[CANCER RESEARCH 26 Part 1, 1185-1188, June 1966]

Host-Virus Relationships in Virus-induced Tumors and Transformed Cells[1]

ANTHONY J. GIRARDI

Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania

Introduction

There are 2 points that were mentioned briefly in Dr. Rauscher's presentation which I would like to comment upon, especially since they may bear on the problems and obstacles in determining the etiologic factors of Hodgkin's disease. They are: (*a*) viral interference and interferon production, and (*b*) development of neoantigens in cells of tumors induced *in vivo*, or cells transformed *in vitro*, by viruses.

Interference and Interferon Production

Dr. Rauscher has presented results of the work of Henle and Henle (16) with the EB2 cells derived from Burkitt lymphoma, stating that interferon is produced by these cells. This may suggest that a viral agent is carried by these cells, and obviously this is a promising lead in attempting to establish an etiologic factor. Several points seem pertinent concerning interferon production, and these may bring to light several important relationships between tumor-inducing agents and cells.

First, interferon or interferon-like substances have been produced by tissues exposed to a variety of agents other than viruses which include nonviral heterologous RNA from cells (26, 28), bacteria and endotoxin (17, 37), yeast (20), anionic polysaccharide (21), and phytohemagglutinin (38). This point is presented only to stress caution in interpreting results obtained from interferon studies as they apply to the problem of etiology and is not meant to challenge the data presented for the Burkitt lymphoma cells.

More important is the fact that interference may occur *without* the production of interferon, and such a situation exists in several virus-induced leukemias and sarcomas. For instance, in chicken cells, the growth of Rous sarcoma virus (RSV) may be inhibited by prior infection of the cells with viruses of the avian leukosis complex, *without production of interferon*; the interference seems to be due to the production of viral coat protein which prevents superinfection by a virus with a similar coat [see Rubin (29)], probably at the level of adsorption or penetration.

To carry this point further, and at the same time to present a method that could be applied to etiologic studies for leukemias, lymphomas, and Hodgkin's disease, I would like to discuss briefly results obtained by Plotkin *et al.* (24) during etiologic studies with human leukemic bone marrow. Earlier studies from our laboratory (19) indicated that the Bryan and Schmidt-Ruppin strains of RSV induced changes in human and simian tissue cultures. The question was then posed: If chicken leukosis viruses are capable of interfering with the growth of RSV in chick tissue culture, would human "leukosis agents" prevent development of RSV-induced lesions in primate tissue culture? In the study reported by Plotkin *et al.* (24), bone marrow aspirates obtained from the iliac crest of leukemic children were inoculated directly into tissue cultures of grivet monkey kidney cells (*Cercopithecus aethiops*). Treated cultures and control cultures were then trypsinized and subcultivated at weekly intervals. After 3 weeks the cultures treated with bone marrow and the control cultures were challenged with RSV and observed for the development of typical lesions. Marrows from 14 children with acute lymphocytic leukemia were studied. Interference of RSV effects were observed in 6 cases. Tests with 2 nonleukemic bone marrows indicated no such interference. The interference phenomenon in this example was not mediated by interferon. Although no claim was made that an agent had been isolated, the study is continuing and offers a method applicable to studying the etiology of lymphomas and leukemia.

Another example of interference in the absence of detectable interferon production occurred in studies with human cells that had been transformed by SV_{40} virus but which were no longer shedding infectious SV_{40} virus (Henle and Girardi, unpublished). Three of 12 transformed cell lines demonstrated interference with growth of poliovirus Type II and vesicular stomatitis virus. None of the cells produced a transmissible interferon.

In viral interference studies it is equally important to consider a shift in the cell population from one of susceptibility to one of resistance but not really mediated by a virus.

In the above examples, virus-induced transformed cells that demonstrated viral interference did so without the benefit of interferon. This fact standing alone probably has little significance. However, several reports indicated inhibition of interferon synthesis in cells treated with chemical and physical carcinogens (2–5, 27). The De Maeyers (2–4) showed that carcinogenic polycyclic hydrocarbons 3-methylcholanthrene, benzo(a)pyrene, and 7,12-dimethylbenz(a)anthracene inhibited interferon synthesis in rat embryo cell cultures. Chemically related but noncarcinogenic compounds, such as benzo(a)pyrene and pyrene had no effect. Rotem *et al.* (27) indicated that 3 lines of hamster embryo cells transformed *in vitro* with 3-methylcholanthrene and 3,4-benzpyrene produced less interferon than normal hamster embryo cells following infection with NDV and EMC viruses.

The De Maeyers (5) extended their studies to include carcino-

[1] This work was supported, in part, by USPHS Research Contract PH-43-62-157 from the National Cancer Institute.

Downloaded from cancerres.aacrjournals.org on April 16, 2021. © 1966 American Association for Cancer Research.

gens of a different nature. They found that ultraviolet light-irradiated rat embryo cell cultures showed depressed interferon production. They suggested the existence of a common target site for the chemical and physical agents, perhaps resulting in an impairment of cellular DNA function, since it has been shown that interferon synthesis is coded by the genome of the cells.

Thus, as a corollary of their ability to induce transformation of cells, chemical, physical, and viral agents may lead to inhibition of interferon synthesis even in situations where viral interference is noted. The absence or depressed synthesis of interferon in malignant tissue might be expected and certainly does not in itself indicate lack of viral etiology. Conversely, the production of interferon by malignant tissue grown *in vitro* could represent an activity of the exceptional tumor-inducing virus, or could be the result of an agent etiologically unrelated to the malignant state of the tissue. In this regard, it is of interest to note that oncogenic viruses which do not induce interferon production in the cells that they transform may be capable of producing interferon in the nontransforming, lytic cell systems. Though the underlying mechanism of this special host-virus relationship in the transformed cell is still obscure, the results of studies with interferon production or interferon inhibition might serve as an important clue toward understanding the initial events during transformation. If, as has been suggested, interferon production may be a feedback mechanism for control of RNA synthesis (38) and may be under the control of the genome of the host cell, its abrogation by either physical, chemical, or viral agents removes an important cellular control mechanism.

One last point concerning the interference phenomenon should be mentioned here, for again it may serve as a guide in etiologic studies in Hodgkin's disease. In the RSV-avian leukosis system, the agents responsible for interference of RSV growth also serve as helpers for maturation of the RSV in cells that carry the virus but do not produce infectious particles. In our studies (19) with RSV-infected human and simian cultures the lesions persisted in cultures subcultivated over long periods of time (now over 18 months), but infectious virus could not be recovered from these affected tissues. Based on what is known in the chick cell-RSV system (15), we posed a 2nd question: Did the RSV persist in the infected primate tissue in a "defective" form requiring a helper virus to complete its maturation? If the RSV in primate cells is in need of a helper virus, the search for the latter might encompass more than the use of avian leukosis viruses that act as helpers for RSV in chick embryo fibroblasts (15). If a defective helper virus system is species-specific, only the exposure of RSV-infected human and monkey kidney cultures to human leukemic material would result in appearance of infectious RSV. This method could demonstrate the presence of a "helper virus" in human leukemia. To date, examination of 10 leukemic marrows for this "helper" activity have been negative.

Neoantigens in Cells of Virus-induced Tumors or Transformed Cells

Investigations to date have established quite conclusively that certain malignant tissues may differ antigenically from those of corresponding normal host tissue. Such differences may result from antigenic simplification or loss, or antigenic fortification or gain. These problems are discussed at length in a review by Haddow (14). In addition, conclusive evidence has been presented indicating that tumors induced by viruses including polyoma, SV_{40}, Rous sarcoma, and adenoviruses possess new and probably nonviral antigens (18). Huebner has suggested the term neoantigen. Certain of these antigens appear to be synthesized by the cell under the influence of the viral genetic code, though they may be made in cells in the absence of infectious virus production. The antigens are detected by the complement fixation (CF) test (18) or through fluorescent antibody technics (25). Though the induced complement fixation antigens (ICFA) have been the ones most frequently examined, studies which measure parameters other than CF ability of cells suggest that more than 1 new antigenic component is present as a result of virus-induced transformation. This suggestion especially pertains to studies with the SV_{40}-induced, virus-free hamster tumors and cell cultures prepared from such tumors, which, following X-irradiation, are highly effective in preventing the occurrence of tumors in hamsters inoculated with SV_{40} at birth (9). Earlier studies with transplantable SV_{40}- and polyoma-induced tumors in mice and hamsters suggested the presence of a new foreign antigen which is specific and of the homograft type, being demonstrable only by resistance to tumor transplant challenge (1, 10, 12, 22, 30–35). Though the evidence might be considered more indirect, since it was obtained in studies in which adult animals were immunized with SV_{40} or polyoma viruses and later challenged with tumor cells, a possible explanation suggested that transformed cells developed as a result of virus inoculation. In turn, these cells produced an antigen that led to the development of tumor resistance. Habel (11) concluded that the homograft-type antigen in polyoma-transformed cells was not identical with polyoma ICFA. Since results of studies with SV_{40} ICFA suggest that it is located primarily in the cell nucleus (6, 25), one might think that the tumor rejection mechanism is directed against, or mediated by, another antigen.

Human cells transformed by SV_{40} possess ICFA, and this antigen is retained after the cultures have recovered from crisis (8, 13) and no longer shed infectious virus. Such SV_{40}-transformed *human* cells, free of infectious virus, were effective in preventing virus-induced tumors in hamsters even though the cells are of a species foreign to the experimental host (7). In these studies hamsters less than 24 hr old were inoculated with the virus while the immunizing cell preparations were injected i.p. during the latent period prior to tumor development. The prevention of virus-induced tumors was achieved with intact cells, whereas frozen and thawed or formalin-treated preparations at equivalent concentrations were not active in reducing tumor incidence. Since frozen and thawed preparations contained SV_{40} ICFA in high titer, an antigenic component other than SV_{40} ICFA, seemed to be responsible for the protective action. Similarly, freezing and thawing as well as formalin-treatment destroyed the immunizing potential of the SV_{40}-induced hamster tumor cells (7), even though in previous experiments the immunizing action of intact cells was unaffected by X-irradiation doses under 4000 r (9). Thus, at least 2 neoantigens (CF and homograft type) and possibly more may be present in cells transformed by virus. The presence of an enhancing phenomenon has been demonstrated in the SV_{40} hamster system (Girardi, in preparation). Sera from SV_{40} tumor-resistant animals were mixed with tumor cells and re-

Downloaded from cancerres.aacrjournals.org on April 16, 2021. © 1966 American Association for Cancer Research.

inoculated into the autologous serum donors. Such inocula led to formation of tumors in the previously "immune" host; a control inoculum of tumor cells and normal hamster serum placed on the opposite flank of the same animal was rejected completely. These results were confirmed by similar studies in which the mixtures of tumor cells and "immune" or normal sera were injected into normal hamsters. In these animals, tumors could grow at both inoculation sites; however, the tumor cell-"immune serum" inoculum led to earlier tumor formation and enhanced growth. It is possible, as suggested by Snell (36) and Möller (23), that surface antigen(s) are coated by the enhancing antibody from sera from immune animals, and this leads to an afferent inhibition of the protective homograft reaction of the host. Fluorescent antibody studies show that these enhancing sera in fact do coat the cells, and this may be due to the presence of surface antigens unrelated to the homograft-type antigen.

Dr. Rauscher referred in his talk to data that suggested the presence of an antigen detected by the fluorescent antibody technic, which might be common to murine and human leukemia cells. Current and future work in our laboratory is directed toward examining human and murine leukemia cells for common antigens of the homograft type by the ability of human leukemia cells to induce resistance in mice to challenge with virus-induced murine leukemia. Such studies could lead to clues concerning etiology, even in the absence of infectious virus in the human leukemic tissues or extracts.

References

1. Defendi, V. Effect of SV_{40} Virus Immunization on Growth of Transplantable SV_{40} and Polyoma Virus Tumors in Hamsters. Proc. Soc. Exptl. Biol. Med., *113:* 12–16, 1963.
2. De Maeyer, E., and De Maeyer-Guignard, J. Inhibition by 20-Methylcholanthrene of Interferon Production in Rat Cells. Virology, *20:* 536–39, 1963.
3. ———. Inhibition by 3-Methylcholanthrene of Interferon Formation in Rat-Embryo Cells Infected with Sindbis Virus. J. Natl. Cancer Inst., *32:* 1317–31, 1964.
4. De Maeyer-Guignard, J., and De Maeyer, E. Effect of Carcinogenic and Noncarcinogenic Hydrocarbons on Interferon Synthesis and Virus Plaque Development. *Ibid.*, *34:* 265–76, 1965.
5. ———. Inhibition of Interferon Synthesis and Stimulation of Virus Plaque Development in Mammalian Cell Cultures after Ultra-Violet Irradiation. Nature, *205:* 985–87, 1965.
6. Gilden, R. V., Carp, R. I., Taguchi, F., and Defendi, V. The Nature and Localization of the SV_{40}-induced Complement-fixing Antigen. *Ibid.*, *53:* 684–92, 1965.
7. Girardi, A. J. Prevention of SV_{40} Virus Oncogenesis in Hamsters. I. Tumor Resistance Induced by Human Cells Transformed by SV_{40}. *Ibid. 54:* 445–51, 1965.
8. Girardi, A. J., Jensen, F. C., and Koprowski, H. SV_{40}-induced Transformation of Human Diploid Cells: Crisis and Recovery. J. Cellular Comp. Physiol., *65:* 69–83, 1965.
9. Goldner, H., Girardi, A. J., Larson, V. M., and Hilleman, M. R. Interruption of SV_{40} Virus Tumorigenesis Using Irradiated Homologous Tumor Antigen. Proc. Soc. Exptl. Biol. Med., *117:* 851–57, 1964.
10. Habel, K. Resistance of Polyoma Virus Immune Animals to Transplanted Polyoma Tumors. *Ibid.*, *106:* 722–25, 1961.
11. ———. Specific Complement-fixing Antigens in Polyoma Tumors and Transformed Cells. Virology, *25:* 55–61, 1965.
12. Habel, K., and Eddy, B. E. Specificity of Resistance to Tumor Challenge of Polyoma and SV_{40} Virus Immune Hamsters. Proc. Soc. Exptl. Biol. Med., *113:* 1–4, 1963.
13. Habel, K., Jensen, F., Pagano, J. S., and Koprowski, H. Specific Complement-fixing Tumor Antigen in SV_{40}-Transformed Human Cells. *Ibid.*, *118:* 4–9, 1965.
14. Haddow, A. Immunology of the Cancer Cell: Tumor-specific Antigens. Brit. Med. Bull., *21:* 133–39, 1965.
15. Hanafusa, H., Hanafusa, T., and Rubin, H. The Defectiveness of Rous Sarcoma Virus. Proc. Natl. Acad. Sci. U. S., *49:* 572–80, 1963.
16. Henle, G., and Henle, W. Evidence for a Persistent Viral Infection in a Cell Line Derived from Burkitt's Lymphoma. J. Bacteriol., *89:* 252–58, 1965.
17. Ho, M. Interferon-like Viral Inhibitor in Rabbits after Intravenous Administration of Endotoxin. Science, *146:* 1472–74, 1964.
18. Huebner, R. J., Rowe, W. P., Turner, H. C., and Lane, W. T. Specific Adenovirus Complement-fixing Antigens in Virus-free Hamster and Rat Tumors. Proc. Natl. Acad. Sci. U. S., *50:* 379–89, 1963.
19. Jensen, F. C., Girardi, A. J., Gilden, R. V., and Koprowski, H. Infection of Human and Simian Tissue Cultures with Rous Sarcoma Virus. *Ibid.*, *52:* 53–59, 1964.
20. Jensen, K. E., Neal, A. L., Owens, R. E., and Warren, J. Interferon Responses of Chick Embryo Fibroblasts to Nucleic Acids and Related Compounds. Nature, *200:* 433–34, 1963.
21. Kleinschmidt, W. J., Cline, J. C., and Murphy, E. B. Interferon Production Induced by Statolon. Proc. Natl. Acad. Sci. U. S., *52:* 741–44, 1964.
22. Koch, M. A., and Sabin, A. B. Specificity of Virus-induced Resistance to Transplantation of Polyoma and SV_{40} Tumors in Adult Hamsters. Proc. Soc. Exptl. Biol. Med., *113:* 4–12, 1963.
23. Möller, G. Studies on the Mechanism of Immunological Enhancement of Tumor Homografts. I. Specificity of Immunological Enhancement. J. Natl. Cancer Inst., *30:* 1153–75, 1963.
24. Plotkin, S., Jensen, F. C., and Koprowski, H. Demonstration of Rous-interfering Agents from Human Leukemic Bone Marrow (abstr.). Federation Proc., *24:* 175, 1965.
25. Pope, J. H., and Rowe, W. P. Detection of Specific Antigen in SV_{40}-transformed Cells by Immunofluorescence. J. Exptl. Med., *120:* 121–28, 1964.
26. Rotem, Z. Production of Interferon in Cells Treated with a Foreign Nucleic Acid. Israel J. Exptl. Med., *11:* 174–78, 1964.
27. Rotem, Z., Berwald, Y., and Sachs, L. Inhibition of Interferon Production in Hamster Cells Transformed *in Vitro* with Carcinogenic Hydrocarbons. Virology, *24:* 483–86, 1964.
28. Rotem, Z., Cox, R. A., and Isaacs, A. Inhibition of Virus Multiplication by Foreign Nucleic Acid. Nature, *197:* 564–66, 1963.
29. Rubin, H. Virus Defectiveness and Cell Transformation in the Rous Sarcoma. J. Cellular Comp. Physiol., *64* (Suppl. 1)*:* 173–79, 1964.
30. Sjögren, H. O. Studies on Specific Transplantation Resistance to Polyoma-Virus-induced Tumors. I. Transplantation Resistance Induced by Polyoma Virus Infection. J. Natl. Cancer Inst., *32:* 361–74, 1964.
31. ———. Studies on Specific Transplantation Resistance to Polyoma-Virus-induced Tumors. II. Mechanism of Resistance Induced by Polyoma Virus Infection. *Ibid.*, *32:* 375–93, 1964.
32. ———. Studies on Specific Transplantation Resistance to Polyoma-Virus-induced Tumors. III. Transplantation Resistance to Genetically Compatible Polyoma Tumors Induced by Polyoma Tumor Homografts. *Ibid.*, *32:* 645–59, 1964.
33. ———. Studies on Specific Transplantation Resistance to

Downloaded from cancerres.aacrjournals.org on April 16, 2021. © 1966 American Association for Cancer Research.

Polyoma-Virus-induced Tumors. IV. Stability of the Polyoma Cell Antigen. *Ibid.*, *32:* 661–66, 1964.
34. Sjögren, H. O., Hellström, I., and Klein, G. Resistance of Polyoma Virus-immunized Mice to Transplantation of Established Polyoma Tumors. Exptl. Cell Res., *23:* 204–8, 1961.
35. ———. Transplantation of Polyoma Virus-induced Tumors in Mice. Cancer Res., *21:* 329–37, 1961.
36. Snell, G. D. The Suppression of the Enhancing Effect in Mice by the Addition of Donor Lymph Nodes to the Tumor Inoculum. Transplant. Bull., *3:* 83–84, 1956.
37. Stinebring, W. R., and Younger, J. S. Patterns of Interferon Appearance in Mice Injected with Bacteria or Bacterial Endotoxin. Nature, *204:* 712, 1964.
38. Wheelock, E. F. Interferon-like Virus Inhibitor Induced in Human Leukocytes by Phytohemagglutinin. Science, *149:* 310–11, 1965.

Downloaded from cancerres.aacrjournals.org on April 16, 2021. © 1966 American Association for Cancer Research.

AACR American Association for Cancer Research

Cancer Research
The Journal of Cancer Research (1916–1930) | The American Journal of Cancer (1931–1940)

Host-Virus Relationships in Virus-induced Tumors and Transformed Cells

Anthony J. Girardi

Cancer Res 1966;26:1185-1188.

Updated version	Access the most recent version of this article at: http://cancerres.aacrjournals.org/content/26/6_Part_1/1185.citation

E-mail alerts	Sign up to receive free email-alerts related to this article or journal.
Reprints and Subscriptions	To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.
Permissions	To request permission to re-use all or part of this article, use this link http://cancerres.aacrjournals.org/content/26/6_Part_1/1185.citation. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.

Downloaded from cancerres.aacrjournals.org on April 16, 2021. © 1966 American Association for Cancer Research.