Antibody Response to Herpes Simplex Virus Type 1 Polypeptides and Glycoproteins in Primary and Recurrent Infection

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Summary

Sequential sera from a patient with primary Herpes Simplex Virus type 1 (HSV-1) encephalitis and a patient with HSV-1 recurrent oral lesions were collected. Sera were analyzed quantitatively by radioimmunoassay and qualitatively by electrophoresis and autoradiography of immune precipitates to determine the sequence of antibody production to specific radiolabeled HSV-1 polypeptide and glycoprotein antigens. The major antibody response in both primary and recurrent sera was against HSV-1 envelope antigens and the major capsid polypeptide. Sequential sera showed a significant correlation between neutralizing antibody titers and quantitative antibody to HSV-1 glycoproteins. Qualitative electrophoretic analysis of primary infection sera showed sequential appearance of antibodies to increasing numbers of HSV-1 polypeptides by the fourteenth day of infection. A corresponding qualitative antibody response to glycoproteins was not seen. Sequential sera obtained before, during, or after a recurrent lip lesion in another patient showed no significant quantitative or qualitative changes in antibodies to either HSV-1 glycoproteins or polypeptides.

Speculation

A qualitative variation in antibody response to Herpes Simplex Virus polypeptides develops following a primary infection. The lack of qualitative variation in antibody response to recurrent infection provides a means of distinguishing primary from recurrent Herpes Simplex Virus infections.

Antibodies to Herpes Simplex Virus type 1 (HSV-1) envelope proteins are important in virus neutralization (19), antibody dependent cellular cytotoxicity, and in complement mediated cellular cytotoxicity (16). Neutralizing antibodies attach to specific HSV glycoproteins on the membranes of HSV infected cells and the HSV envelope (5, 6, 21). Eberle and Courtney (5) have purified and fractionated the HSV-1 glycoproteins gC and gA/gB and shown that these major glycoproteins are expressed on the surface of virions and on infected cell surfaces. Five major HSV-1 glycoproteins have been described by Spear and associates (1, 21). The antibodies to glycoproteins gC, gB, and gD are important in virus neutralization (3, 18, 23). Norrild, *et al.* (16), have shown that antibody to glycoproteins gB, gD, and gC are functional in antibody dependent cell mediated cytotoxicity and antibody dependent complement mediated cytotoxicity. Although some of the functions of antibodies directed to specific HSV-1 glycoproteins have been discovered, little is known about the sequence of their production and even less is known of antibodies directed to non-glycosylated polypeptides.

We have analyzed sequential sera samples from a child with a primary HSV-1 infection and a patient with recurrent infections for antibodies to specific HSV-1 polypeptides and glycoproteins.

Qualitative analyses by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and by radioimmunoassay (RIA) and neutralizing antibody were used to determine the sequence of antibody response to specific HSV-1 antigens during primary and recurrent infections.

MATERIALS AND METHODS

Cells and virus. Human epidermoid carcinoma no. 2 (HEp-2) cells used for viral antigen production were grown in Eagle's minimum essential medium (EMEM) supplemented with 10% fetal calf serum, antibiotics, and NaHCO₃. CV-1 cells used in neutralizing antibody titrations were grown in EMEM supplemented with 5% calf serum, antibiotics, and NaHCO₃. HSV-1 strain 70-1116 was isolated from the brain biopsy of a 14-year-old male with HSV encephalitis and was typed by Dr. Andre J. Nahmias as type 1. This strain has the polypeptide pattern of HSV-1 reference strains (2) by SDS-PAGE analysis and was used for preparation of HSV-1 antigen extracts. HSV-1 strain KOS was used for determining neutralizing antibody titers of sera.

Patient sera and controls. Sequential sera were obtained beginning 5 days after onset of symptoms through 2 years after the acute episode from a child who developed primary HSV-1 encephalitis at 9 months of age. There was no clinical history of previous herpes simplex infections in either mother or baby and sero-conversion between the infant's acute and convalescent sera indicates a primary herpes simplex infection. Brain biopsy contained an HSV isolate which was confirmed as type 1 by Dr. A. J. Nahmias. Sequential sera were also obtained from a young adult male with type 1 recurrent HSV lip lesions. Sera were collected at intervals before, during, and after a recurrent infection. All studies were approved by the Human Subjects Review Committee of the Children's Hospital Research Foundation and written informed consent was obtained. Hyperimmune HSV-1 rabbit serum was used as the positive control serum for this study and was prepared in New Zealand white rabbits by immunization with HSV-1 antigen extract from infected primary rabbit kidney cells grown in EMEM with 5% rabbit serum. Cells were infected with HSV-1 (70-1116) at a multiplicity of 10 plaque-forming units per cell and harvested 20 h postinfection by first washing cell monolayers three times with 0.05 M tris buffer (pH 7.0), freeze-thawing cells, and then centrifuging scraped cell suspension at 1,000 X g. The resulting supernatant antigen extract was inactivated with ultraviolet light and combined with Freund's adjuvants to make 50% emulsions. This emulsion was injected intradermally into the rabbit's backs as follows: four injections at 2 wk intervals, the first two with complete Freund's adjuvant and the second two with incomplete Freund's adjuvant. Two months after the initial injection series a booster series of four injections with incomplete Freund's adjuvant was administered. Sera were obtained by cardiac puncture. Negative control sera used in these experiments were obtained from rabbits immunized in parallel with uninfected

primary rabbit kidney cells, and from adult humans who had no neutralizing antibody to HSV. All control sera were screened before use for presence or absence of HSV-1 antibody by radioimmunoassay, neutralization, and SDS-PAGE analysis.

Neutralizing antibody titrations. Two-fold dilutions of serum were mixed with 30–50 plaque-forming units of HSV-1 (KOS) and incubated for 30 min at 37°C. The mixture was adsorbed to confluent CV-1 cells in 35 mm wells for 1 h at 34°C in a CO₂ incubator. Cells were overlaid with 2% methyl cellulose medium and incubated for 3 days. Cell monolayers were stained with neutral red and plaques counted. Reciprocals of greatest serum dilutions which produced 50% plaque reductions were recorded as neutralizing antibody titers.

Preparation of radiolabeled antigen extracts. Confluent monolayer cultures of HEP-2 cells in 75 cm² flasks were infected with HSV-1 (70-1116) at a multiplicity of 10–20 plaque-forming units per cell, inoculum was adsorbed for 90 min at 37°C, and then decanted. Radiolabeled HSV-1 polypeptides were produced by adding EMEM with 1/10 normal concentration of amino acids and supplemented with 2% fetal calf serum, NaHCO₃, antibiotics, and uniformly labeled [¹⁴C] amino acid mixture (Amersham CFB 0.104) at a concentration of 5 µCi/ml media. Radiolabeled HSV-1 glycoproteins were produced by adding EMEM with ½ normal concentration of glucose and supplemented with 2% fetal calf serum, NaHCO₃, antibiotics, and D-[U-¹⁴C] glucosamine hydrochloride (Amersham CFB 0.142) at a concentration of 5 µCi/ml media. Radiolabeling at 37°C continued until 22 h postinfection when medium was decanted, cell monolayers washed three times with 0.05 M tris buffer (pH 7.0), and following the antigen extraction method of Vestergaard *et al.* (24), buffer containing 0.02 M glycine 2% Triton X-100, and 0.0076 M Tris (pH 8.6) was added (1 ml/flask) before freezing at -70°C. Cell monolayers were thawed, scraped, sonicated on ice, and suspensions centrifuged at 10,000 X g for 30 min at 4°C. Supernatants were divided in aliquots and frozen at -70°C, until used as either [¹⁴C] labeled HSV-1 polypeptide antigen or [¹⁴C] labeled HSV-1 glycoprotein antigen in antibody assays. Uninfected cells were treated in parallel and used as negative control antigens.

Quantitation of HSV-1 antibody by RIA. Test sera diluted 1:10 in phosphate buffered saline (PBS) were reacted with either [¹⁴C] labeled HSV-1 polypeptide antigen or [¹⁴C] labeled HSV-1 glycoprotein antigen for 60–90 min at 37°C. Immune complexes were separated from unbound [¹⁴C] labeled antigens by adding fixed-killed staphylococcal protein A adsorbent (14) and incubating mixture for 15 min at room temperature. Adsorbent-immune complex aggregates were centrifuged at 1000 X g and pellets washed three times with wash buffer (0.5 M NaCl; 0.4% Triton X-100; 10 mM EDTA) and one time with PBS. Immune complexes were eluted from adsorbent and dissociated by adding SDS-PAGE sample buffer (2% SDS, 5% β-mercaptoethanol; 10% glycerol; 0.001% bromophenol blue; 0.05 M Tris pH 6.8) and placing suspension in boiling water bath for 5 min (4). Adsorbent was removed by centrifugation and quantitation of HSV-1 antibody directed against either polypeptide or glycoprotein antigens was determined by liquid scintillation counting of radiolabeled antigens eluted into the supernatant.

SDS-PAGE and autoradiography. The SDS-PAGE system utilized has been described previously (15, 22). An 8.5% (w/v) separating gel and 4.5% (w/v) stacking gel were crosslinked with N, N' diallyltartardiamide (DATD) and used to analyze the viral antigens in a 1.5 mm thick slab gel (Bio Rad Model 220) with discontinuous SDS-glycine buffer system. Dissociated samples in SDS-PAGE sample buffer were obtained from the HSV-1 antibody RIA and electrophoresed at a constant current of 30 mA per gel slab until the bromophenol blue marker reached the bottom of the gel (~2 h). The gel was fixed and stained with 0.1% coomassie brilliant blue in 50% trichloroacetic acid, destained, and dried. An autoradiogram was made on GAF non-screen x-ray film to visualize the [¹⁴C] labeled HSV-1 polypeptides and glycoproteins which had been bound by antibodies in the test sera.

Molecular weight determination. Molecular weight standards

were obtained from Bio-Rad Laboratories and contained Myosin (200,000), β-galactosidase (130,000), phosphorylase (94,000), bovine serum albumin (68,000), and ovalbumin (45,000). Approximately 2 µg of these protein standards were electrophoresed with each run and the relative electrophoretic mobility of HSV-1 antigens calculated from the standard curve obtained from these proteins. The apparent molecular weights of HSV-1 polypeptides and proteins were the average of multiple molecular weight determinations made in 8.5% acrylamide gels crosslinked with DATD. Glycoprotein weights represent the average molecular weight of the diffuse band.

RESULTS

Radiolabeled antigens and control sera. The radiolabeled HSV-1 polypeptide and glycoprotein antigens present in the antigen extracts used for HSV-1 antibody assays are shown in Figure 1. Thirty-two HSV-1 induced polypeptides (Track A) can be seen and molecular weights of these polypeptides are indicated. Viral glycoproteins (Track E) gC, gB (gA/gB), gE, and gD were detected in the [¹⁴C]-glucosamine labeled antigen extract. Glycoproteins labeled gB probably represent a combination of gA and gB not clearly separable in our gel system and have been reported to represent different forms of the same glycoprotein (7). When hyperimmune rabbit serum was reacted with the polypeptide and glycoprotein antigen extracts (Tracks B and F), antibodies to 15 HSV-1 polypeptides and 4 HSV-1 glycoproteins were identified. When negative control serum was reacted with the antigen extracts (Tracks C and G), no antibodies directed against HSV-1 polypeptides or glycoproteins were detected. When PBS was reacted with the antigens (Tracks D and H), nonspecific attachment of HSV-1 antigens to the bacterial adsorbent was not seen.

Fig. 1. Autoradiographic patterns of radiolabeled HSV-1 polypeptides and glycoproteins and HSV-1 antigens complexed by positive and negative control antisera. Track A shows unreacted HSV-1 polypeptide antigen. Tracks B-D illustrate the HSV-1 antigens bound when HSV-1 polypeptide antigen was reacted with (B) hyperimmune rabbit serum, (C) non-immune human serum, and (D) phosphate buffered saline. Track E shows unreacted HSV-1 glycoprotein antigen. Tracks F-H represent the HSV-1 antigens bound when HSV-1 glycoprotein antigen was reacted with (F) hyperimmune rabbit serum, (G) non-immune human serum, and (H) phosphate buffered saline. MW = molecular weight $\times 10^{-3}$. Gly = glycoprotein designation.

When [14 C] labeled uninfected control antigens were reacted with HSV-1 hyperimmune rabbit serum and human serum known to contain HSV-1 neutralizing antibody, no radiolabeled cellular polypeptides or glycoproteins were detected with SDS-PAGE and autoradiography. RIA quantitations of this reaction produced values equal to PBS negative controls.

To evaluate the possibility that some of the HSV-1 antigens detected by autoradiography represented changing solubility of certain HSV antigens during immunologic reactions, two experiments were performed. Goat anti-rabbit albumin and rabbit albumin were reacted in the presence of the radiolabeled HSV-1 extracts to determine if this immunologic reaction decreased the solubility of viral antigens. In this experiment no insoluble HSV-1 antigens were precipitated nor were any detected in a second experiment where HSV-1 antigens extracted from infected Hep-2 cells, were reacted with antiserum to uninfected Hep-2 cells.

Sequential antibody production in primary infection. Serum samples were obtained from the patient with primary HSV-1 enceph-

alitis on days 5, 9, 14, and 26; 14 months and 2 years after onset of illness. Corresponding levels of neutralizing antibody and antibody to HSV-1 glycoproteins measured by RIA (Table 1) showed a correlation coefficient of 0.80 (significant t $P < 0.01$). Antibody measurement by all three quantitative methods, neutralization and glycoprotein and polypeptide RIA, showed parallel increases in antibody levels first detected at day 9.

SDS-PAGE qualitative analysis of immune precipitates formed with sequential sera showed differences in development of antibodies to HSV-1 polypeptides as compared to glycoproteins (Fig. 2). Antibodies to HSV-1 polypeptides showed progressive development of antibodies to increasing numbers of viral polypeptides and relative autoradiographic intensities of precipitated antigen bands. Day 9 serum (Track A) contained low levels of antibody directed to polypeptides with molecular weights of 159,000 and 152,000 and by day 14 (Track B) antibodies to 125,000 and 63,000 molecular weight polypeptides were present as were increased concentrations of antibodies to 159,000 and 152,000 molecular weight polypeptides. Antibody to the 132,000 molecular weight polypeptide, present in trace amounts on day 14, showed an increase on day 26 (Track C), was maximal at 14 months (Track D), and remained through the 2 year serum (Track E). Serum from day 9 (Track F) had low levels of antibodies to HSV-1 glycoproteins and on day 14 (Track G), antibodies against gC, gB (gA/gB), gE, and gD were fully developed and no qualitative changes occurred through 2 years (Track H, I, J). The 132,000 molecular weight polypeptide and glycoprotein gC have similar apparent molecular weights, however, they do not immunoprecipitate at the same rate during the acute stages of the illness. Additional antibodies to lower molecular weight polypeptides (96,000 and 67,000) appeared at 14 months (Track D), however, there was loss of antibody to a 38,000 molecular weight polypeptide at 14 months and loss of antibody to 48,000 molecular weight polypeptide in the 2 year serum (Track F).

Sequential antibody production in recurrent infection sera. Serum samples were obtained from a patient with recurrent HSV-1 infection at 210, 120, and 30 days prior to a recurrent lip lesion. Sera were also drawn at the time of recurrence and at 7, 35, and 300 days after the recurrent infection. Neutralizing antibody titers remained unchanged at 128 in all sera. Quantitative RIA measurements of antibody to HSV-1 glycoprotein antigens showed a

Table 1. Specific antibody quantitation in primary HSV-1 encephalitis sera

Point in illness when serum drawn	HSV-1 neutralizing antibody titer ¹	RIA ² of antibody to:	
		HSV-1 glycoprotein antigen	HSV-1 polypeptide antigen
Day 5	<4	not done	9.0
Day 9	32	16.5	18.6
Day 14	512	91.3	68.5
Day 26	512	109.5	75.5
14 months	256	99.0	90.9
2 years	256	93.0	82.0
Positive control serum	2048	168.7	161.8
Negative control serum	<4	8.0	8.0

¹ Reciprocal of greatest serum dilution which produced 50% plaque reduction.

² Values represent counts per minute $\times 10^{-3}$ precipitated by serum when reacted with specified radiolabeled antigen.

Fig. 2. Autoradiographic patterns of radiolabeled HSV-1 polypeptide and glycoprotein antigens bound by sequential sera from patient with primary HSV-1 encephalitis. Tracks A-E illustrate HSV-1 polypeptides bound by specific antibody in sera collected (A) 9 days, (B) 14 days, (C) 26 days (D) 14 months, and (E) 2 years after onset of encephalitis. Tracks F-J show glycoprotein antigen bound when HSV-1 glycoprotein antigen was reacted with serum collected on (F) day 9, (G) day 14, (H) day 26, (I) 14 months, or (J) 2 years after onset of illness. MW = molecular weight $\times 10^{-3}$. Gly = glycoprotein designation.

Fig. 3. Autoradiogram of antibody response in the sequential sera from a patient with recurrent herpes labialis. Tracks A-G show polypeptides bound when radiolabeled HSV-1 polypeptide antigen was reacted with serum collected (A) 210 days, (B) 120 days, and (C) 30 days before recurrence, (D) on the day of the lesion, and (E) 7 days (F) 35 days, and (G) 300 days after recurrence. Tracks H-N represent the same sera reacted with radiolabeled HSV-1 glycoprotein antigen; (H) 210 days, (I) 120 days, (J) 30 days before lesion, (K) day of lesion, (L) 7 days, (M) 35 days, and (N) 300 days after lesion. MW = molecular weight $\times 10^{-3}$. Gly = glycoprotein designation.

slight decrease in antibody during the recurrence and a slight increase immediately following. These minor fluctuations are not statistically significant. RIA measurements of antibody to HSV-1 polypeptides also showed no significant change over the time studied.

Qualitative analysis by SDS-PAGE of the immune precipitates formed by these sequential sera showed a constant antibody pattern to HSV-1 polypeptides and glycoproteins (Fig. 3). The HSV-1 polypeptides identified with these sera are similar to the 14 month and 2 year sera from the patient with primary infection and include 159,000, 152,000, 132,000, 125,000, 96,000, and 42,000 molecular weight polypeptides and glycoproteins gC, gB (gA/gB), gE, and gD.

DISCUSSION

The analysis of sequential sera from the patient with primary infection showed easily identifiable qualitative variation in antibody to HSV-1 polypeptides from 9–26 days after onset of infection and minor changes up to 2 years after infection. During this early phase there was a serum antibody response to the polypeptides of the HSV-1 envelope, the major capsid polypeptide, a nonstructural β polypeptide, and the HSV-1 glycoproteins (8, 9, 12, 20, 25). The most variable immune response was to the 132,000 molecular weight envelope polypeptide. Antibody to this polypeptide was not present during the first 14 days after onset but was easily identified in all sera collected 26 or more days after onset of infection.

The 132,000 molecular weight polypeptide is reported to be a precursor of glycoprotein gC (21); however, the immune response to these two viral products are different. Antibody to gC is detected early in the course of primary infection and antibody to 132,000 molecular weight polypeptide is not and develops later. The reasons for this difference are not clear. The known excretion of glycoprotein gC from infected cells may provide a more effective stimulation for antibody production (13) than the polypeptide or the polypeptide may be poorly expressed on the surface of HSV infected cells. Similar, although less distinct, late appearances of

antibody to polypeptides of molecular weights 96,000, 67,000, and 48,000 were also seen.

The sequential sera from the patient with recurrent HSV-1 infection showed no qualitative or quantitative changes in antibody to HSV-1 polypeptides or glycoproteins in sequential sera collected before, during and after recurrent infection. The antigen profile immuno-precipitated with all sera included the 132,000 96,000, 67,000, and 48,000 molecular weight polypeptides which were not present in sera collected early in primary infection. Zweerink and Stanton (26) recently observed a similar unchanging antibody response to polypeptides in sera collected from three patients after a recurrent HSV infection. This unchanging response to HSV polypeptides and glycoproteins in recurrent HSV-1 infection suggests that antibody is probably not very important in regulation of these infections.

This original observation of the sequential development of antibody to specific HSV-1 polypeptides has the potential to clearly differentiate primary from recurrent infections. Eberle and Courtney (6) have recently utilized similar methodology to assay antibody to HSV 1 and 2 type specific and common antigens in human sera to identify past type 1 from type 2 infections as another example of the advantage of characterizing the immune response to specific HSV antigens. The qualitative assessment of antibody response to specific antigens of other viruses has also been useful with Epstein-Barr (10) and Hepatitis B (11) virus infections. Application of this approach to HSV infections may prove to be a useful tool in clinical and epidemiologic studies (17).

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