significant effect on Ts cell activity (Fig. 2c). Thus, the activity of antibody to I-J^k was lost specifically by adsorption on I-J^k splenocytes. Cells from naïve animals did not confer susceptibility to the growth of the tumor (Fig. 2d).

As noted above, administration of anti–I-J reagents in vivo retarded primary tumor growth in some systems (*13, 14*). Our results show that this can occur by interfering with the appearance of transferable suppressor cells and attest to the importance of I-J bearing elements in UV radiation–induced tumor-specific Ts cell activity prior to the overt appearance of a tumor. The discovery that administration of CY has a similar effect may be related to earlier observations indicating that the splenic I-J bearing APC's required for effector-suppressor cell activation are CY-sensitive (*15*). Although it is difficult to make quantitative comparisons between the observed effects of antibody to I-J^k compared to those of CY on the basis of these experiments, the greater activity of antibody observed compared to that of CY might be due to interference by the antibody, both at the level of activation of Ts cells by the I-J bearing APC's and at the level of I-J bearing Ts cells, while CY may work by interference only at the I-J bearing APC level. Dose-response titrations may help to address this question. Further, the observation that the activity of monoclonal antibody to I-J^k is lost by adsorption on naïve I-J^k but not I-J^d splenocytes from H-2 congenic mice confirms that the antibody recognizes specifically an H-2 product. Therapeutic approaches to malignancies or other diseases in which Ts cells play a role may therefore be possible through the use of antibodies or other regulatory agents that modify Ts cell activity or generation.

RICHARD D. GRANSTEIN
JOHN A. PARRISH
DANIEL J. MCAULIFFE
Department of Dermatology, Harvard Medical School and *Massachusetts General Hospital, Boston 02114*
CARL WALTENBAUGH
Department of Microbiology and Immunology, Northwestern University Medical School, Chicago, Illinois 60611
MARK I. GREENE
Department of Pathology, Harvard Medical School

References and Notes

1. M. L. Kripke, *J. Natl. Cancer Inst.* **53**, 1333 (1974).
2. C. W. Spellman and R. A. Daynes, *Transplantation* **24**, 120 (1977).
3. M. S. Fisher and M. L. Kripke, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 1688 (1977).
4. ———, *Science* **216**, 1133 (1982).
5. J. A. Drebin, L. L. Perry, J. S. Bromberg, J. T. Nepom, B. Benacerraf, M. I. Greene, in *Immunochemotherapy*, V. E. Nydeggar, Ed. (Academic Press, New York, 1981), p. 99.
6. E. S. Dye and R. J. North, *J. Exp. Med.* **154**, 1033 (1981).
7. R. J. North, *ibid.* **155**, 1063 (1982).
8. D. B. Murphy *et al.*, *ibid.* **144**, 699 (1976).
9. T. Toda, M. Taniguchi, C. S. David, *ibid.* **144**, 713 (1976).
10. M. I. Greene, A. Pierres, M. E. Dorf, B. Benacerraf, *ibid.* **146**, 293 (1977).
11. M. Steinmetz *et al.*, *Nature (London)* **300**, 35 (1982).
12. C. N. Baxevanis, Z. A. Nagy, J. Klein, *J. Immunol.* **131**, 628 (1983).
13. M. I. Greene, M. E. Dorf, A. Pierres, B. Benacerraf, *Proc Natl. Acad. Sci. U.S.A.* **74**, 5118 (1977).
14. J. A. Drebin, C. Waltenbaugh, S. Schatten, B. Benacerraf, M. I. Greene, *J. Immunol.* **130**, 506 (1983).
15. A. Lowy, A. Tominaga, J. A. Drebin, M. Takaoki, B. Benacerraf, M. I. Greene, *J. Exp. Med.* **157**, 353 (1983).
16. R. D. Granstein, A. Lowy, M. I. Greene, *J. Immunol.* **132**, 563 (1984).
17. N. L. Letvin, J. T. Nepom, M. I. Greene, B. Benacerraf, R. N. Germain, *ibid.* **125**, 2550 (1980).
18. C. Waltenbaugh, *J. Exp. Med.* **154**, 1570 (1981).
19. Supported in part by National Research Service Award 1F32 CA07014-102, a grant from the Arthur O. and Gullan M. Wellman Foundation, a grant from the Council for Tobacco Research, and AI 18072.

28 November 1983; accepted 26 January 1984

Arabinosylcytosine Induces Fetal Hemoglobin in Baboons by Perturbing Erythroid Cell Differentiation Kinetics

Abstract. *Arabinosylcytosine, a compound that inhibits DNA synthesis in rapidly dividing cells, stimulates fetal hemoglobin in adult baboons and produces significant perturbations in the pools of erythroid progenitors. It appears that changes in the kinetics of erythroid cell differentiation rather than direct action on the γ genes underlie stimulation of fetal hemoglobin in the adult animals in vivo. These results also suggest that chemotherapeutic agents selected for their low carcinogenic or mutagenic potential could be used for therapeutic induction of fetal hemoglobin in patients with sickle cell anemia.*

Evidence suggests that stimulation of fetal hemoglobin (Hb F) synthesis will have beneficial effects in patients with β-chain hemoglobinopathies. Stimulation of Hb F was recently achieved in baboons and in patients with thalassemia or hemoglobin S (Hb S) disease treated with 5-azacytidine (*1, 2*). Since globin gene expression is correlated with the degree of globin gene methylation (*3*) and since 5-azacytidine inhibits DNA methylation, it was suggested that the stimulation of Hb F in the treated individuals was due to demethylation of γ-globin genes (*1, 2, 4*). We suggested that the induction of Hb F by 5-azacytidine could be attributed to cell kinetic perturbations introduced by the drug (*5*). By inhibiting proliferation of rapidly dividing erythroid cells (late progenitor pools and early normoblasts), 5-azacytidine introduces changes in the differentiation kinetics of erythroid cells that lead to premature commitment to terminal maturation of early erythroid progenitors having an active Hb F program and thus induces production of red cells expressing Hb F (F cells) (*5*). We tested this cell kinetic mechanism of Hb F stimulation in baboons (*Papio cynocephalus*) treated with 1-β-D-arabinofuranosylcytosine (Ara-C). We expected this inhibitor of DNA synthesis to affect cells engaged in DNA synthesis (such as late erythroid progenitors) and induce kinetic perturbations of erythropoiesis but not to directly influence the degree of methylation of γ-globin genes (*6*).

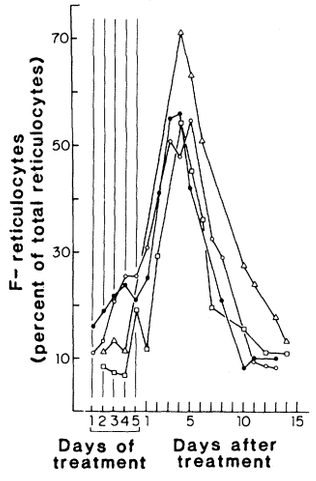

Fig. 1. Stimulation of F-reticulocyte production in three baboons treated with Ara-C (one animal was treated twice). Note the sharp increase in F reticulocytes, reaching a peak 4 to 5 days after the end of treatment.

Anemic baboons (*7*) were given four or five daily doses of Ara-C (2 mg/kg per day). Effects on Hb F were measured with counts of F cells, chemical measurements of Hb F, globin chain biosynthesis, and counts of F reticulocytes (newly produced red cells that contain Hb F) (*5, 8*).

Administration of Ara-C led to sharp increases in F reticulocytes as the percentage of total reticulocytes (Fig. 1). Peak values (50 to 75 percent) were reached 4 to 5 days after the end of treatment. Concomitantly, $\gamma/\gamma + \beta$ biosynthetic ratios increased from 0.01 to

0.02 before treatment to 0.30 to 0.47 after treatment; F cells from 1.5 to 3.4 percent to 35.2 to 57.1 percent; Hb F from 0.4 to 0.9 percent to 5.7 to 18.6 percent; absolute levels of Hb F from 0.05 to 0.11 g/dl to 0.44 to 1.5 g/dl; and absolute F-cell volume (F-cell hematocrit) from 0.6 to 1.3 percent to 11.5 to 13.7 percent. These results show that Hb F can be induced in the adult animal by a compound that does not directly influence γ-gene methylation.

Effects of the treatment on pools of erythroid progenitor cells were assessed with cultures of bone marrow cells in semisolid media (*9*). The perturbations in erythroid progenitors observed in manipulated animals are depicted in Fig. 2. Induction of anemia resulted in the expected expansion of erythroid progenitor pools, especially the late ones [colony-forming units erythroid (CFUe) and erythroid cluster–forming cells (e-clusters)]. Administration of Ara-C at the doses used did not affect the pool of burst-forming units erythroid (BFUe); this was not surprising since the majority of these early erythroid progenitors are not in cycle (*10*). By contrast, Ara-C significantly affected the actively cycling late progenitors (CFUe and e-clusters). There was a profound reduction in the size of late erythroid progenitor pools during or immediately after treatment, followed by a drastic increase (Fig. 2A). Alternatively, there was a mild reduction in size during treatment followed by a striking increase soon after treatment (Fig. 2B).

Animals treated with Ara-C were also treated with identically designed courses of 5-azacytidine. 5-Azacytidine produced waves of F reticulocytes that were similar in the time of their appearance after treatment and in their response curve to those produced by Ara-C (Fig. 3). Assessment of progenitor cell pools revealed perturbations like those described for Ara-C. These results strongly suggest that the two compounds stimulate Hb F synthesis through a common mechanism that affects the animal's erythroid cell differentiation kinetics.

Previous studies in erythroid cell cultures have shown that early erythroid progenitors in the adult have an active Hb F program that is turned off during downstream differentiation (*11*). The perturbations of erythroid progenitor kinetics in baboons treated with Ara-C may explain the stimulation of Hb F. The sudden reduction in the pool of late erythroid progenitors induces premature commitment to terminal maturation of a large number of earlier progenitors in order to meet the continuing erythropoietic demand. The newly formed cells have an active Hb F program and form mature progeny that display that program; this results in the brisk F-reticulocyte response that follows the treatment. The subsequent increase in the size of late erythroid progenitor cell pools (*12*) results in a slowdown in the entry of early progenitors to the late compartment of erythropoiesis, and F-reticulocyte production returns to baseline levels in the anemic animal (*13*).

In addition to their relevance to the cellular mechanism that controls Hb F expression in the adult, these findings have implications for the treatment of sickle cell anemia. If stimulation of Hb F in these patients could be achieved solely by direct γ-globin gene demethylation, therapeutic attempts would have been limited only to 5-azacytidine. If the stimulation of Hb F is mainly indirect and of cell kinetic origin, as the experiments with Ara-C suggest, several other compounds could be used for treatment. Hence, chemicals that are potentially less carcinogenic or mutagenic than 5-azacytidine could be used (*14*), an important consideration in the treatment of persons with the variable life-span of patients with sickle cell anemia.

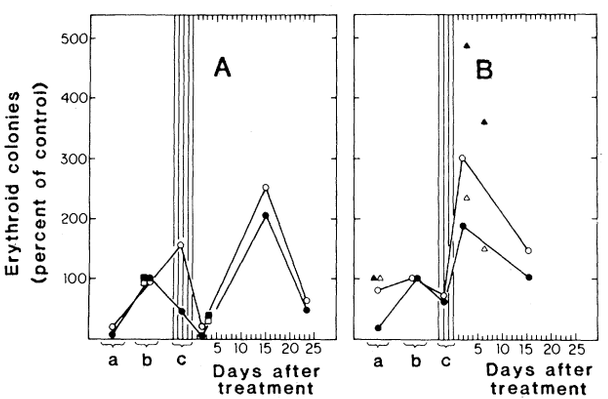

Fig. 2. Perturbations in late erythroid progenitor pools by Ara-C. The number of erythroid colonies (CFUe plus e-clusters) per 10^5 plated bone marrow cells cultured with (open symbols) or without (closed symbols) added erythropoietin was normalized by using the pretreatment value, 100 percent. Results are shown for (a) the normal animal before treatment, (b) the anemic animal before treatment, and (c) during treatment of the anemic animal with Ara-C. (A) Data for animals that were treated for 5 days. There is a profound reduction in late progenitor cell pools after treatment and a sizeable expansion a few days later. (B) Data for an animal that was treated for 4 days (○; ●) and an animal that received Ara-C while it was not anemic (△; ▲). Note the small reduction in late progenitor cell pools during treatment and the striking expansion after treatment. The expansion reflects perturbation of the erythroid progenitor cell pools by Ara-C (*13*).

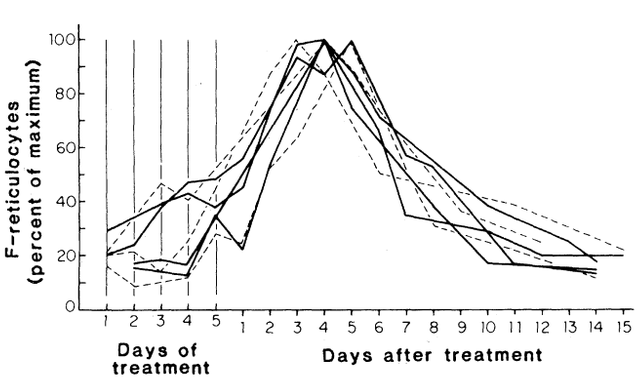

Fig. 3. Production of F reticulocytes in baboons treated with Ara-C (2 mg/kg per day) (continuous lines) or 5-azacytidine (2 mg/kg per day) (dashed lines). Note that the two drugs elicit nearly identical responses.

THALIA PAPAYANNOPOULOU
ANA TORREALBA DE RON
ROBERT VEITH
Division of Hematology, Department of Medicine, University of Washington, Seattle 98195
G. KNITTER
Regional Primate Center, University of Washington, Seattle
G. STAMATOYANNOPOULOS
Division of Medical Genetics, Department of Medicine, University of Washington, Seattle

References and Notes

1. J. DeSimone, P. Heller, L. Hall, D. Zwiers, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 4428 (1982); S. Charache *et al.*, *ibid.* **80**, 4842 (1983).
2. T. J. Ley *et al.*, *N. Engl. J. Med.* **307**, 1469 (1982).
3. L. H. T. vanderPloeg and R. A. Flavell, *Cell* **19**, 947 (1980).
4. However, the ε-globin genes of the patients treated with 5-azacytidine are also demethylated but are not expressed (*2*), indicating that γ-globin gene demethylation alone does not explain the stimulation of Hb F in vivo.
5. A. T. Torrealba de Ron *et al.*, *Blood* **63**, 201 (1984).
6. P. A. Jones and S. M. Taylor [*Cell* **20**, 85 (1980)] showed that, unlike 5-azacytidine, Ara-C does not inhibit DNA methylation.
7. Anemic animals were used because previous studies with 5-azacytidine showed that optimum stimulation of Hb F synthesis requires expanded erythropoiesis. Animals were kept at a packed cell volume of 20 to 25 percent by

frequent removal of 7 to 15 percent of their blood volume. This experimental model closely mimicks conditions in homozygous Hb S disease in vivo, in which there is expanded erythropoiesis and a continuous loss of mature erythrocytes by hemolysis.
8. T. Papayannopoulou, E. Vichinsky, G. Stamatoyannopoulos, *Br. J. Haematol.* **44**, 535 (1980). Since a portion of the animals' blood volume was removed by phlebotomy, the Hb F response after treatment with Ara-C is best measured by the counts of F reticulocytes and the measurements of γ-globin biosynthesis.
9. Burst-forming units erythroid are the earliest cells committed to erythroid lineage. Their progeny are CFUe. Erythroid cluster–forming cells are the most mature progenitors in baboon erythropoiesis. These progenitors were assayed in plasma clot cultures [D. L. McLeod, M. M. Shreeve, A. A. Axelrad, *Blood* **44**, 517 (1974)] or in methylcellulose cultures [N. N. Iscove and F. Sieber, *Exp. Hematol. (Oak Ridge, Tenn.)* **3**, 32 (1975)].
10. N. N. Iscove and L. G. Guilbert, *In Vitro Aspects of Erythropoiesis* (Springer-Verlag, New York, 1978), pp. 3–7; F. C. Monette *et al.*, *Exp. Hematol. (Oak Ridge, Tenn.)* **8**, 484 (1980); A. A. Fauser and H. A. Messner, *Blood* **54**, 1197 (1979).
11. T. Papayannopoulou, M. Brice, G. Stamatoyannopoulos, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 2923 (1977); Th. Papayanopoulou *et al.*, *Science* **199**, 1349 (1978); G. Stamatoyannopoulos and T. Papayannopoulou, *Cellular and Molecular Regulation of Hemoglobin Switching* (Grune & Stratton, New York, 1979), pp. 323–349.
12. The striking increase in the number of late progenitor cells after treatment reflects the animal's attempt to rebuild the late erythroid progenitor cell pools.
13. Another possibility is that Ara-C and 5-azacytidine stimulate F-cell production by directly stimulating early progenitors to downstream differentiation and enhancing the erythroid maturation process. A similar mechanism has been proposed to explain induction of cell differentiation in leukemia patients treated with Ara-C [S. J. Wisch *et al.*, *N. Engl. J. Med.* **309**, 1599 (1983); J. F. Desforges, *ibid.*, p. 1637].
14. Hydroxyurea or methotrexate are considered to have low carcinogenic potential. We have treated baboons with hydroxyurea and found that this compound perturbs the erythroid progenitor cell pools and stimulates Hb F synthesis.
15. Supported by NIH grants HL-20899, GM 15253, HL-07093, and RR00166.

3 November 1983; accepted 22 November 1984

Isolated Chromaffin Cells from Adrenal Medulla Contain Primarily Monoamine Oxidase B

Abstract. *Cultured chromaffin cells from bovine adrenal medulla were found to contain primarily the B form of monoamine oxidase. This monoamine oxidase B enzyme was somewhat distinct from B enzymes from other sources, in that noradrenaline was a much poorer substrate than serotonin. Nonetheless, studies with selective inhibitors of the A form (clorgyline) and the B form [(−)-deprenyl] confirmed that chromaffin cell monoamine oxidase was the B form. The observation that chromaffin cell monoamine oxidase has poor affinity for catecholamines is consistent with physiological needs that require the cell to synthesize and store large amounts of catecholamines.*

Chromaffin cells have the highest concentrations of catecholamines in the body, and an elaborate enzymatic system exists to achieve this end (*1*). Tyrosine hydroxylase in the cytoplasm converts tyrosine to dopa (*2*), which is rapidly converted to dopamine by nonspecific aromatic amino acid decarboxylase (*3*). Dopamine, still in the cytoplasm, is then taken up into the chromaffin granule by an adenosine triphosphate–dependent mechanism (*4*), where it is converted to noradrenaline by dopamine-β-hydroxylase (*5*). Subsequently, noradrenaline returns to the cytoplasm where it is methylated to adrenaline by phenylethanolamine *N*-methyl transferase (*6*) before final uptake into the chromaffin granule for storage.

In spite of the profound flux of catecholamines through the cytosolic portion of the cell, one can detect relatively little evidence of catecholamine deamination by the otherwise quite active adrenal medullary monoamine oxidase (MAO) (*7*). Indeed, we had noted in preliminary studies (*8*) that inclusion of a nonselective monoamine oxidase inhibitor such as tranylcypromine had no apparent influence on measured noradrenaline accumulation either in partially purified chromaffin granules or in isolated chromaffin cells. Pargyline, a nonspecific MAO inhibitor, apparently does not have any profound effects on cytosolic catecholamine levels in pheochromocytoma cells, since neither synthesis nor secretion of catecholamines was affected (*9*). Such a result could be accounted for either by the chromaffin cells having relatively low MAO activity or by the cells having a form of MAO that had a selectively poor affinity for catecholamines.

Monoamine oxidase is found in two forms, A and B, which differ in substrate specificity and inhibitor sensitivity (*10*). For example, MAO-A preferentially deaminates serotonin and noradrenaline (*10, 11*) and is selectively inhibited by clorgyline (*11*). By contrast, MAO-B has a high affinity for phenylethylamine and benzylamine (*10*) and is selectively inhibited by (−)-deprenyl (*12*). Tyramine and dopamine are substrates for both enzyme forms (*11*).

To examine the role of MAO directly, we prepared purified chromaffin cells by collagenase digestion using our modification (*13*) of the method of Livett *et al.* (*14*). Chromaffin cells were separated from contaminating fibroblasts by differential plating (*15*). These cells were frozen and thawed twice at −70°C, then centrifuged at 14,000*g* for 20 minutes at 4°C, and the pellet was washed once in 0.3*M* sucrose. The pellet was finally suspended in 0.1*M* Na-K-PO_4 buffer, *p*H 7.4, and used as a source of MAO enzyme. MAO activity was measured with various substrates by the method of Tipton and Youdim (*16*). As shown in Table 1, chromaffin cell MAO can deaminate monoamines with different efficiencies and relative values of maximum velocity (V_{max}). Nonhydroxylated amines such as phenylethylamine and kynuramine had significantly lower values of the Michaelis constant (K_m) than hydroxylated amines such as tyramine, dopamine, serotonin, and noradrenaline. The K_m of MAO for noradrenaline was 1100 μ*M*, nearly three times that of serotonin and 50 times that of phenylethylamine. Furthermore, the relative V_{max} for noradrenaline, as compared to tyramine, phenylethylamine, and dopamine, was significantly lower. These observations suggested that the MAO in the chromaffin cell was somewhat unusual and was perhaps the B type.

We examined the inhibitor specificity of chromaffin cell MAO with selective inhibitors of MAO-A and MAO-B (*11, 12*). Clorgyline, the selective inhibitor of MAO-A, was a poor inactivator of chromaffin cell MAO when tyramine, phenylethylamine, and serotonin were used as substrates (Fig. 1A). The median inhibitory concentration (IC_{50}) ranged from 10^{-7} to 5×10^{-6}*M*. In addition, with all the three substrates, clorgyline exhibited a simple sigmoid curve, indicating one type of enzyme (*11*).

(−)-Deprenyl also showed a single inhibitory curve, but in contrast to clorgyline, it was a much more potent inhibitor of tyramine, phenylethylamine, and serotonin deamination. The IC_{50} values for (−)-deprenyl ranged from 5×10^{-9}*M* to 5×10^{-8}*M*. These results are similar to the inhibitory potencies of (−)-deprenyl for MAO-B in other tissues such as liver (*17*), human platelet (*18*), brain (*19*), and lung (*20*).

These data thus indicate that the MAO in the chromaffin cell is primarily in the B form of the enzyme. This conclusion is primarily substantiated by the observation that the tyramine inhibition curves with clorgyline and (−)-deprenyl were of simple single form and that (−)-deprenyl was a much better inhibitor of chromaffin cell MAO than was clorgyline. In addition, the relative V_{max} (Table 1) was higher for the type B substrate phenylethylamine than for the MAO-A substrates serotonin and noradrenaline. Ear-