentiate and count immunocompetent cells (it was announced and confirmed at the World Congress of Immunology in Brighton that lymphocytes were not unique). Rosettes (spontaneous and induced) blossomed under our microscopes as visualization of one to two receptor molecules at the surface of T- and B-lymphocytes. Due to the development of monoclonal antibodies during not more than 25 years, we may presently recognize as many as 104 different surface molecules on T-, B-, and NK lymphocytes.

The first working meeting for standardization of tests in clinical immunology was held in the Croatian Medical Association in 1975 (moderator: Dragan Dekaris).

Polyclonal and clonal lymphocyte expansion was even at that time considered to be the external property of immunoreaction to tumors, infections and autoimmune diseases.

We tackled immunoreaction stimulation in malignant diseases in 1976; as others, we underestimated the capability for prostaglandin induction of nonspecific bacterial stimulators (BCG, *Corynebacterium parvum, Propionibacterium acnes,* Picibanil®, a peptidoglycan from *Brevibacterium divarticatum*). Some successful animal experiments were abandoned after we could not, after many years of exerted efforts, confirm them on human model, while we are still persistent with some others as our focus has shifted to synthetic immunomodulators, cytokines, specific receptor ligands.

From »general to individual« occurred in 1978 when we shifted our interest from systemic immunoreaction to the local one (local nasopharynx immunity). At approximately that time (the early 1980s) we ceased to be, almost imperceptibly, the fans of specific immunoreaction. A door of nonspecific immunity gradually begun to open in front of us, with all the attractive components of acute inflammatory reaction: phagocytosis, ADCC and NK-cells and cytokines became our everyday life in 1980.

Despite interdisciplinary character, team members were systematically pushing issues of broad social significance like (unsuccessful) introduction of specialist training in clinical immunology for medical doctors (Academy of Medical Sciences of Croatia, 1978). Due to gradual increase in interest in clinical immunology, we organized in 1982 the first course in clinical immunology. Postgraduate course in Allergology and Clinical Immunology at the School of Medicine, University of Zagreb was introduced in 1986. In the same year, one of the activities of our team was officially recognized: a Referral Center for Clinical Cellular Immunodiagnostics of the Croatian Ministry of Health was founded at the Institute of Immunology.

The second[6] textbook in immunology in Croatia was *Basic Allergology* (published by Školska knjiga) which was written by Dragan Dekaris and published in 1983. Vlatko Silobrčić was the author of the book *How to publish a scientific paper* (published by Jumena). A proposal of standardization of immunological terminology was also published.

Possibly because we ourselves reached a certain age, we started in 1985 to work on immunoreaction in elderly persons. The Croatian Homeland War suddenly shifted us toward the immunology of stress (1992); the stress still represents our mode of living and it seems that it won't ever leave us any more.

Looking back, in addition to great pleasure in work, most of the past time was marked by productive companionship with other Croatian immunologists. I have always considered these people to be members of the Croatian Immunological Society: dealing with immunology and membership in this Society are two inseparable entities. Our weekly (*sic!*) meetings were always well-attended and far from boring. There were no limits in discussions: we were a small group (we live in a small country), and immunology was advancing so fast that each one of us encountered innumerable challenges in their field which were shared with others. Financial situation could be described as moderate, and a scientist's status involved no particular pay but rather esteem. Our professional society was for most of that time functioning well and when the time came I accepted an offer by Branko Vitale to be a secretary of the Society during his term of office as a president. I enjoyed performing this duty with Branko Vitale – he always had brilliant ideas that were to be realized. I considered the time spent in the Society management under the leadership of Branko Vitale as a traineeship period.

The term of office as the Society president (Materials and methods, Results)

In May 1993, Filip Čulo resigned from the head position in the Croatian Immunological Society; for the first time in the Society history, the helm was taken by a female team (Mažuran, Rabatić, Gagro). Inital president's report sums up, in the manner of a typical legalist[7] and a traditionalist, all activities of the Society from its foundation to that moment.

[5] Ivan Roitt: Essential immunology. Croatian translation by D. Dekaris and V. Silobrčić

[6] the first was published in 1977, the authors were Vlasta and Šime Vlahović (Bases of Medical Immunology, Otokar Keršovani, Rijeka)

[7] *legalitas regnorum fundamentum*

During the first six months, most activities of the Small Council were directed toward contacts with umbrella organizations (EFIS[8]), IUIS[9]. In October 1993, I attended the EFIS Annual Assembly in Leipzig where the Croatian Immunological Society membership in EFIS was discussed. I was given moral support but, as it turned out later, we were still far from full membership (Eur J Immunol 1993;23:3054). Only after ICSU[10] included Croatian Immunological Society in its regular membership, IUIS and EFIS automatically did the same (EFIS Meeting, Freiburg, February 1994; announcement at the IUIS Barcelona meeting, April 1994). Nevertheless, even before formal recognition, the benefit of our activities directed toward gaining membership in international associations could already be discerned: EFIS was the sponsor and endorsed financial support for the meeting *Mechanisms of Local Immunity* that was prepared by Dane Rukavina and due in 1994 in Opatija. After exhausting war years, there was a light in the tunnel ahead of us: the first meeting with international participation after 1990.

Generosity was also evident in the domain of scientific publications: Croatian Immunological Society members obtained privileged subscription to the journals *Immunology Letters*, *Current Opinion in Immunology*, and *European Journal of Immunology*.

The IUIS paper *The Immunologist* was sent to the address of the Society without charge.

Yet another important international favour was extended due to the sympathy of the confirmed friend of Croatia, Georg Wick (Innsbruck): an invitation to the Society members to participate at equal footing at the Annual Meeting of the Austrian Society for Allergology and Immunology (ÖGAI, Graz, November 1993; host: Konrad Schauenstein). Thanks to Wick, four scholarships were also offered for young researchers in Austrian laboratories yet, regrettably, we took advantage of only one (Lidija Šmejkal-Jagar). This activity was regularly attended successively for several years but the Society members presently seem to have lost their interest in the closest neighbors.

Of the persons who exerted great efforts in internationalization of Croatian immunology, a mention should certainly be made also of Ivica Valpotić who represented Croatian veterinary immunology through his membership in the IUIS Veterinary Immunology Committee.

At the emergency Society meeting in December 1993, we voted for amendments to the Society Statute. I must say with regret that today, five years later, I am particularly sorry that some of the provisions have never been realized; *e.g.*, according to the section of this Statute related to the Society activity, it involves the right of issuing the title of an immunologist, which is the first step to a potential Chamber.

I left the wind beneath my wings: the beginning was promising.

Although the entire year of 1993 was packed with fast turn of events, and 1994 was even better and financially more successful, our frustrations caused by war and isolation were suppressed after first official visits to Croatian Immunological Society (or Croatia, actually). After Stefan Tierfelder whose intellectual and emotional connections with scientists from the Ruđer Bošković Institute remained unbroken, the top official of the umbrella organization, Klaus Eichman, also paid a visit. The EFIS president arrived in an official visit at the beginning of March 1995 and held an excellent lecture at the Croatian Academy of Sciences and Arts. By this action, he demonstrated without any doubt that Croatia is a safe country with expectations from its scientific potential. A step forward in this direction was also a publication of the Croatian Ministry of Science and Technology: Scientific Research in Croatia (1995; editor: Greta Pifat-Mrzljak) where activities of Croatian immunologists got adequate position due to the efforts made by Croatian Immunological Society Small Council and all members.

The year 1994 was, in brief, successful also regarding scientific activity. Members of the Society increasingly presented their investigations and started to meet and associate as before.

Mechanisms in Local Immunity (September 1994), the second immunological meeting under the auspices of the Alps-Adria Working Community and the first immunological meeting with international participation in independent Croatia gathered about 150 participants. A hundred and thirty-nine reports were registered (one plenary lecture, 42 symposium lectures, 17 selected oral presentations and 79 communications in workshops). Croatian immunologists held three symposium lectures, 12 selected oral presentations, and 49 communications in workgroups (in total, 64 contributions, i.e. 46%). All large immunological centers were represented: Zagreb with 43 active participants, Rijeka with 16, Split with 4, and Osijek with one presentation).

We get together with Austrian immunologists for the second time in Vienna (November 1994). We actively participated in this meeting with 15 presentations. Georg Wick handed over the presidential sash to Marta Eibl whose partiality toward Croatian immunologists had been unquestioned for decades.

[8] European Federation of Immunological Societies
[9] International Union of Immunological Societies
[10] International Confederation of Scientific Societies

There was a change at the helm of the journal Periodicum Biologorum: Vlatko Silobrčić handed over the burden, after 22 years of dedicated work, to Branko Vitale who took up a very serious commitment to return Periodicum biologorum among CC-indexed journals[11]. I have heard these days that he has fulfilled this obligation and I cordially congratulate him.

Everything was going on better – an enthusiasm was felt that had been absent for years, and we decided to make another step forward: after a very long pause, we ventured to put forward in 1995 to our members that the Croatian Immunological Society restored the institution of regular annual meetings. Thanks to Sabina Rabatić, the financial situation was favourable and, encouraged by international support, we thought that it would be advisable if the meetings involved international participation. Thus, in the first issue of the Society information in 1995, we suggested an at a glance program of regular annual Society meeting at the end of November. In April, we forwarded letters to invited lecturers, and the following of them replied: F. Sinigaglia (Milan), N.A. Mitchinson (Berlin), G.Wick (Innsbruck), M.L.Kapsenberg (Amsterdam), K.Havemann (Marburg), U.Kozinowsky (Ulm) i C.R. Stokes (Bristol).

The war broke out in May in Zagreb unexpectedly loud and clear. The words by Francesco Sinigaglia in his letter where he accepted our invitation will remain in memory for all time: »Thank you for your very kind letter inviting me to the next annual meeting on November 23rd in Zagreb. The day your letter arrived the Italian television announced the dropping of bombs in Zagreb. I was shocked and saddened once again. I have good friends living in Croatia and in the past I have visited your contry several times... I am delighted and honoured by your invitation and if the situation permits I will definitely accept your invitation«.

The situation permitted it to happen. We had an excellent meeting[12].

Conclusion

Activity of the Croatian Immunological Society goes deep into the scientific life in Croatia. It has been productive, rich, internationally recognized, and it affected the quality (and quantity) of basic and applied (clinical immunology) science and higher education (graduate and postgraduate). The Society Board must encourage Society members to become recognizable by their intellectual contribution and presence in scientific population. In return, the Society offers much. In the first place, it is identity, professional association and friendly support, while excellent newsletter, quality lectures, meetings, and even a (small) financial assistance also cannot be disregarded.

To be a president of such a society is great responsibility which should be assumed with humility in regard to those who performed this duty before. Supervision of an organization in which a president is elected among members who are ready and capable of coping with possible problems in society's activity rather than wait with folded arms is a unique life experience. It was a great pleasure for me to serve the Society during two and a half years, and I want to express my gratitude to Sabina Rabatić and Alenka Gagro who were my close collaborators in that period. However, I also thank many members who unselfishly came to my assistance whenever necessary. The rule according to which a vice-president takes up the duty of a president allows continuity and good level of information about Society's activities. Thus, it was my pleasure to hand over the office to my vice-president and current president of the Society Sabina Rabatić. I knew that the Society would remain in the same course and that it would serve its members well. And it has happened that way.

1995 – 2000 Sabina Rabatić

In an attempt to leave a written trace to generations that follow on the occasion of such a great anniversary that we celebrate today, I have a wonderful duty and honor to describe what we were dealing with the period mentioned above. It has not been easy for me, as a markedly emotional person, to sum up the thoughts only about the those activities in the Society where I participated and worked during my entire active life. I can still clearly remember the atmosphere during the first regular Society meeting (in December 1976) in the lecture hall of the 3rd wing of the Ruđer Bošković Institute when I was elected first for an associate member of the Society. As known, Croatian Immunological Society has had strict criteria for acceptance of regular members from the very beginning of its activity. One could become a regular member only after defending a MSc thesis in immunology. Well, from that moment right until today, I believe that the reasons why I was absent from any of numerous Society's activities were very rare and always justified. One of my first duties in the Society was to organize famous Wednesday meetings in the then trendily equipped classroom in the basement of the Institute of Immunology. Elderly members may remember those hanging TV monitors which Professor Ikić had had installed in that classroom so that students could, as part of practical classes in immunology, observe, *e.g.*, dissections of laboratory animals. Famous and excellent discussions were taking place in that room that have in a way determined my scientific interest and developed the necessary scientific criticality.

[11] Period biol was in the 1972–1992 period a CC-indexed journal
[12] Period biol **97** (supp 1), 1995.

Also, my propensity to collect money was very soon noticed and during Professor Branko Vitale's term of office as a president, I was entrusted with the honorable duty of the Society's treasurer. I was performing the duty of a treasurer and vice-president of the Society also in the 1992–1995 period, and in November 1995 I took up the responsible function of its president. At that moment the Society was actually on an upturn after a heavy struggle for its regained international recognition. The criteria and tasks that we defined for ourselves were demanding. We started many actions and we were expected to try to continue and develop them. On this occasion, I am going to remind you in a chronological order of some significant activities that we implemented in the period stated above.

1996 In 1996, we organized the second annual meeting of the Society with international participation: »1996 Annual Meeting of the Croatian Immunological Society«, this time in Opatija in September. Three invited lecturers from abroad and 14 selected lecturers from Croatia had their presentations in front of 150 participants. The abstracts from this, as well as all other annual meetings organized by the Society, were published in our paper, the journal Periodicum Biologorum.

It was a good introduction to our second international meeting: Third International Meeting »Mechanisms in Local Immunity«, Opatija, September 25–28, 1996, that takes place every two years in Opatija and has, so to speak, become tradiotional. The Third Meeting of the Alps-Adria Society for Immunology of Reproduction was held at the same time.

Among other international activities, I would like to single out the participation of our members in the annual meeting of the Austrian Society for Allergology and Immunology (ÖGAI) in Vienna, for which the organizers offered three bursaries to members of our Society. It was at the same time the celebration of the 25th anniversary of the Austrian Society, and collaboration with the Croatian Immunological Society was emphasized in many occasional speeches.

The Society organized election and nomination of members for the bursaries offered by the International Union Against Cancer (UICC) in collaboration with Fondazione Callerio Trieste for the 18th UICC Tumour Biology Training Course in Trieste and Rijeka on June 20–26, 1996. Seven of our members obtained bursaries for that course. In that year, the Great Council of Croatian Immunological Society reached a decision on election and announcement of the first honorary member of the Society. It was Prof C.A. Janeway Jr., Yale University, New Haven, USA, who arrived in Zagreb on our invitation and a plaque was awarded to him on that occasion.

We were also honored by lectures of Professor Luka Milas, Houston, Professor J. Stein Streilein, Miami, Professor W. Streilein, Boston, and Professor A.M. Shamsuddin, Baltimore.

Regarding international activity of the Society, it should be mentioned that Croatian Immunological Society forwarded a request to the European Federation of Immunological Societies (EFIS) to organize John Humphrey School in Croatia. The request was granted at the session of the EFIS Board held in Paris on November 12, 1996. The organization was approved of an international immunology school entitled »Effector Functions of Immune Cells« to be held in 1998 in Dubrovnik.

Activities in the country were oriented toward organization of regular monthly lectures (there were 12), admission of potential new Society members (six new members), publication of information (three issues), and the like.

1997 In ever more difficult material circumstances in science, we succeeded in our intention and maintained continuity in organizing annual Society meetings. I believe that all of you remember the meeting in the old City Hall in the Zagreb upper town, entitled: »1997 Annual Meeting of the Croatian Immunological Society«, held on November 6–7, 1997. On that occasion, we had with us very dear guests among whom there was also EFIS president Professor Israel Pecht. We really had eminent lecturers with excellent topics, including also considerable activity of our members.

In 1997, the largest European meeting of immunologists was held, i.e. the 13th European Immunological Congress in Amsterdam. We organized a trip to this congress yet we must state with regret that only 10 of our members were able to participate in it.

EFIS Commission for European Clinical Immunology was set up in that year and I was elected as a Croatian delegate. The Commission convened in Düsseldorf with the aim to prepare a text on the status of profession: immunology in Europe and preparation of a proposal for education and specialist training in immunology. The text has been finished and will soon be printed in the journal Immunology Today. This issue indeed deserves to be broadly discussed by all members of the Society and imposes the need to organize a specific meeting on this topic.

Besides in EFIS, the Society is an active member in one other international professional organization – ECLM (European Confederation for Laboratory Medicine). During 1997, we attended the meeting that was organized in Düsseldorf and the electoral meeting of this Confederation.

Among activities in the country in 1997, we should point out the celebration of the anniversary of the Croatian Society of Natural Sciences (CSNS), dedicated to a great Croatian natural scientist and founder of CSNS Spiridion Brusina. Upon initiative from our Society, honorary lecture was held on that occasion by Prof. Thomas J. Gill. Prof. Gill was awarded the plaque of a honorary member of our Society and a medal with the image of Spiridion Brusina.

We launched a drive to grant the Society's annual award to young members for the best scientific paper in immunology. In 1997, this award was granted to Mirjana Kljajić-Turkalj, MSc. The Society was the proponent for the state science award. After selection procedure, we proposed Prof. P. Lučin. We are glad to be able to point that State awards for science were granted to as many as three of our members (Pero Lučin, Krešimir Pavelić and Alemka Markotić). We are also pleased by the fact that we have been continually rejuvenating. In 1997, we had six new members.

1998 Our 30th anniversary year abounded in activities. First we had – and it was the greatest until then, Fourth International Meeting »Mechanisms in Local Immunity« in Opatija. Over 200 active participants with 130 presentations bear evidence that the team led by Prof. Rukavina exerted great efforts in organization of that meeting which is today still one of the hallmarks of our Society's activities.

What is to make our 30th anniversary memorable is certainly the first international school John Humphrey Course that was organized in Croatia. As you probably know, it was held on October 11–14, 1998 in Dubrovnik. It was organized by CIS and supported by EFIS; the School in Dubrovnik gathered 80 participants from Croatia, Slovenia, Italy, Germany, Great Britain and the USA. Significant contribution to its success was given by excellent lecturers, 13 of them who created during the full four working days such a scientific atmosphere and level that could make many an international meeting envious. Personally, I consider the organization of that meeting the greatest success of this Society's Board and therefore I am particularly sorry that a relatively small number of people used this opportunity to see it for themselves.

We organized the fourth annual Croatian Immunological Society meeting dedicated to our anniversary. On that occasion we began to arrange the data on activities of our Society. and published a sort of an anthology dedicated to the 30th anniversary of Croatian Immunological Society in croatian. We were aware that this was a result only of our first attempts at setting information in order. We listed possible sections, started to set up the list of all members, tried to find their actual addresses. We collected photos and brief curricula vitae of our members who deceased too early. For the first time we brought together the bibliography of all our members related to the field of immunology. An inventory was made of all books and book chapters that were written by our members. We made a list of awards and acknowledgments granted to our members in that period, as well as of the meetings organized by the Society.

The summary of this activity was published in *Immunology Letters* in an article entitled: Croatian Immunological Society – 30 years of organized activities. As stated there, analysis of the collected data shows that immunology has a prominent place among the scientific disciplines that have most intensely developed in Croatia after World War II.[1]

1999 The Society organized the fifth annual meeting in Zagreb, November 25, 1999. Our outstanding guest this year was S. Romagnani, (Italy), U. Koszinwsky (Germany) and A. Rot (Austria). At this occasion Professsor U. Koszinowski was awarded as honorary member of Croatian Immunological Society.

In cooperation with Croatian Academy of Sciences and Arts Croatian Immunological Society organized an international meeting »New insights in posttraumatic stress disorders« in Zagreb. March 1999.

One of the activities done this year in collaboration with Croatian Society for Allergology and Clinical Immunnology was a preparation of the List of diagnostic test in clinical immunology, that are performing in Croatia.

This year we were also honored by lecture of Professor P. Ogra, Galveston, Texas USA.

Again, three of our members were awarded by State awards for science (V. Silobrčić, S. Rabatić, I. Sabolić).

What should I say at the end of this account? I have been active in this Society with much joy and always considered my commitments in it inseparable from my obligations in everyday work. This has been my profession, my vocation, my choice. I have often said that it has been an honor and a privilege to know some of the people that I have worked with. All of them are eminent members of our Society. I am therefore proud and happy that I have myself made a small contribution to the action of the Society for the benefit of all of us.

2000 – 2002 Tanja Marotti

It was my honor and pleasure to be the first president of the Society in 21th century when the new era in immunology starts; it involves proteomics, genomics and metabolomics in immunological investigations as well. The pioneer immunological investigations which involve genomics point up complexibility of immune biology and break many dogmas as »one gene – one protein – one antibody«.

[1] RABATIĆ S 2000 Croatian immunological society-30 years of organized activities. *Immunology Letters 73*: 23–27

Both proteomics and genomics help us to realize better mechanisms involved in T-cell receptor responses and cytokine network interactions. The human genome project, of which draft version was presented to scientific community in 2000, brought to scientists involved in immunological investigations numerous informations. The collateral damage of this great progress were high expenses of investigations, which is accessible only for laboratories with significant fundings.

2000 On Annual Assembly of the Society held on 8th of March 2000 in Zagreb, Croatian Immunological Society presidency proposed new members of working group for specialists in fileld of immunology. The conclusion of the Society Assembly was that criteria for specialization in the field of immunology should be coordinated in accordance to EFIS-a and IUIS criteria.

In December 2000, Annual Meeting of the Society was held at Rudjer Boskovic Institute, in Zagreb.The members presented their work on 18 poster presentations and 15 short oral presentations. All presentations from Annual Meeting were printed in Book of abstracts. More than 100 registrated participitians attended the Meeting.

During 2000, Croatian Immunological Society held 7 monthly seminars. The lectures were: prof. dr. H. Banfić, dr. Rajko Kušec, academician V. Silobrčić, ing. M. Hasan, prof. John Gordon (The Medical School, The University of Birmingham, UK), dr. Nasim Mavaddat (Wolfson College, Cambridge, UK), Prof G. Poli (Department of Clinical and Biological Sciences, Universiti of Torino, Italy).

Also, during 2000 we had three joint seminars together with Croatian Medical Association, Croatian –Austrian Society, and Croatian Physiological Society.

2001 In December 2001, the Society organized 2001 ANNUAL MEETING OF THE CROATIAN IMMUNOLOGICAL SOCIETY. The Meeting was held at Institute »Rudjer Boskovic« in Zagreb.We had 4 plenar lectures, 9 selected oral presentations and 21 poster presentations. All presentations from 2001 CIS Annual Meeting were printed in Book of Abstracts.

During 2001, we have organized 4 monthly seminars and three joint seminars with other societies.

Croatian Immunological Society parcitipates in organization of several international scientific meetings and trainings:

VIII INTERNATIONAL CONGRESS OF REPRODUCTIVE IMMUNOLOGY – academician D. Rukavina. Opatija, Croatia 2.7.– 6.7. 2001.

ICMAN – THE FIRST INTERNATIONAL CONFERENCE ON MECHANISMS OF ACTION OF NUTRACEUTICALS – prof. dr. K. Pavelić. 14.10.–19.10. 2001. Cavtat, Croatia.

2002-2006 Stipan Jonjić

In **2002** the Annual Meeting was held in **Trakošćan** (November 22 – 24). Invited speakers were Gottfried Dohr (Graz), Dirk Busch (Munich), John Gordon (Birmingham), Manolis Pasparakis (Rome), Astrid Krmpotić (Rijeka) and Mario Poljak (Ljubljana). From 46 posters, 16 were elected for oral presentation.

Participants of the Annual Meeting visited castle Trakošćan, and after that the gala dinner was held with poster awards and election of new members. John Gordon was elected an Honorary Member of the Society.

In **2003** the Annual Meeting was held in **Brijuni** (October 17 – 19). Invited speakers were Marko Radić (Memphis), Cristina Cerboni (Rome), Mathias Müller (Wien), Nataša Štrbo (Rijeka), Alenka Gagro (Zagreb), Hugo Oscar Besedowsky (Marburg Hessen), Robert Dantzer (Bordeaux) and Firdaus Dhabhar (Columbus). From 50 posters, 17 were elected for oral presentation.

In **2004** the plan was to organize the Annual Meeting in Vinkovci but in the end, due to some technical problems, it was held in Opatija (October 8 – 10). Invited speakers were Margarita Del Val (Madrid), David Hafler (Boston), Milena Hasan (Rijeka), Francesco Colucci (Cambridge), Siniša Volarević (Rijeka), Alemka Markotić (Zagreb), Pero Lučin (Rijeka), Roberto Biassoni (Genova), Stephen St. Jeor (Reno), Vlatko Silobrčić (Zagreb). From 48 posters, 13 were elected for oral presentation.

In **2005** the Annual Meeting was held in **Božava** (Dugi otok) from September 29 till October 2. That year the Faculty of Medicine, University of Rijeka celebrated its 50 th anniversary. Invited speakers were Peter Ghazal (Edinburgh), William Britt (Birmingham), Joanne Trgovcich (Ohio), Mariastefania Antica (Zagreb), Boris Labar (Zagreb), Tihana Lenac (Rijeka), Ana Marušić (Zagreb), Alojz Ihan (Ljubljana), Martin Stacey (Oxford) and Nazzareno Dimasi (Genova). From 54 posters, 19 were elected for oral presentation.

Every Annual Meeting of the Croatian Immunological Society in that period was a great opportunity for members to meet, exchange ideas and information and learn more about work in different labs. Although the activity of the Society was mostly connected with organization of Annual Meetings it is important to emphasize that Croatian Immunological society was also involved in EFIS activities; mostly in activities regarding the Day of Immunology, traditionally held in April. Members of the Society organized a lot of interesting lectures with speakers – respected scientists – from all over the world.

2006 – Sabina Rabatić

I was honored and became again the president of the Society in June 2006. As this was in the middle of the year it was especially convenient for us to implement the new EFIS (The European Federation of Immunological Societies) idea and we organized the »1st Joint Meeting of European National Societies of Immunology« as a part of 16th European Immunology Congress in Paris September 06–09, 2006. Unfortunately, only 30 of our members with 28 presentations were present in Paris. Still, we were very satisfied that 13 scholarships had been awarded to young students to enable their participation at that Congress.

Under the coordination of EFIS all member states were encouraged to organize activities in connection with the International Day of Immunology. In order to promote the immunology as a discipline our Society organized an open public lecture »Influenza: prevention and dilemma«, delivered by dr. Maja Šantak. In 2006, eight new members of the Society were elected (A. Tešija Kuna, D. Polančec, I. Slavuljica, I. Gašparović, L. Rnjak, M. Ilić, M. Žirović, S. Mandarić). Dr. Milena Hasan was awarded the prestigious EMBO scholarship for postdoctoral improvement in Paris at Institute Pasteur, Department of Immunology, Cytokines and Lymphocyte Development Unit. The newly created web site of the Society (www.hid-zg.hr) enabled us a very good and professional communication and exchange of all relevant information.

1999 A special effort was made to activate more members of the Society to meet on regular monthly bases. We succeeded with this idea and in 2007 our Society organized 9 monthly meetings with very interesting lecturers and topics.

Events within the International Day of Immunology, April 29, 2007

In all member states, under the coordination of EFIS, lectures, TV shows and different manifestations were performed with the goal to improve the knowledge and recognition of immunology as science and profession. The Society joined the action for the immunology popularization on the occasion of the International Day of Immunology this year in the following way:

In collaboration with the editor of the journal GEO the richly illustrated and written in a popular manner the article »Our friend, our enemy – The immunological system« was published in its Croatian edition (April, 2007). The copies of the respective journal were distributed in schools with the idea that the teachers within biology or related courses dedicate a lecture to immunology and the International Day of Immunology. On the same occasion our members delivered lectures within immunology and related courses in the biomedical field at the Universities. On the web pages of Zagreb Medical faculty and the Society, the article »The importance of Immunology« by dr. Danka Grčević, the vice president of the Society was published.

Annual Meeting of Croatian Immunological Society

Annual Meeting was held on the Red Island near to Rovinj October 19–21, 2007. The Meeting was presented *in toto* on the newly established web site (announcement, call for participation, registration, review of abstracts). There were 103 registered participants with 56 presentations, out of which 12 oral presentations were selected. Seven plenary lectures were delivered as well. The Meeting hosted 6 guest lecturers: Peter J M Openshaw (London, UK), Yong-Jun Liu (Houston, USA), James P Di Santo (Paris, France), Ilias I N Doxiadis (Leiden, The Netherlands), Federica Sallusto (Bellinzona, Switzerland), Ulrich Koszinowski (Munich, Germany). Three prizes for the poster presentations were awarded: (Jurica Arapović, Lidija Habjanec and Branko Pevec)

»Spiridion Brusina« medal awarded to prof. Ulrich Koszinowski the Society proposed prof. Ulrich Koszinowski to be awarded with the »Spiridion Brusina« medal for 2006. Prof. Koszinowski, director of the Institute Max Von Pettenkofer University Ludwigs Maximillian in Munich was unselfishly and long-standing helping the development of Croatian science and training of Croatian scientists. The proposal was accepted by Croatian Natural Society and the medal was given to prof. Koszinowski on an award presentation ceremony at the 2007 annual meeting. The medal recipient delivered the »Spiridion Brusina Lecture« entitled »Visualization of virus distribution pathways and the role of host control«, and the article is published in this issue of Periodicum Biologorum.

Award for the best published article of a Society member in 2006 was given to Tihana Lenac for the work: *Tihana Lenac, Matthias Budt, Jurica Arapovic, Milena Hasan, Albert Zimmermann, Hrvoje Simic, Astrid Krmpotic, Martin Messerle, Zsolt Ruzsics, Ulrich H. »The herpesviral Fc receptor fcr-1 down-regulates the NKG2D ligands MULT-1 and H60.« Journal of Experimental Medicine 203: 1843–1850, 2006*.

Several of our members were honored in 2006 with prestigious national and international awards:

Prof. Siniša Volarević: Croatian Academy of Sciences and Arts annual award in the field of molecular biomedicine

Dr. Alemka Markotić and dr. Neven Žarković: National science annual award for scientific achievement in biomedicine.

Prof. Ana Marušić: elected vice president of Council of Science Editors (CSE), association of editors of about 1000 scientific journals from all over the world.

We are especially happy that in 2007, 28 new members joined the Society.

ABOUT OUR JURNAL

Spiridion Brusina founder of the Jurnal

Spiridion Brusina (1845–1908) counts among most renowned Croatian natural scientists. He was first appointed professor of zoology at the University of Zagreb and founder of the Croatian National Zoological Museum. Being a very capable organizer, Brisuina led the first organized Adriatic research expedition in 1894, which was carried out in scientific trips along East Adriatic coast from Trieste to Budva.

A far as his scientific orientation is concerned Brusina belongs among the most enthusiastic propagators of Darwin's evolution theory. For his outstanding scientific discoveries he was electted member of numerous academies such as Zagreb, Budapest, Belgrade, Palermo, Philadelphia and Istanbul.

In his desire to organize Croatian scientists Brusina founded in 1885 Croatian Natural History Society today Croatian Society of Natural Sciences and was his first president 1886 – 1898

Spiridion Brusina (1845–1908)
Portrait made by Vlaho Bukovac

Journal Periodicum biologorum

In 1887 Brusina established the Society's journal GLASNIK and was its first editor-in-chief. The same Journal later become renowned Periodicum biologorum – an interdisciplinary international journal devoted to life sciences.

About purpose of the journal Brusina wrote in 1887: »*It is most important that scientific articles may be written not only in Croatian but also in Latin, Russian, French, English, German and Italian languages. This will ensure that learned world will better aquited with the work of Croatian natural scientists.*«

Spiridion Brusina Award

Paying a tribute to the founder of Croatian Society of Natural Sciences and the founder of the first scientific journal Periodicum biologorum in Croatia Spiridion Brusina, The Presidency of Croatian society of natural sciences inaugurated 1997 Annual Spiridion Brusina award consisting of medal and diploma in Latin and honorary lecture delivered by awardee published in the journal.

Awardees are distinguished scientists from abroad who beside their internationally recognized scientific achievements, deeply contributed to the promotion and development of Croatian science through direct colaboration and/or education of young Croatian scientists and especially for generous material and moral support during the War of independence.

Previous Spiridion Brusina awardees

Thomas Gill II, US
Miroslav Radman, France
Klaus Wandelt, Germany
Krešimir Krnjević, Canada
Rudolf Zahn, Germany
Stanimir Vuk Pavlović, USA
Martin John Bukovac, USA

Werner E.G. Müller, Germany
Laszlo Forrom, Switzerland

2007 Spiridion Brusina Award

For 2007, Croatian immunological society nominated and the Presidency of the Croatian Society of Natural Sciences unanimously elected professor Ulrich Koszinowski as awardee for his outstanding scientific achievements in cellular immunology and virology and for his long lasting fruitful cooperation with Croatian immunologists at the Medical Faculty, University of Rijeka, and for his generous help in supporting scientific reserach and education of young scientists from Rijeka.

In particular The Presidencyy would like to express sincere gratitude to professor Koszinowski for his generous help and moral support of our country during the last war.

Highlights from prof. Koszinowski' s scientific curriculum vitae

During his scientific career prof. Koszinowski served as professor of virology and immunology at Univerity of Ulm and Heidelberg. Since 1966 he is the head of Department of virology at the University of München.

Among his numerous scientific achievements probably the most important is confirmation of Zinkernagel and Doherty description MHC restriction of CTL responses using vaccinia virus. In systematic approach he cloned the MCMV genome and established a physical map of the genome.

Further, he charaterized MCMV IE enhancer promotor, non-structural CTL antigen pp89 and derived nonapeptide, inhibition of antigen presentation and identification of responsible viral genes, and its morphogenesis. Fibaly he generated the first herpesviral full lenght genome BAC and applyied BACmid besed techniques to viral mutagenesis.

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Spiridion Brusina Lecture

Visualization of virus distribution pathways by cell type specific labelling of virus progeny

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Abstract

Cytomegalovirus (CMV), a prototypic β-herpesvirus, is an important human pathogen causing protean clinical manifestations. Virus pathogenesis can be studied in the mouse cytomegalovirus (MCMV) model. Although MCMV has been extensively studied, the contribution of specific cell-types is still elusive. Here, an approach based on the Cre / loxP*-system to investigate MCMV infection at the level of cell types* in vivo *is discussed. An MCMV with an* egfp *reporter-gene which is only expressed in cre-expressing cells was constructed. In cre-transgenic mice this reporter virus produces an EGFP+ virus progeny in a cell type-specific manner. Using this conditional gene expression system the viral productivity of specific cell types and their contribution to viral dissemination* in vivo *were determined. Clearly, virus production by some cell types contributes to virus dissemination whereas the productivity of other cell types does not, although they produce the bulk of virus progeny.*

The conditional virus labelling will also serve to other aspects of virus pathogenesis. Virus mutants that lack specific viral genes can be used to screen for cell type-specificity of the missing gene functions. Such conditional MCMV mutants should allow the dissection of herpesvirus pathogenesis at the level viral genes acting on specific cell types in vivo.

INTRODUCTION

Pathogenesis is what occurs when a virus infects a host. This includes all stages from entry into host tissues, distribution within the host to the major target organs and tissues onto the infection of the next host. Virus pathogenesis may or may be not associated with symptoms of infection. All viral genes probably contribute in one way or the other to viral pathogenesis. Also individuals of a given species differ with respect to genetic susceptibility to infection. Major host determinants are certainly the ability to respond by innate and adaptive immunity. The response to inflammatory signals, density of viral receptors and expression of cell intrinsic proteins which restrict viral protein expression are also parameters of viral pathogenesis. In general the expression of viral and cellular proteins is subject to subtle expression control, which depends on local tissue composition and may change under conditions of inflammation and with age. These many variables may lead to results which are difficult or impossible to predict.

Most studies on viruses use permissive cell lines. Yet, virus infection of permissive tissues and cell lines *in vitro* is only distantly related to virus pathogenesis. Tissue culture cells are highly selected for virus replication. They lack the natural tissue environment which plays a major control during innate and adaptive immunity *(1, 2)*. In addition, for adaptation of *in vitro* growth most cell lines have undergone transformation events. Basically, cell lines *in vitro* can serve for studies on virus morphogenesis and replication but not for studies on virus pathogenesis.

Tissue specific virulence genes?

Viruses differ in the number of genes they encode. Viruses encoding only few genes are in no way simpler than viruses that encode many. Many genes are multifunctional and the smaller the total number of genes is, the more likely are the chances that each gene bears multiple functions. Studies on virus mutants *in vitro* have led to the concept of *essential* and *non essential* viral genes. Many functions become apparent during viral replication in all cell types *in vitro*, and the lack of such genes results in the block of viral replication. Such genes are considered as *essential* genes. The inactivation or deletion of other genes may affect viral replication *in vitro* only to some extent or not at all. Such a gene is considered as *non essential*, meaning that this gene is not essential for replication *in vitro* under the experimental conditions used. It does not exclude essential functions in cell types that have not been tested. Herpesviruses with up to 240 Kb of double stranded DNA encode between 100–200 proteins. Only about 40 proteins are quite conserved between all herpesviruses and are essential in all herpesviruses for virus morphogenesis *(3)*. This leaves a vast majority of proteins with nonessential functions. Several of these genes affect host immune functions with regard to innate and adaptive immunity. Although the mechanistic principle for a number of genes is known it is unlikely that they affect all cells in a uniform fashion. Some CMV genes are known to define cell type preference *(4, 5, 6),* but most cell type preferences cannot be identified by studying virus mutants *in vitro*. Even *in vivo* infection conditions would at best reveal subtle differences in organ virus load. Therefore, much more subtle approaches to virus pathogenesis at the cell type level are needed. This will lead to the detection of new effects, the formulation of hypotheses and explanations of the principles of action.

Conditional virus tagging *in vivo*

Virus pathogenesis requires invasive studies. Therefore, studies are carried out with the human pathogen that can infect animals. The disadvantage is that the host is more or less distantly related to humans and therefore the principles which dictate the host side of viral pathogenesis differ. The alternative is the analysis of a virus related to the human pathogen in its natural host. Here the natural process resulting from co-evolution of the virus with its host is studied. The disadvantage is that virus and host – with the example of herpesviruses of mice and men – have undergone a process of separate co-evolution for at least 50 million years. Both approaches have apparent limitations. For some virus host models virus and host genetics are

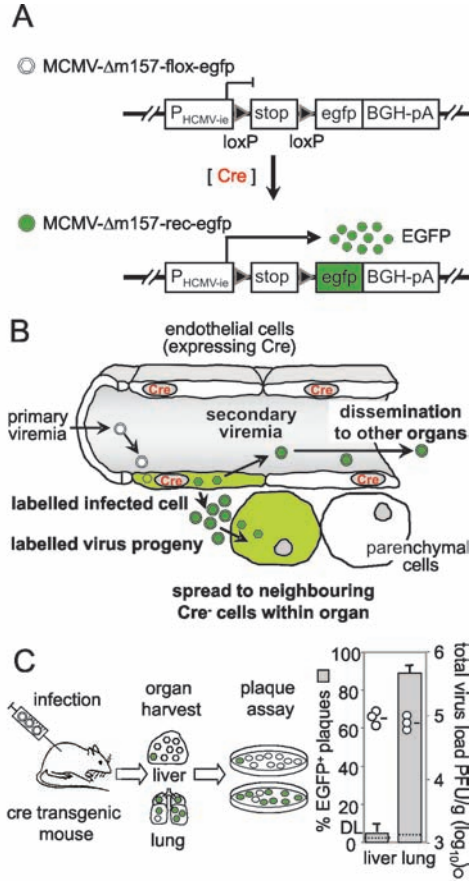

Figure 1. ***Tracing of cell type specific progeny by conditional virus recombination.*** *(A) A Cre inducible expression cassette is inserted into the m157 gene of the MCMV genome resulting in MCMV-Δm157-flox egfp (MCMV-flox). Cre mediated removal of a transcriptional stop sequence flanked by loxP sites (floxed) leads to expression of EGFP under control of the major immediate early promoter of HCMV (MCMV-rec). (B) Cell type specific Cre mediated recombination of viral genomes in cre transgenic mice allows identification and quantification of labelled infected cells and virus progeny produced by defined cell types (e.g. endothelial cells). As the recombined marker is stably maintained in the virus progeny spread of virus to Cre-neighbouring cells or dissemination to other organs can be analysed. (C) Schematic set-up of experiments and analysis. Transgenic mice expressing Cre recombinase under control of a cell type specific promoter are infected with the MCMV-flox reporter virus. After different time points post infection various organs are harvested and the amount of MCMV-rec and MCMV-flox- plaque forming units (PFU) are quantified by plaque assay on mouse embryo fibroblasts (MEF) using fluorescence microscopy. The graph depicts total (MCMV-rec plus MCMV-flox) virus load per gram organ for individual mice as open circles that refer to the logarithmic scale on the right hand side. Horizontal bars mark mean values and dotted lines indicate detection limits of total virus load. Gray columns refer to the linear scale on the left hand side and show the mean percentage of EGFP+ plaques, with the standard deviation indicated by vertical bars.*

(Taken from Sacher et al., Cell, Host and Microbes, 2008)

well advanced. The mouse has been subject to extensive genetic studies and today represents the best studied mammalian host. Allelic variants of important genes which control inflammation, infection and immunity have been characterized. The mouse genome has been the subject of not only targeted but also random mutagenesis approaches which have recently revealed important new principles of host resistance especially to MCMV infection *(7, 8, 9)*.

Studies on viral pathogenesis profit from an array of multiple techniques. Host and viral genetics certainly form the core of research on virus pathogenesis. Only by mutagenesis of defined viral functions can new phenotypes be detected. On the host side the analysis of the type, location, kinetics of appearance of infected areas is possible by immunohistochemistry, cell morphology, electron microscopy, fluorescence activated cell sorting and cell scanning (FACS). Recently microarray techniques for gene expression and systemic proteomics approaches have been developed that can monitor changes of gene and protein expression in cells. In the future these techniques may be applied on a larger scale to *in vivo* studies. This will show to which extent the events described for specific cell types can be generalized. So far only few tissues can be taken from infected animals and processed for the physical isolation of individual cell types such as cells from blood, bone marrow or spleen. For most other tissues the isolation of infected cells is a daunting task. Further, the identification of infected cells by detection of viral protein antigens does not differentiate between productive and abortive infection. Even if a given cell type is prone to productive infection *in vitro* the identification of the infected state *in vivo* does not mean that such cells produce virus. Inflammation caused by infection may increase or decrease virus productivity in an area or may switch virus infection from one to another cell type in the same tissue with or without subsequent virus productivity.

With the mouse cytomegalovirus (MCMV), a member of the ß-herpesvirus family we use a natural pathogen in the natural host. The infection of mice with MCMV represents a model for the analysis of replication, dissemination, and immune control of human CMV (HCMV). We have introduced herpesvirus genome cloning as infectious bacterial artificial chromosomes which permits to mutate (mutation of) any gene of the genome in a targeted or a random fashion *(10, 11)*.

The principles that govern virus spread from the side entry to the major target sites represent a complex aspect of virus pathogenesis, because many different tissues, different cell types and different principles of inflammation and immunity are involved With the exception of viruses that use only nerves for transport, the viral entry into the host through epithelial barriers is usually followed by virus transport to lymphatic organs and subsequent systemic distribution by viremia as cell associated or free virus. Some organs such as liver and spleen usually produce large amounts of infectious virus early during infection. They are thought to disseminate the virus to other sites where manifestations of infection appear later (*12*). This plausible model is generally used to explain systemic virus infections *(13)*. Also MCMV has been described to disseminate from an initial peripheral site of entry to spleen and liver, and secondary viremia is considered to distribute the virus from liver and spleen to other organs *(14)*.

The BAC-cloned MCMV genome *(15)* was used to construct a recombinant MCMV with a Cre-inducible fluorescent reporter EGFP A cassette comprising a loxP-flanked (floxed) transcriptional stop sequence between the HCMV major immediate-early promoter and the EGFP coding sequence replaced parts of the *m157* gene resulting in MCMV-Δ*m157*-flox-*egfp* (MCMV-flox, Figure 1A). The Cre / *loxP*-system is the most frequently used recombination system *(16)*. Cre is the 38 kDa product of the *cre* (cyclisation recombination) gene of bacteriophage P1. The Cre enzyme recognizes a 34 bp sequence called *loxP* (locus of X-over P1) and catalyzes DNA recombination between pairs of directly repeated *loxP* sites, resulting in the excision of the DNA in-between as a covalently closed circle *(17–20)*. The action of cre in cells *in vivo* generates EGFP expressing recombined virus MCMV-Δ*m157*-rec-*egfp* (MCMV-rec) *(21)*. The number of EGFP tagged viruses in organ homogenates of *cre* transgenic mice is determined by plaque assay (Figure 1B). Since the recombined locus is maintained in the viral progeny it is possible to track virus produced by a specific cell type to other cells that do not express Cre. Viral dissemination between different cell types within as well as between organs and the colonization of distant targets can thus be studied.

After the first round of virus replication, the number of $EGFP^+$ plaques represents the productivity of the Cre expressing cell type, and the comparison with the number of $EGFP^-$ plaques reveals its relative contribution to the total virus productivity. This can be tested organ for organ. Furthermore, analysis of $EGFP^+$ cells and of recombined virus progeny over time demonstrates viral spread between different cell types within an organ as well as virus dissemination between organs (Figure 1C).

Cell Type-Specific Labelling of Virus Progeny

Vascular endothelial cells (EC) are a major source of viremic CMV *(22)*. For MCMV tagging in EC we used Tie2-*cre* mice expressing Cre under control of the Tie2 promoter in vascular EC *(23)*. MCMV-flox was administered by an intravenous (i. v.) route to provide conditions of primary viremia. In accordance with the presence of endothelia in all organs MCMV-rec was found on day 3 post infection (p.i.) in all organs tested (Figure 2A). We determined the contribution of the EC-derived progeny to the total virus load in organs. Of course, this contribution of EC to virus production is only safe for the first replication

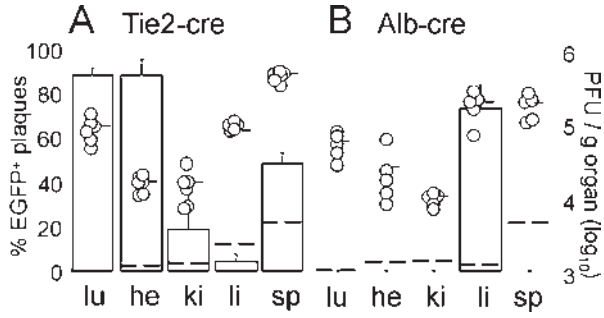

Figure 2. ***Hepatocytes and endothelial cells are crucial cell types for replication of MCMV* in vivo.** *Tie2-cre (A) or Alb-cre (B) mice were infected i.v. with 106 PFU MCMV-Dm157-flox-egfp. Three day post infection organs were harvested and analysed as depicted in Fig. 1C (lu: lung, he: heart, ki: kidney, li: liver, sp: spleen).*

(Taken from Sacher et al., Cell, Host and Microbes, 2008)

round from about 24 to 32 hours post infection. At subsequent replication rounds a high percentage of an EC-derived progeny could mean different results. Either the EC continue to produce virus, other cell types are infected in further rounds by the labelled virus progeny and dominate the infection, or that viruses produced in the first round in other cell types acquire the label upon secondary infection of EC.

Remarkably, organs strongly differ with respect to the proportion of MCMV-rec. A high recombination rate (~80%) was found in lungs and heart, an intermediate rate in spleen and kidney (~50% and ~20%, respectively), and a low rate (<10%) in the liver. Notably, a genetic link between host mutation and MCMV related heart failure was recently identified *(9)*. Infected EC are thought to contribute to viremic dissemination. Chronic CMV infection of vascular tissues is discussed as a potential trigger in the process of atherosclerosis. EC isolated from different vascular beds support lytic and productive CMV replication *in vitro (24)*. We could thus confirm and extend these findings by presenting definite evidence for the productive infection of EC by CMV *in vivo*.

Hematogenous dissemination is a crucial step in many systemic infections. Besides leukocytes, EC are supposed to play an important role for CMV dissemination. Viremic virus can be derived from productively infected EC in the endothelia or from detached circulating EC *(22)*. It can be associated with leukocytes *(25)* that received the virus from EC, or it can move as cell-free virion. We could provide formal evidence for the contribution of EC to secondary viremia (Figure 3d).

Hepatocytes are producers of MCMV but do not disseminate the virus

Hepatocytes are prominent targets of MCMV infection in the liver as defined by immunohistology and in situ hybridization *(26)*. In order to compare the contribution of hepatocytes with that of other liver cells, mice expressing Cre in hepatocytes under control of the albumin promoter (Alb-*cre*; Postic *et al*., 1999) were infected with MCMV-flox. As shown in Figure 2B, ~70% of the total infectivity in the liver represented progeny recombined in hepatocytes, suggesting that hepatocytes are a major producer cell type. MCMV-rec was virtually absent from organs other than the liver on day 3 after infection of Alb-*cre* mice. This finding provided also the reassuring technical information that there was no ectopic expression of Cre recombinase in nonhepatic tissues under infection conditions.

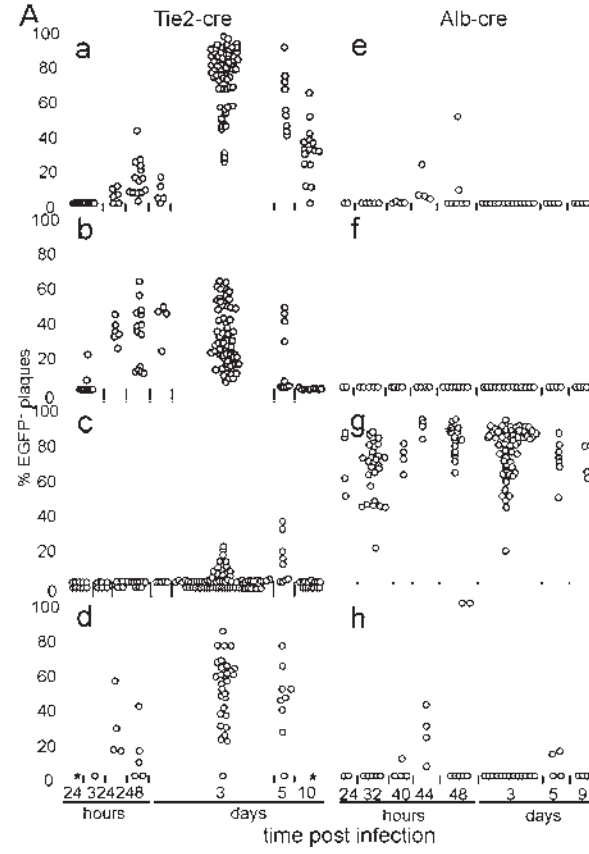

Figure 3. ***Endothelial cells and hepatocytes are primary and productive target cells of MCMV but hepatocyte derived MCMV does not colonize other organs.*** *(A) Tie2-cre (a-d) or Alb-cre (e-h) mice were infected with 106 to 5x106 PFU of MCMV-Δm157-flox-egfp and organs were harvested 24 hours to 10 days post infection. Percentage of MCMV-rec + PFU from lung (a, e), spleen (b, f), liver (c, g) and blood (d, h) was determined by plaque assay on MEF and indicated as open circles. Each open circle refers to the value of an individual mouse. Data of Alb-cre mice were derived from 16 independent experiments and data of Tie2-cre were summarized from 13 independent experiments.*

Hepatocytes are separated from the circulation by the liver endothelium. It was therefore a surprise to consistently find a high proportion of MCMV-rec in Alb-*cre* mice already 24 and 32 hours after i.v. infection (Figure 3g). This implies that MCMV crosses the liver endothelium without a prior replication cycle in EC. In conclusion, these data showed that hepatocytes are a prominent and productive, direct target for MCMV distributed via primary viremia.

EC are present in all organs whereas in the Alb-*cre* model the cellular source of recombined virus is restricted to the liver. This provided a model to study virus dissemination from organ to organ. Hepatocytes represent an early target cell type producing MCMV-rec with rapid kinetics preceding the virus peak in most other organs. The liver is also the main contributor to the total virus load in the mouse (Figure 4).

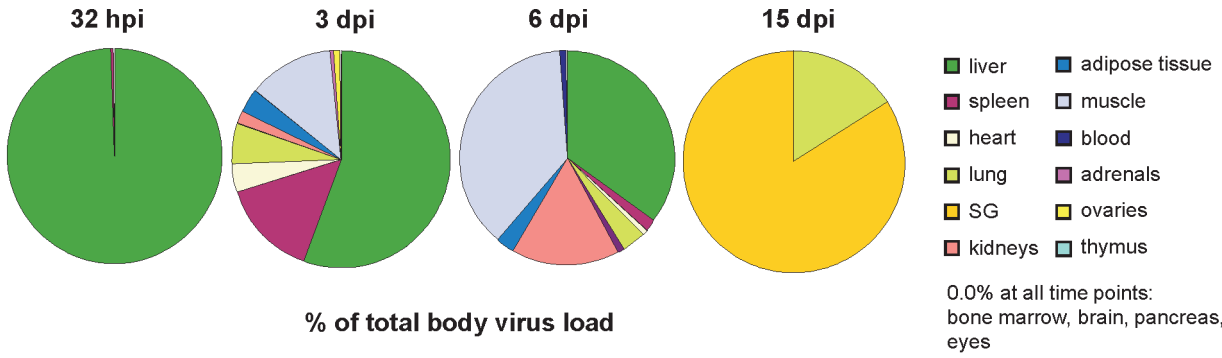

Figure 4. ***Hepatocytes are the main virus producer cells.*** *Tie2-cre an Alb-cre mice per group were infected with 106 MCMV-Δm157-flox-egfp iv and 32 hours, 3, 6 or 15 days post infection organs were harvested and total organ virus load was determined and depicted as pie chart. In the case of fat, muscle, and blood, defined tissue mass or blood volume were tested and the results were extrapolated to the total body values (fat, 15% of total body mass; muscle, 35 % of total body mass; blood, 78ml/kg). Virus load in brain and pancreas were below detection limit at all time points and are not included in the pie charts. During acute infection the liver virus load is dominant. Late during infection virus is found only in salivary gland and lung.*

However, hepatocyte-derived MCMV-rec was only occasionally and transiently released into the circulation in low amounts visible on day 2 p.i. (Figure 3h). and did not colonize spleen and lungs, kidney and other organs (Figure 3e,f) *(21)*. This finding excluded hepatocytes as a source of virus dissemination. The lack of dissemination proved to be independent of virus dose and the possibility that replication in hepatocytes alters the cell-type tropism of the virus progeny by adaptation was ruled out. Also the infection route used for the induction of experimental primary viremia was not responsible for the lack of dissemination. In conclusion, virus generated in hepatocytes was documented not to be disseminated by secondary viremia *(21)*.

Liver and spleen are regarded as major sources for virus dissemination during secondary viremia *(12)*. Our finding was even more surprising as the liver generates the bulk of infectious progeny (Figure 4). Thus, virus productivity in an organ and virus dissemination from this organ are not necessarily linked. The role of the spleen in dissemination of MCMV via secondary viremia remains an open question.

Conclusions and perspectives

Which mechanisms could account for the lack of dissemination of liver-derived MCMV? Inability of MCMV spread between cell types in the liver was not the mechanism, since hepatocyte-derived virus was found to spread efficiently to EC, and likewise, liver EC-derived virus spread to hepatocytes. Occasionally there was even some hepatocyte-derived MCMV dissemination into the blood (Figure. 3h). Thus the export block was not absolute. Yet, the hepatocyte-derived MCMV still did not colonize other organs, even after immunosuppression that increased the virus load in the liver by up to three orders of magnitude. We could also exclude a contribution of adaptive immunity of NK cells, and of radiosensitive mechanisms *(21)*.

We, therefore, assume that during primary viremia most organs are infected almost simultaneously. Accordingly, the generation of a simultaneous local innate inflammatory response to infection has to be expected in all infected tissues. Some tissues produce more progeny than others. This may contribute to secondary viremia if the anatomic position of the infected cell type provides access to the vascular system. Whether secondary viremia suffices to disseminate and to colonize other organs would then depend on the local inflammatory response. Accordingly, mainly tissues with a low inflammatory response due to or associated with low local virus production would be permissive to superinfection during secondary viremia. Experimental secondary viremia led to organ colonization only under a condition of high secondary viremia (Figure 5).

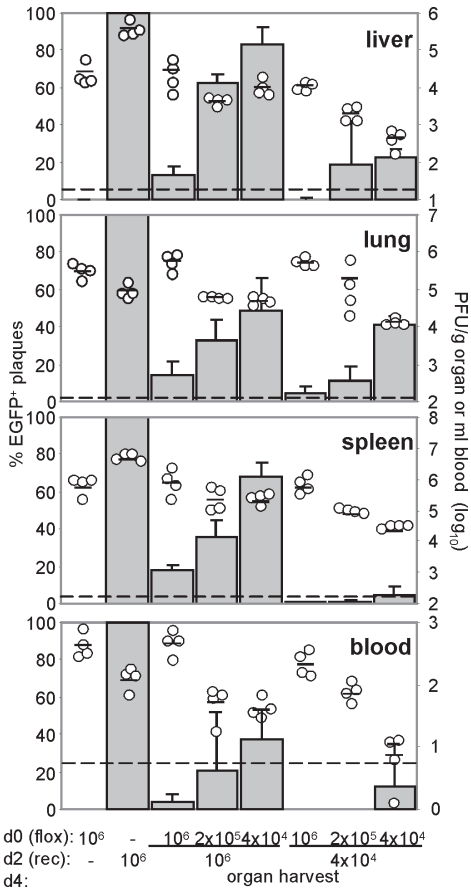

Figure 5. ***Organ colonization requires an excess of virus during secondary viremia.*** *C57BL/6 mice were infected i.v. with MCMV-flox (primary viremia) 2 days later the same mice were infected i.v. with MCMV-rec to artificially mimic secondary viremia. 4 days after the first infection organs were harvested and MCMV-flox and MCMV-rec organ loads were quantified as shown in Fig. 1C. Note that in normal C57BL/6 mice there is no recombination* in vivo. *MCMV -flox and MCMV-rec serve to demonstrate virus colonization of already infected organs.*

Under natural infection conditions this level of secondary viremia was not seen. In fact, inflammatory conditions caused by MCMV are known to affect infection by other pathogens *(27)*. Only specific situations, such as the transplantation of an infected organ into a naive recipient, a local reactivation event from latency, or a genetic deficiency in the cytokine response would predispose an individual to those variable disease and dissemination conditions as are seen in HCMV infection. It shall be attractive will be interesting to exploit the conditional approach in mice deficient for specific functions, such as defects that cause vascular diseases *(28, 29)* associated with HCMV infection.

Viruses, in particular herpesviruses encode numerous non-essential genes, regulatory sequences and also miRNA's the functions of which are poorly understood. These often constitute the major part of the viral genome. Among CMV functions that have been characterized there are genes that control cell tropism *(4, 5, 6),* i.e. apoptosis *(4, 30, 31)* the interferon response *(32),* cytokines/chemokines *(33),* the NK cell response *(34–40),* antibody function *(41),* and regulators of antigen presentation *(42)*. Often several genes code for related functions *(43)*. It appears extremely unlikely that all cell types are affected by these viral modulators in a stereotypic fashion. It appears plausible to assume that these genes govern the virus life cycle in a cell type specific fashion. Tagged virus mutants in mouse lines expressing Cre in different cell types offer new chances to study this hypothesis. We expected to find genes which predominantly act on specific tissues to support individual steps of the viral life cycle. The definition of cell type specific functions of viral genes *in vivo* will foster the understanding of virus pathogenesis.

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