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Anti-Glycoprotein D Monoclonal Antibody Protects against Herpes Simplex Virus Type 1-Induced Diseases in Mice Functionally Depleted of Selected T-Cell Subsets or Asialo GM1$^+$ Cells

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Passive transfer of a monoclonal antibody (MAb) specific for glycoprotein D (gD) is highly effective in preventing the development of herpes simplex virus type 1-induced stromal keratitis. In the present study, we investigated whether animals which had been functionally depleted of T-cell subsets or asialo GM1$^+$ cells would continue to be responsive to MAb therapy. BALB/c mice were depleted of CD4$^+$, CD8$^+$, or asialo GM1$^+$ cells by treatment with anti-L3T4, anti-Lyt 2.2, or anti-asialo GM1 antibodies, respectively. Functional depletion of CD4$^+$ cells was documented by the loss of delayed-type hypersensitivity responsiveness, while CD8$^+$ cell depletion was accompanied by abrogation of cytotoxic lymphocyte activity. Anti-asialo GM1 treatment led to the loss of natural killer cell lytic activity. Mice depleted of the desired cell population and infected on the scarified cornea with herpes simplex virus type 1 uniformly developed necrotizing stromal keratitis by 3 weeks postinfection. A single inoculation of anti-gD MAb (55 μg) given intraperitoneally 24 h postinfection strongly protected hosts depleted of CD4$^+$ cells against stromal keratitis. Likewise, antibody treatment in CD8$^+$ or asialo GM1$^+$ cell-depleted hosts was as therapeutically effective as that seen in non-cell-depleted mice. We also observed that in cell-depleted mice, the virus spread into the central nervous system and caused encephalitis. The CD4$^+$ cell-depleted mice were the most severely affected, as 100% developed fatal disease. Anti-gD MAb treatment successfully protected all (32 of 32) CD4$^+$-, CD8$^+$-, or asialo GM1$^+$-depleted hosts against encephalitis. We therefore conclude that antibody-mediated prevention of stromal keratitis and encephalitis does not require the obligatory participation of CD4$^+$, CD8$^+$, or asialo GM1$^+$ cells. However, when mice were simultaneously depleted of both CD4$^+$ and CD8$^+$ T-cell subsets, antibody treatment could not prevent fatal encephalitis. Thus, antibody can compensate for the functional loss of one but not two T-lymphocyte subpopulations.

Infection of the cornea with herpes simplex virus (HSV) can induce disease ranging from mild epithelial ulcers to severe necrotizing stromal keratitis (4). Indeed, ocular infection with HSV is regarded as the most common cause of infectious blindness in industrialized countries (17). Murine models of herpetic stromal keratitis have been developed, and studies of the pathogenesis of the disease have been conducted with both euthymic and athymic hosts. The collective results strongly support the concept that corneal opacity is due, at least in part, to the host's T-cell immune response to virus infection (10, 19, 24, 29).

HSV type 1 (HSV-1)-induced stromal keratitis can be prevented by the timely administration of monoclonal antibodies (MAbs) specific for the major glycoproteins of the virus (15, 20). However, the mechanism by which passively transferred MAb protects against blinding corneal opacity is not known. It seems unlikely that protection is due solely to neutralization of cell-free virus or antibody-mediated lysis of virus-infected cells, because antibody treatment did not result in rapid virus clearance from the eye (15). In fact, it has been shown that MAbs lacking virus-neutralizing or antibody-dependent cellular cytotoxicity activities can be protective (28). Since HSV-sensitized T cells appear to be needed for the development of stromal keratitis (10, 24), we postulated that passive transfer of antibody protected against this disease because it prevented T-cell sensitization. However, subsequent investigation established that the generation of immune T cells to virus antigens was not suppressed (16). On the contrary, delayed-type hypersensitivity (DTH) responsiveness to HSV-1 antigens was accelerated.

In BALB/c mice, DTH to HSV antigen is mediated by the CD4$^+$ T-cell subset (24). One possibility was that the early appearance of these sensitized cells together with the antibody would favor efficient clearance of viral antigens from ocular tissue. As a consequence, corneal inflammation would be minimized. In the absence of antibody treatment, CD4$^+$ T-cell appearance would not be accelerated and the slower emergence of these cells together with persisting virus antigen could favor an immunopathogenic response, i.e., stromal keratitis.

Accordingly, the present study was undertaken to test the foregoing scenario. The specific question investigated was whether effective antibody therapy required the active participation of HSV-sensitized CD4$^+$ T cells. It is well known that additional types of lymphocytes, such as the CD8$^+$ T-cell subset and natural killer (NK) cells, can lyse HSV-infected cells (2, 36) and release antiviral lymphokines such as gamma interferon (13, 34). We therefore also investigated whether these two types of effector cells played a critical role in antibody-mediated host recovery.

The experimental approach was to selectively deplete mice of one of the above three cell types via the in vivo administration of antibody specific for the appropriate surface antigen and then assess whether cell depletion inter-

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fered with the MAb's capacity to prevent stromal keratitis. During the course of the experiments, it was observed that in cell-depleted hosts ocular infection was followed by migration of virus into the central nervous system (CNS). Therefore, it was also possible to determine whether lymphoid cell participation was required in order for antibody to protect against fatal encephalitis. Our results showed that regardless of which type of cell was depleted, MAb continued to protect against both ocular and CNS disease. Only when the host was depleted of both T-cell subsets did passively transferred antibody fail to prevent death.

MATERIALS AND METHODS

Animals. Four-week-old female BALB/c mice were obtained from Charles River Breeding Laboratories, Wilmington, Mass.

Virus. HSV-1 strain RE, a known stromal keratitis inducer (15, 19), was used. Virus stocks were grown and titrated on Vero cells as previously described (14).

Corneal infection. For corneal infection, mice were anesthetized with 0.2 ml of a 1:10 dilution of sodium pentobarbital (50 mg/ml of stock solution) and one eye of each mouse was scarified by three twists of a 2-mm corneal trephine. A 2-μl volume containing the desired concentration of HSV-1 strain RE was dropped onto the corneal surface and gently massaged into the eye with the eyelids. Eyes were examined weekly for 5 weeks in masked fashion by using a dissecting biomicroscope with a fiber optic light source. Corneal opacity was graded on a scale of 0 to +5 as described elsewhere (20); a score of 0 indicates a clear cornea, whereas a +5 score represents severe necrotizing stromal keratitis. Ocular opacity scores did not change significantly after day 21.

Antibodies. The anti-HSV glycoprotein D (gD) MAb 8D2 was prepared and purified as previously described (15). The GK 1.5 hybridoma was a generous gift from Frank Fitch (University of Chicago, Chicago, Ill.). This cell line secretes a rat immunoglobulin G2b (IgG2b) MAb reactive with the murine L3T4 antigen on the surface of $CD4^+$ cells. GK 1.5 blocks class II major histocompatibility complex antigen-specific functions (5, 35) such as helper or DTH functions. The 2.43 hybridoma was a generous gift from Robert Hendricks (University of Illinois College of Medicine, Chicago, Ill.). The 2.43 cell line secretes a rat IgG2b MAb reactive with the murine Lyt 2.2 antigen on the surface of $CD8^+$ cells. The 2.43 MAb inhibits T-cell-mediated cytolysis (30, 31). Rabbit anti-asialo GM1 antiserum (WAKO Chemicals, Dallas, Tex.) has been shown to reduce NK activity in vivo in BALB/c mice (8).

Lymphocyte depletion. Depletion of $CD4^+$ or $CD8^+$ T cells was performed by using the ascitic fluids described above. To deplete $CD4^+$ cells, 250 μl of anti-CD4 ascitic fluid was given intraperitoneally (i.p.) 2 days before ocular infection. To deplete $CD8^+$ cells, 100 μl of anti-CD8 ascitic fluid was given i.p. 1 day before ocular infection and 1, 7, and 14 days postinfection. The effectiveness of depletion was assessed by testing for removal of functions mediated by the specific cell subsets (DTH or cell-mediated cytolysis of virus-infected cells) and by fluorescence-activated cell-sorting (FACS) analysis. Anti-asialo GM1 was diluted 1:8 in phosphate-buffered saline (PBS), and mice were given 200 μl i.p. 2 days before infection and 1 and 4 days postinfection. The effectiveness of anti-asialo GM1 treatment was determined by testing for a reduction in spleen cell NK activity.

DTH assay. DTH responsiveness was measured by using the ear swelling assay as previously described (14), except that the HSV-1 test antigen was prepared by diluting HSV-1(RE) to a concentration of 10^8 PFU/ml before UV irradiation.

CTL assay. An in vitro cytotoxic T-lymphocyte (CTL) assay was used to determine the lytic activity of cervical lymph node cells. CTL effector cells were generated by removing the cervical draining lymph node 6 days after corneal infection and preparing a single cell suspension in RPMI 1640 medium. Cells were adjusted to a concentration of 2×10^6 viable cells per ml and placed in Iscove's modified Dulbecco's medium supplemented with 10% fetal bovine serum, 10% Nu-Serum IV (Collaborative Research, Inc., Lexington, Mass.), 7×10^{-5} M alpha-thioglycerol, 6 mM glutamine, 100 U of penicillin, 100 μg of streptomycin, and 0.25 μg of amphotericin B (Fungizone; Flow Laboratories, McLean, Va.) per ml. Cells were incubated at 37°C in 5% CO_2 in the presence of UV-irradiated HSV-1(RE) (multiplicity of infection of 0.5) for 3 days and then used as effector cells in a 4-h chromium release assay. BALB/c mammary adenocarcinoma EMT.6 cells (a generous gift from Barry Rouse, University of Tennessee) were grown in Dulbecco's medium supplemented with 5% fetal bovine serum, 5% Nu-Serum IV, 1.8×10^{-2} M $NaHCO_3$, 1.0×10^{-2} M HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid), and antibiotics. A single cell suspension of EMT.6 cells (10^6 cells per ml) was incubated in the presence of 200 μCi of $Na_2{}^{51}CrO_4$ and HSV-1(RE) (multiplicity of infection of 10) for 4 h, washed, and then used as target cells in the chromium release assay. Uninfected EMT.6 cells served as the control. BALB/c effector cells (5×10^5) were added in 100-μl portions to wells of a 96-well plate containing 100 μl of infected or uninfected target cells to give an effector-to-target cell ratio of 50. Spontaneous release was determined by the addition of 100 μl of medium to 100 μl of target cells. Maximum release was determined by the addition of 100 μl of 5% sodium dodecyl sulfate to 100 μl of target cells. The plate was centrifuged for 2 min at $150 \times g$ and incubated at 37°C in 5% CO_2 for 4 h. After incubation, the plate was centrifuged for 5 min at $150 \times g$, and the radioactivity in 100 μl of supernatant from each well was counted in a Beckman gamma counter. Percent specific lysis was calculated as follows: [(experimental release − spontaneous release)/(maximum release − spontaneous release)] × 100. Spontaneous release was always less than 15% of maximum release.

NK assay. A chromium release assay was used to measure NK activity in spleen cells from HSV-infected mice treated or not treated with anti-asialo serum. Spleens were removed from mice and teased apart with sterile needles. The teased tissues were transferred to a conical centrifuge tube, and large pieces of tissue were allowed to settle; the single cells were then transferred to a conical centrifuge tube and centrifuged. The erythrocytes in the pellet were lysed by hypotonic lysis. The remaining cells were adjusted to the appropriate concentration (5×10^6 cells per ml) and used as effector cells in the NK assay; 100 μl of effector cells was added to the same volume of ^{51}Cr-labeled YAC-1 target cells (10^5 cells per ml). The remainder of the assay was performed as for the CTL assay (see above). Spontaneous release was always less than 22% of maximum release.

FACS. The efficiency of T-cell subset depletion was monitored by FACS analysis 6 days postinfection. Individual spleens from antibody-treated and control mice were excised and teased into single cell suspensions. Erythrocytes were removed by lysis with 0.83% ammonium chloride. The cells were washed in FACS diluent (PBS with 0.1% sodium azide

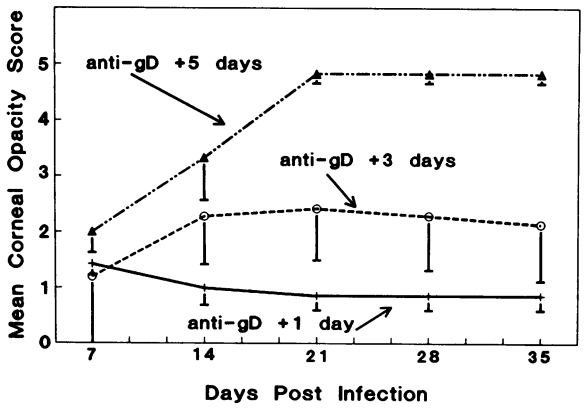

FIG. 1. Influence of time of administration on effective antibody therapy. BALB/c mice were infected on the cornea with 2×10^4 PFU of HSV-1 and given a single inoculation of anti-gD MAb (55 μg) i.p. on day 1, 3, or 5 postinfection. The mean ocular opacity score of the IgG2a-treated controls was 4.0 ± 0.6 on day 21. There were six to seven mice per group.

and 2.0% fetal bovine serum), adjusted to the appropriate concentration, and incubated with a 1:20 dilution of either anti-CD4 or anti-CD8 ascites fluid in FACS diluent for 20 min at 4°C. After being washed twice with FACS diluent, the cells were incubated with fluorescein-conjugated goat anti-rat antibody (TAGO Inc., Burlingame, Calif.) for 20 min at 4°C. The cells were again washed twice and analyzed with a FACS 440 (Becton Dickinson).

RESULTS

Effect of anti-gD MAb given 1, 3, or 5 days postinfection. We investigated when after virus infection anti-gD MAb must be given in order to obtain a significant therapeutic effect. The results are shown in Fig. 1. It can be seen that antibody given 24 h after corneal infection was highly effective ($P < 0.025$) at preventing stromal keratitis. This result is in agreement with previous studies (15, 20). When antibody treatment was delayed until day 3 postinfection, the results were variable within the treated group. The eyes of four mice were protected, but three others developed blinding disease. Anti-gD MAb given on day 5 was not protective for any of the recipients. These data show that under the conditions tested, immunoglobulin must be administered <3 days after infection for optimum therapeutic effectiveness. They also indicate that if effector cell collaboration with antibody is necessary to prevent corneal opacity, such collaboration must begin shortly after the anti-gD MAb is given.

Effect of anti-CD4 treatment on DTH responsiveness to HSV. BALB/c mice infected on the cornea with HSV-1 develop sensitized $CD4^+$ T cells which can mediate DTH (24) (Fig. 2). The inflammatory response was significantly enhanced ($P < 0.01$) when the animals had also been treated with anti-gD MAb. These results confirm our earlier report that passively transferred antibody to gD accelerates host sensitization (16). Figure 2 shows that a single inoculation of anti-CD4 MAb reduced DTH responsiveness in both antibody-treated and untreated HSV-1-sensitized hosts to background levels, i.e., to levels of nonspecific swelling seen in the ears of naive hosts. FACS analysis performed 6 days postinfection showed that $CD4^+$ cells in the spleen were reduced by >95%. This alternative assay provided independent confirmation of the effectiveness of anti-CD4 treatment. The anti-CD4 treatment was selective in that the specific lysis of HSV-1-infected target cells (43%) by $CD8^+$ cells was not abrogated. $CD4^+$ cell depletion was transient in that DTH responsiveness was restored by day 18 postinfection (data not shown).

Effect of $CD4^+$ cell depletion on the therapeutic effectiveness of anti-gD MAb. We next tested whether anti-gD treatment would protect against stromal keratitis in mice functionally depleted of $CD4^+$ cells. Figure 3 shows that untreated $CD4^+$-depleted hosts uniformly developed necrotizing stromal keratitis. Furthermore, the virus spread into the CNS and produced fatal encephalitis in all six recipients by day 18 (Table 1). However, none of the MAb-treated mice developed severe corneal opacity or CNS disease (Fig. 3). Thus, $CD4^+$ T-cell participation was not required in order for the antibody to be protective.

Effect of anti-CD8 treatment on HSV-specific CTL response. It was possible that T cells of the $CD8^+$ phenotype might play a role in antibody-mediated protection. To test

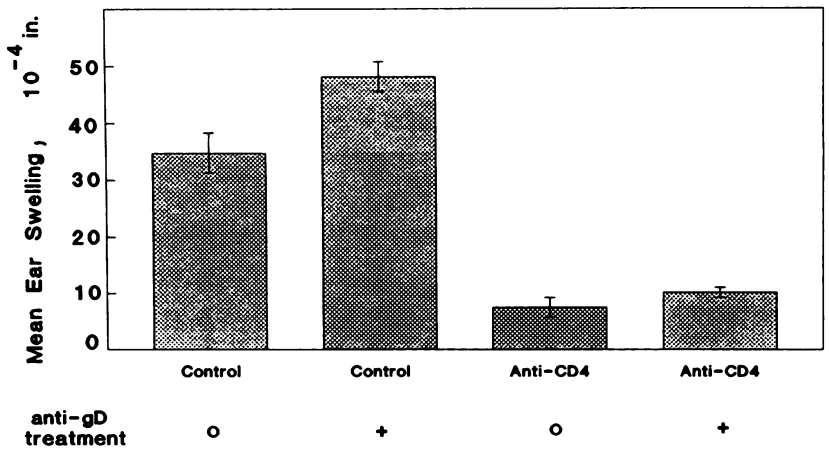

FIG. 2. Effect of anti-CD4 treatment on DTH responsiveness to HSV-1. Control and $CD4^+$ cell-depleted mice were infected on the cornea with 8×10^4 PFU of HSV-1. A portion of the animals were given anti-gD MAb (55 μg) i.p. 24 h later. On day 6 postinfection, DTH testing was done. There were six mice per group.

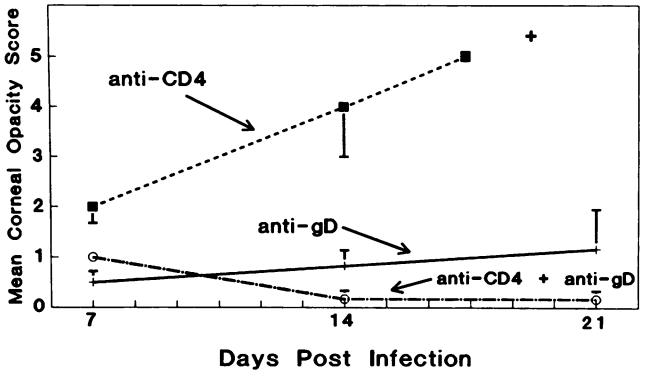

FIG. 3. Effect of CD4$^+$ cell depletion on the capacity of anti-gD MAb to protect against HSV-1-induced stromal keratitis. Anti-CD4-treated and untreated mice were infected on the cornea with HSV-1. Twenty-four hours later, the mice were given anti-gD MAb or IgG2a i.p. and then monitored for development of corneal opacity. There were six mice per group. CD4$^+$ cell-depleted hosts given anti-gD MAb were significantly protected against the development of stromal keratitis ($P < 0.05$).

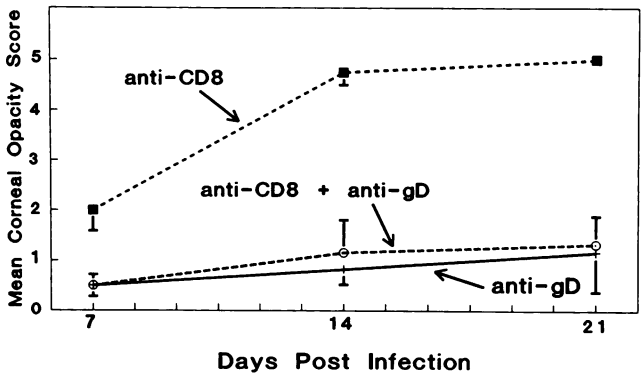

FIG. 4. Effect of CD8$^+$ cell depletion on the capacity of anti-gD MAb to protect against HSV-1-induced stromal keratitis. Anti-CD8-treated and untreated mice were infected on the cornea with 2×10^4 PFU of HSV-1. Mice were given anti-gD MAb or IgG2a i.p. 24 h later and then monitored for the development of corneal opacity. There were six mice in each group. CD8$^+$ cell-depleted hosts given anti-gD MAb were significantly protected against stromal keratitis ($P < 0.005$).

this premise, mice were depleted of this T-cell subset via anti-CD8 treatment. In vivo treatment with anti-CD8 MAb completely abrogated the subsequent generation of CTL activity in cervical lymph node cells taken from HSV-infected hosts 6 days postinfection. Specifically, cervical lymph node cells from control mice lysed HSV-infected target cells with a specific lysis of 18.0 ± 0.04%, while anti-CD8-treated mice had a specific lysis of −1.3 ± 0.4%. There was no lysis of uninfected target cells by cervical lymph node cells from either group. However, anti-CD8-treated hosts still could mount an HSV-specific DTH response ($34.2 \pm 1.7 \times 10^{-4}$ in. [1 in. = 2.54 cm]). FACS analysis indicated that the number of CD8$^+$ cells in the spleen was reduced by 85% and that this level of depletion persisted through day 21 postinfection (data not shown).

Effect of CD8$^+$ T-cell depletion on the therapeutic effectiveness of anti-gD MAb. Mice functionally depleted of CD8$^+$ T cells were infected on the cornea with HSV-1 and given anti-gD MAb 24 h later. Figure 4 shows that anti-CD8 treatment did not impair the antibody's protective effects, as the opacity scores were not significantly different from those for the T-cell-intact, antibody-treated animals. It is also evident that CD8$^+$ cell depletion did not interfere with the development of stromal keratitis in the untreated hosts. Two of six CD8$^+$-deficient mice died of CNS disease, whereas none of the anti-gD-treated hosts developed encephalitis (Table 1). These results indicated that the CD8$^+$ T-cell subset was not required in order for the antibody to protect against corneal disease or encephalitis.

TABLE 1. Incidence of encephalitis in HSV-1-infected BALB/c mice depleted of CD4$^+$, CD8$^+$, or asialo GM1$^+$ cells and treated with anti-gD MAb[a]

Group	Cell depletion	Anti-gD treatment	No. of deaths/total no. of mice in groups (%)
A	None	No	2/32 (6)
B	None	Yes	0/34 (0)
C	CD4$^+$	No	6/6 (100)[b]
D	CD4$^+$	Yes	0/6 (0)[c]
E	CD8$^+$	No	2/6 (33)[d]
F	CD8$^+$	Yes	0/18 (0)[e]
G	CD4$^+$ and CD8$^+$	No	8/8 (100)
H	CD4$^+$ and CD8$^+$	Yes	8/8 (100)
I	Asialo GM1$^+$	No	4/8 (50)[f]
J	Asialo GM1$^+$	Yes	0/8 (0)[g]

[a] Results 21 days post-HSV-1 corneal infection.
[b] Mortality significantly higher than that of group A, $P = 0.001$.
[c] Incidence of survival greater than that of group C, $P = 0.001$.
[d] Mortality significantly higher than that of group A, $P = 0.05$.
[e] Incidence of survival greater than that of group E, $P = 0.01$.
[f] Mortality significantly higher than that of group A, $P = 0.002$.
[g] Incidence of survival greater than that of group I, $P = 0.02$.

Loss of anti-gD MAb protective effect in mice depleted of both CD4$^+$ and CD8$^+$ T cells. Ocular infection of mice simultaneously depleted of CD8$^+$ and CD4$^+$ cells resulted in the deaths of all animals due to encephalitis (Table 1). Passively transferred anti-gD MAb did not prevent death (Table 1), although it did significantly ($P < 0.001$) increase the mean survival time from 9 to 19 days. The mean ocular opacity score in anti-gD-treated, T-cell-depleted mice (1.33 ± 0.56) was significantly lower ($P < 0.005$) than that seen in T-cell-intact control mice (4.5 ± 0.22) at 18 days postinfection. However, since all untreated T-cell-depleted controls had died, it was not possible to determine whether the reduced ocular disease in the T-cell-depleted mice was due to the lack of CD4$^+$ cells, to the effect of the anti-gD MAb, or to a combination of both.

Effect of asialo GM1$^+$ cell depletion on the therapeutic effectiveness of anti-gD MAb. Rabbit anti-asialo GM1 serum was used to selectively deplete mice of those cells, including NK cells, which express this antigen. HSV-1-infected mice given this antiserum had no NK lytic activity in their spleen cells 7 days postinfection, whereas the lytic activity in the spleen cells of untreated infected animals was 8% ($P < 0.04$). Additional evidence that functional depletion had occurred was provided by the finding that the GM1$^+$ cell-depleted hosts exhibited increased susceptibility to HSV-1 corneal infection. Four of eight mice died with encephalitis by day 10 (Table 1), and all surviving animals developed necrotizing stromal keratitis. We found that anti-gD MAb treatment in GM1$^+$-depleted hosts was as effective as that seen in antibody-treated GM1$^+$ cell-intact animals (Fig. 5 and Table 1). These results indicated that anti-gD MAb did not need to

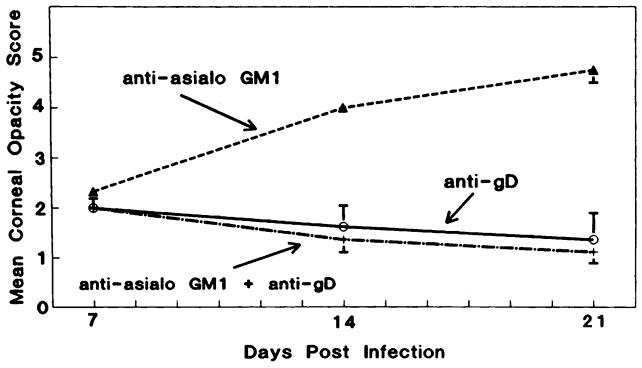

FIG. 5. Effect of anti-asialo GM1 treatment on the capacity of anti-gD MAb to protect against HSV-1-induced stromal keratitis. Anti-asialo GM1-treated and untreated mice were infected with 5×10^4 PFU of HSV-1. Mice were given anti-gD MAb or IgG2a i.p. 24 h later and then monitored for the development of corneal opacity. There were eight mice in each group. Asialo $GM1^+$ cell-depleted hosts given anti-gD MAb were significantly protected against stromal keratitis ($P < 0.005$).

collaborate with asialo $GM1^+$ cells in order to protect against stromal keratitis or CNS disease.

DISCUSSION

Passively transferred MAbs to the major glycoproteins of HSV-1 are remarkably effective at preventing virus-induced stromal keratitis (15, 20). The present study was designed to probe the protective mechanism(s). Specifically, we investigated whether the participation of selected lymphocyte subsets was required in order for the antibody to exert its protective effect. We show here that functional depletion of $CD4^+$, $CD8^+$, or asialo $GM1^+$ cells did not abrogate anti-gD therapy. In fact, antibody treatment in the cell-depleted mice prevented stromal keratitis just as efficiently as in hosts with an intact lymphocyte population. Furthermore, the capacity for antibody to prevent CNS disease also continued unabated in lymphocyte-subset-depleted hosts.

In conducting this work, it was important to establish that functional depletion of the desired lymphocyte population had been achieved. Evidence for $CD4^+$ cell depletion was provided by the demonstration that DTH responsiveness to HSV-1 antigen was abrogated during the first week postinfection, i.e., the time during which putative T-cell–MAb collaboration would be expected to occur (Fig. 1). Since stromal keratitis development in the BALB/c mouse appears to require $CD4^+$ cells (24), it was also important that depletion be transient in nature. Accordingly, only a single treatment with anti-L3T4 was used. Indeed, it was found that $CD4^+$ cell-depleted hosts which survived to day 14 postinfection did develop necrotizing stromal keratitis and that positive DTH tests could be elicited (unpublished observations). Thus, we can conclude that the absence of stromal disease in the depleted mice was due to antibody-mediated protection and not because there were insufficient $CD4^+$ T cells to induce inflammation.

Although transient depletion of $CD4^+$ T cells did not abrogate the development of herpes stromal keratitis, it greatly increased host susceptibility to CNS disease following HSV-1 corneal infection. None of the $CD4^+$ cell-depleted mice survived to 3 weeks postinfection, whereas 94% of T-cell-intact mice survived (Table 1). This result, which provides further evidence of functional $CD4^+$ T-cell depletion, supports the view that $CD4^+$ cells play a critical role in CNS resistance to HSV-1 infection (22, 23, 25). Curiously, in certain other studies (6, 24), the selective depletion of $CD4^+$ cells did not result in increased host susceptibility to CNS disease. The reason for the variable results is not known.

Prolonged depletion of $CD8^+$ cells, the other major T-lymphocyte subset, also failed to interfere with the capacity of anti-gD MAb to prevent stromal keratitis or encephalitis. Evidence for effective functional depletion of these T cells was provided by the finding of increased host susceptibility to CNS disease as well as the abrogation of CTL activity in vitro. The role that $CD8^+$ T cells play in herpes stromal keratitis has been controversial. These cells have been reported to be protective in BALB/c mice infected with the RE strain of HSV-1, as their selective depletion resulted in increased severity of ocular disease (24). In contrast, this T-cell subset was found to contribute to the immunopathological process in the eyes of A/J mice infected with HSV-1 strain KOS (10). Recent studies indicate that the role which this T-cell subset plays in stromal disease is apparently influenced by the strain of virus used for infection (9, 11). We found that vigorous inflammatory reactions occurred in untreated, cell-depleted mice. This supports the view that $CD8^+$ cells are not needed for the development of corneal opacity after HSV-1(RE) infection.

Asialo GM1, a glycosphingolipid, is a component of the NK cell membrane. Although this antigen is not entirely restricted to this lymphocyte subset, it is these cells which appear to be primarily depleted by anti-asialo GM1 treatment (32, 34). In the present study, we found that NK cell-depleted hosts developed necrotizing stromal keratitis and became significantly more susceptible to CNS disease following HSV-1 corneal challenge. The former observation suggests that NK cells are not required for the development of ocular inflammation. The latter results agree with the view that asialo $GM1^+$ cells contribute to host resistance to HSV-1 infection, a concept for which there has been both support (7, 18, 27) and disagreement (1, 3, 33). Administration of anti-gD MAb to asialo $GM1^+$ cell-depleted hosts proved to be strikingly effective in that both stromal and CNS disease were prevented. Thus, analogous to the T-cell depletion results, functional asialo $GM1^+$ cell depletion does not compromise effective anti-HSV serotherapy.

Our study does not rule out the possibility that cells other than those tested may participate in the protective mechanism of anti-gD MAb. However, the present results do not provide support for the hypothesis that antibody and T cells or NK cells collaborate to prevent blinding stromal disease. It is of special interest that little or no histological (unpublished observations) or clinical evidence of inflammation was noted in the cornea despite the fact that vigorous DTH responses to HSV antigens could be elicited systemically. Collectively, our observations raise the intriguing prospect that antibody may prevent stromal keratitis by somehow disrupting the early signals that trigger ocular inflammation. Such a hypothesis would be compatible with the narrow therapeutic window observed for antibody treatment. Efforts are under way to investigate this premise.

We also investigated the effect depletion of both T-cell subsets would have on antibody-mediated protection. It was found that mice depleted of both $CD4^+$ and $CD8^+$ T cells displayed greatly increased vulnerability to HSV-1-induced encephalitis, so much so that none survived longer than 11 days postinfection. Under these conditions, antibody therapy delayed, but did not prevent, death. Six of the anti-gD-

treated dual T-cell-depleted animals survived to day 18. Of these, four had no or very minimal corneal haze. Unfortunately, since all of the untreated infected controls died by day 11, i.e., before stromal keratitis had time to become clearly evident, we are uncertain as to whether the dual T-cell-depleted hosts would develop classical stromal disease. Therefore, the question of whether anti-gD treatment could prevent stromal keratitis in $CD4^+$ plus $CD8^+$ cell-depleted hosts remains unknown.

However, it is quite clear that antibody requires at least some T-cell help to prevent HSV-1-induced encephalitis. Our results confirm and extend earlier studies which showed that passively administered anti-HSV polyclonal antibody is not protective in mice rendered grossly deficient in T cells by artificial means or as a consequence of lymphoid deficiency disease (21, 25). Since anti-gD MAb protects in the absence of $CD4^+$ or $CD8^+$ cells but not both, this suggests that in our model, $CD4^+$ cells can compensate for the loss of $CD8^+$ cells, and vice versa. Evidence that these two T-cell subsets can function independently of each other has also been observed in responses to lymphocytic choriomeningitis virus infection (12).

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REFERENCES

1. **Bukowski, J. F., and R. M. Welsh.** 1986. The role of natural killer cells and interferon in resistance to acute infection of mice with herpes simplex virus type 1. J. Immunol. **136**:3481–3485.
2. **Ching, C., and C. Lopez.** 1979. Natural killing of herpes simplex virus type 1-infected target cells: normal human responses and influence of antiviral antibody. Infect. Immun. **26**:49–56.
3. **Chmielarczyk, W., H. Engler, R. Ernst, U. Opitz, and H. Kirchner.** 1985. Injection of anti-thy 1.2 serum breaks genetic resistance of mice against herpes simplex virus. J. Gen. Virol. **66**:1087–1094.
4. **Dawson, C. R., and B. Togni.** 1976. Herpes simplex eye infections: clinical manifestations, pathogenesis and management. Surv. Ophthalmol. **21**:121–130.
5. **Dialynas, D. P., D. B. Wilde, P. Marrack, A. Pierres, K. A. Wall, W. Havran, G. Otten, M. R. Loken, M. Pierres, and F. W. Fitch.** 1983. Characterization of the murine antigenic determinant, designated L3T4a, recognized by monoclonal antibody GK1.5: expression of L3T4a by functional T cell clones appears to correlate primarily with class II MHC antigen-reactivity. Immunol. Rev. **74**:29–56.
6. **Erlich, K. S., D. Wofsy, R. D. Dix, and J. Mills.** 1989. Effects of selective depletion of $L3T4^+$ T-lymphocytes on herpes simplex virus encephalitis. Clin. Immunol. Immunopathol. **52**:190–201.
7. **Habu, S., K. I. Akamatsu, N. Tamaoki, and K. Okumura.** 1984. *In vivo* significance of NK cell on resistance against virus (HSV-1) infection in mice. J. Immunol. **133**:2743–2747.
8. **Habu, S., H. Fukui, K. Shimamura, M. Kasai, Y. Nagai, K. Okumura, and N. Tamaoki.** 1981. *In vivo* effects of anti-asialo GM1. I. Reduction of NK activity and enhancement of transplanted tumor growth in nude mice. J. Immunol. **127**:34–38.
9. **Hendricks, R. L.** 1991. Role of lymphokines in HSV-1 corneal stromal disease. Invest. Ophthalmol. Visual Sci. **32**:854.
10. **Hendricks, R. L., M. S. P. Tao, and J. C. Glorioso.** 1989. Alterations in the antigenic structure of two major HSV-1 glycoproteins, gC and gB, influence immune regulation and susceptibility to murine herpes keratitis. J. Immunol. **142**:263–269.
11. **Hendricks, R. L., and T. M. Tumpey.** 1990. Contribution of virus and immune factors to herpes simplex virus type 1-induced corneal pathology. Invest. Ophthalmol. Visual Sci. **31**: 1929–1939.
12. **Kasian, M. T., K. A. Leite-Morris, and C. A. Biron.** 1991. The role of $CD4^+$ cells in sustaining lymphocyte proliferation during lymphocytic choriomeningitis virus infection. J. Immunol. **146**: 1955–1963.
13. **Kaufmann, S. H. E., E. Hug, and G. de Libero.** 1986. *Listeria monocytogenes*—reactive T lymphocyte clones with cytolytic activity against infected target cells. J. Exp. Med. **164**:363–368.
14. **Lausch, R. N., W. R. Kleinschrodt, C. Monteiro, S. G. Kayes, and J. E. Oakes.** 1985. Resolution of HSV corneal infection in the absence of delayed-type hypersensitivity. Invest. Ophthalmol. Visual Sci. **26**:1509–1515.
15. **Lausch, R. N., J. E. Oakes, J. F. Metcalf, J. M. Scimeca, L. A. Smith, and S. M. Robertson.** 1989. Quantitation of purified monoclonal antibody needed to prevent HSV-1 induced stromal keratitis in mice. Curr. Eye Res. **8**:499–506.
16. **Lausch, R. N., H. Staats, J. F. Metcalf, and J. E. Oakes.** 1990. Effective antibody therapy in herpes simplex virus ocular infection: characterization of recipient immune response. Intervirology **31**:159–165.
17. **Liesegang, T. J., L. J. Melton, P. J. Daly, and D. M. Ilstrup.** 1989. Epidemiology of ocular herpes simplex. Incidence in Rochester, Minn., 1950 through 1982. Arch. Ophthalmol. **107**: 1155–1159.
18. **Lopez, C., R. Ryshke, and M. Bennet.** 1980. Marrow-dependent cells depleted by ^{89}Sr mediate genetic resistance to herpes simplex type 1 infection in mice. Infect. Immun. **28**:1028–1032.
19. **Metcalf, J. F., D. S. Hamilton, and R. W. Reichert.** 1979. Herpetic keratitis in athymic (nude) mice. Infect. Immun. **26**:1164–1171.
20. **Metcalf, J. F., J. Koga, S. Chatterjee, and R. J. Whitley.** 1987. Passive immunization with monoclonal antibodies against herpes simplex virus glycoproteins protects mice against herpes ocular disease. Curr. Eye Res. **6**:173–177.
21. **Minagawa, H., S. Sakuma, S. Mohri, R. Mori, and T. Watanabe.** 1988. Herpes simplex virus type 1 infection in mice with severe combined immunodeficiency (SCID). Arch. Virol. **103**:73–82.
22. **Nagafuchi, S., I. Hayashida, K. Higa, T. Wada, and R. Mori.** 1982. Role of Lyt-1 positive immune T cells in recovery from herpes simplex virus infection in mice. Microbiol. Immunol. **26**:359–362.
23. **Nash, A. A., and P. G. H. Gell.** 1983. Membrane phenotype of murine effector and suppressor T cells involved in delayed hypersensitivity and protective immunity to herpes simplex virus. Cell. Immunol. **75**:348–355.
24. **Newell, C. K., S. Martin, D. Sendele, C. M. Mercadal, and B. T. Rouse.** 1989. Herpes simplex virus-induced stromal keratitis: role of T-lymphocyte subsets in immunopathology. J. Virol. **63**:769–775.
25. **Oakes, J. E., W. B. Davis, J. A. Taylor, and W. A. Weppner.** 1980. Lymphocyte reactivity contributes to protection conferred by specific antibody passively transferred to herpes simplex virus-infected mice. Infect. Immun. **29**:642–649.
26. **Oakes, J. E., J. T. Rector, and R. N. Lausch.** 1984. $Lyt\text{-}1^+$ T cells participate in recovery from ocular herpes simplex virus type 1 infection. Invest. Ophthalmol. Visual Sci. **25**:188–194.
27. **Rager-Zisman, B., P. C. Quan, M. Rosner, J. R. Moller, and B. R. Bloom.** 1987. Role of NK cell in protection against herpes simplex virus-1 infection. J. Immunol. **138**:884–888.
28. **Rector, J. T., R. N. Lausch, and J. E. Oakes.** 1982. Use of monoclonal antibodies for analysis of antibody-dependent immunity to ocular herpes simplex virus type 1 infection. Infect. Immun. **38**:168–174.
29. **Russell, R. G., M. P. Nasisse, H. S. Larsen, and B. T. Rouse.** 1984. Role of T-lymphocyte in the pathogenesis of herpetic stromal keratitis. Invest. Ophthalmol. Visual Sci. **25**:938–944.
30. **Sarmiento, M., D. P. Dialynas, D. W. Lancki, K. A. Wall, M. I. Lorber, M. R. Loken, and F. W. Fitch.** 1982. Cloned T lymphocytes and monoclonal antibodies as probes for cell surface molecules active in T cell-mediated cytolysis. Immunol. Rev. **68**:135–169.
31. **Sarmiento, M., A. L. Glasebrook, and F. W. Fitch.** 1980. IgG or

IgM monoclonal antibodies reactive with different determinants on the molecular complex bearing Lyt2 antigen block T cell-mediated cytolysis in the absence of complement. J. Immunol. **125**:2665–2672.

32. **Stein-Streilein, J., and J. Guffee.** 1986. *In vivo* treatment of mice and hamsters with antibodies to asialo GM1 increases morbidity and mortality to pulmonary influenza infection. J. Immunol. **136**:1435–1441.
33. **Tamesis, R. R., and C. S. Foster.** 1990. Natural killer cellular cytotoxicity against herpes simplex virus-infected cells in Igh-1-disparate mice. Invest. Ophthalmol. Visual Sci. **31**:2224–2229.
34. **Trinchieri, G.** 1989. Biology of natural killer cells. Adv. Immunol. **47**:187–376.
35. **Wilde, D. B., P. Marrack, J. Kappler, D. P. Dialynas, and F. W. Fitch.** 1983. Evidence implicating L3T4a in class II MHC antigen reactivity: monoclonal antibody GK 1.5 (anti-L3T4) blocks class II MHC antigen-specific proliferation, release of lymphokines, and binding by cloned murine helper T lymphocyte lines. J. Immunol. **131**:2178–2183.
36. **Yasukawa, M., and Y. Kobayashi.** 1985. Inhibition of herpes simplex virus replication *in vitro* by human cytotoxic T cell clones and natural killer cell clones. J. Gen. Virol. **66**:2225–2229.