# PHARMACOLOGY AND TOXICOLOGY

# On the Erythron Response to Inflammation and Its Mechanisms

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Using the mouse model of acute infectious peritonitis caused by *Escherichia coli*, it is shown that the development of inflammation is accompanied by increases in the number of erythrokaryocytes, erythroid colony-forming units, and erythroid hematopoietic islets in the bone marrow and by rises in the activities of supernatants of cultured stimulated adherent and nonadherent myelokaryocytes and of peripheral blood. The results of this study indicate that a characteristic feature of acute inflammation is strong activation of erythropoiesis with the development of hyperplasia of the erythroid marrow.

**Key Words:** *inflammation; erythropoiesis*

The mechanisms underlying the regulation of erythropoiesis in inflammation have been studied inadequately, and the available information about quantitative changes in the erythron during inflammatory processes is scanty and contradictory. There are reports of anemia with a fall in the number of erythroid cells in the bone marrow occurring as early as 24 h after the experimental induction of acute inflammation and peaking on days 6-9 [1,2,8], and erythrocytosis has been found to develop at the same times [6].

The present experiment was undertaken to investigate the response of the erythron to acute inflammation and the mechanisms of this response.

## MATERIALS AND METHODS

The experiment was carried out on 102 male CBA mice weighing 18-20 g. The model of inflammation was infectious peritonitis caused by *Escherichia coli* strain ATCC 25922 inoculated intraperitoneally in 0.3 ml isotonic NaCl solution in a dose equal to one-half of the $LD_{50}$ [4,7]. Mice were killed by decapitation at various times of the inflammatory process, and the total number of myelokaryocytes was counted in the femur and a myelogram prepared. The content of committed erythropoietic progenitor cells - erythroid colony-forming units (CFU-E) [12] and hematopoietic islets (HI) [3,10] - in the bone marrow was determined, as was the erythropoietic activity (EPA) of conditioned media of blood serum and of adherent and nonadherent bone marrow cells. Conditioned media were produced by a 24-h culture of $2\times10^6$ cells/ml adherent or nonadherent myelokaryocytes in complete RPMI-1640 medium containing 10% fetal calf serum in the presence of, respectively, 10 μg/ml of *E. coli* (serotype 0111:B4) lipopolysaccharide (Sigma) or 5 μg/ml of concanavalin A (Sigma).

## RESULTS

The inflammation was accompanied by a short-term rise in the number of bone-marrow erythroid ele-

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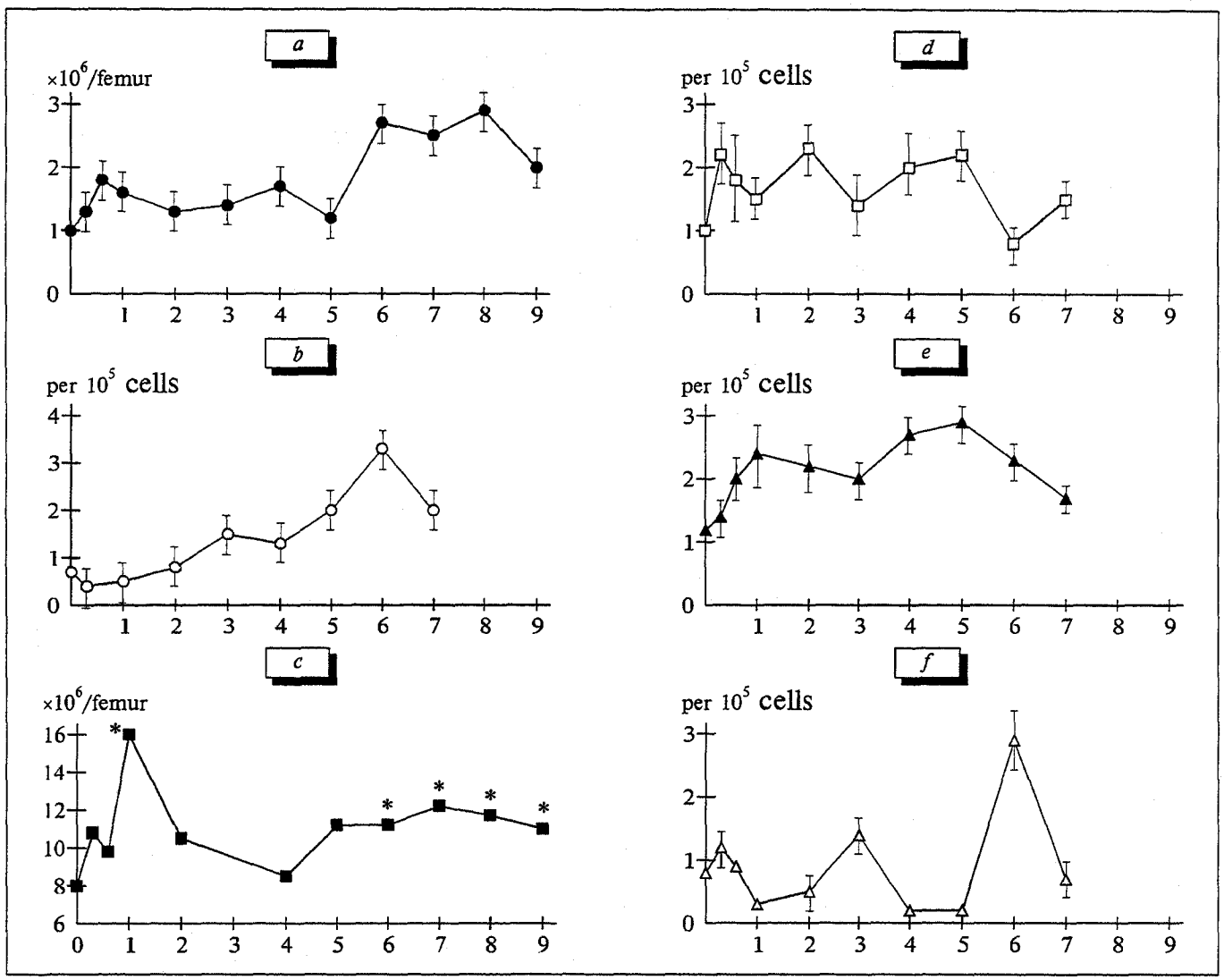


**Fig. 1.** Number of erythroid cells (*a*), CFU–E (*b*), and macrophage–positive hematopoietic islets (HI) (*c*) in the bone marrow of test mice, and erythropoietic activity of their adherent (*d*) and nonadherent (*e*) myelokaryocytes and of blood serum (*f*) on different days (abscissa) of acute infectious peritonitis. Confidence limits at $p=0.05$. The asterisk marks a significant increase in the number of HI over the baseline.

ments by hour 12 of exposure to the phlogogen and by their persistent increase on days 6-9, with a maximum on day 8, when their number exceeded the baseline level 2.5-fold (Fig. 1, *a*), attesting to a hyperplasia of the erythroid series. This hyperplasia coincided with the hyperplasic phase of the granulocytic-monocytic population in the bone marrow during inflammation [4].

The development of hyperplasia was preceded by an increase in the number of CFU-E in the bone marrow. Their number began to rise on day 3 and significantly exceeded the baseline on days 5-7, reaching a maximum on day 6 (4.8-fold increase over the baseline) (Fig. 1, *b*). The intensified release of CFU-E from the marrow preceding the hyperplasia indicates that the latter was due to stimulated proliferation and differentiation of erythroid precursors.

The inflammation was also marked by elevations in the bone marrow of both the total number of HI and the number of macrophage-positive HI, the latter being mainly represented by erythroid islets [3]. Their number was greatly increased on days 1 (2-fold) and 6-9 of inflammation, peaking on day 7, when it exceeded the baseline level 1.6-fold (Fig. 1, *c*). There were also increases in the EPA of the conditioned media of stimulated myelokaryocytes. The EPA of supernatants from lipopolysaccharide-stimulated adherent nucleated cells was elevated at 6 h and on day 5 postinoculation, exceeding the baseline activity 2.8-fold at both these times (Fig. 1, *d*). The EPA of conditioned media from concanavalin A-stimulated nonadherent cells was elevated at 12 h and on days 1, 2, 4, 5, and 6, with maxima on days 1 and 5, when it exceeded the

baseline level 3-fold and 3.5-fold, respectively (Fig. 1, *e*). The increased number of erythroid islets in the bone marrow and the elevated EPA of myelokaryocytes were indications that the proliferation and differentiation of erythropoietic progenitor cells were stimulated as a result of heightened functional activity of the hematopoiesis-inducing microenvironment (HIM) in the bone marrow. In particular, the increased number and altered ratio of HI reflected a structural and functional reorganization of the bone marrow directed toward recreating a particular HIM and eventually at enhancing the proliferation and differentiation of hematopoietic cells. HI, which are structural-functional associations usually consisting of a centrally located macrophage or fibroblast surrounded by hematopoietic cells of different types (erythroid, granulocytic, and mixed erythoid-granulocytic HI have been identified [3]) and having different degrees of maturity, are believed to be the sites where the corresponding hematopoietic cells undergo differentiation from committed to mature forms [10]. The enhanced production of short-range humoral regulators of erythropoiesis (as reflected in the heightened EPA) by adherent (macrophages) and nonadherent (lymphocytes) myelokaryocytes testifies to intensified functional activity of cellular elements in the HIM and to an activation of the HIM as whole since macrophages and lymphocytes are its major components [5].

On day 3 and especially day 6 of inflammation, the EPA of the peripheral blood was also elevated, by factors of 2.4 and 4.3, respectively (Fig. 1, *f*). According to current notions, EPA of the blood is an integrative indicator of various HIM-mediated remote controls of erythropoiesis and depends mainly on erythropoietin production [11,14]. During inflammation, other important contributors to the enhanced EPA of the blood and to the activation of erythropoiesis may be erythropoietin-like substances liberated by activated leukocytes of the inflammatory focus and peripheral blood [9].

Hence, a characteristic feature of acute inflammation is pronounced activation of erythropoiesis with hyperplasia of the erythrocytic series in the bone marrow. This activation is associated with intensified proliferation and differentiation of erythroid precursors due, in turn, to activation of the HIM which mediates the various remote controls over erythropoiesis.

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