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Alteration of Lymphocyte Transformation Response to Herpes Simplex Virus Infection by Acyclovir Therapy

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To evaluate the effect of acyclovir (ACV) therapy on the cellular immune response, we sequentially followed 43 patients with culture-proven first episodes of genital herpes simplex virus (HSV) infection. Twenty-three patients who were treated with ACV and 20 who received placebo had blood obtained weekly during the first 6 weeks after onset of lesions and had their in vitro lymphocyte transformation (LT) response to inactivated HSV antigens measured. The mean stimulation index to HSV antigens at week 3 among patients treated with systemic ACV was 3.5 ± 0.64 compared to 18.4 ± 6.89 in their placebo-treated counterparts ($P < 0.05$). The mean time to the development of the peak LT response to HSV antigens was 4.3 weeks in systemic-treated versus 3.4 in placebo-treated patients ($P < 0.05$). The time to the development of the peak in vitro LT response to HSV antigens and the height of that response were, however, similar between topical ACV- and topical placebo-treated patients. The geometric mean HSV-2-neutralizing titer in convalescent sera was 5.4 in recipients of systemic ACV compared to 10.0 in patients treated with systemic placebo ($P < 0.05$). The LT response to HSV antigen was also measured at the first recurrence in 11 patients. No differences were found in the time to first recurrence, lesion duration, number of lesions, or mean stimulation index response to inactivated HSV antigens between the six patients treated with systemic ACV during their primary episode and the five given placebo during their primary episode. Systemic ACV therapy appears to diminish the peak in vitro LT response to inactivated HSV antigens as well as to delay the time to development of that peak response. However, the cell-mediated immune response to subsequent episodes appears similar.

Cellular immune responses are important in the containment of herpes simplex virus (HSV) infection (11, 14, 18, 19). Impaired cell-mediated immunity is known to increase the frequency, risk of dissemination, and the severity of HSV infections (2, 10, 12, 13, 16, 21). Topical acyclovir has been shown to decrease the duration of pain, viral shedding, and duration of lesions in primary first episodes of genital HSV infections (4, 5, 7). Intravenous and oral acyclovir have been shown to shorten the duration of viral shedding, healing time, and local symptoms as well as to decrease the systemic symptoms and the complications of primary first-episode genital HSV infections (3, 6, 11a). In vitro, the addition of acyclovir to lymphocyte cultures has been shown not to affect the lymphocyte transformation (LT) response to either soluble antigens or mitogens (20). To evaluate the effect of topical and systemic acyclovir therapy on in vitro cell-mediated immune responses of patients with first-episode genital herpes, we compared weekly LT responses to mitogens and inactivated HSV antigen in 43 patients treated with topical and systemic acyclovir.

MATERIALS AND METHODS

During the course of a series of double-blind, placebo-controlled trials of topical, oral, and intravenous (i.v.) acyclovir for first-episode genital HSV infections, all patients consenting to have peripheral blood taken weekly were studied. At entry, all patients had symptoms for 6 days or less, denied previous genital HSV infection, and were followed at least every other day with genital examinations and sequential viral cultures until lesions were healed. Patients were then seen at weekly intervals until 6 weeks. After the episode was resolved patients were instructed to present to the clinic with recurrences. Details of the demographic and clinical results of the studies have been presented elsewhere (4–7, 11a).

Virus isolation, typing, and serological tests. At each clinic visit cultures were obtained with a Dacron swab from the cervix and vulva of women and from the penis and urethra of men and placed into viral transport media. If no lesions were seen, the surface of the penis or vulva was swabbed. Duplicate tubes of fetal tonsil cells were inoculated and examined weekly. These cells and supernatant fluid from cultures exhibiting HSV cytopathic effect were spotted onto glass slides and stained for the presence of HSV antigen by an indirect immunofluorescence assay, using murine monoclonal antibodies specific for HSV type 1 (HSV-1) and HSV-2 (16).

Titers of neutralizing antibody to HSV-1 and HSV-2 were measured on serum specimens from the initial and week 4 visits as previously described (17).

LT assay. LT responses to inactivated HSV antigens and mitogens were assayed weekly as previously described (8). Briefly, mononuclear cells were separated from heparinized blood by Ficoll-Hypaque density centrifugation, washed three times with 25 ml of sterile phosphate-buffered saline, and suspended in Waymouth medium 752 with L-glutamine (GIBCO Laboratories, Grand Island, N.Y.) containing penicillin and streptomycin. The cell suspension was diluted in 10% autologous plasma to a concentration of 10^6 viable lymphocytes per ml. Aliquots of 0.2 ml of the mononuclear cell suspension were placed in flat-bottomed microtiter plates, and 25 μl of the mitogen concanavalin A (120 μg/ml) or pokeweed mitogen (0.5 mg/ml) or *Candida albicans* antigen (13.0 μg/ml; Hollister Stier, Spokane, Wash.) or the undiluted and 10^1 dilutions of prototype HSV-1 and HSV-2 antigens or both were added. All assays were done in quadruplicate. The microtiter plates were incubated in a

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humidified incubator containing 5% carbon dioxide for 6 days and then labeled with 1.0 μCi of tritiated thymidine. Cells were collected 18 to 22 h later with a 24-channel (MASH) tissue culture harvester and counted in a scintillation counter. Five HSV antibody-negative "control" patients were also assayed weekly.

The inactivated HSV-1 and HSV-2 antigens were prepared as previously described. HSV-1 strain E115 and HSV-2 strain 333 were inoculated into HeLa cells; when 4^+ cytopathic effect was present, the cells and supernatant fluid were collected and sonicated. One lot of prototype HSV antigen was utilized throughout the course of this study. Preliminary experiments indicated that 10^8 PFU of stock HSV-1 or HSV-2 antigen, inactivated and incubated for 7 days, provided the optimal blastogenic response.

The lymphoproliferation response to HSV antigen was expressed as a stimulation index (SI), which for HSV antigens was calculated as mean counts per minute with the homologous HSV antigen (e.g., with HSV-1 prototype antigen for subjects from whom HSV-1 was isolated and HSV-2 for those from whom HSV-2 was isolated from lesions) divided by the mean counts per minute with an uninfected HeLa cell control. The SI for the mitogens was calculated as the mean counts per minute with the mitogen divided by the mean in the unstimulated lymphocyte control wells. The highest SI with either the undiluted or the 10^{-1} dilution of the homologous HSV antigen was used in all calculations. All LT assays were performed without knowledge of the cultures or therapy of the patients. Comparisons between groups were evaluated by Mann-Whitney rank sum and Student's *t* test analysis.

RESULTS

Forty-three patients (9 men and 34 women) were studied. HSV was isolated from genital lesions in 42 patients. One patient had characteristic genital lesions from which HSV was not isolated, but seroconverted to HSV-2 in the neutralizing antibody assay. Twenty-three patients received acyclovir and 20 received placebo. Eleven of the 43 patients (1 male, 10 females) studied were treated with 5% acyclovir ointment in polyethylene glycol for 7 days. Nine (four males, five females) were treated with topical (placebo preparation) polyethylene glycol alone for 7 days. Eight patients (one male, seven females) received i.v. acyclovir (5 mg/kg every 8 h for 5 days) and four patients (one male, three females) received oral acyclovir (200 mg five times a day for 10 days). Nine patients (one male, eight females) were given normal saline i.v. three times a day for 5 days, and two patients (one male, one female) were given an oral placebo five times a day. Previous studies have indicated that oral and i.v. acyclovir had similar effects in the acute course of disease, whereas topical acyclovir, while effective, did not affect either systemic symptoms or cervical, urethral viral shedding (5). As such, for the purpose of data analysis, patients were grouped into four categories: (i) topical acyclovir; (ii) topical placebo; (iii) systemic acyclovir (oral and i.v.); and (iv) systemic placebo recipients (oral and i.v.).

Effect of topical acyclovir on LT response. Of the 20 patients who were enrolled in the trial, 18 had HSV-2 isolated whereas 2 had HSV-1 isolated (1 acyclovir, 1 placebo recipient). Nine of the acyclovir and seven of the placebo recipients had first-episode primary (lacked HSV-neutralizing antibody in acute serum) and four (two acyclovir, two placebo recipients) had nonprimary first episodes of genital herpes. Seven of 9 placebo and 10 of 12 acyclovir recipients complained of systemic symptoms (such as fever, headache, stiff neck, or photophobia) on presentation.

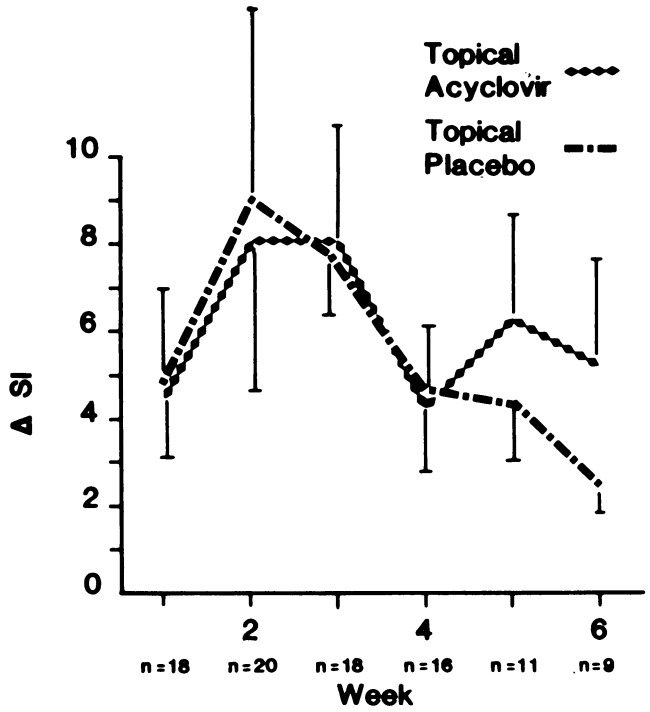

FIG. 1. In vitro LT response to HSV antigens in patients with first-episode genital herpes treated with topical acyclovir or placebo.

The mean lesion areas at the first clinic visit for the topical acyclovir and topical placebo cream recipients were 708 ± 391 and 882 ± 398 mm^2, respectively (not significant). The mean numbers of external genital lesions were 26.7 ± 16.4 and 29.0 ± 16.7, respectively. The mean duration of viral shedding for all external genital lesions and the mean duration of lesions for the 12 patients receiving topical acyclovir were 7.2 ± 0.6 and 17.9 ± 5.9 days, and those for the patients receiving topical placebo were 8.9 ± 1.4 (standard error) and 21.3 ± 10.8 days.

The mean counts per minute in the unstimulated lymphocyte cultures were 960 ± 146 (standard error) and these were similar between placebo- and acyclovir-treated patients. The mean SIs to the mitogen concanavalin A in the topical placebo group were 70.7 ± 78.3, 70.1 ± 92.0, 63.8 ± 53.46, 69.5 ± 67.8, 71.8 ± 102.1, and 73.4 ± 103.2 at weeks 1 to 6, respectively, and these were similar in both topical placebo- and topical acyclovir-treated patients. Similarly, no difference in the LT response to pokeweed mitogen was seen during the course of infection.

The mean SI to inactivated HSV antigens was 4.7 at week 1. The SI peaked at 8.5 during weeks 2 to 3 of disease and then gradually fell over the first 6 weeks (Fig. 1). The mean SIs to HSV antigens were similar between the topical and placebo groups throughout the entire study period. Similarly, the percentages of patients with an SI of >3.0 were 40% at week 1, 58% at week 2, 80% at week 3, 44% at week 4, 40% at week 5, and 33% at week 6. The percentages were similar between the two groups. No control patient had an SI to HSV antigen of >3.0.

Effect of systemic acyclovir on LT responses. Twenty-three patients were evaluated; 21 had HSV-2 and 2 had HSV-1 isolated. Twelve patients were given systemic acyclovir (2 males, 10 females) and 11 were given systemic placebo (2

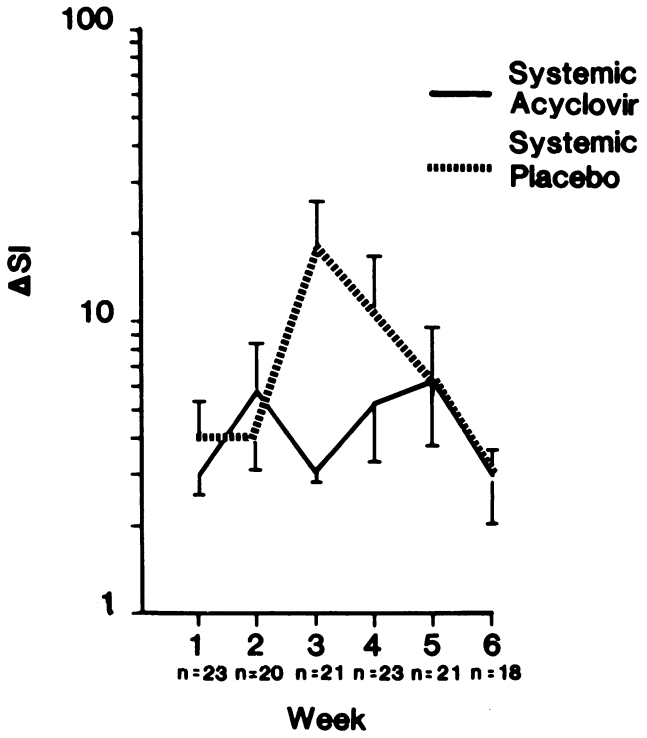

FIG. 2. In vitro LT response to HSV antigens in patients with first-episode genital herpes treated with systemic acyclovir or systemic placebo.

males, 9 females). Twenty-one patients (11 acyclovir, 10 placebo) had first-episode primary and two patients (1 acyclovir, 1 placebo) had nonprimary first-episode genital HSV-2 infections. The mean lesion areas and the mean number of lesions at the onset of therapy in placebo- versus systemic acyclovir-treated patients were similar (418 ± 450 mm^2 and 27.2 ± 18.0 lesions versus 460 ± 367 mm^2 and 23.8 ± 12.5 lesions, respectively). After the onset of therapy, the mean duration of viral shedding and mean duration of genital lesions of systemic acyclovir recipients were 5.0 ± 1.5 and 13.6 ± 2.7 days compared to 14.8 ± 4.1 and 25.4 ± 6.12 days for placebo recipients ($P < 0.001$ for each comparison).

The mean counts per minute in unstimulated lymphocyte cultures were 928 ± 77.5 (standard error) in systemic acyclovir and 743 ± 33 (standard error) in placebo recipients (not significant). Similarly, the mean SIs to the mitogens concanavalin A and pokeweed were similar in acyclovir and placebo recipients and did not vary over the course of disease (data not shown). The lymphoproliferative response to *Candida* antigen was performed on 10 of the 12 patients treated with systemic acyclovir and on 8 of the 11 patients given systemic placebo. In systemic acyclovir recipients the median SIs to *Candida* antigen were 1.4, 5.1, 4.7, 3.9, 2.9, and 4.3 at weeks 1 to 6 of disease. Comparable values in placebo recipients were 3.0, 4.5, 1.8, 2.1, 1.8, and 1.6. No statistically significant differences between these two groups were noted at any of the time points.

In systemic acyclovir recipients the mean SIs to inactivated HSV antigens were 3.0 ± 0.9, 5.8 ± 2.8, 3.1 ± 0.6, 5.1 ± 1.9, 6.2 ± 2.4, and 2.6 ± 0.7 at weeks 1 to 6 of disease. Comparable values in placebo recipients were 4.3 ± 1.1, 4.2 ± 1.2, 18.4 ± 6.9, 10.2 ± 6.5, 6.2 ± 2.8, and 3.2 ± 0.25 (Fig. 2).The mean SI of 18.4 at week 3 in placebo recipients was significantly greater than the mean of 3.05 seen in systemic acyclovir recipients ($P < 0.05$).

The time to development of peak LT to HSV antigen was delayed in systemic acyclovir as compared with placebo recipients (Table 1). The mean time to the development of peak SI to HSV antigen was 4.3 ± 0.4 weeks in patients receiving systemic acyclovir compared to 3.4 ± 0.3 weeks in placebo recipients ($P < 0.05$). The mean time to development of peak SI was similar in topical acyclovir-treated patients (3.3 weeks) compared with their placebo-treated counterparts (3.1 weeks). Whereas the mean peak SI and the range of the SI to HSV antigen (2.4 to 75.5) achieved during the first 6 weeks were highest for the group receiving the systemic placebo and lowest for the group receiving systemic drug (3.1 to 27.7), these differences in peak SI were not statistically significant among any of the four groups tested.

Humoral antibody response. In patients with first-episode primary HSV-2 infection, the geometric mean titers of HSV-2-neutralizing antibody in convalescent sera were 5.4 in systemic acyclovir recipients and 10.0 in placebo recipients ($P < 0.05$). The geometric mean titer of neutralizing antibody to HSV in convalescent sera was 12.30 in topically treated acyclovir patients compared to 12.6 in the placebo-treated counterparts. No statistically significant difference in geometric convalescent mean antibody titer was seen among patients treated with topical acyclovir, topical placebo, or systemic placebo.

LT response during recurrences. Six of the patients receiving acyclovir and five of the patients receiving systemic placebo had their in vitro LT responses to autologous HSV antigen assayed during the first recurrence of disease (Table 2). The mean times to the first recurrence among these patients were 83 and 75 days, respectively, in acyclovir and placebo recipients. The duration of genital lesions (9.6 ± 2.0 versus 8.1 ± 1.4 days) and number of lesions (2.4 ± 0.7 versus 2.9 ± 0.3) of the first clinical recurrence were also similar in the acyclovir- and placebo-treated patients. The mean peak SI assayed during the week of recurrence was

TABLE 1. Time and magnitude of mean peak SI to HSV antigens in acyclovir-treated patients with first-episode genital herpes

Treatment group	Mean peak SI (±SD) during first 6 wk	Mean wk (±SD) of peak SI	Convalescent antibody geometric mean titer[a]
Topical acyclovir (n = 11)	14.50 ± 3.20	3.3 ± 0.4	12.3
Topical placebo (n = 9)	12.07 ± 3.37	3.1 ± 0.4	12.6
Systemic acyclovir (n = 12)	10.90 ± 3.06	4.3 ± 0.4 } $P < 0.05$[b]	5.4 } $P < 0.05$[c]
Systemic placebo (n = 11)	25.60 ± 8.43	3.4 ± 0.3 }	10.0 }

[a] Among patients with primary HSV-2 infection.
[b] Mann-Whitney rank sum test.
[c] Student's t test.

TABLE 2. Clinical and cellular immune responses during subsequent clinical recurrence of disease

Treatment	Mean time to first recurrence (days)	Mean lesion duration (days)	Mean no. of lesions	Mean HSV SI during first recurrence
Systemic acyclovir (n = 6)	82.8 ± 22.4	9.6 ± 2.0	2.4 ± 0.7	7.95 ± 1.80
Systemic placebo (n = 5)	75.0 ± 10.1	8.1 ± 1.4	2.9 ± 0.3	7.63 ± 1.57

similar between patients who had been treated with systemic acyclovir versus placebo-treated patients (7.95 ± 1.80 versus 7.63 ± 1.57, respectively). The mean peak in vitro SI to inactivated HSV-2 antigens during the first recurrence of disease among placebo-treated patients was significantly less than that achieved during the first episode of disease (7.63 ± 1.57 versus 25.6 ± 8.4, respectively; $P < 0.001$).

DISCUSSION

The mechanisms involved in the immune response to HSV infection are largely undefined. Deficient humoral immunity (8) and cell-mediated immunity (9) have been reported as correlates of increasing susceptibility to HSV infection. Some factors influencing the development of humoral and cell-mediated immunity to HSV infection are host specific and genetically determined (15). Other factors that modulate immune response relate to the level and type of antigenic stimulation experienced by the host (1) and might be altered by antiviral chemotherapy.

In this study topical acyclovir treatment for the first-episode genital herpes was not associated with any alterations in the in vitro LT response to inactivated HSV antigens. However, systemic acyclovir therapy was associated with a delay in the peak in vitro LT response to HSV antigen, as well as a reduction in the mean HSV-neutralizing antibody response. That the differences we observed in all patients who received systemic acyclovir were specific for the immune response to HSV and were related to the receipt of acyclovir was substantiated by the similar responses between systemic acyclovir and placebo recipients to the unrelated *C. albicans* antigen and the mitogens concanavalin A and pokeweed. The alterations in the in vitro LT response to inactivated HSV antigen seen in first episodes of disease were not followed by measurable differences in the clinical time to next recurrence or the in vitro lymphocyte blastogenic response with subsequent recurrences.

Several explanations might account for our observations. Although previous studies have shown that topical acyclovir decreases the duration of viral shedding and shortens the duration of external lesions, this effect is less pronounced than that observed with systemic therapy. In addition, the duration of viral shedding from internal lesions (i.e., pharynx and cervix), the duration of systemic symptoms, and the frequency of new lesion formation during the course of first-episode disease are not decreased by topical acyclovir therapy. Data from clinical studies of i.v. and oral acyclovir report plasma concentrations from both preparations well above the minimum inhibition concentration for HSV and also show that both preparations (i) shorten the duration of systemic symptoms, (ii) decrease the duration of viral shedding from internal as well as external lesions, and (iii) inhibit new lesion formation during the primary episode (3, 6, 11a). Both systemic preparations of acyclovir provide a >80% reduction in viral shedding when compared with placebo-treated patients (5). The decreased duration of viral shedding seen in patients treated with systemic acyclovir implicates decreased antigenic stimulation secondary to partial eradication of virus as a possible mechanism for the decrease in SI. The later peak in the SI seen in the patients treated with systemic acyclovir might indicate that viral replication continued after the discontinuation of therapy (two patients formed new lesions within 1 week of discontinuing i.v. acyclovir). This delayed peak could also reflect stimulation from latent virus not affected by acyclovir therapy, or it may merely reflect a delayed peak response of antigen-stimulated cells subsequent to less antigenic stimulation early in the course of disease. Previous studies have indicated that the peak SI to inactivated HSV antigens is slightly higher if autologous serum is used as opposed to heat-activated HSV antibody-negative sera (8). The slight blunting of the humoral response to primary HSV infection in patients given systemic acyclovir also suggests that antibody may have played a role in the decrease of the peak SI seen in these patients. Alternatively, it is possible that as yet undefined serum inhibitors such as drug metabolites or circulating viral products could account for these effects.

In vitro experiments show that the toxic effects of acyclovir on lymphocytes are not implicated as decreasing lymphocyte blastogenic responses. Peak blood levels of acyclovir in the dosages used in this study were 5.8 ± 1.8 μg/ml (range, 3.3 to 10.2 μg/ml) (6). Reports demonstrate that incubation of lymphocytes with up to 20 μg of acyclovir per ml does not inhibit LT response (20). As such, we feel that the antiviral effect of acyclovir is a more likely explanation of our results than is a direct immunosuppressive effect of the medication.

Although we observed alterations in the immune response during the primary episode, these alterations were not followed by measurable clinical alterations of the subsequent untreated recurrence. Hence, systemic acyclovir therapy modifies the time course of the lymphocyte blastogenic response in primary disease but does not appear to prevent the host from mounting a blastogenic response during the first recurrence and does not alter the clinical presentation of the first recurrence.

Further studies of the mechanisms by which systemic acyclovir inhibits cellular immune responses, especially in populations with poor cellular immune responses, will be of interest. Studies to determine whether chronic systemic therapy alters immunological responses and the correlation of these altered responses with clinical response to medication are warranted.

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