Erythropoietin formation during hypoxia in mice with impaired responsiveness to erythropoietin induced by irradiation or 5-fluorouracil injection

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Summary. Plasma erythropoietin levels during continuous exposure to hypobaric hypoxia in mice with marrow aplasia induced by whole body X-irradiation or 5-fluorouracil injection were higher than in control mice similarly exposed. These findings give support to the hypothesis that a relationship exists between erythropoietin production rate and erythroid responsiveness to the hormone.

Fried et al.[2] have reported that plasma erythropoietin (Ep) levels of WWv mice, which show a congenital anemia because of a defect in their multipotential hemopoietic stem cells and a decreased response to Ep, are higher than those of comparably anemic nonmutans (+ +). This difference is no longer present 7 days after transplanting bone marrow cells into WWv mice. At this time, the response of WWv mice to Ep is comparable to that of $^{++}$'s. From these observations the authors have suggested that the rate of Ep production at any level of anemia is modified by the ability of the hemopoietic cells to respond to Ep[2]. Studies done in our laboratory, which can be similarly interpreted, have shown that plasma Ep levels in splenectomized mice, which also show a decreased response to Ep, are much higher and are maintained for a longer time during exposure to continuous hypoxia than in similarly treated normal mice[3].

To clarify the possible relationship between Ep production and response to Ep, we report in this article the changes in plasma Ep levels during hypoxia in 2 experimental models showing bone marrow aplasia and impaired responsiveness to the hormone, which were induced by either whole body X-irradiation (WBI) or 5-fluorouracil (5-FU) injection.

Methods. Adult female mice of the CF_1 strain were used throughout. Measurement of erythropoiesis in non-polycythemic mice was made by the percentage of the dose of ^{59}Fe incorporated into erythrocytes 72 h after injection[4]. Plasma Ep levels were assayed in post-hypoxic polycythemic mice[5]. Plasma (0.6 ml) was administered i.p. on the 5th post-hypoxic day, followed by ^{59}Fe on the 7th day and determination of percent RBC-isotope incorporation was made 48 h later. The 5-FU was given in a single i.p. injection of 150 mg/kg of b.wt. X-irradiation was delivered by a Westinghouse 400 kV unit at a dose rate at 30 cm of 78.3 rad/min. Mice received a lethal dose of 900 rad.

Results. Figure 1 shows the changes of RBC-^{59}Fe incorporation in mice as a function of time after either whole body X-irradiation (open circles) or 5-FU injection (solid circles). Both treatments induced a similar erythropoietic depression. While no recovery was observed in the irradiated mice, radioiron uptake recovered rapidly after the 6th post-injection day in the 5-FU-treated group.

To study the effect of WBI or 5-FU injection on the plasma Ep content during hypoxia, groups of intact mice or mice lethally irradiated or injected with 5-FU 48 h before were put into an altitude chamber at 456 mb. Mice from each group were removed after various intervals and bled immediately, and the plasma from each group was pooled for bioassay of Ep content. The results obtained are shown in figure 2. Plasma taken from each group of mice before hypoxic exposure did not stimulate erythropoiesis in assay mice. Plasma Ep levels rose rapidly during the first 24 h of exposure to hypoxia and then fell in the 3 studied groups, although both irradiated and 5-FU mouse plasma had a much higher titer than the control during the whole exposure period. RBC-^{59}Fe uptakes in assay mice receiving irradiated mouse plasma were almost 3 times, and in those receiving 5-FU mouse plasma almost 2 times greater than those of mice injected with control plasma. The hematocrits

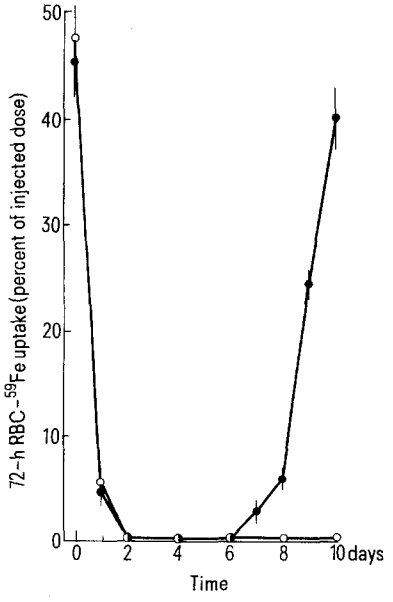

Figure 1. Change in ^{59}Fe activity incorporated into circulating red cells of mice with time following 900 rad whole body X-irradiation (open circles) or 5-fluorouracil injection (150 mg/kg, solid circles). Radioiron was administered i.v. on the days indicated. Vertical lines from the mean incorporation values represent SE.

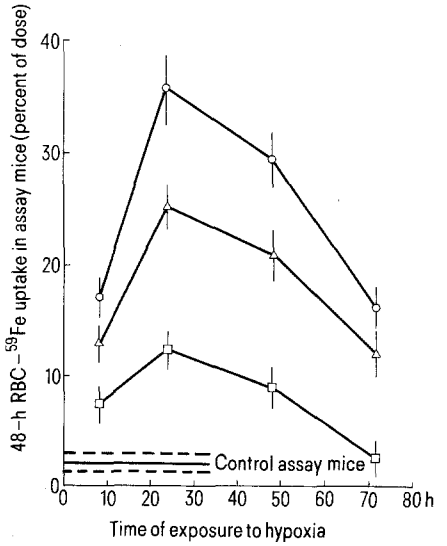

Figure 2. Erythropoietic activity of the plasma of normal (hexagons), 5-fluorouracil-treated (triangles) or irradiated (circles) mice during exposure to hypoxia. Ep activity measured as percent ^{59}Fe incorporation in the post-hypoxic polycythemic mice bioassay. Vertical lines from the mean incorporation values represent SE.

of the control mice after 3 days of hypoxia were significantly greater than at the beginning of the hypoxic exposure (40.1 ± 0.8 vs 44.9 ± 0.6). The hematocrits of both irradiated and 5-FU-injected mice did not change during the exposure to hypoxia.

Discussion. Plasma Ep levels of mammals are related to the oxygen supply to the tissues relative to their oxygen needs[6]. This alone, however, is not apparently enough to explain either the fall in plasma Ep level of animals during continuous exposure to hypoxia before any change in the red cell volume is detectable[7], or the old observation that patients with active erythropoiesis generally have lower plasma Ep levels than comparably anemic patients with erythroid hypoplasia[8]. These observations would indicate that the plasma Ep level is in some unknown manner influenced by the erythroid activity of the marrow, in addition to being regulated by the tissue oxygen needs and supply. From the above observations, Stohlman and Brecher[7] have advanced the view that Ep is utilized by the erythroid marrow. However, studies from this and other laboratories[9,10] suggest that the marrow does not play a significant role in the inactivation of Ep. Ep concentration in the plasma compartment depends on the balance between Ep production rate and Ep disappearance rate. Since the latter is apparently not influenced by the erythroid activity of the marrow, a mechanism could exist through which Ep production is influenced by the rate of erythropoiesis. In this sense, the study of Fried et al.[2] suggests that Ep production is influenced by the ability of the hematopoietic cells to respond to Ep. At present, no methods are available for measuring the rate of production of Ep. Consequently, either plasma titers of Ep or the amount of Ep excreted in the urine are measured and translated to indicate the rate of Ep production. For these measurements to be meaningful, information is required concerning the plasma half-life of Ep. Since plasma Ep half-life has been shown to be within normal values in rats with bone marrow aplasia induced by nitrogen mustard or actinomycin D[10], the changes observed in plasma Ep levels during hypoxia in our experiments represent real changes in Ep production rates. However, the mechanisms by which plasma Ep levels were higher in irradiated than in 5-FU injected rats in spite of a similar erythropoietic depression is not apparent. Gutnizky et al.[11], studying the effect of whole body irradiation in rats with the spleen shielded on Ep production, have proposed radiation as a new and different stimulus to Ep production. Therefore, factors other than the responsiveness to Ep may be operating. Although additional evidence is required, the results of the experiments reported here give support to the hypothesis that a relationship exists between Ep production rate and erythroid responsiveness to the hormone[2].

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Regulation of lymphokine production in peripheral blood mononuclear cell cultures[1]

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Summary. An increase in the production of macrophage migration inhibitory factor, chemotactic factor for neutrophils, and skin reactive factor, was observed in lymphocyte cultures if the cells were allowed to age in culture for 24 h. The increased lymphokine production was reduced by adding concanavalin A-stimulated and mitomycin C-treated suppressor cells. It is suggested that the lymphokine production could be regulated by suppressive mononuclear cells.

After incubation with a mitogen or a specific antigen, the lymphocytes produce a number of soluble factors (lymphokines) with various biological activities. These include macrophage migration inhibitory factor (MIF), chemotactic factor for neutrophils (CFN), skin reactive factor (SRF), etc. These factors play a part in the inflammatory processes of delayed-type hypersensitivity.

Suppressor cells have been implicated in virtually all of the immunologic regulatory mechanisms that are recognized[2,3]. The responsiveness of human peripheral blood mononuclear cells (PBMC) stimulated with suboptimal concentrations of concanavalin A (Con A) is significantly increased if the cells are allowed to age in culture for 24 h. This increased reactivity has been interpreted as indicating a depletion of the suppressor cells during the incubation period[4,5].

In this study the MIF, CFN and SRF productions of freshly-separated and aged PBMC cultures were compared and the effects of Con A-stimulated suppressor cells on the lymphokine production of aged cultures were measured.

Materials and methods. Samples of blood were collected from 35 healthy donors. The PBMC were purified by density gradient centrifugation[6] and 3×10^6 washed PBMC were resuspended in 3 ml Parker medium 199 supplemented with L-glutamine and 10% heat-inactivated fetal calf serum. Immediately after the preparation, the freshly-separated PBMC cultures were stimulated with 30 µg/ml Con A, or in other experiments with 1 µg/ml purified protein derivative of tuberculin (PPD). At the end of the culturing (24 h, 37 °C) the cells were centrifuged and the lymphokine content of the supernatant was detected.

Suppressor cells were depleted by aging. PBMC suspen-