Cell Cycle of Bone Marrow CD34+ Cells during Autoimmune Disease Development in MRLMpJ/lpr Mice

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The development of autoimmune disease in Fas-deficient MRLMpJ/lpr mice is associated with a relative decrease in the content of bone marrow CD34+ cells, which can attest to intensification of migration of early hemopoietic precursors from the bone marrow. The intensity of CD34+ apoptosis is high in young healthy MRLMpJ/lpr mice in comparison with the control, but decreases during the development of autoimmune disease. Proliferative activity of CD34+ cell population surpassed the control in all mouse groups, except AID2. The detected shifts in quantitative and qualitative parameters of CD34+ cells attest to an important role of stem hemopoietic precursors in the formation of autoimmune disease in MRLMpJ/lpr mice.

Key Words: *MRLMpJ/lpr mice; CD34+ cells; hemopoietic precursors; autoimmune disease; cell cycle*

Fas-deficient MRLMpJ/lpr mice carrying mutant *lpr* gene are characterized by disturbed apoptosis of immunocompetent cells, which leads to the appearance of autoreactive populations and numerous T-lymphocyte subpopulations specific for autoimmune processes ($CD4^-CD8^-$), which results in the formation of severe lymphadenopathy, associated with the production of various autoantibodies, immune complexes, and glomerulonephritis development [9,12].

Fas/FasL interactions play an important role in hemopoiesis regulation. The content of CFU increased significantly in the peripheral blood and spleen of Fas(lpr)- and FasL(gld)-defective mice during the fetal and postnatal periods [10], as well as the content of granulocytic macrophageal CFU precursors in the bone marrow [3]. According to other authors, the level of spontaneous apoptosis of granulocytes is virtually the same in B6, B6(lpr), and B6(gld) mice and Fas/FasL interactions are unimportant for modulation of the content of committed myeloid precursors and peripheral blood pool of mature granulocytes or the capacity of these cells to spontaneous apoptosis [6].

Some authors regard systemic and organ-specific autoimmune diseases (AID) as stem cell disease [7]. Stem cells of MRLlpr mice are much more radioresistant that stem cells of normal mice; splenic CFU count increases with aging in this mouse strain [8]. The development of AID in MRLMpJ/lpr mice is often associated with stimulation of the erythroid stem [11].

The study of the quantitative and functional characteristics of stem hemopoietic cells (SHC) in autoimmune diseases is important for clinical practice, for example, for improving the technology of SHC transplantation.

We studied quantitative and functional characteristics of CD34+ cells during the development of AID in MRLMpJ/lpr mice.

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MATERIALS AND METHODS

Stable presence of protein in the urine (>3 mg/ml) was considered as the main diagnostic criterion of glomerulonephritis development [1]. Three groups of MRLMpJ/lpr mice were formed in accordance with the stages of AID formation: normal animals (n=14; aged 4-5 weeks); mice at the stage of predisease (n=10; 24 weeks); and mice with pronounced proteinuria (n=27; 24 weeks). The values obtained in studies of $CD34^+$ cell characteristics in 10 $(CBA\times C57Bl/6)F_1$ mice served as the control.

Urinary protein was measured colorimetrically with Coumassi brilliant blue stain; calibration curve was plotted using BSA (Sigma; 100-1000 μg/ml).

The percentage of $CD34^+$ population among the bone marrow cells was evaluated by flow cytofluorometry (FACSCallibur, Becton Dickinson); cell cycle was evaluated by DNA content in cells stained with propidium iodide (4 μg/ml; Sigma) [4] using monoclonal cells pre-treated with FITC-labeled anti-CD34 monoclonal antibodies (PharMingen). The percentage of cells with diploid and hyperdiploid DNA set (cells in the G_0/G_1 and S/G_2M phases of the cell cycle, respectively) among $CD34^+$ cells (green fluorescence) was evaluated. Apoptotic cells with fragmented DNA formed a characteristic hypodiploid peak (orange fluorescence).

RESULTS

Two subgroups of mice with proteinuria differing significantly by the percentage of proliferating cells (cell cycle S/G_2M phases), were formed after evaluation of quantitative and functional characteristics of $CD34^+$ cells. In subgroup AID1 (n=15) this parameter considerably surpassed the mean for control group (12.66%), and in AID2 subgroup (n=12) the content of proliferating cells was below 12.66%. Disease development was associated with a decrease in the percentage of $CD34^+$ cells in the bone marrow (Fig. 1, *a*): this value in AID1 mice was significantly lower than in all other groups, except AID2 (AID1 and AID2 subgroups virtually did not differ by this parameter).

The level of $CD34^+$ cell apoptosis was significantly increased in young healthy MRLMpJ/lpr mice in comparison with the control, but decreased with the formation of AID reaching significant differences in AID2 mice in comparison with $CD34^+$ cell apoptosis in other groups (Fig. 1, *b*).

Proliferative activity (cell cycle S/G_2M phases) of $CD34^+$ cells was increased significantly in comparison with the control in MRLMpJ/lpr mice of all groups, except AID2 subgroup (Fig. 2). A significant decrease in the percentage of proliferating $CD34^+$ cells in some animals with AID (AID2) together with their decreased count and reduced level of apoptosis can indicate exhaustion of resources maintaining functional activity of this cell population during the disease development.

The percentage of $CD34^+$ cells in the cell cycle G_0/G_1 phases was reduced significantly in young and AID1 mice in comparison with the control (Fig. 2). This parameter was close to the control in AID2 group and significantly higher than in other groups.

The detected changes in the quantitative and qualitative parameters of $CD34^+$ cells attest to an important role of SHC in the formation of AID. A decrease in the percentage of $CD34^+$ cells in mice with AID can indicate intensification of migration of early hemopoietic precursors from the bone mar-

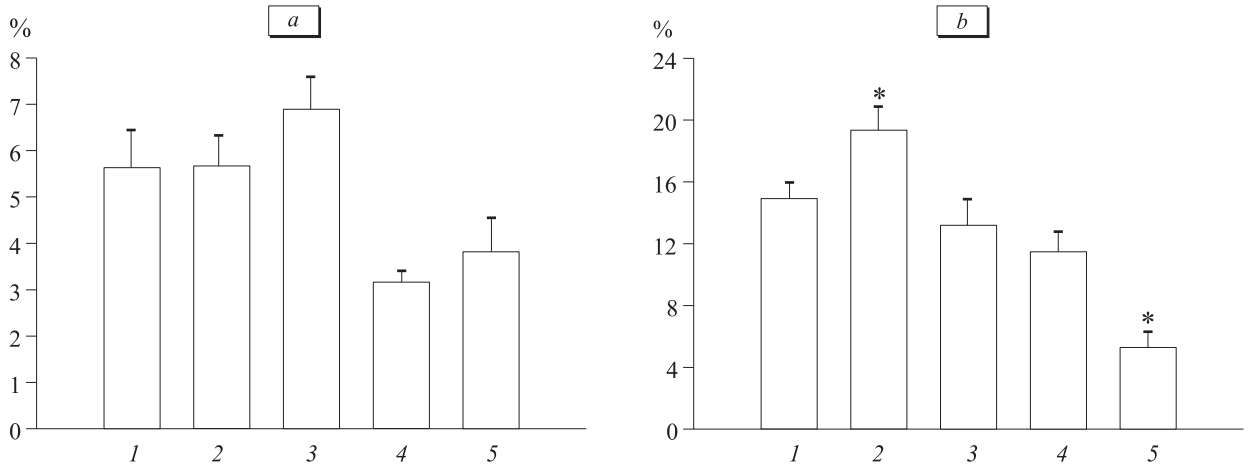

Fig. 1. Percentage of $CD34^+$ cells in the bone marrow (*a*) and of apoptotic cells (*b*) in $CD34^+$ cell population in the bone marrow of $(CBA\times C57Bl/6)F_1$ hybrids (*1*), normal MRLMpJ/lpr mice (*2*), MRLMpJ/lpr mice at the stage of predisease (*3*), AID1 (*4*), and AID2 groups (*5*). Here and in Fig. 2: *p<0.05 compared to the control.

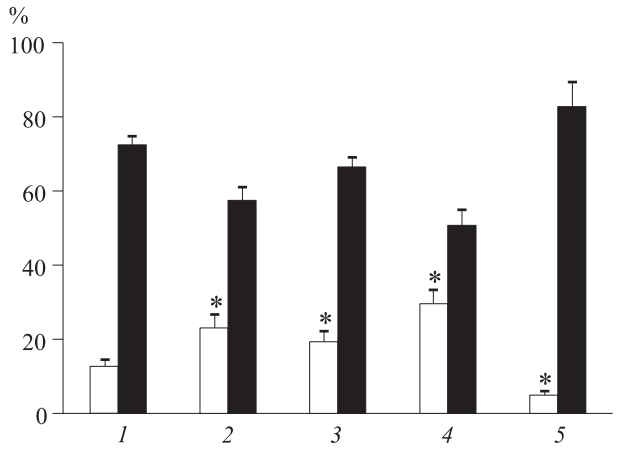

Fig. 2. Percentage of CD34⁺ cells in cell cycle S, G_2/M (light bars) and G_0/G_1 phases (dark bars) in (CBA×C57Bl/6)F_1 hybrids (*1*), normal MRLMpJ/lpr mice (*2*), MRLMpJ/lpr mice at the stage of predisease (*3*), AID1 (*4*), and AID2 groups (*5*).

row. One of the major chemoattractants for SHC is SDF-1 [2]. This chemokine participates in the progress of autoimmune nephritis in NZB/W mice [5]; presumably, high migration activity of SHC contributes to the formation of AID in MRLMpJ/lpr mice. It is obvious that the population of CD34⁺ cells is involved in the formation of autoimmune pathological process characterized by impaired apoptosis of immunocompetent cells in AID: decreased level of apoptosis of these cells with increase in their proliferative activity during disease development indicate deep disorders in functional activity of hemopoietic precursors.

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