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Use of Monoclonal Antibodies for Analysis of Antibody-Dependent Immunity to Ocular Herpes Simplex Virus Type 1 Infection

JAMES T. RECTOR, ROBERT N. LAUSCH, AND JOHN E. OAKES*

Department of Microbiology and Immunology, College of Medicine, University of South Alabama, Mobile, Alabama 36688

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Monoclonal antibodies specific for the five major glycoproteins of herpes simplex virus type 1 (HSV-1) were tested for their capacity to mediate immunity to ocular HSV-1 infection. The specificity of the immunoglobulin made by each monoclone was determined by immunoprecipitation of [^{14}C]glucosamine-labeled polypeptides from detergent-solubilized HSV-1-infected cells. Of the five monoclonal antibodies studied, two immunoprecipitated glycoproteins gA/B, one immunoprecipitated glycoprotein gC, one immunoprecipitated glycoprotein gD, and one immunoprecipitated glycoprotein gE. All five were effective in passively transferring immunity to mice when they were given 4 to 24 h after HSV-1 infection on an abraded cornea. Four of the monoclonal antibodies were also evaluated for their capacity to neutralize HSV-1 and to promote complement-mediated cell lysis and antibody-dependent cellular cytotoxicity. It was found that none of these in vitro assays correlated with the protective activity of the antibodies in vivo. In fact, one of the monoclonal antibodies was unreactive in all three immunological reactions, even though it was highly effective in promoting recovery from HSV-1 induced ocular disease in vivo. The results suggest that antibodies can interact in vivo with virus-specific glycoproteins gA/B, gC, gD, and gE to initiate recovery from HSV-1-induced ocular disease, and that the therapeutic effectiveness of a specific monoclonal antibody does not correlate with its immunological reactivity in vitro.

Herpes simplex virus type 1 (HSV-1) is a leading cause of corneal opacities in the United States (10). In laboratory animals, ocular HSV-1 infection results in the spread of HSV-1 from the sites of infection along sensory nerves to the central nervous system. Once in the central nervous system, HSV-1 can replicate in the brain and kill the host (5, 12).

It has been reported that the passive transfer of HSV-1-specific antibody as late as 48 h postinfection can promote recovery from ocular HSV-1 infection by inhibiting the spread of the virus within the brain after its entry into the central nervous system (5). However, the mechanisms by which antibody inhibits virus spread within the central nervous system is not clear. Previous studies have shown that virus-specific antibody can interact with glycoproteins gA/B, gC, and gD at the surfaces of HSV-1-infected cells in vitro to mediate immunocytolysis in the presence of either complement or peripheral blood leukocytes (2, 13, 16, 17, 29, 30). In addition, these glycoproteins, when at the surface of HSV-1 virions, can serve as targets for neutralizing antibody (7, 9). At present, it is not known which of these glycoproteins or combination of glycoproteins can interact with antibody in vivo to initiate recovery from ocular HSV-1 infection. It is also not clear whether immunocytolysis and virus neutralization mediated by each of these glycoproteins in vitro contribute to the clearance of HSV-1 in vivo.

To investigate these questions, we have established hybridoma cell lines producing antibodies against major glycoproteins specified by HSV-1. All of these monoclonal antibodies were effective in passively transferring immunity to ocular HSV-1 infection despite the fact that some of these monoclonal antibodies did not have the capacity to mediate virus neutralization or immunocytolysis in vitro.

MATERIALS AND METHODS

Cells and media. Vero cells were grown in minimal essential medium (MEM) supplemented with 5% newborn calf serum (NBCS), sodium bicarbonate, and antibiotics. The P3X63Ag8.653 cell line (obtained from John Kearney, Birmingham, Ala.) is a subclone of

P3X63Ag8 and does not express any detectable immunoglobulin chains. The P3X63Ag8.653 cell line, hybrids of this line, and mouse lymphocytes were grown in Dulbecco MEM supplemented with 5% NBCS, 5% fetal calf serum, 10 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid) buffer, $NaHCO_3$, glutamine, and antibiotics.

Preparation of virus. HSV-1 (strain KOS), HSV-2 (strain 333), and HSV-1 (strain MP) (which lacks gC) were originally obtained from Fred Rapp (Hershey, Pa.) and have been maintained in our laboratory by passage on Vero cells (Flow Laboratories, Inc., McLean, Va.) at a multiplicity of infection of 0.1 PFU per cell. Virus was harvested from infected cells by three cycles of freezing and thawing. The lysate was clarified by centrifugation at $1,000 \times g$ for 10 min and assayed on Vero cells for virus titer as previously described (18).

Production and selection of anti-HSV-1-producing hybrids. SJL/J mice (Jackson Laboratory, Bar Harbor, Maine) were immunized in a rear footpad with 5×10^6 PFU of HSV-1 (KOS) per ml. After 2 to 3 weeks, the footpad was inflamed by injecting 0.05 ml of 10% saline. After 6 h, the inflamed footpad was abraded with an emery board, and virus was dropped onto the abraded area. Mice were sacrificed 3 to 5 days later, and their spleens and draining lymph nodes were removed and fused with P3X63Ag8.653 myeloma cells by adding polyethylene glycol. The cells were selected in a culture medium containing 0.1 mM hypoxanthine, 10 mM aminopterin, and 30 mM thymidine for 2 to 3 weeks. Culture supernatants from hybridizations were screened for antibody production by an enzyme-linked immunosorbent assay. This assay was performed with hybridoma screening reagent (Bethesda Research Laboratories, Inc., Gaithersburg, Md.) according to the directions of the manufacturer. HSV-1-infected Vero cells were attached to 96-well polystyrene tissue culture plates and used as a target antigen (11). Cells from positive wells were cloned twice by limiting dilutions in 96-well tissue culture plates. Dilutions were made so that the multiplicity of seeding was less than 0.1 cell per well. Supernatants from wells containing any one discernible colony were rescreened for antibody production. Clones that continued to produce antibodies were expanded in liquid culture and used to produce ascites tumors in pristane-primed F1 mice (SJL/J × BALB/c). The ascites and serum were collected and used as sources of monoclonal antibodies throughout this study. In addition to the monoclonal antibodies generated in our laboratory, monoclonal antibodies of known specificity to HSV-1 glycoproteins were obtained from M. Zweig (31) and L. Pereira (23, 24) and used to establish specificities of our antibodies and to complete our panel of monoclonal antibodies to all the major HSV-1 glycoproteins.

Immune precipitation. The specificitics of monoclonal antibodies were examined by immune precipitation of [^{14}C]glucosamine-labeled detergent extracts of HSV-1-infected Vero cells. Vero cells grown in 150-cm^2 flasks were mock-infected or infected with HSV-1 (KOS) at a multiplicity of 20 PFU per cell. After 1 h of absorption at 37°C, the cells were rinsed with Tris buffer (137 mM NaCl, 5 mM KCl, 7 mM Na_2HPO_4, 0.5 mM $MgCl_2$, 0.9 mM $CaCl_2$, 5 mM Tris [pH 7.4]). Cells were labeled at 5 to 24 h postinfection with 2.0 μCi of [^{14}C]glucosamine (D-[1-^{14}C]-glucosamine; specific activity, 54.2 mCi/mmol; New England Nuclear Corp., Boston, Mass.) per ml in MEM–5% NBCS at 37°C. The cells were then harvested by scraping, washed three times in cold phosphate-buffered saline, and solubilized in lysis buffer (10 mM Tris-hydrochloride [pH 7.2], 0.15 M NaCl, 0.2% NaN_3, 0.5% [wt/vol] Nonidet P-40, 1% sodium deoxycholate, 1% aprotinin [Sigma Chemical Co., St. Louis, Mo.]). The lysate was blended in a Vortex mixer, held on ice for 20 min, and spun at 40,000 rpm with a TY-65 rotor in a Beckman L8-70 centrifuge for 1 h at 4°C. The supernatant was collected and preabsorbed with Pansorbin (Calbiochem, La Jolla, Calif.) and clarified by centrifugation before being mixed with antibody. A 100-μl sample of supernatant was mixed with 10 μl of antibody and incubated overnight at 4°C. A 200-μl portion of washed 10% suspension of Pansorbin in SaC buffer (phosphate-buffered saline containing 0.5% Nonidet P-40, 5 mM potassium iodide, and 0.2% sodium azide) was added to each tube and incubated for 30 min at 4°C with occasional mixing. The Pansorbin immune complexes were collected by centrifugation, washed three times in SaC buffer, and solubilized in 50 μl of solubilizing buffer (0.154 g of dithiothreitol, 0.2 g of sodium dodecyl sulfate [SDS], 5 mg of bromophenol blue, 0.8 ml of 1 M Tris-hydrochloride [pH 6.8], 1.0 ml of glycerol, and 8.0 ml of water). This material was boiled for 2 min and clarified by centrifugation, and the supernatant was analyzed by slab gel electrophoresis with a discontinuous buffer system and an 8.5% acrylamide separating gel cross-linked with 0.47% *N,N*′-diallyltartardiamide. The stacking gel was 5% acrylamide cross-linked with bisacrylamide. The stained gels were processed for fluorography (3) and exposed to Kodak X-Omat AR X-ray film. ^{14}C-labeled molecular weight markers (Bethesda Research Laboratories) were run with each gel to estimate the molecular weights of the bands.

Passive immunity with monoclonal antibody. BALB/c mice 4 weeks old (Charles River Laboratories, Inc., Wilmington, Mass.) were anesthetized with 0.004 mg of phenobarbital per g of body weight. The eye was then scarified by three trephine motions with a microtrephine, and 3.9×10^7 PFU of HSV-1 was dropped on the surfaces of the eye with a tuberculin syringe and massaged into the eye with the eyelid. Mice were then passively immunized at selected times postinfection by intraperitoneal injections of 0.3 ml of diluted ascites. Although densitometric scanning of gels of ascites from SDS-polyacrylamide gel electrophoresis showed that antibody protein varied from 9 to 40 mg, each ascites had enzyme-linked immunosorbent assay titers $> 10^6$ against HSV-1 (KOS)-infected Vero cells. The numbers of survivors in control groups and in groups of mice receiving monoclonal antibody were compared. The significance of the data was determined by negative-exponential transformation of survival times (15).

Measurement of neutralizing antibodies. Neutralizing antibodies were quantified by a modification of the microneutralization test described by Rawls et al. (26). Briefly, antibody was diluted twofold from 1:20 to 1:2,580 with MEM–2% NBCS in 96-well microtiter tissue culture plates. Diluted virus containing about 10^2 PFU in 0.025 ml of MEM–2% NBCS was then added to each well. After 1 h at room temperature, the mixtures

TABLE 1. Characterization of HSV-1-specific monoclonal antibodies

Monoclonal antibody[a]	Isotype[b]	Immunofluorescence staining reaction in membranes of HSV-1-infected cells[c]
D8AB	IgG2B	1:640[d]
F3AB	IgG1	1:1,280
G8C	IgG2B	1:1,280
H7E	IgG1	1:1,280

[a] Source of monoclonal antibody was mice bearing ascites tumors.

[b] Immunoglobulin classes and subclasses were determined by standard double-diffusion agarose gels (1% agarose in 150 mM NaCl–50 mM barbital buffer, pH 9.6). Rabbit anti-mouse immunoglobulin class and subclass sera (Litton Bionetics) were loaded into wells adjacent to antibodies from ascites fluids, and precipitin lines were read after 72 h at room temperature.

[c] HSV-2-infected cells were tested at a 1:40 dilution of monoclonal antibody and showed no reaction.

[d] Dilution of monoclonal antibody.

were added to microtiter wells containing monolayers of Vero cells. The cultures were then incubated at 37°C in 5% CO_2 until cytopathic effects were observable, after which the monolayers were fixed in methanol-acetone and stained with crystal violet (0.15%). The neutralizing titer of the antibody was the reciprocal of the highest dilution of antibody which inhibited cytopathic effects by 50%.

The effect of complement on the neutralization titer was assessed by adding 50 μl of a 1:20 dilution of rabbit low-toxicity complement (Cedarlane Laboratories, Hicksville, N.Y.) to wells containing cells, virus, and antibody.

Assays for immunocytolysis. Assays were carried out in microtiter plates with round-bottomed wells. In the antibody-dependent cellular cytotoxicity (ADCC) assay, the target cells were L929 cells infected in suspension 2 to 3 hours, previously with HSV-1 at a multiplicity of 3 to 6 PFU per cell. After being labeled with 200 μCi of ^{51}Cr (sodium chromate; New England Nuclear) for 60 min at 37°C in a shaking water bath and being washed three times, 10^4 target cells were put into wells in 0.1-ml volumes. Then 50 μl of test or control serum or ascites fluid at the desired dilution was added, followed by 50 μl of effector cells. The effector cells were peritoneal exudate cells obtained from BALB/c mice 7 to 12 days after intraperitoneal inoculation of 2×10^6 to 4×10^6 colony-forming units of *Mycobacterium bovis* strain BCG (25). The effector-to-target ratio ranged from 10:1 to 20:1. After a 6-h incubation period at 37°C in 5% CO_2, the plates were centrifuged to pellet the cells, and 0.1-ml samples of supernatant were drawn off and counted.

For the complement-mediated antibody lysis test, HSV-1-infected Vero cells served as the target. Monolayers of Vero cells infected for 16 to 20 h with HSV-1 at 3 PFU per cell were collected by trypsinization, washed, and labeled with ^{51}Cr as described above. To 0.1 ml, containing 2×10^3 target cells, was added 50 μl of the test material followed by 50 μl of guinea pig serum diluted 1:10. The guinea pig serum which served as the complement source was preabsorbed with Vero cells. The cultures were incubated as above for 1 to 2 h, and then the released label was counted.

In both assays, the extent of cytolysis was calculated as follows: % specific lysis = [(experimental ^{51}Cr release − spontaneous ^{51}Cr release)/(Maximum ^{51}Cr release − spontaneous ^{51}Cr release)] × 100.

Maximum ^{51}Cr release was determined by adding 2.5% SDS. Spontaneous release was the counts released in the presence of medium alone.

Immunofluorescence. Indirect immunofluorescence was performed to determine whether the monoclonal antibodies would bind to virus antigens found on HSV-infected Vero cells. In tests for membrane antigen reactive antibodies, unfixed cells attached to 15-mm glass cover slips were incubated with the test sample for 30 min and then washed and covered with fluorescein isothiocyanate-conjugated rabbit anti-mouse immunoglobulin G (IgG) (Cappel Laboratories, Downingtown, Pa.). The fluorescence of the cells was evaluated with a Leitz orthoplan photomicroscope. Appropriate positive and negative controls were included in each test.

RESULTS

Characterization of monoconal antibodies. Four hybridoma cell lines were established from two independent fusions. Ascites from each of the hybrid lines was assayed for immunoglobulin class specificity by double-diffusion techniques. All of the monoclonal antibodies gave a single precipitin line against class-specific or subclass-specific antiserum, demonstrating that they belonged to either the IgG1 or the IgG2b subclass (Table 1). SDS-polyacrylamide gel electrophoresis analysis of ascites produced by all four hybrid lines showed single bands for both heavy and light chains (data not shown). The monoclonal antibodies also showed differences in the mobilities of their light chains, confirming their independent origin.

The capacity of each monoclonal antibody to bind to infected cells was determined by testing the capacity of the antibodies to react with HSV-1- and HSV-2-infected cells by indirect immunofluorescence. All four of the monoclonal antibodies showed strong immunofluorescence on fixed HSV-1-infected cells. None of the monoclonal antibodies reacted with fixed HSV-2-infected cells. This suggests that all four monoclonal antibodies are specific for HSV-1-infected cells.

Specificity of antibody-antigen interaction. Immune precipitation experiments were carried out to identify the antigens which reacted with monoclonal antibodies prepared in our laboratory. Since we were interested in establishing a panel of monoclonal antibodies reactive with individual glycoproteins of HSV-1, all immune precipitations were done with [^{14}C]glucosamine-labeled extracts prepared from HSV-1-infected Vero cells or mock-infected Vero cells. Labeled extract was reacted with HSV-1-immune mouse serum, normal mouse serum, or individual

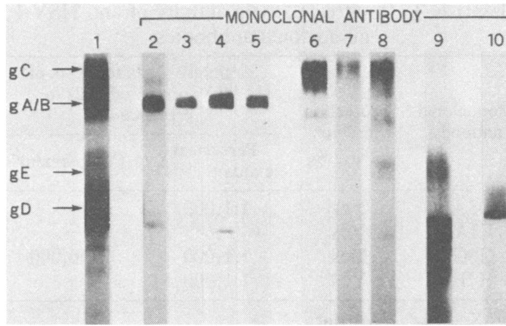

FIG. 1. Fluorograms of immunoprecipitates obtained with monoclonal antibodies or with immune mouse serum. Lysates of HSV-1-infected Vero cells, labeled with [^{14}C]glucosamine 5 to 24 h postinfection, were immunoprecipitated with monoclonal antibodies or with immune mouse serum and electrophoretically separated on SDS-polyacrylamide gels. Lane 1, mouse immune serum; lane 2, D8AB; lane 3, F3AB; lane 4, 3S; lane 5, H233; lane 6, G8C; lane 7, 19S; lane 8, HC1; lane 9, H7E; lane 10, 1S.

monoclonal antibodies. Immune precipitates were collected with *Staphylococcus aureus* protein A, separated on 8.5% acrylamide gels cross-linked with *N,N'*-diallyltartardiamide, and visualized by fluorographic techniques. Polypeptides were not precipitated from mock-infected cells or by immune serum or by any of the monoclonal antibodies, nor did normal mouse serum precipitate polypeptides from HSV-1-infected cells (data not shown). The identities of immune precipitates of our four monoclonal antibodies were determined by comparison on the same slab gel with immune precipitates of monoclonal antibodies of known specificity provided by M. Zweig (31) and L. Pereira (23, 24).

Results reveal four different patterns of electrophoretically separated glycoproteins corresponding to gA/B, gC, gE, and gD (Fig. 1). Monoclonal antibodies D8AB and F3AB precipitated a major band with a molecular weight of approximately 120,000 (120 K), along with several faster-migrating forms from infected-cell extracts. These lower-molecular-weight forms were more apparent on film exposed to the gel for longer periods of time. Identical patterns were obtained with monoclonal antibodies 3S and H233, known to be specific for gA/B (23, 31). At present, it is unclear whether the 120-K doublet represents a gA/gB complex or distinct gA or gB glycoproteins (8, 27, 31). Therefore, we have designated these monoclonal antibodies as anti-gA/B.

Monoclonal antibody G8C immunoprecipitated predominantly two [^{14}C]glucosamine-labeled polypeptides identical to those precipitated by HC1 and 19S, monoclonal antibodies with known specificity to gC (24, 31). The larger glycoprotein has a molecular weight of approximately 130 K and represents the mature form of gC, whereas the smaller glycoprotein has a molecular weight of approximately 90 K. A precursor-product relationship between the 130-K form and a 105- to 110-K glycoprotein has already been established (7, 24, 32). Whether the 90-K protein seen on our gels for all three antibodies represents this precursor form is not known. Besides the indistinguishable immunoprecipitation patterns between G8C, HC1, and 19S, monoclonal antibody G8C reacted with HSV-1 (KOS)-infected cells by the enzyme-linked immunosorbent assay but did not react with gC-deficient HSV-1 (MP)-infected cells. Monoclonal antibodies H7E, D8AB, and F3AB all reacted with both HSV-1 (KOS)- and HSV-1 (MP)-infected cells (data not shown). Based on these results, we have classified monoclonal antibody G8C as being directed to gC.

Monoclonal antibody H7E produced a unique pattern of separated glycoproteins that did not match any of the immunoprecipitation patterns characteristic of monoclonal antibodies specific for gA/B, gC, or gD. This pattern consisted of molecular weight bands of approximately 80 to 90 K and a broad band of lower-molecular-weight forms. The only HSV-1 glycoprotein with similar molecular weight mobilities on SDS-polyacrylamide gels is gE (1, 21, 22). The specificity of H7E to gE was confirmed independently by P. Spear (personal communication).

The final monoclonal antibody 1S was kindly provided by M. Zweig; it immunoprecipitates 59- and 52-K proteins, representing gD and its precursor, respectively (4, 9, 31, 32). The widening of the band in the gD region seen in Fig. 1 is due to comigration of the heavy chain of immunoglobulin with gD.

Effect of passively transferred monoclonal antibodies on host resistance to ocular HSV-1 infection. The four monoclonal antibodies generated in our laboratory and one monoclonal antibody specific for gD (1S), provided by M. Zweig (31), were tested for their capacity to promote recovery from ocular HSV-1 infections. We passively transferred 0.3 ml of each monoclonal antibody to mice 4 to 24 h postinfection. It was found that monoclonal antibodies to each virus-specific glycoprotein significantly protected animals from ocular HSV-1 infection ($P < 0.01$; Fig. 2). It was also noted that D8AB was more effective in initiating recovery from ocular HSV-1 infection when passively transferred at 4 h postinfection; monoclonal G8C, on the other hand, was more effective in initiating recovery at 24 h postinfection. Monoclonal antibodies F3AB, 1S, and H7E were as effective in protecting mice from ocular infection at 4 h postinfection as they

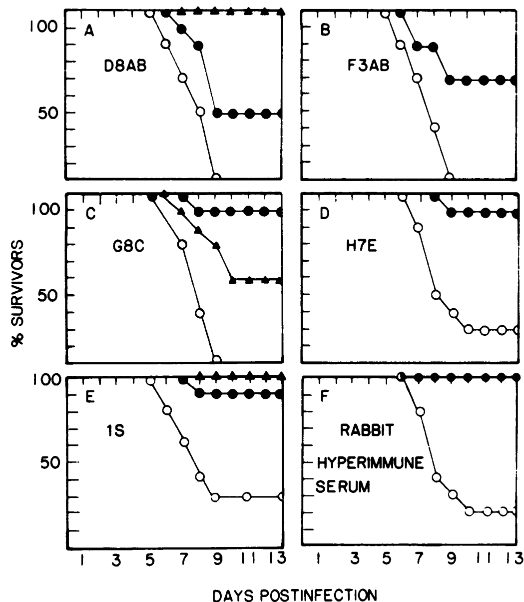

FIG. 2. Effect of monoclonal antibodies on survival of mice after ocular inoculation of 3.9×10^7 PFU of HSV-1. In panels A, C, and E, antibody was given 4 h (▲) and 24 h (●) postinfection. In panels B, D, and F, antibody was given 24 h postinfection (●). Some mice were given normal rabbit serum (○). There were 10 mice per group.

were at 24 h postinfection (data at 4 h postinfection not shown for F3AB and H7E).

Immunological reactivity of monoclonal antibodies in vitro. Neutralization experiments were performed to test the capacity of four monoclonal antibodies generated in our laboratory to neutralize HSV-1 in the presence or absence of complement. For these experiments, ascites fluids were diluted serially in microtiter plates and mixed with 100 PFU of HSV-1. The virus-antibody mixtures were incubated either in the presence or absence of complement for 1 h before being added to Vero cell monolayers in

TABLE 2. Neutralization of HSV-1 by monoclonal antibodies

Monoclonal antibody[a]	Neutralization titer[b]	
	Without complement	With complement
D8AB	<1:20	1:40
F3AB	<1:20	<1:20
G8C	1:640	>1:2,560
H7E	<1:20	<1:20

[a] Source of monoclonal antibody was ascites-bearing mice.
[b] Determined by microneutralization.

TABLE 3. In vitro cytolytic activity of anti-HSV-1 monoclonal antibodies

Monoclonal antibody	Protection in vivo[a]	Antibody-mediated lysis of HSV-1-infected cells in presence of:	
		Peritoneal exudate cells[b]	Complement[c]
D8AB	Yes	1:1,000	–
F3AB	Yes	–	–
G8C	Yes	1:1,000	1:50,000
H7E	Yes	1:1,000	–

[a] See Fig. 2 for data.
[b] Each monoclonal antibody was tested at the dilution of 1:50, 1:100, or 1:1,000. The titers are expressed as the highest of these three dilutions giving ^{51}Cr release greater than the ^{51}Cr release obtained with peritoneal exudate cells plus control serum at the 0.05 level of significance, as determined by the Student's *t* test. A minus (–) indicates no lysis at 1:50 dilution of antibody.
[c] The titer is expressed as the highest dilution of antibody giving ^{51}Cr release greater than the ^{51}Cr release obtained with complement alone at the 0.05 level of significance, as determined by the Student's *t* test. A minus (–) indicates no lysis at 1:50 dilution of antibody.

96-well microtiter plates. In the absence of complement, only G8C caused a reduction in the number of plaques. However, monoclonal antibody D8AB reduced the number of plaques, provided that complement was included in the incubation mixture (Table 2). Thus, virus neutralization by G8C occurred independently of complement, but virus neutralization by D8AB was complement dependent. Monoclonal antibodies F3AB and H7E did not neutralize HSV-1 in our tests.

Each monoclonal antibody was then tested for complement-mediated cell lysis and ADCC. For these experiments, ^{51}Cr-labeled HSV-1-infected cells were incubated with appropriate dilutions of each monoclonal antibody in the presence of complement or of peritoneal exudate cells activated by *Mycobacterium bovis* BCG. Only G8C had the capacity to lyse HSV-1-infected cells in the presence of complement (Table 3). However, monoclonal antibodies G8C, D8AB, and H7E all lysed HSV-1-infected cells in the presence of activated peritoneal exudate cells.

DISCUSSION

In this report, the biological activities of monoclonal antibodies directed against epitopes present on glycoproteins of HSV-1 are described. The specificity of each monoclonal antibody was determined by immunoprecipitation of extracts of HSV-1-infected cells. Two monoclonal antibodies specific for glycoproteins gA/B,

one specific for glycoprotein gC, one specific for glycoprotein gD, and one specific for glycoprotein gE were identified. All five preparations have been found to react with HSV-1-infected cells, but not with HSV-2-infected cells, in immunofluorescence tests (31; Table 1). Thus, the five monoclonal antibodies used in this study appeared to recognize type-specific epitopes on each glycoprotein. Recently, Dix et al. (6) reported that neutralizing monoclonal antibodies to a type-common epitope on glycoprotein gD and a type-specific epitope on glycoprotein gC were both protective when given 24 h after subcutaneous virus infection. In our study, monoclonal antibodies specific for gA/B and gE, in addition to monoclonal antibodies specific for gC and gD, protected mice from ocular HSV-1 infection. Thus, antibodies directed to all the known major glycoproteins of HSV-1 can promote recovery from ocular disease induced by this virus.

Did the monoclonal antibodies protect primarily by neutralizing virus infectivity? This possibility seems quite unlikely for several reasons. First, several of the antibodies that protected mice from ocular HSV-1 infection failed to demonstrate any virus-neutralizing activity in vitro. Second, the antibody was not passively transferred until 24 h after virus infection, i.e., at a time when most of the virus would be located within the cells and so would not be susceptible to antibody. Finally, previous studies in our laboratory have shown that $F(ab')_2$ fragments obtained from rabbit anti-HSV-1 serum which can neutralize virus in vitro are not protective in vivo (19). It also is very unlikely that activation of the classical complement pathway was needed for recovery since several of the antibodies which protected were of the IgG1 class, an isotype which does not readily fix complement (20). In addition, it is known that heterologous immune serum will protect C5-deficient mice from both ocular and subcutaneous HSV infection (19).

If virus neutralization and complement-mediated lysis are not the principal mechanisms by which the monoclones promote recovery from HSV-1 infection, what other mechanism might be operative? In experiments by Kohl et al., a combination of human leukocytes and a subneutralizing dose of antibody protected infant mice from intraperitoneal HSV-1 infection (14). Since neither cells nor antibodies protected by themselves, it was suggested that ADCC was responsible for the protection seen. ADCC was also considered as a possible mechanism to account for the observation that the Fc component of the antibody molecule was needed to resolve intracellular infections in vivo (19).

In the present study, monoclonal antibodies D8AB, G8C, and H7E could mediate lysis of HSV-1-infected L929 cells in collaboration with activated mouse peritoneal exudate cells. These results extend the findings of Norrild et al. (17) by showing that type-specific epitopes on glycoprotein gE, in addition to gA/B, gC, and gD, can serve as targets in ADCC tests. It is of interest that monoclonal antibody F3AB failed to mediate lysis. Thus, protection in vivo was not correlated with ADCC activity in vitro, a finding which has also been observed in a simian virus 40 tumor model (25). Nevertheless, since a variety of cell types, including platelets (28), can participate in ADCC, and since target cells vary greatly in their susceptibility to being lysed by this mechanism, it is clearly premature to rule out ADCC as a possible mechanism by which any of the monoclonal antibodies initiated recovery in vivo. Since the monoclonal antibodies varied in their in vitro biological activities, it is possible that each monoclonal antibody may not have mediated recovery by the same mechanism. Differences in the biological activities of monoclonal antibodies in vivo may be related to the topographical location or temporal expression of viral glycoproteins on infected cells against which a particular monoclonal activity binds. This may explain why D8AB protected a greater number of recipients when it was passively transferred at 4 h postinfection, whereas G8C protected better at 24 h.

In conclusion, it has been shown that HSV-1-specific glycoproteins gA/B, gC, gE, and gD can all elicit the production of antibodies capable of interfering with virus spread in vivo after ocular HSV-1 infection. An important finding was that one of these monoclonal antibodies did not mediate virus neutralization, complement-mediated lysis, or ADCC in vitro. The fact that this monoclonal antibody protected in the absence of these reactions may reflect the fact that antibody interacts with infected tissues by a complex mechanism that may involve the participation of additional factors or cells which are not available in vitro. However, it is clear that antibody reactivity in these three in vitro tests does not correlate with the ability to passively transfer immunity against ocular disease induced by HSV-1.

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