Neutralizing mechanisms of two human monoclonal antibodies against human cytomegalovirus glycoprotein 130/55

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The neutralization of human cytomegalovirus (HCMV) after adsorption to the cell surface at 4 °C was studied using two neutralizing monoclonal antibodies (C-23 and C-41) recognizing glycoprotein 130/55. HCMV adsorbed to cells was neutralized by C-23 (complement-independent), but not by C-41 (complement-dependent). Furthermore, the virus remained sensitive to C-23 for 120 min after shifting up from 4 °C to 37 °C, suggesting that C-23 might block an early stage of virus penetration into cells, and also that transition from virus attachment to virus penetration might be quite slow. The cell-to-cell infection of HCMV was also blocked only by C-23, and not by C-41. On the basis of the results presented here, we suggest that C-41 blocks the attachment of virus to the cell surface whereas C-23 prevents the penetration of virus into the cell.

Recently, several electrophoretically unique envelope glycoproteins have been shown to elicit neutralizing monoclonal antibodies (MAbs) against human cytomegalovirus (HCMV). A glycoprotein complex, comprising a 50K to 55K species and a 130K to 160K precursor, has been reported which induces complement (C')-dependent neutralizing antibodies in mice and guinea-pigs (Pereira et al., 1982; Britt, 1984; Rasmussen et al., 1985). Another HCMV glycoprotein complex, comprising proteins of 47K to 52K, has been found to elicit a C'-independent neutralizing response (Kari et al., 1986; Gretch et al., 1988). A glycoprotein of 86K has also been shown to induce C'-independent neutralizing antibodies in mice and guinea-pigs (Rasmussen et al., 1985). Masuho et al. (1987) developed two different human neutralizing MAbs (C-23 and C-41) directed to the glycoprotein complex of 130K and 55K (gp130/55) of HCMV. The neutralizing activity of C-23 was C'-independent and that of C-41 was C'-dependent (Masuho et al., 1987; Tomiyama et al., 1990). However the neutralizing mechanisms of these MAbs operating during the process of virus infection are still unclear. In the present study, neutralization of HCMV after adsorption to the cell surface was investigated using the above two MAbs (C-23 and C-41).

Human embryonic fibroblast (HEF) cultures were grown in Eagle's MEM (EMEM, Gibco) supplemented with 10% foetal calf serum (FCS, Gibco) and antibiotics [10 μg/ml of streptomycin and 100 international units (IU)/ml of penicillin]. The maintenance medium (MM) consisted of EMEM with 2% FCS and antibiotics. HEF cultures infected with the Davis strain of HCMV were sonicated at 10 Hz, and, after centrifugation at 2000 r.p.m. for 30 min, the supernatant of this sonicated preparation was used as the virus preparation (Tanaka & Numazaki, 1979). Virus preparations were stored at −80 °C after adding 10% DMSO.

Two different human MAbs, C-23 (2000 μg/ml) and C-41 (2000 μg/ml), were prepared as described previously (Tomiyama et al., 1990). Both MAbs are of the IgG1 isotype and both recognize gp130/55 on the HCMV envelope (Tomiyama et al., 1990). They have been reported to neutralize laboratory strains, including AD169 and Davis, as well as clinical isolates of HCMV (Masuho et al., 1987; Tomiyama & Masuho 1990).

The neutralizing activities of these MAbs for virus already adsorbed to cells at 4 °C were determined by measuring the reduction in the number of infectious centres (ICs) using an indirect immunofluorescent antibody technique (IFA). Fifty μl of HCMV (100 to 200 p.f.u./50 μl) was inoculated into each well of HEF monolayers on eight-well tissue culture chamber slides (Nunc) and adsorbed at 4 °C for 120 min. After washing three times with cold PBS, 400 μl of MM with C-41 in the presence of 10% C' (Kyokuto Co.) or with C-23 in the
Fig. 1. Adsorption of the virus and treatment with MAbs at 4 °C. Neutralizing activities of C-23 (with heated C'; ○) and C-41 (with fresh C'; ■) after virus adsorption were assayed by IFA at 1:16 and 1:64 concentrations. Control experiments were carried out using C-7 (with C'; □) (non-neutralizing MAb directed to a 64K protein of HCMV) and C' alone (△).

Fig. 2. Adsorption of the virus at 4 °C and treatment with MAbs at 37 °C. HEF cultures adsorbed with HCMV at 4 °C for 120 min were shifted up to 37 °C with normal MM. After incubation for various periods at 37 °C, 1:16 dilutions of either C-23 (with heated C'; ○) or C-41 (with fresh C'; ■) were added to the cultures and the reduction of ICs was counted on day 3.

presence of heat-inactivated 10% C' was added to each well of the cultures and the culture slides were incubated at 4 °C for various periods in 5% CO₂–95% air. After washing again three times, the medium was changed to normal MM and the cultures were shifted up to 37 °C. On day 3 of incubation, the cultures were washed with PBS, fixed in acetone and stained by indirect IFA using a 1:10 dilution of seropositive human serum (anti-late antigen titre 1:128, anti-early antigen titre 1:32) and fluorescein isothiocyanate-conjugated goat anti-human IgG (Dako). Finally, IFA-positive ICs (single cells and foci consisting of several infected cells) were counted under a fluorescence microscope.

The numbers of ICs were markedly reduced by treatment with C-23, but not with C-41; negative controls in the assay included C-7, a non-neutralizing MAb to a 64K protein of HCMV, and C' alone as reported by Masuho et al. (1987) (Fig. 1). The maximum reduction of ICs was 90% after 90 to 120 min of the treatment using both a 1:16 and a 1:64 dilution of C-23. The different neutralizing activities of C-23 and C-41 for pre-adsorbed virus suggested that neutralization by C-41 might be related to blocking of virus attachment and that by C-23 to prevention of virus penetration.

We studied the length of time C-23 was able to maintain neutralizing activity after virus attachment. HEF cultures in chamber slides and adsorbed with HCMV at 4 °C for 120 min were washed three times and shifted to 37 °C in normal MM. After incubation for various periods at 37 °C, a 1:16 dilution of C-23 (with heat-inactivated C') or C-41 (with fresh C') was added to the cultures. A reduction of ICs was demonstrated using C-23, but not using C-41 (Fig. 2). The magnitude of the effect depended on the incubation period before adding C-23; the result was a 90% reduction in ICs if MAb was added immediately after shifting up to 37 °C, and a 75% reduction if MAb was added after 60 min, but the effect soon disappeared after that time. These results suggested that C-23 might block an early stage of virus penetration into cells.

The replication pattern of CMV is similar to that of herpes simplex viruses (HSV). HSV is known to enter cells by envelope-cell membrane fusion and this process can be blocked by a C'-independent mechanism with MAbs directed to glycoprotein B (gB) or glycoprotein H (gH) on the HSV envelope (Cai et al., 1988; Fuller et al., 1989). As HCMV gB is homologous to the HCMV gp130/55 (Cranage et al., 1986), it is suggested that the C'-independent MAb C-23 specific for HCMV gB might inhibit a virus envelope–cell membrane fusion step. However, further study is required to confirm this.

The transition from virus attachment to virus penetration is fast for many viruses. However the above results suggested a slow transition for HCMV. We need further
Table 1. Inhibition of cell-to-cell infection with MAbs

<table>
<thead>
<tr>
<th>MAb</th>
<th>C-23 (without C')</th>
<th>C-41 (with C')</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dilution</td>
<td>1:4</td>
<td>1:16</td>
<td>1:64</td>
</tr>
<tr>
<td>No. of ICs</td>
<td>181</td>
<td>205</td>
<td>208</td>
</tr>
<tr>
<td>(Single) (%)</td>
<td>168</td>
<td>125</td>
<td>65</td>
</tr>
<tr>
<td>Foci* (%)</td>
<td>13</td>
<td>80</td>
<td>143</td>
</tr>
</tbody>
</table>

* Foci consisting of several infected cells.

Table 2. Effect of MAb on the number of infected cells (ICs)

<table>
<thead>
<tr>
<th>MAb</th>
<th>No. of ICs</th>
<th>Percentage of ICs as foci</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Single</td>
<td>Foci</td>
</tr>
<tr>
<td></td>
<td>(%)</td>
<td>(%)</td>
</tr>
<tr>
<td>C-23</td>
<td>168</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>125</td>
<td>80</td>
</tr>
<tr>
<td>C-41</td>
<td>65</td>
<td>143</td>
</tr>
</tbody>
</table>

studies to determine whether the results are peculiar to the Davis strain used in this study or have general relevance.

CMV is a highly cell-associated virus, and infection spreads via cell-to-cell transmission as well as via free virions. Tomiyama & Masuho (1990) reported that C-23 blocks the cell-to-cell transmission of HCMV; its suppressive effect on virus spread is dependent on its concentration and is greater when the antibody was added sooner after infection. However, the mechanism of this effect was not clear, and hence we studied the mechanism of inhibition by using immunofluorescence rather than plaque assay. In the culture infected with HCMV, most of the infected (IFA-positive) cells were single and the number of these ICs increased up to 3 days following inoculation. On day 4, however, foci consisting of several infected cells were formed by cell-to-cell contact. The number of ICs (single cells and foci) did not change. Therefore the numbers of single infected cells and of foci were analysed on day 4 of incubation in this study. As shown in Table 1, the total number of ICs (single cells and foci) was almost the same in all cultures tested. However, there was a marked difference in the number of foci, indicative of cell-to-cell infection, between the C-23- and C-41-treated groups. The number of foci consisting of several infected cells was reduced by C-23 in a MAb dose-dependent manner. Thus only 7% of ICs were foci in the presence of C-23 at a 1:4 dilution, whereas 69% of ICs were foci with C-23 at a 1:64 dilution. In contrast, treatment with MAb C-41 had no effect upon the number of ICs appearing as foci. Hence cell-to-cell infection of HCMV was blocked by C-23 as reported by Tomiyama & Masuho (1990), but not by C-41. This can be explained if released viruses in the intercellular space rapidly attach to the neighbouring cell surface and become insensitive to blockage by C-41, whereas such attached viruses would remain sensitive to C-23 because of the slow transition from virus attachment to virus penetration.

References


(Received 14 January 1992; Accepted 9 June 1992)