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-

Lymphokine production in PBMC cultures

	n	Freshly-separated cultures[a]	Aged cultures[a]	Suppressor-reconstitued cultures[a]	Control cultures[b]
MIF activity[c] (migration index)					
Undiluted supernatants	11	0.76±0.08	0.64± 0.11[d]	0.85±0.10[e]	1.03±0.06
1:4 diluted supernatants	11	0.83±0.11	0.77± 0.07[d]	0.97±0.07[e]	0.98±0.04
CFN activity[f] (cells/HPF)	14	9.08±5.69	30.27±18.71[d]	15.01±8.33[e]	1.8 ±0.8
SRF activity[g] (mm)	10	6.0 ±0.83	10.4 ± 1.2	7.2 ±0.45[e]	1.23±0.8

Data given as mean±SD; p was calculated by Student's t-test. [a] See in 'Materials and methods'. [b] PBMC suspension was cultured for 2 days. After a centrifugation 30 µg/ml Con A or 1 µg/ml PPD was added to the supernatants, respectively. [c] MIF production was induced by PPD, the activity was tested in triplicate cultures. [d] Significantly higher activity than in freshly separated cultures (p0.01). [e] Significantly lower activity than in aged cultures (p0.01). [f] CFN production was induced by Con A, the activity was tested in duplicate. [g] SRF production was induced by PPD, the activity was tested in 3 guinea-pigs.

sions were incubated at 37 °C for 24 h, then washed and resuspended (aged cultures). Such cultures were stimulated with Con A or PPD, and the lymphokine content of the medium was estimated after culturing for 24 h.

Suppressor cells were activated by adding Con A. Freshly-separated PBMC were cultured with 50 µg/ml Con A for 2 days, and the cells were then harvested. The pooled cells were treated with 50 µg/ml mitomycin C for 60 min at 37 °C, washed 3 times and resuspended in Parker medium.

In the examination of the effects of suppressor cells on the lymphokine production of aged cultures, 1.5×10^6 activated suppressor cells were added to the aged cultures (suppressor-reconstituted cultures); these were stimulated with Con A or PPD, and, after culturing, the lymphokine content of the medium was estimated.

MIF activity was measured by a conventional in vitro assay, using guinea-pig peritoneal exudate cells packed in capillary tubes[7]. CFN activity was detected in a Boyden chamber, using 10^7/ml granulocytes[8]. In the measurement of SRF activity, undiluted supernatants were drawn into tuberculin syringes and injected intradermally into 3 normal guinea-pigs. The diameter of the erythematous skin reaction was observed 10 h following the injection[9].

Results. The results are summarized in the table. In the freshly-separated PBMC cultures, PPD induced MIF production in 9 of the 11 subjects examined. With the aged cultures, a lower migration index was found for all of the 9 MIF-producing volunteers than in the supernatant of the freshly-separated PBMC culture. The addition of Con A-stimulated suppressor cells to the aged cultures decreased the MIF production substantially; in these cultures PPD did not induce MIF production at all in 5 cases.

As regards the SRF, the intradermal injection of guinea-pigs with 0.1 ml supernatant from the PPD-stimulated freshly-separated PBMC cultures resulted in an erythematous area measuring 6±0.83 mm for all 10 donors. The supernatant of the aged cultures led to a significantly larger erythema. The SRF content of the suppressor-reconstituted cultures virtually coincided with that of the freshly-separated PBMC cultures.

Similar results emerged from the study of the CFN content of the Con A-stimulated cultures. The supernatant of the aged cultures exhibited a significantly higher CFN activity, which was decreased considerably by the addition of Con A-activated suppressor cells to the culture.

Discussion. These investigations showed that the MIF activity of the aged cultures was 4 times higher than that of the freshly-separated PBMC cultures, and their CFN and SRF activities too were significantly higher (table). This enhancement effect of aging does not appear to be antigen-specific, since the MIF and SRF productions were examined in PPD-stimulated PBMC cultures, and the CFN production in Con A-stimulated PBMC cultures.

The inhibition of macrophage migration has been assumed to depend upon the release of MIF[10]. In other experiments, however, a migration stimulation factor was observed in cultures of lymphocytes[11]. Thus, the result of a migration inhibitory assay depends upon the balance of inhibitory and stimulatory activities. Fox and Rajaraman presumed that the migration stimulation factor is released from the suppressor cells, and they therefore consider it possible that the enhanced migration inhibitory effect of the aged cultures might be due to a decrease in the macrophage stimulating activity[12]. However, it is not probable that the higher CFN and SRF activities of the aged cultures are caused by a diminished production of mediators of opposite effect, for antiphlogistic or chemotaxis-decreasing lymphocyte products are not known.

Another possible explanation is that the PBMC acquired an increased capacity to secrete lymphokines if the cells were cultured for 24 h. An elevated ^{3}H-thymidine incorporation was earlier observed in Con A-stimulated preincubated PBMC cultures[4,5].

A more likely possible explanation for the increased response of preincubated cells is that some suppressive influence, which might be activated in freshly-stimulated cultures, is lost or diminished during incubation. This is suggested by the observation that when cells were cultured with preactivated cells (suppressor-reconstituted cultures), the increased responsiveness was significantly inhibited. It seems very likely that these added cells replaced suppressor cells whose function had been altered in the cultures.

This in vitro model suggests that the mononuclear suppressor cells are able to regulate the delayed-type hypersensitivity via inhibition of lymphokine synthesis too.

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