ACTA
STOMATOLOGICA
CROATICA
www.ascro.net

Acta Stomatol Croat. 2007;41(1):13-22.

IZVORNI ZNANSTVENI RAD
ORIGINAL SCIENTIFIC PAPER

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Potklase salivarnog IgA i IgG kod bolesnika s oralnim Lihen planusom - Ogledna studija

Salivary IgA and IgG Subclass Levels in Patients with Oral Lichen Planus - A Pilot Study

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Sažetak
Oralni lihen planus (OLP) kronična je upalna bolest koju karakterizira imunoreaktivnost usmjerena prema bazalnim keratinocitima i posredovana je T-limfocitima. Svrha istraživanja bila je odrediti potklase salivarnog imunoglobulina A1 (IgA1) i IgA2 te IgG1,2,3,4 kod bolesnika s retikularnim oblikom OLP-a tijekom akutne faze i remisije te u kontrolnoj skupini. U ukupnoj nestimuliranoj slini kod 19 bolesnika s OLP-om u dobi od 30 do 72 godine, srednje dobi od 58 godina, u akutnoj fazi i tijekom remisije te kod 21 kontrolnog ispitanika u dobi od 20 do 52 godine, srednje dobi od 35 godina, određene su potklase salivarnog IgA-a radijalnom imunodifuzijom i potklase salivarnog IgG-a uz pomoć enzimskog imunotesta. Između bolesnika u akutnoj fazi i kontrolnih ispitanika nije bilo znatnih razlika u IgG-u1 i 2 IgA-a1 te IgA-a2 ($p>0,05$). Bolesnici u akutnoj fazi imali su znatno veće vrijednosti IgG-a3 i IgG-a4 te proteina sline ($p=0,021$; $p=0,004$; $p=0,029$) u odnosu prema kontrolnoj skupini. Između bolesnika u akutnoj fazi i u remisiji nije bilo većih razlika u vrijednostima IgG-a1,2,3,4 i IgA-a1, a znatno su bile povišene vrijednosti IgA-a2 ustanovljene u akutnoj fazi ($p=0,049$). Između bolesnika u fazi remisije i kontrolne skupine nije bilo razlika ni u jednoj salivarnoj potklasi - bilo IgA-a bilo IgG-a ($p>0,05$). Iz svega navedenoga može se zaključiti da u akutnoj fazi raste IgA2, što bi mogao biti utjecaj pojačane aktivnosti sekretorne imunosti, a možda je i posljedica mikrobne stimulacije koja se vidi u akutnoj fazi lihena u odnosu prema fazi remisije.

Zaprimljen: 20. rujna 2006.
Prihvaćen: 2. veljače 2007.

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Ključne riječi
slina; imunoglobulin G; imunoglobulin A; lichen planus

Uvod

Oralni lihen planus (OLP) jedna je od najčešćih dermatoza te može zahvatiti i sluznicu usne šuplјine, a javlja se u nekoliko različitih oblika. Uzrok OLP-a je nepoznat, ali ispitivanja pokazuju da imunološke reakcije imaju važnu ulogu (1). Dokazano je da se u patogenezi lihena događa proces koji posreduju T-stanice, ponajviše zato što tkivo lihena sadržava lokalni infiltrat aktiviranih T-limfocita

Introduction

Oral lichen planus (OLP) is one of the most common dermatoses affecting the oral mucosa, which may appear in different forms. The cause of OLP is still not clear, but studies indicate that immunologic reactions may play a significant role (1). There is a substantial evidence that the pathogenesis of lichen planus (LP) involves a T-cell mediated process, particularly as LP lesional tissue exhibits a local

zajedno s povećanom ekspresijom citokina i promijenjenom ekspresijom adhezijskih molekula. Osim toga terapija usmjerena prema supresiji imunog odgovora koji je stanično posredovan, smanjuje limfocitni infiltrat i dovodi do kliničkog poboljšanja promjena vezanih za lihen. Ima dokaza da humoralna imunost također ima svoj udjel u nastanku i perpetuaciji lihena. IgM, IgG, IgA, fibrin, fibrinogen, C3 i C4 te C5 mogu biti u zoni bazalne membrane unutar promjena i oko promjena tkiva idiopatskog lihena (2). Femiano i suradnici (3) predložili su teoriju prema kojoj je ključni događaj u nastanku lihena promjena površinskog antigena na bazalnim keratinocitima koju vlastiti imuni sustav ne prepoznaje, pa počinje lančana reakcija koja završava uništenjem keratinocita. Ispitivanja serumskih imunoglobulina i analize tkiva, uz uporabu imunofluorescentnih tehnika, monoklonalnih protutijela i imunokemije, upozorila su na imunološku disfunkciju u patogenezi lihen planusa (4). Imunoglobulin G, A i M, zatim C3 te fibrinogen, mogu se naći deponirani u području bazalne membrane u lihenu. Ipak, svi ti proteini upozoravaju na nespecifičnu poliklonalnu reakciju povezanu s vaskularnom ozljedom (5). Serumske vrijednosti IgG-a znatno su povišene kod osoba s virusom hepatitisa C (HCV) infekcijom i lihenom u usporedbi sa skupinom koja je imala samo lihen te s kontrolnom skupinom, iako je prosječna vrijednost skupine HCV-a također bila povišena u usporedbi s kontrolnom skupinom. Koristeći se direktnom imunofluorescencijom, autori ipak nisu otkrili depozite protutijela unutar bilo kojeg tkiva samog lihena ili lihena povezanog s HCV-om, što bi moglo upućivati na to kako je malo vjerojatno da lokalizirani humoralni imuni odgovor ima glavnu ulogu u patogenezi bilo kojeg oblika bolesti (6). Outschoorn i suradnici sugerirali su kako promjene u potklasama IgG-a mogu odražavati poremećaj u imunoregulatornoj aktivnosti i da su te promjene u potklasama imunoglobulina specifične za svaku bolest. Također se pretpostavlja da promijenjene vrijednosti potklasa IgG-a mogu odražavati mikrobnu antigenu stimulaciju u usnoj šupljini (8). Dosad je dokazano da su salivarne potklase IgA-a i IgG-a bile povećane kod bolesnika s OLP-om, ali na malom broju ispitanika. Nadalje, pretpostavljalo se kako potklase IgG-a mogu reflektirati stadij bolesti, tj. akutnu fazu nasuprot razdoblju remisije – takav su primjer bolesnici s buloznim pemfigoidom (9). Ipak, kod oboljelih od OLP-a nije bilo istraživanja koja bi se odnosila na mjerenje tih potklasa u akutnoj fazi i u fazi remisije. Svrha ovog ispitivanja bila je odrediti pot-

infiltrate of activated T lymphocytes, with increased local expression of cytokines and altered expression of adhesion molecules. Furthermore, therapies that suppress cell-mediated immune response reduce the lymphocyte infiltrate and cause clinical improvement of LP lesions. Some evidence indicates that humoral immunity may also play a part in the development and perpetuation of LP. IgM, IgG, IgA, fibrin, fibrinogen, C3, C4 and C5 may be present at the basement membrane zone within lesional and perilesional tissue of idiopathic LP (2). Femiano et al. (3) as well as others have proposed that the key event in the pathogenesis of OLP is a surface antigen change on the basal keratinocytes, which is not recognized by one's own immune system and consequently starts a chain reaction which finishes in the destruction of the keratinocytes themselves. Serum immunoglobulin studies and tissue analyses utilizing immunofluorescent techniques, monoclonal antibodies, and immunochemistry have implicated immunological dysfunction in the pathogenesis of lichen planus (4). The immunoglobulin G, A, and M, C3 and fibrinogen all may be found variably deposited in the basement membrane zone in lichen planus. However, the presence of all such proteins suggests a nonspecific polyclonal reaction associated with vascular injury (5). Serum IgG levels were significantly elevated in the HCV (hepatitis C virus)-associated OLP group compared with the OLP and normal groups, although the average level of the HCV group was also elevated compared with controls. These results indicate that while increased serum IgG is apparently associated with HCV infection, serum IgG levels are nevertheless higher in patients with OLP as well as HCV. Using direct immunofluorescence, however, authors did not detect any deposition of antibodies within any of the OLP and HCV-associated OLP lesional tissues examined, suggesting that a localised humoral immune response is unlikely to play a major role in the pathogenesis of either form of the disease (6).

Outschoorn et al. (7) suggested that alterations in IgG subclasses may reflect the underlying immunoregulatory dysfunction in autoimmunity and that these immunoglobulin subclass alterations are disease specific. Also, it has been postulated that altered IgG subclass levels might reflect microbial antigenic stimulation in the oral cavity (8). So far, salivary IgA and IgG subclasses have been shown to be increased in patients with OLP, but on a small number of patients. Furthermore, it has been postulated that IgG subclasses might reflect disease stage, i.e. acute

klase salivarnog imunoglobulina A i G u ukupnoj nestimuliranoj slini bolesnika s oralnim lihen planusom u akutnoj fazi i tijekom remisije, kako bi se ustanovio točan profil tih parametara u slini te odredilo postoje li bilo kakve razlike u vrijednosti potklasa s obzirom na stadij bolesti te u odnosu prema kontrolnoj skupini. Također se očekivalo da će potklase salivarnog IgA-a i IgG-a biti povišene u akutnoj fazi oboljelih od OLP-a u odnosu prema fazi remisije i kontrolnoj skupini.

Ispitanci i postupci

Od svakog je ispitanika, prije nego što je uključen u istraživanje, dobiven informirani pristanak u skladu s Helsinškom deklaracijom II. Sudionike istraživanja pregledao je samo jedan specijalist oralne medicine. Ispitna skupina sastojala se od 21 ispitanika s retikularnim oblikom lihena u akutnoj fazi (hiperkeratoza s upalom koja se očitovala kao crvenilo sluznice) i remisiji (hiperkeratoza, ali bez upale). U istraživanje su uključeni samo bolesnici s bilateralnim bukalnim lezijama OLP-a (ne-erozivnima) s eritemom i hiperkeratozom, a zahvaćale su područje između 3 i 9 cm2 (strane 1x3 cm do 3x3 cm2, što je mjereno parodontalnom sondom). Svi su bili upućeni na naš Zavod prije početka bilo kakvog liječenja. Razlika između dviju faza određena je na temelju kliničke slike. Skupinu bolesnika činilo je 9 muškaraca i 12 žena u dobi između 30 i 72 godine - srednje dobi od 58 godina. Od ukupno 19 bolesnika, samo 7 nije uzimalo nikakve lijekove, 7 ih je uzimalo antihipertenzive, 8 antacide, a jedan je bio na inzulinu. Dijagnoza lihena potvrđena je patohistološkim nalazom. Kontrolna skupina sastojala se od 21 ispitanika i oni nisu imali ni oralne ni sistemske bolesti te dva mjeseca prije uključivanja u istraživanje nisu uzimali lijekove. Kontrolna se skupina sastojala od 10 žena i 11 muškaraca u dobi od 20 do 52 godine - srednje dobi od 35 godina. Kontrolnu skupinu činili su zdravi zaposlenici našeg Zavoda, studenti i laboratorijsko osoblje. Ni jedan od ispitanika nije odustao od sudjelovanja u istraživanju, ali od dvoje bolesnika nismo dobili slinu u stadiju remisije, pa je ukupan broj bolesnika s OLP-om bio 19.

phase versus remission period, as for exemple seen in patients with bullous pemphigoid (9). However, there are no published studies regarding IgA or IgG subclass switching in OLP patients during the acute stage and remission period.

The aim of our study was to determine salivary immunoglobulin A and G subclasses in the whole resting saliva in patients with oral lichen planus in acute stage as well as during remission period in order to assess exact profile of these parameters in saliva and to evaluate whether there are any differences in the subclasses levels with regard to the stage of the disease and to compare them with healthy controls. Increased salivary IgA and IgG subclasses are expected in patients with OLP during acute stage in comparison to the ones during remission period as well as to the healthy controls.

Material and Methods

Prior to investigation informed consent according to Helsinki II was obtained from each participant. All the participants were examined by the same clinician. The study population consisted of 21 patients with reticular form of OLP in acute phase (hyperkeratosis with inflammation, seen as redness of the mucosae) and during remission period (hyperkeratosis but no inflammation). Only patients with bilateral buccal (non-erosive) OLP lesions with erythema and hyperkeratosis affecting areas between 3-9 cm^2 (sides 1x3 cm to 3x3 cm^2, as measured by periodontal probe) were randomly recruited, before any therapy was given to them. The difference between two phases was established on the basis of clinical appearance. In the patient group there were 9 males and 12 females, age range 30-72, mean 58 years. Out of 19, only 7 patients did not take any medication, wheras 7 were taking antihypertensives, 8 antacids, 1 was taking insuline. Diagnosis of OLP was confirmed histopatologically. Control group consisted of 21 participants who were our department's healthy stuff members, students and laboratory stuff, free of any oral or systemic disease, who were not taking any medication 2 months prior to this investigation. Control group consisted of 10 females and 11 males, age range 20-52, mean 35 years. None of the participants declined to participate. Two out of initial patients with OLP were not taken into account as we did not obtaine their saliva samples during remission period.

Skupljanje sline

Postupak su opisali Wu-Wang i suradnici (10). Kako bi se izbjegle dnevne varijacije, slina svih ispitanika skupljala se od 10 prijepodne do 13 sati. Svi sudionici zadržavali su slinu u ustima 5 minuta bez gutanja i zatim su ju ispljunuli u čistu plastičnu epruvetu. Određena je bila i količina sline. Uzorci su odmah pohranjeni u zamrzivač na -20 stupnjeva C te su se tamo čuvali do početka analize.

Određivanje podrazreda IgG-a

Koncentracije podrazreda salivarnog IgG-a (IgG1,2,3,4) određivale su se "sendvič"-enzimskim imunotestom (Human IgG subclass Combi EIA kit s 4x8 WELL STRIPS, The Binding Site, Velika Britanija) čija je varijabilnost unutar testa ("intra-assay") bila između 2,61 i 6,03%, između testova ("inter-assay") između 5,16 i 9,54%, a osjetljivost, redom: 0,42, 7,35, 0,29 i 0,44 μg/l za IgG1, IgG2, IgG3 i IgG4. Uzorci sline razrijeđeni su 1:100 za određivanje IgG-a1 i 1:25 za IgG2, -3 i -4. Odgovarajuće razrijeđeni standardi i uzorci dodani su u bunariće mikrotitarske pločice, prije toga obložene specifičnim antitijelima na ljudski IgG1, -2, -3 i -4. Nakon ispiranja, kako bi se uklonili nevezani proteini, dodan je pročišćeni peroksidazom označen ovčji konjugat antihumanog IgG-a. Nevezani konjugat uklonjen je ispiranjem, a vezani je vizualiziran dodavanjem supstrata 3,3´,5,5´-tetrametilbenzidina koji daje plavi reakcijski proizvod. Optička se gustoća očitavala čitačem ELISA za mikrotitarske pločice Dynatech MRX pri 450 nm, a konačne su koncentracije imunoglobulina izražene u μg/L sline, uzimajući u obzir razrjeđenje uzoraka sline.

Određivanje podrazreda IgA-a

Koncentracije podrazreda salivarnog IgA-a (IgA1 i IgA2) određivale su se radijalnom imunodifuzijom (Human IgA subclass NL Combi RID Kit, The Binding Site, Velika Britanija). Odgovarajući volumeni (2x5 μl za IgA1 i 10 μl za IgA2) nerazrijeđene sline te odgovarajuće razrijeđen standard i kontrolni uzorak pipetirani su u bunariće ploče za imunodifuziju u agaroznom gelu, koji sadržava odgovarajuće monospecifično antitijelo. Ploče su zatim ponovno zatvorene u originalnu ambalažu i inkubirane u vlažnoj komori. Nakon 96 sati inkubacije na sobnoj temperaturi, draguljarskim se povećalom mjerio promjer precipitacijskog kruga s točnošću od 0,1 mm. Koncentracije imunoglobulina očitane su iz priložene referentne tablice i izražene u μg/L sli-

Saliva collection

The procedure was as described by Wu-Wang et al.(10). To avoid daily variations, the saliva sample was collected between 10 a.m. and 1 p.m. for all volunteers. All participants were instructed to collect saliva in their mouths for 5 min without swallowing and to spit into a clean plastic container. Salivary flow rate was determined. The pooled samples were immediately placed in a -20ºC freezer until ready for analysis.

Determination of IgG subclasses

Salivary IgG subclasses (IgG1,2,3,4) were determined by the "sandwich" enzyme immunoassay (Human IgG subclass Combi EIA kit with 4x8 well strips for each subclass, The Binding Site, England) having intra-assay variability between 2.61 and 6.03% and inter-assay variability between 5.16 and 9.54% and with analytical sensitivity of 0.42, 7.35, 0.29, and 0.44 µg/L for IgG1, IgG2, IgG3 and IgG4, respectively. Saliva samples were diluted 1:100 for IgG1 and 1:25 for other subclasses determinations. Appropriately diluted calibrators and samples were added to the microplate wells pre-coated with specific antibodies to human IgG1, IgG2, IgG3 and IgG4, respectively. After washing to remove the unbound proteins, purified peroxidase labelled sheep anti-human IgG conjugate was added. The unbound conjugate was removed by washing and the bound conjugate was visualised by 3,3', 5,5'-tetramethylbenzidine substrate which gives a blue reaction product. The optical densities were read by the Dynatech MRX Microplate Reader at 450 nm, the final subclass concentrations were calculated according to the dilutions of saliva samples and expressed in µg/L of saliva.

Determination of IgA subclasses

Salivary IgA subclasses (IgA1 and IgA2) were determined by radial immunodiffusion (Human IgA subclass NL Combi RID Kit, The Binding Site, England). Appropriate volumes (2x5 µl for IgA1 and 10 µl for IgA2) of nondiluted saliva as well as of appropriately diluted calibrator and control sample were placed into agarose gel immunodiffusion plates containing the appropriate monospecific antibody. The plates were then tightly closed, re-sealed in their original pouches and placed in the moist chamber. After 96 hour incubation at room temperature the precipitation ring diameters were measured to the nearest 0.1 mm using a jewellers eyepiece, the antibody concentrations were read from the reference table enclosed, and expressed in µg/L of sa-

ne, uzimajući u obzir dvostruki volumen uzorka koji se koristio za određivanje IgA-a1. Promjeri precipitacijskih krugova standarda i kontrolnog seruma uvijek su bili u očekivanom rasponu, tj. njihove su koncentracije uvijek bile u sklopu 10% koncentracija navedenih na naljepnici bočica.

Proteini su određeni prema Bradfordovoj metodi (11).

Statistička analiza obavljena je statističkim softverom SPSS 10,0 za Windowse. Također je napravljena deskriptivna statistika i normalnost distribucije, a testirana je jednosmjernim Kolmogorov-Smirnovim testom. Budući da je test pokazao da su podaci normalno distribuirani, analiza podataka obavljena je parametrijskim testovima. Analiza varijance (ANOVA) i usporedbe parova (post hoc Scheffeov test) koristile su se kako bi se ispitale razlike u potklasama salivarnih imunoglobulina A i G te proteina između različitih skupina ispitanika. P vrijednosti od 0,05 smatrale su se znatnima.

Rezultati

U vrijednosti salivarnih potklasa IgG-a1 i IgG-a2 između bolesnika u akutnoj fazi i kontrolnoj skupini nije bilo znatnih razlika (p=0,952 i p=0,207). Između bolesnika u akutnoj fazi i kontrolnoj skupini postojale su razlike u vrijednostima IgG-a3 i IgG-a4 (p=0,021 i p=0,004). Zatim, nije bilo razlika u vrijednostima salivarnih potklasa IgA-a1 i IgA-a2 između bolesnika u akutnoj fazi i kontrolnoj skupini (p>0,05). Također su ustanovljene znatne razlike u proteinima sline između bolesnika u akutnoj fazi i kontrolnoj skupini (p=0,029).

Između bolesnika u akutnoj fazi i fazi remisije nije bilo znatnih razlika u vrijednostima IgG-a1,2,3,4 i IgA-a1. Znatne razlike između bolesnika u akutnoj fazi i u fazi remisije nađene su u vrijednostima IgA-a2 (p=0,049).

Između bolesnika u fazi remisije i kontrolne skupine nije bilo razlika ni u jednoj salivarnoj potklasi, bilo IgA-a ili IgG-a (p>0,05).

Na kraju nije bilo statistički znatnih razlika u količini izlučene sline (p>0,05).

liva, taking into account the double sample volume used for IgA1 determination and the fact that all saliva samples were nondiluted. The ring diameters of the calibrator and control serum were always in the expected range, i.e. their concentrations were always within 10% of the concentrations stated on the vial label.

Salivary proteins were determined according to the Bradford (11).

Statistical analysis was performed by means of statistical software SPSS 10.0 for Windows. Additionally descriptive statistics was made and normality of distribution was tested by one-way Kolmogorov-Smirnov test. As the test showed that the data were normally distributed, subsequent data analysis was done with parametric tests. Analysis of variance (ANOVA) and pair comparisons (post hoc Scheffe's test) were used in order to examine differences in salivary immunoglobulin subclasses A and G levels as well as proteins between different groups of participants. P values 0,05 were considered as significant.

Results

There were no significant differences in salivary IgG1 and IgG2 subclasses (p=0,952 i p=0,207) between patients in acute phase and controls. Significant differences in salivary IgG3, IgG4 and proteins were found between patients in acute phase and controls (p=0,021; p=0,004; p=0,0029). Furthermore, there were no significant differences in salivary IgA1 and IgA2 subclasses between patients in acute phase and controls (p>0,05). Between patients in acute phase and ones in the remission period there were no significant differences in salivary IgG1,2,3,4 i IgA1 subclasses, however significant difference was found in IgA2 levels (p=0,049).

In patients during remission period and controls there were no significant differences in any salivary IgA or IgG subclass levels (p>0,05).

There were no significant differences between the tested groups regarding quantity of saliva (p>0,05).

Tablica 1. Potklase salivarnog imunoglobulina G 1,2,3,4 i A1 i 2, zajedno s proteinima sline kod bolesnika s akutnim oralnim lihen planusom tijekom faze remisije i u kontrolnoj skupini.
Table 1 Salivary immunoglobulin G1-4, and A1-2 subclasses together with proteins in patients with acute oral lichen planus, during remission period and in the controls.

		Broj • N	Sr. vrijed. • Mean	St. dev. • SD	St. pog. • SE	95% intervali pouzdanosti • 95% Confidence interval for Mean		Min.	Max.
						Donja granica • Lower bound	Gornja granica • Upper bound		
IgG1	akutna • acute	19	53,95	42,46	9,74	33,48	74,41	5,38	153,80
	remisija • remission	19	23,80	13,00	2,98	17,54	30,07	4,39	53,30
	kontrole • control	21	48,42	82,85	18,08	10,71	86,13	3,80	345,80
IgG2	akutna • acute	19	336,10	293,67	67,37	194,56	477,65	64,57	1038,20
	remisija • remission	19	280,50	221,64	50,85	173,67	387,33	14,00	908,50
	kontrole • control	21	185,57	271,56	59,26	61,96	309,19	12,50	1111,30
IgG3	akutna • acute	19	41,38	42,59	9,77	20,85	61,91	0,56	176,83
	remisija • remission	19	29,24	22,03	5,05	18,62	39,86	3,14	95,62
	kontrole • control	21	15,00	16,94	3,70	7,28	22,71	1,20	50,40
IgG4	akutna • acute	19	84,46	94,82	21,75	38,75	130,16	0,00	306,58
	remisija • remission	19	41,19	40,08	9,20	21,87	60,51	4,26	118,50
	kontrole • control	21	18,34	19,30	4,21	9,55	27,12	1,30	73,80
IgA1	akutna • acute	19	205,16	310,37	71,20	55,57	354,76	32,00	1294,60
	remisija • remission	19	82,31	47,56	10,91	59,39	105,24	12,95	192,16
	kontrole • control	21	91,34	52,53	11,46	67,43	115,25	37,50	247,50
IgA2	akutna • acute	19	211,49	171,82	39,42	128,68	294,31	51,96	649,20
	remisija • remission	19	119,22	70,80	16,24	85,09	153,34	33,58	284,40
	kontrole • control	21	122,29	66,17	14,44	92,17	152,41	50,00	339,60
Proteini • Proteins	akutna • acute	19	1,25	0,69	0,16	0,91	1,58	0,26	2,55
	remisija • remission	19	1,11	0,67	0,15	0,78	1,43	0,10	2,37
	kontrole • control	21	0,75	0,31	0,07	0,61	0,89	0,24	1,36

N-number of subjects; SD-standard deviation; SE-standard error

Tablica 2. Usporedba potklasa salivarnih imunoglobulina G1,2,3,4 i A1 i 2 te proteina sline u trima ispitivanim skupinama (ANOVA).
Table 2 Statistical analysis (ANOVA) of salivary immunoglobulin G1-4, and A1-2 subclasses and proteins.

	Zbroj kvadrata odstupanja • Sum of Squares	Stupanj slobode • Degrees of freedom	Srednji kvadrat odstupanja • Mean Square	F-vrijednost • F-value	Razina znatnosti • Significance
IgG1	9863,41	2	4931,70	1,59	,211
IgG2	233099,88	2	116549,94	1,66	,198
IgG3	6980,06	2	3490,03	4,14	,021
IgG4	44548,97	2	22274,48	6,29	,003
IgA1	180507,05	2	90253,52	2,76	,072
IgA2	105977,79	2	52988,89	4,18	,020
Proteini • Proteins	2,69	2	1,34	4,04	,023

Tablica 3. Usporedba potklasa salivarnih imunoglobulina G1,2,3,4 i A1 i A2 te proteina sline kod bolesnika s akutnim oralnim lihen planusom, s nalazom u fazi remisije i s kontrolnom skupinom (post hoc Scheffeov test).
Table 3 Comparison of salivary immunoglobulin G1-4, and A1-2 subclasses together with proteins in patients with acute oral lichen planus, during remission period and in the controls performed with Scheffe's test.

			Srednja pogreška • SE	Znatnost • Sig	95% intervali pouzdanosti • 95% Confidence interval	
					Donja granica • Lower bound	Gornja granica • Upper bound
IgG1	akutna • acute	remisija • remission kontrole • control	18,0211 17,5868	,255 ,952	-15,17 -38,69	75,4598 49,7517
IgG2	akutna • acute	remisija • remission kontrole • control	85,7468 83,6804	,811 ,207	-160,02 -59,90	271,2261 360,9618
IgG3	akutna • acute	remisija • remission kontrole • control	9,4122 9,1854	,441 ,021	-11,53 3,28	35,8047 49,4796
IgG4	akutna • acute	remisija • remission kontrole • control	19,3023 18,8371	,090 ,004	-5,2727 18,7479	91,8064 113,4874
IgA1	akutna • acute	remisija • remission kontrole • control	58,6480 57,2345	,121 ,148	-24,63 -30,10	270,3295 257,7518
IgA2	akutna • acute	remisija • remission kontrole • control	36,5122 35,6322	,049 ,051	,4597 -,400	184,0940 178,8087
Proteini • Proteins	akutna • acute	remisija • remission kontrole • control	,1871 ,1826	,755 ,029	-,330 -,041	,6111 ,9598

SE-standard error; sig.-significance

Rasprava

Uloga salivarnog Ig-a, posebice potklasa salivarnih imunoglobulina u imunopatogenezi i kliničkom tijeku oralnih bolesti, nije sustavno ispitana. Dosadašnji podaci o serumskim imunoglobulinima u lihenu kontroverzni su i primarno se sastoje od podataka na bolesnicima s lihenom kože, dok su skromni podaci o salivarnim imunoglobulinima, posebice u potklasama IgA i IgG. Mahood (12) je izvijestio da postoji vjerojatno manji poremećaj u serumskim imunoglobulinima tih bolesnika koji nestaje kako se koža oporavlja. Isti autor je ustanovio jasno povišenje serumskog IgG-a i IgM-a, s manje znatnim povišenjem IgA-a. Lundström (13) je istaknuo da su srednje vrijednosti serumskog IgG-a bile povišene kod bolesnika s lihenom u usporedbi s kontrolnom skupinom, a nije bilo znatnih razlika u serumskim IgA-u i IgM-u. Ono što se vidi kod većine bolesnika s lihenom jest blagi do umjereni poliklonski porast IgG-a koji nije tipičan za bilo koju drugu dosad poznatu bolest. Kao sekundarni efekt, stanicama posredovani antigeni i citotoksične reakcije, mogu utjecati na humoralnu imunost i tada dovesti do povišenja uglavnom IgG-a. Gandolfo i suradnici (14) izvijestili su o povećanju prosječnih vrijednosti IgG-a u serumu bolesnika s atrofično-erozivnim oblikom OLP-a u usporedbi s bolesnicima s retikularnim oblikom OLP-a, te tako potvrđuju ulogu humoralne imunosti u patogenezi OLP-a. Griffith i suradnici (15) predložili su da povišena razina se-

Discussion

The role of salivary immunoglobulins, especially immunoglobulin subclasses in the immunopathogenesis and the clinical course of oral mucosal diseases, have not been yet studied comprehensively. So far, data on serum immunoglobulins in LP are controversial and concern primarily patients with skin lesions, and data upon salivary immunoglobulins, especially IgA and IgG subclasses are sparse. Mahood (12) reported that there is some, probably minor, disturbance of serum immunoglobulins which reverts to normal as the skin recovers. The same author found clear rise in serum IgG and IgM levels, with less significant rise in IgA levels. Lundström (13) found that mean levels of serum IgG were elevated in the OLP patients when compared to the control group together, but there were no significant differences in serum IgA and IgM. The pattern seen in most of the OLP patients with immunological changes is slight to moderate polyclonal increase of IgG which is not typical for any specific disease known hitherto. As a secondary effect cell-mediated antigenic and cytotoxic reactions may also influence the humoral immunity and then lead mainly to elevations of serum IgG. Gandolfo et al. (14) reported increase in the average serum IgG level in patients with atrophic-erosive form of OLP when compared to the patients with reticular form of OLP, confirming the role of humoral immunity in OLP pathogenesis. Griffith et al. (15) suggested that

rumskog IgG-a može predstavljati sekundarnu infekciju tijekom erozije sluznice, a Lundström (13) smatra da se radi o stalnoj autogenoj proizvodnji topljivih antigena. Rabinovich i suradnici (16) također su ustanovili povišenu razinu serumskog IgG-a i ona je bila gotovo statistički znatna kod bolesnika s OLP-om te su zaključili da je uloga humoralne imunosti u patogenezi OLP-a vjerojatno sekundarna na reakciju protiv bazalnih keratinocita koja je posredovana stanicama. Mi smo pretpostavili da povišenje serumskog IgG-a, koje je ustanovljeno u drugim istraživanjima, može biti objašnjenje za povišene vrijednosti salivarnog IgG-a u našoj studiji. Ipak, simultano određivanje IgG-a u slini i serumu dalo bi precizniji odgovor. Neki autori, poput Sistiga i suradnika (8) te Lozade-Nura i Mirande (17) istaknuli su važnost IgG-a u imunosti sluznica. Smatra se da IgG nađen u slini većinom dolazi iz seruma te da se prenosi pasivnom difuzijom kroz sluznice. Možemo pretpostaviti da povišena razina salivarnog IgG-a može dolaziti iz seruma. Možemo također pretpostaviti da je sistemski imuni odgovor koji je vođen uglavnom kroz IgG potaknut kod bolesnika s OLP-om. Malo je vjerojatno da je sistemska infekcija potaknula preferencijski porast u potklasama IgG-a, zato što ni jedan od bolesnika nije imao sistemsku infekciju. Također je malo vjerojatno da je lokalna infekcija dovela do povišenja IgG-a, jer naši bolesnici nisu imali lokalne infekcije. Čini se da je fiziološka uloga potklasa IgG-a općenito nepoznata, osim što se zna da blokira protutijela u alergijskim reakcijama (18). IgG4 obično reagira na alergene, posebice polisaharide. Također se smatra da IgG4 može djelovati kao patognomično protutijelo u tkivima i da su njegove potklase povišene u tkivu drugih tkivnih dermatoza, poput onih pemfigus vulgaris, folijaceus, bulozni pemfigoid, pemfigoid mukoznih membrana i lihen sklerozus (9,18-21). Na kraju su neki autori pretpostavili kako promjene u potklasama IgG-a mogu odražavati aktivnost bolesti, kao što je akutni stadij nasuprot razdoblju remisije kao što se vidi kod bolesnika s buloznim pemfigoidom kod kojih IgG1 prelazi na IgG4 (19). Ipak, kod naših ispitanika s oralnim lihen planusom nije bilo razlika ni u jednoj potklasi IgG-a između bolesnika u akutnoj fazi i onih u remisiji.

Crago i suradnici (22) te Brown i Mestecky (23) pretpostavljaju da promjene u koncentraciji IgA-a1 i/ili IgA-a2 mogu biti posljedica različite antigene stimulacije u usnoj šupljini koja potiče ili IgA1 ili IgA2 da proizvode protutijela iz plazma stanica.

Iz svega navedenoga može se zaključiti da u

elevated serum level of IgG may represent a secondary oral infection during mucosal erosion, while Lundström (13) suggests that it might represent a continous autogenous production of soluble antigens. Rabinovich et al. (16) also found increased levels of serum IgG approaching statistical significance in patients with OLP and concluded that the role of humoral immunity in the pathogenesis of OLP is probably secondary to the cell mediated reaction against basal keratinocytes. We assumed that the rise in serum IgG found in other studies could be the explanation for increased salivary levels of IgG subclasses in our study. However, simultaneous determination of IgG in saliva and sera would more precisely solve the problem in the future. Some authors (8,17) advocated an important role of IgG in mucosal immunity. IgG found in saliva has been reported to originate mainly from serum and is transported by passive transmucosal diffusion. We can only speculate that the increased levels of salivary IgG 2,3,4 subclasses in both phases of the disease could be a result of increased IgG levels in serum of patients with OLP. At this point we can assume that a systemic immune response conducted mainly through IgG is triggered in patients with OLP. It is very unlikely that a systemic infection, which otherwise might give a preferential rise in IgG subclasses, is responsible for these differences seen in our patients with OLP, because at the time of this investigation none of the patients with OLP had any systemic infection. Also it is very unlikely that local infection would give rise to IgG 2, 3, 4 subclass levels as our patients were free of any oral infections. So far it seems that the physiological role of the IgG subclasses in general is unknown, except their role of blocking the antibodies in allergic reactions (18). IgG4 usually responds to allergens, especially to polysaccharides. Also, it has been postulated that IgG4 can act as a pathogenic antibody in the tissues and its subclasses are elevated in the tissues of other similar dermatoses such as pemphigus vulgaris, foliaceus, bullous pemphigoid, mucous membrane pemphigoid and lichen sclerosus (9, 18-21). Finally, some authors postulated that changes in IgG subclasses might reflect disease activity such as acute stage *versus* remission period as it was seen in bullous pemphigoid patients where switching from IgG1 to IgG4 occurs (19).

However, in our participants with oral lichen planus there were no differences in any IgG subclass levels between the patients in the acute phase and the ones in the remission period. Crago et al. (22)

akutnoj fazi raste IgA2, što bi mogao biti utjecaj pojačane aktivnosti sekretorne imunosti, a možda i posljedica mikrobne stimulacije u akutnoj fazi lihena u odnosu prema fazi remisije. Na kraju treba istaknuti da su na rezultate ovog istraživanja utjecali moguća prisutnost parodontitisa, dobna razlika između ispitne i kontrolne skupine, pušenje te prisutnost mikroba. Budući da nismo imali referentne vrijednosti normalnih salivarnih obiju potklasa, kontrolnu skupinu činili su zdravi mlađi ljudi. Vjerojatno su najvažniji čimbenik koji ima utjecaja na rezultate u obliku povišenja IgA-a2 mikrobne stimulacije. Nažalost to u ovom istraživanju nije učinjeno i svakako bi u budućima i taj čimbenik trebalo uzeti u obzir. Isto tako velika standardna devijacija i 95%-interval pouzdanosti pokazuju da su ispitivane skupine premale, ali je ovo ispitivanje obavljeno kao ogledna studija kako bi se ispitale razlike u tim skupinama.

as well as Brown and Mestecky (23) suggested that changes in IgA1 i/ili IgA2 might result from different antigenous stimulation in the oral cavity which triggers either IgA1 or IgA2 antibody production from plasma cells. We might conclude that acute phase is characterized with increase in IgA2 which might reflect microbial stimulation seen in acute phase in comparison to the remission period. Finally, this study's possible confounding factors that could bias the obtained results were possible presence of periodontitis, age difference between OLP group and controls, smoking status, as well as microbial presence. As we did not have any reference values regarding normal salivary Ig levels, we recruited the control group from healthy younger adults. Probably the most important possible confounding factor in elevation of IgA2 could be the microbial stimulation. Unfortunately, assessment of microbiological load and diversity has not been studied in this study and of course this would then clearly show whether rise in certain subclass was a result of microbial preferential stimulation. Also, SD and 95% mean confidence intervals are large suggesting the study groups were too small, but this study was designed as a pilot study in order to see whether any differences exist at all.

Zahvala

Autori su zahvalni gđi Blaženki Ladika-Davidović koja im je izradila enzimski imunotest.

Acknowledgment

The authors are thankful to Ms. Blazenka Ladika-Davidović, MS, for her excellence in performing ELISA.

Abstract

Oral lichen planus (OLP) is a chronic inflammatory disease which is characterized by an immunoreactivity directed against basal keratinocytes and mediated by T/lymphocytes. However, it is well known that salivary immunoglobulins have important role in the protection of mucosal surfaces. The aim of this study was to determine salivary immunoglobulin A1 (IgA1) and IgA2, together with IgG 1,2,3,4 subclass levels in patients with oral reticular lichen planus during acute stage and remission period as well as in comparison to the controls. In the whole resting saliva of 19 patients with OLP, age range 30-72, mean 58 years in acute phase and during remission period, and in 21 controls, age range 20-52, mean 35 years, salivary IgA and IgG subclasses were determined with radial immunodiffusion and enzyme immunoassay respectively. There were no significant differences in salivary IgG1 and IgG2 as well as IgA1 and IgA2 between patients in acute phase and controls (p>0.05). Patients in acute phase had significantly increased IgG3, IgG4 and proteins in comparison to the controls (p=0,021; p=0,004; p=0,029). No significant differences could be found between patients in acute phase and during remission period in IgG1,2,3,4 and IgA1 while IgA2 was significantly increased in acute phase in comparison to the remission period (p=0,049). Between patients in remission period and controls there were no significant differences in any IgA or IgG salivary subclasses (p>0,05). We can conclude that acute phase is characterized with increase in IgA2 which might reflect increased activity of secretory immunity as a possible result of microbial stimulation seen in acute phase in comparison to the remission period.

Received: September 20, 2006
Accepted: February 2, 2007

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Key words
Saliva; Immunoglobulin G;
Immunoglobulin A; Lichen Planus

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