Protection against Zosteriform Spread of Herpes Simplex Virus by Monoclonal Antibodies

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The in vivo protective role of herpes simplex virus (HSV-1)–specific antibody was analyzed using monoclonal antibodies (MAbs) reactive with discrete antigenic sites on glycoproteins B, C, and D (gB, gC, gD) in the murine zosteriform spread model of HSV-1. All of the anti-gC and anti-gD MAbs, and one of four anti-gB MAbs (B6) were protective. The in vitro abilities of the MAbs to neutralize HSV-1 and mediate antibody-dependent cellular cytotoxicity (ADCC) against HSV-1–infected cells were examined as potential mechanistic correlates to in vivo protection. All animals given MAbs at high ADCC unit doses were protected. Some but not all mice given MAbs at high ADCC unit doses were protected. These studies designate specific epitopes recognized by protective antibodies and indicate that protection from the neurologic spread of HSV may be related to neutralization, ADCC, or both. The actual contribution of ADCC and neutralization to in vivo antibody-mediated protection remains unclear.

The immune response to a primary mucocutaneous herpes simplex virus (HSV) infection is multifarious, relying predominantly on T cell–mediated immunity to clear the initial site of infection and antibody produced by B cells to limit the amount of virus invading the local sensory ganglia [1–3]. Both mice and humans generate an antibody response to a wide range of both structural (envelope and core) and nonstructural (regulatory) HSV proteins during infection, although in humans the structural proteins, especially glycoproteins B, C, and D (gB, gC, and gD), appear to be the main antibody targets [4–6]. However, the antibody response arises too late during primary infection to prevent colonization of the local sensory ganglia and seems unable to prevent recurrent infections in a significant portion of individuals harboring latent HSV. In mice, passive transfer experiments have demonstrated that monoclonal antibodies (MAbs) specific for HSV glycoproteins gB, gC, gD, and gE can protect naive mice against a lethal HSV challenge [7–9]. The ability of polyclonal immune sera and gD-specific MAbs to protect in a model of recurrent disease has also been demonstrated [10].

The means by which HSV-specific antibody mediates protection in vivo remains uncertain. Other than the extracellular neutralization of virions, the mechanisms by which antibody may effect antiviral immunity depend on the binding of viral antigens expressed on the infected cell surface; these mechanisms include the triggering of the classical complement cascade (and infected cell lysis via antibody-dependent complement-mediated cytotoxicity [ADCMC]), the activation of Fc receptor–bearing killer cells (antibody-dependent cellular cytotoxicity [ADCC]), and the suppression of viral replication (mediated by the viral protein–antibody interaction at the cell surface) [11]. Earlier reports indicated that either neutralization or ADCC was a principal mechanism of protection against lethal HSV challenge [7, 10]. Others, however, found the protective ability of passively transferred antibody to have no correlation with the in vitro activities of neutralization, ADCMC, or ADCC [12]. Finally, previous accounts of antibody-mediated protection against HSV in vivo have relied on anti-gB, -gC, -gD, and -gE-reactive MAbs of undefined epitope specificity and as a result have yielded no indication as to the areas of the respective glycoproteins important in the generation of a protective or nonprotective antibody response.

The murine zosteriform spread model mimics the course of human herpetic disease in that infection is initiated at a cutaneous site and spreads to the peripheral nervous system, from which the virus reemerges and infects regions of the epithelium remote from the inoculation site. We examined the ability of a representative panel of monoclonal antibodies, specific for discrete antigen sites on gB, gC, and gD, to protect against the zosteriform spread of HSV-1. We also examined each MAb's affinity for the challenge virus and ability to neutralize HSV-1_{BK} and mediate ADCC against infected cells in vitro.

Materials and Methods

Viruses. HSV-1 KOS 321 and a clinical isolate of HSV-1 (HSV-1_{BK}) were propagated in HEp-2 cells and maintained on McCoy's 5A medium with 10% donor calf serum (DCS; GIBCO, Grand Is-

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land, NY) as previously described [13]. Infectious particles were recovered after three freeze-thaw cycles, divided into aliquots, and stored at −80°C. Titration on Vero cell monolayers revealed titers of 1.8×10^9 $TCID_{50}$/ml for KOS 321 and 5.6×10^8 $TCID_{50}$/ml for $HSV\text{-}1_{BK}$.

MAbs. The production of the HSV-specific MAbs B3, B4, B6, B8, C3, C4, C8, D2, D7, and D8 has been described previously [14]. Each was produced as mouse ascites fluid, concentrated by ammonium sulfate precipitation, suspended in PBS, and stored at −80°C. The previously reported properties of the MAbs [9, 15, 16] are summarized in table 1.

Mice and inoculations. Male C3H/TEN (University of Tennessee Memorial Research Center Hospital, Knoxville) and C3H/HeJ (Jackson Laboratory, Bar Harbor, ME) mice, 6 weeks old, were infected as described by Simmons and Nash [17]. One day before infection, their dextral flanks were shaved and depilated with Nair (Carter Products, New York); 2 h before infection, 100 μl of a MAb (as concentrated ascites fluid) was administered intravenously in 200 μl of Hanks' balanced salt solution (HBSS). Control animals received 300 μl of HBSS alone. The mice were then coded and mixed so that their treatment was not known at the time of infection. After anesthetizing the mice with Metofane (Pitman-Moore, Washington Crossing, NJ), each was percutaneously inoculated with a 28-gauge needle on the mid-flank region by scratching through a 10-μl drop containing 2.8×10^5 $TCID_{50}$ of $HSV\text{-}1_{BK}$, leaving an abraded area of ~9 mm^2. This inoculation scheme yielded a zosteriform band in ~90% of untreated control mice on day 5 after inoculation.

Determination of protection. Monoclonal antibody–mediated protection was determined by examining the banding pattern of the infected mice for up to 2 weeks after infection. Almost all mice that formed a zosteriform band died or were sacrificed after symptoms of encephalitis. Protection was recorded as positive when no zosteriform spread was evident. Significant protection was determined using χ^2 contingency table comparison (Statview 512+ MacIntosh software; Brainpower, Calabasas, CA) of each treatment group with the respective number of control mice.

Affinity determination. Individual MAb affinities for $HSV\text{-}1_{BK}$ were determined using an ELISA variation of the method of Frankel and Gerhard [18]. Briefly, $HSV\text{-}1_{BK}$ virions were purified by 4-h, 80,000-*g* centrifugation (L3–50 ultracentrifuge; Beckman, Palo Alto, CA) over a 20%–50% continuous potassium tartrate (Aldrich Chemical, Milwaukee) gradient in 25 × 89 mm centrifuge tubes (Beckman) and used as antigen. Binding curves were determined by coating the ELISA plate wells with a constant amount of purified antigen and adding a series of 12 twofold dilutions of each antibody (in quadruplicate). Total antibody able to bind $HSV\text{-}1_{BK}$ antigen (for the determination of binding constants) was quantitated using a constant amount of antibody (equivalent to the last relevant dilution of the antibody-binding curve) and reacting it with various amounts of prebound antigen. Absorbance was read at 490 nm on an automated reader (model EL310; Bio-Tek Instruments, Burlington, VT). Comparison to a standard curve (A_{490} by nanograms of gamma globulin) generated by coating ELISA plates with known amounts of mouse gamma globulin (Accurate Chemical & Scientific, Westbury, NY) allowed the precise conversion of each A_{490} reading into nanograms of antibody bound. The affinity constants were determined from the linear part of the Scatchard analysis of the MAb binding curves.

Reactivation of latent HSV-1. Mice surviving infection were kept for 2–3 months, sacrificed, and dissected. Dorsal root ganglia innervating the inoculation site were explanted into 1 ml of McCoy's 5A medium with 5% DCS in 2-ml tubes (Corning Glass Works, Corning, NY). After 3–5 days of incubation at 37°C, this culture was transferred to a media-evacuated 25-cm^2 flask that had been seeded with 1×10^6 Vero cells the previous day. After 2–4 hours, 5 ml of McCoy's 5A medium with 2% DCS was added. Flasks were observed daily for cytopathic effect (CPE) for 7 days. Afterwards, two rounds of cell pelleting, three freeze/thaw cycles, and replating of the supernatant fluid on a fresh 25-cm^2 flask of Vero cells (observed for another 7 days for CPE) were done to assay for slowly replicating virions.

Neutralization assay. The ability of each MAb to neutralize $HSV\text{-}1_{BK}$ was examined by preincubating 80 $TCID_{50}$ of $HSV\text{-}1_{BK}$ with series of twofold dilutions of each MAb in the presence of complement (Low-Tox-M rabbit complement; Accurate Chemical) at a final dilution of 1:10, in a total volume of 75 μl in 96-well microtiter plates, for 3 h at 37°C. Aliquots (25 μl) of the mixture were then transferred in triplicate to confluent monolayers of Vero cells (maintained in McCoy's 5A medium with 10% DCS). After 1 h of adsorption, three drops of modified Eagle's medium (GIBCO) with 2% DCS and 1.25% low-melting-point agarose (BRL, Gaithersburg, MD) warmed to 37°C was added to each well. After 3 days, the number of plaques was counted. Neutralization units were expressed as the total amount of each MAb initially added to the well in which the 50% end point of plaque reduction occurred.

ADCC assay. MAb-mediated anti-HSV ADCC activity was determined by the procedure of Kohl et al. [19]. Adherent mouse peritoneal cells were prepared from mice that had been injected with 1 ml of thioglycollate broth (BBL Microbiology Systems, Cockeysville, MD) 5 days earlier by peritoneal lavage with 4 ml of ice-cold RPMI 1640 medium (GIBCO) with HEPES buffer (0.025 *M* final), 0.2% sodium bicarbonate, 10% fetal calf serum, penicillin-streptomycin-neomycin antibiotic mixture (GIBCO), 50 μg/ml L-glutamine, and 2-mercaptoethanol (5×10^{-5} *M* final) (complete RPMI). Vero cells, infected with HSV-1 strain KOS 321 at a MOI of 1.5 18 h before use and labeled by incubating 2×10^6 cells in

Table 1. Properties of the monoclonal antibody (MAb) panel.

MAb	Isotype	Antigenic site	Neutralization titer KOS	Neutralization titer HSV-2	Plaque inhibition (KOS)
B3	IgG3	gB I	2560	–	–
B4	IgG3	gB III	10,240	640	–
B6	IgG2b	gB IV	10,240	–	+
B8	IgG2a	gB II	102,400	2560	–
C3	IgG2a	gC IIa	6400	–	–
C4	IgG2a	gC I	6400	–	–
C8	IgG2a	gC IIb	20,480	–	–
D2	IgG3	gD I	81,920	25,600	+
D7	IgG2a	gD IX	4096	2560	+
D8	IgG2a	gD X	5120	2560	+

NOTE. Antigenic site refers to the discrete area of the envelope glycoprotein B, C, or D (gB, gC, gD) of herpes simplex virus (HSV) type 1 recognized by the MAbs. Neutralization titers shown are reciprocal 50% end points for HSV-1 strain KOS 321 and HSV-2 strain 186.111. Plaque inhibition refers to the ability of the MAbs to hinder plaque formation in vitro.

1 ml of complete RPMI with 150 μCi of ^{51}Cr (Amersham; Amersham, UK for 4 h at 37°C, served as targets.

The ability of MAb to mediate ADCC was determined in 96-well round-bottom microtiter plates (Corning) by incubation (16 h, 37°C) of dilutions of each MAb (quadruplicate samples in complete RPMI) to 5×10^3 target cells and $2–2.5 \times 10^5$ adherent cells (an effector-to-target cell ratio of 40–50:1). Percentage of ^{51}Cr release was determined by dividing the amount of ^{51}Cr in the medium by the total amount of label added per well. The percentage of specific release (percentage of ADCC) mediated by the MAbs was determined by subtracting the percentage of ^{51}Cr release of the negative control wells (containing peritoneal cells and targets [no serum control] or peritoneal cells and targets along with dilutions of normal mouse serum [normal serum control]) from the percentage of ^{51}Cr release of the MAb wells. In all experiments, the percentages of ^{51}Cr release for either negative control were equivalent (5%–10% above the spontaneous release by the targets themselves, which ranged from 10% to 15%). The standard errors of the percentage of ^{51}Cr release sample means were ≤10%. Units were determined by titrating each MAb-mediated ADCC from zero to maximum and assigning one ADCC unit value to the amount of each MAb added to the assay that caused 50% of maximum ^{51}Cr release from the targets.

$F(ab')_2$ fragment generation. MAbs C3 and C4 were fragmented by incubating them at a concentration of 2 mg/ml with pepsin (porcine stomach mucosa, 2× crystalline; Calbiochem, La Jolla, CA) at 25 μg/ml in 0.1 *M* citrate buffer (pH 3.8) for 6 h at 37°C. The reaction was terminated by raising the pH above 7 by addition of 3.0 *M* Tris-HCl, pH 8.6, and the mixture was desalted by centrifugation over Centricon-30 microconcentrators (30,000-MW cutoff; Amicon, Danvers, MA) for 30 min at 2500 *g* and 4°C in a Sorvall Superspeed RC2-B with a fixed-angle SS-34 rotor (Du Pont Instruments, Hoffman Estates, IL). Before passive transfer to mice in protection studies, the digestion was confirmed by SDS-PAGE, and the fragments were diluted in HBSS so that each mouse received a 0.5-mg dose in a 300-μl intravenous injection.

Results

MAb protection against the zosteriform spread of HSV-1. We initially examined the ability of MAbs, administered 2 h before infection, to block zosteriform lesion formation. Our results, as summarized in table 2, indicate that MAbs specific for HSV-1 gB, gC, and gD could protect against zosteriform lesion formation (and death). Specifically, mice treated with the MAbs specific for gC and gD (C3, C4, C8, D2, D7, and D8) and one of four of the gB-specific MAbs (B6) had a significantly lower frequency of zosteriform lesion formation than did control mice given HBSS.

The protection data of table 2 represent an additive summarization of several separate experiments in C3H mice. Each trial included a select group of MAbs in treatment groups of four to six mice each. About 90% of the negative control mice, which received HBSS, demonstrated zosteriform band formation 5 days after challenge. As shown, MAb B8 conferred no improvement over HBSS-treated controls, and MAbs B3 and B4 yielded an ~10% improvement over HBSS controls.

Table 2. Monoclonal antibody (MAb) protection against zosteriform spread of herpes simplex virus type 1.

MAb	Affinity (M^{-1})	Dose (mg)	No. (%) of mice with zosteriform spread	*P*
B3	3.3×10^9	5.6	13 (77)	NS
B4	6.7×10^9	1.0	16 (76)	NS
B6	7.9×10^8	1.2	2 (11)	<.001
B8	1.9×10^9	1.3	15 (100)	NS
C3	1.9×10^9	4.0	5 (17)	<.001
C4	3.8×10^9	4.8	3 (21)	<.001
C8	3.3×10^9	0.2	6 (43)	.006
D2	3.9×10^9	0.3	10 (63)	.05
D7	2.8×10^8	7.2	1 (7)	<.001
D8	1.5×10^9	4.6	2 (10)	<.001

NOTE. Results of several protection experiments for each MAb are shown. Zosteriform spread refers to formation of zosteriform bands after challenge. Significance was determined by χ^2 comparison of zosteriform spread-positive antibody-receiving mice with HBSS-treated control mice (~90% positive). NS = not significant ($P > .05$).

MAb D2 yielded nearly 30% protection, and C8 protected 50% of the mice. MAbs C3 and C4 yielded ~70% protection, and MAbs B6, D7, and D8, 80% protection. Two trends were apparent: Antibodies directed against discrete antigenic areas of gC and gD were all significantly protective, and only one of four gB-specific MAbs provided a high and significant degree of protection.

The dorsal root ganglia innervating the site of infection from mice protected by MAb administration were explanted to assay for the reactivation of latent HSV-1. A greater percentage of mice treated with the protective gC MAbs demonstrated the presence of reactivatible virus than of those treated with MAbs B6, D2, D7, and D8. On the whole, 42% of the gC MAb-treated mice were found to harbor reactivatible HSV-1, compared to 56% of the positive control group and none of the mice receiving the gD-specific MAbs or MAb B6.

The relationship between protection and MAb dose and affinity for the challenge virus. We examined several immune parameters of the MAbs to evaluate whether they had a relation to the MAb's ability to protect against zosteriform lesion formation. Initially, 100 μl of concentrated ascites fluid was administered intravenously in 200 μl of HBSS. Quantitation of the amount of gamma globulin in each MAb preparation with an ELISA (using a series of fivefold dilutions of each MAb as antigen and comparing the final A_{490} readings to a standard curve revealed that the initial MAb doses used to determine protection ranged from 0.2 to 7.2 mg (table 2). Additional protection experiments using several dilutions of the more concentrated ascites preparations showed no significant variation in the ability of MAbs B6, C3, C4, D7, and D8 to block zosteriform lesion formation when 0.2–0.3 mg was administered (table 3). Furthermore, MAb D8 was 75% protective at 0.01 mg, while MAb B6 was minimally protective (25%) at a dose of 0.01 mg. On the whole, although the total

Table 3. Protection against zosteriform spread by reduced quantities of monoclonal antibodies (MAbs).

	Dose, mg							
MAb	0.7	0.6	0.5	0.4	0.3	0.2	0.05	0.01
B6		100 (6)	100 (4)		100 (4)		25 (4)	25 (4)
C3			100 (4)			60 (5)		
C4			100 (4)			80 (5)		
D7	100 (4)		100 (4)		100 (6)			
D8			100 (6)	100 (4)		100 (4)	67 (3)	75 (4)
C3 $F(ab')_2$/Fab fragments			0 (4)					
C4 $F(ab')_2$/Fab fragments			0 (4)					

NOTE. Data are percentage protection (no. of mice tested): percentage of mice that did not band after challenge with herpes simplex virus type 1_{BK}. Controls (HBSS-treated) had a background protection of ~8%.

IgG content of each MAb initially administered varied, higher doses of immunoglobulin did not correlate with zosteriform protection, and the protective MAbs were equally effective at a lower dose of 0.2–0.3 mg.

Affinities were determined to assess whether the protective MAbs had a higher affinity for HSV-1_{BK}, used as a challenge virus in this study. Subjecting the points of each of the MAb HSV-1_{BK} binding curves to Scatchard analysis and using the linear portion of the Scatchard plots to determine binding constants (Table 2) indicated that all the MAbs reacted with HSV-1_{BK} with high affinity (ranging from 2.8×10^8 to 6.7×10^9 M^{-1}) and that the affinity of the MAbs for HSV-1_{BK} was not significantly related to their ability to protect against zosteriform spread. MAbs B6, D7, and D8, which gave the greatest protection against zosteriform spread, had the lowest binding affinities for HSV-1_{BK}. The three gC-specific MAbs had somewhat greater affinities for the challenge virus than did MAbs B6, D7, and D8 but were less protective. In addition, the nonprotective MAbs B3, B4, and B8 had higher affinities than did MAb B6.

The role of neutralization and ADCC in protection. To further examine the in vitro activities of the MAbs, each was serially diluted to the nanograms-per-milliliter range and added to neutralization and ADCC assays. As shown in table 4, each MAb could neutralize HSV-1_{BK}, and most could act in anti-HSV ADCC. Units were assigned to the amount of MAb necessary to achieve 50% neutralization and 50% of the maximum amount of ADCC mediated by that MAb (figure 1).

When the neutralization and ADCC units contained in the 0.2-mg doses of the protective MAbs was compared, a division into two general high- and low-activity groups was apparent. The protective MAbs B6, D2, D8 at 0.2 mg neutralized at $1–5 \times 10^4$ neutralization units. The other protective MAbs, C3, C4, and D7, neutralized about 10-fold less well ($1–4 \times 10^3$ neutralization units). Oddly, the nonprotective MAbs B3, B4, and B8, at their original concentrations, had relatively high neutralization values ($1–4 \times 10^4$ neutralization units). A greater division was found among the ADCC values of the protective MAbs at a dose of 0.2 mg: C3, C4, C8, D7, and D8, 2×10^5 to 2×10^6 ADCC units; B6 and D2, $\sim 4 \times 10^3$ ADCC units. Here, the nonprotective MAbs B3 and B4 had relatively low ADCC values at the original dose (<100 and 8.2×10^3 ADCC units, respectively). Nonprotective MAb B8 was unusual in that its 50% maximum ADCC activ-

Table 4. Monoclonal antibody (MAb) neutralization and antibody-dependent cellular cytotoxicity (ADCC) activity for herpes simplex virus type 1.

		Neutralization at			ADCC activity at	
MAb	1 neutralization unit (ng)	Original dose	0.2 mg	1 ADCC unit (ng)	Original dose	0.2 mg
B3	340	1.6×10^4		680	8.2×10^3	
B4	31	3.9×10^4		>10 μg	<100	
B6	18	6.7×10^4	1.1×10^4	55	2.2×10^4	3.7×10^3
B8	78	1.7×10^4		1.3	1.0×10^6	
C3	70	5.7×10^4	2.9×10^3	0.1	4.0×10^7	2.0×10^6
C4	51	9.4×10^4	3.9×10^3	1.0	4.8×10^8	2.0×10^5
C8	130	1.5×10^3	1.5×10^3	0.1	2.0×10^6	2.0×10^6
D2	6.3	4.8×10^4		75	4.0×10^3	
D7	94	7.7×10^4	2.1×10^3	0.1	7.2×10^7	2.0×10^6
D8	17	2.7×10^5	1.2×10^4	0.1	4.6×10^7	2.0×10^6

NOTE. Neutralization and ADCC activity are expressed in neutralization units (amount of MAb causing 50% plaque reduction) and ADCC units (amount of MAb causing 50% maximal ^{51}Cr release), respectively. Original doses are listed in table 2.

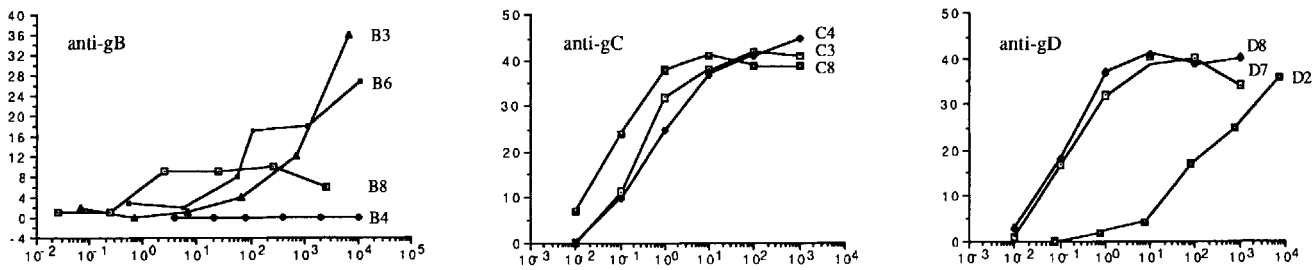

Figure 1. Monoclonal antibody-mediated antibody-dependent cellular cytotoxicity. The amount of ^{51}Cr label released from infected target cells was used to calculate percentage of specific lysis. B3, B4, B6, B8, C3, C4, C8, D2, D7, and D8 are monoclonal antibodies (see table 1). Ordinate, percentage of specific lysis; abscissa, monoclonal antibody (nanograms) per microtiter well.

ity occurred at a low dose (1.3 ng), whereas its maximum ADCC never exceeded 10% specific lysis (figure 1), unlike most other MAbs, which reached maximum specific lysis of 40%. Therefore, although B8 had a high ADCC activity at its original dose, the total ADCC mediated by B8 was less than that mediated by the other MAbs, and its ADCC value was not relevant to those of the other MAbs.

The potentially significant difference between 10^4 and 10^3 neutralization units was apparent when considering the protection mediated by MAbs B6 and D8 at doses of 0.05 and 0.01 mg (table 3). At a dose of 0.2–0.3 mg, both MAbs had total neutralization values $>10^4$. At 0.05 and 0.01 mg, neutralization was $\sim 3 \times 10^3$ and 6×10^2 neutralization units, respectively, and the protective capacity of both MAbs was compromised. MAb B6 showed the greatest loss of protection (from 100% to 25%), and the ability of D8 to protect (75%) at $\leq 10^3$ neutralization units may have been due to its ability to mediate ADCC at a 500-fold greater level than did B6: total ADCC values at 0.01 mg were 1×10^5 ADCC units for D8 and 2×10^2 ADCC units for B6. It is therefore possible that protection mediated solely by neutralizing ability required a minimum of 10^4 neutralization units. However, a dose of this magnitude was not necessarily sufficient in itself for protection, as MAbs B3, B4, and B8 were not protective at this level.

The potential importance of ADCC in protection was suggested by the divergent results of the low-dose transfers of B6 and D8 and was further supported by the protection mediated by the gC-specific MAbs at 0.2 mg, where the total neutralization activity per mouse was $<10^4$ neutralization units and total ADCC activity per mouse, $>10^5$ ADCC units. All mice that received MAbs active at $>10^5$ ADCC units were protected from zosteriform spread (except for B8). Of the mice that received $<10^4$ ADCC units in 0.2 mg of MAb, only those given B6 and D2 were protected (at 0.2 mg, both had activity at $>10^4$ neutralization units). Finally, when the Fc portion of MAbs C3 and C4 was removed by pepsin treatment, and 0.5 mg of the $F(ab')_2$ and Fab fragments was administered to mice before zosteriform challenge, no protection was seen (table 3).

Discussion

Gauging the ability of immune sera to block virus spread from the skin to the nervous system and vice versa is important for determining the ability of HSV-specific antibody to protect from ganglionic colonization and subsequent reactivation. To assess potential antibody-mediated protection from primary infection and spread via the nervous system, we used ten representative MAbs reactive with discrete epitopes of gB, gC, and gD and measured their ability to protect against zosteriform spread after local infection. All MAbs specific for gC and gD and one of the four gB-reactive MAbs (B6) protected against zosteriform lesion formation. Reactivation of HSV-1 did not occur from the ganglia innervating the site of infection in mice given the gD-specific MAbs or MAb B6, suggesting that the administration of these MAbs significantly limited the amount of virus that accumulated in the ganglia during acute infection. Passive transfer of the protective MAbs to infected mice after ganglionic colonization demonstrated that most were also effective in impeding the flow of virus from the infected ganglion to the skin (unpublished data).

Both neutralization and ADCC may be effective methods of antibody-mediated in vivo protection against HSV, since MAbs with high neutralization values (B6 and D8) and MAbs with high ADCC values and low neutralization values (C3, C4, C8, and D7) were protective. Although most protective MAbs mediated both neutralization and ADCC, three results served to differentiate between the two activities. First, all MAbs that mediated a high degree of ADCC were protective (C3, C4, C8, D7, and D8), whereas strongly neutralizing MAbs B3, B4, and B8, which did not mediate a high degree of ADCC, were not. Also, MAb D8 was protective when given at a dose with low neutralizing activity ($<10^4$ units) but high ADCC activity ($>10^4$ units), while MAb B6 was not protective when given at a dose with low neutralizing and ADCC activity. Finally, when the Fc portion, which is required for ADCC, was removed from MAbs C3 and C4 before transfer, no protection was conferred. Other reports of antibody-mediated immunity to HSV also have demonstrated protection by nonneutralizing MAbs [7, 12] and the requirement

of the Fc portion of immunoglobulin for protection [20, 21]. The in vivo relevance of ADCC has been demonstrated in HSV and tumor cell challenge studies in mice [22–24]. While neutralizing antibody may be important in limiting the spread of free virus, it may only function in vivo when present in high titer, and even then may do little to combat viral infection [25, 26]. Conversely, ADCC may be effective at low antibody concentration both in vitro and in vivo [19].

Additional mechanisms that may have contributed to the protective capacity of the MAbs include ADCMC and the inhibition of cell-to-cell spread of HSV-1 (documented for antibody acting alone or in conjunction with immune effector cells [11, 27]), which has been shown to be an activity of the protective MAbs B6, D2, D7, and D8. All MAbs used in this study mediated ADCMC against HSV-1–infected Vero cells in the presence of rabbit complement (unpublished data). Whether they could differentially activate mouse complement in vivo is unknown; however, several other investigators have found no role for in vivo ADCMC as a potential mechanism of MAb-mediated protection [7, 21, 28]. Additional undefined mechanisms may be operative in vivo, and since we have correlated in vivo protection with in vitro–defined antibody activity, the actual protective mechanism in vivo is uncertain.

The role of epitope recognition in antibody-mediated protection was initially suggested by reports of antibody ("sensitizing antibody") that interfered with the protective action of neutralizing antibody in vitro and in vivo [25, 26]. Reports of the inability of polyclonal antibody to protect against infection [29, 30] and recent accounts of epitope-specific MAb-mediated protection lend further support to the importance of epitope specificity in antibody-mediated protection. Our protection results indicated that the epitopes recognized by MAb B6 and all gC- and gD-specific MAbs were important targets for antibody-mediated immunity against HSV-1 and that MAb B6 and the gD-reactive MAbs were most effective in limiting the amount of virus that accumulated in the ganglia during acute infection. The region of gB important in this regard has been localized to the first 233 amino acids of the amino terminus of the glycoprotein [15]. MAb B6 reactivity was mapped to residue 85 within this linear antigenic site [31]. Conversely, gB-specific MAbs reactive to antigenic sites I (amino acid residues 381–441), II (residues 441–727), and III (residues 283–380) did not identify targets of protective antibody-mediated immunity in the zosteriform model, even though these MAbs reacted with the challenge virus with high avidity and had relatively high neutralizing ability in vitro.

Protection against zosteriform spread was achieved with the gC-specific MAbs reactive with antigenic sites I (amino acid residues 307–373) and II (residues 129–147) [32]. The antigenic sites (I, IX, and X) recognized by the protective gD-specific MAbs are incompletely defined [33]. MAb D2 recognized a discontinuous site [34] and its corresponding *mar*D2.1, a single-mutation site at amino acid residue 157 of the mature gD-1 protein, causing a glutamine-to-leucine substitution (unpublished data). The ability of all the gC- and gD-specific MAbs to protect against zosteriform spread indicates that several epitopes exist on these molecules that induce protective immunity, whereas antibody directed to three of the four antigenic sites of gB was ineffective in vivo.

Construction of synthetic immunogens based on epitopes identified by the protective MAbs may be complicated by the discontinuous nature of many of the sites. However, we have made synthetic peptides corresponding to the epitope recognized by MAb B6 and have generated HSV-1–neutralizing, protective antibody using one as an immunogen in mice (unpublished data). It would be of interest to further dissect the epitopes recognized by the gC- and gD-reactive MAbs and evaluate their protective potential as immunogens.

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