nononcogenic adenoviruses such as Ad2 may be destroyed efficiently in vivo by early appearing, nonspecific host effector cells, whereas cells infected with highly oncogenic adenoviruses such as Ad12 may be relatively resistant to such host defenses. This differential susceptibility of Ad-infected hamster cells to host cell–mediated lysis would result in an increase in the number of cells infected with a highly oncogenic adenovirus serotype that could become stably transformed. A further selection in vivo against tumor induction by nononcogenic Ad serotypes and for tumor induction by highly oncogenic Ad serotypes might be exerted by NK's cells, activated macrophages, and CTL after the establishment of transformation.

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References and Notes

1. J. J. Trentin, Y. Yabe, G. Taylor, *Science* **137**, 835 (1962).
2. R. J. Huebner, *Perspect. Virol.* **5**, 147 (1967).
3. P. H. Gallimore and C. Paraskeva, *Cold Spring Harbor Symp. Quant. Biol.* **44**, 703 (1980); A. M. Lewis, Jr., and J. L. Cook, in *The Molecular Biology of Adenoviruses*, W. Doerfler, Ed. (Springer-Verlag, Heidelberg, in press).
4. J. L. Cook and A. M. Lewis, Jr., *Cancer Res.* **39**, 1455 (1979); A. M. Lewis, Jr., and J. L. Cook, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 2886 (1980); *Cancer Res.* **42**, 939 (1982).
5. J. L. Cook, J. B. Hibbs, Jr., A. M. Lewis, Jr., *Int. J. Cancer* **30**, 795 (1982).
6. J. L. Cook, C. H. Kirkpatrick, A. S. Rabson, A. M. Lewis, Jr., *Cancer Res.* **39**, 4949 (1979).
7. J. L. Cook, J. Hauser, C. T. Patch, A. M. Lewis, Jr., A. S. Levine, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 5995 (1983).
8. A. M. Lewis, Jr., and W. P. Rowe, *J. Virol.* **7**, 189 (1971).
9. J. L. Cook, J. B. Hibbs, Jr., A. M. Lewis, Jr., *Proc. Natl. Acad. Sci. U.S.A.* **77**, 6773 (1980).
10. L. A. Feldman and F. Rapp, *Proc. Soc. Exp. Biol. Med.* **122**, 243 (1966).
11. L. Glimcher, F. W. Shen, H. Cantor, *J. Exp. Med.* **145**, 1 (1977); C. W. Reynolds, R. Rees, T. Timonen, R. B. Herberman, in *NK Cells and Other Natural Effector Cells*, R. B. Herberman, Ed. (Academic Press, New York, 1982), pp. 17–23.
12. K. Raska, Jr., and P. H. Gallimore, *Virology* **123**, 8 (1982).
13. R. Bernards *et al.*, *Nature (London)* **305**, 776 (1983).
14. D. S. Nelson, M. Nelson, E. Farram, Y. Inoue, *Aust. J. Exp. Biol. Med. Sci.* **59**, 229 (1981); M. J. Droller, M. U. Schneider, P. Perlmann, *Cell. Immunol.* **39**, 165 (1978).
15. J. H. Pope and W. P. Rowe, *J. Exp. Med.* **120**, 121 (1964).
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Immunologic Inhibition of Ultraviolet Radiation–Induced Tumor Suppressor Cell Activity

Abstract. *Long-term exposure of C3H mice to ultraviolet radiation resulted in the formation of suppressor T cells that recognize ultraviolet radiation–induced regressor skin cancers as a class before the appearance of overt tumors. Administration of monoclonal antibodies to the product of the I-J^k subregion of the major histocompatibility complex or low doses of cyclophosphamide in vivo inhibited the development or activity of these cells. This activity of the monoclonal antibody was eliminated by adsorption on B10.BR (I-J^k) but not B10.D2 (I-J^d) splenocytes. These findings provide evidence that elements expressing the I-J determinant are important in regulating the host response prior to the overt development of ultraviolet radiation–induced skin cancers and suggest novel therapeutic approaches to malignancies or other diseases involving suppressor T cells in their pathogenesis.*

Long-term exposure of C3H mice to ultraviolet (UV) radiation results in the appearance of cutaneous fibrosarcomas and squamous cell carcinomas, most of which regress on transplantation to naïve syngeneic recipients (*1*). This tumor system has several unusual characteristics, among which is the appearance of T suppressor (Ts) cells that abrogate T cell–mediated immune rejection of most UV radiation–induced regressor tumors (*2–4*). These Ts cells appear before the development of overt tumors and have been shown to be crucial to the development and growth of UV radiation–induced skin tumors (*4*). Thus, this system offers the opportunity to study the influence of immunotherapeutic strategies designed to eliminate or modify Ts cells early in the process of carcinogenesis.

In other tumor systems, interference with Ts cell generation or function by various modalities has been shown to result in retarded primary tumor growth

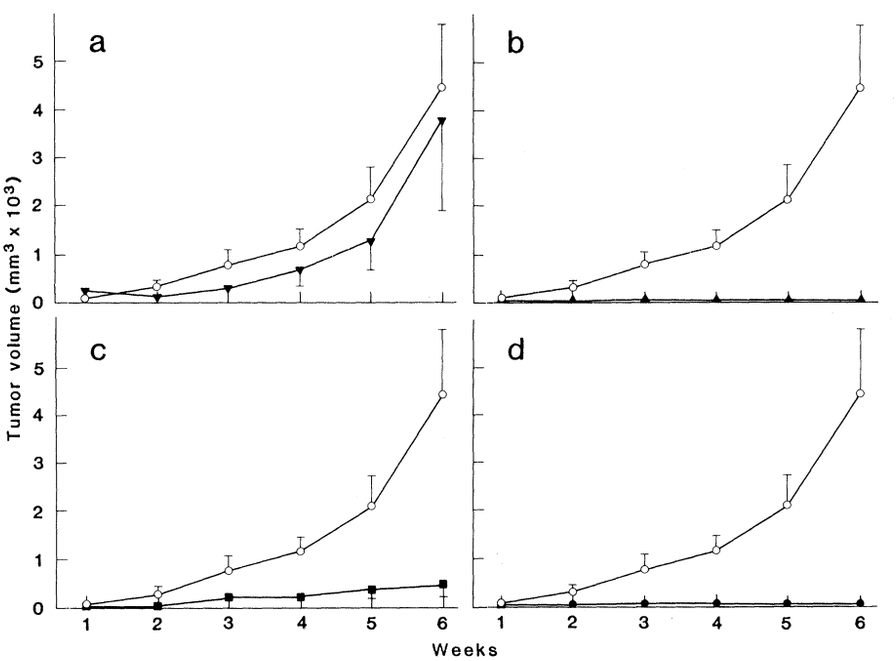

Fig. 1. Results of experiment 1. Eight mice in each group received 10^8 spleen cells intravenously from each category of donor and 2×10^6 cells of UV radiation–induced regressor tumor 2240 subcutaneously. Tumor volume was scored weekly by measuring three dimensions. Mean tumor size in animals that received cells from mice treated with UV radiation alone (positive control) (○) is compared with that in the other groups. (a) Ultraviolet radiation plus 0.2 ml of medium given intraperitoneally twice weekly (▼). (b) Ultraviolet radiation plus 500 ng of monoclonal antibody to I-J^k (WF8.C12.8) given intraperitoneally twice weekly (▲). (c) Ultraviolet radiation plus 20 mg of CY per kilogram of body weight given intraperitoneally twice weekly (■). (d) No treatment of donors (negative control) (●). Tumors regressed with time in some individual animals even in groups in which the mean tumor volume increased progressively. At 6 weeks the percentage of tumor-bearing animals was 75.0 percent (○), 75.0 percent (▼), 0 percent (▲), 25.0 percent (■), and 12.5 percent (●) (N = 8 in all groups). Error bars are ± standard error of the mean (S.E.M.). At 6 weeks there was no significant difference between (○) and (▼) groups (P = 0.360 by rank-sum method). Other groups compared to composite of (○) and (▼) groups as: P = 0.002 (▲), P = 0.012 (■), and P = 0.002 (●). Because of multiple comparisons, the 95 percent significance level for these is P = 0.017.

(*5*) and successful adoptive transfer of tumor immunity (*6, 7*). Determinants encoded by the I-J subregion of the murine H-2 major histocompatibility complex are found on Ts cells (*8*) and Ts cell derived suppressor factors (*9, 10*). The location of the I-J gene is uncertain (*11*), and it has been speculated that the I-J structure may represent a product of the I-A or I-E genes (*12*). Administration of antisera or monoclonal antibody to I-J in vivo has been shown to inhibit tumor growth in primary tumor–bearing mice (*13, 14*). In addition, a set of I-J bearing antigen-presenting cells (APC's) required for effector-suppressor cell activation is present in the spleen (*15*) and skin (*16*) of mice. This cell presents an additional locus at which antibodies to the I-J determinant may exert their effects. Furthermore, this cell has been shown to resist UV radiation in the skin (*16*) and thus may be expected to contribute to the generation of UV radiation–induced tumor-specific Ts cells. In earlier studies, exposure to UV radiation resulted in a systemic loss of functional I-A bearing APC activity (*17*), and we speculated that antigens that arise as a consequence of UV radiation exposure are thus presented selectively by the I-J bearing APC's leading preferentially to Ts cell activity (*16*).

To elucidate the manner in which antibody to the I-J determinant affects the immune response, we studied the effect of administration of monoclonal antibody to I-J^k (*18*) on the generation of Ts cells that arise as a consequence of UV radiation exposure before overt tumor appearance. In addition, because the splenic I-J bearing APC's have been shown to be cyclophosphamide (CY) sensitive (*15*), we also studied the effect of CY on Ts cell generation.

The shaved dorsal surfaces of C3H mice were exposed to 39.6 kJ/m^2 of UV radiation from a bank of FS-40 sunlamps on Mondays and Fridays for 12 weeks. During the period of irradiation, each animal in one group received 500 ng of antibody to I-J^k (WF8.C12.8; protein A purified from supernatant); each animal in another group received 20 mg of CY per kilogram of body weight; and each in another group received only medium (Hanks balanced salt solution containing 0.1 percent fetal calf serum) on Tuesdays and Fridays. Negative control mice did not receive UV radiation. One week after the last irradiation, spleens from all these mice were removed and 10^8 viable nucleated splenocytes were transferred intravenously to each of several naïve syngeneic mice. The recipients were then inoculated subcutaneously with 2×10^6 or 1.5×10^6 2240 UV radiation–induced regressor tumor cells. Growth of this tumor reflects the activity of Ts cells, and rejection of the tumor occurs when suppression is not evident. Tumor volume was determined from the measurement of three dimensions.

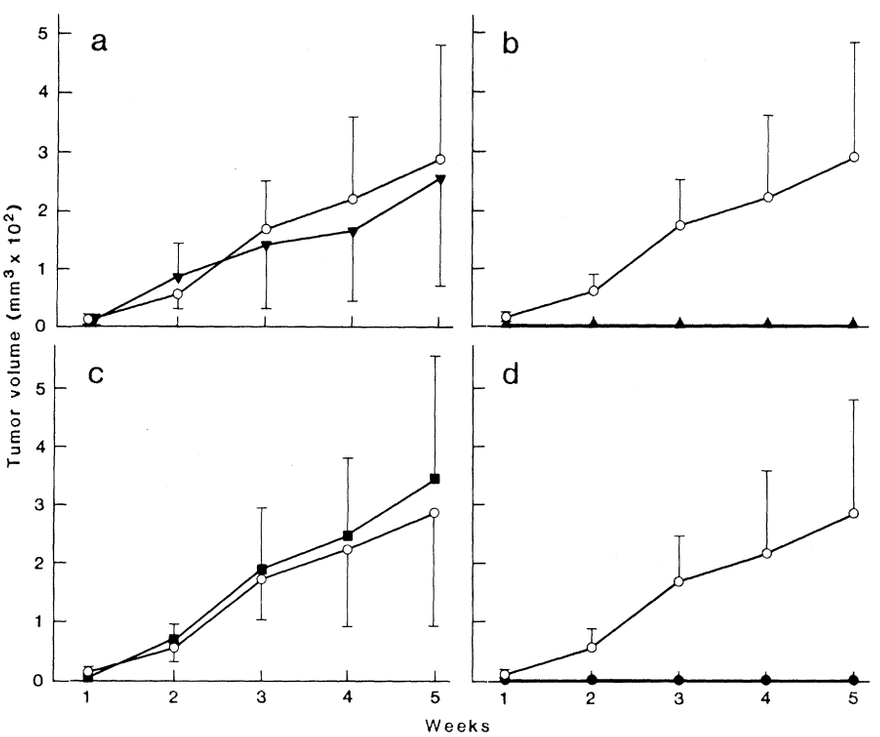

Fig. 2. Result of experiment 2. Eight mice in the positive control group and nine mice in each of the other groups received 10^8 spleen cells intravenously from each category of donor and 1.5×10^6 cells of UV radiation–induced regressor tumor 2240 subcutaneously. Tumor volume was scored weekly by measuring three dimensions. Mean tumor size in animals that received cells from mice treated with UV radiation alone (positive control) (○) is compared with that in the other groups. (a) Ultraviolet radiation plus 0.2 ml of medium given intraperitoneally twice weekly (▼). (b) Ultraviolet radiation plus 500 ng of monoclonal antibody to I-J^k absorbed on B10.D2 (I-J^d) splenocytes given intraperitoneally twice weekly (▲). (c) Ultraviolet radiation plus 500 ng of monoclonal antibody to I-J^k adsorbed on B10.BR (I-J^k) splenocytes given intraperitoneally twice weekly (■). (d) No treatment of donors (negative control) (●). Tumors regressed with time in some individual animals even in groups in which the mean tumor volume increased progressively. At 5 weeks the percentage of tumor-bearing animals was 62.5 percent (○), 44.4 percent (▼), 0 percent (▲), 44.4 percent (■), and 0 percent (●) [$N = 8$ in group (○), 9 in all other groups]. Error bars are ± S.E.M. At 5 weeks there was no significant difference between (○) and (▼) groups ($P = 0.226$ by rank-sum method). Other groups compared to composite of (○) and (▼) groups as: $P = 0.014$ (▲), $P = 0.543$ (■), and $P = 0.014$ (●). Because of multiple comparisons, the 95 percent significance level for these is $P = 0.017$.

The average tumor size in recipients of Ts cells from donors treated with UV radiation increased progressively over 6 weeks (positive control), and the average size of the tumors in this group was not significantly different from that of UV radiation–treated animals given medium alone (Fig. 1a). Administration of monoclonal anti–I-J^k antibody to UV radiation–treated donors during the irradiation period significantly inhibited the development of Ts cell activity (Fig. 1b). Similarly, administration of CY during the irradiation period also significantly inhibited the development of Ts cell activity although to a lesser degree than the antibody to I-J^k (Fig. 1c). The lower activity of CY compared to antibody was confirmed in an additional experiment (not shown). Cells from naïve donors did not confer susceptibility to the growth of UV radiation–induced regressor tumors (Fig. 1d).

Specificity of the antibody to I-J^k was shown by loss of activity caused by adsorption on B10.BR (I-J^k) but not B10.D2 (I-J^d) splenocytes (12 μg of antibody was adsorbed on 10^9 cells for 2 hours on ice) (Fig. 2). The UV radiation–treated donors showed Ts cell activity that was statistically similar to that of UV radiation–treated animals given medium alone (Fig. 2a). Administration of antibody to I-J^k adsorbed on B10.D2 (I-J^d) splenocytes to UV radiation–treated donors during irradiation significantly inhibited the development of Ts cell activity (Fig. 2b). However, administration of antibody to I-J^k adsorbed on B10.BR (I-J^k) splenocytes to UV radiation–treated donors did not lead to a statistically

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.

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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).
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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*).

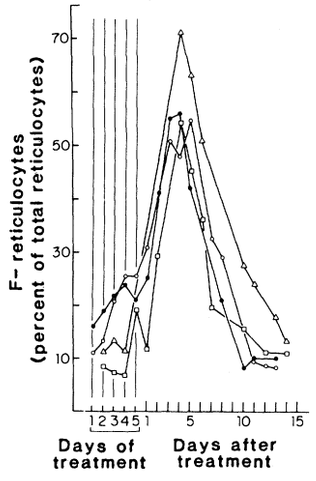

Fig. 1. Stimulation of F-reticulocyte production in three baboons treated with Ara-C (one animal was treated twice). Note the sharp increase in F reticulocytes, reaching a peak 4 to 5 days after the end of treatment.

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