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Fetal Antigen Capable of Inducing Transplantation Immunity against SV40 Hamster Tumor Cells[1]

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Hamster, mouse and human cells transformed by simian virus 40 (SV40) share a common antigen capable of stimulating specific transplantation immunity against SV40-induced tumors in hamsters (1, 2). Cursory consideration of this observation might suggest that the viral genome must code for the common tumor-specific antigen in the different cell species. Knowledge acquired recently about the size of the SV40 genome limits the potential informational complement of this virus to seven to eight genes (3). Available data indicate that at least six genes are required for virus synthesis (3, 4), and radiation studies suggest that one or two different genes are required for transformation (5). It is becoming increasingly difficult to adhere to the concept that the virus codes directly for the tumor-specific transplantation antigen (TSTA) which is incorporated into the membrane of transformed cells unless the TSTA is a small protein and is a pleiotropic viral gene product.

An alternative hypothesis could be that the specificity of the transforming potential of the virus may be accomplished by the specific activation (repression or derepression) of cellular genes. Prehn reported that embryomas might possess antigens which are cross-reactive with antigens of certain methylcholanthrene-induced tumors (6). Ting concluded that mouse fetus injected into syngeneic adult mice did not promote transplantation immunity against polyoma-transformed mouse cells (7). In this and other similar studies (2) presentation of fetal tissue to the adult animal may result in the rapid maturation of the fetal cell under the humoral (hormonal) influence of the mature animal eliminating or masking fetal antigens comparable to those expressed as transplantation antigens in tumor cells. A preliminary examination of the role of fetal antigen in transplantation resistance indicates that irradiated hamster and mouse fetal tissues contain antigens which evoke immunity to SV40-induced tumors in syngeneic hamsters.

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MATERIALS AND METHODS

The results reported here were obtained using the SV40 hamster tumor cell line, F5-1, produced in random-bred Syrian golden hamsters, and also an SV40-induced tumor cell line derived in the inbred LSH/LAK line of hamsters. The F5-1 line has never been observed to yield infectious virus whereas the inbred tumor cell line yields virus in low titer. No histoincompatability has ever been reported for the random-bred hamsters used. Irradiated tumor cell preparations as well as adult control tissue were prepared as previously described (8). Hamster cells were obtained from fetuses of 9, 12 and 14 days gestation. Fetuses at each age were harvested aseptically, rinsed several times in Hanks' balanced salt solution (HBSS) and aspirated gently through a 26-gauge needle. The dispersed cells were then pelleted at 25°C by centrifugation and resuspended in HBSS. The procedure was repeated once and the pellet resuspended in HBSS to contain 5×10^6 viable cells/ml. BALB/c mouse fetal tissue was obtained during the 10th to 14th day of gestation and treated in a similar fashion. All tissue used to immunize 4- to 6-week-old hamsters was subjected to 5000 R x-irradiation as described previously (9) and injected by the intraperitoneal route in volumes of 1 ml. Immunization was administered on three occasions at 1-week intervals with freshly prepared cells. SV40 tumor cells were sealed into diffusion chambers as previously described (9) and implanted into the peritoneal cavity of anesthetized hamsters 10 days following the last immunization. Chambers were left in place 5 days, removed and examined for viable cells by the trypan blue dye exclusion test.

TABLE I

Induction of transplantation immunity to SV40 hamster tumor cells employing hamster fetal tissues as immunogen

Antigen[a]	Average No. of Viable Cells/ Chamber at 5 Days Post-Implant[b]	Percentage Inhibition of Target Cell Growth	Percentage Protection against SV40 Tumor Cell Challenge[c]
Unvaccinated controls	339,400		0
SV40 tumor cells	101,800	70	100
Adult syngeneic kidney cells	330,400	NSD[d]	
Hamster fetal cells			
9-day gestation	145,700	57	70
12-day gestation	163,200	53	60
14-day gestation	315,600	7	0
Mouse fetal cells			
12-day gestation	203,500	40	40

[a] All cells subjected to 5000 R of x-irradiation before intraperitoneal injection.

[b] Chambers originally inoculated with 15,000 viable SV40 tumor cells.

[c] Protective efficacy against cell challenge in animals receiving test preparations compared to tumor incidence in hamsters receiving adult hamster tissue as immunogen.

[d] NSD, not a statistically significant difference from unvaccinated control result as determined by the Wilcoxon test. All other differences significant at 1% confidence level.

Following removal of the chambers, the hamsters were subsequently challenged with 5×10^4 SV40 tumor cells in the right subscapular space. Animals were palpated weekly for tumors.

RESULTS AND DISCUSSION

SV40 tumor cells in diffusion chambers in adult hamsters immunized with 9- or 12-day irradiated hamster fetal cells failed to proliferate to the same extent as did the target cells in diffusion chambers of control animals. The degree of inhibition in a typical experiment is shown in Table I. Previous studies using the diffusion chamber assay indicate that the inhibition results from the binding of specific antibody at the plasma membrane of the target cell (8). Immunization with 14-day hamster fetal cells failed to induce cytostatic antibody. SV40 hamster tumor cells (F5-1) exhibited cytostatis in diffusion chambers in hamsters immunized against irradiated mouse fetal cells. Cell challenge studies from three experiments confirmed the existence of transplantation immunity in those animals which had exhibited cytostatic antibody. The degree of protection against challenge obtained with 9-day fetus from hamsters was 10% in one experiment, 30% in another and 70% in the experiment cited. Variation may result from either difference in fetus (number per mother or size) or in the cell challenge standardization. Similar results to those described in Table I using the random-bred hamster were obtained in the inbred hamster following immunization with 9- and 14-day irradiated hamster fetal cells.

The results reported here suggest that hamster and mouse fetal cells possess an equivalent or at least cross-reactive antigen to that present in SV40 hamster tumor cells as determined by the induction of cytostatic antibody and transplantation immunity. In the hamster fetus the antigen was no longer expressed by the 14th day of gestation. The negative results obtained by Ting (7), Kit *et al.* (2) and ourselves with non-irradiated fetal cells injected into adult animals can most plausibly be explained on the basis of rapid maturation of the fetal cell in the mature environment. These data clearly suggest that transplantation-like antigens are present in the hamster fetus during embryogenesis and are not expressed in the terminal stages of gestation. Duff and Rapp (10) have observed that normal pregnant hamsters develop antibody against SV40 hamster tumor cell S antigen, in agreement with our results. The observation that irradiated mouse fetal tissue was immunogenic against SV40-induced hamster tumors in hamsters is compatible with the fact that mouse and hamster tumor cells induced by SV40 share a common TSTA. It is important in future work that we establish whether the antigens expressed in embryogenesis are, in fact, identical to those present in the virus-transformed cell. Of equal importance is the need to establish the spectrum of tumor transplantation-like antigens present in fetal cells employing a variety of viral and chemically stimulated tumors. Finally, the relationship between human fetal antigens and human tumors must be examined in the light of this observation.

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