Cytokine Profile and Immune Status of Patients with Chronic Glomerulonephritis Concomitant with Chronic Opisthorchiasis

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Decrease in the absolute count of T lymphocytes in the peripheral blood (mainly $CD4^+$ cells), activation of humoral immunity with accumulation of immune complexes, and suppression of the neutrophil metabolic activity were detected in patients with chronic glomerulonephritis in the presence of opisthorchis invasion. Imbalance in the serum concentrations of cytokines produced by T-helper subpopulations (predominance of Th products) was detected.

Key Words: *glomerulonephritis; opisthorchiasis; immunity; cytokines*

The prevalence of chronic glomerulonephritis (CGN), a steadily progressing immunity-mediated inflammatory disease, notably increased during the latest decade [6,8,10]. Immune disorders largely contribute to chronic transformation of the disease and formation of chronic renal insufficiency [7,9,11]. The disease is often concomitant with chronic opisthorchiasis (CO) in West Siberian regions hyperendemic for opisthorchiasis. Opisthorchis invasion involves the immune system in the pathological process in response to antigenic structures of the helminth and some its bioactive products possessing an immunosuppressive effect [4,5]. This aggravates the course of CGN in patients with mixed disease [2]. The problems of immune homeostasis in this complex pathology remain unclear, for example, the concentrations of the key mediators of cellular and humoral reactions, their correlations with each other and with CGN activity parameters, duration and intensity of helminthic invasion in CGN patients with CO. We studied cellular and humoral immunity and cytokine profiles (serum IL-4, IL-6, and γ-IFN) in CGN patients with preserved renal function in the presence of CO.

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MATERIALS AND METHODS

A total of 100 CGN patients concomitant with CO (group 1) and 30 patients with CGN without CO (group 2) aged 15-40 years, all of them with preserved renal function, treated at Nephrology Department of Tomsk Regional Clinical Hospital, were examined. Control group consisted of 25 healthy volunteers.

The diagnosis of Opisthorchis invasion was based on detection of *Opisthorchis felineus* eggs in the bile and feces. Isolated urinary syndrome predominated in the clinical picture in the majority of patients in groups 1 and 2 (51 (51%) and 12 (40%), respectively). Morphologically, 81 (81%) and 25 (83.3%) patients presented with mesangioproliferative variant of CGN, 5 (5%) and 1 (3.3%) with membranous, and 11 (11%) and 4 (13.3%) with mesangiocapillary variants, respectively.

Immunological parameters were evaluated as described previously [1]. Lymphocyte subpopulations were evaluated by immunofluorescent analysis on an EPICS-C flow cytometer (Coultronics) using monoclonal antibodies (Medbiospektr Firm). IgA, IgM, and IgG were measured by radial immunodiffusion, IgE by enzyme-linked immunosorbent assay. The level of circulating immune complexes (CIC) was evaluated by precipitation. The oxygen-dependent metabolism of

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neutrophils was studied by NBT test. Serum levels of IFN-γ, IL-4, and IL-6 were measured by enzyme-linked immunosorbent assay using commercial Pro Con reagent kits (Protein Contour Firm, Russia).

The results were statistically processed by parametrical and nonparametrical methods using Biostatistika 4.03 software.

RESULTS

The study revealed a significant decrease in the absolute counts of leukocytes and $CD2^+$, $CD3^+$, and $CD4^+$ cells in the blood of patients with CGN concomitant with CO and of $CD4^+$ lymphocytes in CGN patients without CO in comparison with the controls (Table 1). The counts of $CD8^+$ cells surpassed the control in both groups ($p<0.01$).

The level of CIC in group 1 patients was higher than in healthy subjects ($p<0.05$), while in group 2 it was below the control ($p<0.05$).

The neutrophil oxygen-dependent metabolism (evaluated by spontaneous NBT test) was decreased in patients with mixed disease, while the stimulated test showed decreased activity in both groups.

The levels of IgM, IgG, and IgE increased significantly in group 1, while IgG level decreased in group 2 ($p<0.05$).

The suppression of T-immunity was more pronounced in patients with invasion longer than 10 years in comparison with patients with shorter invasion. The absolute counts of $CD3^+$ cells were $0.79\pm0.06\times10^9$/liter and $0.98\pm0.05\times10^9$/liter, respectively ($p<0.05$).

Presumably, suppression of cell-mediated immunity is responsible for deceleration of reparative processes in the renal tissue of patients with mixed disease and hence, is essential for the prognosis. We found that the increase in creatinine content and impairment of glomerular filtration and tubular reabsorption were accelerated in patients with mixed disease in comparison with CGN patients without CO.

The function of Th1 and Th2 lymphocyte subpopulations was evaluated by measuring serum IFN-γ and IL-4 levels and the concentration of IL-6 proinflammatory cytokine.

In group 2 patients IL-6 level during the disease exacerbation increased more than 4-fold in comparison with healthy subjects ($p<0.01$; Table 2). During CGN remission the concentration of IL-6 exhibited just a trend to a decrease, not reaching the control level.

The concentration of IFN-γ in both groups virtually did not differ from the control irrespective of activity of the inflammatory process.

The level of IL-4 during exacerbation in group 2 was comparable to that in the controls, while during remission IL-4 concentration was higher than in controls ($p<0.001$) and patients with active CGN ($p<0.001$).

In group 1 patients the level of IL-6 was higher than in healthy subjects, especially during exacerbation (5-fold, $p<0.01$). We detected a correlation between the morphological index of renal inflammation activity and blood concentration of IL-6 in patients of groups 1 and 2 ($r_S=0.79$ and 0.68; $p<0.01$).

The level of IL-4 during exacerbation in patients with mixed disease virtually did not differ from the

TABLE 1. Parameters of Cellular and Humoral Immunity in CGN Patients and Healthy Subjects (*M*±*m*)

Parameter	Control (*n*=25)	CGN+CO (*n*=100)	CGN without CO (*n*=30)
Leukocytes, $\times10^9$	6.21±0.14	5.28±0.34*	5.61±0.48
Lymphocytes, $\times10^9$/liter	2.12±0.18	1.51±0.08*	1.57±0.07
$CD2^+$ cells, $\times10^9$/liter	1.54±0.12	0.86±0.13*	0.92±0.12
$CD3^+$ cells, $\times10^9$/liter	1.42±1.12	0.59±0.11*	0.82±0.15
$CD4^+$ cells, $\times10^9$/liter	0.85±0.11	0.62±0.10*	0.74±0.15*
$CD8^+$ cells, $\times10^9$/liter	0.42±0.10	0.44±0.10*	0.56±0.10*
CD4/CD8	2.01±0.07	1.41±0.09*	1.32±0.05*
$CD72^+$ cells, $\times10^9$/liter	0.26±0.07	0.36±0.08	0.26±0.11
IgA, g/liter	2.31±0.07	3.25±0.43	2.83±0.33
IgM, g/liter	1.37±0.11	1.67±0.24*	1.44±0.09$^+$
IgG, g/liter	12.43±1.21	15.86±0.35*	8.75±0.25*$^+$
IgE, mg/ml	41.42±2.31	55.34±1.13*	44.23±2.12$^+$
CIC, arb. units	72.41±2.81	97.12±4.56*	65.11±4.81$^+$
NBT spontaneous, %	14.84±1.12	9.92±1.11*	11.06±1.01
NBT stimulated, %	28.13±2.83	15.02±0.21*	16.41±1.01*

Note. $p<0.05$ compared to: *control, $^+$group 1.

TABLE 2. Levels of IL-4, IL-6, IFN-γ in CGN Patients and Healthy Subjects (*M*±*m*)

Parameter	Control (*n*=25)	CGN+CO (*n*=100)	CGN without CO (*n*=30)
IL-4, pg/ml	35.0 (30.0, 40.0)	28.35 (24.1, 48.4) 356.4 (193.5, 610.1)*x	31.5 (26.6, 47.1) 476.9 (213.4, 661.2)*+x
IL-6, pg/ml	51.0 (39.0, 63.0)	261.05 (164.0, 307.7)* 215.25 (139.1, 290.3)*	220.6 (115.5, 276.8)* 196.13 (107.6, 259.8)*
IFN-γ, pg/ml	41.8 (31.7, 51.9)	41.8 (27.6, 86.9) 35.77 (16.2, 72.3)	44.4 (20.9, 77.7) 35.2 (13.5, 74.4)

Note. Numerator: values during CGN exacerbation; denominator: values during remission; the median is shown, with 25th and 75th percentiles in parentheses. *$p<0.01$ compared to the control, +$p<0.05$ compared to group 1, and x$p<0.01$ compared to numerator.

control. During CGN remission the concentration of this cytokine surpassed that in donors and patients with CGN exacerbation ($p<0.01$). In group 2 the level of IL-4 during remission was significantly higher than in group 1 ($p<0.05$).

Hence, dysfunction of Th1 and Th2 was revealed in CGN patients. The Th2 immune response predominated in patients with CGN alone and concomitant with CO.

The detected shifts in the immune status and cytokine profiles lead to imbalance between the inflammation and reparation processes in CGN patients with CO and favor the maintenance and progress of the pathological process in the kidneys and in organs of obligate habitation of the helminths, which should be taken into consideration when planning the treatment strategy.

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