Immunologic Manipulation of Metastases due to Herpesvirus Transformed Cells

Abstract. *Herpes simplex virus type 1 (HSV-1), type 2 (HSV-2), and simian virus 40 (SV40) fail to induce immunity in weanling Syrian hamsters to transplant of hamster cells transformed by HSV-2. However, the development of metastatic tumors is markedly enhanced by prior immunization with HSV-1. Immunization with SV40, ultraviolet-irradiated tumor cells, or ultraviolet-irradiated normal hamster embryo cells inhibits the development of metastases. The HSV-hamster system appears a good one for the study of development, prevention, and control of metastases by mammalian cells transformed by a common human virus.*

Human herpes simplex virus type 2 (HSV-2) was originally implicated in human cervical cancer by epidemiological methods (*1*). Recently, HSV-2 antigens have been detected in exfoliated human cells from the genital tract by immunofluorescence techniques (*2*), and infectious HSV-2 has been isolated from human cervical cells (*3*). These results can be interpreted to imply that HSV-2 may play a significant role in the induction and maintenance of cervical cancer. Further evidence that HSV-2 can convert normal cells into oncogenic cells came from in vitro experiments which demonstrated that HSV-2 strain 333 had the ability to transform normal hamster embryo fibroblast (HEF) cells (*4*).

Hamster embryo fibroblast cells which transformed after infection by ultraviolet-irradiated HSV-2 were oncogenic when injected into newborn Syrian hamsters but did not induce tumors when injected into weanling hamsters. However, after one passage in a newborn hamster, the transformed cells became highly oncogenic when injected into weanling Syrian hamsters. In addition to their oncogenic properties, HSV-2 transformed cells contained HSV-2 specific antigens in the cytoplasm and on the cell surface (*5*). Location of these antigens was demonstrated by immunofluorescence techniques. Hamsters that developed tumors as a result of cellular injection also developed HSV-2 neutralizing antibodies which were possibly induced by incomplete HSV-2 particles that have been observed by electron microscopy in a few of the transformed cells (*6*).

Cells transformed by other DNA containing viruses usually develop virus-specific antigens which can induce an immune response in the host animal. This response can mediate the rejection of the tumor cells when the cells are injected into animals that have been previously immunized by the same tumor virus responsible for the cellular transformation (*7*). To determine whether a similar tumor-specific transplantation system was detectable in the HSV-2 transformed cells, weanling Syrian hamsters (2 to 3 weeks old, from the Lakeview hamster colony) were injected subcutaneously with 10^6 plaque-forming units (PFU) of HSV-1 strain 35 at weekly intervals for 3 weeks. One week after the final immunizing injection the hamsters (eight animals at each dose) were challenged with 10^1, 10^2, 10^3, 10^4, or 10^5 viable HSV-2 transformed HEF cells which had been previously passed one time in a newborn hamster (333-8-9 T1). Two other groups of weanling hamsters were used as controls. The first control group was immunized by three injections with 10^6 PFU of simian virus 40 (SV40) per injection and the second control group received three injections of medium 199 instead of a virus suspension. One week after the final injection the medium control and SV40 animals were also challenged with 333-8-9 T1 cells. In contrast to the results obtained with other DNA tumor virus systems, the immunization of animals with HSV-1 strain 35 did not protect the animals from the 333-8-9 T1 cells (Fig. 1). In animals immunized with HSV-1 tumors developed at a rate nearly identical to the rate observed for animals immunized with SV40 or injected with medium 199.

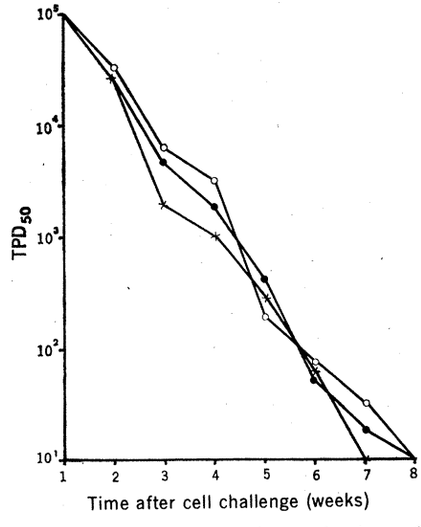

Fig. 1. Effect of prior immunization of weanling hamsters on the rate of the development of primary tumors after injection of hamster embryo cells transformed by herpes simplex virus type 2. Hamsters were immunized by three intraperitoneal injections of 10^6 plaque-forming units of HSV—1 strain 35 (solid circles), 10^6 plaque-forming units of SV40 (open circles), or medium 199 (crosses). Each hamster received three immunizing injections at 1-week intervals. One week after the final immunizing injection hamsters were challenged subcutaneously with 10^5, 10^4, 10^3, 10^2, or 10^1 viable HSV-2 transformed cells (333-8-9 T1). Eight hamsters were injected with each cell number and each immunization procedure. The results are presented as the number of cells required to produce tumors in 50 percent of the animals (TPD_{50}) compared to time after cell challenge.

HSV-1 strain 35 was an attenuated virus which had lost much of its virulence for mice and hamsters (*8*). It was possible that this strain had lost the capability to induce virus-specific transplantation immunity in addition to the loss of virulence. Therefore, nonattenuated HSV-1 (strain Patton) and HSV-2 (strain 333) were also used to immunize weanling hamsters before they were challenged with tumor cells. The injection of either the HSV-1 or the HSV-2 strains into weanling hamsters was fatal if the viruses were not inactivated for 30 seconds by ultraviolet irradiation (42 erg sec^{-1} mm^{-2}) prior to injection. Hamsters immunized by inactivated HSV-1 or HSV-2 did not demonstrate transplantation immunity when compared to animals immunized with SV40 or medium 199. All animals injected with either HSV-1 strain 35, HSV-1 strain Patton, or HSV-2 strain 333 developed HSV neutralizing antibodies. Therefore, the immunized hamsters were not tolerant to HSV. From these results it was concluded that rejection or retardation of the primary tumor could not be induced by prior immunization of hamsters with HSV-1 or HSV-2 under the conditions of the above experiments.

The 333-8-9 T1 cells were observed to metastasize after the development of a primary tumor that was a result of a subcutaneous injection of cells into susceptible weanling hamsters. These metastatic lesions were usually localized in the lungs. Prior immunization of weanling hamsters might induce low levels of protection against 333-8-9 T1 cells, which could be detected if

Table 1. Effect of hamster immunization with SV40 or HSV-1 on the occurrence of lung metastases after injection of HSV-2 transformed hamster embryo fibroblasts.

Immunizing agent*	Number injected†	Percent with primary tumor‡	Number with metastases§	Percent with metastases
HSV-1	28	100	22	78
SV40	30	100	8	27
Medium 199	35	100	20	57

* Each hamster was given three subcutaneous injections at 1 week intervals. Animals immunized with SV40 or HSV-1 received 10^6 PFU diluted in 0.1 ml of medium 199 supplemented with fetal bovine serum (10 percent) and $NaHCO_3$ (0.075 percent). † Immunized hamsters were injected with 10^5 viable HSV-2 transformed cells (333-8-9 T1) 1 week after the third immunizing injection. ‡ Primary tumor is defined as the tumor appearing at the site of the cellular injection. § The number of hamsters with lung metastases was determined by visual examination of the hamsters 10 weeks after tumor cell injection.

inhibition of metastatic tumors was used as a criterion for protection. The following experiment was designed to determine what effect prior immunization would have on the development of metastatic tumors. Weanling Syrian hamsters were first immunized with either HSV-1 strain 35, SV40, medium 199, ultraviolet-irradiated (3 minutes at 42 erg sec^{-1} mm^{-2}) 333-8-9 T1 cells, or ultraviolet-irradiated HEF cells. Each hamster received three immunizing injections at weekly intervals, followed by a subcutaneous injection of viable cells 1 week after the final immunizing injection. Hamsters in each group developed primary tumors at essentially the same rate. Eight animals receiving 10^5 tumor cells were checked for metastases 6 weeks after cell challenge, those receiving 10^4 cells were checked at 7 weeks, 10^3 cells at 8 weeks, 10^2 at 9 weeks, and 10^1 cells at 10 weeks. The results are shown in Fig. 2, solid bars. Hamsters receiving medium 199 developed metastatic lesions in 20 percent of the animals. The number of metastases increased to 47 percent if the animals were first immunized with HSV-1 strain 35. However, the number of metastases was 5 percent in animals immunized with ultraviolet-irradiated HEF cells or 8 percent when immunized with ultraviolet-irradiated 333-8-9 T1 cells. The most interesting and potentially significant observation was that detectable metastatic tumors did not develop when test animals were first immunized with SV40.

A second cell line transformed by ultraviolet-irradiated HSV-2 (333-2-26 T2) was also tested to determine the general nature of the results observed (Fig. 2, hatched bars). The overall results obtained with this cell line were similar to those obtained with the 333-8-9 T1 cell line although the percentage of animals with metastases was generally lower in all groups. HSV-1 again enhanced metastases; and SV40, HEF cells, and 333-2-26 T2 cells inhibited metastases.

To determine whether the enhancement of metastases by HSV-1 and the inhibition of metastases by SV40 would continue to be apparent if the animals were maintained for a longer period of time with a large primary tumor, the following experiments were carried out. Hamsters were first immunized with three injections of either 10^6 PFU of HSV-1 strain 35, 10^6 PFU of SV40, or medium 199 at weekly intervals. These animals were then challenged with 10^5 viable 333-8-9 T1 cells 1 week after the final immunizing injection. Animals were killed 10 weeks after cell challenge and visible metastases were identified (Table 1). The same relation was found as in the previous experiments. Lung metastases developed in 79 percent of the HSV-1 immunized hamsters challenged, in only 27 percent of the SV40 immunized hamsters, and in 57 percent of the medium 199–injected hamsters. Among the challenged hamsters that had been given medium 199 or HSV-1, 20 percent had metastases in more than one organ (kidney, gastrointestinal tract, or liver). No metastases in more than one organ were observed in hamsters immunized with SV40. Ninety percent of the animals that were immunized with HSV-1 or medium 199 showed ten or more distinct metastatic lung tumors each. However, no hamsters immunized by SV40 developed more than two visible metastic lesions in the lung.

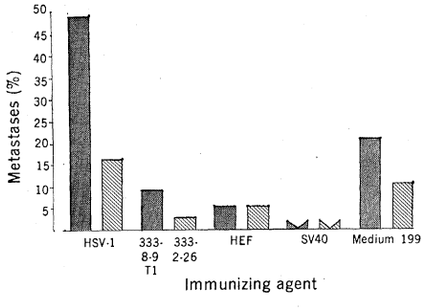

Fig. 2. Effect of prior immunization of weanling hamsters on the development of metastases by subcutaneous injection of cells transformed by herpes simplex virus type 2. See text and Fig. 1 for details of immunization procedure. Solid bars show the development of metastases when the transformed cell line 333-8-9 T1 was used for cell challenge. Hatched bars show the development of metastases when the transformed cell line 333-2-26 T2 was used for cell challenge. Solid bars should be compared only with solid bars and hatched bars should be compared only with hatched bars. The results are presented as the percentage of hamsters that developed visible lung metastases compared to the total number of hamsters developing a primary tumor.

The above results demonstrate that immunization with SV40 can induce a reaction in weanling hamsters, which inhibits metastases of HSV-2 transformed cells. Immunization with HSV-1 does not induce this inhibition but induces an enhancement of metastases. Neither HSV-1 nor SV40 inhibits the induction of primary tumors. Several theories can be postulated to explain this inhibition by SV40. Two theories that have precedent in current research are (i) a nonspecific stimulation of the cellular immune response similar to that induced by BCG (Bacillus Calmette Guerín) (*9*) and (ii) the development of an immune response directed against embryonic antigens on the surface of the HSV-2 transformed cells. It has been demonstrated that SV40-induced tumors contain surface embryonic antigens (*10*). Which, if either, of these hypotheses is true cannot be determined at present. However, the inhibition of metastases by normal HEF cells supports the embryonic antigen hypothesis.

The enhancement of metastases by HSV-1 can best be explained by the blocking antibody theory (*11*). Prior immunization of hamsters by HSV-1 may induce the development of circulatory antibodies directed against virus-specific antigens on the surface of the transformed cells. The adherence of this antibody to the cells protects them against destruction by immunocompetent cells.

From our results, the HSV-2 transformed hamster cells appear to be an ideal system in which to study the characteristics and control of metastases in an experimental animal system.

Results obtained can be used to help determine whether immunological control of metastases is feasible in human cancer.

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Translocation Trisomic Mice: Production by Female but Not Male Translocation Carriers

Abstract. *In man, there is generally a greater chance for a translocation trisomic child to be born if the mother rather than the father is the translocation carrier. The same type of inheritance has occurred in the mouse. Female mice heterozygous for the reciprocal translocation* T(**14;15**)6Ca *have produced a high frequency of translocation trisomic offspring. Male mice heterozygous for the same translocation have produced no translocation trisomic offspring. Thus, the laboratory mouse may provide a model for studying the cause of this phenomenon.*

In man, when the parent carrying a reciprocal or Robertsonian translocation is the mother, the probability is generally increased that a child will be born with a specific type of chromosomal defect, a translocation trisomy (*1*). An example is the high frequency of children born with Down's syndrome from translocation carrier mothers compared to those from translocation carrier fathers (*2*). The reason why this type of chromosomal abnormality tends to be transmitted through females but not males is unknown.

I have found the same phenomenon in mice carrying the reciprocal translocation *T*(**14;15**)*6Ca* (hereafter *T6*). This translocation is a reciprocal exchange between chromosomes 14 and 15 (*3*), such that the rearranged chromosome with the centromere end of 15 and noncentromere end of 14 (15^{14}) is extremely small and cytologically identifiable by conventional staining procedures (*4*, *5*). The piebald (*s*) gene on chromosome 14 is located so close to the *T6* breakpoint that it is used to mark the translocation (*5*, *6*).

Translocation trisomic offspring were produced in crosses that were originally designed to locate the *T6* breakpoint in chromosome 15 in relation to the centromere and the underwhite (*uw*) locus (*7*). In these crosses individual + *T6* +/*uw* + *s* mice were crossed to *uw* + *s*/*uw* + *s* mice. The young were visually scored for *uw* and *s*, and their chromosomes were analyzed for the presence of chromosome 15^{14}. Care was taken to cytologically distinguish *T6*/+ mice that had 40 chromosomes (including 14, 14^{15}, 15^{14}, and 15) from those mice that appeared *T6*/+ but actually had 41 chromosomes (including 14, 14, 15, 15, and 15^{14}), and thus were trisomic for the chromosomal regions contained in chromosome 15^{14} (*5*, *7*). Since all of the mice with 41 chromosomes were phenotypically nonunderwhite piebald, the *uw* locus (*uw*+ allele) is located on chromosome 15^{14} (*7*) and the *s* locus (*s*+ allele) is located on chromosome 14^{15} (*5*).

Table 1. Results of crosses involving *T6* and *uw*.

Phenotype	Total	Progeny analyzed (No.)	Chromosomes (No.)
Cross: ♀ + *T6* +/*uw* + *s* × ♂ *uw* + *s*/*uw* + *s*			
+ *T6* +	58	26	40
uw + *s*	53	18	40
+ *T6 s*	21*	8	41
uw + +	0		
Total	132	52	
Cross: ♀ *uw* + *s*/*uw* + *s* × ♂ + *T6* +/*uw* + *s*			
+ *T6* +	27	7	40
uw + *s*	38	3	40
+ *T6 s*	0		
uw + +	0		
Total	65	10	

* Of the 13 mice whose chromosomes were not analyzed, 7 died before chromosome counts were taken, and the remaining 6 are being tested for fertility. By age 2 weeks the mice with 41 chromosomes were smaller than their normal sibs. The deleterious effect of the *s*/*s* condition, megacolon, appears to be intensified in these mice.

Translocation trisomic offspring were produced only in crosses involving *T6*/+ females, never *T6*/+ males (Table 1). To date, eight *T6*/+ females have produced 21 trisomics in 132 young (15.9 percent); six *T6*/+ males have produced no trisomics in 65 young. This difference is highly significant ($P < .001$).

The translocation trisomics are retarded in development, their viability is greatly reduced, and they have a nervous, shaky behavior (*8*). The testes of the males are small, possibly indicating sterility. However, two presumed translocation trisomic females—which are nonunderwhite piebald, display the nervous, shaky behavior, and were retarded in development—have produced 20 young; nine of these appear to be translocation trisomics.

Study of gametogenesis in female and male *T6*/+ mice may allow us to understand why this chromosomal defect is generally transmitted through mammalian females but not mammalian males.

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