Serological Response Patterns to Herpes Virus Type 2 Early and Late Antigens in Cervical Carcinoma Patients

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ABSTRACT

The frequency of antibodies to herpesvirus type 2 early antigen (AG-4) is significantly greater in women with carcinoma of the cervix than in matched cancer controls. The present study was designed to determine whether AG-4 seronegativity in a minority of women with cervical carcinoma is dependent on time or method of treatment and whether or not the serological profile is correlated with prognosis. Anti-AG-4 complement-fixing antibodies were assayed in 34 newly diagnosed cases of squamous cell carcinoma of the cervix prior to therapy, 1 month after treatment and at 3-month intervals. Clinical outcome was evaluated after 22 to 47 months (mean, 36.4 months). There were 15 AG-4 antibody positive cases at presentation and during the follow-up period (seropositive group). Eleven patients were seronegative at presentation and developed AG-4 antibodies after radiation and/or surgical treatment (seroconversion group). Eight patients lacked AG-4 antibodies at all sample times (seronegative group). The progression-free rate in the three groups was as follows: seropositive, 94%; seroconversion, 73%; and seronegative, 38%. In a subgroup of 23 patients treated with radiation only, the progression-free rates were: seropositive, 100%; seroconversion, 63%; and seronegative, 33%. AG-4 seronegative status was not related to (a) clinical stage at presentation; (b) tumor size at presentation; (c) lack of antibodies to late herpesvirus type 2 antigens; or (d) to T-lymphocytopenia. The results suggest that herpesvirus type 2 AG-4 seronegativity in the minority of cervical cancer patients does not depend on the time of sampling. Seropositivity may have a favorable prognostic significance.

INTRODUCTION

Circumstantial, but not conclusive, seroepidemiological and biochemical evidence has accumulated which implicates HSV-2 and genital herpes infections with carcinoma of the uterine cervix (1, 2, 6, 8, 9, 10, 15, 17, 19-21, 23). Moreover, the oncogenic potential of herpesvirus in other animal hosts suggests the possibility of a similar role in humans (13). Several investigators have reported a greater frequency of antibodies to a putative HSV-2-induced tumor-associated antigen in sera obtained from cervical cancer patients than in matched cancer controls.

Aurelian et al. (3) reported that infection of HEp-2 cells with HSV-2 leads to the production of an early antigen (AG-4) which fixed complement in a high proportion of cervical carcinoma sera. The prevalence of AG-4 antibody was related to the stage of cervical cancer. The possible prognostic significance of anti-AG-4 in their study was unclear. These investigators partially purified AG-4 and identified it as a minor 16,000-dalton internal virion protein (22). They interpreted their data to indicate that AG-4 is weakly expressed during productive HSV-2 infection but is strongly expressed during nonproductive conditions characteristic of neoplastic transformation and during active growth of either primary or recurrent cervical cancer.

Subsequent studies confirmed the preferential reactivity of AG-4 with sera of patients with squamous cell carcinoma, especially of the uterine cervix (7, 11, 16). However, the frequency of AG-4- and HSV-2-neutralizing antibodies varied in different geographic populations. In most of these studies, sera were collected from patients before treatment only. Therefore, it is possible that some patients develop AG-4 antibodies subsequent to treatment or not at all, although they may form antibodies to the major virion antigens. The present investigation was designed to explore this question and to determine whether the AG-4 antibody response was correlated with tumor progression.

MATERIALS AND METHODS

Study Group. Blood specimens were obtained from 34 (26 Caucasian, 8 black) newly diagnosed cases of cervical carcinoma referred to North Carolina Baptist Hospital for treatment. The patients ranged in age from 28 to 94 years (mean, 56.1). The distribution of patients by stage was: Stage I, 21 patients; Stage II, 8 patients; Stage III, 5 patients. Twenty-three patients were treated with radiation alone, 7 patients were treated surgically, and 4 patients received combined therapy. Clinical outcome was evaluated from 22 to 47 months (mean, 36.4 months) after diagnosis. All were classified as squamous cell carcinoma of the cervix. Sera were obtained at the time of presentation, 1 month after completion of therapy, and at 3-month intervals thereafter.

Clinical Staging. The cancers were clinically staged according to the classification of the International Federation of Gynecology and Obstetrics as follows: Stage I, carcinoma confined to the cervix; Stage II, carcinoma involving either the upper two-thirds of the vagina and/or reaching the perineum; Stage III, carcinoma involving the lower one-third of the vagina and/or extending to the pelvic wall.

Clinical Follow-up. At the end of clinical follow-up, each patient was classified into one of 3 categories: no evidence of disease; alive with disease; or died with disease. Clinical follow-up was available on all 34 cases.

Preparation of Antigens. HSV-2 (ANG strain) was grown in HEp-2 cells as described previously (12, 13). Early antigens (AG-4), late antigens (AG-S), and control antigens (AG-H) were
prepared essentially as described by Aurelian and Strnad (4). The specificity of the AG-4 was verified with coded sera kindly provided by Dr. L. Aurelian. To minimize variation in the sensitivity of the assay, the antigen concentration was adjusted to give 50% complement fixation with a positive control serum.

**Antibody Assay.** The quantitative microcomplement-fixation test was used as described by Aurelian et al. (3). Controls in each assay included: AG-4 positive, AG-S positive serum; AG-4 negative, AG-S positive serum; anticomplementary controls for AG-4, AG-S, AG-H and serum; and an indicator control. Sera which fixed greater than 10% complement were considered positive since the AG-H controls always fixed less than 10% of the complement with the equivalent amount of protein measured by the procedure of Lowry et al. (14). All sera were tested in 2-fold dilutions from 1:2 to 1:32 or greater. As a specificity control for AG-4, extracts were prepared from HEp-2 cells infected with rabbit poxvirus. Antibody titers determined on different occasions did not vary more than one dilution.

**Statistical Analysis.** Data were analyzed for significance by one-way analysis of variance, the t test for differences between 2 means, and the χ² statistic for differences among proportions. Differences at p = 0.05 or less were considered significant.

**RESULTS**

**Prevalence of HSV-2 Antibodies.** The proportions of patients with AG-4 and AG-S antibodies at presentation were 44 and 94%, respectively. Antibody prevalence increased to 56% AG-4 seropositive and 97% AG-S seropositive at 9 months posttherapy. By 18 months, 77% of cases were AG-4 seropositive and 100% were AG-S seropositive. Thus, AG-4 antibodies were more prevalent in patients after treatment as compared to the time of presentation. None of the sera reacted with the infected HEp-2 cell extracts (AG-H) or to rabbit poxvirus-infected HEp-2 cell extracts. These results indicate that a proportion of patients who were AG-4 seronegative near the time of diagnosis converted to seropositive during the posttreatment period.

**Antibody Response Profiles.** The patients could be divided into 3 categories depending on their serological reaction patterns to AG-4 (Chart 1). Eight of the 34 patients (24%) were AG-4 antibody negative (seronegative group) at each interval tested; 11 patients (32%) were AG-4 antibody negative at presentation but developed AG-4 antibodies after therapy (seroconversion group), and 15 patients (44%) were AG-4 antibody positive at all sample times (seropositive group). The mean AG-S titers at presentation were significantly higher in the AG-4 seropositive group than in the AG-4 seronegative group (p = 0.05). The results also indicate that AG-4 and AG-S antibodies persisted for at least 18 months in treated patients. The mean age of the seronegative group (63.3 years), the seroconversion group (51.2 years), and the seropositive group (56.7 years) did not differ significantly.

**Antibodies to HSV-2 in Cervical Carcinoma**

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**Antibody Titters as a Function of Tumor Stage at Presentation.** The relationship between antibody titers and the clinical stage of the tumor at the time of presentation is shown on Chart 2. The mean AG-S antibody titers at presentation were significantly correlated with tumor stage (F = 4.0) whereas AG-4 titers at presentation did not differ significantly between the 3 groups (F = 0.79). The possible differences in antibody titers after therapy could not be analyzed statistically.

**Relationship Between Serological Status and Clinical Outcome.** Multiple variables affect survival rate in cancer patients, including the clinical stage of the tumor. Therefore, we took tumor stage into account when analyzing the possible influence of the other variables on patient survival. Table 1 relates survival to AG-4 serology and stage. Only one of the 15 patients in the AG-4-seropositive group died with disease. This 84-year-old patient presented with Stage I cancer and died 24 months later with disease. Two of the 11 AG-4 seroconverters died with disease; they were 55 and 72 years old at diagnosis of Stage II carcinoma of the cervix. In addition, one AG-4 seroconverter (age 72, Stage III) is alive with disease at 35 months. In contrast, of the 8 patients in the seronegative group, 3 patients (2 Stage I, 24 and 83 years, and one Stage III, age 70 years) survived 10 months or less, and 2 patients (Stage I, ages 68 and 72 years) are alive with disease at 46 and 26 months, respectively. The difference in the progression-free rate of the AG-4 antibody producers (i.e., seropositive group plus the seroconversion group) as compared to the AG-4 antibody nonproducers (seronegative group) is significant (P = 0.02). When the subgroup of 23 patients who were treated with radiation were considered, a similar pattern was observed (Table 2). However, the difference was of borderline signifi-
cance (P = 0.05) possibly because of the smaller number of patients.

**Relation between AG-4 Seroreactivity and Immune Status.**
A possible explanation for the low progression-free rate of the AG-4-seronegative group is that these patients were immuno-suppressed because of their age, tumor burden, response to therapy, or other factors. Therefore, we performed T-lymphocyte quantitation both before therapy and at intervals after therapy. As expected, a T-lymphocytopenia was observed which was most marked at about 3 months postradiotherapy and often persisted for a longer period. As shown in Table 3, the number of T-cells at 1 year was 95% of the mean pretreatment value in the surgical treatment group. In contrast, in the radiotherapy group, the number of T-cells at 12 months was significantly lower than that in the surgical group and was only 44% of the mean pretreatment values.

The relationship between T-cell levels and AG-4 seroreactivity is shown in Table 4. The T-cell levels returned to pretreatment levels in the AG-4-seropositive group by 12 months after therapy. In the AG-4-seronegative group, the number of circulating T-cells reached approximately 75% of the level at 12 months; whereas, in the seroconversion group, the number of circulating T-cells represented only 62% of the pretreatment level at 12 months. These results indicate that AG-4 serological status did not correlate with T-cell levels or, by inference, with immunosuppression.

**DISCUSSION**

The frequency of complement-fixing antibodies to HSV-2 late antigens in untreated cervical carcinoma patients in our North Carolina sample (94%) is comparable to that observed in other United States studies (3, 16). The frequency of complement-fixation antibodies to HSV-2 early antigens (AG-4) in our sample of patients before treatment (44%) was similar to that noted in a Japanese study (11) but was lower than that reported in other United States studies (3, 16). However, after therapy, the proportion of AG-4-seropositive patients increased to 77%. The lower prevalence of antibodies to HSV-2 early antigens in untreated patients observed in this study compared to other studies could be explained by differences in: (a) sensitivity of the antibody assays; (b) host factors; (c) herpes simplex virus strains or substrains in the patient population under study; or (d) antigen-antibody-complex formation and removal from the blood circulation. With regard to these possibilities, we observed that the maximum AG-4 antibody titer was significantly correlated with the maximum AG-S-antibody titer (correlation coefficient, 0.56; P < 0.01). This suggests that the antibody responses to HSV-2 early and late antigens are not independent.

### Table 1
Progression-free rate related to AG-4 serological pattern and stage in cervical carcinoma patients treated with radiation and/or surgery

<table>
<thead>
<tr>
<th>Stage</th>
<th>Seropositive</th>
<th>Seroconversion</th>
<th>Seronegative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>10/11</td>
<td>3/3</td>
<td>3/7</td>
<td>16/21 (76)*</td>
</tr>
<tr>
<td>II</td>
<td>2/2</td>
<td>4/6</td>
<td>0/0</td>
<td>6/8 (75)</td>
</tr>
<tr>
<td>III</td>
<td>2/2</td>
<td>1/2</td>
<td>0/1</td>
<td>3/5 (60)</td>
</tr>
<tr>
<td>Total</td>
<td>14/15 (94)</td>
<td>8/11 (73)</td>
<td>3/8 (38)</td>
<td>25/34 (74)</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, percentage.

### Table 2
Cervical carcinoma progression-free rate related to AG-4 serological pattern and stage in patients treated with radiation therapy alone

<table>
<thead>
<tr>
<th>Stage</th>
<th>Seropositive</th>
<th>Seroconversion</th>
<th>Seronegative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>6/6</td>
<td>2/2</td>
<td>2/5</td>
<td>10/13 (77)*</td>
</tr>
<tr>
<td>II</td>
<td>1/1</td>
<td>3/5</td>
<td>0/0</td>
<td>4/6 (67)</td>
</tr>
<tr>
<td>III</td>
<td>2/2</td>
<td>0/1</td>
<td>0/1</td>
<td>2/4 (50)</td>
</tr>
<tr>
<td>Total</td>
<td>9/9 (100)</td>
<td>5/8 (63)</td>
<td>2/8 (33)</td>
<td>16/23 (70)</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, percentage.

### Table 3
Correlation between the form of therapy and posttreatment lymphocyte values in cervical carcinoma patients

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Hysterectomy (N = 10)</th>
<th>Radiation (external and implant) (N = 21)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean RFC/ml</td>
<td>% of RFC</td>
<td>Mean RFC/ml</td>
</tr>
<tr>
<td><strong>Erythrocyte-rosette-forming cells</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pretreatment</td>
<td>1643</td>
<td>71.1</td>
<td>1492</td>
</tr>
<tr>
<td>Posttreatment</td>
<td></td>
<td></td>
<td>1127</td>
</tr>
<tr>
<td>6 mos.</td>
<td>1156</td>
<td>61.0</td>
<td>659</td>
</tr>
<tr>
<td>12 mos.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* NS, not significant.
Our data indicated that a large proportion of cervical carcinoma patients possess antibodies to HSV-2 early and late antigens both before and after definitive treatment. However, approximately one-third of the patients converted to AG-4 seropositive within 3 to 6 months after completion of therapy although AG-S antibodies were present before treatment. A smaller group, approximately one of every 4 patients, was AG-S-seropositive but lacked detectable AG-4 antibodies at any of the observation times. These different response patterns could not be explained by variability of the antibody assay, by differences in T-lymphocyte levels, by method of treatment, by the clinical stage at presentation, or by differences in tumor size. In AG-4-seropositive patients, antibodies persisted for at least 18 months following therapy. We did not observe the loss of AG-4 seroreactivity which was reported by Aurelian et al. (3). Our results are consistent with those of Notter and Docherty (16), who reported persistence of AG-4 antibodies in treated cervical carcinoma patients. Christenson and Espmark (5) also noted an increase in titer of antibodies to membrane antigen(s) of herpes simplex virus-infected cells following treatment. It seems likely that the HSV-2 response patterns may be influenced by (a) removal of an antibody depot, (b) release of HSV-2 antigens, or (c) recovery from an immunosuppressed state. Available evidence does not exclude any of these possibilities.

The results of this study suggest that the lack of an AG-4 antibody response is correlated with an increased risk of an unfavorable clinical outcome (Tables 1 and 2). Proof of this contention requires a follow-up study with a large number of patients to permit matching for other variables which may affect the outcome. If such a study confirms the initial results reported here, herpesvirus serology would acquire new significance.

The clinical outcome seemed to be related to qualitative rather than quantitative differences in the response to AG-4 antigens. If confirmed, this could indicate that differences in tumor-associated antigen and/or host response to such antigens are important in cervical carcinoma.

REFERENCES


20. Rawls, W. E., Tompkins, W. A. F., and Melich, J. L. The association of...
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