Anti-glycoprotein B monoclonal antibody protects T cell-depleted mice against herpes simplex virus infection by inhibition of virus replication at the inoculated mucous membranes

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A monoclonal antibody (MAb 2c) specific for glycoprotein B of herpes simplex virus (HSV) mediated clearance of the virus from the infected mucous membranes. Young adult C57BL/6J mice were inoculated intravaginally with HSV type 1 and injected intraperitoneally either 24 and 72 h or 65 and 265 h post-inoculation with a polyclonal immune serum or the MAb 2c, both adjusted to the same neutralizing capacity. Immunization with the polyclonal immune serum did not alter the duration of virus shedding from the genital mucous membranes although a lethal outcome of infection was clearly prevented. Immunization with the MAb, however, resulted in a rapid clearance of the virus from the genital tract thus completely inhibiting genital inflammation and lethality. The same effects were achieved in mice depleted in vivo of CD4+ T cells although peripheral virus replication continued longer in these mice. In mice depleted of both CD4+ and CD8+ T cells the polyclonal immune serum was no longer able to protect against lethality, and virus replication in the mucous membranes persisted until the mice died. In contrast, after treatment with the MAb peripheral infection was quickly eliminated and all mice survived. These findings indicate that clearance of the virus from the primary site of replication can be mediated by humoral immunity. The relevance of this observation for vaccination against HSV is discussed.

Introduction

It is well established that administration of antiviral antibodies protect mice from a lethal herpes simplex virus (HSV) infection, whether infection occurred subcutaneously, intraperitoneally or into the footpad (Baron et al., 1976; Oakes & Rosemond-Hornbeak, 1978; Worthington et al., 1980; Dix et al., 1981; Balachandran et al., 1982). Lethality is also prevented if the virus is inoculated on non-injured mucous membranes (Eis & Schneweis, 1986). There are several results indicating that protection is achieved by reducing the amount of infectious virus during the acute phase of ganglionic infection resulting in a decreased number of infected ganglia and rapid development of latency (McKendall et al., 1979; Sekizawa et al., 1980; Erlich et al., 1987; Schneweis et al., 1988). Thus, the spread of virus from the peripheral site of infection to the central nervous system is interrupted (Kapoor et al., 1982a; Metcalf et al., 1987). However, virus multiplication at the inoculation site is not reduced, even after administration of large amounts of antibodies (Kapoor et al., 1982b; Eis & Schneweis, 1986; Shimeld et al., 1990). Clearance of the virus from the primary site of replication is the result of cell-mediated immunity, as demonstrated by adoptive transfer experiments and studies with mice depleted of T cells by anti-T cell monoclonal antibodies (MAbs) (Nash et al., 1980, 1987; Kapoor et al., 1982b; Eis & Schneweis 1986; Bonneau & Jennings, 1989, 1990). From these findings it seems that HSV infection in epithelial cells expressing high levels of major histocompatibility complex (MHC) class I antigens is controlled by T cell-mediated immunity, whereas infection in neuronal cells not expressing MHC antigens (Banks & Rouse, 1992) is cleared by humoral immunity.

Previously, we described a MAb, designated 2c, specific for HSV glycoprotein B, which exhibits a different mechanism of protection from that induced by polyclonal immune sera. Transfer of this antibody to immunocompetent mice 24 and 72 h after viral inoculation significantly reduced virus multiplication in mucous membranes of the genital tract (Eis-Hüibinger et al., 1991). Consequently, ganglionic infection was reduced and lethality and local inflammatory lesions were abolished. Here, we report on the activity of MAb 2c in mice depleted of CD4+ (L3T4+) T cells, and in mice
depleted of both CD4+ and CD8+ (Lyt 2+) T cells. The results show that this antibody is able to mediate clearance of HSV from the peripheral site of infection in the absence of cell-mediated immunity.

Methods

Mice. Female C57BL/6J (H-2b) mice were obtained from Charles River Wiga, and used when 33 to 37 days old.

Virus. Herpes simplex virus type 1 (HSV-1) strain 342 hv (Schneweis et al., 1984) was grown on Vero cells in Eagle's minimal essential medium with 10% fetal calf serum (EMEM 10% F). Virus was titrated in microtitre plates on Vero cells, and the TCIDso per 0.05 ml was determined according to the method of Reed & Muench (1938).

Antibodies. The procedures for preparation and characterization of MAb 2c were described in detail elsewhere (Eis-Hübinger et al., 1991). Briefly, MAb 2c was derived by fusing spleen cells from BALB/c mice, hyperimmunized with HSV-1 strain 342 hv, with cells of the myeloma cell line X63-Ag8.653 by adding polyethylene glycol. MAb 2c was determined to be specific for HSV-1 gB by immunoblotting, and belonged to the IgG2a subclass, determined by standard double-diffusion agarose gels using rabbit anti-mouse Ig class and subclass sera. The human standard immune serum preparation used (Beriglobin S; Behringwerke) had a complement-independent neutralizing titre of 1:640 and was diluted twofold to contain the same neutralizing activity as the polyclonal sera. The stock preparation of MAb 2c had a complement-independent neutralizing titre of 1:1280 to HSV-1 in 0.025 ml and was diluted for application fourfold in Iscove's medium. The stock preparation of MAb 2c had a complement-independent neutralizing titre of 1:640 and was diluted twofold to contain the same neutralizing activity as the polyclonal immune serum. The ELISA titre of the applied antibody dilutions was between 10^4 and 10^6 when determined according to the method of Kahlen & Whiteley (1988), except that peroxidase-conjugated rabbit antibodies to mouse and human IgG were used. Hybridoma clones secreting the rat MAbs YTS 191.1 [anti-L3T4 (CD4)] and YTS 169.4 [anti-Lyt 2 (CD8)] (Cobbold et al., 1984) were a generous gift of Dr H. Waldmann, University of Cambridge, U.K., obtained from Dr R. M. Zinkernagel, University of Zurich, Switzerland. Cells were maintained in serum-free Iscove's medium supplemented with Nutridoma-NS (Boehringer). Antibodies were concentrated from the cell-free culture fluid either by ammonium sulphate precipitation followed by dialysis or by ultrafiltration (Diaflow, Amicon). Antibody stocks were stored at -20 °C at a concentration of 10 to 20 mg/ml. Antibody concentration was determined by the Mancini test using anti-rat IgG antiserum and an affinity chromatography-purified rat IgG standard. For controls, equivalent volumes of Iscove's medium were treated in the same manner. [Controls with culture medium were equivalent to controls given a non-HSV-specific MAb (Eis-Hübinger et al., 1991).]

Depletion of T cells. On days -3, -2 and +2 (day 0 representing the day of virus inoculation), mice were injected intravenously with 3.5 mg of stock preparations of YTS 169.4 and/or YTS 191.1. Injections were repeated intraperitoneally on days +8, +15, +22 and +29. Controls were treated with equivalent volumes of precipitated culture medium.

Mouse protection experiments. Mice were inoculated intravaginally with 2 x 10^6 TCIDso of HSV-1 suspended in 0.1 ml EMEM 10% F as described previously (Eis & Schneweis, 1986). Vaginal swabs were taken every second day after inoculation and assayed for virus on Vero cell monolayers. Virus titres were determined in microtitre plates. Mice were examined daily for external skin lesions, neurological illness and death. Fourfold-diluted HSV antiserum (0.5 ml) or twofold-diluted precipitated culture medium (0.5 ml) were injected intraperitoneally either 24 and 72 h or 65 and 265 h post-infection. The bilateral lumbosacral ganglia L4 to S3 of positively infected mice were surgically removed after day +30 and assayed individually for virus. Ganglia were cut into small pieces and cocultivated with human embryonic lung fibroblasts for 30 days. For enhancement of HSV reactivation, culture medium was supplemented for the first 3 days with 10 μg/ml 5-azacytidine as described previously (Whitby et al., 1987; Sienko et al., 1991).

Flow cytometry. Cells of spleen and the para-aortic and inguinal lymph nodes of individual anti-CD4- and anti-CD8-treated mice and controls were obtained on day +11 and analysed for CD4 and CD8 antigens. After lysis of the erythrocytes, 1 ml of the cell suspension was incubated for 45 min either with PE-labelled anti-L3T4 (GR 1.5) or FITC-conjugated anti-Lyt 2 (53-6-7) (both Becton Dickinson). After washing the cells in PBS, a single colour fluorescence analysis (fluorescence-activated cell sorting, FACS) was made by using a FACSscan (Becton Dickinson).

Neutralization test. Blood was obtained by retro-orbital puncture of anaesthetized mice. The neutralization test was performed on Vero cells in microtitre plates. Diluted antibody solutions or sera (0.025 ml) were incubated for 45 min with PE-labelled anti-L3T4 (GR 1.5) or FITC-conjugated anti-Lyt 2 (53-6-7) (both Becton Dickinson). After washing the cells in PBS, a single colour fluorescence analysis (fluorescence-activated cell sorting, FACS) was made by using a FACSscan (Becton Dickinson).

Results

Effect of delayed administration of MAb 2c on peripheral virus replication

Previous studies have shown that administration of MAb 2c 24 and 72 h after infection markedly reduces viral replication in the mucous membranes of immunocompetent mice (Eis-Hübinger et al., 1991). We have...
investigated whether this antibody would be effective when administered after peripheral replication had become established. Consequently, MAb 2c was injected on days +3 and +11 (65 and 265 h after virus inoculation). For comparison, a polyclonal immune serum was used. The results are shown in Fig. 1. It can be seen that the polyclonal immune serum did not suppress viral multiplication in the mucous membranes. However, treatment with MAb 2c resulted in a rapid clearance of the virus from the mucous membranes, indicating that MAb 2c is also effective when administered after peripheral infection has reached its plateau.

We next investigated whether MAb 2c would suppress virus multiplication in mice depleted of CD4+ cells. For that purpose mice were injected with anti-CD4 MAb according to the depletion protocol described in Methods. FACS analysis of spleen and lymph node cells performed on day +11 showed a profound depletion of the CD4+ cells, typically more than 95%. The antibody response to HSV in mice depleted of CD4+ cells was completely inhibited (neutralization titre with complement < 1:5), when measured with blood samples collected on days +8, +16 and > +30.

Fig. 2 shows that in CD4+-depleted mice virus multiplication in the mucous membranes continued for a further 6 days compared with their immunocompetent littermates. Administration of the polyclonal immune

### Table 1. Effect of transfer of polyclonal immune serum and MAb 2c on clinical outcome of HSV infection and viral latency in immunocompetent and T cell-depleted mice

<table>
<thead>
<tr>
<th>T cell depletion</th>
<th>Immunization</th>
<th>No. of mice with genital inflammation/</th>
<th>No. of dead mice/</th>
<th>No. of ganglia with HSV reactivation/</th>
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<tr>
<td></td>
<td></td>
<td>no. of mice tested (%)</td>
<td>no. of mice tested (%)</td>
<td>no. of ganglia tested (%)</td>
</tr>
<tr>
<td>None</td>
<td>None*</td>
<td>20/28 (71)</td>
<td>14/26 (54)</td>
<td>61/138 (44)‡</td>
</tr>
<tr>
<td>None</td>
<td>PIS*</td>
<td>2/11 (18)</td>
<td>0/10 (0)</td>
<td>36/120 (30)</td>
</tr>
<tr>
<td>None</td>
<td>MAb 2c*</td>
<td>0/6 (0)</td>
<td>0/6 (0)</td>
<td>9/60 (15)§</td>
</tr>
<tr>
<td>None</td>
<td>MAb 2c</td>
<td></td>
<td></td>
<td>0/5 (0)</td>
</tr>
<tr>
<td>CD4+</td>
<td>None*</td>
<td>8/18 (44)</td>
<td>15/18 (83)</td>
<td>13/36 (36)</td>
</tr>
<tr>
<td>CD4+</td>
<td>PIS*</td>
<td>7/17 (41)</td>
<td>1/16 (6)</td>
<td>67/167 (40)‡</td>
</tr>
<tr>
<td>CD4+</td>
<td>MAb 2c*</td>
<td>0/6 (0)</td>
<td>0/6 (0)</td>
<td>5/72 (7)</td>
</tr>
<tr>
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<td>MAb 2c</td>
<td></td>
<td></td>
<td>0/5 (0)</td>
</tr>
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<td></td>
<td></td>
<td>0/10 (0)</td>
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<tr>
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<td>MAb 2c</td>
<td></td>
<td></td>
<td>0/11 (0)</td>
</tr>
</tbody>
</table>

* Immunization was performed 65 and 265 h post-inoculation.
† Some cultures were lost by bacterial contamination.
‡ PIS, Polyclonal immune serum.
§ One mouse died after day +30.
¶ Immunization was performed 24 and 72 h post-inoculation.
†† Results for eight positively infected mice. In three additional mice HSV infection was prevented by MAb 2c treatment. Virus was not found in the ganglia of these mice.
serum 65 and 265 h after inoculation only slightly enhanced virus elimination from the mucous membranes. In contrast, in MAb 2c-treated mice virus multiplication was suppressed in CD4+-deficient animals as well as in immunocompetent mice.

To investigate the effect of early administration of MAb 2c in CD4+-depleted mice, antibody was transferred 24 and 72 h after inoculation. As shown in Fig. 3, there was no difference in viral clearance between immunocompetent mice and anti-CD4-treated mice. These experiments show that MAb 2c is effective in CD4+-depleted mice and does not require any cooperation with actively produced antibodies or activated CD4+ T cells.

Effect of MAb 2c on clinical outcome of infection in immunocompetent mice and mice depleted of CD4+ cells

As shown in Table 1, 71% of the untreated immunocompetent mice developed genital inflammatory lesions and more than half of the mice died. After administration of the polyclonal immune serum genital inflammatory lesions were reduced (P = 0.003) and none of the mice died (P = 0.003). Depletion of the CD4+ cells resulted in a lower percentage of mice displaying genital inflammatory lesions (P = 0.067) but lethality was increased (P = 0.042). Surprisingly, administration of the polyclonal immune serum did not reduce the number with genital lesions in CD4+-depleted mice. However, the polyclonal immune serum was still able to prevent the lethal outcome of infection in these mice (P < 0.001). As expected from the restricted virus replication in the mucous membranes, MAb 2c-treated mice were completely protected from the formation of genital lesions (immunocompetent mice, P = 0.001; CD4+-depleted mice, P = 0.046) and fatal encephalitis (P = 0.017, P < 0.001, respectively).

Latent infection was found in 44% of the ganglia of surviving immunocompetent mice and in 30% of the ganglia from immunocompetent mice treated with the polyclonal immune serum (P = 0.019). In the ganglia of CD4+-depleted mice, latent virus infection was found slightly reduced, probably the result of selecting only moderately infected mice. In the ganglia of CD4+-depleted mice given the polyclonal serum, viral latency was in the same range as that of the normal untreated mice, although viral shedding from the mucous membranes was short-lived in the latter. In MAb 2c-treated mice latent infection was rarely observed (immunocompetent and CD4+-depleted mice, P < 0.001).

Effect of MAb 2c in mice depleted of both CD4+ and CD8+ cells

To investigate whether the presence of CD4+ and CD8+ cells is required at all for the protective effect of MAb 2c, mice were simultaneously depleted of CD4+ and CD8+ cells. Treatment with anti-CD8 MAb resulted in a reduction of CD8+ cells in the same range as for the CD4+ cells, as measured by flow cytometric analysis. As shown in Fig. 4, all mice depleted of both T cell subsets and not passively immunized continued with virus shedding until they died within 11 days. Passive transfer of polyclonal immune serum 24 and 72 h post-inoculation did not protect against death, although the survival time of the mice was increased. Only one out of 10 mice survived, eliminating the virus at day +22. [In a blood sample from this mouse, taken 13 weeks post-inoculation HSV-specific antibody (neutralization titre with complement 1:20) was found, indicating that T cell depletion was not total in this mouse. All MAb 2c-treated mice proved HSV antibody-negative when tested 13 weeks post-inoculation.] In contrast, all MAb 2c-treated mice survived (P < 0.001), most probably due to the reduction of viral replication in the mucous membranes. In three
MAb 2c-treated mice, viral inoculation did not result in infection, whereas all controls and polyclonal immune serum-treated mice became infected. Genital inflammatory lesions were completely absent in all mice simultaneously depleted of both CD4+ and CD8+ cells, regardless of their state of immunization (Table 1). In accordance with the restricted viral replication in the mucous membranes, the incidence of latent ganglionic infection was low in MAb 2c-treated mice (Table 1).

Discussion

Recovery from HSV infection involves both cell-mediated and humoral immunity. However, they are not equally engaged in the different target tissues of infection. Antibodies are important in restricting ganglionic infection, thus inhibiting the spread of virus to the central nervous system, whereas cell-mediated immunity is important in clearing the virus from the primary infection site (Kapoor et al., 1982a, b; Nash et al., 1987; Simmons & Nash, 1987; Blacklaws & Nash, 1990). Recently, we described a MAb against HSV-1 gB (MAb 2c), which mediates clearance of infectious virus from the mucous membranes of immunocompetent mice following antibody transfer on days 1 and 3 after viral inoculation. In continuing this work, we now show that virus replication at the epithelial site is also decreased when the antibody is administered at a later stage of infection. This result indicates that MAb 2c acts very rapidly.

The results with CD4+-depleted mice demonstrate that the protective effect on peripheral virus multiplication is mediated by MAb 2c alone and does not require the presence of other antibodies or activated CD4+ cells. The experiment with simultaneous depletion of CD4+ and CD8+ cells shows that the MAb is still effective in mice severely deficient in T cell-mediated immunity. In contrast, a conventional immune serum did not significantly protect these mice against death, although a prolongation of the survival time was achieved. Our findings with the polyclonal immune serum are very similar to those recently reported by Staats et al. (1991). These workers show that a neutralizing MAb, specific for glycoprotein D, was effective in protecting CD4+ cell-depleted mice against fatal encephalitis after corneal infection but was incapable of promoting recovery in mice depleted of both CD4+ and CD8+ cells. As in to our study, the mean survival time was significantly increased. Furthermore, kinetic studies have shown that treatment with this MAb did not significantly accelerate virus clearance from the eye (Lausch et al., 1989).

The mechanism for protection by MAb 2c is rather unclear. The simplest explanation would be that protection is achieved by neutralization of extracellular virus. However, this explanation is not very plausible since antibodies were administered after HSV had entered the cells and HSV can spread directly to contiguous cells without being exposed to the intercellular fluid environment. Furthermore, the neutralizing capacities of MAb 2c and the polyclonal immune serum were adjusted to the same level. Inoue et al. (1991) have shown that an anti-gD MAb suppressed dissemination of the virus in newborn rabbits by neutralization of the virus inoculum at the skin site and by inactivation of HSV-infected mononuclear cells in blood. However, the same effect was achieved by a polyclonal immune serum (Friedlaender et al., 1987). Another explanation for the protective mechanism of MAb 2c could be an unusual transudation of the antibody. It has been reported that systemically administered antibodies fail to protect intravaginally infected mice against death due to the lack of antibody transudation into the vaginal secretions (McDermott et al., 1990). However, this mechanism is very unlikely since we could not detect the antibody in the genital secretions. Furthermore, generation of virus-specific cytotoxic T lymphocytes by the administered antibody as postulated by Sethi (1983) is also excluded since the MAb 2c is effective immediately after its intraperitoneal administration and also in CD8+-depleted mice.

We therefore favour the idea that the MAb identifies an epitope of gB not usually recognized. Since MAb 2c was still effective when mixed with polyclonal antiserum (Eis-Hübing er et al., 1991), antibodies with the relevant epitope specificity are presumably not, or only slightly, represented in polyclonal immune sera. However, caution is necessary when interpreting the data in this way. On the basis of our study the possibility cannot be strictly excluded that adding a high-affinity antibody to polyclonal sera might increase its activity. Work is in progress in our laboratory to localize the epitope of MAb 2c.

Our experiments do not identify the mechanism of antibody-mediated immunity. It is possible that MAb 2c protects directly or cooperates with other cellular effector systems such as macrophages or other Fc receptor-positive cells (ADCC). Since it has been shown that lysis of HSV-infected cells by ADCC acts very rapidly (for review see Kohl, 1991), cooperation with these cells could be possible. On the other hand, it is likely that the presence of MAb 2c in mucous membranes alone is sufficient to suppress viral replication since MAb 2c is able to inhibit completely cell-to-cell spread of HSV and HSV-induced cell fusion in cell culture monolayers (Eis-Hübing er et al., 1992). However, in considering the exact site of action of MAb 2c, it has to be discussed whether MAb 2c inhibits viral replication in mucous membranes or reduces zosteriform spread of the virus by affecting the skin–ganglion circuit (Simmons & Nash, 1985).
Several studies have shown that a close correlation exists between the degree of virus replication at the primary site of infection and the frequency and severity of peripheral diseases as well as the incidence of ganglionic infection (Tullo et al., 1982a, b; Eis & Schneweis, 1986). It is obvious that the data obtained in an animal model cannot be directly applied to human infection. However, as already mentioned by others (Blacklaws & Nash, 1990), the main therapeutic focus for human HSV infection is to clear the virus quickly from the peripheral site of infection and to stop latent infection. The experiments reported here show that these points could be fulfilled in the mouse model by an HSV-specific antibody, even in mice deficient in T cells. Since in humans only humoral immunity can be passively transferred, the finding of an antibody that is as effective as cell-mediated immunity may be of protective or therapeutic significance.

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References


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