

Augmentation of Regulatory T Cells (CD4+CD25+Foxp3+) Correlates with Tumor Stage in Patients with Colorectal Cancer

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ABSTRACT

Recent evidence suggests that decline of regulatory T cells (Tregs) play a critical role in the prevalence of autoimmune diseases inhibiting the maintenance of peripheral self tolerance, while its augmentation leads to insufficient antitumor response, accompanied with poor prognosis in various malignancies. Increased number of Tregs (CD4+CD25+FoxP3+) were noticed in peripheral blood mononuclear cells (PBMCs), tumor-infiltrating lymphocytes (TILs) and/or regional lymph nodes lymphocytes (LNLs) of patients with gastrointestinal tumors. The aim of our study was to investigate the correlation between the percentage of Tregs in peripheral blood of patients with colorectal carcinoma, using flow cytometric technique and tumor stages, classified as Dukes' A, B, C or D and by stage of differentiation. Peripheral blood venous samples were obtained from 92 patients with colorectal cancer and from 30 healthy adult volunteers. Statistical analysis: Linear regression equations were generated using a least-squares method and analyzed for differences of covariance. Statistical significance was calculated by Mann Whitney U-test. Our data has shown that 15% patients with colorectal cancer were classified as Dukes' A, 41% were Dukes' B, 35% were Dukes' C and 9% were Dukes' D. 54% patients with CRC were well differentiated, 11% were poorly differentiated, 20 were moderately differentiated, stage, 4% were mucinous carcinoma and rest of 11% were partly good differentiated with mucinous components. The increased percentage of Tregs in colorectal cancer patients correlates with tumor stage. These results indicate a possible involvement of regulatory T cells in disease progression. New strategies using inhibition or depletion of Tregs are necessary to elucidate the complexity of defective tumor immunity.

Key words: colorectal cancer, Dukes' classification, innate immunity, regulatory T cells (Tregs).

Introduction

Regulatory T cells (Tregs) represent a subpopulation of suppressor T cells that mediate immune tolerance by suppressing autoreactive T cells. Tregs control tumor expansion at the priming, as well as at the effector's phase of T-cell immune responses. Immune system's capacity to distinguish between innocuous and harmful foreign antigens is controlled by mechanisms of central and peripheral tolerance. Mechanisms of peripheral tolerance involve induction of cell death or the development of a non-responsive state (anergy) of T cells. Regulatory T (Treg) cells have active suppression mode and may induce peripheral tolerance. These immunoregulatory mechanisms of Treg cells are the subject of intensive investigation. Dendritic cells (DCs) maturation and/or activation

are important in the induction of peripheral tolerance and may control peripheral tolerance inducing the differentiation of Treg cells. As professional antigenpresenting cells (APC), dendritic cells initiate a primary T cell immune response. Immature dendritic cells are able to stimulate Tregs and may act as a stabilizer in which effector cells and Tregs may be a physical act¹⁻⁴. An important mechanism for activation of Tregs is by immature dendritic cells. Stimulation activity of dendritic cells is highly dependent on their maturity level/activation and environmental factors such as proinflammatory cytokines and active components of Toll like receptor (TLR) Dendritic cells (DC) from the intestinal epithelium, induced antigen from the external environment

and can migrate to mesenteric lymph nodes (MLN) and encouraging the development of Tregs thereby preventing inflammation colon secretion of immune suppressive cytokines⁵⁻⁸. Development and function of Tregs depend on the transcription factor FoxP3, and can be reprogrammed during the selection in the thymus or induced in peripheral tissues. Although there are many literature findings on Treg cells (22, 23), their inhibiting activities, as well as contributing to the control of T and B cell activity in defense against foreign antigens in vivo conditions, it is not fully clarify their functions. It is well known that human Treg cells express perforin and granzyme, although the functional significance of granulocyte/ egzocyte pathway at this time has not yet been fully elucidated. It is believed that an additional suppressive mechanism mediated by Treg cells, could be earned by killing autologous cell targets through the perforin/granzyme-dependent way. Generally we can conclude that the lack of Tregs may contribute to the development of autoimmune syndrome: insulin-dependent diabetes, thyroiditis, colitis, arthritis, gastritis, and allergic diseases, while their activation prevents the immune response against tumors and intracellular pathogens^{9,10}.

Subjects and Methods

We analyzed the peripheral blood lymphocytes, as well as the lymphocytes from the irrigating areas of lower mesenteric vein (taking during the operation) by flow cytometric technique (FACSCalibur) for determine the number of T, B cells, NK cells, regulatory T cells (Tregs) and NKT cells of patients with colorectal cancer on systemic and local level, comparing to healthy volunteers (peripheria).

Results

All the patients were operated on the surgical clinic of KBC Rijeka. Here we present the characteristics of those patients who have voluntarily agreed to have their data used for scientific research. In Figure 1 we can see the characteristic localization of colon cancer. We note that the largest percentage of patients had rectum cancer.

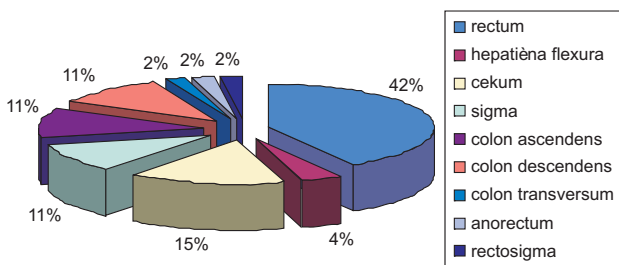


Fig. 1. Localisation of tumor

Our patients are analyzed depending on Dukes' classification. The largest number of patients was in a group of Dukes' B (41%). (Figure 2).

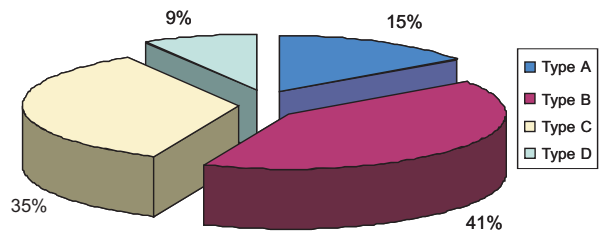


Fig. 2. The distribution of tumors, depending on the classification of the Dukes'.

It is very important differentiation of the tumor and Figure 3 shows the epidemiological characteristics of patients with colorectal cancer with respect to differentiation.

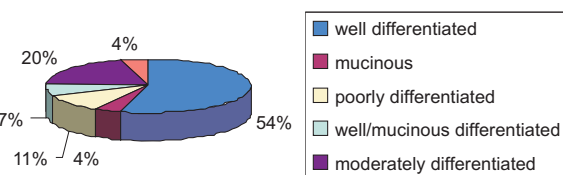


Fig. 3. The distribution of tumors with respect to differentiation.

Analyzing the immune parameters we did not find statistical differences between cancer in the right and left colon, but in many tested values statistically are observed significant differences in systemic and local levels.

In Figure 4 note statistically significant increased of T reg cells in patients with colorectal cancer ($p < 0,01$), and this increase was also expressed at the local level (Fig. 5). ($p < 0,05$).

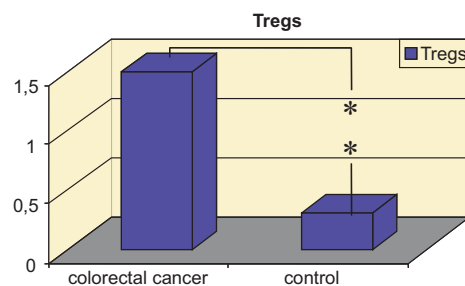


Fig. 4. Tregs in patients with colorectal cancer.

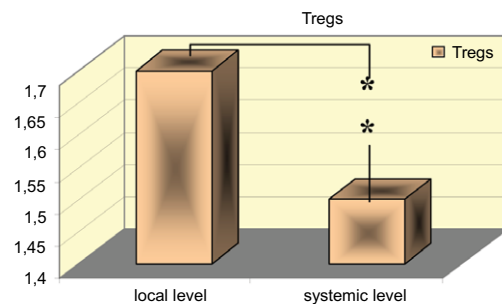


Fig. 5. Ratio of Tregs in patients with colorectal cancer on systemic and local level.

Figure 6 shows the distribution of Tregs depending on the classification of the Dukes'. We note that percentage of Tregs increases due to higher cancer grade, but paradoxically, in Dukes' type D is reduced probably due to general failing of the immune system.

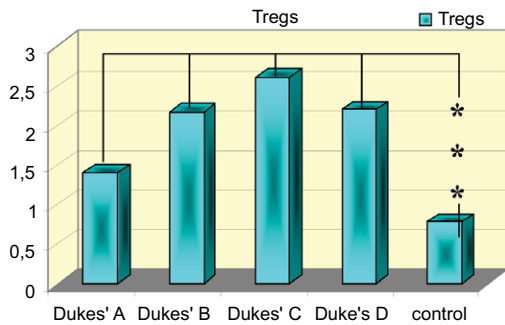


Fig. 6. Distribution of Tregs depending of the Dukes' classification.

On Figure 7 is shown the distribution of Tregs depending of differentiation, without statistical significance.

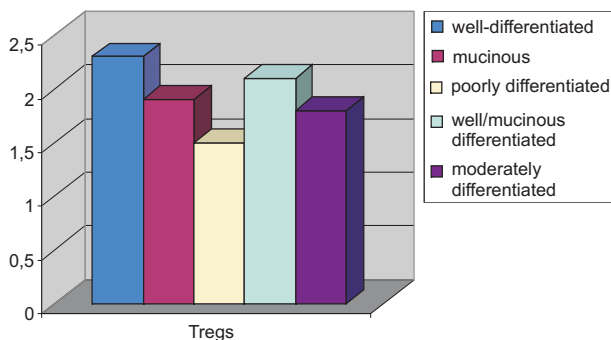


Fig. 7. Distribution of Tregs depending of differentiation.

Discussion and Conclusion

Immune cells may contribute to tumor development and progression through the complex interplay of different mechanisms and mediators. A variety of cytokines, chemokines and growth factors that are present at the site of inflammation may stimulate tumor growth, angiogenesis and metastasis. Particularly in patients with acute ulcerative colitis were observed elevated levels of Tregs in the colon compared with healthy control. Although no reliable evidence confirming the link between elevated Tregs in chronically inflamed tissue and the risk of developing malignant disease, the more hypotheses emphasize that local suppression of the chronic inflammatory process contributes to tumor growth by preventing the successful early immunologic survival¹². An increasing number of literature sources highlights the role of Tregs, if not in the tumor initiation process, then at least in the prevention of immunity in an already well-known tumor. Our results have indicated a significant fact. The number of regulatory T cells was elevated in all forms of cancer with emphasis that the number of Tregs was higher in the higher grade of tumor. Also it is very interesting results that at the local level the number of Treg cells were higher than in the peripheral blood of patients. Today it is considered that both subpopulations Tregs: innate (nTregs) and induced (iTregs) are involved in tumor immunity, but the right mechanisms of action still remains unrecognized¹³. Tregs containing FoxP3 +, and gliokortikoid-induced TNF receptor, and cytotoxic T lymphocyte associated antigen-4, can suppress effector T cells in the periphery. Tregs are present in high numbers within the tumor-infiltrated lymphocytes, peripheral blood lymphocytes and / or within the lymph nodes of patients with cancer and their frequency strongly correlates with tumor progression and the efficacy of treatment^{14,15}.

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POVEĆANJE REGULACIJSKIH T STANICA KORELIRA SA STADIJEM TUMORA KOD PACIJENATA S KOLOREKTALNIM KARCINOMOM

S A Ž E T A K

Noviji podaci pokazuju da snižavanje regulacijskih T stanica (Tregs) igra ključnu ulogu u učestalosti pojavljivanja autoimunih bolesti održavanja periferne tolerancije, dok je njihovo povećanje povezano s pojavom nedovoljnog anti-tumorsog odgovora, i lošijom prognozom u raznim malignim bolestima. Povećan broj Tregs (CD4 + CD25 + FoxP3 +) je primijećen u mononuklearnim stanicama periferne krvi (PBMCs), tumor-infiltriranim limfocitima (TILs) i / ili limfocitima iz regionalnih limfnih čvorova (LNLs) bolesnika s tumorima probavnog sustava. Cilj našeg istraživanja bio je istražiti korelaciju između postotka Tregs u perifernoj krvi bolesnika s karcinomom debelog crijeva, koristeći tehniku protočne citometrije i usporediti vrijednosti sa stupnjevima bolesti (po Dukes') i po stupnju diferencijacije. Uzorci periferne venske krvi su dobiveni od 92 pacijenata s karcinomom debelog crijeva i od 30 zdravih odraslih dobrovoljaca. Statistički značaj izračunat je Mann Whitney U testa. Naši podaci pokazuju da 15% bolesnika s rakom debelog crijeva su klasificirani kao Dukes' A, 41% su bili Dukes' B, 35% su bili Dukes' C i 9% su bili Dukes' D. 54% bolesnika s CRC je bilo dobro diferencirano, 11% su bili slabo diferencirani, 20% je bilo umjereno diferenciranih, a 4% mucinoznih karakteristika, dok je preostalih 11% bilo dijelom dobro diferencirano s mucinoznim osobitostima. Povećani postotak Treg stanica u karcinomima debelog crijeva korelira s težinom klasifikacije tumora. Ovi rezultati ukazuju na moguću ulogu regulacijskih T stanica u napredovanju bolesti. Neophodna su daljnja istraživanja koja bi koristila inhibiciju ili depleciju Tregs kako bi se razjasnila složenost njihova djelovanja i uloga u imunološkoj otpornosti na tumor.