Effects of Monoclonal Antibodies Directed against Herpes Simplex Virus-specific Glycoproteins on the Generation of Virus-specific and H-2-restricted Cytotoxic T-Lymphocytes

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(Accepted 10 June 1983)

SUMMARY

Passive transfer of monoclonal antibodies specific for herpes simplex virus type 1 (HSV-1) glycoproteins gC or gD resulted in the generation of highly potent HSV-specific and H-2-restricted primary cytotoxic T-lymphocytes (CTL) in the lymph nodes of recipients provided they were injected at 12 h post-infection. When the same antibody was injected at 3 h before HSV infection, the recipients failed to generate primary anti-HSV CTL. Rather, lymph node cells from such donors proved unsuitable for the initiation in vitro of a secondary anti-HSV CTL response by restimulation.

Available experimental data have suggested the important role of thymus-derived (T)-lymphocytes in resistance to herpes simplex virus (HSV) infections (Zisman & Allison, 1976; Howes et al., 1979). Studies concerned with conventional cytotoxic T-lymphocytes (CTL), which are virus-specific and restricted by the major histocompatibility complex (H-2 in mouse and HLA in man), have revealed that they can be generated to high levels when primed lymphocytes are secondarily stimulated in vitro both in murine (Sethi & Brandis, 1977; Pfizenmaier et al., 1977; Lawman et al., 1980; Nash et al., 1980; Kastrukoff et al., 1981; Sonoda et al., 1981) and human (Sethi et al., 1980) systems. However, it has been difficult to demonstrate anti-HSV CTL during the course of primary infection in vivo and the reasons for this lack of CTL response remain unexplained. In one study (Pfizenmaier et al., 1977), this apparent unresponsiveness was overcome by pretreating mice with cyclophosphamide which presumably eliminated the suppressor T-cell subset and thus created conditions that led to the generation of anti-HSV CTL.

Recent reports (Dix et al., 1981; Rector et al., 1982; Balachandran et al., 1982) that passively administered monoclonal antibodies directed against major HSV-encoded glycoproteins (gC, gA/B, gD and gE) can effectively protect the recipients against fatal HSV infection led me to consider the possibility that virus-specific CTL may be generated under these circumstances. The data presented here showed that a substantial level of primary CTL was induced in mice that were protected against fatal footpad HSV infection by passively injecting monoclonal antibodies to at least two of the HSV-1-encoded major glycoproteins (gC and gD) at 12 h post-infection. However, although administration of these monoclonal antibodies at 3 h before HSV infection protected the recipients from death, a primary anti-HSV CTL response was not detected. On the contrary, such a treatment also failed to elicit detectable precursors of CTL suitable for subsequent secondary in vitro restimulation. The data highlight the fact that complex interaction(s) between virus antigen(s) and specific monoclonal antibodies may determine the outcome of CTL responses during the course of HSV infections.

HSV-1 (strain KOS), when infected in the right rear footpad of female BALB/c (H-2d) mice at a dose of $5 \times 10^5$ p.f.u., routinely killed 80 to 85% of the animals (mean survival time 10 to 14 days). The infected animals showed signs of neurological illness and virus could be consistently recovered from their brains. Hybridoma-derived monoclonal antibodies designated 19-S (IgG2a) and 1-S (IgG2a) specific for HSV-1 glycoproteins gC and gD respectively were tested for...
Table 1. Protection of BALB/c mice against HSV-1 infection mediated by monoclonal antibody directed against virus-specified glycoprotein gC

<table>
<thead>
<tr>
<th>Treatment with monoclonal antibody (designation)</th>
<th>Time of injection (h)</th>
<th>Survivors/total (on day 14)‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSV-1-infected mice (group)*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>19-S</td>
<td>−3</td>
</tr>
<tr>
<td>2</td>
<td>19-S</td>
<td>+12</td>
</tr>
<tr>
<td>3</td>
<td>VSV-27</td>
<td>−3§</td>
</tr>
</tbody>
</table>

* Mice in different groups (10 mice/group) were injected with $2 \times 10^6$ p.f.u. of HSV-1 (KOS) in the hind footpad.
† Diluted (1:2) ascitic fluid containing monoclonal antibody 19-S (virus neutralization titre in presence of complement 1:800) was injected i.p. (1 ml/mouse) either 3 h before ($-3$ h) or 12 h after infection ($+12$ h). Control mice received the same amount of ascitic fluid containing monoclonal antibody VSV-27 directed against VSV at 3 h before infection.
‡ No further mortalities were observed up to 65 days post-infection.
§ Administration of antibody 12 h after infection gave identical results.

A total of 30 male BALB/c mice were injected in hind footpads with HSV-1 ($2 \times 10^6$ p.f.u./mouse) and divided into three groups of 10 mice each. Mice in group 1 had received, 3 h before infection, intraperitoneal (i.p.) injections (1.0 ml/mouse) of 19-S. Mice in group 2 were injected with identical amounts of 19-S at 12 h post-infection. Group 3 animals serving as controls, received at 3 h before infection, i.p. injections of ascitic fluid containing anti-vesicular stomatitis virus (serotype New Jersey; VSV<sub>NJ</sub>) antibody designated VSV-27 (Sethi & Brandis, 1980, 1981). The results (Table 1) showed that 19-S antibody effectively protected mice from HSV-induced death, irrespective of whether it was injected at 3 h before or at 12 h after infection. All the control mice (group 3) had died by 2 weeks. Similar results (not recorded) were obtained with gD-specific monoclonal antibody, 1-S as well as with several other independently isolated monoclonal antibodies directed against gC or gD. These observations are in accord with the work of others utilizing anti-HSV monoclonal antibodies or conventional antisera (Dix et al., 1981; Rector et al., 1982; Balachandran et al., 1982). Since these monoclonal antibodies possessed the capacity to neutralize HSV-1 in vitro, the most plausible explanation of the protection conferred by the antibodies when injected 3 h before infection would be the direct neutralization of injected virus per se. However, it is inconceivable that the above mechanism would account for the protective effect of the antibody when injected 12 h after infection at which time the input virus had already infected the host tissues. In this situation, the proposed alternative mechanism(s) such as antibody-dependent cell-mediated cytotoxicity (Shore et al., 1974; Norrild et al., 1980) or antibody-dependent complement-mediated lysis of infected cells (Norrild et al., 1979) may have been responsible for the protective effect; unequivocal in vitro evidence in support of such mechanism(s) is, however, lacking.

The question of whether mice protected against HSV infection by passively injected monoclonal antibodies generate anti-HSV CTL was examined next. Groups of mice were treated with monoclonal antibodies 19-S, 1-S or VSV-27 and infected with HSV-1 as in the above experiment and their draining lymph nodes removed at the indicated time intervals to assess CTL activities. The results of a typical experiment (Table 2) showed that lymph node cells from mice receiving monoclonal antibodies at 12 h after infection had substantial lytic activity for HSV-infected, H-2 compatible P815 (H-2<sup>b</sup>) cells when tested at day 7 post-infection; lymph node cells obtained at day 21 failed to exert significant lysis. The HSV-specific lytic activity of lymph node cell populations was eliminated on pretreatment with anti-Thy 1.2 antiserum in the presence of complement (data not shown), indicating the T-cell nature of the effectors. The specificity controls showed that these effectors were HSV-specific and H-2-restricted, since at equivalent effector/target cell ratios, significant amounts of $^{51}$Cr were not released from HSV-
Table 2. Generation of primary HSV-specific and H-2-restricted CTL in virus-infected BALB/c mice under the influence of specific monoclonal antibodies

<table>
<thead>
<tr>
<th>Treatment of HSV-infected mice with monoclonal antibody (designation)</th>
<th>Time of monoclonal antibody injection (h)†</th>
<th>Specific 51Cr release‡ (%), from targets by effectors collected at day 7 after infection with HSV</th>
</tr>
</thead>
<tbody>
<tr>
<td>19-S</td>
<td>-3</td>
<td>P815/HSV</td>
</tr>
<tr>
<td></td>
<td>+12</td>
<td>32-6§</td>
</tr>
<tr>
<td></td>
<td>-3</td>
<td>5-3</td>
</tr>
<tr>
<td>1-S</td>
<td>+12</td>
<td>39-9</td>
</tr>
<tr>
<td>VSV-27 (control)</td>
<td>-3</td>
<td>4-6</td>
</tr>
<tr>
<td></td>
<td>+12</td>
<td>3-8</td>
</tr>
</tbody>
</table>

*, † See footnotes to Table 1.
‡ Draining lymph nodes of infected mice were excised and non-adherent cell suspensions, usually a pool of two mice, used as the effectors in cytolysis assays carried out as described previously (Sethi & Wolff, 1980). Briefly, target cells [L-929 (H-2k); P815 (H-2a)] were infected with HSV-1 at a m.o.i. of 5 or with VSV-Nj (m.o.i. 20). After virus adsorption (37°C for 1 h), cultures were washed and 10⁶ cells incubated with 1 mCi Na₂⁵¹CrO₄; mock-infected (including natural killer cell-sensitive YAC-1) cells were incubated with 5¹Cr as usual. After 2 h (HSV-infected) or 8 h (VSV-Nj-infected), cells were washed and suspended in growth medium (GM) consisting of RPMI 1640 (Flow Laboratories), 10⁻² heat-inactivated foetal calf serum, 20 mM-HEPES, 20 mM-glutamine, 5 × 10⁻⁵ M-2-mercaptoethanol and antibiotics. Aliquots (100 µl; 10⁴ cells) of test targets were mixed in round-bottomed plastic tubes with 100 µl of appropriate effectors to give an effector (E) to target (T) cell ratio of 30:1. After centrifugation (50 g, 10 min) the tubes were incubated for 3 h at 37°C. The tubes were re-centrifuged (400 g, 10 min) in the cold and from each tube, 100 µl of supernatant was counted in a gamma counter. The results are expressed as percentage specific ⁵¹Cr release according to the formula: 100 × (E - S)/M - S, where E = release in test, S = spontaneous release in presence of normal lymph node cells, and M = maximum release (ct/min) in 1% Triton X-100. The tabulated values are means of quadruplicate assays in a typical experiment with standard errors < 2%, and are omitted. Spontaneous ⁵¹Cr release (3 h) values in the presence of normal lymph node cells ranged from 8 to 16% for uninfected and 14 to 22% for infected targets. The experiment was performed three times with concordant results and higher E:T ratios (50:1 or 75:1) showed an identical trend.
§ Significant specific lysis of ⁵¹Cr-labelled YAC-1 targets was not observed. When effectors were generated in C3H (H-2a) mice, they showed specific lysis for HSV-1-infected L-929 (H-2k) but not P815 (H-2a) cells.
|| NT, Not tested.

infected, H-2-incompatible L-929 (H-2a) cells or from VSV-Nj-infected or uninfected syngeneic P815 targets. Under identical test conditions, lymph node cells from HSV-infected mice which had received monoclonal antibodies at 3 h before infection or those derived from controls (given VSV-27 monoclonal antibodies) showed no appreciable lytic activity specific for HSV-infected P815 cells. Thus, passive administration of 19-S or 1-S at 12 h after infection but not at 3 h before infection induced a significant primary anti-HSV CTL response in the recipients.

In separate experiments, when lymph node cells from protected mice which had received monoclonal antibodies at 12 h were collected at day 21 after infection and restimulated in vitro, highly potent HSV-specific and H-2-restricted CTL were generated in such cultures (40-6% specific ⁵¹Cr release from HSV-infected P815 cells compared to 4-6% of HSV-infected L-929 and 3-0% of uninfected P815 targets). Secondary restimulation of lymph node cells from HSV-infected mice that had received no pretreatment also generated a detectable CTL response (mean specific ⁵¹Cr release from HSV-infected appropriate targets ranged from 36-5 to 40-0% compared to control values of less than 5%). Strikingly, secondary in vitro restimulation did not generate a detectable CTL response when the responders were lymph node cells from virus-infected mice which had received monoclonal antibodies at 3 h before infection (mean specific ⁵¹Cr release being 5 to 8%, which was equivalent to the control values).

The inability of mice to elaborate CTL-precursors (CTL-P) when the monoclonal antibodies were given shortly before infection (3 h) is reminiscent of similar findings with influenza virus (Greenspan & Doherty, 1981). However, an apparent anomaly in the present situation is that unlike the observations with influenza virus (Greenspan & Doherty, 1981), late administration of monoclonal antibodies (i.e. 12 h after infection) could induce a potent CTL response. The precise mechanism(s) by which monoclonal antibodies effect the generation of CTL responses
remain obscure. The inability to detect HSV-specific CTL-P in mice receiving monoclonal antibodies shortly before infection may be due to low levels of (or inadequately primed) CTL-P generated by antibody-neutralized virus preparations as reported for influenza virus (Greenspan & Doherty, 1981). Presumably, inadequate helper cell activity, which is a prerequisite for the development of antigen-specific CTL-P (Wagner & Röllinghoff, 1978) is elicited in this situation. Interestingly, anti-HSV titres were significantly elevated in the sera of these mice (data not shown), which suggests that anti-HSV CTL and humoral responses may be under the control of different regulatory mechanism(s). It is pertinent that mice exposed to heat-inactivated HSV fail to generate CTL but develop elevated serum antibody titres (Naylor et al., 1982).

REFERENCES


*(Received 4 February 1983)*