vate guanylate cyclase and increase tissue cyclic GMP is of obvious interest. Goldberg *et al.* (*20*) reported that phorbal myristate acetate, the biologically active component of croton oil, increases cyclic GMP in cultured fibroblasts, but data are otherwise lacking. Conversely, questions arise concerning the mutagenic and carcinogenic potential of other agonists of guanylate cyclase, such as NH_2OH and nitrite, whose effects on the enzyme appear quite similar to those of the nitrosamines. While administration of nitrite to intact animals has failed to induce tumors (*19, 22*), this agent has been reported to transform cells in culture (*23*). With regard to the action of NH_2OH on guanylate cyclase, it is pertinent to note that the carcinogenic activity of some aromatic amines depends on their metabolism to *N*-hydroxylamine derivatives and that NH_2OH is mutagenic (*24, 25*). Further examination of the relationship between chemical carcinogenesis and the guanylate cyclase–cyclic GMP system is indicated.

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References and Notes

1. N. D. Goldberg, M. K. Haddox, S. E. Nicol, D. B. Glass, C. H. Sandford, F. A. Kushl, R. Estensen, *Adv. Cyclic Nucleotide Res.* **5**, 307 (1975).
2. Z. Miller, E. Lovelace, M. Gallo, I. Pastan, *Science* **190**, 1213 (1975).
3. P. N. Magee and J. M. Barnes, *Br. J. Cancer* **10**, 114 (1956).
4. W. Lijinsky and S. E. Epstein, *Nature (London)* **225**, 21 (1970).
5. I. Berenblum, in *Frontier of Biology: Carcinogenesis as a Biological Problem*, A. Neuberger and E. L. Tatum, Eds. (North-Holland, Amsterdam, 1974), pp. 67–201.
6. H. Kimura, C. K. Mittal, F. Murad, *J. Biol. Chem.* **250**, 8016 (1975).
7. ———, *Nature (London)* **257**, 700 (1975)
8. F. R. DeRubertis and P. A. Craven, *J. Biol. Chem.* **251**, 4651 (1976).
9. G. Schultz and J. G. Hardman, *Adv. Cyclic Nucleotide Res.* **5**, 339 (1975).
10. J. G. Hardman and E. W. Sutherland, *J. Biol. Chem.* **244**, 6363 (1969).
11. C. Nagata, Y. Ioki, M. Kodama, Y. Tagashira, M. Nakadate, *Ann. N.Y. Acad. Sci.* **222**, 1031 (1973).
12. F. R. DeRubertis, R. Chayoth, J. B. Field, *J. Clin. Invest.* **57**, 641 (1976).
13. P. A. Craven and F. R. DeRubertis, *Anal. Biochem.* **72**, 455 (1976).
14. T. D. Chrisman, D. L. Garbers, M. A. Parks, J. G. Hardman, *J. Biol. Chem.* **250**, 374 (1975).
15. F. R. DeRubertis and P. A. Craven, *Metabolism*, in press.
16. Kimura *et al.* (*6*) examined NH_2OH-responsive guanylate cyclase activity in the 100,000*g* supernatant fraction of hepatic homogenates and reported much smaller increases with this agent. However, we have found that 50 to 75 percent of the NH_2OH-responsive enzyme activity in whole homogenates of liver and kidney is lost on fractionation of these homogenates at 100,000*g*. Since the resultant particulate fractions are also poorly responsive to NH_2OH, loss of a particulate or labile tissue component necessary for expression of the action of NH_2OH on soluble enzyme activity may be involved. The lower nitrite responsiveness observed by Kimura *et al.* (*6*) when this agonist was tested at 1 m*M* appears to be due to a steep concentration-response relation and the higher concentration (20 m*M*) used in our studies. When we tested nitrite at 1 m*M*, we noted changes in hepatic enzyme activity similar to those previously described (*6*).
17. M. F. J. Blokzijl-Homan and B. F. van Gelder, *Biochim. Biophys. Acta* **234**, 493 (1971).
18. A. Imamura and C. Nagata, *Gann* **65**, 417 (1974).
19. B. Commoner, J. C. Woolum, B. H. Senturia, J. L. Ternberg, *Cancer Res.* **30**, 2091 (1971).
20. N. D. Goldberg, M. K. Haddox, R. Estensen, J. G. White, C. Lopez, J. W. Hadden, in *cAMP, Cell Growth and the Immune Response*, W. Brown, L. M. Lichtenstein, C. W. Parker, Eds. (Springer-Verlag, New York, 1974), pp. 247–262.
21. E. W. Thomas, F. Murad, W. B. Looney, H. P. Morris, *Biochim. Biophys. Acta* **297**, 564 (1973).
22. M. Greenblatt and W. Lijinsky, *J. Natl. Cancer Inst.* **48**, 1389 (1972).
23. H. Tsuda, N. Inue, S. Takatama, *Biochem. Biophys. Res. Commun.* **55**, 1117 (1973).
24. J. W. Cramer, J. A. Miller, E. C. Miller, *J. Biol. Chem.* **235**, 885 (1960).
25. J. H. Weisburger, *Cancer* **28**, 60 (1971).
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Diamond-Blackfan Syndrome: Lymphocyte-Mediated Suppression of Erythropoiesis

Abstract. *Peripheral blood lymphocytes from six patients with congenital hypoplastic anemia suppressed erythroid cell formation by normal human bone marrow cells in response to erythropoietin in vitro. The results suggest that the anemia in these children has an immunologic basis.*

Congenital hypoplastic anemia (CHA, or Diamond-Blackfan syndrome) is a disorder of childhood; it is characterized by progressive anemia beginning early in infancy, and is sparing of the white blood cells and platelets (*1*). In the bone marrow, erythroid precursors are virtually absent. Since its initial description in 1938 (*2*), the etiology of CHA has remained unknown. The production of erythropoietin (Ep) (*3*), and the numbers of erythroid progenitor cells (*4*) capable of proliferation and differentiation into red cells appear to be adequate. In contrast to pure red cell aplasia (PRCA) acquired by adults (*5*), a serum inhibitor of erythropoiesis is not present in CHA (*6*). Peripheral blood lymphocytes from an adult with common variable hypogammaglobulinemia and PRCA can inhibit erythropoiesis in vitro (*7*). We now present evidence that peripheral blood lymphocytes from patients with CHA inhibit red cell production by normal bone marrow cells, an indication that CHA may have an immunologic basis.

Peripheral blood lymphocytes from six patients with clinically established CHA were separated from heparinized whole blood by means of a Ficoll-Hypaque density gradient (*8*). Cells from 24 randomly selected normal subjects, five individuals who were multiply transfused with blood products for a variety of other disorders, and three adults with acquired PRCA, were used in this study. The control group included individuals matched as to sex and age to the patients with CHA. Control studies were performed in parallel with experimental studies. Normal human bone marrow cells were obtained by aspiration from the posterior iliac crest of 20 individuals with a normal hematological profile. Varying numbers of peripheral blood lymphocytes (0.5×10^5, 1×10^5, 2×10^5, and 4×10^5 cells) were cultured with 6×10^5 bone marrow cells in the plasma clot culture system (*9*) in

Table 1. Effect of human peripheral blood lymphocytes on erythroid colony formation by normal human bone marrow cells in response to erythropoietin in vitro. Each value represents the mean ± 1 standard error of the mean of results obtained from all studies. The numbers in parentheses indicate the number of studies conducted.

Additions to culture	Number of erythroid colonies per 6×10^5 bone marrow cells at lymphocyte concentrations of			
	0.5×10^5	1×10^5	2×10^5	4×10^5
None	0	0	0	0
Normal lymphocytes	0	0	0	0
2 I.U. of Ep*		*422 ± 21*		
+ Normal lymphocytes (24)	576 ± 18	612 ± 32	594 ± 17	572 ± 26
+ Lymphocytes, transfused patients (5)	632 ± 68	608 ± 72	576 ± 53	575 ± 49
+ PRCA lymphocytes (3)	486 ± 47	509 ± 55	617 ± 89	603 ± 77
+ CHA lymphocytes (12)	46 ± 9	12 ± 6	22 ± 7	110 ± 15
+ F-T CHA lymphocytes†	‡	‡	494 ± 72	‡

*Human urinary erythropoietin used in these studies was collected and concentrated by the Department of Physiology, University of Northeast, Corrientes, Argentina, and further processed and assayed by the Hematology Research Laboratories, Children's Hospital of Los Angeles, under NIH grant HE-10880. †Lymphocytes were subjected to repeated freeze-thawing (F-T) prior to use. ‡Not determined.

Table 2. Effect of human serum on erythroid colony formation by normal human bone marrow cells in response to erythropoietin in vitro. Each value represents the mean ± 1 standard error of the mean of the results obtained in all studies. The number of studies are shown in parentheses.

Additions to culture	Number of erythroid colonies per 6×10^5 bone marrow cells at serum concentrations of			
	0.05 ml	0.10 ml	0.15 ml	0.20 ml
None	0	0	0	0
2 I.U. of Ep*		*481 ± 76*		
Normal serum (6)	467 ± 39	412 ± 42	449 ± 32	414 ± 54
PRCA serum (3)	29 ± 5	6 ± 2	0	0
CHA serum (6)	600 ± 74	723 ± 46	832 ± 88	661 ± 74

*See legend to Table 1.

the presence or absence of 2 I.U. (international units) of Ep for 5 to 7 days in a humidified atmosphere of 3 percent CO_2 in air. Only colonies composed of eight or more erythroid cells were considered in the calculation of the results. Erythroid colony growth of normal human bone marrow in the plasma clot is dependent on the presence of Ep (Table 1). No colonies were formed in the absence of Ep, while 422 ± 21 colonies were produced in its presence. Addition of normal human lymphocytes to the bone marrow in the presence of Ep resulted in a statistically significant increase in the number of colonies formed ($P < .05$). Lymphocytes alone do not give rise to erythroid colonies even in the presence of Ep, so that this appears to be a direct enhancement of erythroid colony growth. By contrast, lymphocytes from all six patients with CHA suppressed the formation of erythroid colonies by these bone marrow cells in the presence of Ep (Table 1). Each of the CHA patients was studied on two separate occasions, and in every instance inhibition of erythropoiesis was found. Table 1 also shows that prior repeated freeze-thawing of these lymphocytes removed their capacity to suppress erythroid colony formation, suggesting the need for cellular integrity. The results (Table 2) demonstrate that serums from patients with CHA did not exhibit erythropoietic-inhibitory activity. In fact, the addition of CHA serum resulted in a significant increase in the number of colonies formed. This stimulatory effect of the CHA serum is due to the presence of Ep in these serums (Table 2). Serums from patients with PRCA significantly inhibit erythroid colony formation (Table 2), but lymphocytes from these patients did not suppress erythroid colony formation (Table 1). Since our patients with CHA had received multiple blood transfusions throughout the course of their disease, the possibility existed that the suppression of erythropoiesis by CHA lymphocytes might be due to prior sensitization by foreign histocompatibility antigens. However, as can be seen in Table 1, when lymphocytes from five patients, who had been receiving prolonged transfusion of blood products as part of their therapy, were cultured together with normal bone marrow in this system, no inhibition of colony formation was observed. These results indicate that a component of peripheral blood lymphocytes from patients with CHA may be the primary agents in the production of anemia in this disorder. This is in contrast to PRCA of the adult where a serum-borne inhibitor of erythropoiesis has been implicated as the cause of anemia (*5*). It is not clear at present whether the lymphocytes that suppress erythropoiesis reside in the bone marrow or arrive there as part of the recirculating lymphocyte pool which has free access into and out of the bone marrow parenchyma (*10*).

About 40 to 50 percent of patients with CHA undergo clinical remission on corticosteroid therapy (*11*). Pharmacologic doses of steroids have been shown to cause lymphocytopenia (*10*), alter lymphocyte migration patterns (*12*), and protect against T-lymphocyte cell-mediated cytotoxicity (*13*). All but one of the CHA patients, all of the patients with PRCA, and one of the multiply transfused patients were receiving corticosteroid therapy at the time of our study. The lymphocytes from the patients receiving corticosteroids did not inhibit erythroid colony formation by normal bone marrow cells. The fact that lymphocytes from patients with CHA, but not those from PRCA or multiply transfused individuals, suppressed erythropoiesis in this system indicates that (i) corticosteroid therapy does not lead to the preferential selection of a population of lymphocytes capable of inhibiting erythropoiesis, and (ii) the ameliorative effects of corticosteroids may be mediated through an effect on the erythroid precursor cell. In this regard, Stavy *et al.* (*13*) have presented in vitro evidence that corticosteroids protect target cell mouse fibroblasts from T-lymphocyte killer activity, but do not alter the functional capacity of these cells when tested in the absence of steroids.

In normal hematopoiesis, cell-cell interactions have been shown to play a possible regulatory role in white blood cell production (*14*). Our studies indicate that such interactions may be important for red cell formation as well. The presence of suppressor-helper lymphocyte populations has been demonstrated in animals and man (*15*); the relative ratios of the two populations appear to be critical. Recently, suppressor or killer cells have been implicated in a variety of disorders including diabetes mellitus (*16*), polymyositis (*17*), and common variable immunodeficiency (*18*) in man. Lymphocytic choriomeningitis (*19*) and Theiler's virus–induced demyelinization (*20*) have similar mechanisms in mice. Thus, CHA may be an additional disorder to be added to the growing list of lymphocyte autoaggressive diseases.

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References and Notes

1. L. K. Diamond, D. M. Allen, F. B. MaGill, *Am. J. Dis. Child.* **102**, 149 (1961).
2. L. K. Diamond and K. O. Blackfan, *ibid.* **56**, 464 (1938).
3. D. Hammond and L. K. Diamond, *ibid.* **100**, 466 (1960).
4. M. H. Freedman, D. Amato, E. F. Saunders, *J. Clin. Invest.* **57**, 673 (1976).
5. S. B. Krantz and V. Kao, *Proc. Natl. Acad. Sci. U.S.A.* **58**, 493 (1967).
6. G. Geller, W. Krivit, R. Zalusky, E. D. Zanjani, *J. Pediatr.* **86**, 198 (1975).
7. E. D. Zanjani, S. D. Litwin, R. Zalusky, *Blood* **46**, 113 and 1038 (1975).
8. A. Boyum, *Scand. J. Clin. Lab. Invest. Suppl.* **21** (97), 77 (1968).
9. K. Goldstein, H. D. Preisler, J. D. Lutton, E. D. Zanjani, *Blood* **44**, 831 (1974).
10. A. S. Fauci and D. C. Dale, *J. Clin. Invest.* **53**, 240 (1974).
11. D. M. Allen and L. K. Diamond, *Am. J. Dis. Child.* **102**, 416 (1961).
12. A. S. Fauci, *Immunology* **28**, 669 (1975).
13. L. Stavy, I. R. Cohen, M. Feldman, *Cell. Immunol.* **7**, 302 (1973).
14. M. T. Aye and E. A. McCulloch, *Blood* **45**, 485 (1975).
15. R. K. Gershon, *Contemp. Top. Immunobiol.* **3**, 1 (1974); *Transplant. Rev.* **26**, 170 (1975).
16. S. W. Haung and N. K. Mclaren, *Science* **142**, 64 (1976).
17. R. L. Dawkins and F. L. Mastaglia, *N. Engl. J. Med.* **288**, 434 (1973).
18. T. A. Waldman, S. Broder, R. M. Blaese, M. Durm, M. Blackman, W. Strober, *Lancet* **1974-II**, 609 (1974).
19. G. A. Cole, D. H. Gilden, A. A. Monjan, N. Nathanson, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **30**, 1831 (1971).
20. H. L. Lipton and M. C. Dal Canto, *Science* **192**, 62 (1976).
21. Supported by PHS grants CA-10728 and CA-18755. We thank G. Lurinsky for technical assistance.
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