Combined Disorders in Erythro- and Immunopoiesis in (C57Bl/6×DBA/2)F1 Mice with Immunodeficiency Induced by Graft-Versus-Host Reaction and Immunocomplex Glomerulonephritis

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Disorders in erythro- and immunopoiesis were studied in B6D2F1 mice with immunodeficiency induced by graft versus host reaction and with immunocomplex glomerulonephritis. By the 6th-7th months of the disease the animals developed anemia accompanied by enchanced phagocytic activity of macrophages and production of tumor necrosis factor. Macrophage dysfunction is presumed to be a cause of anemia.

Key Words: *immunodeficiency; glomerulonephritis; erythropoiesis; tumor necrosis factor-α; mice*

Study of the relationship between immuno- and hemo (erythro-)poiesis is an important approach to investigation of the mechanisms of immunodeficiency. The effect of erythron on the immunity is studied mainly under physiological conditions [3]. Some authors consider dysfunction of erythropoiesis as a component in the pathogenesis of immune disease. Combined disorders of erythro- and immunopoiesis are neglected in experimental studies of immunopathological diseases, such as systemic lupus erythematosus or AIDS or only individual stages of erythron differentiation are investigated.

Induction of chronic graft-versus-host reaction (GVHR) in (C57Bl/6×DBA/2)F1 (B6D2F1) mice leads to the development of immunodeficiency (ID) and autoimmune disease associated with the anemic syndrome and similar to systemic lupus erythematosus in humans [4]. Here we proceeded with investigations of combined disorders of erythro- and immunopoiesis in B6D2F1 mice.

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MATERIALS AND METHODS

Experiments were performed on B6D2F1 mice from Experimental Animal Clinic of Siberian Division of the Russian Academy of Medical Sciences.

GVHR was induced in B6D2F1 by intravenous injection of lymphoid cells from parental DBA/2 mice [10]. After 6-7 months group 1 animals developed ID without proteinuria and group 2 developed immunocomplex glomerulonephritis (ICG) confirmed clinically and morphologically.

Primary humoral immune response was evaluated by the number of antibody-producing cells in the spleen [8].

Reticulocytes were counted in peripheral blood smears stained with azur II. The content of stem hemopoietic cells in the bone marrow was evaluated by the number of colony-forming units in the spleen (CFUs) of sublethally irradiated syngeneic recipients at various terms after transplantation of the bone marrow [14].

Phagocytic activity of peritoneal macrophages incubated with 2.5% opsonized sheep erythrocytes was estimated spectrophotometrically by hemoglobin in content cells treated with a detergent [12].

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The content of tumor necrosis factor-α (TNF-α) in macrophage supernatant was measured using TNF-α-sensitive L-929 cells [9]. Supernatant collected routinely and diluted 1:100 was added into wells and activity of TNF-α was evaluated by supravital crystal violet staining of cell monolayer, estimating the percentage of dead cells.

Results were statistically processed by nonparametrical Wilcoxon *U* test, analysis of correlations was carried out using Spearman test.

RESULTS

Both groups of B6D2F1 mice with GVHR developed ID, as evidenced by suppression of primary humoral immune response. Splenomegalia was found in all mice, but the splenic index (spleen to body weight ratio) increased only in animals with ICG. Erythrocyte sedimentation rate increased while body weight decreased in both experimental groups (Table 1).

The spleen, lymph nodes, liver, and kidneys were taken for morphological examination. Manifestations of ID (atrophy of lymphoid organs) were observed in all animals. Focal mononuclear infiltration, degeneration and atrophy of the parenchyma were found in examined organs. Changes in the kidneys were the most pronounced in mice with ICG and minor in animals with ID: negligible focal lymphohistiocytic infiltration of the stroma and moderate degeneration of parenchymatous cells. Similar morphological changes in the liver, kidneys, and immune organs were observed in patients with systemic lupus erythematosus. The degree of morphological changes in renal tissue in mice with ICG and ID correlated with protein concentration in the urine (Table 2) [5]. Moreover, ascites in mice with ICG together with high proteinuria indicated the development of the nephrotic syndrome.

We previously showed enhanced production of interleukin-1 in mice with ID and ICG [4]. In this study macrophage status was evaluated by activity of phagocytosis and production of TNF-α.

Phagocytic activity of macrophages increased in mice with ID and ICG in comparison with the control (Table 1). In animals with ID, spontaneous production of TNF-α was lowered and lipopolysaccharide (LPS)-stimulated production of this factor was virtually the same as in the control. In mice with ICG, spontaneous production of TNF-α did not differ from the control, while LPS stimulation considerably increased TNF-α production in comparison with the control (Table 2).

We previously showed that apart from immunocompetent cells and some cytokines, erythrokaryo-

TABLE 1. Immunopoiesis and Parameters of Red Blood in B6D2F1 Mice with ID and ICG (*M±m*)

Parameter	Control (*n*=20)	Mice with ID (*n*=15-20)	Mice with ICG (*n*=10-15)
Body weight, g	26.1±0.8	22.40±0.75*	21.3±0.7*
ESR, mm/h	1.3±0.4	3.6±0.4*	11.2±3.3*
Splenic index, %	1.05±0.40	1.2±0.2	1.90±0.25*
Number of antibody-producing cells in spleen	45 600±1234	17 200±1599*	12 600±1120.5**
Phagocytic activity, arb. units	280.0±15.2	502.7±20.1*	1029.4±34.2*
Hemoglobin, g/liter	199.2±3.6	169.6±1.4*	140.3±6.9*
Hematocrit, %	49.4±0.4	44.90±0.14*	38.8±1.4*
Reticulocytes, %	10.20±1.32	15.9±2.4*	21.3±3.4*

Note. *$p<0.01$, **$p<0.05$ *vs.* the control.

TABLE 2. Renal Function, Status of Macrophages, and Number of Exogenous Splenic Colonies in Mice with ID and ICG (*M±m*)

Parameter		Control (*n*=20)	Mice with ID (*n*=15-20)	Mice with ICG (*n*=10-15)
Proteinuria, g/liter		0.30±0.06	0.54±0.30	6.6±0.5*
Production of TNF-α by peritoneal macrophages, %	spontaneous	12.20±0.45	4.1±1.6*	10.66±5.00
	LPS-induced	40.8±3.9	41.0±4.2	54.8±4.0**
Number of exogenous splenic colonies	CFUs-5/spleen	10.4±1.5	13.1±2.1**	8.0±1.5**
	CFUs-8/spleen	12.8±1.5	18.1±2.5*	12.3±1.9

cytes possessed immunosuppressive activity. Erythrocytic suppressor factor suppresses humoral immune response [3] and can participate in the development of immunodeficiency in mice.

Therefore, we studied erythropoiesis in sick animals. Mice with ID and ICG developed anemia with increased content of erythrokaryocytes and erythroid burst-forming bone marrow units [4]. In both groups anemia was accompanied by reticulocytosis (Table 1), which correlated with the severity of the anemic syndrome and attested to preserved regeneration of the bone marrow. On days 5 and 8, the number of exogenous splenic colonies in experimental animals changed: the number of CFUs-5 and CFUs-8 increased in mice with ID and the number of CFUs-5 decreased in mice with ICG (Table 2). CFUs-5 are unipotent erythroid precursors with a lower self-maintenance capacity compared to CFUs-8. In NZB mice, the number of CFUs-5 an CFUs-8 in the bone marrow is increased [6].

Hence, our findings and published reports indicate that experimental mice developed autoimmune anemia associated with enhanced hemolysis. This is confirmed by the presence of antierythrocyte autoantibodies [10] and increased IgG content in the peripheral blood of B6D2F1 mice with chronic GVHR [11].

Dysfunction of the macrophage component of immunity plays an important role in the development of anemic syndrome in mice. TNF-α and interleukin-1 regulating erythropoiesis are involved in the pathogenesis of autoimmune hemolytic anemia [2]. Cytokines stimulate proliferation of preactivated B lymphocytes and affect the production of immunoglobulins [1], specifically, IgG. Increased production of IgG in patients with systemic lupus erythematosus is explained by high production of interleukin-1 [2]. In NZB mice the fraction of antierythrocyte antibodies consists of $IgG_{2\alpha}$ [13], whose secretion is stimulated by TNF-α [1]. We detected an inverse correlation between hematocrit and production of interleukin-1 in mice with ICG (-0.94, Spearman test, $p<0.05$).

TNF-α and interleukin-1 not only mediate immune reactions, but also act as antiinflammatory cytokines: they induce the production of $O_2^{\cdot}$ and H_2O_2 in macrophages, leukocytes, and mesangial cells by activating lipid peroxidation with subsequent destruction of renal tissue [1]. Interleukin-1 and TNF-α impair arachidonic acid metabolism and increase the production of prostaglandin E_2, thromboxane A, and other metabolites, thus stimulating inflammatory process in the kidneys [7].

The enhanced cytokine production in mice with ICG is paralleled by degenerative changes in the kidneys and high proteinuria, which is in line with published reports.

Moreover, TNF-α mediates the development of cachexia by affecting adipocytes and enhancing catabolic processes in the body [1]. Increased production of TNF-α is responsible for body weight loss in mice with ICG.

We previously assumed that hyperplasia of the erythroid hemopoietic stem in mice with ID and ICG contributes to suppression of the humoral immune response. But no correlation between the number of erythrokaryocytes in the bone marrow and primary humoral immune response was found.

Hence, our experimental data provide better understanding of the pathogenesis of secondary ID and anemia in humans.

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