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Received Augst 24, 1966. P.S.E.B.M., 1966, v123.

Prevention and Interruption of SV40 Induced Transplantation Immunity with Tumor Cell Extracts.* (31558)

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Adult hamsters immunized with papovavirus SV40 resist transplantation of cells transformed by the homologous virus(1-4). These rejection studies have suggested the presence of virus specific transplantation antigens which are presumably located at the surface of transformed cells. Recently, the presence of new surface antigens in cells transformed by SV40 has been demonstrated using immunofluorescence(5,6) and cytotoxic tests(7,6).

Animals inoculated with antigens in neonatal life often fail to respond immunologically in adult life to the same antigens(8). This report describes successful attempts to induce a state of immunological unresponsiveness in hamsters against SV40 transplantation antigens by inoculation of cell surface material from SV40-transformed cells. It also describes experiments which revealed that the protection induced by SV40 against cells transformed by the homologous virus could be reduced by treatment of the animals with cell extracts from the virus-transformed cells.

Materials and methods. Viruses. Simian virus 40 was the Baylor reference strain described previously(9). The virus was grown in African green monkey kidney (GMK) cultures maintained in lactalbumin hydrolysate medium (M-H) without serum.

Cell lines. The H-50 cells were derived from a hamster tumor induced by SV40(10). The cells synthesize the intranuclear SV40 tumor (T) antigen(11) and produce tumors in unvaccinated weanling hamsters but not in hamsters vaccinated with SV40(1). The cells also contain a specific surface (S) antigen which can be detected with sera from SV40 vaccinated hamsters resisting transplantation of cells transformed by SV40(5). Specific surface antigens have also been demonstrated by cytotoxic tests using heterologous rabbit serum(7). The cells are free of infectious SV40(12).

The cells were grown in Eagle's medium supplemented with 10% heat-inactivated calf serum, 100 units of penicillin, and 100 μg of streptomycin per ml.

Preparation of cell ghosts. Cell ghosts were prepared according to the method of Haughton(13). Briefly, 2.5×10^8 cells were dispersed with 0.2% trypsin and washed with tris buffered saline (TBS), pH 7.4. The cells were extracted successively with 100 ml of 3, 6, and 15% sodium chloride in the cold. Final wash was carried out in distilled water. No viable cells remained when tested by dye exclusion test(7). The ghost cell preparations were suspended in 4 ml of TBS.

Preparation of ruptured cells. H-50 cells were removed from 16-oz bottles with the help of a rubber policeman. The cells were washed with TBS, sedimented by low speed centrifugation and resuspended in 1 ml of TBS per 16-oz bottle. The cells were frozen and thawed 2 times and treated for 30 seconds in a Raytheon sonic oscillator at 12 kc per second.

Animal experiments. Two sets of experiments were carried out:

* This work was supported in part by USPHS research grant CA-04600 from Nat. Cancer Inst., research grant AI-05382 and training grant 5 T1 AI 74 from Nat. Inst. of Allergy & Infect. Dis., Nat. Inst. Health.

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TABLE I. Effect of Inoculation of Cell Ghosts into Newborn Hamsters on Subsequent Development of SV40-Induced Transplantation Immunity.

Group	Treatment as newborns	Treatment at 30 days	H-50 challenge No. of cells 10^5	10^4	10^3	10^2 Response*	TPD_{50} (No. of cells)
A	None	SV40 (3 inoculations, 1 wk apart)	1/4	1/4	0/4	0/4	>100,000
B	Cell ghosts (H-50)	"	3/4	1/4	3/4	1/4	3,160

* Numerator = No. of animals developing tumors; denominator = No. of animals inoculated.

H-50 = hamster cells transformed by SV40.

1) Newborn hamsters were divided into 2 groups. Group A animals served as controls and remained uninoculated. Animals in Group B were injected subcutaneously with 0.1 ml of ghosts prepared from H-50 cells. The hamsters were weaned 30 days after birth, when they were all vaccinated with SV40. The animals were given 3 injections of the virus at one-week intervals. One week after the last SV40 inoculation, the hamsters were challenged with varying doses of H-50 cells. The doses consisted of 10^5, 10^4, 10^3, and 10^2 H-50 cells. Four animals were used for each challenge dose. The animals were checked each week for 12 weeks for tumor development. The 50% tumor producing dose (TPD_{50}) was calculated using the Reed and Muench formula(14).

2) The second experiment was carried out in weanling hamsters. Hamsters were divided into 5 groups. Hamsters in groups A, B, and E were vaccinated twice with SV40 (2 weeks apart). Animals in group B also received 7 subcutaneous injections of ruptured H-50 cells. The first injection was given a day before the initial inoculation of SV40. The remaining 6 injections were administered on days 1, 3, 5, 7, 10, and 20 after the initial inoculation of SV40. Group C hamsters were treated like those of group B except that they were never vaccinated with SV40. Hamsters of group D served as controls and received 7 inoculations of Eagle's medium. Animals of group E received 5 subcutaneous injections of ruptured H-50 cells on days 20, 24, 27, 30, and 34 after the initial inoculation of SV40. Animals of all 5 groups were then challenged with 10^5, 10^4, 10^3, and 10^2 H-50 cells one week after the second inoculation of SV40. Eight animals were used per challenge dose. The challenge cells were inoculated at a site different from that used to inoculate the ruptured H-50 cells. The hamsters were checked for tumor development each week for 12 weeks.

Results. The effect of ghost cell preparations on the subsequent ability of hamsters to respond immunologically to vaccination by SV40 against SV40 transformed cells is shown in Table I. More than 100,000 cells were required to produce subcutaneous tumors in 50% of the animals previously vaccinated with SV40 (Group A). The hamsters of this group did not receive any treatment as newborns. This degree of resistance following inoculation of SV40 is in agreement with previous studies carried out in this(1,15) and other(2-4) laboratories. The TPD_{50} required to induce tumors in animals inoculated with H-50 ghost material in neonatal life and later vaccinated with SV40 dropped to 3,160 cells (Group B); this represents a greater than 97% decrease in protection when compared to the TPD_{50} of Group A.

The interruption of previously initiated SV40-induced transplantation immunity in weanling hamsters is shown in Table II. As in the previous experiment, the SV40-immunized hamsters of Group A resisted the challenge of 100,000 H-50 cells. The protection afforded by SV40 should be compared to Group D which received only Eagle's medium; in the latter group, the TPD_{50} was 1,740 H-50 cells. Hamsters that received 2 inoculations of SV40 but also received 7 inoculations of ruptured H-50 cells during SV40 immunization (Group B) failed to resist the H-50 cell transplant; only 6,920 cells were

TABLE II. Effect of Inoculation of Ruptured Transformed Cells on SV40-Induced Transplantation Immunity in Weanling Hamsters.

Group	Inoculation of SV40 (days)	Inoculation of ruptured cells (days)	H-50 challenge No. of cells 10^5	10^4	10^3	10^2 Response*	TPD_{50} (No. of cells)
A	2, 16	None	1/8	2/7	0/7	0/8	>100,000
B	2, 16	1, 3, 5, 7, 9, 12, 22	1/3	3/7	2/6	2/8	6,920
C	None	"	6/7	2/6	1/2	0/1	17,000
D	"	None†	4/8	7/8	2/2	2/8	1,740
E	2, 16	22, 26, 29, 32, 36	0/2	3/8	0/5	0/4	46,800

* Numerator = No. of animals developing tumors; denominator = No. of animals inoculated.
† Animals inoculated with Eagle's medium on days 1, 3, 5, 7, 9, 12, and 22.

required to produce tumors in 50% of the animals. This is a significant reduction in protection when compared to the TPD_{50} of Group A (> 100,000 cells). These results indicate that the ruptured transformed cells, when given during SV40 immunization, can interrupt the establishment of virus-induced transplantation immunity. Inoculation of only ruptured H-50 cells (and no SV40) failed to induce significant protection (Group C) against H-50 cells; 17,000 cells were needed for the TPD_{50}. Once SV40 mediated immunity had been established, inoculation of ruptured cells had no significant effect on transplantation immunity (Group E). Almost 47,000 tumor cells were required to produce tumors in 50% of the treated animals.

Discussion. New cellular antigens unrelated to capsid antigens are induced by SV40 during transformation of cells *in vitro* or *in vivo*(1-4,9,11,16,17). Complement-fixation (16) and immunofluorescence tests(11,17) have located one such antigen in the nucleus. A transplantation antigen, presumably located at the cell surface, has been demonstrated *in vivo* by transplantation rejection procedures (1-4). Surface antigens have also been demonstrated *in vitro* by fluorescence(5,6) and cytotoxic tests(6,7), but the relation of these antigens to the transplantation antigen is presently unknown. However, it is certain that the intranuclear and the surface antigens detected by immunofluorescence are serologically unrelated(5).

This report offers evidence for the presence of virus-specific transplantation antigens at the surface of transformed cells. These conclusions are based on the assumption that if an animal is exposed to an antigen before the animal has developed the capacity to react against it, the development of an immune response against this antigen upon later exposure is delayed or postponed(18). Immunological tolerance has also been used to demonstrate transplantation antigens in carcinogen-induced tumors(19). Mice rendered immunologically tolerant by inoculation of killed tumor cells in neonatal life failed to react as adults to immunization against tumor cell transplants. The ghost cells derived from SV40 transformed cells (H-50) possess SV40 transplantation antigens and, when inoculated into newborn hamsters, induce a state of immunological tolerance. As a consequence, these animals fail to respond to SV40 immunization and subsequently fail to resist challenge with autologous SV40-transformed cells. Our results strongly suggest that the transplantation antigens in SV40-transformed cells are present at the surface of the tumor cells and they may be similar to the antigens previously detected by immunofluorescence(5).

Hamsters are susceptible to SV40 tumorigenicity only in neonatal life. The development of tumors in hamsters inoculated with SV40 in neonatal life can be prevented by repeated inoculation of the adult animals with the homologous virus(20). Irradiated nonmultiplying SV40 transformed hamster cells have also proven effective in checking the development of tumors(21).

The present study reveals that the inoculation of ruptured transformed cells during the process of vaccination with SV40 can interrupt the SV40-induced immune response of hamsters leading to rejection of SV40-transformed cells. There are 2 possible explanations for this finding: 1) antigens in

ruptured cells may combine with antibody or with antibody-forming cells and thereby help in the establishment of a successful transplant, and 2) ruptured cells may induce the synthesis of antibodies which play a role in protecting the transplant from the SV40-induced immune response. The second explanation would be a type of immunological enhancement.

Summary. Newborn hamsters inoculated at birth with ghosts prepared from cells transformed by papovavirus SV40 and subsequently vaccinated as weanlings with the virus, failed to reject later challenge of the autologous tumor cells. In other experiments, the establishment of transplantation immunity conferred by inoculation of the virus into normal weanling hamsters could be interrupted with tumor cell extracts injected during the course of virus immunization. Once immunity had been established, however, inoculation of ruptured tumor cells failed to significantly alter the immune status of the animals.

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Received August 24, 1966. P.S.E.B.M., 1966, v123.

Effects of Conditioning upon Stress Responses in the Rat.* (31559)

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In a study(1) of the role of histamine-mediation of pituitary-adrenal activation by a variety of drugs and other stressful stimuli such as cold exposure in the rat, the problem of obtaining stable control values for plasma corticosterone levels was evident. The widespread use of stress as a tool for studying induction of enzyme synthesis and conclusions regarding enzyme regulation drawn therefrom, suggested the desirability of a controlled study to determine optimal methods of animal handling during such studies. Adaptation to handling and intravenous injection of saline was assessed by measuring plasma corticosterone elevation caused by subsequently administered histamine or saline. The combined effect of housing and conditioning to injection

* Support for this work was provided by USPHS grants MH-2405 and AM-08752.

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