Immunoregulatory disorders in irritable bowel syndrome

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Abstract

Irritable bowel syndrome (IBS) is a chronic idiopathic alimentary tract disease of a functional nature. The original cause of IBS remains unknown. However, the functional intestinal disorders (associated with neurogenic and inflammatory factors) seem to usually result from complex general reasons, thus IBS pathogenesis should be investigated from the general – rather than local – perspective of the organism. The understanding of actual functions of the immune system, which exceed by far the defense role, has placed it in one line with endocrine and nervous systems, as guards of the homeostasis of the organism. Regulatory ($CD4^+$ $CD25^+$) T lymphocytes can inhibit in an antigen-nonspecific manner the proliferation and activity of effector $CD4^+$ and $CD8^+$ T lymphocytes, this way preventing the occurrence of auto-immune and inflammatory and allergic reactions. The aim of this study was an attempt in addressing the questions: are there any detectable differences in the magnitude of activation of regulatory T cells in IBS, as measured by the expression of selected receptor molecules? The test group was composed of 17 patients diagnosed in the Clinic of Gastroenterology and Metabolic Diseases in the Medical University of Warsaw, due to the irritable bowel syndrome. The material for cytometric testing was venous blood. The following parameters were subjected to immunological evaluation: percentage values of mononuclear peripheral blood cells [$TCD4^+$ and $TCD8^+$ lymphocytes with co-expression of CD28, CTLA-4 (CD152), $CD69^+$, $CD25^+CD62L^+$, $CD25^+$GITR receptor] and concentration of IL-1β, IL-1Ra (interleukin 1 receptor antagonist), IL-10 and TGF-β as determined by ELISA method. The observed quantitative and functional disorders of Tregs need confirmation and further testing on much broader panel of patients, in order to detect alterations specific for the 3 distinct forms of IBS (diarrhea, constipation-related and mixed).

Key words: *irritable bowel syndrome, ($CD4^+$ $CD25^+$) T lymphocytes, $TCD4^+$ and $TCD8^+$, CD28, CTLA-4 (CD152), $CD69^+$, $CD25^+CD62L^+$, $CD25^+$GITR, IL-1β, IL-1Ra, IL-10, TGF-β, Tregs.*

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Introduction

Irritable bowel syndrome (IBS, *spastic colon*) – is a chronic (lasting at least three months) idiopathic alimentary tract disease of a functional nature, which is characterized by abdominal pains and irregular defecations, not evoked by organic or biochemical alterations [1, 2].

This syndrome was described in detail by Osler back in 1892, and was named by him as *mucous colitis*. This disease affects around 20% of population in the age between 30 and 40 years in the developed societies, with a three-fold higher incidence rate in women. The functional disorders in this ailment include visceral hypersensitivity and malfunctions of intestinal movement activity. The main symptom of IBS is painful cramps of high magnitude, which cease only after a few minutes' time, and have a tendency to relapse after a remission which can sometimes last several hours. The cramps are often accompanied by diarrhea or constipation.

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Therefore, this condition was divided into two forms: IBS-D and IBS-C. These two classes can be also mixed (IBS-M). The original cause of IBS remains unknown. The patho-physiological factors, which can be bound to the onset of the disease, are: post-infection risks (e.g. dysentery) – in these patients the increase in number of intestinal endocrine cells with elevated intracellular levels of serotonine was observed, excessive growth of gut bacterial flora (up to 84% of cases), disorders of visceral sensitivity and motoric bowel activity, as confirmed by: low rectal pain threshold in baloon-insufflation test, enhanced motoric response of large intestine to chosen drugs (prostygmine), hormones (cholecystokinine) or foods, mental problems (in 70-90% of IBS patients personality disorders, anxiety and depression arise), low fiber diet, the role of central nervous system has not been fully resolved – recent studies show alterations in activity of brain cortex areas responsible for pain sensitivity [3-5].

The lack of knowledge regarding causes of this disease creates significant diagnostic and therapeutic problems. There is no direct link between infection and development of IBS [6, 7]. Still, many researchers emphasize the correlation between the genetically-defined predisposition for chronic inflammatory infiltrate of the gut wall, associated with release of mediators sustaining the neurogenic inflammatory process, with the bowel functional disorders [1, 2]. The studies of Gonsalkorate *et al*. [1, 8] have shown, that in a group of patients with IBS the genotype predefining lower anti-inflammatory cytokine production is significantly less frequent than in control group. However, the functional intestinal disorders (associated with neurogenic and inflammatory factors) seem to usually result from complex general reasons, thus IBS pathogenesis should be investigated from the general – rather than local – perspective of the organism. It mostly concerns the progressive, long lasting and periodically exacerbating processes, where the clinical course can suggest the disorders of general defense and homeostatic mechanisms. Therefore, there is a sound base to consider the immunopathogenetic mechanisms as a crucial factor in opening the way to IBS development.

The understanding of actual functions of the immune system, which by far exceed the defense role, has placed it in one line with endocrine and nervous systems, as guards of the homeostasis of the organism [3, 9, 10]. This homeostatic function is introduced by the immune system through its activity in defense, tolerance to normal tissues, supervision of metabolism and genetic correctness of the tissues and participation in regeneration processes. A properly functioning system does not allow the aggression against itself, correct structures (auto-aggression) or neutral factors of external origin (allergy), streamlining the destructive power against pathogenic infectious factors. On the other hand, the protrophic influence is directed to self tissues, supporting their regeneration. The efficiency of these mechanisms is secured, providing that cellular supply processes follow the emerging needs [11-13].

The competence of the immune system, based on the aforementioned defensive and regenerative roles, remains preserved as long as the cellular resources – constantly exploited and exhausted by the system – can be replenished with sufficient pace. Mucosa-associated lymphoid tissue (MALT), the regional structure of the immune system first described by Bienenstock in the nineteen seventies, executes its immune functions within the mucosal membrane environment. In this manner GALT – gut associated lymphoid tissue – is bound to the alimentary tract [5, 14-17]. The composition of the system reveals the full range of cellular and humoral lymphoid elements, with 40-60% of T lymphocytes, 20-40% of B cells and IgA-producing plasmacytes, 10% of macrophages, 1-3% of mast cells and 5% of granulocytes incl. eosinophils. These constituents define the ability of MALT system to cope with the challenges, resulting from the exposition of mucosal membranes to immunogenic environmental factors. The cellular supplementation of MALT springs out from the general immune cell supplies of the body. Peyer's patches (lymphatic follicles being the aggregation of T lymphocytes) and M cells compose the important elements of the local immune system of the mucosa. The role of M cells, mediated by their surface glycocalyx and sIgA molecules, is to intercept the microorganisms and to transport them further to the internodular zones. Lymphocytes migrate via the regional mesenterial lymph nodes, efferent lymphatic vessels and thoracic duct to the blood circulation system. Infection of both viral or bacterial origin activates to a different degree the elements of innate and acquired immunity. Macrophages, natural killer cells and neutrophils constitute the important elements of innate immunity, and produce cytokines of anti-inflammatory activity. Activated dendritic cells, which stimulate the differentiation of naive T lymphocytes to specific $CD4^+$ and $CD8^+$ cells, constitute the elements of the acquired immunity; IFN-γ – producing $CD4^+$Th1 cells activate CD8+ lymphocytes to synthesize the pro-inflammatory cytokines [18-20].

The mechanisms of cellular and humoral immunity mentioned above assure with high probability the elimination of pathogens in the acute phase of the reaction. The inefficient elimination of infectious agent can bear severe consequences for the organism, in a form of prolonged inflammation with resulting tissue damage (e.g. intestines) or of developing auto-immune reactions. The organism protects itself against such a scenario by evolving the mechanisms which prevent self-destruction. One of these mechanisms is the immunosuppressive function of regulatory T cells [14, 20, 21].

The cellular supply of GALT comes from the general body resources of immune cells. Under physiological conditions, the equilibrium between Th1 and Th2 response is maintained thanks to the presence of thymus-derived, regulatory T lymphocytes (Treg), forming on average 5-10% of peripheral $CD4^+$ T lymphocytes.

Regulatory ($CD4^+$ $CD25^+$) T lymphocytes, by means of IL-10 and TGF-β production, can inhibit in an antigen-nonspecific manner the proliferation and activity of effector $CD4^+$ and $CD8^+$ T lymphocytes, this way preventing the occurrence of auto-immune reactions. The overall immunoregulatory efficiency of peripheral regulatory T-cells is determined not only by their number, but also by their activity. This activity, manifested by IL-10 and TGF-β production, represents the important mechanism of regulation of the immune response, and prevents the allergic and auto-immune reactions [22-25].

Rationale and aim of the study

The difficulties in predicting the course of IBS, together with unsatisfying results of its treatment, bring inspiration for the scientific community to investigate the mechanisms responsible for this process. One of them, claimed to inhibit the efficient anti-inflammatory response of effector T lymphocytes, is the activity of regulatory T cells. The progress of inflammatory and allergic states are some of processes attributed to the hyper-activity of these cells. Their contribution has been also assessed in pathogenesis of several auto-immune and neoplastic conditions.

Taking this into consideration, the aim of this study was an attempt in addressing the following questions:

1. Is the prolongation of inflammatory state in course of IBS accompanied by the quantitative changes in the regulatory T-lymphocyte population?
2. Are there any detectable differences in the magnitude of activation of regulatory T cells in IBS, as measured by the expression of selected receptor molecules?

Material and methods

The test group was composed of 17 patients of both genders, diagnosed in the Clinic of Gastroenterology and Metabolic Diseases in the Medical University of Warsaw, due to the irritable bowel syndrome. These 17 patients were included into the study according to Rome III criteria. Their age ranged between 24-81 years, with a mean value of 51.8; the mean age of men was 59.5 and of women – 50.7 years. Three women aged 40-80 (mean 55) suffered from the constipation form of the disease (IBS-C); 5 persons (4 women aged 24-53, mean value 44 years, and 1 man aged 53) were affected with diarrhea form (IBS-D); 9 patients (8 women aged 35-80, mean value 52.5 and 1 man 66 years old) suffered from the mixed form (IBS-M). The length of the disease ranged between 1 and 30 years, with the mean of 6.7 years. The control group consisted of 20 healthy volunteers, according to WHO criteria.

The material for cytometric testing was venous blood. The following parameters were subjected to immunological evaluation:

- percentage values of mononuclear peripheral blood cells, as determined by the monoclonal antibodies in cytofluorometric test (Cytomics FC500, Beckman Coulter):
  - percentage of $TCD4^+$ and $TCD8^+$ lymphocytes,
  - percentage of $TCD4^+$ and $TCD8^+$ lymphocytes with co-expression of CD28 receptor,
  - percentage of $TCD4^+$ and $TCD8^+$ lymphocytes with co-expression of CTLA-4 (CD152) receptor,
  - percentage of $TCD4^+$ lymphocytes with co-expression of $CD25^+$ and $CD69^+$ receptors,
  - percentage of $TCD4^+$ lymphocytes with co-expression of $CD25^+CD62L^+$ and $CD25^+$GITR receptors;
- the magnitude of proliferative response of PBMC in microculture to PHA and ConA;
- concentration of IL-1β, IL-1Ra in serum and supernatants of PBMC cultures as determined by ELISA method;
- concentration of IL-10 and TGF-β in serum and supernatants of isolated $CD4^+$ cells (ELISA kits of Quantikine R&D System, USA).

The mean values of analyzed immunological parameters were taken into account, upon registration of the patient to the Clinic. These results were compared to the values evaluated in the control (healthy) group. The statistical analysis was conducted according to ANOVA/MANOVA procedures. In case of lack of normal distribution, the differences of mean values between individual groups were calculated – independently for each evaluated parameter – by means of non-parametrical U Mann-Whitney and Wilcoxon's test, with significant difference at $p < 0.05$ level.

Results

In the analysis of percentage of $CD3^+CD4^+$ cells and Th/Ts proportion in tested group, significantly lower mean percentage values of $CD3^+CD4^+$ lymphocytes were observed as compared to the analogical values in control group. Such significant differences were not noted between IBS and healthy control group with regard to the mean values of $CD3^+CD8^+$ lymphocytes and percentage values of $CD3^+CD8^+$/CD28 and $CD3^+$CD4/CD28 lymphocytes (Table 1).

The mean values of $CD3^+CD4^+$ cells with co-expression of CTLA-4 receptor in the patients' group were significantly higher than the values evaluated in control group. Such significant differences were not noted between IBS and healthy control group with regard to the percentage values of $CD3^+CD8^+$ lymphocytes with co-expression of CD152 (CTLA-4) receptor.

In the next stage of investigation, the percentage values of $CD4^+$ lymphocytes with co-expression of CD25, CD69 and FoxP3 receptors were measured. The results are presented in Table 2.

In the performed evaluation, no significant differences were seen concerning the mean percentage values of $CD4^+$CD25highFoxP3$^+$ cells between the patients' and con-

Table 1. Percentage values of $CD3^+CD4^+$, $CD3^+CD8^+$lymphocytes with Th/Ts proportion and percentage values of $CD3^+CD4^+$ and $CD3^+CD8^+$ lymphocytes with co-expression of CD28 and CTLA-4 (CD152) receptors in the IBS patients' and control groups

Tested parameter	Patients' group 1 – IBS	Control group K	Statistical analysis
$CD3^+CD4$%	29.7 ±4.9	43.7 ±5.1	1 vs. K; $p < 0.01$
$CD3^+CD8$%	30.2 ±10.4	26.5 ±4.9	ND
Th/Ts	1.0 ±0.4	1.6 ±0.6	1 vs. K; $p < 0.03$
$CD3^+CD4/CD28$%	60.9 ±7.3	68.9 ±13.9	ND
$CD3^+CD8/CD28$%	34.9 ±6,8	32.1 ±10.3	ND
$CD3^+CD4/CD152$%	6.7 ±4.2	3.2 ±2.6	1 vs. K; $p < 0.04$
$CD3^+CD8/CD152$%	9.9 ±4.7	9.3 ±4.2	ND

ND – no data

Table 2. Percentage values of $CD4^+$ lymphocytes with co-expression of $CD25^+$ and FoxP3, $CD25^+CD62L^+$ and $CD25^+GITR^+$ receptors in evaluated groups

	$CD4^+$					
Evaluated groups	**$CD25^{high}FoxP3^+$**	**$CD25^{high}$**	**$CD25^{high}CD69^+$**	**$CD62L^+$**	**$CD25^{high}CD62L^+$**	**$CD25^{high}GITR^+$**
Control K	77.2 ±7.7	3.5 ±1.4	0.78 ±0.2	68.8 ±10.1	68.1 ±1.09	26.8 ±11.2
IBS	85.7 ±17.1	11.2 ±4.9	4.1 ±1.3	66.3 ±11.2	49.3 ±13.9	26.6 ±4.2
Statistical analysis	ND	IBS vs. K; $p < 0.001$	IBS vs. K; $p < 0.001$	ND	IBS vs. K; $p < 0.02$	ND

ND – no data

Table 3. Mean percentage values ± standard deviation of $CD4^+CD25$highcells with co-expression of $CD134^+$ and $CD95^+$ in IBS and control groups

	CD4+		
Evaluated groups	**$CD25^{high}CD134^+$ (%)**	**$CD25^{high}CD95^+$ (%)**	**$CD25^{high}CD152^+$ (%)**
IBS	5.5 ±1.6	5.8 ±2.3	29.8 ±2.7
Control K	3.8 ±1.7	7.9 ±2.6	23.1 ±2.6
Statistical analysis	IBS vs. K; $p < 0.05$	IBS vs. K; $p < 0.05$	ND

ND – no data

trol group. With regard to the $CD4+CD25^{high}$ cells and $CD4^+CD25^{high}CD69^+$ lymphocytes, their mean percentage values were significantly higher in the IBS vs. control group.

Regarding $CD4^+CD62L^+$ and $CD4^+CD25^{high}$GITR lymphocytes, their mean percentage values did not differ significantly between the two tested groups. However, a significant difference ($p < 0.05$) was observed with concern to the $CD4^+CD25^{high}CD62L^+$ and $CD4^+CD25^{high}$ $CD134^+$cells, with their mean percentage values higher in the IBS than in control group (Table 3).

The mean percentage value of $CD4^+CD25^{high}CD95^+$ cells was significantly lower than in control group. With regard to the $CD4^+CD25^{high}$ lymphocytes with co-expression of CD152 (CTLA-4) receptor, no significant differences were observed between the groups.

In context of proliferative response to PHA, the mean value measured in the IBS group was significantly lower than in control (Table 4).

Concerning the proliferative response to ConA, no significant differences of mean values between the groups were observed. The lower mean value of P/C proportion was noted in the IBS group.

The evaluation of IL-10, TGF-β, IL-1β and IL-1Ra concentrations in serum and supernatants from PBMC cultures stimulated with ConA is presented in Table 5. No significant differences were observed between the mean values of IL-10 concentration measured in patients' serum as compared to the control group. In contrast, the IL-10 concentration values in supernatant from cultures of isolated $CD4^+$ cells were significantly lower in IBS vs. control group.

No significant differences in mean values of TGF-β concentration were seen between serum and supernatants from cultures of isolated $CD4^+$ cells in IBS patients; accordingly, these values did not differ from the relevant ones in control group.

With regard to mean values of IL-1β and IL-1Ra concentration in supernatants, they were significantly higher in IBS group. In serum, IL-1β concentration was significantly higher, and IL-1Ra significantly lower than in control group.

Discussion

The immune response to versatile antigen and allergen stimulation is determined on one hand by the level and extensiveness of damage which evokes the inflammatory reaction, and the defense potential of the host on the other. The correct process leads to constraining of inflammation by recruitment of cells, which synthesize several humoral factors with anti-inflammatory activity. The next step of correct response depends on restoration of altered quantitative and qualitative immune parameters, characteristic for the healthy state of organism. The mechanisms, which allow the cells active in inflammatory process to mislead the immunological supervision and in consequence to avoid destruction, concern the pro-inflammatory cells themselves (diminution of expression of surface histocompatibility antigens or adhesion molecules), but are also attributed to the cells which are supposed to supervise the inflammatory process – T lymphocytes (induction of anergy or clonal deletion, disruption of transduction signaling, secretion of immunosuppressive cytokines) [26-28].

In the middle of the nineteen nineties, the subset of cells with $CD4^+$ phenotype and suppressive activity was characterized, which were called T regulatory cells (Tregs). The accumulated knowledge on T regulatory cells seems to argue for their potential role – among others – in patho-

Table 4. Mean values ± standard deviation of PBMC response to PHA and ConA in tested groups

Evaluated groups	PHA (dpm × 10^3/hod)	ConA (dpm × 10^3/hod)	P/C
IBS	31.9 ±8.9	24.7 ±9.9	1.2 ±0.5
Control K	59.6 ±7.01	25.3 ±5.8	2.2 ±0.4
Statistical analysis	IBS vs. K; $p < 0.02$	ND	IBS vs. K; $p < 0.05$

ND – no data

Table 5. Mean values ± standard deviation of IL-10, TGF-β, IL-1β and IL-1Ra concentrations measured in serum and supernatants of cell cultures of IBS patients and healthy individuals

Evaluated groups	IL-10 (pg/ml)		TGF-β (pg/ml)		IL-1β (pg/ml)		IL-1Ra (pg/ml)	
	serum	PBMC culture supernat	serum	PBMC culture supernat	serum	PBMC culture supernat	serum	PBMC culture supernat
IBS	16.8 ±3.1	820 ±210	25.9 ±4.1	2500 ±351	14.9 ±10.5	339 ±114	468 ±224	2643 ±1476
Control group K	19.0 ±3.6	1100 ±340	28.1 ±6.0	2670 ±390	1.9 ±1.8	170 ±80	829 ±323	900 ±810
Statistical analysis	ND	IBS vs. K; $p < 0.05$	ND	ND	IBS vs. K; $p < 0.001$	IBS vs. K; $p < 0.001$	IBS vs. K; $p < 0.01$	IBS vs. K; $p < 0.04$

ND – no data

genesis of neoplastic, auto-immune and infectious diseases. In light of the data presented herein, the participation of T regulatory cells in the pathogenesis of IBS seems probable, with the resulting assumption that their correct function could restrain the progress of the disease.

The aim of this study was to evaluate the selected quantitative and functional exponents characteristic of the course of the disease in patients with IBS. The investigations focused mainly on the quantitative parameters and activation indices of T cell population. The functions of studied subpopulations were evaluated by means of measurement of pro- and anti-inflammatory cytokines in the supernatant of cultured PBMC. The next element of analysis was the characteristics of T regulatory cells, by evaluation of co-expression of CD152 and CD62L molecules, with CD95 apoptotic receptor and CD69 activation receptor also included.

Before the introduction of treatment, in group of IBS patients the mean percentage values of CD3+ lymphocytes were significantly lower than in control group. The confirmation of ongoing inflammatory process in group of patients is the elevated concentration of pro-inflammatory cytokine IL-1β, with lowered IL-1Ra values in serum.

The aggravated immunogenic activity, accompanied by insufficient competence of immunological response, is predominantly observed in chronic infections and is a factor of primary importance in evoking the chronic inflammatory states. The immunogenic properties of the immune system were tested in PBMC cultures and in patients' serum. The increased ratio of pro-inflammatory IL-1β and its antagonist IL-1Ra, above the values observed in healthy individuals, reflects the increased immunogenic activity and accompanies the developing inflammatory reaction, while the decrease of mentioned ratio gives evidence of lowered immunogenic activity and immunological reactivity, which correlates with ceasing of inflammation and improvement of clinical condition.

Another step of immunogenic activity testing was to evaluate the concentration of chosen cytokines, representing both pro- (IL-1β) and anti-inflammatory (IL-1Ra) activity, followed by anti-inflammatory cytokines characterizing T regulatory cells, namely IL-10 and TGF-β. The concentrations of cytokines were measured in supernatants of cultured PBMC and isolated $CD4^+$ cells. The measurements performed in culture supernatants, in the closest vicinity of cultured cells, allow to link accurately the obtained results with activity of potential cytokine producers. Serum, on the other hand, as a liquid environment distant from the cytokine-producing cells, reveals the level of cytokines which is a net result of their production, local consumption and elimination, thus creating difficulties in interpretation.

The evaluation of correlation between IL-1β and TGF-β seems particularly interesting. In the tested group of patients, the significantly elevated IL-1β concentration in culture supernatants was observed, which was not balanced with changes in TGF concentration (in the IBS group the TGF-β values were comparable to the control group). Transforming growth factor β paradoxically inhibits or stimulates the activity of T lymphocytes. The way of reaction depends on the level of differentiation, presence of other cytokines or growth factors and surface expression of co-stimulating receptors. With this in mind, TGF-β can inhibit the proliferation of T lymphocytes, activation of macrophages and dendritic cells. In the animal experimental model, blocking of TGF-β release or its removal from the environment results in escalation of inflammatory process. As to IL-10, its broad spectrum of influences includes immunosuppressive and anti-inflammatory actions. Some of the anti-inflammatory IL-10 effects are mediated by inhibition of transcription factor NF-κB, though the inhibition of IL-5 synthesis is NF-κB independent [29]. Because IL-10 also inhibits the antigen-presenting function of APC, the excess of IL-10 observed in supernatants of patients, with concomitant normal level in serum, can be the reason of diminished effectiveness of macrophages in suppressing the proliferative response of T lymphocytes and of elevated consumption of this cytokine in the affected area of the bowel [17, 25, 30].

Disturbed equilibrium between pro- and anti-inflammatory factors can lead to escalation of immune response and in effect to destruction of self-defense mechanisms. The excessive pro- or anti-inflammatory reaction can also cause the immune defects and anergy, clinically manifested by enhanced susceptibility to infectious agents and onset of auto-immune and neoplastic diseases.

Interleukin 10, along with TGF-β, is a cytokine elaborated by regulatory T cells. It inhibits the function of autoreactive T lymphocytes, lowers the ability to synthesize Th1 cytokines like TNF-α, IL-12 and IL-18, but also reduces apoptosis and extends the life-span of T and B cells by influencing the expression the anti-apoptotic Bcl-2 protein. The involvement of cells in the process of recognition, processing and presentation of antigen gives rise to the cascade of events described as inflammatory response. In addition to participation in T cell response, TGF-β synthesized in platelets also takes part in the early stage of inflammation.

Some of the studies show the independence between the pro-inflammatory activity of TNF-α, IL-1 or IL-8 and anti-inflammatory function of soluble forms of TNF-β or IL-1Ra receptors. In addition, IL-10 can enhance the synthesis of IL-1Ra. Our results seem to confirm these observations (elevated levels of IL-10 and IL-1Ra were observed in supernatants of patients in the IBS group). The reports on the potential role of TGF-α in mediating the activity of Treg lymphocytes are equivocal. It remains an open question, to what extent the suppressive activity of regulatory T lymphocytes depends on the expression of TGF receptor, or on collaboration with IL-10.

Other studies have proven, that the potential role of IL-10 in response to chronic inflammation can be the inhi-

bition of pro-inflammatory activity of Th1 lymphocytes. These studies however have not resolved the question whether Tregs participating in the inhibition of the inflammatory reaction are nTregs cells of thymic origin, or iTregs recruited from peripheral T lymphocytes with low expression of CD25 receptor.

The increase in number and activity of $CD4^+CD25^+$ lymphocytes from the blood of IBS patients can be responsible for the suppression of T lymphocyte response. To investigate this process in our own studies, the percentage of $CD4^+$, $CD8^+$ and co-expression of their CD28 and CD152 molecules (indicative of the cell immunomodulatory potential) were evaluated. In the patients' group, the statistically significant diminution of percentage of $CD4^+$ helper-inducer lymphocytes was shown as compared with the control group, along with lowered $CD4^+/CD8^+$ ratio and unchanged percentage of cytotoxic $CD8^+$ lymphocytes. It is difficult to resolve, whether the noticed quantitative deficit of $CD4^+$ cells is the cause or effect of the observed clinical symptomatology. It seems that this deficit can be caused by the inflammatory state itself, where the escalation, and in particular the chronicity of stimulation with unknown factor exerts the immunosuppressive effect. The increased activity of Tregs can be also of importance in this context. This can also be a reason of lowered $CD4^+$: $CD8^+$ ratio and diminished proliferative abilities of T lymphocytes (drop in response to PHA in the patients' group).

The expression of CD28 receptor is indicative of the stimulating, and of CD152 – inhibitory ability of lymphocytes towards other cell types, including Tregs [31]. In the group of IBS patients, the statistically significant higher percentage of double-positive $CD4^+CD28^+/CD152^+$ cells was noted when compared with the control group. In case of co-stimulating molecules, a specific competition takes place to activate or inhibit the effector functions of stimulated cells. The pool of double-positive CD28CD152 cells can serve as a certain safety mechanism against the non-controlled progress of inflammatory state in the course of IBS. Such a cell hypothetically could, depending on the strength of the stimulating signal, undergo the diversification into the activating $CD28^+$ or inhibiting $CD152^+$ type. In the group of IBS patients, the statistically significant elevation of percentage of $CD4^+CD152^+$ lymphocytes was observed, which could suggest the prevalence of inhibitory processes exerted by $CD3^+CD4^+$ on the active Tregs cells, with simultaneous lack of such impact from $CD8^+$ cells. This can be one of the reasons for the observed deficit of inducer-immunostimulatory cells in the group of IBS patients, leading – together with intact cytotoxic cell number – to lowering of the $CD4^+/CD8^+$ ratio.

The experiments performed in the animal model proved the role of Tregs in inhibiting the inflammatory reaction mediated by Th1 lymphocytes. The depletion of the latter ones during the acute phase of infection can prevent both the retention of antigen and shift to the chronic phase. It seems plausible, that in future the modulation of Tregs function can improve the effectiveness of the therapy, becoming its valuable enhancement. The findings of the last decade have confirmed the earlier suppositions, that thymus-dependent process of supplementation of continuously exploited, multi-task T-lymphocyte population is – or at least should be – continued throughout the whole life time of the organism [5, 15, 20, 24]. The impairment of this process brings about the deficits in T cell population, disproportions between immunogenic and immuno-competent abilities of the immune system, disruptions in immuno-regulatory functions and lowering of the defense potential. It seems that introduction of complex treatment, involving all the stages of the immune response and balancing the complementary mechanisms of the second – specific phase, can be beneficial for the effects of commonly applied therapy.

Conclusions

The results of investigations presented in this study allow us to put forth the following claims:

1. In patients suffering from IBS, the immuno-regulatory disorders take place, with meaningful increase of activity of pro-inflammatory cytokines, decreased activity of anti-inflammatory and immuno-regulatory cytokines and reduction in number and function of regulatory T lymphocytes.
2. The observed quantitative and functional disorders of Tregs need confirmation and further testing on much broader panel of patients, in order to detect alterations specific for the 3 distinct forms of IBS (diarrhea-, constipation-related and mixed).

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