# Effect of Mast Cells on T-Cell Mechanisms of Hemopoiesis Regulation in Inflammation

E. D. Gol'dberg, A. M. Dygai, N. A. Klimenko, B. Yu. Gumilevskii, I. V. Bogdashin, and E. Yu. Sherstoboev

UDC 616.155.36:612.119:616.155.32] – 002

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 115, № 6, pp. 602–604, June, 1993
Original article submitted February 19, 1993

**Key Words:** *inflammation; hemopoiesis; T lymphocytes; mast cells*

Our previous investigations demonstrated the role of T lymphocytes which have migrated from the lymphoid tissue to the bone marrow in the regulation of hemopoiesis during inflammation, and also the modulatory effect of mast cells (MC) from an inflammation focus on hemopoiesis [1,2,4,]. The aim of the present study was to explore the possible realization of the MC modulatory effect on hemopoiesis by T lymphocytes in inflammation.

## MATERIALS AND METHODS

The experiments were carried out on 532 male CBA mice weighing 18-20 g (Rassvet nursery, Tomsk) in the fall and winter in the morning. The inflammation model was acute infectious peritonitis, performed with i.p. injection of $1/2LD_{50}$ *E. coli* (strain ATSS 25922) 24-h culture medium in 0.3 ml saline [2,4]. The animals were decapitated at different times of inflammation. The content of T lymphocytes in the bone marrow [3] and their effect both on the release from the bone marrow to the culture medium of colony-forming units of granulocyte-macrophages (CFU-GM) and erythrocytes (CFU-E) and on the level of colony-stimulating factor (CSF) and erythropoietin activity (EPA) in adhesive myelokaryocyte- and nonadhesive myelokaryocyte-conditioned medium were assayed with monoclonal anti-Thy-$1,2^+$ (antibodies kindly placed at our disposal by V. I. Seledtsov, Research Institute of Clinical Immunology, Siberian Branch of the Russian Academy of Medical Sciences, Novosibirsk). The cloning of CFU-GM and CFU-E was performed in a modified methylcellulose tissue culture [9,11]. CSF and EPA were assessed by the ratio of colony-forming intensity to $10^5$ myelokaryocytes [11]. Conditioned media were prepared by cultivation of $2\times10^6$ cells/ml adhesive and nonadhesive myelokaryocytes in complete culture medium with 10 μg/ml LPS *E. coli* strain 0.111B4 (Sigma, USA) or 5 μg/ml ConA (Sigma, USA) respectively, during 24h at 37°C, 100% humidity, in an atmosphere with 5% carbon dioxide. The functional activity of T lymphocytes was also judged according to the synthesis of macrophage-activating factor (MAF) by the bone marrow nonadhesive cells [6]. MC removal was induced by i.p. injection of 1.8-2.0 ml sterile destilled water 10 days before peritonitis was induced [5,10].

## RESULTS

Inflammation without MC in its focus was characterized by a pronounced accumulation of T lymphocytes (Thy-$1,2^+$ cells) in the bone marrow, just as during the natural development of peritonitis [1,2]. In this case the dynamics of Thy-$1,2^+$ cells was in general similar to that with the usual course

Research Institute of Pharmacology, Tomsk Scientific Center of the Russian Academy of Medical Sciences; Kharkov Medical Institute.

0007-4888/93/0006-0670$12.50 

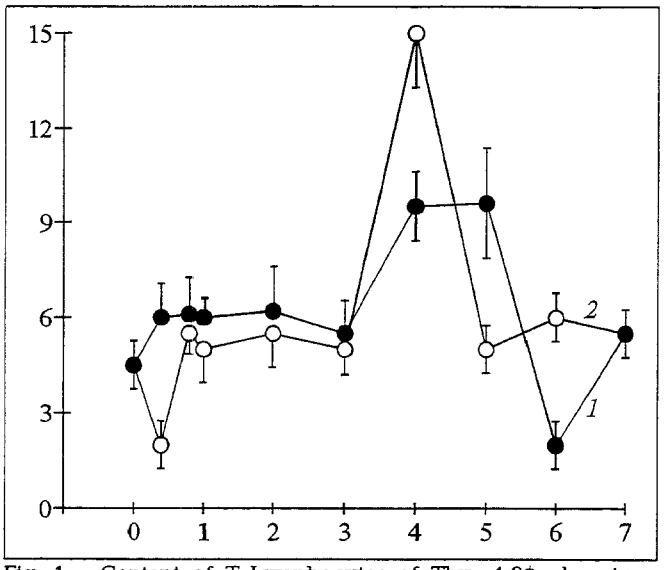


Fig. 1. Content of T Lymphocytes of Thy−1,2⁺ phenotype (%) in mouse bone marrow in the course of acute infectious peritonitis (days) under natural conditions (*1*) and for removal of MC (*2*).

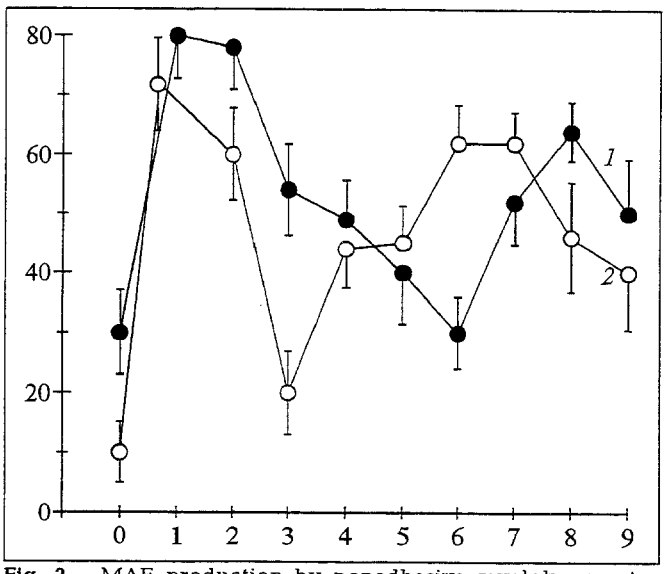


Fig. 2. MAF production by nonadhesive myelokaryocytes (conventional units) in the course of acute infectious peritonitis (days)under natural conditions (*1*) and for removal of MC (*2*).

of inflammation, but the number of Thy-1,2⁺ lymphocytes was reliably higher on the 4th day (when a significant increase of their number was observed in both experimental series) than that with the typical course of inflammation (Fig. 1).

The removal of MC affected MAF production by ConA-stimulated nonadhesive myelokaryocytes. Thus, while in the natural development of inflammation the cytotoxic activity of macrophages treated with nonadhesive bone marrow cell-conditioned medium increased on the 1th, 2nd and 8th day, the same activity in the absence of MC was raised after just 6 h and 12 h, as well as on the 3rd, 4th, 6th, and 7th days. Moreover, the degree of augmentation of MAF production was also higher in the second case (Fig.2).

The absence of MC also influenced Thy-1,2⁺ cell-mediated hemopoiesis regulation. During the ordinary course of inflammation, treatment of adhesive

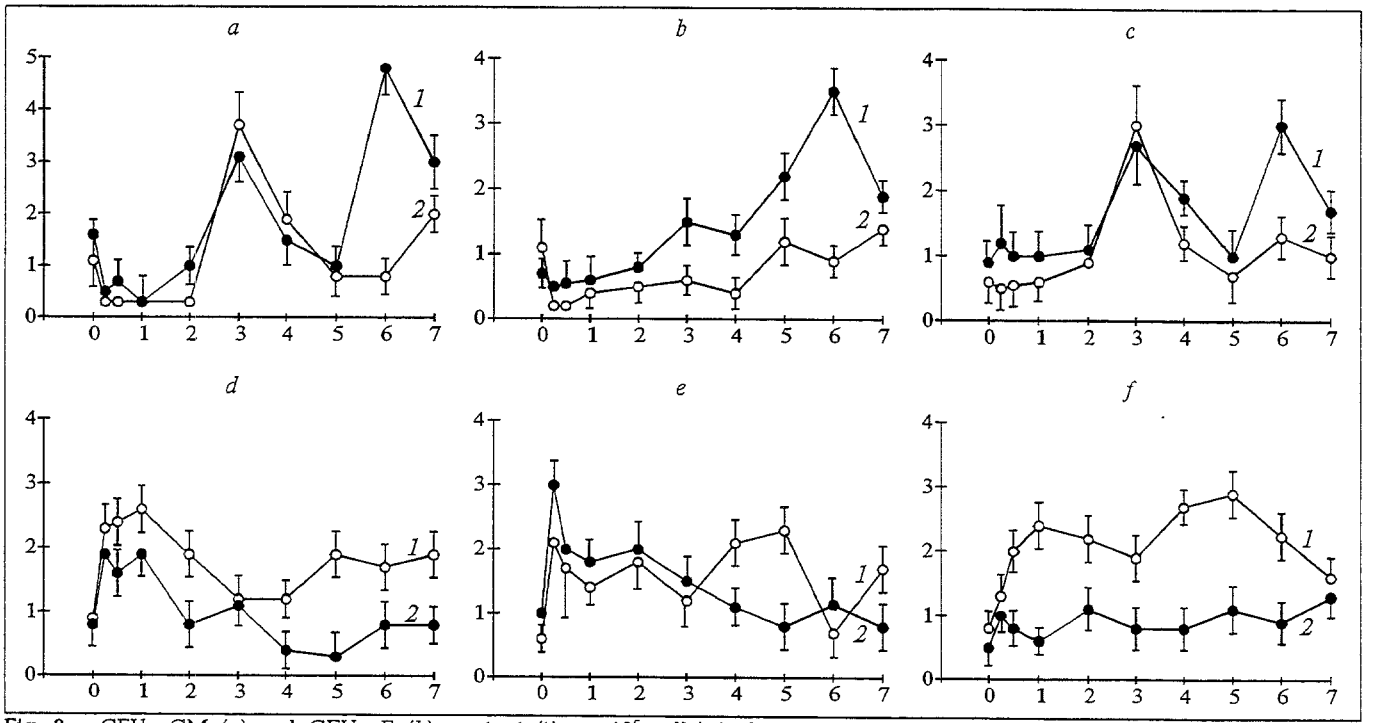


Fig. 3. CFU−GM (*a*) and CFU−E (*b*) content (times $10^5$ cells) in bone marrow, and CSF and EPA of adhesive (*c, d*) and nonadhesive (*e, f*) myelokaryocytes (times $10^5$ cells) in the course of acute infectious peritonitis (days) in mice without MC before (*1*) and after (*2*) treatment of bone marrow myelokaryocyte suspension with anti−Thy−1,2⁺.

karyocytes with anti-Thy-1,2$^+$ markedly reduced CSF production only on the 6th day, but when the MC population was depleted this drop took place as early as the 4th day. The addition of monoclonal antibodies to a cell culture of nonadhesive myelokaryocytes from animals without MC resulted in a CSF decrease observed not only on the 5th day (as with natural inflammation) but also after 6 hours. Whereas anti-Thy-1,2$^+$ diminished EPA production by adhesive cells on the 5th day and by nonadhesive cells after 12 h, 1 day and 6 days in the case of natural inflammation, in the absence of MC this was observed after 6 h, 1 day, 5 days, and 6 days, respectively (Fig. 3).

At the same time, the removal of MC did not affect significantly the influence of monoclonal antibodies upon CFU-GM and CFU-E cloning. Preventive treatment of myelokaryocytes with anti-Thy-1,2$^+$ resulted in the abolishment of the 2nd CFU-GM peak (on the 6th day), regardless of the MC disappearance, and in a significant decrease of CFU-E on the 4th and 6th day with the natural course of inflammationand on the 5th and 6th day without MC (Fig. 3).

The intensified accumulation of T lymphocytes in the bone marrow and MAF production by nonadhesive myelokaryocytes, and the earlier suppression of CSF and EPA production by the bone marrow nucleated cells for T-cell elimination from the myelokaryocyte suspension (obtained from animals with MC removed) testified to a stimulation of T-cell activity in comparison with the usual course of inflammation. These results demonstrated the suppressive effect of MC on T lymphocytes, which are hemopoiesis regulators is (activated, accordingly, by the removal of MC), and showed that the modulating effect of MC on hemopoiesis in inflammation is realized largely via the T cells. The earlier suppression of CSF and EPA production by myelokaryocytes (adhesive or nonadhesive) for T-lymphocyte elimination from the bone marrow cell suspension (obtained from animals without MC) indicates that both the direct and the bone marrow macrophage-mediated effect of lymphocytes on hemopoiesis are preserved regardless of the course of inflammation development [1,2]. That the removal of MC has no effect on T-lymphocyte-mediated CFU-GM and CFU-E cloning shows that the modulating influence of MC on lymphocytes is probably manifested in the effect of the latter at the level of more mature bone marrow cells.

The results obtained are in agreement with some published data on the effect of the biologically active substances of MC on T lymphocytes *in vitro*. Thus, it was shown that histamine inhibits T-cell proliferation, lymphokine production, and T-cell cytotoxicity via the H2 receptors. The presence of H2 receptors has been demonstrated in cytotoxic T lymphocytes , natural killers, lymphokine-producing T cells, and in T suppressers [8,12,15]. It is assumed that, depending on the animal species and subpopulation, T lymphocytes contain both H2 and H1 receptors, and stimulation of the latter exhibits the opposite effects [14]. In respect to serotonin, it is known, to stimulate T-cell migration and lymphokine secretion the via S2 receptors [7,13]. This evidence suggests, that the modulating effect of MC on lymphocytes, and, accordingly, on hemopoiesis in inflammation depends to a large degreeon H2-mediated effects of histamine.

## REFERENCES

1. E. D. Gol'dberg, A. M. Dygai, N. A. Klimenko, *et al., First Congress of Russian Immunologists*, Synopses of Reports, Novosibirsk (1992), p. 115.
2. A. M. Dygai, N. A. Klimenko, B. Yu. Gumilevskii, *et al., Byull. Eksp. Biol.*, **113**, № 5, 514-516 (1992).
3. R. H. Kenneth, T. J. McKearn, and K. B. Bechtol, (Eds.), *Monoclonal Antibodies; Hybridomas: A New Dimension in Biological Analyses,* Plenum Press, New York (1980).
4. N. A. Klimenko, A. M. Dygai, E. V. Abramova, *et al., Byull. Eksp. Biol.*, **112**, № 9, 305-307 (1991).
5. R. U. Lipshits, and N. A. Klimenko, *Ibid.*, **84**, № 12, 660-664 (1977).
6. V. I. Seledtsov, A. P. Suslov, and B. D. Brondz, *Biol. Membrany*, **4**, № 12, 1313-1318 (1987).
7. J.-C. Ameisen, M. Robin, and P. W. Askenase, *J. Immunol.*, **142**, 3171-3179 (1989).
8. C. S. Henney, H. R. Bourne, and L. M. Lichtenstein, *J. Immunol.*, **108**, 1526-1534 (1972).
9. N. N. Iscove, F. Sieber, and K. N. Winterhalter, *J. Cell. Physiol.*, **83**, 309-315 (1974).
10. Y. Kanakura, A. Kurui, N. Waki, *et al., Blood*, **71**, 573-580 (1988).
11. D. Metcalf, *Haemopoietic Colonies*, Berlin (1977), p. 227.
12. C. Oh, H. Okamoto, and K. Nakano, *Agr. Biol. Chem.*, **53**, 377-382 (1989).
13. I. Paegelow and H. Werner, *Wiss Beitr. M. Luther-Univ.*, Halle-Wittenberg, R, 114-123 (1987).
14. M. Plaut and L. M. Lichtenstein, in: Pharmacology of Histamine Receptors, Ed. C. R. Ganellin and M. E. Parsons, Bristol (1982), pp. 392-481.
15. R. E. Rocklin, *J. Clin. Invest.*, **57**, 1051-1058 (1976).