Virus-Induced Transplantation Immunity to Human Adenovirus Type 12 Tumors of the Hamster and Mouse.* (31009)

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Tumor transplantation immunity in completely isologous systems was originally demonstrated in methylcholanthrene-induced sarcomas(1), and has since been demonstrated in tumors induced by other chemicals and by several viruses, including polyoma virus (2,3,4,5), SV40(3,4,5), Gross leukemia virus (6), Friend virus(7,8), Shope papilloma virus (9), Moloney virus(10,11), the Graffi virus (12), and the mammary tumor agent of Bittner(13,14).

For the virus tumors, transplantation immunity may be induced by preimmunization of adults with either live virus or with viable but histo-incompatible tumor cells induced by the same virus in a different strain or species of animal, or with isologous tumor cells rendered non-viable by preirradiation. The extent of tumor immunity is relative and is usually limited to one or two logs of tumor cell inoculum, *i.e.*, requires 10 to 100 times as many tumor cells to achieve the same level of transplantability in the "immune" animals as in the isologous controls.

The tumor immunity for methylcholanthrene sarcomas has been shown to be based on a new cellular antigen present in the tumor tissue but not in the normal tissues of the isologous strain of origin, *i.e.*, a cancer specific antigen(14a).

In the case of the tumors induced by polyoma virus, evidence has been presented indicating that the tumor immunity is based on a new cellular antigen induced by the virus, rather than on virion antigen(15).

With the demonstration of the oncogenic activity of human adenovirus type 12 in hamsters(16) and mice(17), it became of interest to determine whether resistance to transplantation of these tumors could be induced by preimmunization of adult hamsters or mice with live virus. Preliminary studies indicated that both hamsters and mice could be immunized against transplantation of tumors induced by this virus(18). These studies were extended and are reported herein in greater detail.

Materials and methods. The Syrian hamsters and inbred C3Hf/Gs mice used in these experiments were bred in this laboratory from breeding stock obtained originally from the National Institutes of Health and from Dr. Ludwik Gross, respectively. Human adenovirus type 12, prototype strain Huie, was obtained originally from the American Type Culture Collection and was propagated in our laboratory in HeLa or KB cells. The tumors used were induced by injection of this virus into newborn hamsters and C3Hf/Gs mice, and maintained by serial trocar-transplantation in adult hamsters and C3Hf/Gs mice respectively. The mouse tumors used were in the 3rd and 4th transplant generations. The hamster tumors used were in the 16th, 17th, and 18th transplant generations.

Adult male hamsters and both male and female mice (beyond the age of susceptibility to tumor induction) were "immunized" by 3 or 4 subcutaneous or intraperitoneal injections of 0.5 ml of undiluted adenovirus type 12 (A-12) at intervals of from 5 to 30 days. The virus titer was $10^{3.5}$ $TCID_{50}$/ml as determined by 5-day reading of HeLa tubes for cytopathic effect. (Titers determined by infectivity for human embryonic kidney cells are approximately 4 logs higher.) Within 2 to 15 days after the last injection, equal numbers of immunized and non-immunized animals were injected with serial 10-fold dilutions of A-12 tumor cells of their own species (hamster tumor into hamster, mouse tumor into mouse).

Tumor cell suspensions were prepared by mincing the tumor finely with scissors, aspirating in and out of a 10 ml syringe about 4 times, passing through a 50 mesh stainless steel screen mounted in a Swinny filter on a

* This investigation was supported by USPHS Grants CA-06941 and K6-CA-14219 from Nat. Cancer Inst.

TABLE I. Transplantation of Adeno-12-Induced Hamster Tumor Tissue in Adeno-12 Immune and in Control Male Hamsters.

Tumor inoculum	A-12 immune				Non-immune controls					
	Takes/total	% Takes	Avg latent period in days	Total No. regressions	Takes/total	% Takes	Avg latent period in days	Total No. regressions	No. days palpable	% Takes less regressions
Trocar	22/33	67	24	0	30/35	86	21	1	27	83
1×10^9	17/20	85	19	0	20/20	100	19	5	7, 15, 15, 21, 22	75
1×10^8	15/35	43	26	0	23/36	64	25	3	18, 28, 31	56
1×10^7	6/36	17	38	0	12/36	33	44	0		33
1×10^6	0/36	0			2/34	6	25	0		6
1×10^5	0/25	0			0/23	0				0
1×10^4	0/5	0			0/8	0				0
1×10^3	0/8	0			0/8	0				0

10 ml luer-lock syringe and flushing the screen with approximately an equal volume of Gey's solution (containing 1000 units of penicillin G and streptomycin sulfate equivalent to 1 mg of streptomycin base per ml). The mixture was passed again through a fresh 50 mesh screen into a large centrifuge tube, allowed to stand in the refrigerator for approximately 20 minutes, then filtered through a double layer of gauze. The viable (unstained) cell count of the filtrate was determined using a white blood cell diluting pipette, 0.5% nigrosin in Ringer's solution as diluting fluid, and counting in a hemocytometer.

The unstained viable cells usually constituted 80 to 90% of the total. By further dilution with Gey's solution, the cell concentration was adjusted to contain 1×10^9 viable cells per 0.5 ml (inoculum size). From this, 10-fold serial dilutions were prepared. Tumor cell suspensions (0.5 ml) were injected subcutaneously in the dorsal region of equal numbers of A-12 "immune" and non-immune control animals of the same species, sex, and age. Some groups received trocar transplants of pieces of tumor of approximately 0.7×0.7 mm. The transplantation site was inspected by palpation twice a week for tumor growth.

Results. In the hamster system (Table I) the "immune" animals gave a slightly lower percentage of tumor takes, which difference was easily overcome by a 10-fold increase in tumor cell inoculum. The magnitude of the difference was reduced by 9 spontaneous late regressions of tumors that initially grew in the non-immunized control animals inoculated with larger tumor cell doses. No such regressions were observed in the virus immunized group. Whether these regressions represent a basic histoincompatibility of both normal and tumor tissues among different individuals of these non-inbred hamsters or immunization by transplanted tumor specific antigens, or both, cannot be concluded with certainty. The fact that no such spontaneous regressions were observed in the virus immunized group suggests that they were not the result of basic tissue incompatibility, which should apply in both groups. The spontaneous regressions following larger tumor cell inocula in hamsters not preimmunized by virus may there-

TABLE II. Transplantation of Adeno-12-Induced Mouse Tumor Tissue in Adeno-12 Immune and in Control Mice.

	A-12 immune				Non-immune controls			
Tumor inoculum	Takes/total	% Takes	Avg latent period in days	Total No. regressions	Takes/total	% Takes	Avg latent period in days	Total No. regressions
Trocar	8/12	67	12	0	12/12	100	14	0
1×10^9	8/11	73	15	0	11/11	100	15	0
1×10^8	4/20	20	39	0	13/22	59	29	0
1×10^7	3/21	14	35	0	10/19	53	21	0
1×10^6	2/22	9	38	0	9/21	43	38	0
1×10^5	0/19	0			3/13	23	75	0
1×10^4	0/7	0			2/6	33	80	0
1×10^3	0/7	0			0/8	0		
1×10^2	0/4	0			0/7	0		

fore be the result of strong intrinsic tumor antigenicity.

Fortunately the question of normal tissue antigen incompatibility is ruled out in the histoisogenic mouse system (Table 2). In the non-immunized control mice, tumor takes were obtained with smaller tumor cell doses than in the hamster system, and a greater relative resistance on the part of virus preimmunized mice is evident, requiring as much as 2 or 3 or even 4 logs more cells (depending on the cell dose administered to the controls) to establish the same percentage of takes as in the non-immunized controls. The high cell dose levels required to achieve high percentages of tumor takes even in non-immunized animals is of interest. Whereas some tumor takes were obtained in control mice with as little as 10^4 cells, it required 10^9 cells to obtain 100% takes. This suggests that a significant degree of tumor immunity is operative even in the absence of viral preimmunization.

Discussion. The results indicate that tumors induced by human adenovirus type 12 in hamsters and mice are similar to those induced by several other animal viruses, in that virus preimmunized hosts develop a resistance to transplantation of the tumor, which immunity can usually be overcome by increasing the dose of tumor cells inoculated. The demonstration of tumor immunity raises the question of the nature of the antigen(s) responsible for such immunity. Infectious virus has not yet been recovered from the tumors induced by adenovirus type 12, in spite of extensive attempts in many laboratories(16, 19,20). The type specific complement fixing adenovirus antigen (C of Pereira or E of Ginsberg) has been found in tumors induced by adeno-12(21,22). However, in greater abundance in such tumors is a new non-virion "tumor" antigen which can be demonstrated by complement fixation(23,24), immunofluorescence(25,26) and immunodiffusion (22). This "tumor" antigen is identical to an "early" antigen appearing before any viral structural antigen can be demonstrated in cells infected *in vitro*(22,23,26). In a wide variety of cell types (human, monkey, hamster, mouse, rabbit, rat, chick) inoculated with A-12 *in vitro*, the early "tumor" antigen appeared without reference to whether or not the cells underwent complete cytolytic infection, whether or not viral structural antigen was subsequently demonstrable, and whether or not malignant transformation was induced (26). Some A-12 transformed cell lines (hamster, rabbit) retained the early antigen, while in others (rat) it was not demonstrable except after "superinfection." In addition to the above two antigens, immunodiffusion has revealed at least a third new (D) antigen in the A-12 hamster tumor(22).

Which of these new antigens in the tumor cell, if any, is responsible in whole or in part for the transplantation immunity reported herein is not at this time known. One might assume that such tumor antigenicity is due to virion antigen contained in the tumor. Yet evidence has been published indicating that in the case of polyoma tumors the tumor antigenicity is more likely due to new cellular antigen(s) induced by the virus rather than to virion antigen(15). Thus, the relative resistance of the adult as compared to the new-

born, is presumed to be based on the recognition and rejection, by the immunologically competent adult, of induced tumor cells containing new cellular antigen(s). This interpretation may well apply to the tumors induced by adenovirus type 12, which like polyoma virus induces tumors only in newborn animals, not in immunologically competent adults. However, since detection of the above 3 new antigens of the A-12 tumor, both virion and non-virion, is based on the use of serum of tumor bearing animals, and since transplantation immunity to solid tumors is more often based on cell mediated rather than serum mediated immunity, it is possible that the transplantation antigenicity of the A-12 tumors may be unrelated to any of the above 3 antigens.

Summary. Adult Syrian hamsters and C3Hf/Gs mice, beyond the age of susceptibility to tumor induction, were immunized by 3 or 4 injections of human adenovirus type 12. These animals, together with equal numbers of non-immunized controls of the same species, sex, and age, were inoculated with 10-fold serial dilutions of tumor cells induced by adenovirus type 12 in newborn animals of the same species and strain. In both the hamster system and the mouse system preimmunization with virus increased the resistance to subsequent transplants of A-12 tumor cells. The resistance was relative rather than absolute. In the hamster it was overcome by a 10-fold increase in tumor cell inoculum. In the mouse system, an inoculum of 100 to 1000 or more times as many tumor cells was required to overcome the induced resistance. The hamster and mouse tumor cells used have previously been shown to be free of infectious adenovirus. Tumors induced by this human virus in these two species of rodents appear to behave similarly to tumors induced by several animal viruses in that they contain a new virus-induced transplantation antigenicity.

The authors wish to thank Dr. G. L. Van Hoosier, Jr., and Miss Carolyn Gist for technical assistance.

1. Foley, E. J., Cancer Res., 1953, v13, 835.
2. Sjögren, H. O., Hellström, I., Klein, G., Exp. Cell Res., 1961, v23, 204.
3. Habel, K., Eddy, B. E., Proc. Soc. Exp. Biol. and Med., 1963, v113, 1.
4. Koch, M. A., Sabin, A. B., ibid., 1963, v113, 4.
5. Defendi, V., ibid., 1963, v113, 12.
6. Klein, G., Sjögren, H. O., Klein, E., Cancer Res., 1962, v22, 955.
7. Old, L. J., Boyse, E. A., Lilly, F., ibid., 1963, v23, 1063.
8. Steeves, R. A., Axelrad, A., Proc. Am. Assn. Cancer Res., 1965, v6, 61.
9. Evans, C. A., Gorman, L. R., Ito, Y., Weiser, R. S., J. Nat. Cancer Inst., 1962, v29, 277.
10. Sachs, L., ibid., 1962, v29, 759.
11. Glynn, J. P., Bianco, A. R., Goldin, A., Cancer Res., 1964, v24, 502.
12. Pasternak, G., Graffi, A., Brit. J. Cancer, 1963, v17, 532.
13. Weiss, D. W., Faulkin, L. J., Jr., DeOme, K. B., Cancer Res., 1964, v24, 732.
14. Morton, D. L., Proc. Am. Assn. Cancer Res., 1964, v5, 46.
14a. Klein, G., Sjögren, H. O., Klein, E., Hellström, K. E., Cancer Res., 1960, v20, 1561.
15. Habel, K., J. Exp. Med., 1962, v115, 181.
16. Trentin, J. J., Yabe, Y., Taylor, G., Science, 1962, v137, 835.
17. Yabe, Y., Samper, L., Bryan, E., Taylor, G., Trentin, J. J., ibid., 1964, v143, 46.
18. Trentin, J. J., Bryan, E., Proc. Am. Assn. Cancer Res., 1964, v5, 64.
19. Huebner, R. J., Rowe, W. P., Turner, H. C., Lane, W. T., Proc. Nat. Acad. Sci., 1963, v50, 379.
20. Kitamura, I., Van Hoosier, G., Jr., Samper, L., Taylor, G., Trentin, J. J., Proc. Soc. Exp. Biol. and Med., 1964, v116, 563.
21. Huebner, R. J., Pereira, H. G., Allison, A. C., Hollinshead, A. C., Turner, H. C., Proc. Nat. Acad. Sci., 1964, v51, 432.
22. Berman, L. D., Rowe, W. P., J. Exp. Med., 1965, v121, 955.
23. Hoggan, M. D., Rowe, W. P., Black, P. H., Huebner, R. J., Proc. Nat. Acad. Sci., 1965, v53, 12.
24. Van Hoosier, G., Jr., Stinebaugh, S., Trentin, J. J., Fed. Proc., 1964, v23, No. 2, 130.
25. Pope, J. H., Rowe, W. P., J. Exp. Med., 1964, v120, 577.
26. Levinthal, J. D., Ahmad-Zadeh, C., Van Hoosier, G., Jr., Trentin, J. J., Proc. Soc. Exp. Biol. and Med., 1966, v121, 405.

Received December 20, 1965. P.S.E.B.M., 1966, v121.