ON THE NATURE OF TRANSPLANTATION IMMUNITY IN THE ADENOVIRUS TUMOR SYSTEM*

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During the course of experimental viral oncogenesis, a number of tumor cell components have been found to elicit antibody responses in the host animal, thus being recognised as foreign. These antigenic materials have been shown to be specific for each particular oncogenic virus, even when a particular virus is known to transform cells of different species. A variety of antigens may be detected in tumors produced by the DNA viruses of cubic symmetry: *a*) complete infectious virus particles produced in polyoma mouse tumors (1), rabbit papillomata (2), and in small quantities in primary SV40 hamster tumors (3); *b*) structural virion subunits produced in hamster tumors induced by adenovirus types 12, 18, and 31 (4, 5); *c*) nonstructural "T" or "neoantigens", probably coded by the viral genomes, which also appear during early phases of the lytic cycle, and are found in the adenovirus (5, 6), SV40 (6, 7), and polyoma systems (8); and *d*) nonstructural cellular surface antigens, possibly coded by the viral genome, normally stimulating primarily a cellular antibody response, and appearing in the polyoma (9, 10), SV40 (11, 12, 13), and adenovirus tumor systems (14, 15). Although the relationship between these classes of antigens and their possible role in oncogenesis has been studied in a number of previous investigations (15, 16, 17), many points have yet to be resolved. Among these are the circumstances under which the host-antibody response can be deviated to respond predominantly to any particular class of antigen, the precise nature of the nonstructural antigens, and the possible influence of antibody response to each class of antigen upon response to other classes of antigens and resistance to tumor growth.

Although the adenovirus tumor system has been most extensively studied from the standpoint of the humoral antibody response to the structural virion and T antigens (4, 5, 18, 19), there has been relatively little work reported on the transplantation antigen(s) (14, 15). More information on the latter would complement the studies on virion and T antigens, the extensive investigations

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of adenovirus morphology, and the biochemistry of the lytic cycle. The present report describes experiments on the adenovirus transplantation tumor antigen(s) in circumstances by which immunity can be produced, on the relationship between immunity to other antigens and the transplantation antigen(s), and on the specificity and strength of the antigen(s).

Materials and Methods

Experimental Animals.—These consisted of randomly bred golden Syrian hamsters and inbred CBA strain mice from the colonies of the National Institute for Medical Research, London, England. Young adult female animals 4–8 months old were used as routine for transplantations.

Viruses.—Strain 1131 of adenovirus type 12 was used throughout. This strain was originally isolated by Dr. M. S. Pereira from human material and has since been passed continuously in human embryonic kidney (HEK) cells. Inocula prepared from concentrating and disrupting infected cells at 4^+ cytopathogenicity routinely titered $10^7 -> 10^8$ $TCID_{50}$/1 ml on HEK monolayers. In addition, other viruses used included the prototype strains of adenovirus types 18, 5, and 7A; SV40; and the influenza strain A2/Taiwan/1/64 (used as allantoic fluid harvest). Virion subunits of adenovirus type 12 were prepared by stepwise elution on DEAE-cellulose columns as described previously (20). Doubly chromatographed preparations of antigens A (hexon) and C (fiber) were utilized (21).

Tumors.—Adenovirus type 12 tumors were obtained by subcutaneous inoculation of newborn CBA mice with 0.05 ml of virus inocula. Typical adenovirus type tumors first appeared after 2 to 3 months, and at 1 yr most inoculated animals developed tumors. The CBA/A1 tumor line with which most of these experiments were performed was derived from a tumor induced by Dr. R. Taylor in a neonatally thymectomized animal. Four other tumor lines, B1, C1, D1, and E1, were induced by the inoculation of nonthymectomized newborn CBAs. Hamster tumors induced by adenovirus type 12 (strain 1131), the Bryan strain of Rous sarcoma virus (RSV), and the Harvey strain of murine sarcoma virus (MSV) were also used.

Transplantation.—All tumor lines were maintained by serial subcutaneous implantation by trocar of minced tumor fragments. Tumor challenges to elicit transplantation resistance were carried out by intracerebral inoculation of trypsinized single cell suspensions in 0.02 ml volumes. Subcutaneous inoculations of tumor cell suspensions were also occasionally performed. At the 16th transplant passage, a large number of aliquots of a cell suspension of tumor A1 were frozen in dimethylsulfoxide (DMSO) into liquid nitrogen. Most subsequent challenges with this tumor were performed by inoculating individual samples of thawed cells.

Experiments were terminated as routine 1 month after the last animal appeared with tumor. Mean latent periods of tumor development were calculated by averaging the time of tumor appearance for all animals of a given experimental group, excluding animals that did not develop tumors.

Immunizations.—Experimental animals were inoculated with virus preparations subcutaneously or intraperitoneally. Some immunizations were carried out with 1:1 or 1:2 emulsions of virus in complete Freund's adjuvant, or with virus and adjuvant preparations separately administered subcutaneously into opposite flanks. Immunizations were also carried out with subthreshold doses of live isologous tumor cells and suspensions of ultraviolet-irradiated ($1.0–2.0 \times 10^7$ ergs of irradiation) or X-irradiated (3,600 R in a Cobalt 60 bomb) isologous tumor cells. Trocar implantation of heterologous hamster adenovirus type 12 tumors and hamster RSV and MSV tumors were also used. Immunization was also performed with subcellular fractions of isologous mouse tumors. Crude aqueous tumor extracts (20% v/v) were prepared by mincing and grinding viable tumor tissue in phosphate-buffered saline followed by freezing and thawing several times. Immunizing subcellular fractions consisted of supernatant

and resuspended, washed sediment after clarification of crude extracts at 2000 rpm for 1 hr. Other fractions consisted of resuspended, washed sediments obtained after centrifugation of the initial clarified extract at 20,000 rpm for 1 hr, and at 40,000 rpm for 16 hr. Top and bottom fractions of the supernatant material were also used.

Sera, Lymph Node and Spleen Cells.—Sera from tumor-bearing and immunized animals were collected by retroorbital puncture. Lymph node and spleen cells of immunized animals were collected by mincing nodes and spleens and washing tissue through a fine wire mesh.

Adoptive Transfers.—These were performed by incubating mixtures of tumor cells with lymph node cells, spleen cells, or serum from immunized animals at 37° for 1 hr before inoculating the mixtures intracerebrally or subcutaneously into experimental animals. Prior to incubation, sera were either heated at 56°C for 30 min or fortified with equal volumes of addi-

TABLE I

Incidence of Intracerebral and Subcutaneous Takes in CBA Mouse Adenovirus Tumor Line A1

Passage	Cell dose				
	Intracerebral Dosage				
	10^5	10^4	10^3	10^2	
8	2/2	2/2	2/2	0/2	
9		4/4	2/2	0/2	
11		8/8	3/3	2/4	
10		5/5	4/6	0/5	
12		4/4	6/6	1/4	
14		4/4	4/4	4/4	
16		5/5	5/5	3/5	
19		10/10	10/10	7/8	
	Subcutaneous Dosage				
	10^6	5×10^5	10^5	10^4	10^3
8	2/2		2/2	0/2	0/2
28		16/19	5/20	0/19	

tional fresh guinea pig complement. In the case of tumor-serum incubations, an extra dose of serum was administered intraperitoneally 1 wk after the initial inoculation.

Complement Fixation Tests.—These were performed by a dropping method in standard plexiglass hemagglutination trays. The method has been described previously (22). After overnight incubation at 4°C of antigen-antibody-complement mixtures, plates were brought to 37°C and the hemolytic system added.

Virus Neutralization Tests.—These were performed by incubation at 37°C of serum-virus mixtures at appropriate serum dilutions for 1 hr before addition to tubes of HeLa cell monolayers. The highest serum dilution showing clear inhibition of cytopathogenic effect (CPE) compared with virus controls was recorded as the neutralizing titer.

RESULTS

Stability of tumor lines. The A1 tumor line has been carried through 29 passages in CBA mice. Table I shows the results of cell titrations at various passages by the subcutaneous and intracerebral routes. The tumori-

genicity was quite stable throughout all passage levels tested. However, there was a greater than 100-fold difference in the sensitivity of intracerebrally inoculated mice over subcutaneously inoculated animals. To minimize variations due to possible heterogeneity in a large inoculum, the intracerebral route was chosen for challenge in most of the transplantation immunity experiments.

Fig. 1 shows a mouse exhibiting the typical signs of intracerebral pressure due to tumor growth. The animal is hunched and listless, the fur ruffled. Other animals occasionally showed progressive paralysis of the limbs, bulging of the cranial cavity, anterior orbital displacement, lateral rotation of normal head position, or hyperexcitability. The earliest onset of these symptoms occurred 3 wk after inoculation with 10^4

TABLE II

Incidence of Intracerebral Takes in CBA Mouse Adenovirus Tumor Line A1

DMSO frozen passage 16 cells stored in liquid N.

Days post freezing	Cell dose		
	10^4	10^3	10^2
37 UW*	5/5	4/4	3/5
37 W‡	6/6	4/5	2/5
45 UW	5/5	5/5	4/5
116 UW	5/5	6/6	1/6
153 UW	5/5	5/5	4/6
235 UW	6/6	6/6	5/6
262 UW	5/5	7/7	0/7
272 UW	7/7	8/8	9/9
298 UW	7/7	6/6	1/6
345 UW		8/8	

* UW, unwashed cells.

‡ W, washed cells.

cells, and once symptoms appeared animals usually died within a few days. This provided a sharp, uniform end point for titrations. Prolonged survival or tumor regression was never observed once symptoms appeared. When 10^4 or 10^3 tumor cells were administered to unimmunized control animals, most tumors appeared in 30–50 days. However, the appearance of growing tumor was delayed in animals given 10^2 cells and in some immunized animals. Tumors in the latter groups of animals would occasionally take over 100 days to make their appearance. Fig. 2 shows the cross-section of the brain of an animal with symptoms similar to those shown in Fig. 1. The central area is replaced by a large tumor mass, which on higher magnification exhibited the characteristic small-cell adenovirus tumor type histology (23).

It was reasoned that a greater uniformity of tumor challenge could be achieved by continually using cells from the same tumor preparation. This was achieved by freezing multiple aliquots of a single tumor cell suspension in DMSO at a concentration of 5×10^5 cells/ml. Vials of cells were stored in liquid nitrogen and thawed rapidly before

use. Table II shows the viability of frozen stored tumor cells as a function of the time of storage. At the 37th day of storage, tumor cells, unwashed and washed free of the DMSO, were titrated. No significant difference in titer was found. Thereafter, all challenges were performed with unwashed cells. It is also apparent that the tumorigenicity of these cells was essentially unaltered after 345 days of storage.

Attempts to Demonstrate Immunity by Immunization with Virus.—Habel-Sjögren (9, 10) type experiments were performed by immunization with serial dilutions of adenovirus type 12. Results are shown in Table III. Mice immunized with 10^7 infectious units of virus were well protected against challenge with both dilutions of A1 tumor cells. Five of five tested sera from this group of mice also contained complement-fixing antibody against adeno-

TABLE III

Transplantation Immunity Induced by Varying Doses of Adenovirus 12

Immunizing dose*	Intracerebral challenge	Incidence of tumors, days after tumor challenge					
		20	30	40	50	60	80
10^7‡	Either 10^3 or 10^4 cells	0/11	0/11	0/11	0/11	0/11	0/11
10^5		0/12	0/12	2/12	4/12	6/12	9/12
10^3		0/11	5/11	9/11	11/11	11/11	11/11
10^1		0/12	2/12	11/12	12/12	12/12	12/12
None		0/10	2/10	7/10	10/10	10/10	10/10

Challenge: 10^3 or 10^4 A1 mouse tumor cells. Treatment: 100-fold serially diluted 1131 adenovirus 12. Initial titer: 10^7 $TCID_{50}$.

* $TCID_{50}$ in human embryonic kidney cells.

‡ Sera from five mice in this group 4 days prior to challenge had complement fixation (CF) titers of 1/10, 1/40(2), 1/80, and 1/160 against a crude adenovirus 12 preparation.

virus type 12. Immunization with a 100-fold diluted inoculum also gave protection that was manifested chiefly by a prolongation in the latent period. 10^{-4} and 10^{-6} dilutions of virus were nonimmunizing.

Since immunization was demonstrated with whole homologous virus, the effect of soluble antigens was next studied. Table IV shows that, although there was some effect when 10^3 cells were used for challenge, only whole mature virus protected upon challenge of 10^4 A1 cells. DEAE-cellulose–chromatographed structural antigens, and early harvests of infected HeLa cells containing little mature virus but abundant T antigen, were much less effective.

The specificity of immunization was also studied by immunizing with various viruses related or unrelated to adenovirus type 12. Table V shows that adenoviruses types 5 and 7, SV40, and influenza A2 did not induce resistance against tumor transplantation. In Experiment XXVIII, though no great difference in tumor incidence was found, transplantation immunity with adenovirus

12, and to a lesser extent with adenovirus type 18, could be detected through a prolongation of the mean latent period.

The specificity of tumor challenge and comparative antigenicity of tumors

TABLE IV

Effect of Immunization with Viral Antigens on Resistance to Adenovirus Mouse Tumor A1

Immunization	Intracerebral cell dose	
	10^4	10^3
None	5/5	3/6
Complete Ad. 12	0/4	0/4
Ad. 12 A antigen	3/4	0/4
" " C "	4/4	0/3
20 hr HeLa cell Ad. 12 harvest	4/4	0/2
44 hr HeLa cell Ad. 12 harvest	3/3	0/4
72 hr HeLa cell Ad. 12 harvest	0/4	0/4
None	5/5	4/6

TABLE V

Effect on Resistance to Mouse Adenovirus 12 Tumor Line by Prior Immunization with Various Viruses

Experiment	Immunization	Cell dose	Mean latent period
		10^2	*days*
XXVIII	None	9/9	55
	Ad. 12 (× 1)	5/7	66
	" (× 3)	5/8	79
	Ad. 18 (× 3)	7/7	63
	Ad. 5 (× 3)	7/7	54
		10^2 *or* 10^1	
XVIII	None	9/10	
	Ad. 12 (× 1)	3/10	
	Ad. 7 (× 1)	10/10	
	SV40 (× 1)	8/9	
	Influenza A2 (× 1)	8/9	

was studied by challenging adenovirus 12–immunized mice with five separate lines of CBA adenovirus 12–induced tumor. Table VI shows that all five tumor lines were inhibited by adenovirus type 12 immunization, and presumably each was carrying the transplantation antigen. However, each tumor exhibited its own distinctive growth rate and antigencity, the two factors varying inversely.

The time for maximum immunizing effect to occur was found by varying the interval between virus administration and tumor challenge. Table VII

shows that although there was some immunization after 1 wk, manifested by a delay in the mean latent period, it required at least 2 wk for the full immunizing effect to be achieved. Table VIII shows, however, that immunization with

TABLE VI

Resistance of Adenovirus 12 Immunized Mice to Five CBA Mouse Adenovirus Tumors

Tumor line	Transplant passage	Immunization	Intracerebral tumor cell dose	
			10^3	10^2
A1	19	Ad. 12	4/5* (75)‡	0/5 —
		None	5/5 (40)	5/5 (38)
B1	3	Ad. 12	2/5 (110)	0/5 —
		None	5/5 (50)	4/4 (63)
C1	3	Ad. 12	4/5 (98)	0/5 —
		None	5/5 (35)	4/5 (33)
D1	3	Ad. 12	0/5 —	0/3 —
		None	2/4 (60)	3/5 (70)
E1	3	Ad. 12	1/5 (50)	1/5 (80)
		None	5/5 (44)	5/5 (56)

* Incidence of tumor development.

‡ Mean latent period (days).

TABLE VII

Protection Against Tumor Challenge Following Varying Periods of Adenovirus Immunization

Weeks between immunization* and tumor challenge‡	Days after tumor challenge					
	20	30	40	50	60	80
4	0/4	0/4	0/4	0/4	0/4	0/4
3	0/4	0/4	0/4	0/4	0/4	1/4
2	0/4	0/4	0/4	0/4	0/4	0/4
1	0/4	0/4	0/4	1/4	3/4	4/4
Untreated control	0/5	4/5	5/5	5/5	5/5	5/5

* Immunization, 0.2 ml strain 1131 adenovirus 12 intraperitoneally.

‡ Challenge, 10^4 adenovirus 12 A1 mouse tumor cells intracerebrally.

emulsions of complete Freund's adjuvant and virus had less effect than immunization with virus alone. This is illustrated in Text-fig. 1 which shows that heated virus or virus administered with Freund's adjuvant, either in an emulsion or separately, does have an immunizing effect, although considerably less than that of infectious virus alone.

Adoptive and Passive Transfer.—To investigate the nature of the immune

response, mice were immunized with virus or tumor and, after a suitable period of immunization, spleen cells, lymph node cells, and serum were recovered and incubated in vitro for short periods of time with A1 tumor cells before

TABLE VIII

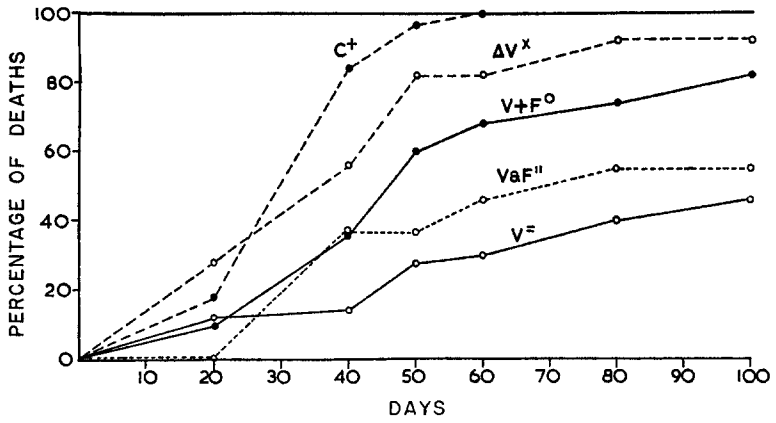
Effect of Freund's Adjuvant on Transplantion Immunity to Mouse Adenovirus Tumor Induced by Adenovirus Immunization

Immunization	Intracerebral tumor cell dose		
	10^4	10^3	10^2
V*	1/6	1/6	0/5
V + F‡	4/6	3/5	4/6
V & F§	3/5	4/6	5/7
Control	6/6	6/6	5/6

* Adenovirus 12 alone, one dose.

‡ Adenovirus 12; adjuvant emulsion, one dose.

§ Adenovirus 12 and adjuvant administered separately at different sites.



TEXT-FIG. 1. Effect of Freund's adjuvant on transplant immunity to mouse adenovirus tumor induced by adenovirus immunization. Percentage of deaths plotted against time after challenge with 10^3 and 10^4 intracerebral adenovirus mouse tumor cells. +, untreated controls; 29 animals. ×, immunized with heated (Δ) adenovirus 12 (56°C, 30 min); 11 animals. ○, immunized with adenovirus 12-adjuvant emulsion; 34 animals. ||, immunized with adenovirus 12 and adjuvant at separate sites; 11 animals. =, immunized with adenovirus 12; 37 animals.

intracerebral inoculation of the mixtures into recipient mice. Results of these experiments showed that immune cells of animals sensitized by adenovirus 12, or to a lesser extent with subthreshold doses of A1 tumor cells, were able, upon incubation with tumor cells, to limit subsequent tumor development in the intact animal. Lymph node cells were much more potent than spleen cells in this regard. Table IX, which demonstrates the effect of sensitized lymph node

cells on the A1 tumor line, shows agreement with the results on complete in vivo assay (to be described) in that lymph node cells of virus-immunized animals showed a much greater protective effect than cells of animals immunized with live homologous tumor cells. Also consistent with the previously described results was the fact that lymph node cells from animals immunized with virus plus Freund's adjuvant showed no demonstrable protective action. This was true even though animals given virus-adjuvant emulsions were actively im-

TABLE IX

Adoptive Transfer of Adenovirus 12 Transplantation Immunity by Sensitized Lymph Node Cells

Cell mixtures*	Immunization of donors	Days after tumor challenge**						
		20	30	40	50	60	70	80
10/1	Ad. 12‡	0/7	0/7	0/7	0/7	0/7	0/7	0/7
	Ad. 12 + Fr§	0/7	0/7	7/7	7/7	7/7	7/7	7/7
	Tumor‖	0/4	0/4	0/4	1/4	1/4	1/4	2/4
	Control	0/6	0/6	4/6	6/6	6/6	6/6	6/6
1/1	Ad. 12‡	0/8	0/8	0/8	0/8	0/8	0/8	0/8
	Ad. 12 + Fr§	0/8	0/8	7/8	7/8	7/8	7/8	7/8
	Tumor‖	0/8	0/8	2/8	7/8	7/8	8/8	8/8
	Control	0/5	0/5	3/5	5/5	5/5	5/5	5/5

* Ratio of lymph node cells to tumor cells.

‡ Two doses of subcutaneous (S.Q.) adenovirus 12. Serum titer, 1/160 versus adeno 12 viral antigen; negative versus adeno 12 T antigen. Neutraling antibody titer, 1/640.

§ Two doses of adenovirus 12 in complete Freund's adjuvant S.Q. Serum titer, 1/1280 versus adeno 12 viral antigen; negative versus adeno 12 T antigen. Neutraling antibody titer, 1/1280.

‖ Three subthreshold doses of adenovirus 12 mouse tumor cells S.Q. Serum titer, 1/10 versus adeno 12 viral antigen; negative versus adeno 12 T antigen. Neutraling antibody titer, 1/10.

** Challenge, 10^3 A1 frozen mouse tumor cells mixed with lymph node cells from immunized donors and inoculated intracerebrally.

munized against adenovirus 12, showing both complement-fixing and neutralizing antibody. Complement fixation tests also revealed the complete absence of demonstrable anti-T antibody in the sera of all experimental groups of animals regardless of whether lymph node cells showed protective action or not.

Table X shows the effect of repeated immunizing doses of virus upon the sensitization of lymph node cells. Sensitization of lymph node cells was not achieved unless three immunizing doses of virus were administered, in spite of the fact that one dose was sufficient to immunize in the complete in vivo experiments previously described.

Numerous tests were performed to investigate the effect of various sera

directly on tumor cells or via passive transfer. Sera used included those of donor animals listed in Table IX, plus a hyperimmune anti-adenovirus type 12 rabbit serum, and an adenovirus tumor-bearing rat serum that contained a high titer of anti-T antibody. In no case was there any unequivocal evidence of either cytotoxicity or enhancement. This included tumor-serum incubations done in the presence of added complement, and those done with heated serum in the absence of complement.

Attempts to Demonstrate Resistance by Immunization with Cells or Subcellular Fractions.—Table XI shows results of immunization with one dose of various preparations of whole isologous tumor cells or subcellular fractions. From these

TABLE X

Effect of Continued Antiviral Immunization on Development of Cellular Immunity to Adenovirus Mouse Tumor A1 by Adoptive Transfer

Experiment	Immunization	Days after challenge*						Mean latent period
		20	30	40	50	70	90	
								days
XXIV	Control	0/7	2/7	2/7	3/7	6/7	6/7	53
	Ad. 12 (× 1)	0/7	0/7	0/7	6/7	7/7	7/7	51
XIX	Control	0/6	4/6	4/6	6/6	6/6	6/6	37
	Ad. 12 (× 3)	0/7	0/7	0/7	0/7	0/7	0/7	—
XXVIII	Control	0/10	2/10	8/10	8/10	10/10	10/10	42
	Ad. 12 (× 1)	0/9	4/9	6/9	8/9	9/9	9/9	41
	Ad. 12 (× 3)	0/10	0/10	1/10	3/10	6/10	7/10	59

* 10/1 ratio of incubated lymph node cells to tumor cells given intracerebrally (10^4 lymph node cells/10^3 tumor cells).

results it can be seen that, although there was no essential difference in the incidence of tumors in immunized groups, there was some prolongation of the latent period in many of the immunized groups at the 10^4 and 10^3 cell dosages. However, at the 10^2 cell dosage, there was actual evidence of enhancement in practically all of the immunized groups as compared with the controls. In addition, the mean latent period of some of the immunized groups was quite prolonged, indicating that at least some of the enhanced tumors were appearing very late.

Since no immunity was demonstrated upon immunization with dead cells, subthreshold doses of live cells, or subcellular material, it was of interest to ascertain whether immunization could be accomplished by large doses of viable adenovirus tumor cells. It was also of interest to detect any cross-reaction between mouse and hamster adenovirus 12 transplant antigens, such as has been

TABLE XI

Effect of Immunization by Homologous Whole Cells and Subcellular Fractions on Transplantation Immunity to CBA A1 Mouse Adenovirus Tumor

Experiment	Immunizing agent	Intracerebral cell dose					
		10^4		10^3		10^2	
XX	Subthreshold dose (2×10^5 live tumor cells)	5/5*	(42)‡	3/3	(48)	3/3	(67)
	Heated tumor cells (56°C 30 min)	5/5	(37)	7/7	(40)	6/6	(48)
	Control (untreated)	5/5	(32)	6/6	(45)	1/6	(42)
II and XX	1.0–2.0 $\times 10^7$ ergs UV-irradiated (10^6 cells)	10/12	(40)	6/6	(44)	2/4	(48)
	Control (untreated)	9/9	(30)	6/6	(45)	1/6	(42)
XXXIV	X-irradiated tumor cells (10^6 cells) 3600 R Co^{60} irradiation	7/7	(38)	7/7	(75)	2/5	(88)
	Control (untreated)	7/7	(32)	6/6	(45)	1/6	(37)
XX and XXVII	Suspension of crude cell debris	10/12	(45)	11/11	(47)	6/12	(78)
	Control (untreated)	10/10	(31)	13/13	(41)	1/13	(42)
I, IV, and XX	Crude cell extracts	8/11	(66)	10/12	(59)	11/13	(53)
	Control (untreated)	9/9	(29)	9/9	(41)	3/10	(58)
XXVII	Various fractions of crude cell extracts obtained by differential centrifugation	28/30	(35)	28/29	(43)	15/29	(71)
	Control (untreated)	5/5	(30)	7/7	(36)	0/7	(—)

* Incidence of tumors.

‡ Mean latent period of tumor growth, days.

TABLE XII

Effect of Immunization by Various Hamster Tumors on Resistance of CBA Mice to A1 Adeno 12–Induced Tumor

Experiment	Immunizing tumor	Intracerebral cell dose A1 tumor		
		10^4	10^3	10^2
III	Hamster Ad. 12	7/8		
	Control	4/4		
XIII	Hamster Ad. 12	5/6	5/5	4/5
	Hamster RSV*	2/4	5/6	5/5
	Hamster MSV‡	5/5	6/6	5/5
	Control	5/5	5/6	3/5

* Bryan strain, Rous sarcoma virus.

‡ Harvey strain, murine sarcoma virus.

demonstrated between rat and mouse in the Rous system (24). Accordingly, CBA mice were implanted with minced fragments of various virus-induced hamster tumors, and subsequently challenged with the A1 tumor cells. Some of the hamster tumors grew to a size of approximately 1 cm before being rejected. The results are given in Table XII. No immunity was produced by heterotransplantation with the adenovirus 12 hamster tumors, or with the control Rous sarcoma or murine sarcoma virus hamster tumors.

DISCUSSION

The present findings verify the existence of a virus-induced, virus-specific transplantation antigen present in CBA mouse cells that have undergone neoplastic transformation by adenovirus type 12. In agreement with findings in the polyoma system (25, 26), the present results indicate that the antigen is probably a virus-induced cellular antigen, and not a structural virion antigen. However, the adenovirus antigen is comparable to a weak histocompatibility antigen, and in distinction to the polyoma and Rous sarcoma systems (27, 28), immunization could be more readily effected by virus than by homologous cellular material. Indeed, at lower doses of tumor challenge, immunization with one dose of cellular material appeared to lead to possible tumor enhancement rather than rejection.

The phenomenon of enhancement is well known in several experimental tumor systems (29, 30). It is conceivable that in most if not all natural and experimental tumor systems, the mechanisms leading to both rejection and enhancement come into play, and that a delicate balance between these two alternatives may sometimes exist. In the present series of experimental results, in cases where immunization was effected by homologous adenovirus type 12, rejection was clearly the ultimate result. This might be explained by the effect of large dosage, widespread dissemination, and persistence of antigen. If it is postulated that inoculated virus infects cells, producing the transplantation antigen which then immunizes, it is not difficult to conceive that inoculation of a high-titered virus preparation will infect many more cells than are present in a cellular inoculum, and will disseminate in the host animal to a much greater extent, infecting cells in remote parts of the body, and producing more extensive contact with the cells of the reticuloendothelial system.

Inoculation of cellular or subcellular material, on the other hand, provides antigen which is of a potentially smaller quantity than that generated by virus and which has more of a tendency to remain localized. Under these circumstances immunization is weaker and slower, and the stimulus to rejection not so pronounced. Factors leading to enhancement may become dominant under these conditions, especially where the inoculum of tumor is small and the initial antigenic stimulus even less.

The effect of Freund's adjuvant on the immune response was also quite

noteworthy. Although it might be postulated that adjuvant would localize the virus, thus preventing the massive initial infectious cycle described above, inoculations of virus and adjuvant at separate sites had the same effect in depressing the immune rejection response. It must be noted, however, that evidence of actual enhancement when virus was administered with adjuvant was never observed.

Administration of oncogenic viruses with adjuvant or adjuvant-like materials to newborn hamsters has resulted in increased incidence of tumor in the case of Rous sarcoma virus (31)[1], or decreased incidence of tumors in the case of adenovirus type 12.[2] Administration of Bjorklund type adjuvant-like extracts of homologous SV40 tumor in the latent period after neonatal inoculation of SV40 has been described as enhancing tumor development (32, 33), while administration of SV40 or adenovirus type 12 to neonatally SV 40-inoculated hamsters (34, 35, 36) has diminished the incidence of tumors. It appears that the effects of adjuvant on the immune tumor response in mice and hamsters is variable, and the effects of adjuvant in general on the cellular immune response of mice and hamsters is poorly understood. It is clear, however, that adjuvant has a depressive effect on the antitumor homograft type immune response in adult mice challenged with adenovirus-induced tumor. This occurs even though the adjuvant-virus–inoculated mice were actively immunized against virion structural antigens, producing even higher titers of complement-fixing and virus-neutralizing antibody than mice immunized with virus alone and showing transplantation immunity.

Antibody against the adenovirus type 12 T antigen was not found in mice, whether they showed immunity against transplantation or not. This, coupled with the findings that immunization with homologous tumor extracts and early infected human cellular extracts containing abundant T antigen failed to produce immunity, would indicate that the T antigen as well as virion antigens plays little or no role in transplantation immunity. It would also confirm the fact that T antigen is a weak antigen, and that continuous massive doses in the form of an actively growing tumor mass are needed to maintain immunization.[3]

The results of the adoptive and passive transfer experiments establish the fact that the bulk of transplantation immunity, at least in the system described above, is mainly mediated through immune lymphoid cells, and that serum plays no major role, either by cytotoxicity or enhancement. However, the techniques employed for demonstrating serum cytotoxicity or enhancement were not very sensitive, and minor degrees of reactivity might have been undetected. Lymph node cells were more effective than spleen cells in their antitumor

[1] Allison, A. C. and L. D. Berman. Unpublished observations.

[2] Berman, L. D., A. C. Allison, and H. G. Pereira. In preparation.

[3] Huebner, R. J., L. D. Berman, and W. T. Lane. Unpublished observations.

activity. Appreciable transplantation immunity was not achieved by immunization with heated virus, structural subvirion antigens, or unrelated viruses. This would imply that infectious homologous virus was necessary for immuization, and that the reaction was specific for adenovirus type 12 and the closely related type 18. Failure to achieve immunization with adenoviruses type 5 and 7 are contrary to results obtained elsewhere (37, 38) and might perhaps be explained by the difference in experimetal systems employed.

The present experiments show that it takes at least 2 wk for immunization to be fully developed, although some effects were noted after 1 wk. This is in line with the expected initiation of a primary homograft response (39). However, they fail to shed any light on the questions of possible cross-reaction between hamster and mouse transplant antigens.

The systems used in the present experiments offer many advantages in the study of transplantation immunity. Although intracerebral challenge precludes the advantage of being able to measure tumor size as an index of resistance, it offers the advantage of working with smaller tumor challenge doses and provides a relatively sharp end point for titrations. The banking of DMSO frozen tumor cells in liquid nitrogen combines the advantages of a perpetually uniform population of cells with great ease in handling, especially where tissue culture of transplant lines might be difficult to maintain. These experiments show that the A1 tumor cells could be stored frozen for 345 days without any loss of tumorigenicity, and that the DMSO medium in the quantities used is not injurious to the host animal or growing tumor cells.

SUMMARY

The existence of a virus-induced, virus-specific transplantation antigen in adenovirus 12–induced CBA mouse tumors was demonstrated. The antigen is virus-specific, but not related to structural virion or T antigens. It is a weak antigen, and required immunization with whole, infectious adenovirus 12 to produce considerable immunity. Comparable immunity could not be achieved with homologous cellular or subcellular materials, but some indication of enhancement was produced with low tumor dose. Immunization required at least 2 wk and was mediated by immune lymphoid cells. Serum of immunized animals showed no demonstrable cytotoxicity or enhancement. Animals immunized with virus and Freund's adjuvant showed diminished transplantation immunity, although these animals were actively immunized against adenovirus type 12 structural virion antigens.

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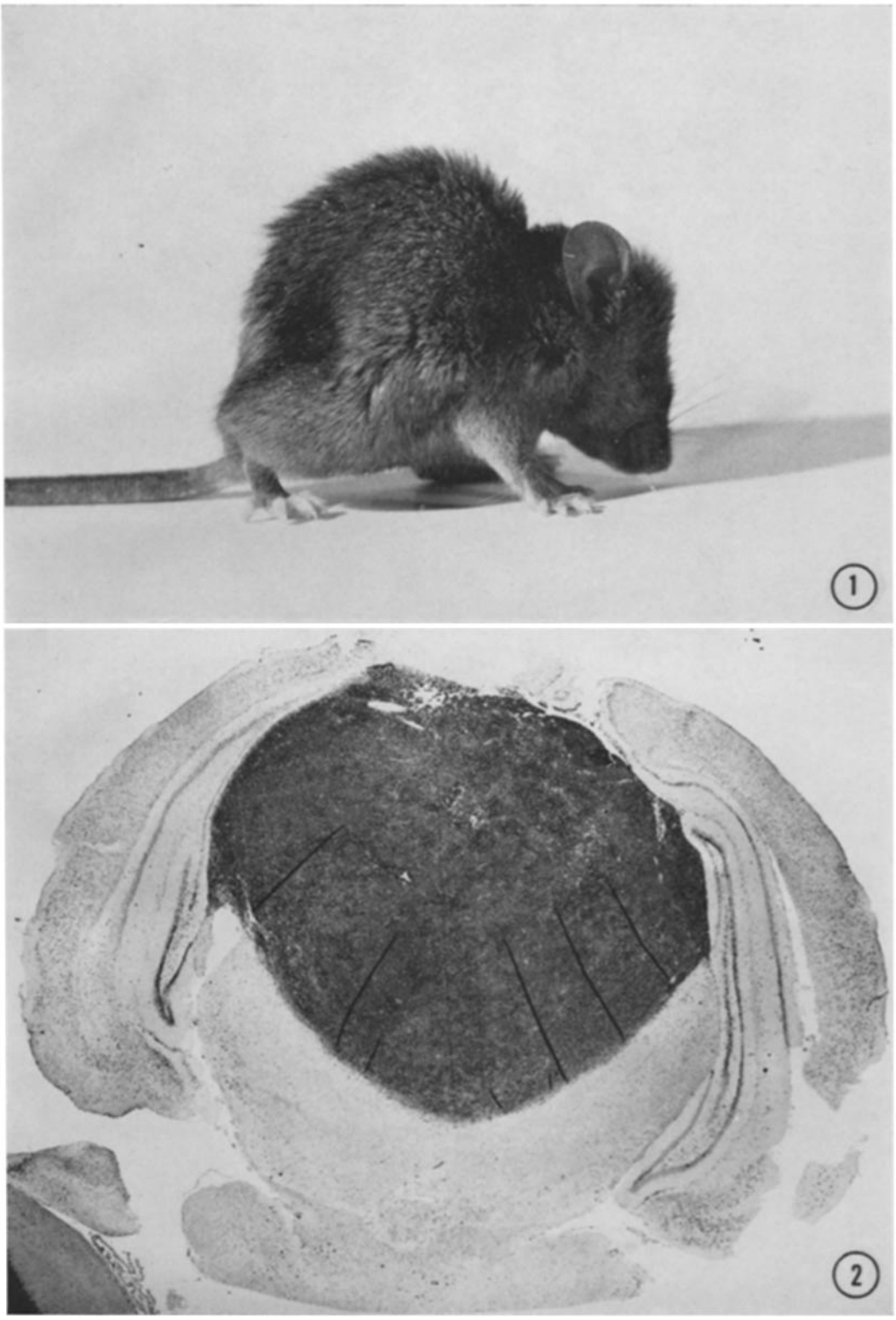
EXPLANATION OF PLATE 104

FIG. 1. Mouse with expanding intracranial tumor showing ruffling of fur and hunching of the back.

FIG. 2. Section of brain of mouse similar to that in Fig. 1. The entire central area has been replaced by an expanding tumor mass. × 15.



(Berman: Adenovirus tumor system)