IMMUNOLOGY AND MICROBIOLOGY

Reaction of CD117$^+$ Cells to Renal Lesion under Conditions of Phagocytic Mononuclear System Stimulation

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The content of CD117$^+$ cells in the kidneys and CD45^{low}CD117$^+$ cells in the bone marrow and blood of mice were studied after partial nephrectomy and under conditions of macrophage stimulation with 3-aminophthalhydrazide. The counts of tubular CD117$^+$ epitheliocytes sharply increased and the content of CD45^{low}CD117$^+$ cells in the bone marrow decreased after renal damage. Injection of 3-aminophthalhydrazide stimulated the expression of CD117 by renal epitheliocytes and led to reduction of CD45^{low}CD117$^+$ cell counts in the bone marrow and blood. Macrophages stimulated proliferative processes in the kidney and differentiation of stem cells in the bone marrow due to synergic effects of their cytokines and stem cell factor.

Key Words: *macrophages; 3-aminophthalhydrazide; regeneration; kidneys*

According to modern concepts, macrophages within the framework of the phagocytic mononuclear system (PMS) play an important role in tissue regeneration and remodeling [3]. They release a variety of cytokines and growth factors and stimulate proliferation of mature organ cells and stem cells. Due to their high plasticity macrophages can transdifferentiate into other cells: endotheliocytes, myofibroblasts, muscle cells, neuronal cells, and hepatocytes. This process can be realized in the kidneys [8].

Synergic effect of macrophage cytokines and stem cell factor (SCF) produced by fibroblasts on cell proliferation in response to injury has been described [7], a hemopoiesis regulator modulating precursor cells through interactions with CD117 receptors (c-kit). CD117 is transmembrane tyrosine kinase receptor of the CSF-1/PDGF receptor kinase family. The CD117–SCF interaction regulates not only hemopoiesis, but also other physiological processes (gametogenesis, neurogenesis, melanocyte formation). The effect of SCF on hemopoiesis consists in triggering of proliferation and differentiation of hemopoietic stem cells and regulation of their migration and homing [4,5]. However, CD117 is not a unique marker of exclusively stem cells and precursor cells, because it is also expressed by mature cells, for example, by renal tubular epitheliocytes [6]. However, the role of CD117$^+$ cells in this organ remains unclear.

It can be hypothesized that the involvement of macrophage monocytes in the regulation of regeneration processes is to this or that measure mediated through proliferation of CD117$^+$ cells, while PMS stimulation initiates the reaction of these cells located in the damaged organ and in other places.

We studied the reaction of CD117$^+$ cells in the bone marrow, blood, and kidneys of mice after partial nephrectomy under conditions of PMS stimulation with 3-aminophthalhydrazide.

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MATERIALS AND METHODS

The experiments were carried out on outbred albino mice kept on standard vivarium rations. Three groups of animals were formed, 10 per group. Group 1 were intact animals, group 2 were animals with partial resection of the kidney (one-third of the left kidney was resected), and group 3 were mice intramuscularly injected with 3-aminophthalhydrazide (immunomodulator stimulating functional and metabolic activity of macrophages) in a dose of 2 mg/kg for PMS stimulation 1 h before the operation [1,2]. The surgery was carried out under ether narcosis. The reaction of $CD117^+$ cells was evaluated 24 h after surgery, which corresponded to the time preceding the peak of unfolding regeneratory processes in the kidneys.

Cells carrying CD117 antigen were identified on sections of the left and right kidneys by standard indirect immunohistochemical staining (monoclonal anti-mouse CD117, clone ACK2, Millipore). The measurements were carried out in 10 visual fields with semiquantitative interpretation of the results (from 0 to +++). The immunohistochemical index (IHI) was calculated from these results.

$CD117^+$ cells in the blood and bone marrow were identified by direct immunofluorescent staining with monoclonal anti-CD117-PE (BD Biosciences), IgG2b, and a relevant "isotypical" control (rat IgG2b-PE, BD). Erythrocyte lysis was carried out using FACS Lysing Solution (BD Biosciences). The leukocyte gate was verified by monoclonal antibodies to common leukocytic antigen CD45 (anti CD45-PerCP-Cy5.5, IgG2b; isotypical control: rat IgG2b-PerCP-Cy5.5; BD). Cytofluorometry was carried out on an FC500 flow cytofluorometer (Beckman Coulter); at least 100,000 events were recorded.

$CD117^+$ cells with the morphology of a minor lymphocyte (by forward and small angle light scatter) with low expression of CD45 marker ($CD45^{low}CD117^+$ phenotype) were regarded as stem cells and precursor cells. The percentage of $CD45^{low}CD117^+$ cells in the total population of $CD45^+$ cells was estimated.

The data were statistically analyzed using Statistica 6.0 software (StatSoft). Presumable homogeneity of two independent samples was verified using Mann–Whitney's *U* test. The data were presented as the mean±standard error of the mean, the differences were considered significant at $p<0.05$.

RESULTS

Immunohistochemical studies of the kidneys from experimental animals showed positive reaction to CD117 (fine granular staining of different intensity of tubular epithelium mainly in the cortical layer). The renal body cells and stromal cells remained immunonegative in all sections. In intact mice, weak positive reaction was found in 25% tubular epitheliocytes; 2% of these cells exhibited medium expression of CD117. No tubules with intensive expression were found. The greater part (73%) of tubular epitheliocytes was immunonegative. Analysis of the sections of the left (operated) and right kidneys showed an increase in the counts of tubular epitheliocytes with medium expression of CD117 24 h after partial resection; cells with intensive expression of this antigen were identified in both kidneys, this resulting in an increase of IHI (Fig. 1). It is noteworthy that PMS stimulation with 3-aminophthalhydrazide led to an increase in the counts of tubular epitheliocytes with medium (38 and 27%) and intensive expression of the antigen in the left and right kidneys (12 and 3%, respectively). Hence, partial resection of the left kidney led to reaction of $CD117^+$ cells in the resected and symmetrical organ. The reaction manifested in an increase in the count these cells and more intensive expression of the antigen by individual epitheliocytes. Stimulation of PMS led to stimulation of these processes, more pronounced in the resected kidney. Important, $CD117^+$ cells were located mainly perivascularly in all the studied groups. Presumably, cytokines produced by the organ and transported with the blood modulated the expression of CD117.

Cytofluorometry of the blood and bone marrow of mice showed that 24 h after partial resection of the kidney the count of $CD45^{low}CD117^+$ cells in the bone marrow sharply decreased, while the content of these cells in the blood remained unchanged in comparison with the parameter in intact animals. The count of

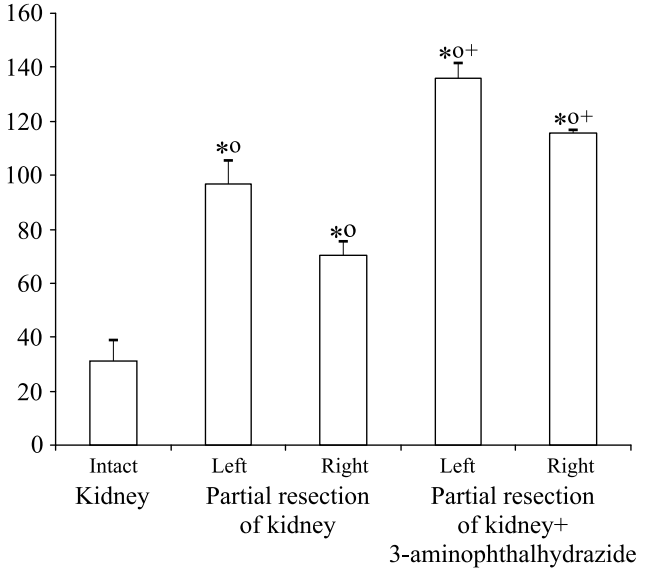

Fig. 1. Changes in IHI in renal injury and under conditions of PMS stimulation. $p<0.05$: *in comparison with intact kidney; °between left and right kidneys in the same group; +in comparison with partial resection of the kidney.

TABLE 1. Content of $CD45^{low}CD117^{+}$ Cells in Mouse Bone Marrow and Blood (*M*±*m*)

Count of $CD45^{low}CD117^{+}$ cells	Group 1 (intact mice)	Group 2 (partial resection of kidney)	Group 3 (partial resection of kidney+3-aminophthalhydrazide)
Bone marrow, 10^3/femur	23.69±3.36	13.14±3.58*	7.07±0.95*
Blood, mm^3	2.96±0.38	3.54±0.52	1.04±0.25*+

Note. $p<0.05$ in comparison with: *group 1, +group 2.

$CD45^{low}CD117^{+}$ cells decreased in both tissues under conditions of PMS stimulation (Table 1).

According to the data of *in vitro* studies of stem cells and precursor cells, SCF promotes their further differentiation [5]. This fact suggests that *in vivo* interaction of SCF and $CD45^{low}CD117^{+}$ cells leads to their differentiation into committed precursor cells and loss of CD117 receptor, which explains the reduction of the count of cells of this phenotype in the studied tissues, most pronounced under conditions of PMS stimulation.

Hence, renal injury causes a reaction of $CD117^{+}$ cells of different location. The count of tubular $CD117^{+}$ epitheliocytes in the kidney increased, which, considering the mitogenic effect of SCF reactions with this receptor, can be associated with the development of reparative regeneration of the kidneys at the expense of tubular cell hyperplasia. In the bone marrow, the reduction of $CD45^{low}CD117^{+}$ cell count was presumably caused by their starting differentiation. Stimulation of PMS with 3-aminophthalhydrazide stimulated these processes due to synergic effects of macrophageal cytokines and SCF on the target $CD117^{+}$ cells.

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