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Levels of Cytokines and Galectin 1 in Patients with Common Variable Immunodeficiency from South-Eastern Serbia

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Heterogenous clinical presentation in Common variable immunodeficiency (CVID) is contributed by alterations of cytokine and signaling pathways. Recently, immunosuppressive, and immunomodulatory functions of Galectin 1 (Gal-1) have been described in experimental models, malignant and autoimmune diseases, but never in patients with CVID. The levels of Gal-1, IFNγ, IL-2, IL-4, IL-10 and IL-17 were determined, using the ELISA method, in serum of 5 patients with CVID and 8 healthy volunteers who made up the control group. Comparative analysis of the levels of Gal-1, Il-2, Il-4, IL-10, IL-17 and IFNγ in patients with CVID did not show statistically significant differences compared to the control. Although higher activities of both IFNγ and IL-10, did not reach significant levels in our cohort, this finding was frequent and significant in larger series of patients with CVID. Increased levels of IFNγ and secretion of immunosuppressive IL-10 may be involved in modulating of the immune response in our cohort. Levels of Gal-1 showed no difference between our cohort and healthy population. Further studies of Gal-1 levels in larger series of CVID patients, will provide more data regarding the activity and role of this molecule in the pathogenesis of CVID.

***Key words**: common variable immunodeficiency, galectin-1, cytokines*

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Nivoi citokina i galektina 1 u pacijenata sa urođenom varijabilnom imunodeficijencijom iz Jugoistočne Srbije

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Izmenjena sekrecija citokina i signalnih puteva doprinosi heterogenoj kliničkoj prezentaciji Urođene varijabilne imunodeficijencije (*CVID*). Poslednjih godina imunosupresivne i imunomodulatorne funkcije Galektina 1 (Gal-1) su opisivane u eksperimentalnim modelima, malignim i autoimunim bolestima, ali nikada kod pacijenata sa *CVID*-om. Nivoi Gal-1, IFNγ, IL-2, IL-4, IL-10 i IL-17 su određivani ELISA metodom kod pet pacijenata sa *CVID* i osam zdravih dobrovoljaca, koji su činili kontrolnu grupu. Upoređivanjem nivoa Gal-1, IL-2, IL-4, IL-10, IL-17 i IFNγ između *CVID* pacijenata i kontolne grupe, nije nađena statistički značajna razlika. Iako povećane aktivnost IFNγ i IL-10 nisu bile značajane u našoj kohorti, takav nalaz je bio čest i značajan u većim serijama pacijenata sa *CVID*-om. U našoj grupi, povišeni nivoi IFNγ i sekrecija imunosupresivnog IL-10 mogu biti uključeni u modulaciju imunog odgovora. Nivoi Gal-1 u ispitivanoj kohorti se nisu razlikovali u odnosu na zdravu populaciju. Dalja istraživanja nivoa Gal-1 na većim serijama *CVID* pacijenata će dati više podataka o aktivnosti i ulozi ovog molekula u patogenezi *CVID*-a.

Ključne reči: *uobičajena varijabilna imunodeficijencija, galektin-1, citokini*

Introduction

Common Variable Immunodeficiency (CVID) is the most common symptomatic immunodeficiency in adults. Its frequency ranges from 30.2/100,000 in the USA (1) to 0.7-1.3/100,000 in Europe (2). Although the clinical picture is very heterogeneous, the common feature of all patients is hypogammaglobulinemia and the presence of recurrent, predominantly bacterial infections. In addition, CVID is characterized by many non-infectious disorders, which are often accompanied by severe and chronic infections, autoimmune phenomena, and the appearance of malignancy (3). The basis of these impairments is insufficiently elucidated and probably includes cytokine and cellular inflammatory pathways, disorders in the maturation of B lymphocytes, impairments of immune tolerance mechanisms of T and B lymphocytes (4). Insights in some of the disturbances mentioned above, could be obtained through findings of aberrant cytokine expression in the serum of patients with CVID (5). For example, an increased concentration of interferon gamma (IFNγ) was associated with activity of a set of genes present in Th1 cells, macrophages, and the innate immunity lymphocytes (6). Conversely, there are also findings indicating an exaggerated Th2 response in CVID with findings of increased IL-4 and IL-10 and low or normal IFNγ (7). The complexity of these interactions is increased by findings of existence and physiological role of galectin-1 (Gal-1) in activation, differentiation, survival, and cytokine production of effector T lymphocytes (8). Within the immune system, Gal-1 is secreted by activated B and T cells and $CD4^+CD25^+$ regulatory T lymphocytes (9). By interacting with glycosylated receptors, including CD45, CD43 and CD7, Gal-1 controls T cell survival (9). Gal-1 has properties of T cell receptor (TCR) antagonist, limiting T cell activation and can inhibit T cell adhesion to extracellular matrix glycoproteins (8).

For the reasons given above, the authors of this study believed that determining the level of Gal-1 in the serum of patients would provide new information about its role on the pathogenesis of CVID. In the existing literature, there is no work dealing with this issue. Also, determining the levels of IFNγ, IL-10, IL-2, IL-4 and IL-17 in serum with Gal-1, would give an insight to complex interactions between segments of immune response and Gal-1, in the studied population.

Aim

In this pilot study levels of serum Galectin-1, IL-2, IL-4, IL-10, and IL-17, will be determined in patients with CVID. Analysis of their levels will give possible clues about characteristics and

aberration of the immune response in this sensitive population of patients from the Southeast of Serbia.

Material and Methods

Patients

At the Clinic for Hematology Allergology and Clinical Immunology of the University Clinical Center in Nis, in the period from June 2000 until February 2025, there were 12 patients with primary agammaglobulinemia. According to European Association for Primary Immunodeficiency and the Pan American Group for Immunodeficiency (ESID/PAGID) criteria, CVID was diagnosed in 10 patients, while one patient had X-linked agammaglobulinemia, and another Niemegen-Breakage syndrome (10). Only 5 CVID patients signed the informed consent and their clinical data and biological material were available for the study. The average age of the patients was 31.8 years (22-42 years) at the time of making diagnosis, while at the time of analysis it was 36 years (19-54 years). All patients had symptoms of immunodeficiency and one among them also exhibited allergic phenomena in the form of dyshidrosis. Splenomegaly, defined by imaging methods, as an interpolar diameter of the spleen >14 cm, was recorded in 3/5 patients. After splenectomy, a Marginal Zonal Non-Hodgkin's Lymphoma was found in the spleen in one patient, with no need for further treatment. Unicentric Castelman's disease was diagnosed in another patient after an axillary lymph node biopsy (Table 1).

Table 1. Characteristics of patients with Common Variable Immunodeficiency

Initials	D.Z.	S.P.	R.P.	S.A.	O.I.
Spleen enlargement	Yes	Yes	No	No	Yes
Sex	M	M	F	F	F
Immune dysregulation	/	/	/	/	Dyshidrosis
Malignancies	/	MZ NHL	/	/	/
Infections	Lower resp tract	Ch. Sinusitis	Ch. Tonsilitis	Ch. Sinusitis Diarrhea	Ch. Sinusitis
Age at the moment of Dg (years)	23	34	22	42	38
Delay in Dg (years)	6	25	1	7	1
Substitutional therapy Ig	I.V.	S.C.	S.C.	S.C.	S.C.

Abbreviations: M, male; F, female; MZ NHL, Marginal Zone Non-Hodgkin lymphoma; I.V., intravenous; S.C., subcutaneous; Dg, diagnosis

The control group consisted of 8 healthy volunteers with an average age of 38.8 years, who signed an informed consent to participate in the study. The Ethics Committee of the University Clinical Center in Niš approved the performance of this study (decision number 35110/11), within the internal project of the Faculty of Medicine, University of Niš, Serbia.

Determination of Galectin-1 and cytokines

Serum from healthy donors and serum obtained from CVID patients were used in the study. Quantikin ELISA kit for Human Galectin-1 (R&D Systems, Minneapolis, Minnesota, USA) was used to determine the level of Gal-1 in serum, in accordance with the manufacturer's instructions. Serum cytokine levels were determined according to the manufacturer's instructions by the ELISA method, using the following reagents: Human IL-2 Quantikine ELISA Kit, Human IL-4 Quantikine ELISA Kit, Human IL-10 Quantikine ELISA Kit, Human IL-17 Quantikine ELISA Kit and Human IFN-γ Quantikine ELISA Kit (all manufactured by R&D Systems, Minneapolis, Minnesota, USA).

Statistical Analysis

All statistical analyzes were performed with the IBM SPSS v26.0 program (SPSSInc., Chicago, IL, USA). Results are presented as means ± standard deviation (SD). Intergroup differences were determined by the Mann-Whitney U test, when the findings did not exceed the normality test, or by the T-Test for two independent samples. All *p* values less than 0.05 were considered statistically significant.

Results

Serum levels of cytokines in patients with CVID and the level of Gal-1 did not show statistically significant deviations from the values found in the control group (Table 2). Although there is no statistical significance, higher values of IFN γ and IL-10 were registered in the serum of patients with CVID compared to the control group. At the time of sample collection, none of the subjects suffering from CVID had an active infection, nor elements of an autoimmune or malignant disease.

Table 2. Presentation of serum levels of cytokines in patients with CVID compared to control group

Variable	Groups	Average	St Dev	*P*
Gal-1 (ng/mL)	CVID	31,34	9,65	0,884*
	Control	29,87	9,09	
IFNγ (pg/mL)	CVID	12,47	11,55	0,271*
	Control	4,73	4,74	
IL-4 (pg/mL)	CVID	11,40	2,01	0,546**
	Control	12,06	1,77	
IL-17 (pg/mL)	CVID	7,41	2,69	0,555*
	Control	6,24	2,21	
IL-10 (pg/mL)	CVID	21,76	25,32	0,143*
	Control	5,64	3,59	
IL-2 (pg/mL)	CVID	30,33	2,79	0,271*
	Control	34,84	10,65	

*Mann-Whitney-U test; ** T-T test for two independent samples

Discussion

The results of this study did not find statistical differences between the healthy controls and the analyzed patients, in terms of serum cytokine levels. However, a closer analysis showed higher levels of IFNγ and IL-10 in the serum of CVID patients compared to controls. Higher levels of IFNγ, were accompanied with splenomegaly, in our cohort, that was also described in another study of 35 CVID patients from Serbia. In that study, splenomegaly was more often associated with the finding of the +874T/A IFN gene polymorphism (11).

An elevated level of IFNγ is common in patients with CVID and is a consequence of the activation of a set of genes typical for IFN signature, which was demonstrated in a proteomic analysis in 91 patients with CVID. The activation of genes that are part of the IFN response was more pronounced in CVID patients with inflammatory complications, granulomatous and autoimmune phenomena (6). Higher activity of the immune pathway mediated by IFNγ has also been described in a subgroup of CVID patients with lower lymphopoiesis in the thymus and bone marrow, a reduced number of memory B lymphocytes and quantitative and qualitative disorders of T regulatory lymphocytes (12,13, 14). The aforementioned and other impairments of adaptive immunity in patients with CVID can potentiate the response of innate immunity, with the expansion of inflammatory innate lymphoid cells type 3, followed by an increase in the level of IFN (17). Overall, it is reasonable to say that the elevated values of IFNγ in our study are an expression of continuous dysregulation and impairment of the immune response at different levels.

The analysis of the level of serum IL-2 in this work aimed to indirectly assess the T lymphocyte response to an immune stimulus. Disorders of IL-2 secretion have been described in patients with CVID (5). Lower levels of serum IL-2 or disorders of the IL-2 receptor are most often found, although there are also works, without significant deviations, which is in agreement with the findings in our series of patients (5,15, 16).

There is considerable controversy regarding the roles of different T lymphocyte subpopulations in the pathophysiology or development of the clinical features of CVID. Thus, an increased level of IL-4 in the serum was detected, as an expression of the dominant activity of Th2 cells (7, 18). On the other hand, the Th17 subpopulation and their major cytokine IL-17 are downregulated in CVID (16, 19). Reduced number and function of TH17 cells in the germinal center may be related to the expansion of activated $CD21^{low}$ B cells (2). Disorders in the maturation of B lymphocytes are one of the main characteristics of CVID. Impairments in maturation have been noted in all stages of B cell development, with consequent disturbances in the number and phenotype of all B cell populations (20). These disorders did not bypass group B regulatory lymphocytes, which are important controllers of the immune response and sources of the immunosuppressive cytokine IL-10. The level of these cells in CVID is decreased, which results in a decrease in the secretion of IL-10 from this population (21). However, frequent findings in literature, of elevated levels of IL-10 in the serum of CVID patients are explained by production from Th2 cells (5, 7). This mechanism is probably responsible for the finding of slightly higher IL-10 values in our series of patients. Considering the inhibitory effect of IL-10 on the activation of CD8+ and CD4+ T lymphocytes, directing the immune response towards the Th2 type as well as regulating the innate immune response, it can be assumed that the elevated value of serum IL-10 in this study is associated with the dominant immunodeficient phenotype in our series of patients.

Along with the mentioned cytokines, galectins are also important regulators of the immune response. So far, 15 members of the galectin family have been described in the human species, among which Galectin 1 is the most widespread (22). In their composition, these molecules contain at least one domain capable of recognizing carbohydrates (carbohydrate recognition domain -CRD) (23). The specificity and plasticity of the CDR domain is different; therefore, each galectin recognizes a different set of glycosylated proteins or lipids on the cell surface, extracellular matrix or inside the cell. The physiological consequence of this activity is the ability of galectins to act as regulators of vital cellular processes, such as survival, apoptosis, cell cycle, transcription and mRNA processing, cell adhesion, and migration (23). This diversity carries over

to immune system cells and their functions. Thus, Gal-1 in cooperation with Gal-8 helps the maturation of B lymphocytes into plasma cells and their survival, while the influence of Gal-1 on memory IgM+ B lymphocytes and T lymphocytes in inflammatory states is proapoptotic (24). Gal-1 shapes the immune repertoire by influencing the negative selection of T lymphocytes during maturation in the thymus by stimulating activation-induced death. The immunosuppressive and tolerogenic effects of Gal-1 are manifested by their secretion from dendritic cells and CD4+CD25+ T lymphocytes, after which cell death mediated by the FAS signaling mechanism is induced (25, 26). Suppression is also reflected in the selective deletion of Th1 and Th17 effector cells, the promotion of the tolerogenic and pro-migratory phenotype of dendritic cells, as well as the expansion of regulatory T lymphocytes (27). In the list of numerous functions Gal-1 is the ability to adjust the process of acute inflammation, by controlling the activation, extravasation, chemotaxis and turnover of neutrophils, monocytes, and macrophages (28, 29). Therefore, it can be said that Gal-1 represents an important regulator of immunological tolerance, inflammatory response and achieves a significant link between acquired and innate immune response.

Although the level of Gal-1 in our study group did not differ compared to the control, it is an unequivocal fact that this molecule plays a significant role in the pathogenesis of CVID. The authors of this work believe that this negative finding indicates the need for a different method of examining Gal-1 levels with a larger number of patients.

Conclusion

The elevated level of IFNγ and IL-10 in the serum of patients with CVID indicates a pronounced activation of the immune pathway mediated by IFNγ, which together with IL-10 determines the immune response in CVID. In patients with CVID, the level of Gal-1 does not differ significantly compared to the healthy population. The many important roles of Galectin 1 in the regulation of the immune response require studies of the level and function of this molecule in larger series of CVID patients, in order to obtain more precise answers about the role of this molecule in the pathogenesis of CVID and the potential benefit for the application of targeted therapy directed at this molecule.

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