Thiol regulation of erythrocytic development in vitro

Conditions of incubation	Number of experiments	Number of clusters (2 cells or more)	Number of clusters (4 cells or more)	Total erythroblasts	Erythroblasts per cluster
Without EPO, without thiol	7	6.2± 9.1	1.1± 2.6	18± 29.2	1.4±1.4
Without EPO, with thiol	5	14 ± 21	2.0± 2.8	33± 50.1	1.8±1.0
With EPO, without thiol (EPO control)	9	29 ± 26	13 ±13.4	120± 119	3.9±0.9
With EPO, with 2-ME (2-ME+EPO control)	9	290 ±216*	148 ±120*	1500±1140*	5.4±1.8**
With EPO, with L-CY	8	170 ±141**	78 ± 37	800± 440*	5.7±2.6
With EPO, with DTT	4	290 ±135	150 ± 98	1500±1050	4.9±1.8
With EPO, 2-ME and pHMB 5×10^{-6} M	4	22 ± 20***	7 ± 5.1	70± 61	3.4±0.5
With EPO, 2-ME and pHMB 10^{-5} M	4	8.5± 11.6	3.5± 4.4	30± 35	2.8±0.9

EPO = erythropoietin 0.2 IU/ml; 2-ME = 2-mercaptoethanol; L-CY = L-cysteine; DTT = dithiothreitol; pHMB = p-hydroxymercuribenzoate of sodium. * p-values significantly higher than corresponding EPO control in paired observations ($p \leq 0.01$). ** p-values significantly higher than corresponding EPO control in paired observations ($p \leq 0.05$). *** p-values significantly lower than 2-ME+EPO control in paired observations ($p \leq 0.05$). t-test showed significant differences between erythropoietin controls and the 3 thiol groups ($p \leq 0.01$). In the 4 experiments in which pHMB was incorporated into thiol-containing media erythrocytic development was not significantly different from EPO controls ($p \simeq 0.50$).

5×10^{-5} M concentration, L-CY at either 10^{-4} M or 5×10^{-4} M and DTT at 5×10^{-4} M. Another thiol, glutathione, and a diol, ethylene glycol, were ineffective at doses of 10^{-6} to 10^{-3} M.

In 4 experiments the thiol inhibitor, p-HMB, was incorporated into media containing EPO and 5×10^{-5} M 2-ME and incubated at 37 °C for 30 min before adding the bone marrow cells. At concentrations of 10^{-7} or 10^{-6} M, the number of erythroid clusters was similar to that of 2-ME + EPO controls. However, at the concentrations of 5×10^{-6} and 10^{-5} M erythropoiesis fell to EPO control levels ($p \simeq 0.50$) with no adverse effect on granulocytic proliferation. Nonspecific toxicity had to be ruled out because growth with p-HMB + EPO without thiols was found not to be significantly different from EPO controls (t-test leads to a p-value = 0.325).

These data provide evidence that certain SH compounds enhance the in vitro response of erythropoietin-sensitive precursors from polycythemic rat bone marrow. Preliminary studies show that this is also the case for normal or anemic rat bone marrow (unpublished observations, N. P-S). This enhancement of erythropoiesis is dose-related and can be reversed by specific thiol inhibition using p-HMB, indicating that it is effectively the SH moiety that is active in the in vitro system. Ohmiya and Nakai[9] have recently provided evidence that p-HMB inhibits membrane SH in human erythrocytes (50%) with minimal hemolysis (15%). It is interesting to note that another sulfhydryl inhibitor, iodoacetate, administered to mice causes a dose-related increase in the number of CFU-c but no observable effect on CFU-s[10]. More detailed studies will be required to confirm the importance of membrane SH in hemopoiesis, and particularly to determine whether these in vitro findings have any correlation with the in vivo situation.

1 Correspondence to N. Pourreau-Schneider, Laboratoire de Radiobiologie Clinique, Institut Gustave-Roussy, F-94800 Villejuif, France.
2 C.W. Gurney and C. Pan, Proc. Soc. exp. Biol. Med. *98*, 789 (1958).
3 N. Mohandas and M. Prenant, Blood, in press (1979).
4 C. Mize, M. Prenant, N. Pourreau-Schneider and M. Bessis, Nouv. Revue fr. Hémat. *18*, 627 (1977).
5 M. Bessis, C. Mize and M. Prenant, Blood Cells *4*, 155 (1978).
6 N.N. Iscove, F. Sieber and K.H. Winterhalter, J. cell. Physiol. *83*, 309 (1974).
7 N.N. Iscove and F. Sieber, Exp. Hemat. *3*, 32 (1975).
8 N. Pourreau-Schneider, J.N.C.I. *55*, 1467 (1975).
9 Y. Ohmiya and K. Nakai, Japan J. Pharmac. *27*, 596 (1977).
10 J. Shellhaas, J. Filippi and M. Rheins, Biomedicine *27*, 335 (1977).

Alterations in bone-marrow cellularity following thymectomy in rats[1]

Helen L. Lipscomb and J.G. Sharp

Department of Anatomy, University of Nebraska Medical Center, Omaha (Nebraska 68105, USA), 7 May 1979

Summary. The number of nucleated marrow cells was decreased following neonatal thymectomy in rats, and was corrected by administration of syngeneic lymphoid cells, or by implantation of a syngeneic testis. These results suggest that, in the rat, as has been shown previously in the mouse, lymphoid cells exert parital control over bone marrow cellularity and this effect may be further modulated by sex steroids.

It has been known for several years that the addition of thymocytes enhances both the growth of transplanted bone marrow cells in the mouse[2,3] and the size of granulocyte progenitor colonies in (CFC-GM) agar[4], but the mechanism of this effect is uncertain. It has also been shown that the number of nucleated marrow cells is decreased following neonatal thymectomy, and that thymectomy reduces the ability of bone marrow cells (CFU-s) to produce spleen colonies in the Till and McCulloch assay[5]. This latter effect, possibly due to an effect on the growth fraction of CFU-s, can be corrected by incubation of the bone marrow cells with thymic humoral factor[6]. It has been suggested that a thymus-derived cell sensitive to cytotoxic killing with anti-theta (Thy-1) serum plus complement is involved in the

control of bone marrow growth[7,8]. The absence of this antitheta sensitive regulatory cell (TSRC) has been suggested as a defect in the W/W^v anemic mouse[7-9]. It is difficult to assess if a similar cell is present in species other than the mouse because of the lack of information concerning alloantigen specifically present on the surface of thymocytes, and limitations on the application of the Till and McCulloch spleen colony technique[10] in species other than the mouse. Recently evidence has been presented that a suppressor T-cell might be involved in some instances of poor growth of bone marrow in T-cell depleted mice[11]. The present report describes observations of femoral cellularity in sham-operated and thymectomized rats, in thymectomized rats treated with cells from spleen, thymus, or lymph nodes, and in sham-operated and thymectomized rats implanted with a syngeneic testis.

Fischer 344 female rats were sham-thymectomized (Sham) or thymectomized (TMX) at 5 days of age using the technique of Hard[12]. For cell transfer experiments, thymus, spleen and inguinal and mesenteric lymph nodes were aseptically removed from syngeneic adult or young donors. These tissues were minced in a small volume of RPMI 1640 tissue culture medium, and then pressed through and 80-mesh wire screen. Cell counts were performed, and groups of TMX rats were injected at 14 days of age with 10^5 or 10^7 cells. For the testicular transplant experiment, at 5 days of age, groups of Sham or TMX rats were transplanted s.c. in the neck over the area of the jugular vein with 1 testis from a syngeneic, age-matched donor. All rats were maintained under carefully controlled environmental conditions until sacrifice by decapitation at 3 months of age. Right femora were removed and dissected free of surrounding tissues. The ends of the bones were removed, and contents of the marrow cavity were flushed into a vial of isotonic diluent. Red blood cells were lysed, and the marrow nucleated cellularity determined utilizing a Model Z_{BI} Coulter Counter electronic particle counter.

Results of these determinations are presented in the table. Data are expressed as 10^6 nucleated cells per femur. Compared to Sham rats, femoral cellularity was decreased in TMX rats, and increased by the addition of lymphoid cells from a variety of sources. In addition, transplantation of a testis to a Sham rat led to increased cells per femur, and this effect was enhanced even further in TMX rats with a testis transplant.

These results indicate that, in the rat, as has been previously observed in the mouse, neonatal thymectomy results in a decrease in bone marrow cellularity. This deficit in TMX rats can be corrected by administration of lymphoid cells from a variety of sources as well as by the presence of a syngeneic testis transplant. The latter effect is in good agreement with previous reports of stimulation of hematopoiesis following administration of androgens[13], of an increase in spontaneous spleen colony formation in mice treated with testosterone before irradiation[14], and of greater impairment of CFC-GM in thymectomized female as compared to thymetomized male mice[5]. These observations provide at least indirect evidence that cells from lymphoid tissues control to some degree femoral bone marrow cellularity in the rat, and that the influence of sex steroids on this process may be altered following thymectomy.

The effect of sham-thymectomy (Sham) and thymectomy (TMX), and the injection of cells or transplantation of a syngeneic testis on the number of nucleated cells per femur in Fischer 344 female rats

Treatment	Number of rats	Nucleated cells/femur $\times 10^{-6}$ (mean $\pm$ SEM)
Sham	34	57.9 $\pm$ 1.3
TMX	36	52.7 $\pm$ 1.6
TMX + cells	85	65.7 $\pm$ 0.8
Sham + testis	10	68.2 $\pm$ 6.6
TMX + testis	8	75.6 $\pm$ 8.0

$F = 14.51$; $p < 0.01$.

1 Supported in part by NSF-RIAS 77-06922, NIH AM21137 and the Morseman Foundation.

2 J.W. Goodman and S.G. Shinpock, Proc. Soc. exp. Biol. Med. *129*, 417 (1968).
3 B.I. Lord and R. Schofield, Blood *42*, 395 (1973).
4 D. Metcalf J. Cell Physiol. *72*, 9 (1968).
5 P. Resnitzky, D. Zipori and N. Trainin, Blood *37*, 634 (1971).
6 D. Zipori, in: Biological Activity of Thymic Hormones, p. 233. Ed. D.W. van Bekkum. Kookyer Scient. Publ, Rotterdam 1975.
7 J. Sharkis, W. Wiktor-Jedrzejczak, A. Ahmed, G.W. Santos, A. McKee and K.W. Sell, Blood *52*, 802 (1978).
8 S.J. Sharkis, A. Ahmed, L.L. Sensenbrenner, W.W. Jedrzejczak, A.L. Goldstein and K.W. Sell, in: Experimental Hematology Today, p. 17. Ed. S.J. Baum and G.D. Ledney, Springer-Verlag, New York 1978.
9 W.W. Jedrzejczak, S.J. Sharkis, A. Ahmed, K.W. Sell and G.W. Santos, Science *196*, 313 (1977).
10 J.E. Till and E.A. McCulloch, Radiat. Res. *14*, 213 (1961).
11 J.W. Goodman, S.G. Shinpock and N.L. Basford, Exp. Hematol. *7*, 17 (1979).
12 G.C. Hard, Lab. Anim. *9*, 105 (1975).
13 C.W. Gurney and W. Fried, J. Lab. clin. Med. *65*, 775 (1965).
14 J.C. Marsh, D.R. Boggs, P.A. Chervenick, G.E. Cartwright and M.M. Wintrobe, J. Cell Physiol. *71*, 65 (1968).

Effect of dihydroergocristine infusion on tolbutamide-induced insulin secretion in man

F. Caviezel, M. Poli, A.M. Girardi and G. Pozza[1]

Chair of Medical Pathology and Chair of Experimental Endocrinology, University of Milano, Ospedale San Raffaele, I-20090 Milano-Segrate (Italy), 11 December 1978

Summary. The insulinemic response to 1 g of tolbutamide i.v. is greatly enhanced (+ 145%) after a 60-min infusion of the α-lytic dihydrogenated ergot alkaloid, dihydroergocristine (83.3 µg/min, corresponding to a total dose of 5 mg) in 7 healthy subjects. No differences are observed in the glycemic responses.

Several drugs able to act on adrenergic receptors, consistently interfer with insulin secretion: β-adrenergic stimulating[2,3] and α-blocking[4,5] agents increase insulin secretion, whereas β-blocking[2,6] and α-stimulating[5,7] ones seem to have an inhibitory effect. The dihydrogenated alkaloids of the ergot possessing an α-blocking activity[8] have been shown to increase tolbutamide-induced insulin secretion in dog[2,11] and glucagon-induced insulin secretion in man[12]. It has recently been found that in man that a 7 days i.m. pretreatment with 0.3 mg b.i.d. of dihydroergocristine