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Changes in the Hemopoietic System of Mice Deficient for Tumor Necrosis Factor or Lymphotoxin-α

N. I. Drize, M. S. Drutskaya,** L. P. Gerasimova, T. E. Manakova, I. L. Chertkov, R. L. Turetskaya,* D. V. Kuprash,* and S. A. Nedospasov*

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Hemopoietic and stromal precursor cells were studied in mice deficient for tumor necrosis factor or lymphotoxin-α. In normal hemopoiesis the main characteristics of hemopoiesis in knockout mice did not differ from those in wild-type mice. Implantation of bone marrow cells from mice deficient for tumor necrosis factor onto irradiated sublayer of a long-living bone marrow culture led to a notable increase in the number of mature cells and granulocytic-macrophage precursor cells. This can be due to the fact that tumor necrosis factor inhibits proliferation of hemopoietic precursor cells, while in the absence of this factor precursor cells actively proliferate. On the other hand, cell composition and number of colony-forming units of granulocytes-macrophages are significantly decreased in cultures onto which bone marrow cells from lymphotoxin-α-deficient mice were implanted. This can be explained by impaired expression of adhesion molecules in these animals. In addition, the number of stromal precursor cells was changed in mice deficient by genes of the tumor necrosis factor cluster.

Key Words: *stem hemopoietic cell; tumor necrosis factor; lymphotoxin-α; hemopoietic precursor cells; focus of ectopic hemopoiesis; cong-term bone marrow culture*

Purposeful removal of genes (so-called knockout) is now widely used for investigating the physiological functions of genes and proteins. This method helped to determine biological functions of many transcription factors, signal molecules, cytokines, chemokines, their receptors, *etc.*

Tumor necrosis factor (TNF) and lymphotoxin-α (LT-α) are two closely related cytokines playing an important regulatory role in the immune response and inflammation [2]. Their genes are closely linked [9] and form a cluster of 3 genes TNF/LT, including the LT-β gene [3]. *In vitro* activities of TNF and LT-α are similar and mediated by the same TNF receptor p55. *In vivo* LT-α in a complex with LT-β can transmit an additional signal via LT-β receptor [4], which determines essential differences in the physiological functions and manifests in TNF- and LT-α-deficient mouse phenotypes, though both phenotypes are characterized by disorders in the development of secondary lymphoid organs and humoral immune response [5,7,8, 10]. Effects of both factors on hemopoiesis are different. TNF increases the number of early hemopoietic precursors, which was demonstrated *in vivo* on mice deficient by TNF receptor gene p55; by contrast, *in vitro* it suppresses the proliferation of precursors [16]. TNF can both induce and inhibit apoptosis of early hemopoietic precursors [6,15]. TNF is involved in stro-

Hematology Research Center, Russian Academy of Medical Sciences; *V. A. Engel'gardt Institute of Molecular Biology, Russian Academy of Sciences; A. N. Belozerskii Institute of Physicochemical Biology; **Biological Faculty of M. V. Lomonosov Moscow State University, Moscow.
Address for correspondence: ndrize@blood.ru. Drize N. I.

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mal regulation of early precursors initiating a persistent culture [12]. It was recently demonstrated that cells maintaining hemopoiesis in p55 TNF receptor-deficient mice for a long time loose their repopulation activity with age and their number may decrease 3-4-fold [11]. However hemopoietic and stromal precursor cells in mice deficient by each TNG/LT cluster gene have never been studied in detail.

MATERIALS AND METHODS

Wild type C57Bl/6 mice (WM) of both sexes and TNF-[5,7] or LT-α deficient [10] knockout mice (KM) aged 6-18 weeks were used. The mice were bred at the vivarium of the Institute of Bioorganic Chemistry. These KM derived from sv129/C57Bl/6 were mated 10 times (for KM LT-α) and twice (for KM TNF) with inbred C57Bl/6 strain.

The concentration of splenic colony-forming units (CFUs) was evaluated by Till's and McCullogh's methods. Bone marrow cells ($4\text{-}6\times10^4$) from KM or WM were injected into the caudal vein to lethally irradiated animals in a dose of 10 Gy (2 fractions at 3-h intervals on a ^{137}Cs device IPK). After 8 and 11 days the spleens of irradiated recipients were fixed in Bouin's fluid and hemopoietic colonies were counted. The concentrations of granulocyte macrophage CFU (CFU-GM) and burst-forming erythroid units (BFUe) were counted in methyl cellulose with conditioned medium from WEHI-3B and L929 cells and erythropoietin by the standard method. Colonies were counted on days 7 and 14, respectively. For evaluating of the number of stromal precursor cells capable of transferring hemopoietic microenvironment, bone marrow from the femur of KM and WM mice was implanted under the renal capsule of C57Bl/6 mice [1]. After 1.5 months the size of the focus of ectopic hemopoiesis was evaluated by the number of hemopoietic cells, and osteogenic activity of stromal precursors was evaluated by the weight of newly formed bone. Long-living bone marrow culture was maintained by Dexter's method. Bone marrow from one femoral bone was weighed as rough suspension in 10 ml complete nutrient medium (Fisher's medium, 2 mM glutamine, antibiotics, 10^{-6} M hydrocortisone, and 20% fetal calf and equine serum, 1:2) and cultured in a 25-cm^2 flask. Half medium was replaced once a week.

The data were statistically processed using Student's t test.

RESULTS

The content of hemopoietic precursor cells was virtually the same in adult KM and WM with normal hemopoiesis (Table 1). Therefore, hemopoiesis is normally stable in both mouse strains, despite the defects in KM-TNF and KM-LT-α.

In long-term bone marrow culture from KM a normal sublayer of adhesive cells was formed, and *in vitro* hemopoiesis was virtually the same as in WM (data not presented). However implantation of bone marrow cells from KM onto irradiated cultures from WM mice revealed essential differences in the maintenance of hemopoiesis with regard to both the number of adult cells (Fig. 1, *a*) and the content of CFU-GM (Fig. 1, *b*). The number of mature cells and CFU-GM increased in KM-TNF cultures, which might be due to the fact that TNF not only activated proliferation and differentiation of early precursors in culture [12], but also eliminated CFU-GM [13]. The absence of TNF expression in bone marrow cells can lead to reduction of apoptosis and activation of precursor proliferation. Unexpectedly in response to lipopolysaccharide KM-TNF produced less granulocytic-macrophagal colony-stimulating factor than WM cultures [7]. The number of mature cells and production of CFU-GM were decreased in cultures from KM-LT-α in comparison with the control (Fig. 1). The formation of secondary lymphoid organs and spleen structure were impaired in KM-LT-α, which was partially caused by aberrant expression of some chemokines and adhesion molecules [14]. Changes in adhesive properties of hemopoietic cells can decrease cell production in culture.

On the other hand, no appreciable changes in bone marrow stromal precursor cells capable of transferring hemopoietic microenvironment were observed in KM-LT-α. The size of ectopic hemopoiesis focus formed by KM-LT-α bone marrow was virtually the same as WM focus (Table 1), and therefore, the number of

TABLE 1. Hemopoiesis in WM and KM and Capacity of Stromal Precursor Cells to Transfer of Hemopoietic Microenvironment ($M\pm m$, n=3-5)

Parameter	WM	KM-TNF	KM-LT-α
Number of CFUs/10^5 bone marrow cells	24±2	23.1±5.7	25.2±3.6
Number of CFU-GM+BFUe/10^5 bone marrow cells	56.6±18.2	86.3±8.4	31.2±16.7
Number of nucleated cells/focus, $\times10^6$	2.2±0.7	0.5±0.2	1.8±1.0
Bone weight, mg	1.65±0.30	0.6±0.3	1.1±0.2

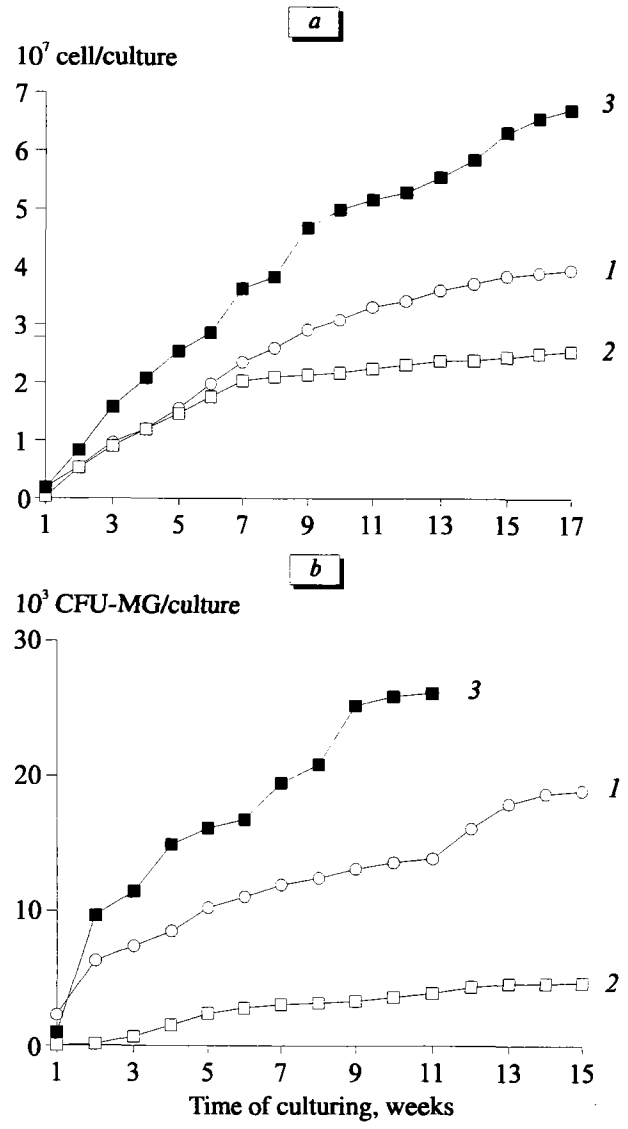

Fig. 1. Cumulative number of cells (*a*) and macrophage granulocyte colony-forming units (CFU-GM, *b*) in culture. *1*) wild type mice; *2*) mice defective for lymphotoxin-α; *3*) mice defective for tumor necrosis factor.

stromal precursor cells in the bone marrow of KM was normal. Presumably, the lymphoid-stromal cell relationships in KM-LT-α disordering the development of secondary lymphoid organs do not involve bone marrow stromal precursor cells.

Bone marrow cultures derived from TNF-deficient mice produced far smaller ectopic hemopoietic foci (Table 1). Small foci of ectopic hemopoiesis in KM-TNF either reflect the physiological effect of TNF or may be due to immune rejection of precursors, because KM-TNF used in these experiments were not completely transferred to the genetic basis of C57Bl/6. This fact deserves further investigation on mice more completely mated with pure C57Bl/6 mice, but experiments with implantation of the bone marrow from mice deficient by all three genes of TNF cluster and having a C57Bl/6 genetic basis demonstrated that the resultant focus of ectopic hemopoiesis is 3-4-fold smaller than after bone marrow transplantation from WM (data not presented). LT-α is not responsible for the formation of ectopic hemopoiesis focus (Table 1), and therefore this effect can be regulated by TNF, though the role of LT-β is formally possible.

Hence, TNF and LT-α playing an important role in the immune response and inflammation are also involved in the regulation of hemopoietic system. These data prove that TNF cluster genes are involved in the complex system regulating proliferation, apoptosis, and relationships with the stromal microenvironment. Further investigation of their role in hemopoiesis during stress and fine molecular mechanisms may open new vistas in the understanding of hemopoiesis regulation at the molecular level.

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