Effect of Acyclovir Treatment of Primary Genital Herpes on the Antibody Response to Herpes Simplex Virus

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Abstract. Sera from patients with first episode primary genital herpes infections who were treated with the antiviral drug acyclovir were studied to determine the effect of therapy on the immune response to herpes simplex virus (HSV) glycoproteins and polypeptides. 63 patients were evaluated, 35 patients received acyclovir: 11 intravenously, 12 orally, and 12 topically, while 28 received placebo. Topical application of acyclovir had no effect on the immune response to HSV infection. However, both oral and intravenous acyclovir were associated with later development of antibodies to two glycoproteins (of 80,000 and 60,000 mol wt [IIg80 and gD, respectively]) and one nonglycosylated polypeptide of 66,000 mol wt (vp66). Antibody to IIg80 was present in convalescent phase serum in 13/23 systemic acyclovir recipients vs. 18/19 placebo recipients ($P = 0.01$) and antibody to gD was detected in 8/23 oral or intravenous acyclovir recipients vs. 11/19 placebo recipients ($P = 0.06$). The mean time to seroconversion to IIg80 (39.0 d) and gD (55.5 d) was significantly longer for systemic acyclovir recipients than for the placebo controls, 23.4 and 18.5 d, respectively ($P < 0.05$ for each comparison). 7 (30%) of 23 systemic acyclovir recipients compared with 100% of the placebo recipients had antibody to vp66 by 30 d after onset of the primary episode ($P < 0.001$).

Subsequent untreated recurrences of genital herpes were associated with seroconversion to gD, IIg80, and vp66. Patients who lacked antibody to both gD and vp66 in sera taken before their first clinical recurrence of disease experienced a longer duration of the recurrent episode (10.8 d) than those who possessed antibody to both vp66 and gD (6.3 d) ($P < 0.05$). In addition, the mean duration of lesions, number of lesions, and mean lesion area were greater in patients who lacked antibody to vp66 but had anti gD, as compared with those who had anti-p66 but lacked anti-gD; suggesting that antibody to vp66 correlated more closely with subsequent disease severity than did antibody to gD.

Acyclovir therapy appears to influence the frequency and time of development of antibody to a number of different HSV-specific polypeptides. Further studies of the effects of antiviral therapies on the immune response to these proteins may help clarify the role of these polypeptides in the pathogenesis of disease.

Introduction

Previous studies evaluating host immune responses in primary genital herpes simplex virus (HSV)[1] infection have shown that antibody responses to viral glycoproteins with molecular weights of 130,000 and 115,000, and the nonglycosylated major nucleocapsid protein of 148,000 mol wt can be detected within the first 10 d of disease in all patients with primary first episode genital HSV-2 infection (1, 2). Antibodies to glycoproteins of 60,000 and 80,000 mol wt and to a nonglycosylated polypeptide of 66,000 mol wt subsequently appear by 21–28 d after onset of symptoms in sera of untreated patients with primary genital HSV-2 infection (1, 2).

The antiviral agent acyclovir has been shown to be a clinically effective treatment for primary first episode genital herpes in immunocompetent patients (3–8). In clinical trials, intravenous, oral, and topical acyclovir decreased the duration of viral shedding by 85, 80, and 50%, respectively, as compared with similarly

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1. *Abbreviations used in this paper:* HSV, herpes simplex virus; HSV-2 glycoproteins and their molecular weights (pgD, 50,000; gD, 60,000; IIg80, 80,000; gE, 70,000–80,000; gF, 80,000; gAB, 115,000; IIg130, 130,000); FET, Fisher's exact test; nonglycosylated proteins and their molecular weights (vp66, 66,000; vp88, 88,000; vp148, 148,000).

followed placebo recipients (4, 5, 8). In the trials of intravenous and oral acyclovir, neutralizing antibody titers to HSV-2 in convalescent phase sera were lower in acyclovir-treated as compared with placebo-treated patients (5, 8). In contrast, anti-HSV-2 neutralizing antibody titers in convalescent-phase sera of patients using topical acyclovir recipients were similar to the neutralizing-antibody titers in placebo recipients (3, 4). To analyze the effect of systemic acyclovir treatment on the humoral response to HSV-specific polypeptides, we evaluated sequential sera from 63 patients with primary HSV-2 genital infections who were enrolled in trials of intravenous, oral, and topical acyclovir.

Methods

Patient population. Patients were entered into placebo-controlled trials of intravenous, oral, and topical acyclovir at the University of Washington Herpes Research Clinic located at the Harborview Medical Center Seattle, WA. All patients presented to the clinic within 7 d after developing genital lesions, all had HSV-2 isolated from their genital lesions, and none had evidence of antibody to either HSV-1 or HSV-2 in their acute-phase sera (i.e., were experiencing "primary first episode" genital HSV-2 infection). During their acute episode, patients were followed with serial genital exams, and lesions were sampled for viral cultures every other day until healing occurred (4, 5, 8). Patients were then seen at 3–6 wk intervals and during subsequent clinical recurrences. Sera were drawn at the first clinic visit, posttherapy, day 21, at 6 wk, and during each recurrence.

Serology. Neutralizing antibodies to HSV were measured by microneutralization using serial dilutions of sera from 1:4 to 1:128. The titer of antibody was the highest dilution of sera inhibiting 50% of the viral input as determined by the Reed-Meunch formula (3, 9). In calculating the mean titer of neutralizing antibody, titers of <1:4 were arbitrarily given a value of 1:2.

Radioimmunoprecipitation of virus-specified polypeptides. Patients' sera were reacted with [^{35}S]methionine-labeled antigen that was prepared from cytoplasmic extracts of infected human embryonic tonsil cells. Briefly, confluent monolayers were incubated 2 h with HSV-2 (strain 333) at a multiplicity of infection of 1.0 plaque-forming unit per cell. Cells were washed and incubated an additional 5 h in methionine-free minimal essential media before 20 μCi/ml of [^{35}S]methionine (1,100 Ci/mmol; New England Nuclear, Boston, MA) was added. Viral glycoproteins were radiolabeled by addition of 10 μCi/ml of [^{14}C]glucosamine (54.2 Ci/mmol; New England Nuclear) at 7 h post-infection. 16 h later cells radiolabeled with ^{35}S or ^{14}C were washed and suspended in lysis buffer (10 mM Tris, pH 8.0; 0.1 mM EDTA, 0.1 M NaCl, 1% Nonidet P-40; 1% sodium deoxycholate) for 30 min on ice. The cytoplasmic fraction was obtained by low-speed centrifugation and insoluble proteins were pelleted at 430,000 *g* for 30 min. The resulting cytoplasmic extract was diluted 1:40 and reacted with human sera that was diluted 1:4 in Tris 0.01 M, pH 8.0. Antigen-antibody complexes were collected on SPA-sepharose beads (Sigma Chemical Co., St. Louis, MO), washed, denatured, and run on 9% polyacrylamide gels in 1% sodium dodecyl sulfate. Radioimmunoprecipitated proteins and glycoproteins were visualized by fluorography with 1 M sodium salicylate and exposed to Kodak XAR film (Eastman Kodak Co., Rochester, NY) for 2–10 d. Each serum sample was reacted with HSV-2 and with mock-infected cell antigen. Each antigen preparation was tested against seronegative human sera and was preadsorbed to that sera, if necessary.

Statistical methods. Comparison between groups was performed by Fisher's exact test (FET), chi square analysis, or *t* test, as specified.

Viral glycoproteins. Recent reports have indicated that the HSV-1 glycoprotein at 130,000 mol wt (gC) and the HSV-2 80,000 mol wt glycoprotein designated gF (10) are related immunologically (11) and map to colinear regions of the respective genomes. Further, the reported molecular weight of gF (10, 11) is similar to that of HSV-2 gE (70,000–80,000 mol wt) (12). A glycoprotein of 50,000 mol wt is immunoprecipitated by monoclonal antibody to gD (our unpublished data) and has been suggested as a precursor of gD (13). For the purpose of reporting our results, we have used the following nomenclature for HSV-2 glycoproteins which are immunoprecipitated in our system by human sera: IIg130 (mol wt = 130,000), gAB (115,000) (14, 15), IIg80 (80,000), gD (60,000), and pgD (50,000) (Fig. 1).

Results

63 patients (43 women and 20 men) with primary genital HSV-2 infection were evaluated. 35 received acyclovir: 11 intravenously (5 mg/kg every 8 h for 5 d), 12 orally (200 mg five times daily for 10 d), and 12 by topical application of 5% acyclovir in polyethylene glycol to external genital lesions six times daily for 7 d. Of the 28 placebo-treated patients, 10 were enrolled in the intravenous trial and nine each were enrolled in the oral and topical acyclovir trials. The mean duration of viral shedding from the onset of lesions to the last positive viral culture was 7.0 d in those treated with intravenous acyclovir, 5.5 d in those treated with oral acyclovir, and 7.0 d in patients treated with

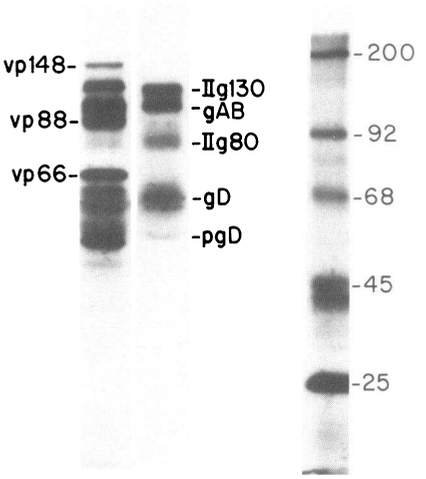

Figure 1. Immunoprecipitation of HSV-2 glycoproteins and polypeptides. HSV-2 antigens radiolabeled with [^{35}S]methionine (*left* lane) or [^{14}C]glucosamine (*right* lane) were precipitated by serum antibody from a patient with recurrent genital herpes due to HSV-2. Immunoprecipitated proteins were electrophoresed into polyacrylamide gels and radiolabeled bands were visualized by fluorography and exposure to x-ray film. ^{14}C-labeled proteins of known molecular weight are shown at the far right with their molecular weight designations in kilodaltons. Lanes were run on the same gel but cut out and rearranged for presentation.

topical acyclovir. Placebo-treated patients shed HSV from genital lesions for a mean of 15.0 d. The mean time from the onset of lesions to the first serum sample was 3.7 d in acyclovir- and 4.0 d in placebo-treated patients (range 1–7 d) in all three studies. The mean time from last sexual exposure to the acute serum sample was 7.3 d for both acyclovir and placebo recipients.

Antibodies to viral glycoproteins and polypeptides in acute-phase sera. While all patients lacked HSV-neutralizing antibody in their first serum sample (acute phase), 60 of 63 patients had antibody detected by radioimmunoprecipitation to the glycoproteins IIg130 and gAB (Table I and Fig. 2, lanes A and D) and the major nucleocapsid polypeptide of 148,000 mol wt (vp148) (Fig. 2). 48 patients (63.2%) had antibodies to a nonglycosylated viral polypeptide of 88,000 mol wt (vp88), while 7 (11.1%) had antibody to another nonglycosylated polypeptide of 66,000 mol wt (vp66). 13 patients had antibody to IIg80, and 4 of the 63 patients (6.3%) had antibody to gD in acute-phase sera. No significant differences in the frequency of antibody were noted in acute-phase sera in patients randomized to acyclovir or placebo treatment (Table I) or between patients enrolled in the intravenous, oral, or topical studies.

Neutralizing antibodies in convalescent-phase sera. All 28 placebo and 31 of 35 acyclovir-treated patients developed anti-HSV-2-neutralizing antibody in sera drawn 14–30 d after the onset of symptoms (convalescent-phase sera). The mean titers of neutralizing antibody to HSV-1 and HSV-2 were 9.7 and 10.6, respectively, in intravenous acyclovir recipients; 14.7 and 16.7, respectively, in oral acyclovir patients, and 16.8 and 28.8 respectively, in topical acyclovir recipients. The mean titers of neutralizing antibody to HSV-1 and HSV-2 in convalescent-phase sera in the 28 placebo-treated patients were 18.7 and 19.1, respectively. The mean neutralizing antibody titer to HSV-2 in convalescent-phase sera was 19.5 in placebo recipients enrolled in the oral and intravenous trials as compared with 11.2 in patients with first episode primary genital HSV-2 infection treated with oral or intravenous acyclovir ($P < 0.05$; t test).

Antibodies to viral glycoproteins and polypeptides in convalescent-phase sera. All 63 patients had demonstrable antibody to vp148, IIg130, and gAB in their convalascent-phase sera (Table I, Fig. 2, lanes B and E). 56 (89%) of 63 patients developed antibody to vp88 by 28 d after onset of symptoms (Table I).

The frequency of detectable antibody to IIg80 and vp66 in day 28 sera differed between acyclovir and placebo recipients. 24 acyclovir recipients (68.6%) vs. 25 placebo recipients (89.3%)

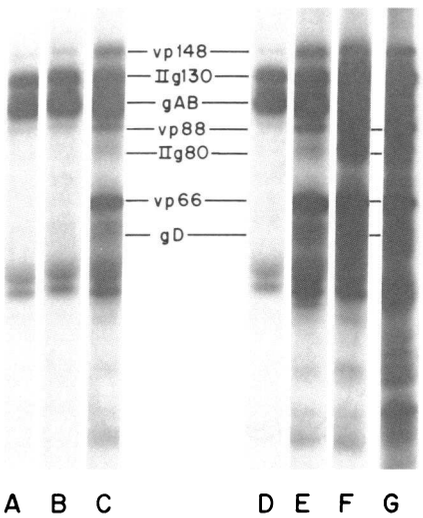

Figure 2. Electrophoretic profiles of [^{35}S]methionine labeled HSV-2 proteins precipitated by sequential sera from a patient receiving oral acyclovir (lanes *A, B, C*) or oral placebo (lanes *D, E, F*). Sera were drawn on day 5 (lanes *A, D*) day 28 (lanes *B, E*) and days 80 and 66 (lanes *C* and *F*, respectively) after onset of symptoms. The acyclovir patient had his first clinical recurrence at day 76. The placebo patient recurred at day 60. The HSV-2 antigen preparation used in these radioimmunoprecipitations is shown in lane G. Note that while the acute-phase sera are similar with respect to the proteins precipitated, convalescent-phase sera from day 28 from the acyclovir patient and from the placebo patient differ. The placebo serum has antibodies to IIg80, vp66, and gD. Antibody to IIg130, gAB, vp148, and vp88 was demonstrable in all sera.

Table I. Frequency of Antibody to HSV-2 Glycoproteins and Polypeptides in Acyclovir (ACV)- and Placebo-treated Patients

	Acute phase sera		Day 28 sera		Follow-up sera*	
Protein	ACV (n = 35)	Placebo (n = 28)	ACV (n = 35)	Placebo (n = 28)	ACV (n = 35)	Placebo (n = 28)
vp148	34	26	35	28	35	28
IIg130	34	26	35	28	35	28
gAB	34	26	35	28	35	28
vp88	25	23	30	26	32	28
IIg80	9	4	24	25	33	26
vp66	3	4	18	28	33	28
gD	2	2	15	17	26	20

n = number of patients in whom antibody to the designated protein was detected in serum sample. * Sera were drawn within 10 d of the first clinical recurrence or between 35 and 90 d of onset of the first episode from those patients who did not have clinical recurrences.

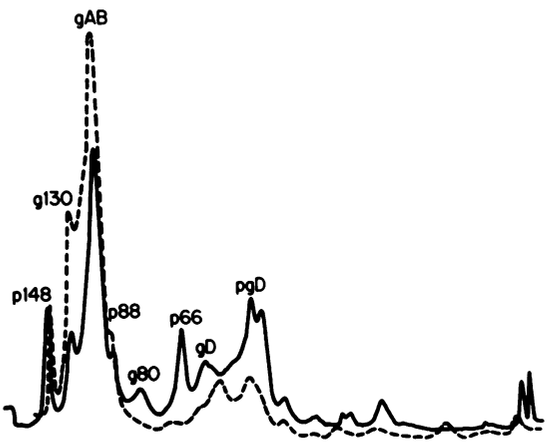

Figure 3. Densitometer scans of fluorograms of [^{35}S]methionine labeled HSV-2 proteins precipitated by convalescent phase sera from an oral acyclovir-treated patient (- - -) and from an oral placebo-treated patient (——. While no difference in levels of precipitated vp148 (p148) and vp88 (p88) are apparent, levels of precipitated IIg130 (g130) and gAB are higher in the acyclovir patient's profile. Lower levels of precipitated gD and pgD and lack of precipitated IIg80 (g80) and vp66 (p66) are apparent in the profile generated using the acyclovir patient's serum. Note that in comparing tracings of other acyclovir- or placebo-treated patients' profiles, relative levels of precipitated vp148, IIg130, gAB, and vp88 were not consistently different nor were levels of precipitated proteins of <60,000 mol wt. The only consistent differences between acyclovir and placebo patients' convalescent sera were in the frequency or levels of precipitated IIg80, vp66, and gD (Tables I–III).

had antibody to IIg80 ($P = 0.05$; chi square), while all of the placebo patients vs. 18 (62.9%) acyclovir recipients had antibody to vp66 ($P < 0.001$; chi square).

In addition to the difference in frequency of seroconversion to IIg80 and vp66 between acyclovir and placebo-treated patients, densitometry tracings revealed that the amounts of IIg80 and gD immunoprecipitated by convalescent-phase sera were less in the acyclovir-treated group (Fig. 3). While the frequency of seroconversion to gD by day 28 was similar in acyclovir (42.9%) and placebo recipients (60.7%) (Table II), the amount of gD immunoprecipitated by seropositive sera from acyclovir patients was consistently less than the levels of gD precipitated by placebo patients' sera (Fig. 3). Relative levels of immunoprecipitated vp148, IIg130, gAB, and vp88 varied between individuals (2) and were not consistently different between acyclovir and placebo-treated patients.

Antibodies to IIg80, gD, and vp66 in convalescent-phase sera of systemic acyclovir recipients. The differences in the frequency and amount of precipitating antibody to IIg80, gD, and vp66 in convalescent-phase sera between acyclovir and placebo recipients were seen only in intravenous or oral acyclovir recipients and not those treated with the topical preparation. Antibody to IIg80 was detected in convalescent-phase sera in 7 of 12 oral acyclovir and in 6 of 11 intravenous acyclovir recipients, compared with 18 of the 19 placebo-treated patients enrolled in these two studies ($P = 0.01$, FET). The mean time required for seroconversion to IIg80 was 43.0 and 35.5 d in intravenous and oral acyclovir recipients vs. 29.7 and 18.6 d in their placebo-treated counterparts ($P < 0.05$, t test). In contrast to systemic acyclovir-treated patients, antibody to IIg80 was demonstrated by 28 d after onset of lesions in 11 of 12 topical acyclovir recipients compared with 7 of the 9 placebo-treated patients enrolled in this study. The mean time to seroconversion was also similar between topical acyclovir and placebo recipients; 22.0 and 22.4 d, respectively.

Antibody to gD was detected in convalescent-phase serum of 17 (61%) placebo recipients compared with 4 of 11 patients

Table II. Effect of Acyclovir (ACV) on Development of Antibody to gD

Treatment group	No. who developed antibody to gD by 30 d into illness	No. who developed antibody to gD in follow-up sera*	Mean time to seroconversion	Range
			d	*d*
IV-ACV $n = 11$	4 (36%)	4	76.8 ($P < 0.05$)‡	11–210
IV-placebo $n = 10$	6 (60%)	1	18.8	7–34
Oral-AV $n = 12$	4 (33%)	5	55.2 ($P < 0.05$)‡	11–137
Oral placebo $n = 9$	5 (56%)	1	18.2	10–34
Topical ACV $n = 12$	7 (58%)	2	16.2	4–37
Topical placebo $n = 9$	6 (67%)	1	22.8	6–35

* Sera were drawn within 10 d of the first clinical recurrence or within 90 d of onset of the first episode from those patients who did not have clinical recurrences. ‡ The t test.

treated with intravenous acyclovir, 4 of 12 treated with oral acyclovir, and 7 of 12 treated with topical acyclovir (Table II) ($P = 0.06$; FET for comparison between oral and intravenous acyclovir-treated and placebo-treated patients). The mean time to seroconversion to gD was 76.8 d and 55.2 d in intravenous and oral acyclovir recipients compared with 18.8 days and 18.2 d in their placebo-treated counterparts ($P < 0.05$ for comparison between oral and intravenous acyclovir and placebo-treated patients (Table II).

The frequency and time course of development of antibody to vp66 was also affected by systemic acyclovir treatment. While all placebo recipients had detectable antibody to vp66 in convalescent-phase sera, only 4 of 11 intravenous acyclovir recipients and 3 of 12 oral acyclovir recipients had detectable antibody to vp66 in these sera ($P < 0.001$, FET) (Table III). Antibody to vp66 was detected in convalescent-phase sera in 11 of the 12 patients treated with topical acyclovir.

To determine whether the lack of antibodies to vp66, IIg80, or gD was due to an artifact such as degradation of these polypeptides by factors in the sera of acyclovir recipients, supernatants from the radioimmunoprecipitates were reacted with fresh serum that had previously been shown to immunoprecipitate vp66, IIg80, and gD. In all cases tested, all three polypeptides could be detected in the resulting precipitates. In addition, to evaluate whether these patients had antibodies to these polypeptides that were not bound by protein A, the experiment was repeated using sheep antihuman Fab as the secondary system. The results confirmed the lack of detectable antibody to vp66, IIg80, and gD in the sera in question.

Effect of recurrence on antibody development. Almost all the patients who lacked antibody to IIg80, gD, and/or vp66 in convalescent sera seroconverted to these polypeptides during follow-up; usually in association with their first untreated clinical recurrence of genital herpes (Table I). For example, 10 (9 acyclovir and 1 placebo) of the 14 patients lacking antibody to IIg80 in their day 28 sera developed antibody to IIg80 during follow-up. 5 of the 9 acyclovir recipients seroconverted to IIg80 within 10 d of the first clinical recurrence. The remaining five patients seroconverted within 90 d of the first episode without apparent association with a recurrent episode of disease. 14 of the 31 patients (11 acyclovir and three placebo recipients) who lacked antibody to gD in their day 28 sera seroconverted during follow-up. 9 of these 14 patients, all acyclovir recipients, seroconverted within 10 d of the first clinical recurrence.

15 of the 17 acyclovir-treated patients who lacked antibody to vp66 in their day 28 sera seroconverted to vp66 during follow-up. Seroconversion to vp66 was associated with the first clinical recurrence of genital herpes in 14 of these 15 patients. The 15th patient had antibody to vp66 detected in sera taken at a routine visit 111 d after her primary episode. Two patients (both intravenous acyclovir recipients) did not develop anti-vp66 during the course of follow-up. One did not develop anti-vp66 in sera 210 d after onset of her illness, despite a single symptomatic recurrence of genital herpes at day 103. The other patient had

Table III. Effect of Acyclovir (ACV) on Development of Antibody to vp66

Treatment group	No. who developed anti-vp66 in convalescent sera	No. who developed anti-vp66 after untreated recurrence	Mean time to seroconversion to vp66
IV-ACV ($n = 11$)	4	5	91.6 (±24.2) $P < 0.01$*
Placebo ACV ($n = 10$)	10	NA‡	16.0 (±2.8)
Oral ACV ($n = 12$)	3	8	74.6 (±13.6) $P < 0.01$*
Oral placebo ($n = 9$)	9	NA‡	16.1 (±2.8)
Topical ACV ($n = 12$)	11	1	23.3 (±2.5)
Topical placebo ($n = 9$)	9	NA‡	23.0 (±3.1)

* The *t* test.
‡ NA, not applicable; all placebo-treated patients had anti-vp66 before their first clinical recurrence.

no reported recurrences and no evidence of anti-vp66 before being lost to follow-up 57 d after enrollment into the study.

The 93% seroconversion rate to vp66 in association with an overt recurrence of disease was higher than the 64 and 50% seroconversion rates with a recurrence seen with gD and IIg80 ($P = 0.08$ and 0.03 (FET), respectively, for comparison between vp66 and gD and IIg80).

Association of antibody to vp66 and gD and severity of the first clinical recurrence. To evaluate the association between the presence of antibodies to IIg80, gD, and vp66 and disease course, we compared the severity and times to first clinical recurrence in patients who lacked one or more of these antibodies to those who possessed antibodies to these polypeptides in convalescent-phase sera. The median time to first clinical recurrence (83.5 d) was not affected by the presence or absence of demonstrable antibodies to vp66, gD, and/or IIg80 in day 28 sera.

Information on the clinical manifestations of the first untreated clinical recurrence of disease was available on 47 of the patients who recurred during the follow-up period. Of these 47 patients, 12 lacked antibody to both gD and vp66 in sera taken before the recurrence, 9 lacked gD but had anti-vp66, 3 possessed antibody to gD but lacked anti-vp66 and 23 had detectable amounts of antibody to both gD and vp66 before the first clinical recurrence.

The mean duration of lesions during the first clinical recurrence was 10.8 d in the 12 patients who lacked antibody to gD and vp66 compared with 6.3 d in those who were seropositive for both these polypeptides ($0.01 < P < 0.02$, *t* test) (Table IV).

Table IV. Relationship between the Presence of Antibody to gD and vp66 in Sera Taken before the First Clinical Recurrence and Clinical Severity of Disease

Present	A: both anti gD anti vp66 (n = 23)	B: anti-gD absent anti vp66 present (n = 9)	C: anti-gD present anti vp66 absent (n = 3)	D: both anti gD & anti vp66 absent (n = 12)
Mean duration of lesions*	6.3	8.0§	14.3	10.7‖
	(3.3)	(3.4)	(4.9)	(6.8)
Mean number of lesions‡	2.9	3.0§	5.8	6.1
	(2.2)	(1.9)	(1.1)	(9.7)
Mean lesion area (mm^2)*	17.4	16.5¶	52.0	37.9
	(18.6)	(25)	(6.1)	(44)

Standard deviation is given in parentheses. * $P < 0.01$ (The t test for comparison between groups A & C). ‡ $0.02 < P < 0.05$ (The t test for comparison between groups A & C). § $0.02 < P < 0.05$ (The t test for comparison between groups B & C). ¶ $P = 0.08$ (The t test for comparison between groups B & C). ‖ $0.01 < P < 0.02$ (The t test for comparison between groups A and D).

The mean number of lesions (6.1) and mean lesion area (36.0 mm^2) also tended to be larger in patients who lacked both gD and vp66 in sera before the recurrence as compared with those who possessed both gD and vp66 (2.9 lesions and 17.44 mm^2) but these differences were not statistically significant.

The mean duration of the first clinical recurrence was 11.5 d in 15 patients who lacked antibody to vp66 in sera taken before the recurrence compared with 6.8 d for the 32 patients who possessed detectable anti-vp66 in their sera ($0.02 < P < 0.05$ t test). Similarly, the mean number of lesions (6.0) and mean area of lesions (39.8 mm^2) was greater in the 15 patients who lacked antibody to vp66 as compared with the 32 patients who possessed antibody to vp66 before the recurrence (2.9 lesions and 17.2 mm^2) ($P < 0.05$ t test). The clinical manifestations of the recurrence were similar in those who were seropositive or seronegative for gD before the recurrent episode. The mean duration of lesions (9.6 d), mean number of lesions (4.8), and mean lesion area (28.1 mm^2) were similar for the 21 patients who lacked antibody to gD before their first clinical recurrence as compared with the 26 patients who possessed antibody to gD in sera before the recurrence (7.2 d, 3.2 lesions, and 21.4 mm^2, respectively, $P > 0.05$). The clinical severity of disease was also similar between the 9 patients who lacked only gD as compared with the 23 who were seropositive for both gD and vp66. The mean duration of lesions, mean number of lesions and mean lesion area were also greater in the 3 patients who lacked antibody to vp66 but had gD-specific antibody than in those who either had antibody to both these polypeptides or to vp66 alone (Table IV).

No apparent association between presence or absence of antibody to IIg80 and severity of the first clinical recurrence was noted. However, as lack of antibody to vp66 or gD and IIg80 were not related, the number of patients for subset analysis was small. For example, 10 of the 17 patients who lacked antibody to vp66 in convalescent sera had demonstrable antibody to IIg80. Similarly, of the 30 patients who lacked antibody to gD, 18 had antibody to IIg80. Presence or absence of antibody to IIg80 did not appear to be a covariable with antibodies to vp66 and/or gD in severity of the recurrence.

Discussion

Our study indicates that systemic acyclovir treatment of first episode primary genital HSV-2 infection influences the subsequent development of antibodies to HSV-specified polypeptides. Patients who were treated with oral or intravenous acyclovir within 7 d of onset of their first episode of primary genital HSV demonstrated (*a*) lower neutralizing antibody titers to HSV-2 in convalescent-phase sera, (*b*) lower frequency and delayed seroconversion to gD and IIg80, and (*c*) decreased frequency of seroconversion to vp66. Acyclovir treatment may also affect the frequency of time of seroconversion to other minor viral proteins. However, the variable response of patients to minor proteins precluded drawing conclusions from the populations studied. In contrast, topical acyclovir application to external genital lesions was not associated with alteration of the neutralizing antibody response or to any change in frequency or amount of precipitating antibodies directed to HSV-specified polypeptides.

Convalescent-phase sera of patients treated with oral or intravenous acyclovir had lower neutralizing antibody titers and decreased frequency of precipitating antibodies to IIg80 and vp66. Decreased levels of precipitating antibody to gD were apparent in these sera. However, the frequency and levels of precipitating antibodies to IIg130 and gAB appeared to be unaffected by acyclovir therapy. Numerous reports have indicated that virion surface glycoproteins are associated with neutralization (16–18). In particular, mouse monoclonal antibodies to gAB have been shown to neutralize infectivity of HSV-1 (16) and HSV-2 (17), while antibodies to HSV-1 gC can neutralize

HSV-1 infectivity (19, 20). Mouse monoclonal antibodies to HSV-1 gD have also been shown to have neutralizing activity in vitro (19) and in vivo (20). Our findings using human sera suggest that optimal neutralization of HSV in humans may require participation of antibodies directed against more than one surface glycoprotein and/or even the presence of antibodies to nonglycosylated polypeptides; a hypothesis that could be addressed with direct functional assays of antibodies to these proteins.

The mechanism by which systemic acyclovir influences the subsequent development of HSV-specific polypeptides is not clear. The difference in the immune response between systemic and topically treated acyclovir recipients may be due to the greater effect of systemic acyclovir on the duration of viral shedding. In addition, topical acyclovir therapy does not affect the duration of viral shedding at mucosal sites such as the cervix, throat, and urethra; sites infected in >90% of persons with primary first episodes of genital herpes. Follow-up of oral and intravenous acyclovir-treated patients who did not seroconvert to gD, IIg80, or vp66 after their primary infection revealed that almost all subsequently developed antibodies to these viral polypeptides. 93% of those who subsequently seroconverted to vp66, 64% of those who subsequently seroconverted to gD, and 50% of those who subsequently seroconverted to IIg80 did so in association with an untreated recurrence of disease. It should be remembered that asymptomatic shedding of virus from the cervix, vulva, or urethra does occur, is of short duration, and would not have resulted in the patient reporting to our clinic. (21, 22). The late seroconversions to these polypeptides not associated with overt recurrences may have been in response to asymptomatic episodes of viral shedding. Whether host immune responses to viral polypeptides; in particular vp66, IIg80, and gD differ between asymptomatic vs. symptomatic periods of disease will require further study.

The high frequency of seroconversion to vp66 and gD in conjunction with the first untreated recurrence of genital herpes does, however, suggest that the delayed development of antibody to these polypeptides after the first episode of genital herpes is related to the effect of acyclovir on viral replication rather than to a failure of the host-immune response to these specific polypeptides. Systemic acyclovir treatment markedly shortens the duration of viral shedding during the primary episode (5, 8). Virus may be present in inadequate amounts or for too short a period of time for immune stimulation by these viral polypeptides. Alternatively, acyclovir treatment may produce noninfectious particles lacking these polypeptides or may cause an alteration in the antigenicity of these viral polypeptides. While acyclovir has been shown to produce noninfectious defective particles in vitro, a similar effect in vivo has not been shown.

Evaluation of the severity of the subsequent clinical recurrences after systemic acyclovir therapy indicated that patients who lacked antibody to both gD and vp66 had a significantly longer duration of lesions than those who had antibodies to both these proteins in sera taken before the recurrence. While the number of patients who lacked only one of these two antibodies was small, patients who were seronegative to vp66 but seropositive to gD tended to have more lesions, larger lesion areas, and a longer duration of lesions as compared with those who lacked only antibody to gD. Thus, while acyclovir therapy affects a variety of viral polypeptides, these data suggest that antibody to vp66, in particular, may be an important marker of clinical severity of infection. Development of specific quantitative assays for antibodies to vp66 and other HSV polypeptides should allow further evaluation as to the role they play in the clinical expression of symptomatic and/or asymptomatic genital HSV infections.

While systemic acyclovir therapy and its apparent effect on antibodies to viral-specified polypeptides appears to affect the first untreated clinical recurrence of genital herpes, our data should not be construed to discourage the use of systemic acyclovir treatment for primary first episode genital herpes. The clinical benefit of systemic acyclovir therapy in primary disease does, in our opinion, offset the slight increase in severity of the first subsequent recurrence of disease, seen in the 40–60% of systemic acyclovir-treated patients who have delayed seroconversion to gD and/or vp66 (4, 8). Detailed monitoring of the effect of systemic acyclovir therapy on the long-term natural history of the clinical and immunologic responses of patients with genital herpes is likely to provide valuable insights into host and virion factors that influence disease expression and/or severity.

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