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Hydroxyurea-Induced Erythroid Differentiation[1] (38956)

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It is well established that red cell production is primarily governed by the hormone erythropoietin (EP) (1). Although it is clear that EP effects the differentiation of responsive hemopoietic stem cells into morphologically recognizable erythroid elements, the precise mechanism by which this is accomplished is unknown (2). It has been suggested, however, that EP acts as a gene derepressor, thus allowing the transcription of the m-RNA responsible for directing hemoglobin synthesis (3, 4). To date, several lines of investigation suggest that EP is not the sole regulator of erythropoiesis. In support of this view are the reports that (i) certain steroids have a direct stimulatory action on hemopoietic tissues (5, 6); (ii) EP does not enhance hemoglobin synthesis in the yolk sac of embryonic mice (7); (iii) EP does not seem to be primarily involved in the pathogenesis of polycythemia vera (8); (iv) EP is not implicated in the mechanism of viral-induced polycythemia in mice (9–11). The data presented herein suggest that differentiation of hemopoietic precursor cells (i.e., EP-responsive stem cells) into the erythroid series can likewise be induced in the absence of EP by the cytotoxic agent hydroxyurea (OHU). This contention derives support from the inability of anti-EP to influence OHU-induced erythroid differentiation in hypertransfused mice.

Materials and Methods. Twelve-week-old female CF1 mice were hypertransfused ip with 1.0 ml of saline-washed homologous red cells on 2 consecutive days, in order to suppress red cell production. In our first two experiments, groups of mice were injected iv with OHU (900 mg/kg), 5 days after their last transfusion. Thereafter, groups were sacrificed at four consecutive 24-hr intervals. The % 18-hr ^{59}Fe incorporation into red cells was performed as previously described (12). Hematocrit and reticulocyte determinations were performed on cardiac blood; animals with an hematocrit less than 59% were excluded. Femoral marrow smears were prepared by a brush technique and stained with Wright's and Giemsa. Tibial nucleated cellularity was measured by electronic particle counting as described by Fruhman (13). Based on prior ^{59}Fe uptake studies we have shown that tibial marrow from OHU-treated mice does not have altered bone adherence properties from normal marrow; hence 95% of the tibial contents are readily flushed by this technique. The % erythroid precursors were estimated from differential counts on 1000 nucleated cells from two slides/mouse and expressed in absolute numbers/tibia. In our second two experiments, groups of 5–11 hypertransfused mice were injected iv with human urinary EP (1.5 units), hydroxyurea (OHU) (900 mg/kg), and anti-EP (4.0 units),[6] 5 days after their last transfusion. The above substances were administered singularly or in combination with each other. Two days later, all animals were sacrificed and the absolute numbers of erythroid precursors/tibia were measured as described above. Composite data are reported in Tables I and II.

Results. Hypertransfusion virtually abolishes erythropoiesis in mice (Table I). Marrow erythroid precursors numbered 0.04 ×

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TABLE I. ERYTHROPOIETIC RESPONSE OF HYPERTRANSFUSED MICE TO A SINGLE INJECTION OF HYDROXYUREA (OHU) (900 mg/kg)[a]

Day after OHU	Number of mice	Erythroid precursors/tibia ($\times 10^{-6}$)	Reticulocytes %	18-hr ^{59}Fe red cell incorporation (%)
Controls	8	0.04 ±0.02	0	0.03 ±0.01
1	4	0.09 ±0.01	0	0.05 ±0.02
2	8	0.20 ±0.02	0	0.01 ±0.01
3	9	0.18 ±0.01	0.01 ±0.01	0.02 ±0.01
4	16	0.06 ±0.02	0.01 ±0.01	0.06 ±0.02

[a] Results are expressed as the group mean ± standard error.

TABLE II. ERYTHROPOIETIC RESPONSE OF HYPERTRANSFUSED MICE TWO DAYS AFTER IV INJECTION OF HUMAN URINARY ERYTHROPOIETIN (EP) (1.5 UNITS), HYDROXYUREA (OHU) (900 mg/kg) AND ANTI-ERYTHROPOIETIN (ANTI-EP) (4.0 UNITS)

Group	Number of mice	Erythroid precursors/tibia[a] ($\times 10^{-6}$)
Controls	10	0.04 ± 0.01
EP	5	1.94 ± 0.17
Anti-EP	5	0.06 ± 0.01
EP + Anti-EP	6	0.07 ± 0.01
OHU	10	0.18 ± 0.02[b]
OHU + Anti-EP	11	0.16 ± 0.02[c]
EP + OHU	5	1.58 ± 0.22
EP + OHU + Anti-EP	6	0.11 ± 0.01

[a] Results are expressed as the group mean ± standard error.

[b] $P < 0.01$ when compared to control group.

[c] $P < 0.02$ when compared to Anti-EP control group.

10^6 per tibia and no reticulocytes were detectable in the peripheral circulation. When plethoric mice were given OHU, a small but significant wave of erythroid differentiation ensued. The maximal erythroid response, as judged by tibial erythroid precursors, was noted on day 2. Although a slight peripheral reticulocyte response occurred thereafter as demonstrated by the slight rise on days 3 and 4, this low-level erythropoiesis was not detected by a significant rise in ^{59}Fe uptake into red cells.

The effects of EP, OHU, and anti-EP on tibial erythroid precursors, 2 days after injection, are shown in Table II. The administration of 1.5 units of EP produced a 60-fold rise in tibial erythroid precursors. When given simultaneously with anti-EP, the effect of EP was virtually blocked. In contrast, anti-EP was without effect in reversing the fivefold rise in tibial erythroid precursors produced by OHU. Simultaneous injection of EP and OHU resulted in a response which was approximately 80% of that produced by EP alone. When anti-EP was given in combination with EP and OHU, the response was comparable to that elicited by OHU alone.

Discussion. The data presented herein clearly indicate that the administration of OHU to plethoric mice is followed by the differentiation of a small number of hemopoietic precursor cells into the erythroid series; an effect which does not appear to be mediated by EP. This conclusion is based on the inability of anti-EP to block the erythroid response to OHU in the hypertransfused mouse. In this regard, since an overloading of hypertransfusion-induced plethoric mice with anti-EP did not further reduce base line erythropoiesis (Table II) (unlike the situation in exhypoxic rodents, 14), it is unlikely that OHU-induced erythroid differentiation was mediated by residual EP or by low level EP production.

In the past, we have shown that OHU exerts a lethal effect on erythroid cells in DNA synthesis (15) and when administered simultaneously with EP to hypertransfused mice, a 20% reduction in erythroid response was noted (12, 16). From this latter observation it was suggested that the precursor cells responsive to EP were in cell cycle. In the present study, the administration of OHU not only killed a portion of EP-responsive cells but, in addition, effected the differentiation of a segment of surviving precursor cells. The mechanism for this occurrence is not clear. Although speculative, a plausible explanation is that OHU may have inhibited the repression of that part of the committed stem cell genome responsible for induction of the enzymes governing hemoglobin synthesis. Although the precise mechanisms regu-

lating genetic expression are unknown, the experiments of Huang and Bonner (17) have demonstrated the importance of histones in rendering DNA inactive as a template by virtue of their ability to complex with this macromolecule. That OHU may perhaps derepress genetic information appears all the more attractive from the standpoint that OHU has been shown to inhibit histone synthesis in actively dividing cells (18, 19).

It is noteworthy that the magnitude of erythroid differentiation induced by OHU was barely detectable by peripheral parameters, thus demonstrating the sensitivity of marrow erythroid quantitation for low levels of erythropoiesis.

Summary. Hydroxyurea, a cytotoxic agent which destroys cells in DNA synthesis, has been shown to evoke the differentiation of a small number of hemopoietic precursor cells in the erythroid series of erythropoietically suppressed hypertransfused mice. This effect does not appear to be mediated by erythropoietin (EP) since the simultaneous injection of anti-EP did not alter this response.

1. Gordon, A. S., and Zanjani, E. D., *in* "Regulation of Hematopoiesis" (A. S. Gordon, ed.), Vol. 1, p. 413. Appleton-Century-Crofts, New York (1970).
2. Stohlman, F., Jr., *in* "Regulation of Hematopoiesis" (A. S. Gordon, ed.), Vol. 1, p. 317. Appleton-Century-Crofts, New York (1970).
3. Galien-Lartigue, O., and Goldwasser, E., *Biochim. Biophys. Acta* **103,** 319 (1965).
4. Krantz, S. B., and Goldwasser, E., *Biochim. Biophys. Acta* **103,** 325 (1965).
5. Reisner, Jr., E. H., *Blood* **27,** 460 (1966).
6. Jacobson, W., Sidman, R. L., and Diamond, L. K., *Ann. N.Y. Acad. Sci.* **149,** 389 (1968).
7. Cole, R. J., Hunter, J., and Paul, J., *Brit. J. Haemat.* **14,** 477 (1968).
8. Stohlman, F., Jr., *Sem. Hemat.* **3,** 181 (1966).
9. Mirand, E. A., Steeves, R. A., Lange, R. D., and Grace, J. T., Jr., *Proc. Soc. Exp. Biol. Med.* **128,** 844 (1968).
10. Sassa, S., Takaku, F., and Nakao, K., *Blood* **31,** 758 (1968).
11. Tambourin, P., and Wendling, F., *Nature (New Biology)* **234,** 230 (1971).
12. Morse, B. S., Rencricca, N. J., and Stohlman, F., Jr., *Blood* **35,** 761 (1969).
13. Fruhman, G. J., *Life Sci.* **3,** 737 (1964).
14. Schooley, J. C., and Mahlmann, L. J., *in* "Regulation of Erythropoiesis" (A. S. Gordon, M. Condorelli and C. Peschle, eds.), p. 167. The Publishing House "Il Ponte," Milan, Italy (1972).
15. Morse, B. S., Rencricca, N. J., and Stohlman, F., Jr., *Proc. Soc. Biol. Med.* **139,** 986 (1969).
16. Morse, B. S., Rencricca, N. J., and Stohlman, F., Jr., *in* "Hemopoietic Cellular Proliferation" (F. Stohlman, Jr., ed.), p. 160. Grune and Stratton, New York (1970).
17. Huang, R. C., and Bonner, J., *Proc. Nat. Acad. Sci.* **48,** 1216 (1965).
18. Yarbro, J. W., *Biochim. Biophys. Acta* **145,** 531 (1967).
19. Yarbro, J. W., *Cancer Res.* **28,** 1082 (1968).

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