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Virus-Specific Antibodies in Sera from Patients with Genital Herpes Simplex Virus Infection

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Virus-specific antibodies against a number of herpes simplex virus type 2 antigens were determined by radioimmunoprecipitation assays in sequential serum samples obtained from 12 patients with initial genital herpes simplex virus infection. The progressive appearance of antibodies to virus-specific antigens was observed; antibodies against a 130,000-molecular-weight glycoprotein complex appeared first, followed by antibodies against the major nucleocapsid polypeptide and then antibodies against a number of other viral antigens, including a polypeptide with a molecular weight of 62,000. Patients who developed a wide variety of antibodies to viral polypeptides shortly after resolution of their initial episode seemed to experience more severe initial infections and more recurrences than did those who reacted poorly with these virus-specific antigens. This was most apparent with respect to antibodies to virus-specific polypeptides with molecular weights between 30,000 and 43,000. Antibody specificity did not change during the course of follow-up regardless of whether serum samples were taken shortly before, during, or after recurrent episodes. Glycoprotein-specific antibodies were quantitated with the purified 130,000-molecular-weight glycoprotein material. No significant fluctuations in these antibody titers were observed before or after recurrences of the disease.

Recurrences of both oral-labial and genital herpes simplex virus (HSV) infections in humans occur frequently. In over 60% of patients with initial genital HSV type 2 (HSV-2) infections, the infection recurs within 6 months, and patients with recurrent genital disease have a median of five recurrences per year (6, 16).

Both humoral and cellular immune responses are important in controlling experimental and human HSV infections. In mice, virus-specific antibodies prevent the spread of virus from the site of primary infection to the spinal cord (16) and reduce the extent of viral replication in sensory ganglia (22). Furthermore, antiviral immunoglobulin G prevents the appearance of infectious virus in transplanted latently infected ganglia (21). The importance of cellular immunity in preventing HSV recurrence is suggested by the increased incidence and severity of herpetic infection among immunosuppressed patients (1, 2, 14, 18). However, patients with both oral and genital infections experience recurrences despite high levels of neutralizing antibodies and virus-specific cellular immunity (7, 12, 17). To explain this paradox, detailed analyses of the specificities of the immune responses during primary and recurrent infections are needed. This is especially important if recurrences are caused by temporal deficiencies in one or more of the many immune parameters associated with herpesvirus infections.

Recently, we compared sera from patients with recurrent facial infections with those from seropositive individuals without recurrences for antibodies against herpesvirus antigens (26). However, this study did not evaluate persons with primary HSV infections. We report here findings on 12 patients with initial genital herpes infection who were followed prospectively for a long period of time and from whom a large number of serum samples were obtained soon after the initial infection as well as immediately before and after recurrences of the disease. These samples were used to determine the sequential appearance of antibodies against a number of HSV-2-specific antigens and the persistence of these antibodies as recurrent infections developed.

MATERIALS AND METHODS

Cells and virus. Primary rabbit kidney cells and primary mouse embryo fibroblasts were grown as described previously (26).

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The Curtiss strain of HSV-2 was obtained from A. Friedman, Merck Institute for Therapeutic Research, West Point, Pa. It was plaque purified three times in primary rabbit kidney cells under carboxymethylcellulose (19) and then grown in primary rabbit kidney cells (for details, see reference 26).

Clinical isolates were obtained from the vesicular fluid of primary and recurrent genital lesions. Vesicular lesions were opened with a 25-gauge needle; the vesicular fluid was collected and the base was scraped with a calcium alginate swab (Inolex Corp., Glenwood, Ill.), and this was placed in Eagle minimal essential medium (EMEM). Within 4 h of collection, 0.25 ml was inoculated into duplicate tubes of fetal tonsil cells in EMEM plus 1% fetal calf serum (24). Cultures were examined three times a week for cytopathic effect, and the cells and supernatant fluids from positive cultures were stored at −70°C. Virus was plaque purified twice, and stocks were prepared as described above. Alternatively, stocks were prepared without plaque purification by infecting primary rabbit kidney cells at a multiplicity of infection of 0.01.

Neutralizing antibody determination. Methods have been described for measuring complement-fixing (CF) antibodies to HSV-2 (24), complement-independent neutralizing antibodies (15, 23), and complement-dependent neutralizing antibodies (26).

Labeling of infected cells. Primary rabbit kidney or mouse embryo fibroblast cells (in 75-cm^2 flasks [Falcon Plastics, Oxnard, Calif.]) were infected at a multiplicity of infection of 0.1 in EMEM without fetal calf serum. When cells began to round up (generally 12 to 16 h postinfection, when most host protein synthesis had ceased), medium was removed, and the cells were washed with phosphate-buffered saline and incubated with 5 ml of EMEM without methionine per flask. Thirty minutes later, 100 μCi of [^{35}S]methionine (Amersham Corp., Arlington Heights, Ill.; 500 Ci/mmol) was added. After 4 h, the cells were washed twice with complete EMEM, incubated for 1 h in EMEM, and removed from the surface of the flask with 0.02% EDTA and 0.05% $NaHCO_3$ in phosphate-buffered saline containing 0.005 M KCl. The infected cell suspension was washed once in phosphate-buffered saline and stored at −70°C as pellets containing 10^6 cells. Unlabeled uninfected cells were harvested similarly and stored as pellets containing 3×10^6 cells.

Immune precipitation and analysis of precipitated viral antigens. Briefly, a soluble extract was prepared from primary rabbit kidney or mouse embryo fibroblasts infected with HSV-2 and labeled with [^{35}S]methionine. Samples were incubated with the appropriate serum samples, and antigen-antibody complexes were isolated with *Staphylococcus aureus* bearing protein A (9), followed by their dissociation in sodium dodecyl sulfate and 2-mercaptoethanol. [^{35}S]methionine-labeled precipitated viral antigens were separated by electrophoresis in 10% discontinuous slab gels (13), and they were visualized by fluorography (3). (For a detailed description of the procedure, see reference 26.)

Quantitation of glycoprotein-specific antibodies. Extracts from [^{35}S]methionine-labeled infected cells were fractionated on columns with the lectin from *Lens culinaris* coupled to Sepharose 4B (for details, see references 25 and 26). Glycoprotein material that absorbed to the lectin and eluted with α-methyl-D-mannoside and glucose was dialyzed and diluted to contain 2,000 cpm/20 μl. This was incubated with 20 μl of serum at room temperature for 2 h. A 50-μl amount of *S. aureus* bearing protein A (10% vol/vol) was added, and incubation was continued for another 15 min, followed by centrifugation at 6,500 × *g* for 2 min and one wash of the pellet in 0.05 M Tris buffer (pH 7.4) containing 0.15 M NaCl and 0.05% Triton X-100. Radioactivity in the combined supernatants was determined by adding the combination to 10 ml of Aquasol (New England Nuclear Corp., Boston, Mass.). Pelleted material was suspended in 0.2 ml of Tris buffer containing 2% sodium dodecyl sulfate and added to 10 ml of Aquasol. Percent precipitation was expressed as counts per minute in the pellet divided by counts per minute in the pellet plus counts per minute in the supernatant.

Statistical methods. Results were analyzed by using either the rank sum or Student's *t* test.

RESULTS

Clinical course and serological responses. Sera and viral isolates were obtained at the University of Washington genital HSV clinic from 12 patients (four males and eight females) without previous symptoms of genital HSV infection. The mean age of patients was 25.8 years (range, 19 to 33 years), and all were Caucasian. Serological data and a summary of the clinical symptoms of these patients are shown in Tables 1 and 2.

Acute-phase sera and the initial clinical HSV isolates were obtained from all patients within 11 days of the onset of symptoms or lesions or both (mean, 7.4 days). Eight patients (2, 3, 6, 7, 8, 9, 11, and 12) did not have CF or neutralizing antibodies in their acute-phase sera (Table 1), and presumably they experienced a primary infection (15). The other four patients (1, 4, 5, and 10) had herpesvirus-specific antibodies in their acute-phase sera. The high antibody levels in patients 4 and 5 clearly suggest previous exposure to HSV. Acute-phase sera from patients 1 and 10 were obtained relatively late after the onset of initial lesions (11 days) compared to the other patients, and they lacked complement-independent HSV-2-neutralizing antibodies (patient 10) or CF antibodies (patient 1). Thus, the nature of the infection (primary or secondary) could not be established with certainty for these patients. Immunoprecipitation analyses (see below) did suggest that patient 1 had a primary infection and that patient 10 had been exposed to HSV at an earlier date.

Table 2 shows that patients with primary infections had extensive genital disease, with a mean duration of viral shedding of 13.5 days (range, 4 to 31 days) and a mean duration of lesions of 23.5 days (range, 8 to 41 days). Five of the eight patients experienced symptoms of fever, headache, myalgia, photophobia, or neck stiffness.

TABLE 1. Antibody titers in acute-phase, early convalescent-phase, and late-phase sera

Patient no. (sex)	No. of days after onset of initial lesions[a]	CF antibody titer (HSV-2)	Complement independent neutralizing antibody titers		Complement enhanced neutralizing antibody titer (HSV-2)
			HSV-1	HSV-2	
1 (M)	11 (A)	<8	<8	64	64
	19 (C)	16	8	64	128
	86 (L)	16	16	32	128
2 (F)	8 (A)	<8	<8	<8	≤16
	27 (C)	8	<8	<8	256
	237 (L)	<8	<8	32	ND[b]
3 (M)	3 (A)	<8	<8	<8	<16
	31 (C)	16	<8	64	64
	59 (L)	<8	<8	32	64
4 (M)	3 (A)	64	128	128	128
	35 (C)	32	128	128	128
	72 (L)	16	256	128	256
5 (M)	10 (A)	128	256	128	512
	31 (C)	128	128	64	512
	45 (L)	ND	128	128	256
6 (F)	3 (A)	<8	<8	<8	<16 (16)[c]
	60 (C)	<16	<8	<8	<16 (32)[c]
	67 (L)	<8	<8	<8	<16 (64)[c]
7 (F)	9 (A)	<8	<8	<8	≤16
	32 (C)	32	<8	<8	256
	514 (L)	32	128	64	512
8 (F)	4 (A)	<8	<8	<8	<16
	45 (C)	128	<8	<8	128
	379 (L)	≥512	<8	32	64
9 (F)	5 (A)	<8	<8	<8	<16
	19 (C)	128	<8	<8	512
	115 (L)	128	<8	16	512
10 (F)	11 (A)	64	<8	<8	256
	29 (C)	128	32	64	256
	465 (L)	64	64	128	512
11 (F)	9	<8	<8	<8	ND
	14 (A)	64	<8	16	128
	49 (C)	64	<8	>32	128
	584 (L)	16	8	8	128
12 (F)	8 (A)	<8	<8	<8	≤16
	50 (C)	64	16	32	256
	395 (L)	32	32	8	512

[a] A (acute), C (early convalescent), and L (late) refer to the serum samples as they were used in experiments described in the text.

[b] ND, Not done.

[c] Complement-dependent neutralizing antibodies against the patient's own viral isolate.

CF and complement-enhanced HSV-2-neutralizing antibodies were present in all early convalescent-phase serum samples except that from patient 6. Late convalescent-phase serum samples, except that from patient 6, contained complement-dependent and complement-independent HSV-2-specific antibodies. Except for patient 7, all eight patients with primary infections, and patient 1, with a possible primary infection, had higher HSV-2-specific than HSV-1-specific complement-independent neutralizing antibody titers. Therefore, these patients were likely to be infected with HSV-2.

Patient 6 did not demonstrate neutralizing antibodies to the laboratory strains of HSV-1 or HSV-2, but did develop neutralizing antibodies to the HSV strain isolated from her initial genital lesions. Restriction endonuclease analysis with *Eco*RI and *Hpa*I showed that this isolate was HSV-1 (11; data not shown).

Of the 12 patients, 10 experienced a recurrence of disease during the follow-up period. The mean time from the onset of the initial episode to the first recurrence was 159 days (range, 41 to 630 days), and the rate of recurrence per 100 patient days of prospective follow-up ranged from 0.22 (one recurrence per 476 days) to 3.25 (one recurrence per 31 days) (Table 2).

Virus-specific antibodies in patient sera. Antibodies against individual herpesvirus polypeptides were identified by incubating the serum

TABLE 2. Clinical signs and symptoms of disease

Patient no.	Symptoms and signs of initial episode				Follow-up data		
	Presence of systemic symptoms[a]	Nature of infection	Duration of viral shedding from external lesions (days)	Duration of lesions (days)	Time to first recurrence (days)	Duration of follow-up (days)	Recurrence rate per 100 follow-up days
1	Yes	Probably primary	21	29	41	292	2.73
2	Yes	Primary	8	17	NR[b]	433	0.00
3	No	Primary	12	28	92	234	1.28
4	No	Secondary	2	7	NR	309	0.00
5	No	Secondary	8	21	83	115	1.74
6	Yes	Primary	4	9	381	918	0.22
7	Yes	Primary	9	15	63	1,380	2.03
8	No	Primary	10	19	80	521	2.30
9	No	Primary	13	14	630	971	0.22
10	No	Probably secondary	21	32	75	1,108	3.25
11	Yes	Primary	21	40	68	909	2.09
12	Yes	Primary	31	41	80	1,175	1.72

[a] Fever, headache, myalgia, photophobia, or neck stiffness.
[b] NR, No recurrence.

samples listed in Table 1 with extracts prepared from mouse embryo fibroblast cells infected with HSV-2 (Curtiss) in the presence of [^{35}S]methionine. Precipitated antigens were identified after electrophoresis in sodium dodecyl sulfate-polyacrylamide gels.

The immunoprecipitation patterns (Fig. 1) differed for the various sera, with some precipitating more than 18 viral polypeptides and others being similar to control seronegative serum. [^{35}S]methionine was added to infected cells late in the replication cycle, when host protein synthesis had ceased, and it is likely that all polypeptides visible on the gels were virus coded. Furthermore, polypeptides were not precipitated by immune serum from radiolabeled extracts of uninfected cells (data not shown), and control serum reacted only slightly with only one polypeptide (see the last track in Fig. 1B). The largest major precipitated polypeptide (labeled C in Fig. 1) is the major constituent of viral nucleocapsids (4, 20). Next there is a group of at least two polypeptides that are glycoproteins (labeled GP130 in Fig. 1) with an approximate molecular weight of 130,000 (4). They are referred to here as GP130. The nature of the other polypeptides, including a major precipitated polypeptide of 62,000 molecular weight (P62), was not determined.

Virus-specific antibodies during initial genital herpes infections. Acute-phase sera of five of the seven patients without significant CF or neutralizing antibody titers (patients 2, 3, 6, 8, and 9) demonstrated immunoprecipitation patterns similar to that of the seronegative control; there was no reaction or a weak reaction with the glycoprotein and no reaction with the nucleocapsid polypeptide (Fig. 1). Acute-phase sera from the two other patients with primary infections (patients 7 and 12) reacted only with the GP130 complex. Two acute-phase serum samples were taken from patient 11. The first one, which lacked CF antibody and complement-independent neutralizing antibodies, was not available for immunoprecipitation analyses. Another sample taken 5 days later, which had measurable CF and neutralizing antibodies, precipitated a number of viral antigens, including GP130, the nucleocapsid, and P62 antigens.

Acute-phase serum from patient 4, with evidence of prior HSV infection, contained a wide range of antibodies to viral polypeptides, whereas that from another patient with a nonprimary initial infection (patient 5) reacted with the nucleocapsid polypeptide and GP130, but only weakly with other HSV-2 antigens.

Virus-specific antibodies in convalescent-phase sera. All late convalescent-phase sera (labeled L in Fig. 1) contained antibodies that were reactive with the nucleocapsid polypeptide, the GP130 complex, and P62, although in patients 3 and 6 the reactivity with the latter antigen was weak (late convalescent-phase serum from patient 5 was not available). Reactivity with a number of other antigens was more variable. For example, late convalescent-phase serum from patient 1 reacted strongly with a polypeptide with a molecular weight of 72,000 and a number of polypeptides with molecular weights between 30,000 and 43,000, whereas convalescent-phase sera from patients 2 and 3 were much less reactive with these antigens. Patients with secondary infections who had antibodies to a wide range of viral antigens in their acute-phase sera (patients 10 and 11) demonstrated similar immunoprecipitation patterns in their convalescent-phase sera. Early convalescent-phase sera (labeled C in Fig. 1) from patients with primary infections showed

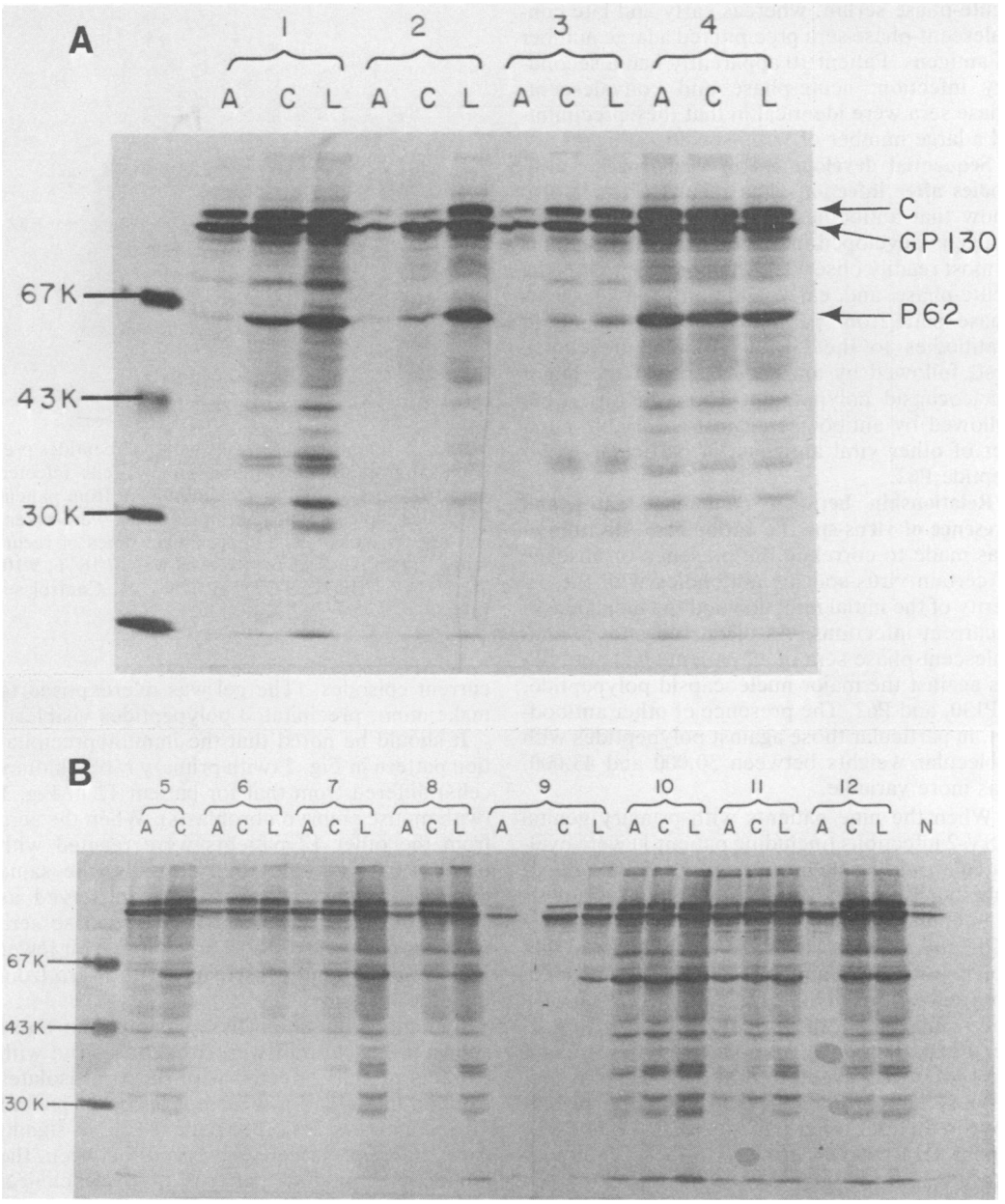

FIG. 1. Fluorograms of gels with polypeptides precipitated from cytoplasmic extract of mouse embryo fibroblasts infected with HSV-2 (Curtiss) by three different serum samples (acute [A], early convalescent [C], and late [L]; see Table 1) from 12 patients with genital herpes infection and from an individual who was seronegative for HSV-1 and HSV-2 (N). (A) Patients 1 through 4; (B) patients 5 through 12 and the seronegative individual.

immunoprecipitation patterns that were intermediate between those of acute-phase and late convalescent-phase sera. Interestingly, serum from patient 6 that failed to neutralize HSV-2 (Curtiss) did react with the nucleocapsid polypeptide, GP130, and P62 that were synthesized by this HSV-2 strain.

Measurements of CF and neutralizing antibodies in the sera of patients 1 and 10 could not establish whether infections were primary or secondary (see above). The immunoprecipitation patterns (Fig. 1) suggest that patient 1 had a primary infection: a few antigens (nucleocapsid and GP130 polypeptides) were precipitated by

acute-phase serum, whereas early and late convalescent-phase sera precipitated a large number of antigens. Patient 10 apparently had a secondary infection: acute-phase and convalescent-phase sera were identical in that they precipitated a large number of virus-specific antigens.

Sequential development of virus-specific antibodies after infection. The results (Fig. 1) also show that antibodies against the various HSV antigens developed in a specific sequence. This is most readily observed when one compares the acute-phase and early and late convalescent-phase sera from patients 2, 6, 7, 8, and 9. Antibodies to the GP130 complex developed first, followed by antibodies against the major nucleocapsid polypeptide. These in turn were followed by antibodies against a variable number of other viral antigens, in particular, polypeptide P62.

Relationship between recurrence rates and presence of virus-specific antibodies. An attempt was made to correlate the presence or absence of certain virus-specific antibodies with the severity of the initial infection and the incidence of recurrent infections. As discussed above, convalescent-phase sera of all patients had antibodies against the major nucleocapsid polypeptide, GP130, and P62. The presence of other antibodies, in particular those against polypeptides with molecular weights between 30,000 and 43,000, was more variable.

When the nine patients with primary genital HSV-2 infections (including patient 1) were evaluated as a separate group, a trend (although not statistically significant) was observed. Patients 2, 3, 6, and 9, whose sera were weakly reactive with the 30,000- to 43,000-molecular-weight polypeptides, had a mean duration of viral shedding and lesions of 8.3 ± 1.7 and 17.0 ± 4.0 days and a subsequent mean recurrence rate of 0.43 per 100 patient days, compared with 18.4 ± 4.1 days of virus shedding, 28.8 ± 5.3 days of lesions, and 2.17 recurrences per 100 patient days in the four other patients (7, 8, 11, and 12), whose sera reacted strongly to these viral antigens.

To evaluate whether virus-specific antibodies varied over the course of the disease, sequential serum samples from patients 10, 11, and 12 were reacted with cytoplasmic extracts of primary rabbit kidney cells infected with HSV-2 (Curtiss). Figure 2 shows the results for patient 12 (similar results were obtained from the other two patients). Once a full complement of virus-specific antibodies was present (in this case, early convalescent-phase serum), no significant changes in the immunoprecipitation pattern in the sequentially obtained serum samples were apparent, regardless of whether they were obtained immediately before, after, or during recurrent episodes. (The gel was overexposed to make minor precipitated polypeptides visible.)

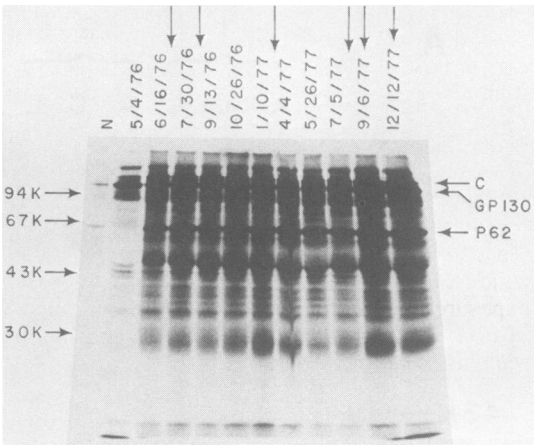

FIG. 2. Fluorograms of gels with polypeptides precipitated from primary rabbit kidney cells infected with HSV-2 (Curtiss) by sequential sera from patient 12. Dates indicate when serum samples were taken, and arrows indicate the approximate times of recurrence. Exact dates of recurrences were 7/16/76, 9/10/76, 2/14/77, 7/10/77, 9/5/77, 12/12/77. N, Control serum.

It should be noted that the immunoprecipitation pattern in Fig. 2 (with primary rabbit kidney cells) differed from that for patient 12 in Fig. 1 (with mouse embryo fibroblasts). When the sera from the other 12 patients were reacted with primary rabbit kidney cell extracts, the same differences among patients were observed as shown in Fig. 1; i.e., convalescent-phase sera from patients 2, 3, 6, and 9 precipitated far fewer HSV-2-specific antigens than did the sera from the other patients.

Immunoprecipitation assays similar to those shown in Fig. 1 and 2 were also carried out with extracts of cells infected with the viral isolates from an initial and a recurrent lesion of patient 12 and an initial lesion of patient 10. No significant differences were observed between the immunoprecipitation patterns of these clinical isolates and the HSV-2 (Curtiss) strain.

Quantitation of antibodies against the GP130 complex in patient sera. The immune response to the GP130 glycoprotein complex was quantitated with material from HSV-2 (Curtiss)-infected primary rabbit kidney cells that specifically bound to the lectin from *L. culinaris*. It has been found that more than 85% of the bound radiolabeled virus-specific polypeptides consist of the GP130 complex (25).

To evaluate whether fluctuations in the titer of antibody to this glycoprotein complex varied over the course of disease, we assayed 10 sequential serum samples obtained from patient 12

over a 20-month period. The results in Fig. 3 show that there was little difference between the early convalescent-phase and subsequent serum samples in antibody titers to the GP130 complex. Specifically, transient decreases in antibodies to the GP130 complex were not detected in specimens taken shortly before recurrences. In fact, there was a trend toward increased reactivity in samples taken late in the course of the disease. This could represent hyperimmunization of this individual because of repeated exposure to the virus during recurrent episodes of disease. Similar results were obtained with sequential serum samples from the other patients.

Glycoprotein fractions were also prepared from primary rabbit kidney cells infected with clinical isolates from patients 10 and 12, and they were used for titration experiments. Results similar to those in Fig. 3 were obtained (data not shown).

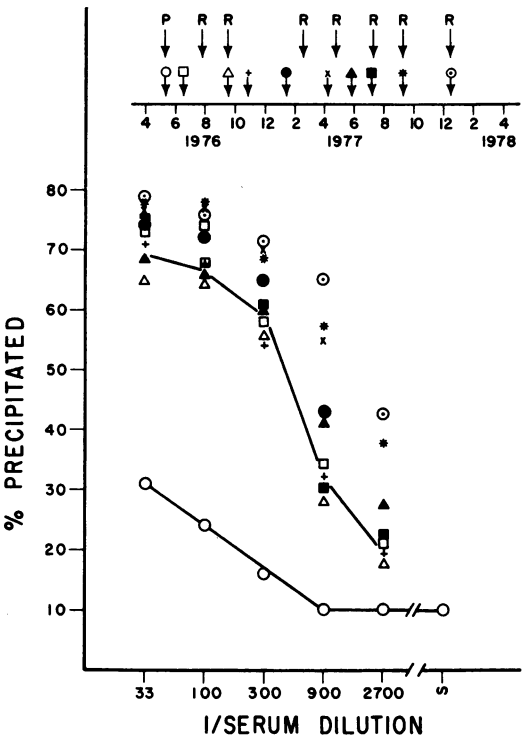

FIG. 3. Titration of antibodies in 10 sequential serum samples from patient 12 against the lectin-purified GP130 complex. The time scale at the top shows when serum samples were taken; corresponding symbols are used in the titration curves. The time scale also shows when recurrent lesions appeared. P, Primary infection; R, recurrent infection. The serum samples were the same as those used in Fig. 2, except that the acute-phase serum sample taken on 5/4/76 was used, and the late-phase sample taken on 7/30/76 was omitted.

DISCUSSION

Studies of antibody responses to virus-specific polypeptides in patients with initial genital HSV infections were undertaken in patients who presented early in the course of infection and who were prospectively followed with frequent virus isolations, periodic serum samplings, and regular clinical observation. Radioimmunoprecipitation assays were used to determine the sequential development of HSV-2-specific antibodies during initial genital herpes infections and to evaluate whether antibodies persisted immediately before, during, and after recurrent episodes.

Our data indicate a sequential evolution in the antibody response to virus-coded polypeptides during primary genital herpes. Antibodies against a high-molecular-weight (130,000) glycoprotein complex appeared first, followed by antibodies against the major 150,000-molecular-weight nucleocapsid polypeptide. Next antibodies appeared against a number of other viral antigens. Among the latter was a polypeptide with a molecular weight of 62,000. This does not appear to be a surface glycoprotein (no incorporation of [^{3}H]glucosamine, no binding to lectin from *L. culinaris*, and no iodination in the presence of lactoperoxidase; data not shown), and therefore it is not one of the major type-common viral envelope glycoproteins (5, 8). All patients with serological evidence of prior HSV-1 or HSV-2 infection had antibodies reactive with both the GP130 and nucleoprotein antigen in serum drawn early in the initial genital infection.

Because antibodies against relatively heavily labeled polypeptides are easiest to detect, antibodies against a number of polypeptides with little [^{35}S]methionine may appear early along with those against the nucleocapsid and core antigens without being detected at that time. It was of interest that patient 6, without neutralizing antibodies against HSV-2 (Curtiss), did demonstrate reactivity with HSV-2 (Curtiss)-specific polypeptides in his convalescent-phase serum. This case illustrates the apparent utility and increased sensitivity of these immune precipitation reactions compared to standard serological tests as a measure of immunological responsiveness to HSV-2.

The data suggest but do not prove an association between the immune response to virus-specific polypeptides during initial genital infections and the severity of the initial infection, as well as the subsequent recurrence rate of disease. Only nine patients with primary genital infections were studied. Among them, six (patients 1, 3, 7, 8, 11, and 12) experienced frequent recurrences, with a mean recurrence rate of 2.03 per 100 patient days of follow-up, and three (patients 2, 6, and 9) experienced either no

recurrence or infrequent recurrence, with a mean recurrence rate of 0.14 per 100 patient days of follow-up. The early convalescent-phase sera from the six patients who had frequent recurrences reacted strongly with the nucleocapsid polypeptide, the GP130 complex, and a large number of minor viral antigens, especially those with molecular weights between 30,000 and 43,000 (Fig. 1). Sera from patients who had infrequent recurrences did not react well with the 30,000- to 43,000-molecular-weight polypeptides. These data suggest that the host immune response to initial genital infection is a determinant of subsequent recrudescent disease. It should be remembered, however, that there is great variability in the clinical course of initial genital HSV infection, and extension of these observations to more patients is needed.

In previous studies of larger cohorts of patients, we have shown that patients with serological evidence of prior HSV-1 infection tend to have a shorter course of initial genital disease than do patients without prior exposure to HSV (6). To date, follow-up has indicated no difference in subsequent recurrence rates between these two groups of patients (19a; unpublished data). In addition, among patients with primary genital HSV-2 infection, a correlation between high titers of complement-independent neutralizing antibody to HSV-2 titer in early convalescent-phase serum and the subsequent rate of recurrence was observed (Reeves et al., in press). Similarly, in mice a correlation was found between the severity of initial genital infection, the level of neutralizing antibody in convalescent-phase serum, and the ability to demonstrate latent virus in sacral nerve root ganglia (10). Continued prospective evaluation of intensively followed patients with these radioimmunoprecipitation techniques, including attempts to quantitate the amount of virus associated with the initial episode of disease in all the infected anatomical areas, should provide more definitive data concerning the causal relationship between severity of illness, host immune response to initial infection, and rates of recurrence.

Sequential serum samples taken from a number of individuals during the course of follow-up indicated that antibody specificity to the virus-specific polypeptide did not change regardless of whether samples were taken shortly before, during, or after recurrent episodes of disease. These data agree with an earlier observation (26) that virus-specific antibodies do not fluctuate during recurrent facial herpes infections. Although these data appear to suggest that fluctuations in the levels of antibodies are not a factor in recrudescent disease, it should be remembered that these immunoprecipitation techniques may not detect all relevant viral antigens. In addition, each antigen contains numerous antigenic sites and their corresponding antibodies. Within these populations there may be changes that correlate with recurrent infections, and the immunoprecipitation assays used would not detect these changes. Monoclonal antibodies directed against specific regions of relevant antigens should allow for a more accurate evaluation of the possible role of transient fluctuations of specific antibody levels in recurrent infections. Further development of these reagents should also allow the quantitation of antibody responses to virus-specific antigens other than the GP130 complex, especially those antigens with molecular weights between 30,000 and 43,000.

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ADDENDUM IN PROOF

The type specificity of the viral isolated from the 12 patients was confirmed by the indirect immunoperoxidase method (D. Benjamin, Appl. Microbiol. **28**:568–571, 1974). HSV isolated from patient 6 was type 1, the other isolated were type 2.

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