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Human Antibody Response to Herpes Simplex Virus-Specific Polypeptides after Primary and Recurrent Infection

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Human antibody responses to specific polypeptides of herpes simplex virus types 1 and 2 (HSV-1 and HSV-2, respectively) were assessed in serial serum specimens from 18 infected patients by immunoblot technology. Nine patients had HSV-1 infections (six genital and three oral) and nine had HSV-2 genital infections. Antibodies to homologous and heterologous HSV antigens were studied and correlated with total microneutralization and enzyme-linked immunosorbent assay antibodies as well as correlated directly to purified glycoproteins. The data indicated a sequential appearance of antibodies to specific polypeptides, according to virus type and site of infection. After HSV-1 infection, the initial response was to glycoprotein B, but the same was not true for HSV-2 infection, where the initial response appeared to be to the type-specific glycoprotein G. A difference in sequential appearance of antibodies for the two viruses indicated greater reactivity to lower-molecular-weight polypeptides after genital infection, irrespective of type, in contrast to nongenital HSV-1 infections. The antibody responses for selected sera to purified glycoproteins B and D were verified by enzyme-linked immunosorbent assay antibody determinations.

Most humans have antibodies to herpes simplex virus (HSV), one of the most ubiquitous infectious agents (9, 16). Only a few seropositive individuals experience recurrences of HSV type 1 (HSV-1) or type 2 (HSV-2). Severe, life-threatening diseases such as HSV encephalitis or neonatal HSV infections are not uncommon. Prospective clinical, epidemiologic, virologic, and immunologic investigations have attempted to determine the basis for symptomatic versus asymptomatic infection as well as factors which influence recurrences. Host antibody responses to HSV-1 and HSV-2 have been a focus for study, including assessment of neutralization, passive hemagglutination, immunofluorescence, complement fixation, radioimmunoassay, and enzyme-linked immunosorbent assay (ELISA) antibodies (1, 6, 13, 14, 22, 24). Antibody responses to HSV-specific polypeptides have been determined with Western blot or immunoblot assays (4, 5, 10, 11). Antibodies to the HSV-1 glycoprotein gpB, gpC, gpD, gpE (8), and gpG (130,000 molecular weight) of HSV-2 (21), have been of particular interest, particularly their potential protective effects. The correlation of polypeptide-specific antibodies to those determined by conventional methods and clinical design has been limited (4). In addition, the potential problem of antigen denaturation during protein transfer has not been addressed.

Thus, immunoblot antibodies were determined for both homologous and heterologous HSV and compared with total microneutralization (MNT) and ELISA antibody responses by disease state. In addition, purified gpB and gpD were used to evaluate antibody response to exclude possible denaturation of antigens during transfer as a cause of spurious results.

MATERIALS AND METHODS

Virus and cells. HEp-2 cells were infected with strain F of HSV-1 and strain G of HSV-2 for the immunoblot assay and ELISA. BSC-1 cells (African green monkey kidney cells) were employed for MNT antigen preparation. Viral typing of patient isolates was performed with monoclonal antibodies as previously reported (19).

Reagents. Chemicals for polyacrylamide gel electrophoresis were purchased from Bio-Rad Laboratories, Richmond, Calif. Nitrocellulose paper was obtained from Schleicher & Schuell, Inc., Keene, N.H.; peroxidase-labeled anti-human immunoglobulin G (IgG) from Acccurate Chemicals, San Diego, Calif.; the substrate orthodianisidine from Sigma Chemical Co., St. Louis, Mo.; and [^{35}S]methionine (1,000 Ci/mmol) from Amersham Corp., Arlington Heights, Ill.

Immunoblot procedure. Confluent monolayers of HEp-2 cells were infected (multiplicity of infection = 10) with either the F or G strain of HSV. At 24 h postinfection, the cells were scraped into cold phosphate-buffered saline (PBS), pelleted, and solubilized. The acrylamide was linked with *N,N'*-diallyltartardiamide. Antigens from the acrylamide gel were transferred to nitrocellulose membranes according to the procedure described by Towbin et al. (23). Similar intensities of bands after transfer by autoradiography indicated the equivalent transfer of antigens. These gels were used for all specimens. After determination of equivalent transfer, the nitrocellulose was removed; strips (5 mm in width) were cut, soaked in PBS with 0.5% Nonidet P-40, and incubated overnight at room temperature. The strips were washed and incubated in PBS–10% horse serum (100 ml) for 3 h at 37°C to block nonspecific binding sites.

Serum specimens (30 μl) were diluted 1:100 and reacted with both F and G antigen strips at 37°C overnight. Monospecific rabbit antisera to gpB and gpD (kindly provided by B. Norrild, Copenhagen, Denmark) were used on two strips as markers. Antisera to infected cell protein 4 (ICP4) were provided by R. Courtney Shreveport, La. After incubation, strips were washed twice in PBS–3% horse serum–0.5% Nonidet P-40 and once with PBS–3% horse serum. The reacted strips were incubated with a 1:400 dilution of anti-human IgG (peroxidase labeled) in PBS and 10% horse serum and washed with prewarmed PBS. Finally, 5 ml of the substrate orthodianisidine in PBS was added to each strip at 37°C in a darkroom. The substrate was prepared with 1%

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orthodianisidine in methanol and with 30% H_2O_2 to produce final concentrations of 0.02 and 0.12%, respectively.

MNT assays and ELISA. Quantities of neutralizing HSV-1 and HSV-2 antibodies were determined by routine procedures (17, 20). Virus-serum mixtures were incubated at 37°C for 1 h and inoculated in triplicate into flat-bottomed, 96-well microtiter plates containing cofluent monolayers of BSC-1 cells. After 3 days, plates were fixed and stained with 0.5% methylene blue. Antibody titers were expressed as the reciprocal of the highest dilutions found to have 50% neutralizing activity. The ELISAs were performed with crude antigen preparations as described by Coleman et al. (7). HEp-2 cells infected with HSV-1 (F strain) and HSV-2 (G strain) were harvested with a 90% cytopathic effect. Cells were sonicated in Tris-glycine buffer, clarified, and stored at −70°C. Uninfected HEp-2 cells, harvested identically, were used as a control antigen. Antigens were diluted in 0.05 M carbonate buffer (pH 9.6) and used to coat wells in an Immulon II 96-well plate (Dynatech Laboratories, Inc., Alexandria, Va.) at 4°C overnight. After sera were incubated for 2 h at 37°C, the plates were washed, and goat anti-human IgG peroxidase conjugate was added. The substrate was *o*-phenylenediamine · 2HCl. This assay was used to screen sera for total HSV antibodies.

Purified gpD and gpB of HSV-1 were used in an ELISA to determine the relative significance of protein denaturation which could occur during the transblotting procedure. Antigens were generously supplied by L. Pereira (12) and used as described by Coleman et al. (7). Antigens were diluted 1:1,000 in PBS. Twofold dilutions of sera, beginning at 1:50, were prepared in PBS–0.05% Tween 20–1% bovine serum albumin. The endpoint for positivity was the reciprocal of the highest serum dilution which yielded an optical density twice that of control wells without antigens. Sera proven to be positive or negative in the ELISA were also included in each assay as controls.

RESULTS

Study population. Fifty-four sera from 18 patients with virologically confirmed HSV infection were examined. Patients were classified as having primary, initial (prior infection with heterologous virus), or recurrent disease according to history, clinical presentation, and antibody status. Nine patients had HSV-1 infections: six had genital infections (three primary and three initial), and three had oral infections (two primary and one recurrent). An acute serum specimen was obtained within 7 days of the onset of primary or initial disease from seven of eight patients. The first serum specimen from one initial patient was obtained at day 10. Serum from the patient with recurrent disease was obtained 15 days after the most recent episode. Follow-up serum specimens were obtained over a period of 1 to 24 months. There were no interim recurrences. Nine patients had genital HSV-2 infections (four primary and five recurrent). Acute sera were available within 1 week of onset from four HSV-2-infected patients with primary infections. The remaining five patients were entered because of the frequency of recurrences. Specimens were collected over a period of 2 to 5 months, when patients had recurrences. Data were gathered and tabulated for the types of infection, relationship of disease onset to phlebotomy, ELISA and MNT titers, and the sequential antibody responses to major polypeptides (Table 1).

Antibodies to specific polypeptides by immunoblot. (i) HSV-1 infections. The acute sera of patients A, B, and C (Table 1) with primary genital HSV-1 infections were devoid of immunoblot, MNT, and ELISA antibodies. The sequential immunoblot serum antibodies of these patients were determined (Fig. 1). An antibody response to gpB was observed early (15, 22, 25, 26), followed by an antibody response in the 145,000 to 155,000-molecular-weight range as has been reported previously (11, 26). These antibodies appeared to be against the viral nucleocapsid proteins to the VP5, VP6, and VP7 (15). At different times (ranging from weeks to months) appeared antibodies to gpC, gpD, and a number of lower-molecular-weight polypeptides (35,000 to 49,000), including the p35 complex. Follow-up serum specimens demonstrated antibodies to an 80,000-molecular-weight protein, presumably gpE. Increasing quantities of antibodies, indicated by scanning densitometry, were observed for all previously recognized polypeptides. These antibodies remained unchanged in the serum specimens over the follow-up period.

Three patients (D, E, and F) had HSV-1 that was isolated from an oral site. Two patients had primary gingivostomatitis, and another, originally presumed to have a primary infection because of disease presentation, had preexisting antibodies. Similar sequences of polypeptide antibodies were present in these patients (Fig. 1, lanes D1 to 4). Notably, antibodies to ICP4 (molecular weight, 175,000) were prominent in these patients, compared with those with genital infections. The antibodies against ICP4, like antibodies to gpB, the 96,000-molecular-weight protein, and p35, persisted for at least 14 months after the primary infection. Labial recurrences led to boosting of antibody responses to ICP4, gpB, gpC, and gpD in particular, while there was no apparent increase in response to the p35 complex (Table 1).

Patients G, H, and I had a history of labial HSV infection and related antibodies at the time of the initial genital HSV-1 infection. Antibodies to the studied antigens were present in the initial serum (Table 1). No more than a two- to fourfold increase in the antibody response to each polypeptide was demonstrated in follow-up serum specimens.

(ii) HSV-2 infections. Patients K, L, Q, and R were considered to have primary genital HSV-2 infections. Antibodies were either absent at clinical presentation or low in specimens obtained within a week of disease onset. All patients had clinical findings indicative of primary infections. Subsequent sera demonstrated antibodies to the major glycoproteins, especially gpB and gpD; antibodies to proteins in the range of 75,000 to 96,000 molecular weight, presumably including gpE; and antibodies to the 130,000-molecular-weight glycoprotein, presumably gpG (21). The late serum specimens revealed strong reactivity to both the p35 complex and ICP4. Subsequent sera demonstrated a further increase in the quantity of antibodies to all polypeptides, particularly the 66,000-molecular-weight protein, probably VP16.

Sequential sera specimens of patients with recurrent genital herpes (J, M, N, O, and P) were analyzed because of differences in the frequency of recurrences. Examples of immunoblots from two of these patients (Fig. 2) reveal existing antibodies to most previously noted polypeptides. Large quantities of antibodies to ICP4 were present in serum samples from patients O and P, who had greater than 12 recurrences per year (Fig. 2). Patients with recurrences less than three times per year (patients J, M, and N) had no antibodies to ICP4.

Immunoblotting was performed on all the serum specimens against heterologous antigens. The data (not presented) indicated a diminished response to each polypeptide.

Antibodies to purified glycoproteins. Sera from four pa-

TABLE 1. Primary, initial, and recurrent infections of HSV types 1F and 2G

Patient by HSV type	Day after onset	ELISA titer[a]	MNT	Fold increase in antibody response to polypeptide:[b]					
				ICP4	gpC or gpG	gpB	gpE	gpD	p35 complex[c]
1[d]									
A	17	<50	<10						
	710	12,800	960		2.5	2.5	1.0	1.5	6.0
	780	12,800	1,280		2.5	2.5	1.0	1.5	6.2
B	15	<50	<10						
	30	400	120		1.5	5.0		2.0	1.0
	150	6,400	240			7.5		3.0	
C	3	<50	<10						
	150	1,600	240	1.0	2.5	2.0	2.5	5.0	25.0
	210	1,600	320	2.5	3.5	2.5	5.0	15.0	40
D	1	<50	<10						
	30	3,200	640	2.5	3.5	5.5	1.0	2.0	3.0
	300	6,400	1,280	4.0	5.5	6.5	2.5	4.0	4.5
	425	12,800	1,280	5.5	7.0	8.0	4.0	5.5	6.5
E	6	<50	<10						
	10	800	60	1.0	4.0	6.0		4.2	8.0
	27	800	60	2.0	5.0	7.0	1.0	5.0	5.0
	33	800	60	2.0	5.0	7.0	1.0	5.0	5.0
	90	6,400	640	6.0	7.0	9.0	1.0	6.0	3.0
F	3	1,600	640	2.3	3.6	4.6	1.0	2.0	3.6
	5	12,800	960	2.7	5.3	6.0	1.6	2.7	2.7
	12	12,800	1,280	4.0	6.6	10.3	2.6	3.6	1.6
G	3	100	40		2.0	3.0		2.0	7.0
	5	200	320	1.0	4.0	5.0	1.0	3.0	8.0
	12	800	640	2.0	8.0	9.0	2.0	3.0	11.0
H	37	800	320	1.0	6.0	9.0	2.0	3.0	12.0
	210	1,600	640	2.0	8.0	10.0	3.0	5.0	16.0
I	5	1,600	640	1.0	5.0	6.0	1.0	2.0	7.0
	33	2,800	1,280	4.0	8.0	9.0	2.0	3.0	11.0
2[e]									
J	300	1,600	640		1.3	1.0		1.3	5.6
	510	1,600	640		1.3	1.6		1.0	6.3
	540	2,800	1,280		1.6	1.6		1.0	5.0
K	1	<50	<10						
	3	<50	<10						
	10	3,200	160	1.0	1.2	2.4	1.2	2.0	5.2
	60	6,400	320	2.0	5.4	5.2	1.2	2.0	9.2
L	4	200	40						
	49	6,400	320	1.0	6.0	3.0	2.0	6.0	32.0
	79	6,400	320	5.0	14.0	7.0	4.0	10.0	48.0
M	690	800	160			1.0			
	720	12,800	640		1.0	6.5			8.0
	750	12,800	1,280		1.5	4.5			4.5
N	90	1,600	640		1.0	1.0	1.0	3.0	8.0
	210	12,800	960		2.0	3.0		1.0	34.0
	240	12,800	1,280		4.0	5.0		5.0	36.0
O	990	12,800	1,280	2.0	6.0	3.0	1.0	2.0	8.0
	1,050	12,800	1,280	3.0	9.0	4.0	1.8	3.0	12.0
P	1,170	12,800	1,280	5.0	9.0	6.0		1.0	12.0
	1,260	12,800	1,280	7.0	12.0	10.0	4.0	5.0	19.0
Q	4	<50	<10						
	60	800	160	1.0	7.5	10.0	5.0		65.0
	120	6,400	320					10.0	
R	10	<50	<10						
	24	400	320	3.0	7.0	4.0	1.0	4.0	16.0
	135	25,600	1,280	7.0	11.0	11.0	2.0	8.0	30.0

[a] Crude antigen.

[b] Individual immunoblot strips were scanned with a zeineh soft laser beam densitometric scanner (Biomed Instruments, Inc., Chicago, Ill.). Individual polypeptides were cut and weighted. The weights were converted into comparative numerical ratios of each antibody response of each serum sample.

[c] P35 complex: series of proteins ranging from 34,000 to 49,000 molecular weight; the major immunoreactive band is 39,000.

[d] Includes patients with gingivostomatitis (D. E, and F) and patients with type 1 primary (A, B, and C) and initial and recurrent (G, H, and I) genital infections.

[e] Includes primary (K, L, Q, and R) and initial and recurrent (J, O, P, M, and N) genital infections.

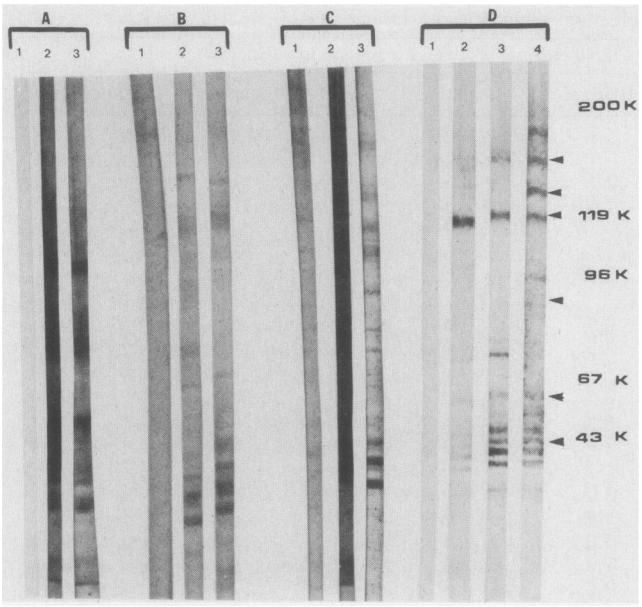

FIG. 1. Immunoblot profiles of sera from patients with HSV-1 infections. Patients A, B, and C had primary genital infections, and D had primary gingivostomatitis. Lanes marked 1 to 3 or 1 to 4 show sequential serum specimens. Arrows identify the proteins ICP4, gpC, gpB, gpE, gpD, and the p35 complex, respectively, from top to bottom. K, Molecular weight $\times 10^3$.

tients were assayed for antibodies to gpB and gpD with purified glycoproteins. The acute sera from patient A, who had a primary HSV-1 genital infection, was negative (titers of <50) for either gpB or gpD antibodies, while the convalescent sera obtained 2 years postinfection had titers of 400 to 800 against gpB and gpD antibodies, respectively. A similar response was demonstrable in patient E, whose convalescent sera (obtained 3 months after primary gingivostomatitis) had antibody titers of 6,400 against gpB and 3,200 against gpD. The same titers of antibodies were also in sera obtained from patient J, who had a recurrent genital HSV-2 infection. For a primary HSV-2 genital infection, patient K had an antibody response similar to those obtained after a primary HSV-1 infection. No antibodies against either gpB or gpD were detected in the acute sera, while titers of 800 against gpB and 400 against gpD were present 10 days after onset. These titers remained unchanged in the sera obtained 60 days postinfection.

DISCUSSION

The definition of host response to primary and recurrent HSV infections has been and remains of major interest to investigators in this field. Until recently, serologic assays failed to unequivocally distinguish HSV-1 and HSV-2 infections or to allow dissection of host response for precise correlation with disease pathogenesis. Within the last 3 years, several investigators have demonstrated the utility of immunoblotting and immunoprecipitation for assessing specific aspects of host antibody response to HSV (5, 10–12). Such studies have broadly characterized antibodies to both soluble and insoluble antigens, assessed the relative type specificity of particular polypeptides, and more recently, correlated the effects of antiviral therapy on subsequent antibody response. When compared to prior reports, the present study emphasizes the variability of host antibody responses to specific polypeptides, particularly with reference to antibodies produced to the major glycoproteins and ICP4. Furthermore, antigen denaturation in this study did not influence the detection of antibodies to gpB and gpD in, albeit, a limited number of specimens.

In spite of the limited numbers of patients evaluated, the overall pattern of response for HSV-1 and HSV-2 primary infections was quantitatively and sequentially defined. Notably, the data indicate a difference in the sequence of appearance of antibodies for the two virus types, which may be a function of their intratypic variability (18). For primary HSV-1 infections, whether genital or oral, the initial host response is directed against gpB, followed by antibodies to the 145,000- to 155,000-molecular-weight structural component of the virus. Later, antibodies to other glycoproteins and to the nucleocapsid p35 complex appear. While gpB, gpD, and ICP4 were identified by monoclonal antibodies, the 80,000-molecular-weight antibody was presumed to be gpE. Surprisingly, the three patients with oral infection all developed large quantities of ICP4 antibodies after primary infection.

Patients with primary genital herpes caused by HSV-2 develop antibodies first to an apparent gpG. Subsequently, antibodies to the other polypeptide complexes like gpB, gpD, gpE, p35, and ICP4 appear. This result is somewhat in contrast to data reported previously, where an initial response to the p35 complex was observed in primary genital infections, followed by an antibody response to the glycoproteins (10).

These observations have relevance for the initiation of several HSV vaccine trials both in the United States and abroad. While purified glycoprotein vaccines have demonstrated efficacy in animal model experiments (3), such vaccines may be less useful than those which induce broader

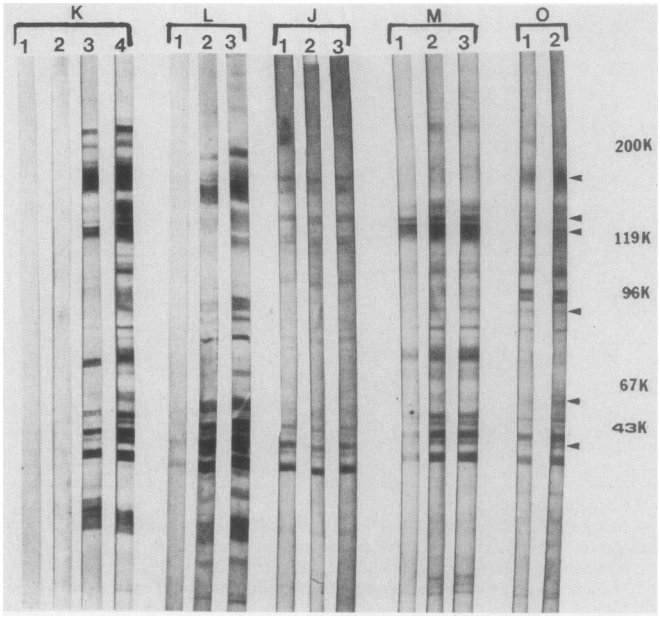

FIG. 2. Immunoblot profiles of sera from patients with HSV-2 genital infections. Patients K and L had primary genital infections, J and M had recurrent infections, and O had a frequent recurrent infection. Arrows identify the proteins ICP4, gpG, gpB, gpE, gpD, and the p35 complex, respectively, from top to bottom. K in vertical scale (right side) is molecular weight $\times 10^3$.

immunity. The availability of immunoblot technology provides an assay by which humoral antibody response to vaccines can be defined, especially in seronegative individuals. Clearly, a marker for natural infection in vaccine recipients is necessary, and immune blotting may help provide one. This was especially true in our study, given the relative quantitative difference in host response between primary and either initial or recurrent infections. Recurrent infections or initial genital infections led to a lower magnitude of antibody responses both acute and chronic, perhaps paralleling the diminished quantity of virus or antigenic load at the site of infection.

The role of antibodies to the nucleocapsid complex in genital versus nongenital infections is worthy of comment. Oral HSV-1 infections showed no increase in these antibodies over time, in contrast to genital infections, in which persistent increase in these antibodies were observed. This distinction from a pathogenic standpoint will require follow-up. Genital infections, in general, appear to result in greater reactivity to lower-molecular-weight polypeptides, although not in the sequence reported (11). Why there are differences in the sequential appearance of antibodies is unclear.

One specific polypeptide has attracted interest of late as it relates to antiviral therapy, namely the 66,000-molecular weight protein or VP16 (2). From the presented immunoblots, detection of these antibodies is possible promptly after primary infection, but their persistence over time is less than that of other protein markers.

An inate problem with the use of immunoblot assays is the destruction of noncontinuous or conformational epitopes by sodium dodecyl sulfate, urea, and mercaptoethanol during the electrophoresis and transfer of antigens to nitrocelluose. Therefore, only the subset of HSV-specific IgG which reacts with denatured antigens is detected by this assay. Two previous reports by Eberle et al. have acknowledged this limitation and suggested the use of a complementary assay with nondenatured antigens (10, 11).

Our data suggest that the use of antigens not denatured by sodium dodecyl sulfate does not appreciably increase the sensitivity of detecting antibodies to these antigens. Acute sera from two patients which had failed to react with gpB and gpD by immunoblot were also negative when purified glycoproteins were used as antigens. In addition, there was a good correlation between the quantity of antibodies against gpB and gpD in both assays. In the five sera which were positive in both assays, immunoblot data suggested the antibodies against gpB were more abundant than those against gpD in all sera. The titer of antibodies to purified gpB was also higher in four of these sera when assayed by the ELISA. The finding that antigens in our immunoblots appeared to be broadly reactive may have resulted from renaturation of conformation epitopes during the overnight incubation with PBS and Nonidet P-40 before use.

The present study enabled characterization of the antibody response to both HSV-1 and HSV-2 infections. In addition, differences were observed in the host immune response to primary and recurrent infections, as well as in genital versus nongenital infections. The relative importance of these differences remains for further elucidation as, at least for the present, they are not indicative of frequency or severity of recurrences.

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LITERATURE CITED

1. **Arvin, A. M., C. M. Koropchak, A. S. Yeager, and L. Pereira.** 1983. Detection of type-specific antibody to herpes simplex virus type 1 by radioimmunoassay with herpes simplex virus type 1 glycoprotein C purified with monocloal antibody. Infect. Immun. **40**:184–189.
2. **Ashley, R. L., and L. Corey.** 1984. Effect of acyclovir treatment of primary genital herpes on the antibody response to herpes simplex virus. J. Clin. Invest. **73**:681–688.
3. **Berman, P. W., T. Gregory, D. Crase, and L. A. Lasky.** 1985. Protection from genital herpes simplex virus type 2 infection by vaccination with cloned type 1 glycoprotein D. Science **227**: 1490–1492.
4. **Bernstein, D. I., E. Garratty, M. A. Lovett, and Y. J. Bryson.** 1985. Comparison of western blot analysis to microneutralization for the detection of type-specific herpes simplex virus antibodies. J. Med. Virol. **15**:223–230.
5. **Bernstein, D. I., M. A. Lovett, and Y. J. Bryson.** 1984. The effects of acyclovir on antibody response to herpes simplex virus in primary genital herpetic infections. J. Infect. Dis. **150**:7–13.
6. **Buddingh, G. J., D. I. Schrum, J. C. Lanier, and D. J. Guidry.** 1953. Studies of the natural history of herpes simplex infections. Pediatrics **11**:595–609.
7. **Coleman, R. M., L. Pereira, P. D. Bailey, D. Dondero, C. Wickliffe, and A. J. Nahmias.** 1983. Determination of herpes simplex virus type-specific antibodies by enzyme-linked immunosorbent assay. J. Clin. Microbiol. **18**:287–291.
8. **Compton, T., and R. J. Courtney.** 1984. *In* F. Rapp (ed.), Herpesviruses. UCLA Symposium on Molecular and Cellular Biology, vol. 21. Alan R. Liss, Inc., New York.
9. **Douglas, R. G., and R. B. Couch.** 1970. A prospective study of chronic herpes simplex virus infection and recurrent herpes labialis in humans. J. Immunol. **104**:289–295.
10. **Eberle, R., S. W. Mou, and J. A. Zaia.** 1984. Polypeptide specificity of the early antibody response following primary and recurrent genital herpes simplex virus type-2 infections. J. Gen. Virol. **65**:1839–1845.
11. **Eberle, R., and M. Siao-Wen.** 1983. Relative titers of antibodies to individual polypeptide antigens of herpes simplex virus type 1 in human sera. J. Infect. Dis. **148**:436–444.
12. **Eisenberg, R., M. Ponce de Leon, L. Pereira, D. Long, and G. H. Cohen.** 1982. Purification of glycoprotein gD of herpes simplex virus types 1 and 2 by use of monoclonal antibody. J. Virol. **41**:1099–1104.
13. **Forghani, B., T. Klassen, and J. R. Baringer.** 1977. Radioimmunoassay of herpes simplex virus antibody: correlation with ganglionic infection. J. Gen. Virol. **36**:371–375.
14. **Glorioso, J. C., and J. W. Smith.** 1977. Immune interactions with cells infected with herpes simplex virus: antibodies to radioiodinated surface antigens. J. Immunol. **118**:114–126.
15. **Heine, J. W., P. G. Spear, and B. Roizman.** 1972. Proteins specified by herpes simplex virus. VI. Viral proteins in the plasma membrane. J. Virol. **9**:431–439.
16. **Nahmias, A. J., and B. Roizman.** 1973. Infections with herpes simplex viruses 1 and 2. N. Engl. J. Med. **289**:667–674.
17. **Pauls, F. P., and W. P. Dowdle.** 1967. A serologic study of herpes virus hominis strains by microneutralization tests. J. Immunol. **98**:941–947.
18. **Pereira, L., D. V. Dondero, D. Gallo, V. Devlin, and J. D. Woodie.** 1982. Serological analysis of herpes simplex virus types 1 and 2 with monoclonal antibodies. Infect. Immun. **35**:363–367.
19. **Pereira, L., T. Klassen, and J. R. Baringer.** 1980. Type-common

and type-specific monoclonal antibody to herpes simplex virus type 1. Infect. Immun. **29**:724–732.
20. **Rawls, W. E., K. Iwamtot, E. Adam, and J. L. Melnick.** 1970. Measurement of antibodies to herpesvirus type 1 and 2 in human sera. J. Immunol. **104**:599–606.
21. **Roizman, B., B. Norrild, C. Chan, and L. Pereira.** 1984. Identification and preliminary mapping with monoclonal antibodies of a herpes simplex virus 2 glycoprotein lacking a known type 1 counterpart. Virology **133**:242–247.
22. **Skinner, G. R. B., C. Hartley, and J. P. Whitney.** 1976. Detection of type-specific antibody to herpes simplex virus type 1 and 2 in human sera by complement-fixation tests. Arch. Virol. **50**:323–333.
23. **Towbin, H. T., T. Staehelin, and J. Gordon.** 1979. Electrophoresis transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA **79**:4350–4354.
24. **Yoshino, K., T. Morishima, and Y. Aoki.** 1971. Complement requirement of neutralizing antibodies appearing after primary and booster immunizations with herpes simplex virus. J. Microbiol. **15**:53–62.
25. **Zweerink, H. J., and L. Corey.** 1982. Virus-specific antibodies in sera from patients with genital herpes simplex virus infection. Infect. Immun. **37**:413–421.
26. **Zweerink, H. J., and L. W. Stanton.** 1981. Immune response to herpes simplex virus infections: virus-specific antibodies in sera from patients with recurrent facial infections. Infect. Immun. **31**:624–630.