

# Studies of Tumor-specific and Herpesvirus Nonvirion Antigens<sup>1</sup>

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## Summary

Tumor-associated cell antigens have been identified for certain squamous carcinomas. One group of tumor antigens present on these cells is herpesvirus-induced nonvirion antigens. Another group of tumor cell antigens is also identified. Both groups of antigens produce cell-mediated immune reactions. Studies of their possible value as immunogenic and immunodiagnostic agents are indicated.

## Introduction

There are previous reports of the presence of group- and type-specific stable and unstable DNA adenovirus-induced nonvirion "T" antigens (6, 13, 18-20) and virus-induced nonvirion cell membrane tumor-specific transplantation antigens (5, 10) present in animal tumors. After developing further techniques, it was of interest to identify the nonspecific and specific normal and tumor-associated factors present on the cell membranes of fetal and adult normal human cells and of human cancer cells in order to determine their role in eliciting cell-mediated immune responses in the host. At first we chose to work with a direct test of skin reactions to various types of carefully separated cancer, fetal, and normal cell membrane antigens and to seek the very specific delayed hypersensitive reactions, analyzed by several criteria. Using these techniques we have been able to identify certain antigens such as those associated with intestinal cancer (7, 11) and leukemia (2, 8, 14).

## Materials and Methods

Materials and methods used for our work with herpesvirus nonvirion antigens have been described (9, 12, 15-17). A summary of recent procedures is shown in Table 1.

## Results and Discussion

As seen with several other types of cancer, crude cancer cell membrane sonicates do not produce delayed hypersensitive skin reactions, because of the presence of a blocking

factor (2), but a partially separated fraction may induce a specific (7, 8) response. The pellet of the insoluble sonically disrupted membrane material remaining is skin test negative when stepwise sequential low-frequency sonication at different intervals is used.

The partially separated skin-reactive cervical cancer cell membrane Sephadex G-200 fraction reacted in CF<sup>3</sup> tests for herpesvirus type 2-related antigens (9, 12). Tests with separated antigens of normal vaginal tissues and separated intestinal cancer antigens were negative. After further separation by gradient polyacrylamide gel electrophoresis using 4 stacked gels, it was found that gel Region 3-eluted material (see Chart 1) of these further separated fractions could be tested accurately with serial dilutions of cervical cancer sera (9).

Skin tests were conducted on groups of guinea pigs sensitized to herpesvirus types 1 and 2 or to cell control preparations (3, 4). Herpesvirus type 1 (KOS strain) produced a delayed hypersensitive skin reaction both in guinea pigs sensitized to herpesvirus type 2 (SAV) and in guinea pigs sensitized to herpesvirus type 1, whereas herpesvirus type 2 seemed to be more specific, using these particular strains.

Herpesvirus-induced nonvirion antigens have been described (28). Type and strain specificity studies are necessary (25, 26) with many herpesvirus isolates, and we continue to study the nonvirion antigens associated with newer strains. In these studies, we note that there appear to be differences between concentrations of the crude nonvirion preparations required for detection by CF and for production of antibodies.<sup>4</sup> In addition, several strains of herpesviruses types 1 and 2 were used in HEP-2, HEK, and fetal brain cell cultures, respectively, and the CF-reactive nonvirion antigens were compared.<sup>4</sup>

An association of herpesvirus type 2 with cervical cancer and of herpesvirus type 1 with lip cancer has been suggested by clinical and laboratory studies (21-24, 30). From pathologically evaluated lip cancer cells, the structurally intact components were gently removed from the membranes by sequential low-frequency sonication and the material was further separated by gel filtration and electrophoretic techniques. Again a specific antigen gave positive skin tests for delayed hypersensitive reactions. The lip and cervical carcinoma antigens were found to react specifically

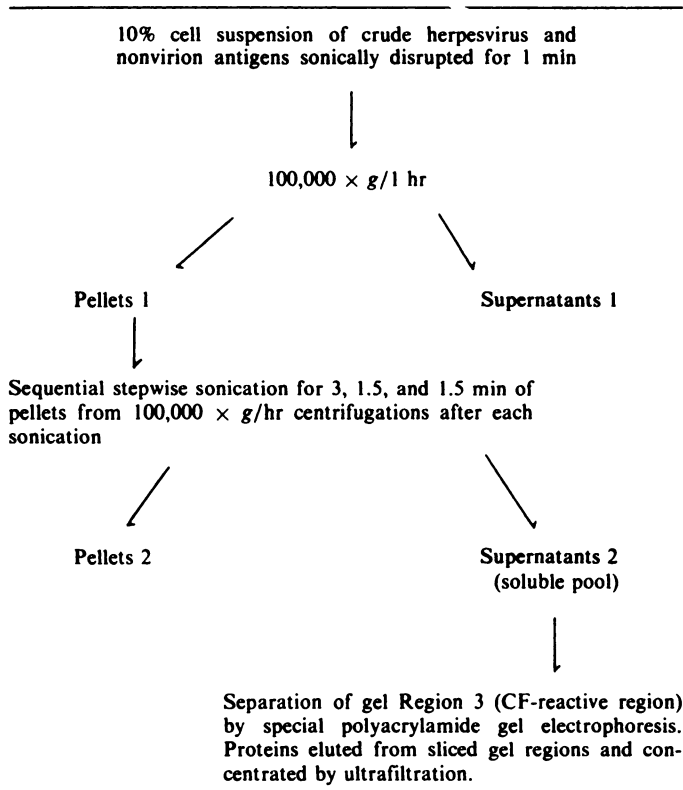
<sup>1</sup> Presented at the International Symposium on Human Tumors Associated with Herpesviruses, March 26 to 28, 1973, Bethesda, Md.

<sup>2</sup> Presented by.

<sup>3</sup> The abbreviation used is: CF, complement fixation.

<sup>4</sup> G. Tarro and A. Hollinshead, manuscript in preparation.

**Table 1**  
*Separation of CF-reactive components of herpesvirus nonvirion antigens from crude, early harvests of superinfected cell cultures*



in CF tests with antibody to herpesvirus nonvirion antigens (16). Conversely, human cervical cancer sera and matched controls, selected for lack of detectable antibody for herpesviruses by immunofluorescence, CF, and neutralization tests, were found to react with herpesvirus nonvirion antigens (16, 27) and with cervical carcinoma-separated antigens but not with the separated normal vaginal antigens (16); controls were nonreactive.

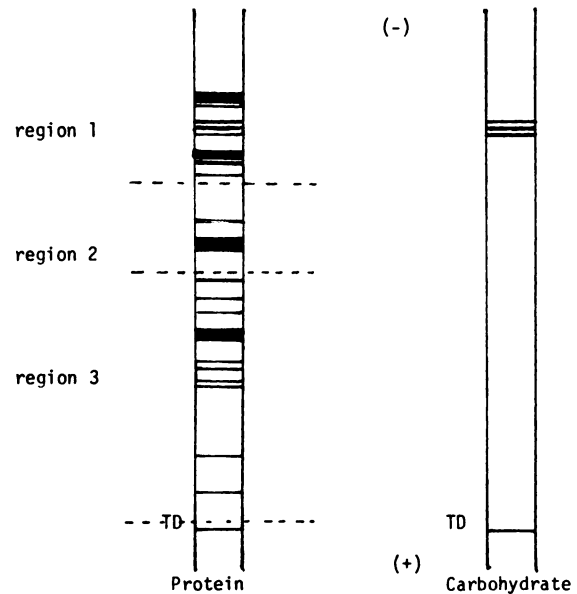
We acquired a larger tumor specimen and were able to have enough primary cancer cells for absorption of 2 of these cancer sera with the cancer cells. We also prepared herpesvirus type 2-infected HEp-2 cells, stored at 4° for 2 weeks so that the preparation would have virion antigens but be free of nonvirion antigens. We absorbed aliquots of 2 other cancer sera with this material, just in case we had not detected all of the structural virion components in these sera. The 2 sera absorbed with the cancer cells did not react with the nonvirion antigens and did not react with the cancer antigen. There was not enough sera for absorption with normal cells for control purposes. One of the sera absorbed with the 2-week-old crude virion preparation proved to be anticomplementary, but the other serum again reacted with the cervical cancer antigen.

Although the soluble portions of the herpesvirus nonvirion antigens and the cervical cancer antigens were quite different in composition, they did have components of identity (polyacrylamide gel electrophoresis Region 3)

which were necessary for CF reactivity (16). The gels are divided into 3 regions (1, 2, and 3; see Chart 1) for further analysis of these components. Many gels were electrophoresed, monitored by Coomassie brilliant blue staining of 1 gel for every 6 separated, carefully sliced, eluted, dialyzed, and tested according to our standard procedures.

Initially, 3 age- and sex-matched patients were selected for skin testing prior to radiation therapy. A patient with Stage 2A epidermoid carcinoma of the cervix, a patient with Stage 1 endometrial adenocarcinoma, and a patient with Stage 1 breast cancer were not allergic to skin tests with mumps and streptokinase-streptodornase. These patients were further skin tested with material eluted from Regions 1, 2, and 3 of the separated cervical cancer antigens and normal vaginal antigens. Positive tests of >5 mm induration at 48 hr were seen in the cervical cancer patient to Region 2 and Region 3 but not to Region 1 cervical cancer antigens. Tests of the 3 regions in the other 2 patients were negative. All tests to vaginal Regions 1, 2, and 3 were negative. Subsequent autologous and allogeneic skin tests of 15 randomly selected patients with squamous cervical carcinomas and 8 control patients with ductal breast carcinomas have verified these original observations.

As shown in Chart 1, periodic acid-Schiff staining for carbohydrates revealed the presence of glycoproteins in Region 1. Glycoproteins are absent from Regions 2 and 3. Since it would be difficult to test fetal counterpart cervical tissues, we cannot say whether these glycoproteins might indicate the presence of carcinoembryonic antigens, seen in a similar area in gel separations of fetal intestines and adult



**Chart 1.** Separation by gradient polyacrylamide gel electrophoresis using layered gel solutions of 10, 7, 4.75, and 3.5%. Tracking dye (TD) was bromphenol blue. Eighty- $\mu$ g protein aliquots of cervical cancer cell membrane sonicate Sephadex Fraction II were separated on each stacked gel preparation and stained with Coomassie brilliant blue for protein bands and with periodic acid-Schiff stain for carbohydrates. Unstained gels were sliced into 3 regions, as shown, for other tests (see text).

intestinal cancer antigens (11). Such studies are being conducted using fetal lip tissues.

The gel regions were routinely CF tested in order to determine which regions from these same preparations would react with herpesvirus nonvirion antisera. Cervical cancer gel Regions 3 were positive at 24 and 48 hr for CF reactivity with the antisera specific for herpesvirus nonvirion antigens. In earlier tests, crude fetal intestinal preparations occasionally gave a 24-hr positive CF test but were negative at 48 hr. However, purified gel regions of fetal and adult intestinal and breast cancer and normal vaginal, breast, and intestinal tissues were negative. Many tumors and cancer sera have now been analyzed for presence or absence of nonvirion antigens and antibodies, respectively.<sup>4</sup>

Therefore, cancer antigens share a common component with the nonvirion antigens, and this component is necessary for CF reactivity. It may be that this is a genetic marker for the presence of the virus genome within the cell (1). However, we also show that the activated virus gene expressions are accompanied by other new expressions in the cancer cell; namely, that there are other components involved in producing specific cell-mediated reactions in the cancer patient. This activation of other expressions occurring during oncogenesis must be studied in detail. If entire chromosome segments are activated, it is possible that some expressions may be incidental. The use of such markers will permit a further understanding of the role of the several herpesviruses in human cancer.

With this in mind, we are studying the immunogenicity of many tumor- and viral-associated antigens. We are also conducting many tests of the crude and purified nonvirion antigens for reactivity with a series of carefully chosen cancer and control sera.

One group of studies with herpes simplex virus-induced nonvirion antigens included 2 large-scale coded tests with National Cancer Institute sera and 1 large-scale test with Baylor Medical Center sera (15, 17). The persistent immune defects seen in squamous cell carcinomas of the cervix and the head and neck (29) may be explained by the role of herpesvirus in direct immunosuppression (17).

## References

1. Frenkel, N., Roizman, B., Cassai, E., and Nahmias, A. DNA Fragments of Herpes Simplex 2 and Its Transcription in Human Cervical Cancer Tissue. *Proc. Natl. Acad. Sci. U. S.*, *69*: 3784-3789, 1972.
2. Herberman, R., Lepourhiet, A., Hollinshead, A., Char, D., McCoy, J., and Leventhal, B. Humoral and Cell-mediated Immunity in Human Acute Leukemia. *In*: 26th Annual Symposium on Fundamental Cancer Research 1973, in press.
3. Hollinshead, A. Conference and Workshop on Cellular Immune Reactions to Human Tumors. *J. Natl. Cancer Inst.*, *37*: 206-208, 1972.
4. Hollinshead, A. Antigeni Solubili della Membrana Cellulare Specifica dei Tumori. *Aggiornamenti*, *8*: 8-16, 1972.
5. Hollinshead, A., and Alford, T. Identification of a Soluble Transplantation Antigen from the Membrane Fraction of Adenovirus Tumor Cells. *J. Gen. Virol.*, *5*: 411-418, 1969.
6. Hollinshead, A., Alford, T., Orozlan, S., Turner, H., and Huebner, R. Separation and Description of Adenovirus 12-induced Cellular Antigens Which React with Hamster Tumor Antisera. *Proc. Natl. Acad. Sci., U. S.*, *59*: 385-389, 1968.
7. Hollinshead, A., Glew, D., Bunnag, B., Gold, P., and Herberman, R. Skin Reactive Soluble Antigen from Intestinal Cancer-cell Membranes and Relationship to Carcinoembryonic Antigens. *Lancet*, *1*: 1191-1195, 1970.
8. Hollinshead, A., and Herberman, R. Identification and Characterization: Cell Membrane Antigens Specific for the Blast Phase of Leukemia. *In*: Proceedings of the Fourth International Symposium on Comparative Leukemia Research, Japan, 1973, in press.
9. Hollinshead, A., Lee, O. B., McKelway, W., Melnick, J. L., and Rawls, W. E. Reactivity between Herpesvirus Type 2-related Soluble Cervical Tumor Cell Membrane Antigens and Matched Cancer and Control Sera. *Proc. Soc. Exptl. Biol. Med.*, *141*: 688-693, 1972.
10. Hollinshead, A., McCammon, J. R., and Yohn, D. S. Immunogenicity of a Soluble Transplantation Antigen from Adenovirus 12-induced Tumor Cells Demonstrated in Inbred Hamsters (PD-4). *Can. J. Microbiol.*, *18*: 1365-1369, 1972.
11. Hollinshead, A., McWright, C., Alford, T., Glew, D., Gold, P., and Herberman, R. Separation of Skin-reactive Intestinal Cancer Antigen from the Carcinoembryonic Antigen of Gold. *Science*, *177*: 887-889, 1972.
12. Hollinshead, A., Melnick, J. L., and Rawls, W. E. Reactivity between Herpesvirus Type 2-related Soluble Membrane Antigens in Cells from Carcinomas of the Cervix, Vulva, and Vagina and Matched Cancer and Control Sera. *Intern. Virol.*, *2*: 105, 1971.
13. Hollinshead, A. C., Alford, T. C., Turner, H. C., and Huebner, R. J. Adenovirus Antibody and Antibody to "T" Antigen (Neoantigen): Location in Gamma Globulin. *Nature*, *211*: 423-424, 1966.
14. Hollinshead, A. C., and Herberman, R. B. Separation of the Major Histocompatibility Antigens from Other Antigens Present on Human Leukemic and White Blood Cell Membranes. Fifth International Symposium on Comparative Leukemia Research, Padova, Italy, *Bibliotheca Haematol.*, *39*: 828-837, 1971.
15. Hollinshead, A. C., Lee, O. B., Chretien, P. B., Tarpley, J. L., Rawls, W. E., and Adam, E. Antibodies to Herpesvirus Nonvirion Antigens in Squamous Carcinomas. *Science*, *182*: 713-715, 1973.
16. Hollinshead, A. C., and Tarro, G. Soluble Membrane Antigens of Lip and Cervical Carcinomas: Reactivity with Antibody for Herpesvirus Nonvirion Antigens. *Science*, *179*: 698-700, 1973.
17. Hollinshead, A. C., Tarro, G., Rawls, W., and Chretien, P. Herpesvirus Nonvirion Antigens: Relation to Squamous Cell Carcinomas. *In*: E. Kurstak (ed.), *Viral Immunodiagnosis*. New York: Academic Press, Inc., in press.
18. Huebner, R. J., Pereira, H. G., Allison, A. C., Hollinshead, A. C., and Turner, H. C. Production of Type-specific "C" Antigen in "Virus Free" Hamster Tumor Cells Induced by Adenovirus Type 12. *Proc. Natl. Acad. Sci. U. S.*, *51*: 432-439, 1964.
19. Huebner, R. J., Rowe, W. P., and Lane, W. T. Oncogenic Effects in Hamsters of Human Adenovirus Types 12 and 18. *Proc. Natl. Acad. Sci. U. S.*, *48*: 2051, 1962.
20. Huebner, R. J., Rowe, W. P., Turner, H. L., and Lane, W. T. Specific Adenovirus Complement-fixing Antigens in Virus-free Hamster and Rat Tumors. *Proc. Natl. Acad. Sci. U. S.*, *50*: 379, 1963.
21. Josey, W. E., Nahmias, A. J., and Naib, Z. M. Genital Infection with Type 2 Herpesvirus Hominis: Present Knowledge and Possible Relation to Cervical Cancer. *Am. J. Obstet. Gynecol.*, *101*: 718, 1968.
22. Kvasnicka, A. Relationship between Herpes Simplex and Lip Carcinoma. III. *Neoplasma*, *10*: 199, 1963.
23. Kvasnicka, A. Relationship between Herpes Simplex and Lip Carcinoma. IV. Selected Cases. *Neoplasma*, *12*: 61, 1965.
24. Rawls, W. E., Laurel, D., Melnick, J. L., Glicksman, J. M., and Kaufman, R. H. A Search for Viruses in Malignant Cervical Tissue. The Isolation of Herpes Viruses with Distinct Antigenic Properties. *Am. J. Epidemiol.*, *87*: 647, 1968.

25. Tarro, G. Studio Attuale e Futuro Sigli Antigeni Non Strutturali di Herpesvirus. *Giorn. Mal. Infettive Parassitarie*, 23: 752, 1971.
26. Tarro, G. Herpesvirus Nonvirion Antigens and Oncogenesis. *Proceedings of the Seventh Meeting of the European Tumor Virus Group*. Zierikzee, The Netherlands, 1972.
27. Tarro, G., and Battista, A. Presence of Antibody for Herpes Nonvirion Antigens in Sera of Cancer Patients. *In: Proceedings of the Second International Symposium on Cancer Detection and Prevention*, Bologna, Italy (*Excerpta Med.* 275 (abstr.): 120, 1973), in press.
28. Tarro, G., and Sabin, A. B. Virus-specific, Labile, Nonvirion Antigen in Herpesvirus-infected Cells. *Proc. Natl. Acad. Sci., U. S.*, 65: 753, 1970.
29. Twomey, P., Catalona, W., and Chretien, P. Cellular Immunity in Cured Cancer Patients. *Cancer*, in press.
30. Wyburn-Mason, R. Malignant Change following Herpes Simplex. *Brit. Med. J.*, 2: 615, 1957.

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*Cancer Res* 1974;34:1122-1125.

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