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Tumor Antigens in Hamsters with Sarcomas Associated with Herpesvirus Type 2[1] (38681)

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Tumor-associated antigens (TAA) have been demonstrated in several animal and human cancers by a variety of in vitro and in vivo techniques (1, 2). In animals, TAA have been detected in tumors induced by physical and chemical agents, as well as by various viruses. It was therefore of interest to attempt the detection of TAA in sarcomas produced after inoculation of newborn hamsters with herpes simplex virus type 2 (3), a virus related to human urogenital cancers (4, 5). The immunoadsorption in-gel method, employed earlier for the demonstration of virus-specific antigens and antibodies (6), was used to demonstrate, purify and isolate TAA and anti-TAA in hamsters with herpesvirus-associated sarcomas.

Materials and Methods. Tumors. Two herpes simplex virus type 2 (HSV-2) associated hamster tumors were used: (1) OT-1, a chondrosarcoma first noted at the site of inoculation 5 mo after intrathoracic injection of the MS strain of HSV-2 into newborn hamsters, and (2) OT-11, a hemangiosarcoma, first observed after 16 mo at the site of inoculation of the Pitts strain of HSV-2 into newborn hamsters (3). The tumors were transplanted into inbred golden Syrian hamsters, strain LSH (Lakeview). Subcutaneous injection of 0.5 ml containing 10^5 tumor cells into 3- to 4-wk old weanling hamsters induced tumors within 30 days. Tumors were harvested aseptically and used for further transplants or for antigen preparation. Tumor-bearing hamster sera (TBHS) were also obtained within 2–4 wk after appearance of the tumors.

Antigens. Antigens were prepared from the OT-1 and OT-11 tumors, and from liver and muscle tissues of uninoculated hamsters. Pooled normal hamster sera (NHS) and pooled TBHS were also used as antigens. Tumors or normal tissues were removed aseptically, rinsed thoroughly and washed 3X in Earle's basic salt solution containing penicillin and streptomycin (EBSS). A 20% tissue suspension in EBSS, homogenized in a chilled Lourdes blender by intermittent runs of 1 min for several cycles, was centrifuged at 10,000 rpm for 30 min at 4° to yield supernate 1 and pellet 1. Pellet 1 was resuspended in EBSS to one tenth of the original volume and the suspension was rapidly frozen and thawed 3X in acetone-dry ice bath. This mixture was centrifuged at 5000 rpm for 30 min at 4° to yield supernate 2 and pellet 2. Supernates 1 and 2 were pooled and concentrated tenfold by dialysis against carbowax. The retentate was dialyzed against phosphate buffered saline (PBS) pH 7.2 for 24 hr in the cold. The supernate pool was added to pellet 2 and the mixture was sonicated (Branson sonifier) for 2 min in the cold.

Antisera. Antisera were prepared in albino rabbits against each of the aforementioned antigens. At least two rabbits were used for each immunogen. The inoculum consisted of 2 ml of immunogen emulsified in an equal volume of complete Freund adjuvant. Each rabbit received 4–5 im inoculations at 10 day intervals. Serum samples were obtained before immunization and 10 days after the last injection.

Serological methods. Immunodiffusion (ID) and immunoelectrophoresis (IE) were used to demonstrate TAA and anti-TAA. The immunoadsorption-in-gel method detailed by Ibrahim (6), was employed to eliminate cross-reactivity between tumor and normal tissue antigens. In brief, the appro-

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priate adsorbing antigen preparation (normal liver, normal muscle or NHS) was placed in the central well, allowed to diffuse for 2 hr at 37°, then replaced with the antiserum to be adsorbed (antiserum to tumor antigens or to TBHS). Unadsorbed and adsorbed antisera were also tested by immunoelectrophoresis (7), the adsorption being performed in the trough of the agar-coated slide.

Purification of anti-TAA and TAA. Immunoadsorption-in-gel was adapted for this purpose. Control slides were always used with unadsorbed serum and with adsorbed serum, as described above, for the demonstration of the site of the precipitin line(s) representing the TAA. For the purification of anti-TAA, the central well of several agar-coated slides was filled with the adsorbing antigens (normal tissue antigen preparation). After diffusion of the adsorbing antigens for 2 hr at 37°, the residual was replaced with rabbit anti-tumor serum and incubated at 37° for 24 hr without filling the peripheral wells with any antigen preparations. When the precipitin line(s) representing the TAA appeared in the control slides, the gel in similar areas of the test slides was cut off. To elute the anti-TAA, the gels were homogenized in PBS pH 7.2, incubated overnight at 4°, centrifuged at 10,000 rpm for 30 min and the supernate concentrated 10X.

Essentially the same techniques were applied to purify the tumor-associated antigens with minor modifications. Adequate adsorption-in-gel was possible only after performing two successive adsorptions in tubes by mixing the tumor antigen preparations with rabbit anti-normal tissue sera, incubating the mixtures overnight at 4°, and sedimenting the antigen–antibody complexes by centrifugation. The supernate, containing the partially purified TAA, was further purified by the adsorption-in-gel technique, similar to that described above for the purification of the anti TAA.

Results. Preimmunization rabbit sera failed to react with any of the test antigens. The ID reactions of anti-OT-11 serum unadsorbed (A) and adsorbed with normal tissue antigens (B) are shown in Fig. 1. Although the unadsorbed antiserum demonstrated extensive cross-reactivity with normal tissue preparations, additional precipitin line(s) could be observed with the tumor preparations. The adsorbed anti-OT-11 serum was found to react only with the homologous tumor antigen. This reactivity was eliminated by adsorption of the antiserum with the homologous tumor antigen preparations (not shown).

Figure 2 is a schematic representation of several experiments showing that the anti-OT-11 serum, when purified by the immunoadsorption in-gel method reacted with OT-1 and OT-11 antigens, as well as with the tumor-bearing hamster serum; no reaction was noted with normal hamster tissues or serum. These findings indicate the successful purification of anti-TAA, the antigenic identity of the OT-1 and OT-11 tumors and the demonstration of circulating TAA in sera of tumor-bearing hamsters.

Figure 3 demonstrates a line of identity between the purified TAA of OT-11 and antisera to OT-11, OT-1 and to TBHS; no

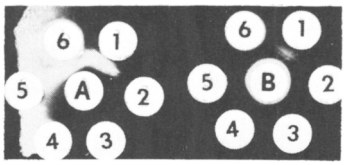

FIG. 1. Immunodiffusion patterns: Central wells contain rabbit anti-OT-11 serum: (A) unadsorbed and (B) adsorbed with normal liver antigen preparation. The peripheral wells contain antigen preparations of: (1) OT-11 tumor; (2) normal muscle (NM); (6) normal liver (NL).

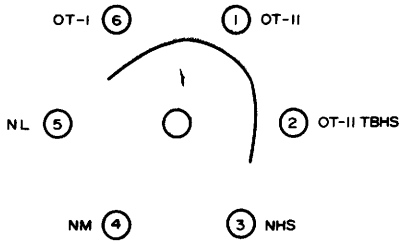

FIG. 2. Immunodiffusion patterns: Central well contains the purified anti-OT-11 serum. The peripheral wall contain antigen preparations of: (1) OT-11 tumor; (2) OT-11 tumor-bearing hamster serum (TBHS); (3) normal hamster serum (NHS); (4) normal muscle (NM); (5) normal liver (NL); (6) OT-1 tumor.

reaction was observed with antisera to normal liver, normal muscle, or normal hamster serum. These results indicate the successful isolation and purification of OT-11 TAA and confirm the presence of circulating TAA in sera of tumor-bearing hamsters. Further evidence for the specificity of this reaction was obtained when anti-TBHS, adsorbed with normal hamster serum, was found to react with OT-11 and TBHS, but not with normal hamster serum or normal tissues (Fig. 4).

Some of the above-mentioned experiments were repeated using immunoelectrophoresis with essentially similar results.

Discussion. Various physicochemical methods have been applied for the preparation and purification of tumor-associated antigens and specific antisera. The immunoadsorption-in-gel technique was found to be a simple method for the demonstration of TAA in HSV-2 associated hamster sarcomas (Fig. 1). In addition, this method was found useful in the purification of anti-TAA of the hamster tumors (Fig. 2). Purification of the TAA, however, required tube adsorptions in addition to in-gel adsorption.

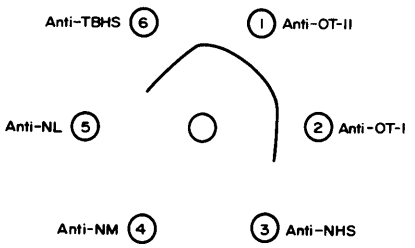

FIG. 3. Immunodiffusion patterns: Central well contains purified TAA of the OT-11 tumor. The peripheral wells contain antisera to: (1) OT-11; (2) OT-1; (3) NHS; (4) NM; (5) NL, (6) TBNS.

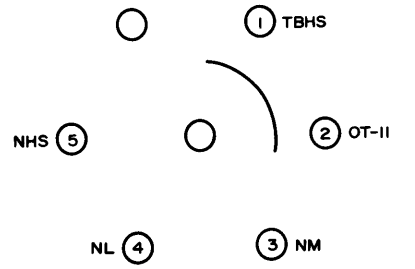

FIG. 4. Immunodiffusion patterns: Central well contains rabbit anti-TBHS adsorbed with NHS. The peripheral wells contain antigen preparations of: (1) TBHS; (2) OT-11; (3) NM; (4) NL; (5) NHS.

The demonstration of circulating TAA in sera from tumor-bearing hamsters was achieved by three procedures. The purified anti-TAA reacted with TBHS showing a pattern of identity with the TAA of OT-1 and OT-11 (Fig. 2). The unadsorbed antiserum to TBHS reacted with the purified TAA of OT-11 (Fig. 3). Furthermore, the same antiserum adsorbed with NHS reacted with TBHS as well as with the crude OT-11 tumor antigen preparation but not with NHS (Fig. 4). Circulating tumor-associated antigens have been detected in sera of individuals with various human cancers (1, 2). Many of these antigens are also expressed in fetal tissue—"embryonic antigens" (2, 8). Investigations are in progress to determine if the circulating TAA in the serum of tumor-bearing hamsters share common antigens with fetal hamster tissues. The possible relationship of the TAA of the hamster sarcomas obtained after inoculation of HSV-2 to the viral antigens is also being actively pursued.

The present studies with HSV-2 associated hamster tumors have provided a model in which the immunoadsorption in-gel method has been developed and evaluated for the demonstration, purification and isolation of TAA and anti-TAA and the detection of circulating TAA. The adsorption in-gel technique may prove of value in the detection of circulating TAA in human cancers and be of potential diagnostic or prognostic use.

Summary. Tumor-associated antigens (TAA) were demonstrated in preparations of hamster sarcomas associated with Herpes simplex virus type 2, as well as in the sera of tumor-bearing hamsters. An immunoadsorption-in-gel method was employed to demonstrate and purify the TAA and anti-TAA. These results suggest the potential use of this technique for the demonstration of TAA or of anti-TAA in humans or animals with cancer.

1. Hellstrom, K. E., and Hellstrom, I. Adv. Cancer Res. **12**, 167 (1969).
2. Cinader, B., Med. Clin. N. America **56**, No. 3, 801 (1972).
3. Nahmias, A. J., Naib, Z. M., Josey, W. E., et al. Proc. Soc. Exp. Biol. Med. 134, 1065 (1970).

4. Nahmias, A. J., Roizman, B., New Engl. J. Med. **289,** 667 (1973).
5. Symposium "Herpesvirus and Cervical Cancer." Cancer Res. **33,** 1345 (1973).
6. Ibrahim, A. N., Proc. Soc. Exper. Biol. Med. **130,** 784 (1969).
7. Ibrahim, A. N., and Hammon, W. McD., J. Immunol. **100,** 93 (1968).
8. Laurence, D. J. R., and Neville, M. A., Brit. J. Cancer **26,** 335 (1972).

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