The attempt to analysis of chosen anti-inflammatory cytokines concentration in serum of patients with active and non-active celiac disease

Running title: Anti-inflammatory cytokines in celiac disease

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Summary

AIM: The aim of study was determining concentrations of chosen anti-inflammatory cytokines in serum of patients with celiac disease (CD) and assessing its correlation to clinical parameters in active and non-active CD.

METHODS: Serum levels of IL-4 and IL-10 were determined in 56 patients with active and 54 with non-active celiac disease and in control group. Manufactured sets of ELISA test were used in study. The results were subjected to statistical analysis.

RESULTS: There was not a difference in concentrations of IL-10 in compared groups and levels of IL-4 in all patients were undetectable.

CONCLUSION: From shown data it follows that the aberrant profile of IL-10 secretion is not associated with clinical parameters and suggests an inherent defect in IL-10 secretion in CD and healthy individuals.

Key words: celiac disease, anti-inflammatory cytokines, interleukin-4, interleukin-10.

INTRODUCTION

The celiac disease (CD) is a chronic inflammatory disease which depends on expression of genes related or not related with the HLA system, where the activity of environmental factors is critical to release the onset of disease. Along with the development of molecular and immune methods the perception of celiac disease pathogenesis was changed.

The celiac disease has been currently recognized by many investigators as an immune background disease (1, 2). Among the others, in favor of that, speaks the presence in majority of patients of so called autoimmune haplotype of histocompatibility antigens, the presence of specific antibodies (AGA, ARA, EmA, ssDNA, dsDNA and against CL cardiolipin), identification of auto-antigens against which antibodies are directed (among the others, tissue transglutaminase [tTG], calreticuline) or co-existence of other diseases with autoimmune background in celiac patients (type I diabetes, autoimmune thyreoiditis, collagenous diseases, autoimmune alopecia, hepatitis or biliary inflammation) (3, 4).

The significant discovery which fixed the direction for studies was the statement, that immune reactions of small intestine mucosa form background of celiac disease. In 1964 Ferguson started those studies proving the presence of increased cytokine levels in small intestine biopsies from celiac patients (5). From that moment the investigators gave their attention to cytokine's role in a cascade of changes under gluten influence.

Numerous studies proved the participation of pro-inflammatory cytokines (mainly IL-2, IL-6, IFN-γ and TNF-α) in pathogenesis of disturbances observed in active celiac disease. However, there are only few reports on anti-inflammatory cytokines role, mainly the IL-4 and IL-10 in celiac patients, who complied or not to gluten-free diet.

Interleukin-4 is a glycoprotein, with mass ranging from 15 to 19 kDa (6). It is coded by gene situated on the long arm of the chromosome 5 in q23-31 segment, being in association with genes for the IL-3, IL-5, IL-13 and the GM-CSF (7). Its production depends in great part on the Th2 cells. It is blocked by the IL-2, IFN-γ and lymphotoxin (TNF-β) (6). IL-4 modulates the release and function of other cytokines, for example together with the IL-10 inhibits the release of IFN-γ by the Th1 lymphocytes (8).

Interleukin-10 coded by gene situated on the chromosome 1 is a glycoprotein with molecular mass 18.5 kDa (9). It is mainly released by activated Th2 lymphocytes but also the B cells, monocytes, macrophages and keratinocytes. It has many functions which result in inhibition of immune response of cellular-type inflammatory reaction as well as it expressed immunosuppressive role (10). To its main functions belong the inhibition of cytokine production by the Th1 (especially IFN-γ, IL-2, IL-3), and the Th2 lymphocytes, monocytes and macrophages (IL-1β, IL-6, IL-8, IL-12, G-CSF, GM-CSF, TNF-α) (11).

Abnormal levels of anti-inflammatory cytokines were demonstrated locally in small intestine mucosa in celiac patients. In reply to local activation of immune system the number of circulating cells which produce cytokines also increased. Previous data pointed out the correlation between intensity of intestinal changes and cytokines concentration in serum. Further investigations showed the differences in cytokine profile between celiac patients and the control group. The stimulation with gliadin in celiac patients caused release of the IFN-γ, IL-2, IL-4, IL-6 and the IL-10 by blood cells. In the control group after the stimulation, the secretion of IFN-γ and IL-4 was not observed but merely the IL-2, IL-6 and the IL-10.

MATERIALS AND METHODS

The levels of the IL-4 and IL-10 were determined in 110 blood samples obtained from celiac patients (studied group) and in the comparable group (group III, n = 43). On the basis of results of anti-endomysial antibodies titration (EmA) the subjects were divided into two groups: I -EmA (+) patients who not complied to gluten-free diet, with active disease (n = 56) and II -EmA(-) patients who complied to gluten-free diet, with non-active form of celiac disease (n = 54). The sample for determinations consisted of ulnar vein blood in amount of 3 ml. To separate serum and cells the blood was centrifuged for 5 -7 minutes with speed of 4000 rpm. The level of cytokines in serum was determined by ELISA (Bender MedSystems) on microplates coated with mice monoclonal antibodies against given cytokine. The sensivity of both tests was 2.0 pg/ml. The obtained results were statistically analyzed with rank sum of Kruskal-Wallis, t-Student test, Pearson correlation index and χ^2 test, and in case of insufficient class numbers (< 6) with accurate Fisher test.

RESULTS

According to statistical analysis of age, body weight and height it was stated that both examined groups were homogenous regarding height, but different regarding age and body mass (tab. 1, 2).

In all examined groups serum IL-4 levels were undetectable (as in healthy persons).

The detectable IL-10 levels were found in serum of 22 patients (undetectable in healthy persons) (fig. 1).

In statistical analysis no differences of mean values of IL-10 cytokine in compared groups were found (tab. 3).

DISCUSSION

The celiac disease is caused by genetically determined immune response to ingested gluten, resulting in local activation of immune system in small intestine mucous membrane and growth in number of cytokines producing cells in peripheral blood. According to Przemiosło et al., the source of cytokines could be both the T cells of lamina propria and intraepithelial ones, in group of which γ/δ of TcR lymphocytes would be responsible for the process (12). According to others the presence of cytokines in epithelium is only the evidence of their quick transfer from lamina propria where they were produced. The most recent data allow assuming that the source of cytokines beside the T lymphocytes and macrophages are also mast cells and enterocytes (13, 14).

The levels of cytokines found locally in the intestine express the relation with serum concentration of cytokines. The dependency between activation of the immune system cells in intestine and peripheral blood is explained by constant recirculation of activated immune cells between organ where the inflammatory reaction takes place and periphery (15, 16). With the use of flow cytometry, it was proved that gliadin in vitro caused activation of isolated mononuclear cells of peripheral blood (PBMC), expressed by activation of the HLA-DR and the IL-2R markers combined with cytokines secretion. The induction was observed in 5-10% of mononuclear cells both in celiac patients and healthy subjects (17). The activation could be blocked by adding anti-HLA-DR antibodies, what proves the fact that HLA system particles are engaged in the PMBC gliadin peptides presentation.

One could not exclude the other reaction independent from intestine type immune response, which takes place in peripheral blood similar but not identical with observed in mucous membrane. There are also reports that a kind of antigen used to

stimulate the TCC could have the crucial influence on cytokines production. It is probable that gliadin peptides prefer the Th0 response in peripheral blood cells both in celiac patients and healthy subjects.

Lahat et al. proved that small intestine mucous cells in celiac disease produce cytokines of the Th1/Th0 profile with the IFN-γ predominance (18). Beside interferon in biopsies, the secretion of the TNF-α, IL-6, more rarely IL-4, IL-5 and IL-10 (19, 20) was also found. The results of study in which mRNA expression for chosen cytokines was determined in isolated T cells from biopsies of healthy children and celiac ones at different stages of diagnosis and treatment showed that in healthy children intestinal T cells revealed the expression of the mRNA for IL-2, IFN-γ, TNF-α and TGF-β, and also not high levels of the mRNA for IL-10, what points out for persistence in small children mucosa so called stage of „inflammation under control" (21). In further part of the mentioned study, in children with active celiac disease significantly high levels of the IFN-γ and IL-10 produced by intraepithelial lymphocytes (IELs) were found. The rise of the TNF-α, TGF-β, IL-2 or IL-4 was not observed. It is worthy, to quote the results of the other study in which in lamina propria of intestinal mucosa in celiac patients apart from increased levels of the IL-10 there was also observed the rise in E_2 prostaglandin (PGE_2) concentration, which also expressed non-specific immunosuppressive activity (22).

The majority of investigators points out the Th1 type of immune response of mononuclear cells of peripheral blood, although there are also reports mentioning the Th1/Th0 type (12, 23, 24, 25, 26).

In few studies investigators showed the significant role of inflammatory cytokines (IL-4 and IL-10) in immune response to gluten. After gliadin stimulation in majority of biopsies taken from celiac patients and healthy subjects, the expression of mRNA for

IL-4 was observed (27). It was suggested that rapid growth of the IL-4 is related to mastocytes degranulation under influence of cytokines of the Th1 type (25). From the other side, it could be related with increased level of active B cells. It could contribute to explain the increased level of autoantibodies specific for celiac disease. Inconsistent studies results do not allow for univocal support of the IL-4 presence in peripheral blood cells in patients with active celiac disease. In the light of these facts our results seem to be relevant - in all subgroups no detectable levels of the IL-4 in serum were observed.

Some interesting conclusions were presented by investigators who studied immune response in patients with isolated gluten-sensitive enteropathy (GSE) and dermatitis herpetiformis (DH), which is currently recognized as dermal form of celiac disease. They found that disease presentation depends on the kind of cells in pathogenesis as well as on profile of cytokines they produce. In the GSE CD8+ lymphocytes are predominant, while in the DH CD4+, in patients with the GSE the production of IFN-γ as well as IL-2 and small amounts of IL-4 were found. In patients with the DH significantly increased level of the IL-4 and only slightly increased of the IFN-γ were found (28, 29).

In the studies where the IL-10 levels were determined in both patients with active and treated form of celiac disease in both groups the increased levels of the Il-10 in mucous membrane were found, although the rise in number of the Il-10 producing cells in blood of subjects exposed to gluten and in active celiac disease comparing to control group was not found (26). It complies with our own results in which while in 22 patients detectable levels IL-10 were found, no difference in concentration among the groups was found. The aberrant profile of IL-10 secretion is not associated with clinical parameters and suggests an inherent defect in IL-10 secretion in CD and

healthy individuals. On the other hand, the statement of detectable levels of the IL-10 can point for presence of spontaneous IL-10 secretion reported by some authors. They suggested the source of the IL-10 could be other cells than lymphocytes or their production results from activation by other factors, i.e. bacterial products (lipopolysacharides).

The indirect proof of the IL-10 lack of part in celiac disease pathogenesis are results of clinical trials to treat with recombinant human IL-10 which were undertaken in patients with so called resistant celiac disease (*refractory celiac disease*) at the end of nineties. Interleukin 10 had to inhibit the immune response of the T lymphocytes, monocytes and also the B lymphocytes (11). Moreover, by regulation of gene expression it could block secretion of the other cytokines with pro-inflammatory profile, such as the TNF-α, IL-1, or IL-6. However, the results obtained do not evoke an enthusiasm. The total histological remission was obtained in only 1 of 8 patients who underwent the therapy. During the treatment the adverse events were frequent (mainly headaches and thrombocytopenia), and their aggravation forced the patients to discontinue therapy (30).

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TABLES

Table 1. The mean values of age, weight and height percentile in particular groups.

	n	Age		Weight percentile*		Height percentile*		GFD
		mean	SD	mean	SD	mean	SD	
Group I	**56**	13.4	5.1	4.1	1.6	3.8	1.7	**-**
Group II	**54**	12.9	3.5	4.1	1.7	3.7	1.8	**+**
Group III	**43**	10.7	3.8	2.5	1.9	3.1	2.1	**-**

GFD – gluten-free diet

* for percentile channels a number values were attributed, e.g. channel < 3 percentile – 1, channel 3-10 percentile – 2 etc.

Table 2. The statistical comparison of age, weight and height in particular groups.

	Groups I and II		Groups I and III		Groups II and III	
	t	p value	*t*	p value	*t*	p value
Age	0.59	*ns*	2.88	*<0.005*	2.93	*<0.005*
Weight percentile	0.0	*ns*	4.50	*<0.0001*	4.32	*<0.0001*
Height percentile	0.59	*ns*	1.81	*ns*	1.51	*ns*

Table 3. The comparison of mean IL-10 concentrations for patients with active and non-active celiac disease and the control group.

IL-10 pg/ml	Groups I and II		Groups I and III		Groups II and III	
n	9	10	9	3	10	3
min	3,530	2,956	3,530	3,900	2,956	3,900
max	33,748	21,704	33,748	4,450	21,704	4,450
mean	7,715	8,536	7,715	4,240	8,536	4,240

SD	9,786	5,807	9,786	0,300	5,807	0,300
median	4,380	6,508	4,380	4,380	6,508	4,380
***t*-statistics**	-0.21		0.51		1.19	
p-value	*ns*		*ns*		*ns*	

FIGURES

Fig. 1. The mean serum IL-10 levels for particular groups.