IMMUNOLOGY AND MICROBIOLOGY

Regulation of Functional Activity of Bone Marrow Hemopoietic Stem Cells by Erythroid Cells in Mice

S. V. Sennikov, T. V. Inzhelevskaya, L. V. Eremina, and V. A. Kozlov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 130, No. 12, pp. 639-642, December, 2000
Original article submitted August 30, 2000

Transplantation of erythroid and bone marrow cells to irradiated mice stimulated exogenous colony formation. Pretreatment of erythroid cells with specific rabbit antiserum to erythroblasts abolished this effect. The reverse transcriptase polymerase chain reaction revealed the presence of mRNA for interleukin-1α, interleukin-1β, interleukin-3, interleukin-6, and granulocyte-macrophage colony-stimulating factor in erythroid cells. Granulocyte-macrophage colony-stimulating factor was found in the conditioned medium from erythroid cells. Thus, erythroid cells stimulated colony-forming activity of bone marrow cells, which was probably mediated via cytokine synthesis (*e.g.*, granulocyte-macrophage colony-stimulating factor).

Key Words: *erythroblasts; cytokines; gene expression; hemopoietic stem cell*

Previous studies showed that immune cells, including monocytes and T and B lymphocytes, are involved in the feedback regulation of proliferation and differentiation of hemopoietic stem cells [2,3]. This regulatory effect is realized via cell-to-cell interaction and production of humoral factors. The absolute content of erythroid cells is maximum in the bone marrow [1]. However, their role in the regulation of stem cell proliferation and differentiation is poorly understood. Here we studied the effects of erythroid cells on colony-forming activity of bone marrow cells (BMC) and expression of interleukin-1α (IL-1α), IL-1β, IL-3, IL-6, and granulocyte-macrophage colony-stimulating factor (GM-CSF), which stimulate proliferation and differentiation of stem hemopoietic and progenitor cells [8,11,13,16].

MATERIALS AND METHODS

Experiments were performed on (CBA×C57Bl)F_1 and (DBA×C57Bl/6)F_1 mice. Hemolytic anemia was induced with phenylhydrazine (Fisher Scientific Company) injected intraperitoneally in doses of 1.2 and 0.6 mg on days 1 (17.00) and 2 (9.00 and 17.00), respectively. Spleens were taken 4 days after phenylhydrazine administration. Nucleated erythroid cells (NEC) fraction of splenocytes was isolated on a Ficoll-Verografin density gradient (1.076, Loba Feinchemie). Macrophages were removed by adhesion. B cells were removed by panning with high-affinity rabbit antimouse antibodies (Biosan). T cells were removed using monoclonal anti-Thy1.2 antibodies (Saderlain) and by panning on Petri dishes with high-affinity rabbit antimouse antibodies. The purity of NEC population (>90%) was confirmed morphologically by the method of Nocht-Maksimov [10]. The NEC-enriched population (10^6 cells/ml) was cultured with 5 U/ml erythropoietin in a CO_2 incubator at 37°C for 48 h.

Colony-forming activity of BMC was estimated as described elsewhere [15].

The population of BMC enriched with hemopoietic stem cells was obtained using a bovine serum albumin (BSA) density gradient (fractions 2 and 3) [9]. The cells forming antibodies (IgM) to sheep erythrocytes (AFC) were assayed as described previously [7].

Institute of Clinical Immunology, Siberian Division of the Russian Academy of Medical Sciences, Novosibirsk. ***Address for correspondence:*** ici@online.nsk.su. Sennikov S. V.

0007-4888/00/0012-1159$25.00

The NEC-enriched population was treated with 35 mg/ml mitomycin C (Sigma) at 37°C for 2 h.

The antierythroblast antiserum was synthesized at the Institute of Clinical Immunology [5]. The cells were successively incubated with serum at 4°C for 30 min (dilution 1:8) and then with freshly prepared guinea pig complement at 37°C for 45 min.

The NEC-enriched population (10^6 cells/ml) was cultured in a serum-free RPMI-1640 medium containing 4 mM L-glutamine, 2 g/liter $NaHCO_3$, and 20 mM HEPES in 24-well plates (Linbro) for 24 h; the supernatant was isolated by centrifugation at 1500 rpm.

Total RNA was extracted as described elsewhere [6]. mRNA was reverse transcribed to cDNA and amplified using polymerase chain reaction (RT-PCR) to assay cytokine mRNA [14]. The following oligonucleotide primers were used: sense primer AGTATCAGCAACGTCAAGCAACGGGA and antisense primer AGCGCTCACGAACAGTTGTGAATCTGA (IL-1α), TCATGGGATGATGATGATAACCTGCT and CCC ATACTTTAGGAAGACACGGATT (IL-1β), ATGG TTCTTGCCAGCTCTACCACCA and GATAAGACATTTGATGGCATAAAGGA (IL-3), CTGGTGACAACCACGGCCTTCCCTA and ATGCTTAGGCA TAACGCACTAGGTT (IL-6), ATGTGGCTGCAGA ATTTACTTTTCCT and TGGGCTTCCTCATTTTT GCCTGGT (GM-CSF), and GTGGGCCGCT CTAGGCACCAA and CTCTTTGATGTCACGCACGATT TC (β-actin).

RT-PCR products were assayed by electrophoresis in 2% agarose gel. Sau3AI hydrolysate of Puc18 plasmid (Sibenzim) was used as a molecular weight marker.

The content of GM-CSF was measured by electrochemiluminescence on an Origen Analyzer (IGEN). Polyclonal antibodies (Peprotech) labeled with sulfosuccinimidyl-6-(biotinamido)hexanoate (IGEN) and monoclonal antibodies to mouse GM-CSF (R&D Systems) labeled with ruthenium(II) tris(dipyridyl) chelate N-hydroxysuccinimide ester (IGEN) served as specific antibodies. The calibration curve was constructed using recombinant mouse GM-CSF (Peprotech).

RESULTS

It was previously shown that adoptive transfer of erythroid cells suppresses the primary IgM response to T cell-dependent and independent antigens and the secondary IgG response to T cell-dependent antigens [4], but has no effect on T cell reactions. Transplantation of BMC from C57Bl/6 mice (5×10^4 cells) and syngeneic erythroid cells pretreated with mitomycin C (2×10^7 cells) to lethally irradiated $(CBA\times C57Bl/6)F_1$ mice 2-fold increased exogenous colony formation on days 8-9 (Fig. 1). The NEC-enriched population decreased the content of AFC in the spleen of control mice immunized with sheep erythrocytes ($p<0.05$). Pretreatment of the NEC-enriched population with specific rabbit antiserum to erythroblasts abolished these effects.

The ability of various hemopoietic cells to regulate proliferation and differentiation of stem (initial stages) and other hemopoietic cells plays an important role in hemopoiesis. The majority of hemopoietic and immune cells, including erythroid cells, possess this property. Studies of immunoregulatory properties of erythroid cells showed that their effects on B lymphopoiesis are realized via protein synthesis and partially mediated through enhanced production of soluble factors [4]. Production of immunoregulatory proteins and cytokines is the major mechanism underlying the regulatory effect of hemopoietic and immune cells [8,11,13,16]. IL-1, IL-3, IL-6, and GM-CSF modulate functional activity of stem hemopoietic cells. RT-PCR revealed the presence of mRNA for IL-1α, IL-1β, and IL-6 in the NEC-enriched population (Fig. 2). IL-3 and GM-CSF mRNA were not found. The NEC-enriched population was cultured with erythropoietin for 48 h to activate and increase the content of erythroid cells. Morphological assay showed that the test population contained only erythroblasts. Apart from IL-1α, IL-1β, and IL-6 mRNA, the cells treated with erythropoietin contained mRNA for IL-3 and GM-CSF. Therefore, erythropoietin activated cytokine gene expression in NEC. The presence of cytokine mRNA in

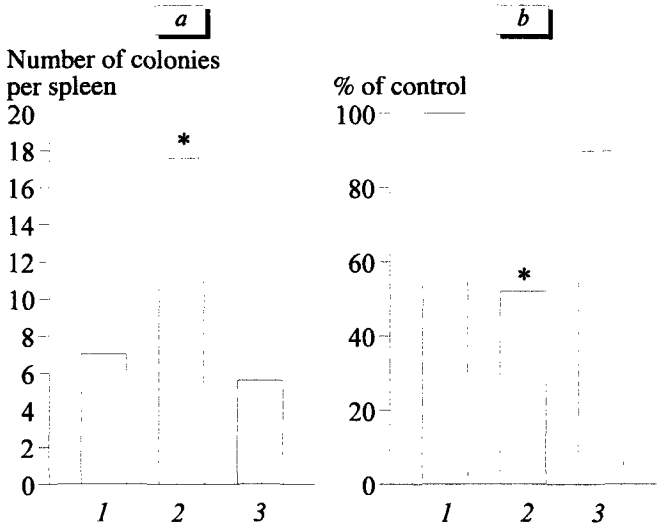

Fig. 1. Effects of nucleated erythroid cells (NEC) on colony formation of bone marrow stem hemopoietic cells (BMC, 5×10^4 cells, fractions 2 and 3) isolated in a BSA density gradient (*a*) and accumulation of antibody-forming cells in the spleen of mice immunized with sheep erythrocytes (*b*): *1*) control (*a*: BMC; *b*: immunization); *2*) addition of NEC treated with mitomycin C; *3*) addition of NEC treated with mitomycin C and antiserum against erythroblasts. NEC: 2×10^7 (*a*) and 10^7 (*b*). $p<0.05$ compared to other groups.

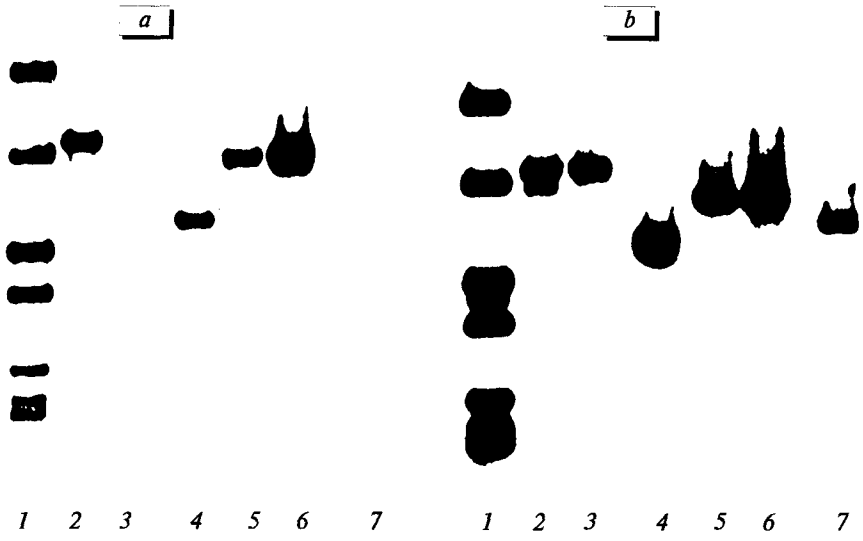

Fig. 2. Electrophoretograms of RT-PCR for cytokine mRNA in mouse nucleated erythroid cells treated with phenylhydrazine (*a*) and incubated with erythropoietin for 48 h (*b*): marker (*1*, Puc18 plasmid Sau3AI hydrolysate), IL-6 (*2*), IL-3 (*3*), IL-1α (*4*), IL-1β (*5*), β-actin (*6*), and GM-CSF (*7*).

the NEC-enriched population is consistent with previous studies of NEC colonies [14]. These data suggest that the regulatory effect of NEC on colony-forming activity of BMC is mediated via expression of IL-1, IL-6, and GM-CSF. Since the presence of cytokine mRNA does not necessarily indicate protein translation and secretion, we measured the content of a corresponding protein. Electrochemiluminescence with labeled antibodies showed the presence of 4201.80± 1671.24 pg/ml GM-CSF in NEC conditioned medium from mice treated with phenylhydrazine.

Thus, erythroid cells regulate not only clonal expansion of immunocompetent B cells, but also the initial stages of proliferation and differentiation of the stem hemopoietic cell. We hypothesized that stimulation of colony-forming activity of the stem hemopoietic cell with erythroid cells is mediated via cytokine gene expression (*e.g.*, GM-CSF production).

REFERENCES

1. I. G. Antipov, G. V. Karpova, V. V. Novitskii, and O. S. Golosov, *The Use of Laboratory Animals for Elaboration, Production, and Control of Biological Medicinal Preparations* [in Russian], Moscow (1976), pp. 207-208.
2. E. D. Gol'dberg, A. M. Dygai, and G. V. Karpova, *Role of Lymphocytes in the Regulation of Hemopoiesis* [in Russian], Tomsk (1983).
3. V. A. Kozlov, I. N. Zhuravkin, and I. G. Tsyrlova, *Stem Hemopoietic Cell and Immune Response* [in Russian], Novosibirsk (1982).
4. S. V. Sennikov, L. V. Gus'kova, D. M. Samarin, *et al.*, *Immunologiya*, No. 2, 32-35 (1997).
5. I. G. Tsyrlova, V. V. Cheglyakova, and V. A. Kozlov, *Ontogenez*, **16**, No. 2, 143-148 (1985).
6. P. Chomozynski and N. Sacchi, *Anal. Biochem.*, **162**, No. 1, 156-159 (1987).
7. A. J. Cunningham and A. Szenberg, *Immunology*, **14**, No. 4, 599-600 (1968).
8. T. M. Dexter, C. M. Heyworth, E. Spooncer, and I. L. Ponting, *Phil. Trans. Roy. Soc. (Lond.)*, **327**, 85-98 (1990).
9. K. A. Dicke, G. Tridonte, and D. W. van Beckum, *Transplantation*, **8**, 422-429 (1969).
10. T. G. I. Hayhoe and R. J. Fleamans, *Atlas of Hematological Cytology*, London (1969), p. 316.
11. J. Moreb and J. R. Zucali, *Leuk. Lymphoma*, **8**, 267-275 (1992).
12. L. J. Obenauer-Kutner, S. J. Jacobs, K. Kolz, *et al.*, *J. Immunol. Methods*, **206**, 25-33 (1997).
13. M. Peters, A. M. Mьller, and S. Rose-John, *Blood*, **92**, 3495-3504 (1998).
14. S. V. Sennikov, L. V. Eremina, D. M. Samarin, *et al.*, *Eur. Cytokine Netw.*, **7**, 771-774 (1996).
15. J. E. Till and E. A. McCulloch, *Rad. Res.*, **14**, 213-222 (1961).
16. E. de Wynter, T. Allen, L. Coutinho, *et al.*, *J. Cell Sci.*, **106**, No. 3, 761-769 (1993).