

Common Antigens of Herpes Simplex Virus 2, Associated Hamster Tumors, and Human Cervical Cancer¹ (39393)

ADLY N. IBRAHIM, MARSHA RAY, JUDITH MEGAW, RICK BROWN,
AND ANDRE J. NAHMIA

Department of Biology, Georgia State University, Atlanta, Georgia 30303; and Department of Pediatrics, Emory University, School of Medicine, Atlanta, Georgia 30303

In a previous communication (1), we reported on the demonstration of tumor-associated antigens (TAA) in sarcomas obtained after direct inoculation of newborn hamsters (2) with herpes simplex virus type 2 (HSV-2). The antigens were detected in the sarcomas, as well as in the sera of tumor-bearing hamsters, by the immunoadsorption-in-gel method. The purpose of this investigation was to determine the antigenic interrelationships between HSV-2, the HSV-2 associated sarcomas and human cervical cancer, related by other methods to HSV-2 (3).

Materials and methods. Viruses. The MS of HSV-2 and the VR₃ strain of HSV-1 were propagated in HEp-2 cells grown in Eagle's MEM with 2% fetal calf serum (FCS).

Tumors. The two HSV-2 associated hamster sarcomas, OT-1 and OT-11, studied earlier (1) were employed. For control purposes, Para (defective SV40)-adenovirus (Para 7) hamster tumor (obtained from Dr. F. Rapp, Hershey Medical Center) were used. Cervical cancer biopsy tissues (Ca Cx) and a cervical cancer cell line (C4-II) were obtained from Dr. E. Franklin, Emory University School of Medicine, and Dr. N. Auersberg, University of British Columbia, Vancouver, respectively.

Antigens and antisera. The methods for preparation of tumor or normal tissue antigens and of rabbit hyperimmune sera (listed in Table I) have been reported earlier (1).

Purification of TAA and anti-TAA of OT-II tumors. The immunoadsorption-in-gel technique, described previously (1), was used.

Serological methods. (i) Immunodiffusion tests: Antisera to the virus or tumor prepa-

rations were reacted, after adsorption with appropriate antigens, with homologous and heterologous antigen preparations. The adsorption-in-gel procedure, previously employed (1), consisted essentially of placing the adsorbing antigen preparation in the central well, allowing it to diffuse for 2 hr at 37°, then replacing it with the antiserum to be adsorbed. The peripheral wells were filled with the homologous and heterologous test antigens, including the adsorbing antigens as controls. (ii) Passive hemagglutination (PHA) tests: The micro PHA procedure was employed, as described earlier (4). Each experiment was repeated at least three times.

Results. Table II presents the immunodiffusion results obtained when the antisera, adsorbed with appropriate antigens, were reacted with the homologous and heterologous antigens. Positive reactions indicate one or more precipitin lines. None of the adsorbed antisera reacted with the adsorbing antigens; neither did preimmunization sera react in these assays nor in any other serological test used in these studies.

Antiserum to HSV-2 reacted with HSV-2, HSV-1, OT-1, OT-11, and Ca Cx (Table II). A precipitin line of partial identity was noted between HSV-2, OT-1, OT-11, and Ca Cx (Fig. 1). When the anti-HSV-2 serum was adsorbed with HSV-2, no immunoprecipitin lines were formed with the previously positive homologous or heterologous antigens. Anti-HSV-1 serum reacted only with the two HSV antigens. No reaction appeared with homologous adsorption whereas adsorption with the heterologous HSV-2 antigen eliminated the reaction with HSV-2 only (Fig. 2).

Antisera to cervical cancer tissues and to C4-II cervical cancer cell line reacted with HSV-2, OT-11, Ca Cx, and C4-II with a

¹ Supported by an NSF Institutional Grant to Georgia State University, the Urban Life Center, NCI Grant (CA 16806) and NCI Contract (NOICP 43393).

TABLE I. ANTIGENS AND ANTISERA USED IN IMMUNODIFFUSION AND PASSIVE HEMAGGLUTINATION STUDIES.

Test and/or adsorbing antigens	Homologous anti-sera prepared	Comments
Herpes simplex virus type 2 (HSV-2)	yes	MS strain
Herpes simplex virus type 1 (HSV-1)	yes	VR ₃ strain
OT-1 tumor	yes	HSV-2 (MS strain)-associated hamster chondrosarcoma
OT-11 tumor	yes	HSV-2 (Pitts strain)-associated hamster hemangiosarcoma
OT-11 TAA	yes (purified)	Purified tumor-associated antigens of OT-11
Para-7 tumor	yes	Para (defective SV40)-adenovirus 7 hamster tumor
Normal hamster liver, muscle, and serum	no	Obtained from normal uninoculated inbred hamsters
C4-II cell culture	yes	Developed from human invasive cervical cancer tissue
Cervical cancer tissue	yes	Pooled cervical cancer tissues
Normal human liver and serum	no	Pools of 9 livers and of 12 human sera from noncancer individuals
Fetal calf serum	yes	Used for propagation of HSV-1, HSV-2, and C4-II
HEp-2 cells	yes	Used for propagation of HSV-1 and HSV-2

TABLE II. IMMUNODIFFUSION RESULTS WHEN VARIOUS ANTISERA, AFTER ADSORPTION WITH THE APPROPRIATE ANTIGEN(S), WERE REACTED WITH THE HOMOLOGOUS AND HETEROLOGOUS ANTIGENS.

Anti-sera to:	Adsorbing antigens	Test antigens						
		HSV-2	HSV-1	OT-1	OT-11	Para-7	Ca Cx	C4-II
HSV-2	FCS ^a + HEp-2	+ ^b	+	+	+	0	+	0
	HSV-2	0	0	0	0	0	0	0
	HSV-1	+	0	+	+	0	0	0
HSV-1	FCS + HEp-2	+	+	0	0	0	0	0
	HSV-1	0	0	0	0	0	0	0
	HSV-2	0	+	0	0	0	0	0
Ca Cx	NHuL ^c + NHuS ^d	+	0	+	+	0	+	+
	Ca Cx	0	0	0	0	0	0	0
	OT-1	0	0	0	0	0	+	0
	OT-11	0	0	0	0	0	+	0
C4-II	FCS + NHuL	+	0	0	+	0	+	+
	OT-1	0	0	+	+	+	+	0
OT-1	OT-1	0	0	0	0	0	0	0
	Para 7	0	0	+	+	0	+	0
	Ca Cx + NHL + NHS	0	0	+	+	+	0	0
	NHS + NHL	0	0	+	+	+	+	0
	OT-11	0	0	0	0	0	0	0
Para 7	Para 7	0	0	+	+	0	+	0
	Ca Cx + NHL + NHS	0	0	+	+	+	0	0
	NHS + NHL	0	0	+	+	+	0	0
	Para 7	0	0	0	0	0	0	0
	OT-1	0	0	0	0	+	0	0
	OT-11	0	0	0	0	+	0	0

^a Fetal calf serum.

^b + = one or more immunoprecipitin lines.

^c Normal human liver.

^d Normal human serum.

^e Normal hamster liver.

^f Normal hamster serum.

pattern of partial identity in one of the precipitin lines. Adsorption with Ca Cx removed homologous, as well as heterologous

reactions, whereas adsorption of anti-Ca Cx serum with OT-1 or OT-11 did not eliminate reactivity with Ca Cx (Fig. 3).

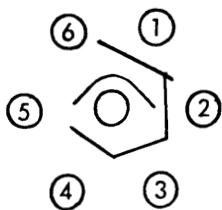


FIG. 1. Immunodiffusion patterns: central well contains rabbit anti-HSV-2 serum adsorbed with HEP-2 cells and FCS. The peripheral wells contain antigen preparations of: (1) HSV-2; (2) OT-1; (3) OT-11; (4) Ca Cx; (5) HEP-2 cells with FCS; (6) HSV-1.



FIG. 2. Immunodiffusion patterns: central wells contain rabbit anti-HSV-1 serum = (A) adsorbed with HEP-2 cells and FCS, and (B) adsorbed with HSV-2. The peripheral wells contain antigen preparations of: (1) HSV-1; (2) OT-1; (3) OT-11; (4) Ca Cx; (5) HEP-2 cells and FCS; (6) HSV-2.

Antisera to OT-1 and OT-11 reacted with Ca Cx and with the three hamster tumor preparations, including Para-7. When these antisera were adsorbed with Para-7, two immunoprecipitin lines were noted with the homologous antigens, one of which showed partial identity with Ca Cx (Fig. 4). Adsorption of the anti-OT-1 and OT-11 sera with Ca Cx removed the reactivity with Ca Cx only. Anti-Para-7 serum showed a common precipitin line with all three hamster tumor antigens, which was abolished after adsorption with either OT-1 or OT-11 (Fig. 5).

The TAA of OT-11 and antiserum to the TAA had been purified earlier (1) and these preparations were tested by passive hemagglutination assays. As noted in Table III, 1:8 to 1:32 titers were obtained when antisera to OT-11, HSV-2, and C4-II were reacted with the purified TAA of OT-11, whereas antisera to HSV-1, HEP-2 cells, and fetal calf serum were nonreactive. When purified antiserum to TAA of OT-11 was tested with various antigen preparations, positive reactions of 1:2 to 1:64 were obtained with HSV-2, OT-11, and C4-II, whereas no reaction was demonstrated with antigen preparations of HSV-1, HEP-2

cells, FCS, and normal hamster liver (Table IV).

Discussion. Following the demonstration of tumor-associated antigens in sarcomas obtained after inoculation of HSV-2 in newborn hamsters (2), it was of interest to ascertain whether HSV-related antigens could be detected in these tumors. As noted in Table II, cross-reactivity was found between anti-HSV-2 sera and the HSV-2 associated hamster tumors (OT-1 and OT-11). Anti-HSV-2 serum also reacted by passive hemagglutination with the purified TAA of OT-11. Anti-HSV-1 serum did not react by either assay with any of the hamster tumors. The antisera to either OT-1 or OT-11 failed

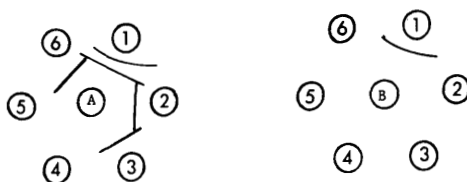


FIG. 3. Immunodiffusion patterns: central wells contain rabbit anti-Ca Cx serum = (A) adsorbed with $\text{NH}_4\text{L} + \text{NH}_4\text{S}$, and (B) adsorbed with OT-1 or OT-11. The peripheral wells contain antigen preparations of: (1) Ca Cx; (2) OT-11; (3) C4-II; (4) Para-7; (5) $\text{NH}_4\text{L} + \text{NH}_4\text{S}$; (6) HSV-2.

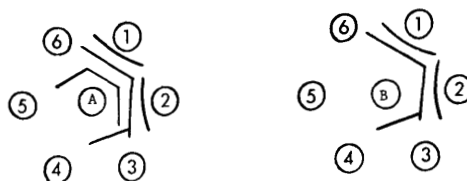


FIG. 4. Immunodiffusion patterns: central wells contain rabbit anti-OT-1 serum = (A) adsorbed with NHL + NHS, and (B) adsorbed with Para-7. The peripheral wells contain antigen preparations of: (1) OT-1; (2) OT-11; (3) Ca Cx; (4) C4-II; (5) NHL + NHS; (6) Para-7.

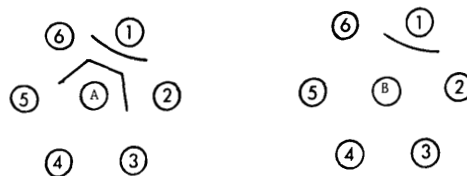


FIG. 5. Immunodiffusion patterns: central wells contain rabbit anti-Para-7 serum = (A) adsorbed with NHL + NHS, and (B) adsorbed with OT-1 or OT-11. The peripheral wells contain antigen preparations of: (1) Para-7; (2) OT-1; (3) Ca Cx; (4) C4-II; (5) NHL + NHS; (6) OT-11.

TABLE III. PASSIVE HEMAGGLUTINATION TITERS OBTAINED WITH PURIFIED TAA OF OT-11.

Antisera to:	Reciprocal titers obtained with purified TAA of OT-11
OT-11	16
HSV-2	32
C4-II	8
HSV-1	<2
HEp-2	<2
FCS	<2

TABLE IV. PASSIVE HEMAGGLUTINATION TITERS OBTAINED WITH PURIFIED ANTISERUM TO TAA OF OT-11.

Antigens	Reciprocal titers obtained with antiserum to TAA of OT-11
OT-11	8
C4-II	64
HSV-2	2
HSV-1	<2
HEp-2	<2
FCS	<2
NHL	<2

to react in immunodiffusion tests with HSV-2, but a very weak reaction was obtained between the purified anti-TAA of OT-11 and HSV-2 in the PHA test (Table IV). The anti-HSV-2 serum failed to react with the Para-7 hamster tumor. Adsorption of the anti-HSV-2 serum with HSV-2 removed homologous reactivity, as well as heterologous reactivity with the HSV-2 associated tumors, implying the presence of common antigens between HSV-2, OT-1, and OT-11.

The hyperimmune rabbit anti-HSV-2, but not the anti-HSV-1, serum also reacted with cervical cancer tissue antigens (Ca Cs). The converse was also observed, i.e., that anti-Ca Cx serum reacted with HSV-2, but not with HSV-1. Adsorption of the anti-HSV-2 serum with HSV-2 eliminated the reaction with Ca Cx, suggesting that the common antigen may be specific to HSV-2. Antiserum to Ca Cx reacted with the HSV-2 associated hamster tumors and C4-II cervical cancer cell line, but not with the Para-7 hamster tumor. Adsorption of anti-Ca Cx serum with the homologous antigens removed both homologous and heterologous reactivity. On the other hand, adsorption of the anti-Ca Cx serum with OT-1 or OT-11 did not abolish reactivity with Ca Cx. Anti-

OT-1 or OT-11 sera adsorbed with Ca Cx still retained their reactivity with the hamster tumor preparations, suggesting that both common and specific antigens are present in the human and hamster tumors. It is likely that these common and specific antigens are different from those found between the HSV-2-associated hamster tumors and the non-HSV-2 related Para-7 tumor. Common reactivity between other HSV-related hamster tumors and Para-7 have also been found by other workers using other serologic tests (5). The antigen shared by the HSV-2 and the Para-7 associated hamster tumors may be explained by the possible existence of a fetal antigen. Such antigen may appear in the neoplasms as result of derepression of the gene in the malignant cell with reappearance of the embryonic antigen. Indeed, the presence of such antigen in SV₄₀-induced tumors in hamsters has been previously reported (6, 7). Attempts to elucidate the nature of the common antigen will be investigated in the near future.

Reactivity of anti-TAA of OT-11 with the C4-II cervical cancer cell line was only demonstrated by passive hemagglutination. The anti-OT-11 serum failed to react by immunoprecipitation with C4-II. Unfortunately, further tests were not possible with C4-II as the cell line was lost.

The above results indicate antigenic relationships between HSV-2, the HSV-2 associated hamster sarcomas and cervical cancer. Antigenic relationships between HSV-2 and the HSV-2 associated hamster tumors were suggested earlier by anticomplement immunofluorescent (ACIF) tests (8) which also pointed to a relation between HSV-2 and cervical cancer. Using different serological tests, other workers have also found antigenic relationships between this virus and human cancer (9, 10). Further work is in progress to characterize the antigens common to the virus and the hamster or human tumors.

Summary. Using the immunodiffusion-adsorption-in-gel and/or the passive hemagglutination techniques, an antigenic relationship has been found between Herpes simplex virus type 2 (HSV-2), cervical cancer tissue antigen preparations, a cell culture derived from cervical cancer, and two

HSV-2 associated hamster sarcomas. No cross-reactive antigens were detected between HSV-1, and the HSV-2 related hamster sarcomas or with human cervical cancer.

1. Ibrahim, A. N., Ray, M., and Nahmias, A. J., Proc. Soc. Exp. Biol. Med. **148**, 1025 (1975).
2. Nahmias, A. J., Naib, Z., Josey, W., *et al.*, Proc. Soc. Exp. Biol. Med. **134**, 1065 (1970).
3. Nahmias, A. J., and Roizman, B., New Eng. J. Med. **289**, 667 (1973).
4. Schneeweis, K. E., and Nahmias, A. J., J. Immun.-Forsch. Bd. **141**, 471 (1971).
5. Laux, D., and Lausch, R., J. Immunol. **112**, 1900 (1974).
6. Coggin, J. H., Ambrose, K. R., and Anderson, N. G., J. Immunol. **105**, 524 (1970).
7. Duff, R., and Rapp, F., J. Immunol. **105**, 521 (1970).
8. Nahmias, A., Ibrahim, A., and Del Buono, I., in "Herpesviruses and Oncogenesis" (H. Zur Hausen and G. De The, eds.), Vol. 2. Lyon International Agency for Research on Cancer (1975).
9. Hollinshead, A., Lee, C. B., and McKelway, W., *et al.*, Proc. Soc. Exp. Biol. Med. **141**, 688 (1972).
10. Aurelian, L., Cancer Res. **33**, 1939 (1973).

Received December 29, 1975. P.S.E.B.M. 1976, Vol. 152.