Virions and Antigens of Herpes Virus Type 2 in Cervical Carcinoma

Laure Aurelian

Departments of Laboratory Animal Medicine and Microbiology, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

Summary

Herpesvirus type 2 (HSV-2) has been associated with squamous cancer of the human cervix on the basis of sero-epidemiological data. Findings providing information on the basis of the association of the HSV-2 genome with cervical cancer cells are presented. A herpesvirus isolated from degenerated cervical tumor cells grown in vitro is identified as a type 2 on the basis of biological and immunological properties. Surface antigens, HSV-2-specific, are observed in a small proportion of cells from a second culture of cervical tumor cells. Exfoliated but not biopsied tumor cells from patients with cervical carcinoma contain HSV-2 antigens, but virus particles are not observed. Finally, 90% of patients with cervical carcinoma have antibody to a HSV-2-induced antigen different from neutralizing antigens. This antibody, present only in 10% of a matched control group, appears to be of prognostic significance. The results are discussed in terms of the virus-host cell interaction. The data suggest that cervical tumor cells harbor the viral genome in a partially repressed state characterized by the expression of only some viral functions. Under conditions of stress, such as high pH, complete expression of the viral genome may occur resulting in the formation of viral components and/or infectious virus.

The search for the etiology of human tumors is complicated by the nature of the host. Evidence that viruses could cause tumors in man has been entirely dependent on the knowledge accumulated from work with animal models. The recent association of two human herpesviruses with tumors has opened new vistas in the study of virus-induced oncogenic processes in man.

Man harbors at least two (9) and possibly more (38) types of herpesvirus. It has been demonstrated repeatedly that the virus associated with genital infections HSV-2, differs from that isolated from eruptions on other parts of the body, HSV-1, with respect to physical, biological, and immunological properties (9). Infection with HSV-1 occurs between 6 months and 5 years of age and is usually inapparent. Primary infection with HSV-2 occurs later in life and is transmitted by sexual contact (25). The infection is generally inapparent and recovery occurs without serious consequences. Three factors make these infections unique. First, some of the individuals with primary infections are afflicted for the rest of their lives, despite the almost invariable presence of circulating antibody, with recurrent eruptions of the lip, cornea, or genitals. Second, recrudescences occur following a specific physical or emotional stress such as fever resulting from infection, exposure to sunlight or wind, menstruation, hormone treatment, or even psychotherapy. Lastly, recent data have indicated that latency (6, 37) is a function of the ability of the viral genome to persist in a repressed state.

The observation that cervical carcinoma essentially behaves like a venereally transmitted disease (32, 39), with a relatively long latency period (the mean age for preinvasive lesions is approximately 20 years younger than that of invasive cervical cancer) has led to the suspicion that HSV-2 might be its causative agent. The problem of the association of this virus with squamous carcinoma of the human cervix constitutes the focus of this presentation.

Clues Pointing to the Oncogenicity of Herpes Simplex Virus

The clues pointing to the oncogenicity of herpes simplex virus fall into four categories summarized in Table 1.

The first clue comes from work with cells in vitro. Thus, a high percentage of aneuploidy (14) and specific structural aberrations of the chromosomes (7) have been described in patients with preinvasive and invasive cervical cancer. In this context it is of particular significance that UV-attenuated HSV-2 can cause polyploidy in diploid human cervical cells but not in lymphoid cells (K. P. Katayama, L. Aurelian, and H. W. Jones, in preparation). Furthermore, it has been reported that cells infected with herpes simplex virus acquire the capacity to synthesize an antigen (designated G) characteristic of some human tumors as well as of cell lines of malignant origin (19). G antigen has not been found in cells of a variety of nonmalignant adult and embryonic human tissues (20). Second, there is guilt by association. Herpes-type particles have recently been shown to give rise to renal adenocarcinoma in frog embryos (22), to Marek’s disease of chickens (29), and to lymphoproliferative disease of monkeys (21). Likewise, they were found in association with cells from human lymphomas (11) described by Burkitt (Epstein-Barr virus). Thirdly, HSV-2, attenuated by UV irradiation, has been shown to transform hamster cells in vitro (10). Finally, both HSV-1 and HSV-2 have been associated with human tumors. Patients with...
The Etiology Hypothesis and Its Predictions

real diseases argues against the promiscuity interpretation or cocarcinogenic, played by HSV-2. Arguing against the cervical cancer as resulting from the causative role, direct cervical cancer groups should have a significantly higher disease rates, all or a large proportion of cases of invasive carcinoma are preceded by carcinoma in situ and invasive cancer (4, 33). Presence of DNA fragment of HSV-2 in an invasive cervical carcinoma (12).

severe and frequently recurring labial infections were reported to develop squamous cell carcinoma of the lip (40), and HSV-2 has been associated with cervical cancer on the basis of seroepidemiological studies (4, 25, 30, 33) indicating a significantly higher frequency of antibody to HSV-2 in patients than in control groups. The three possible interpretations of this association have been discussed previously (2). Briefly, the preferential hypothesis suggests that infection with the virus follows the development of the neoplastic lesion. Since, as indicated by studies of incidence and prevalence rates, all or a large proportion of cases of invasive carcinoma are preceded by carcinoma in situ and atypia (14, 16), it could be inferred from the preferential hypothesis that the prevalence of antibody to HSV-2 in patients with early neoplastic lesions is lower than that in invasive cervical cancer cases. The promiscuity hypothesis envisions the association of HSV-2 with cervical cancer as resulting from the promiscuous nature of the cervical cancer population (32, 39), which by definition is more apt to have all venereal diseases. Accordingly, compared to control populations, cervical cancer groups should have a significantly higher incidence of venereal diseases other than HSV-2. Lastly, the etiology hypothesis interprets the association of HSV-2 with cervical cancer as resulting from the causative role, direct or cocarcinogenic, played by HSV-2. Arguing against the preferential hypothesis is the observation that the frequency rate of antibody to HSV-2 is identical in preinvasive and invasive cervical cancer (4, 33), whereas the lack of association in our population between cervical cancer and other venereal diseases argues against the promiscuity interpretation (33).

The Etiology Hypothesis and Its Predictions

On the basis of in vitro work, it has been suggested that herpesvirus persistence in infected cells depends on the early arrest of virus multiplication (31). The rationale for assuming the arrest must occur early is that it must take place before expression of those functions causing the inhibition of host macromolecular synthesis, an essential prerequisite for the synthesis of structural components of the virus (3). A sufficient explanation for neoplastic transformation of cells harboring this persistent viral genome would be that the neoplastic character of the cell depends directly on the presence of virus-specific protein(s) coded by the latent viral genome and designated Ca-protein. Tumor formation would be a function of secondary effects on the course of differentiation and evolution of the new cell type. The concept that evolves from these interpretations of the mechanisms of association of HSV-2 with cervical epithelial cells is that of a viral genome at different levels of transcription (Table 2). Accordingly, latently infected cells are expected to harbor the viral genome in a completely repressed state. Attempts at virus isolation from women with cytopathological evidence of HSV-2 infection in the interim between recrudescences failed to yield virus (L. Aurelian and H. J. Davis, unpublished data), and viral structural components could not be detected in these cells (34). Finally, Ca-protein is not expected in these cells. Primary infection and recurrences are at the other end of the spectrum; they result from a totally derepressed viral genome, suggesting that infected cervical cells contain virus structural components (proteins and DNA) as well as infectious virus. In contrast to the situation observed in the interim between recrudescences, virus can be isolated with relative ease from women displaying cytopathological evidence of herpesvirus infection (L. Aurelian and H. J. Davis, unpublished data). Finally these concepts predict that virus-induced transformation would be associated with either (a) DNA sequences corresponding to only part of the HSV-2 genome or (b) a partially transcribed HSV-2 genome. In either case tumor cells are expected to contain viral DNA as well as virus-specific RNA and Ca-protein(s).

It is well established that recurrences are induced by a variety of stress conditions. A priori, it would be expected that since stress can induce the expression of the genetic potentiality of a totally repressed genome, it should also be able to induce that of a partially repressed one, if that be the HSV-2 genome present in tumor cells. Thus, viral structural components should be present in stressed but not in unstressed tumor cells.

HSV-2 Isolated from Cervical Tumor Cells Grown in Tissue Culture

Two lines of cervical tumor cells were studied, S332G, established from a biopsy of an intraepithelial cervical lesion, and 614, established from a biopsy of an invasive tumor. Electron microscopy of thin sections revealed large epithelioid cells with elaborate microvilli and packed fibrils, 3 to 8 nm in diameter and consistent with tonofibrils of squamous cells. Three series of experiments were done in order to test the predictions of the etiology hypothesis and establish evidence for the persistence of the viral genome in these cells. First, extracts of the cervical tumor cells were serially
S332G cells at the same transfers in duplicate cultures revealed virus particles with specific herpesvirus morphology at the 10th, 12th, 15th, and 18th transfers. The degenerated and complete or incomplete virions. Cellular degeneration evidence of virus, cytoplasmic and surface viral antigens, filamentous intranuclear structures 20 to 22 nm in diameter and characteristic nuclear alterations (Fig. 1). The relatively high number of unenveloped cytoplasmic particles and the filamentous intranuclear structures 20 to 22 nm in diameter were consistent with those reported for HSV-2 infected cells (35). S332G cells at the same transfers in duplicate cultures that had not degenerated (viable) did not react with Ra-2 sera, failed to induce cytopathogenic effects and yield virus when passed on HEp-2 cells, and virus particles were not observed on electron microscopic examination. The immunological specificity of the four isolates from the degenerated S332G cells as HSV-2 was further ascertained by the observation that Ra-2 sera were more effective in neutralizing the isolates than anti-HSV-1 sera (6).

Since spontaneous degeneration was consistently associated with an increase in the pH of the medium, an experiment was designed to inquire into the effect of pH on virus induction. S332G cells were grown for 3 weeks in the presence or absence of a 5% CO₂ atmosphere. The pH of the medium was determined at 2-hr intervals for the first 10 hr after transfer and once a day during the next 5 days. Normal growth and an even pH of 7.2 to 7.3 were characteristic of the cultures grown under CO₂ atmosphere, whereas extreme pH ranges (7.7 to 8.7) and poor growth were observed in cultures grown without CO₂. At the end of 3 weeks, typical cytopathogenic changes were observed in cultures grown without CO₂, and the cells stained preferentially with Ra-2 serum. Virus isolated from these cells on HEp-2 cultures was identical to HSV-2 in terms of plaque morphology and immunological specificity as determined by plaque reduction neutralization tests. However, similar results were not obtained with 614 cells. Studied at the 3rd and 8th transfers, 614 cells failed to show evidence of infectious virus, as well as virus particles and cytoplasmic virus antigens. However, 2% of 614 cells contained surface HSV-2

### Table 2

#### Predicted HSV-2 components in squamous cells of the human cervix

<table>
<thead>
<tr>
<th>Virus components</th>
<th>Normal squamous cells</th>
<th>Tumor cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Productive infection</td>
<td>Nonproductive infection</td>
</tr>
<tr>
<td></td>
<td>primary or recurrence</td>
<td>&quot;latency&quot;</td>
</tr>
<tr>
<td>1. Infectious virus</td>
<td>+⁺</td>
<td>-⁻</td>
</tr>
<tr>
<td>2. Particles (complete or incomplete)</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>3. Structural antigens</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>4. Ca-protein(s)</td>
<td>(?)⁺</td>
<td>-</td>
</tr>
<tr>
<td>5. Viral RNA</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>6. Viral DNA</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

* This prediction is based on the hypothesis that explains virus persistence in latently infected cells as dependent on the arrest of virus multiplication at some early point before it expresses the functions responsible for inhibition of host macromolecular synthesis (32). Under this hypothesis, virus can be made to manifest itself in the latently infected cells by exposure of the cells to conditions of stress. Exfoliated tumor cells, having been exposed to alkaline medium (23) and complete or partial malnutrition, are expected to contain virus particles and even infectious virus. Unsettling in terms of these predictions are 2 clinical observations. First, herpetic lesions are not observed together with cervical neoplasia, and, second, herpetic inclusion bodies are never observed in exfoliated tumor cells but only in normal squamous cells (27). Indeed, electron microscopy and isolation studies failed to show the presence of virus particles or infectious virus in exfoliated tumor cells (Ref. 5; Fig. 3).

* A sufficient explanation for the induction of tumors in people with latent HSV-2 infection would be that the neoplastic character of the cell depends on the presence of virus-specific protein(s) coded by the latent viral genome. These protein(s), designated Ca-protein(s), would be virus- and tumor-specific, raising speculation as to the possibility of their being synthesized also during the productive infection. If Ca-protein(s) result from the expression of information different from the one expressed in the productive infection, they should be associated only with tumor cells; on the other hand, if Ca-protein(s) result from the unregulated expression of the same genetic information, they should be present in the productive infection as well, provided this is studied at the time interval of maximum synthesis. Studies with oncogenic DNA viruses have indicated that virus- and tumor-associated antigens are also made during the productive infection. It should be pointed out, however, that despite their presence in transformed cells, the role these antigens play in oncogenesis is still unknown; the crucial question of the nature of the viral gene function responsible for transformation has yet to be answered. The studies described in this paper indicate that some complement-fixing antigen(s), tumor- or virus-specific, also appear to be made early in the productive infection.

⁺, present; ⁻, absent.
antigens (Fig. 2) as determined by membrane fluorescence (24) using a human serum containing antibody to HSV-2. Staining was not obtained with a human serum without antibody to either HSV-1 or HSV-2. To date, 614 cells were transferred only 12 times; spontaneous degeneration with concomitant virus release was not observed and virus could not be induced by exposure to media of high pH or containing halogenated pyrimidines.

Critical to the interpretation of our data is the failure to detect the presence of herpesvirus in viable S332G cells either prior to the onset of cell degeneration or in replicate cultures which continued to grow beyond the time of appearance of cell degeneration. In none of the viable cultures tested from the 3rd to the 50th passage were herpesvirus antigens detectable by fluorescent antibody staining; nor was there any cytopathogenic effect on HEp-2 cells or evidence of virus by electron microscopy after 5 serial passages of S332G extracts in such cells. Therefore, there was no evidence for contaminating herpesvirus in the original specimen and no indication of a "chronic" infection (13). The most likely interpretation of our findings is that S332G cervical carcinoma cells are "latently" infected with HSV-2; under certain conditions (such as high pH) virus replication is induced and infectious virus made. This interpretation implies that some or all of the S332G cells harbor the viral genome in a repressed state. However, the presence of HSV-2 specific surface antigens in 2% of 614 cells that are not stressed suggests that even though the HSV-2 genome persists in at least a small number of 614 cells, its association with those cells differs from that observed in S332G cells.

HSV-2 Antigens and Cervical Tumor Cells in Vivo

The difference in the function of animal cells in the artificial in vitro environment of cell culture and in the whole animal (31) raises the question of the nature of the association of HSV-2 with cervical neoplastic cells in vivo. This study is made possible by the observation that prior to exfoliation, tumor cells on the surface of the neoplastic lesions are exposed to suboptimal conditions consisting of partial malnutrition and exposure to glandular secretions of relatively high pH (23). Thus, tumor cells in biopsy specimens, frozen immediately upon collection, constitute unstressed tumor cells whereas exfoliated tumor cells are their stressed counterparts. Frozen sections and impressions of biopsy specimens (biopsied tumor cells) were obtained from 29 patients with preinvasive and invasive cervical carcinoma. Exfoliated cells were collected from the same patients at the same time by the impression of a small wet sponge directly to the surface of the neoplastic lesion or by the irrigation method of Davis (8) using 0.1 M PBS, pH 7.1. Slides were fixed in cold methanol (−40°) for immunofluorescent staining and 4% glutaraldehyde for electron microscopy. To inquire into the presence of HSV-2 antigens in the cervical tumor cells, slides were stained in duplicate with Ra-2 sera and PBS instead of antibody. Fluorescence was not observed in biopsied neoplastic cells from all patients studied in this series (4). Furthermore, electron microscopy of cells from patients 553 and 565 (Table 3), did not reveal virions and cytoplasmic changes previously shown to be associated with synthesis of herpesvirus antigens (28). On the other hand, tumor cells on the surface of the neoplastic lesion (2) as well as exfoliated tumor cells (5) from 25 of the 29 (86%) patients studied in this series reacted with Ra-2 sera. The proportion of reactive cells was independent of the stage of the disease but varied with the patient and ranged between 0 and 40%. Fluorescence appeared as a diffuse cytoplasmic mass, sometimes with granules; nuclear fluores-
The reaction appears to be specific for both HSV-2 and cervical tumor cells. Exfoliated cervical cells from control subjects did not show evidence of herpesvirus antigens unless they were diagnosed as herpetic cervicitis (34). Negative cases also included squamous metaplasia of the cervix, mesodermal tumor of the endometrium, cells from pleural effusions from lymphosarcoma, ovarian embryonal cell carcinoma and embryonal rhabdomyosarcoma, and, finally, impressions of 4 cases of breast carcinoma. The reaction was considered specific for HSV-2 antigens since (a) Ra-2 sera reacted with HSV-2-infected but not uninfected HEp-2 cells; (b) staining was not observed with PBS, preimmunized rabbit sera adsorbed with uninfected HEp-2 cells, and antisera to adenovirus 18 and Mycoplasma orale, whereas it was present in cells stained with antisera to HSV-1 displaying the expected cross-reactivity (9); (c) anaplastic cells reacted with 2 human sera from subjects without cancer but with neutralizing antibody to HSV-2 and did not react with a human serum devoid of antibody to either HSV-1 or HSV-2, and (d) adsorption of Ra-2 sera with HSV-2-infected HEp-2 cells, until they failed to stain HSV-2-infected cells, removed their reactivity for atypical cells. Nonspecific staining was absent in 90% of patients studied in this series. Considering the likely hypothesis that the only difference between exfoliated tumor cells and those in biopsy specimens obtained from the same patients at the same time is the exposure of the tumor cells on the surface of the neoplastic lesions to conditions of stress (23), the presence of HSV-2 antigens in exfoliated but not biopsied tumor cells suggests that the cells harbor the viral genome in a repressed state. This interpretation is consistent with the observation that cells removed from the tumor biopsy and grown in culture do not show the presence of HSV-2 antigens unless maintained in medium of high pH (6).

Twenty anaplastic cells (Patients 565, 593A, and 1149) containing fluorescent antigens and examined by electron microscopy did not reveal the presence of complete or incomplete virus particles (5). However, changes associated with the synthesis of herpesvirus antigens (28) and absent from biopsies, were seen. These consisted of cytoplasmic vesicles, fragmentary membrane-like structures and irregular electron-dense granules (Fig. 3) as well as masses of closely packed osmiophilic bodies probably representing the fluorescent cytoplasmic granules. Thus, unlike the culture situation (6), nuclear fluorescence and complete or incomplete virus particles are absent from exfoliated anaplastic cells. Priori, the absence of virus particles in cells containing virus antigens is unexpected. One possible interpretation is that a complete viral genome does not persist in these cells; alternatively, in atypical cells migration of the virus proteins to the nucleus does not occur. Thus, arginine deprivation of herpesvirus-infected cells causes the absence of nuclear fluorescence and inhibits the formation of virus particles (7), although the major viral proteins are synthesized and accumulate in the cytoplasm; their migration to the nucleus does not occur (18).

### Reactivity of Human Sera with “Early” Antigens Induced by HSV-2

The rationale for studying the presence in human sera of antibody to early HSV-2 antigens is twofold. First, the close similarity of genital and oral strains of the virus has greatly complicated the task of evaluating the results of those seroepidemiological studies indicating an association between HSV-2 and cervical carcinoma; the relatively high percentage of women without cancer but with antibody to HSV-2 has raised doubts further about the validity of this association. Second, according to the etiology hypothesis, the neoplastic character of the cells is a direct function of the presence in tumor cells of virus-coded proteins designated Ca-protein(s). Priori, it seems reasonable to assume that if Ca-protein(s) are not unique components, they are similar to “early” antigens (Table 2) the synthesis of which occurs before onset of DNA replication (31). To test these interpretations, a modification of the micro-
Laure Aurelian

Fig. 3. A, exfoliated tumor cells from Patient 1149 with invasive carcinoma of the cervix stained with Ra-2 serum display diffuse cytoplasmic fluorescence. Normal squamous exfoliated cells (N) do not stain. × 480. B, electron micrograph of one of the fluorescent atypic cells in A. Note cytoplasmic vesicles, disorganized membranous structures, and dense granules. C, cytoplasm; N, nucleus. × 18,500.

Quantitative complement fixation test of Wasserman and Levine (17) was adapted to the herpesvirus system. The antigens used were: AG-4, a crude extract of HEp-2 cells infected with HSV-2 for 4 hr, and a control antigen (AG-H) consisting of a similarly prepared extract of uninfected HEp-2 cells. Sera were obtained from 80 patients diagnosed as atypia (20 subjects), carcinoma in situ (18 subjects), and invasive cervical cancer (42 subjects) and 80 control subjects from age 20 to 80 years, admitted to the Johns Hopkins Hospital for illness unrelated to cervical cancer and having a negative history of cancer. These were matched to the carcinoma groups for age, race, and socioeconomic class according to economic deciles of the resident census tracts for the City of Baltimore. Of the invasive cancer patients, 21 women were untreated at the time of blood collection, whereas 21 had therapy 2 months to 19 years prior to blood collection. Only one of these (Patient 60) had histological evidence of recurrent neoplastic disease. A third group consisted of women with cancers at sites other than the cervix. At the time of blood collection, all subjects were free of active genital herpetic infection as determined by Papanicolaou smears and pelvic examination. The test was as previously described (L. Aurelian, B.S. Schumann, R. L. Marcus, and H. J. Davis, Science, in press). Essentially adapted to a reaction volume of 0.350 ml, it includes an extra incubation period (20 min at 37°C) for added sensitivity. Results, expressed as percentage fixation, are calculated by subtracting the A of reaction mixture from the antibody control A to establish the fixed A. Percentage fixation is computed by dividing the fixed A by the antibody control A. A reaction is considered positive if more than 10% of complement is fixed. It has previously been shown that sera from patients with various chronic diseases have antibody to nucleic acids; furthermore, 17% of all hospitalized patients are positive for antibody to denatured DNA (15). Accordingly, all sera reacting with both AGH and AG-4 were considered negative for AG-4; sera positive for either 1 of the 2 antigens were recorded. These interpretations appeared warranted as they increased the specificity of the test. Among the 200 subjects studied in this series only 35 (15%) had antibody to AGH. Of these, 73% reacted with both A-4 and AGH.

Two features emerge from the results summarized in Table 4. First, the prevalence of antibody to AG-4 was significantly higher in patients with invasive carcinoma (90%) than in a matched control group (10%). It is of particular significance that although the frequency of antibody to HSV-2 (as determined by neutralization tests) is higher in cases than controls, the prevalence of this neutralizing antibody in control women (67%) is significantly higher than that of antibody to AG-4 in the same control groups. The 2nd feature emerging from the results summarized in Table 4, is the absence of antibody to AG-4 in patients with cervical cancer treated between 2 months and 19 years (mean of 4 years) prior to blood collection; treatment did not affect the presence of antibody to HSV-2 in these patients. A priori, it would be expected that antibody to HSV-2 would be reduced by hysterectomy and eventually completely disappear. It is not clear, therefore, whether the presence of neutralizing antibody in these patients is a function of the
HSV-2 and Cervical Carcinoma

relatively shorter interval between therapy and blood collection (mean of 2.5 years) or the presence of latent infection at sites other than the uterus.

These results suggest that (a) antibody to AG-4 differs from neutralizing antibody which is directed against the virus itself and (b) antibody to AG-4, unlike the neutralizing antibody, is tumor specific. The tumor specificity of AG-4 antibody is further ascertained by the observation that its prevalence shows the expected gradation of the development of cervical neoplasia (14, 15). Thus, of 20 women with cervical atypia 7 (35%) and of 18 women with carcinoma in situ, 12 (67%) had antibody to AG-4, as compared to 90% of women with invasive cancer (Table 5). Antibody to AG-4 was not observed in all matched control women, nor in women with tumors at sites other than the cervix.

Is the antigen responsible for inducing antibody to AG-4 in women with cervical cancer the product of expression of a latent HSV-2 genome? Two observations argue in favor of the viral nature of this antigen. First, sera positive for AG-4 do not react with the antigen from uninfected cells, and, second, AG-4 is made in vitro after infection of HEp-2 cells with HSV-2; synthesis of AG-4 starts at 4 hr after infection and lasts for 14 hr (L. Aurelian, manuscript submitted for publication). This evidence, however, does not exclude the possibility that, even in vitro, AG-4 is a derepressed cellular antigen and the antibody detected in cancer patients is essentially directed towards a cellular-coded antigen, such as the carcinoembryonic antigens. Arguing against this interpretation is the absence of antibody to AG-4 in sera from patients with adenocarcinoma of the stomach and pancreas (Table 5) both of which contain carcinoembryonic antigens. The exact nature of AG-4 is not clear at present; it seems reasonable to assume that it is a mixture of antigens of possibly different activities, the individual role of which in the reactivity of patients sera must await further elucidation. Studies now in progress in our laboratory are directed towards purification and characterization of AG-4 in an effort to answer some of these questions.

### Table 4

<table>
<thead>
<tr>
<th>Group</th>
<th>No. tested</th>
<th>Mean age</th>
<th>Mean economic decile</th>
<th>Positive for AG-4</th>
<th>Positive for AG-H</th>
<th>Positive for HSV-2</th>
<th>Positive for HSV-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Un-treated invasive cancer</td>
<td>21</td>
<td>52</td>
<td>4.4</td>
<td>19</td>
<td>90</td>
<td>0</td>
<td>21</td>
</tr>
<tr>
<td>Matched controls</td>
<td>21</td>
<td>50</td>
<td>4.7</td>
<td>2</td>
<td>10</td>
<td>5</td>
<td>24</td>
</tr>
<tr>
<td>Irradiation</td>
<td>12</td>
<td>53</td>
<td>3.8</td>
<td>1</td>
<td>8</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>Hysterectomy</td>
<td>9</td>
<td>46</td>
<td>4.7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total treated</td>
<td>21</td>
<td>50</td>
<td>4.3</td>
<td>1</td>
<td>5</td>
<td>1</td>
<td>5</td>
</tr>
</tbody>
</table>

### Table 5

<table>
<thead>
<tr>
<th>Group</th>
<th>No. tested</th>
<th>Mean age</th>
<th>Mean economic decile</th>
<th>Positive for AG-4</th>
<th>Positive for AG-H</th>
<th>Positive for HSV-2</th>
<th>Positive for HSV-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atypia</td>
<td>20</td>
<td>34</td>
<td>3.0</td>
<td>7</td>
<td>35</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Matched controls</td>
<td>20</td>
<td>31</td>
<td>3.2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Carcinoma in situ</td>
<td>18</td>
<td>39</td>
<td>3.4</td>
<td>12</td>
<td>67</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Matched controls</td>
<td>18</td>
<td>35</td>
<td>3.4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Invasive cancer</td>
<td>21</td>
<td>52</td>
<td>4.4</td>
<td>19</td>
<td>90</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Matched controls</td>
<td>21</td>
<td>50</td>
<td>4.7</td>
<td>2</td>
<td>10</td>
<td>5</td>
<td>24</td>
</tr>
<tr>
<td>Total cases</td>
<td>59</td>
<td>42</td>
<td>3.6</td>
<td>38</td>
<td>64</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total controls</td>
<td>59</td>
<td>39</td>
<td>3.8</td>
<td>2</td>
<td>3</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>Carcinoma of other sites</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vagina</td>
<td>1</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Vulva</td>
<td>1</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pancreas</td>
<td>1</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Stomach</td>
<td>1</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Others</td>
<td>14</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>18</td>
<td></td>
<td></td>
<td>0</td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Conclusions and Perspectives

This paper dealt with the association of HSV-2 with cervical carcinoma. The persistence of herpes simplex virus in man as a model system for the interaction of herpesviruses with their host cells has been discussed in detail by Roizman (31). Considerably less information exists as to the mechanisms of association of herpesvirus with tumor cells. The data presented in this paper support our interpretation (Table 2) that some or all tumor cells harbor the viral genome in a partially repressed state resulting in the presence in these cells of cancer-associated protein(s), and, under stress, in virus-specific components. Biopsied cervical tumor cells in vivo or grown in tissue culture (S332G cells) do not show evidence of virus presence unless previously exposed to certain conditions of stress such as high pH. This unity in behavior is not tarnished by apparent differences. Thus, HSV-2-specific surface antigens are observed in unstressed 614 cells. Furthermore, whereas in S332G cells virus induction consists in the synthesis of virus structural components as well as the formation of infectious virus and is accompanied by the destruction of the host cells, virus cannot be induced in 614 cells, and, in vivo, virus induction does not proceed beyond the stage of synthesis of structural viral proteins. Active herpetic lesions of the cervix are rarely if ever observed in association with cervical neoplasia (H. J. Davis, personal communication), and herpes-induced cellular changes associated with virus formation have never been observed in exfoliated cervical tumor cells (27, 34). These observations are in agreement with the data indicating the presence of herpesvirus antigens but not virus particles in exfoliated cervical tumor cells. It is possible that the abortion of the virus replicative cycle beyond synthesis of virus antigens is due to the short life-span of the exfoliated cells, or that, as shown (18) for arginine-deprived herpesvirus-infected cells, migration of the proteins to the nucleus does not occur in exfoliated tumor cells, thus precluding virus formation. Finally, the possibility cannot be excluded that only a portion of the viral genome persist in cervical tumor cells; in this event virus could not be induced in either exfoliated tumor cells or in 614 cells. DNA sequences corresponding to part of the HSV-2 genome were indeed reported by Frenkel et al. (12), in an invasive cervical tumor. It might be significant, therefore, that whereas S332G cells originate from a case of carcinoma in situ 614 cells were derived from an invasive cervical lesion. The possibility that neoplastic development might be a function of the state of persistence of the viral genome should be given serious consideration. Experiments, now in progress in our laboratory, are directed towards detection of viral DNA in these cells and elucidation of some of these questions.

The findings presented in this paper strengthen the association of HSV-2 with cervical carcinoma; however, is HSV-2 responsible for the tumor in which it is found? The answer is not clear, and the evidence other than that which has been cited is very meager; it is based on the apparently prognostic significance of the antibody to AG-4. Three patients with invasive cervical cancer and one with carcinoma in situ were followed prior to and after cesium therapy. The results summarized in Chart 1 clearly indicate that 2 patients who recovered, i.e., do not show evidence of recurrent neoplasia (102 and 20), do not have antibody to AG-4, whereas recurrent neoplastic disease is associated with continuous presence of antibody to AG-4. The interval that must elapse before antibody to AG-4 disappears is unknown at present and requires further investigation.

Whatever the ultimate interpretation of the studies on the nature of AG-4, it would be quite difficult to explain how changes in clinical status, particularly those of prognostic value, would be provided by antibody to a passenger virus, if this is the role played by HSV-2 in cervical carcinoma. On the other hand, HSV-2 is widespread and readily isolated throughout the world, yet cervical cancer is not quite that common and varies in frequency rates in different populations (41). The possibility cannot be excluded that the specific cell harboring the virus, and the expression of those viral genes needed to transform cells, even if they contain information for an antigen similar or identical to AG-4, are singly or both determined by the genotype of the host.

Acknowledgments

Deepest gratitude is due Dr. R. L. Marcus for his continued advice and help with the complement fixation test, to Dr. H. J. Davis and Dr. C. Julian for collection of sera and cervical tumor samples, and to Dr. J. D. Strandberg and Dr. L. V. Meléndez for their help and collaboration. The expert technical support of B. Schuman and P. Reed are gratefully acknowledged.
References

Virions and Antigens of Herpes Virus Type 2 in Cervical Carcinoma

Laure Aurelian

Cancer Res 1973;33:1539-1547.

Updated version  Access the most recent version of this article at: http://cancerres.aacrjournals.org/content/33/6/1539

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, contact the AACR Publications Department at permissions@aacr.org.