Human Monoclonal Antibodies Neutralizing Human Cytomegalovirus

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SUMMARY

Hybridomas producing human monoclonal antibodies (MAbs) against human cytomegalovirus (CMV) were generated by fusion of human spleen cells and mouse myeloma cells. Two of the six MAbs obtained neutralized viral infectivity even at concentrations lower than 1 μg/ml. One MAb required complement for neutralization but the other did not. Both MAbs recognized viral proteins of M, 130000 and 55000. Furthermore, these neutralizing MAbs bound to the surface membrane of CMV-infected cells. These results suggest that human MAbs may provide a new means of passive immunization against CMV infection in humans.

Cytomegalovirus (CMV) has been associated with significant morbidity and mortality in immunocompromised hosts such as organ transplant recipients (Meyers et al., 1983) and patients with acquired immunodeficiency syndrome (Quinnan et al., 1984). Condie & O'Reilley (1984) showed that high doses of human serum immunoglobulin with a high antibody titre to CMV completely protected bone marrow transplant recipients from fatal CMV pneumonia. Their results suggest that if a human monoclonal antibody (MAb) with an even higher antibody titre was available, it would have a potent protective effect against CMV infection. Many laboratories have attempted to develop human MAbs, but to date there has been only marginal success (Cole et al., 1984; Burnett et al., 1985; Sawada et al., 1985). The major obstacle is the difficulty in obtaining human B lymphocytes from individuals highly immunized with a pertinent antigen. To overcome the restriction on human immunization, we recently established a method for efficient generation of hybridomas that produce human MAbs against a predefined antigen (Masuho et al., 1986; Matsumoto et al., 1986). In this study, we describe human MAbs capable of neutralizing CMV infectivity.

Hybridomas were generated by the fusion of human lymphocytes and mouse myeloma cells as described previously (Matsumoto et al., 1986). In brief, human B lymphocytes were obtained from the spleen of a CMV-seropositive patient with idiopathic thrombocytopenic purpura. These were cultured for 6 days with 50 ng protein/ml of inactivated CMV and 1/10 vol. of B cell growth factor (Cellular Products Inc., Buffalo, N.Y., U.S.A.) and fused with mouse myeloma P3-X63-AgU1 cells. The fused cells were grown in selection medium containing hypoxanthine, aminopterin and thymidine. Four weeks after cell fusion, macroscopic hybridoma colonies appeared in most of the culture wells and 20 to 50% of them were positive for IgG antibody to CMV. We succeeded in cloning six different hybridomas stably producing antibodies by limiting dilution and obtained human MAbs from their culture fluids. All of these MAbs, designated C1, C3, C4, C7, C23 and C41, bound to CMV strains but not to other herpesviruses such as herpes simplex virus and varicella-zoster virus. Their isotypes were found all to be IgG1. C23 had a κ chain and the others had λ chains.

The ability of each MAb to neutralize viral infectivity was determined by measuring the reduction in the number of viral plaques. C23 and C41 neutralized viral infectivity (Fig. 1) but
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Fig. 1. Dose responses in CMV neutralization by (a) C23 and (b) C41. Reduction of viral plaques (strain AD169) was assessed in the presence (●) and absence (○) of guinea-pig complement by the method described previously (Matsumoto et al., 1986).

Fig. 2. Autoradiographs of CMV antigens recognized by human MAbs. Human embryonic lung (HEL) cells were infected with the AD169 strain (lanes 1, 4, 7, 8, 9, 10 and 11) or the Hi-1 strain (lanes 3 and 6), or were mock-infected (lanes 2 and 5); all HEL cells were labelled with [35S]methionine. Cell lysates were immunoprecipitated with C23 (lanes 2 to 4), C41 (lanes 5 to 7), C1 (lane 8), C3 (lane 9), C4 (lane 10), C7 (lane 11) and serum from the donor of the lymphocytes used for the hybridomas (lane 1). The precipitated antigens were analysed by SDS-PAGE as described by Laemmli (1970) and autoradiographed with a Kodak X-ray-Omat film.

The other MAbs did not, even at concentrations higher than 75 µg/ml. Addition of complement did not affect the neutralization by C23 (Fig. 1a) but greatly augmented the neutralization by C41 (Fig. 1b). The concentrations of C23 and C41 necessary for 50% reduction in the viral
There were no differences in the immunofluorescence profiles of the two neutralizing MAbs or the four non-neutralizing MAbs. Photomicrographs for C23 and C3 are shown as the representatives of these two groups. Cells fixed with acetone and methanol (1:1) were allowed to react sequentially with C23 (a) or C3 (c), guinea-pig complement and fluorescein-labelled anti-guinea-pig complement. In the case of unfixed cells, the reactions of C23 (b) and C3 (d) were visualized with fluorescein-labelled anti-human IgG.

Table 1. Neutralization of CMV clinical and laboratory strains by C23 and C41*

<table>
<thead>
<tr>
<th>CMV strain</th>
<th>Tissue culture passage</th>
<th>P.f.u. in control</th>
<th>Plaque reduction (%)</th>
<th>C23</th>
<th>C41</th>
</tr>
</thead>
<tbody>
<tr>
<td>AD169</td>
<td>&gt; 100</td>
<td>42</td>
<td>95</td>
<td>83</td>
<td></td>
</tr>
<tr>
<td>Hi-1</td>
<td>38</td>
<td>150</td>
<td>98</td>
<td>99</td>
<td></td>
</tr>
<tr>
<td>No. 12</td>
<td>8</td>
<td>30</td>
<td>95</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Onoda</td>
<td>8</td>
<td>49</td>
<td>98</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>Omine</td>
<td>6</td>
<td>54</td>
<td>94</td>
<td>94</td>
<td></td>
</tr>
<tr>
<td>YAN-3</td>
<td>5</td>
<td>89</td>
<td>97</td>
<td>64</td>
<td></td>
</tr>
</tbody>
</table>

* CMV strains were neutralized with 10 μg/ml of C23 or C41 and guinea-pig complement.

plaque count were 0.5 μg/ml and 0.3 μg/ml, respectively, in the presence of complement. It is known that CMV strains possess different antigenicities (Rasmussen et al., 1984). Thus, the neutralization by C23 and C41 was examined using five clinical CMV isolates (Table 1). C23 reduced the plaque counts of all the tested CMV strains by more than 90%. On the other hand, C41 showed considerably less plaque reduction in the cases of the No. 12 and YAN-3 strains. Even when the concentration of C41 was increased to 50 μg/ml, the plaque counts of the No. 12 strain were reduced by only 20%.

CMV antigens recognized by these human MAbs were determined by immunoprecipitation (Fig. 2). Both of the neutralizing MAbs, C23 and C41, precipitated two proteins of Mr 130000 (130K) and 55K from CMV-infected cell lysates but not from a mock-infected cell lysate. On the
other hand, all four of the non-neutralizing MAbs precipitated a protein of 64K, which may be a tegument protein (Farrar & Oram, 1984; Pereira, 1984). Serum antibodies from the donor whose lymphocytes were used in the production of these MAbs precipitated much larger amounts of 64K protein than of 130K or 55K proteins (Fig. 2, lane 1). These results suggest that the minor antigens of 130K or 55K rather than the major 64K antigen have important epitopes recognized by neutralizing antibodies.

Binding of the human MAbs to CMV-infected cells was examined by an immunofluorescence test described previously (Kettering et al., 1977). Both of the neutralizing MAbs C23 and C41 bound to the surface membrane of infected cells (Fig. 3b). On the other hand, none of the four non-neutralizing MAbs bound to the surface membrane, though they bound to the nuclei and cytoplasm of infected cells (Fig. 3c, d). These results imply that the epitopes on the 130K or 55K proteins recognized by C23 and C41 are exposed on the surface of infected cells and that the epitopes on the 64K protein recognized by the non-neutralizing MAbs are in internal, non-exposed portions of infected cells.

We have described human MAbs capable of neutralizing CMV infectivity. Both C23 and C41 had a very high neutralization activity. From the viewpoint of passive immunization, however, C23 is more useful than C41 because C23 neutralized all the CMV strains tested but C41 did not strongly neutralize some of the strains. These MAbs recognized 130K or 55K antigens, which have an electrophoretic profile almost identical to that of the envelope glycoprotein complex characterized using mouse MAbs (Pereira, 1984; Rasmussen et al., 1985; Britt & Auger, 1986). In addition, C23 and C41 were capable of binding to the surface membrane of infected cells. Therefore, these MAbs may protect a host from CMV infection not only through viral neutralization but also through antibody-dependent cellular cytotoxicity and complement-mediated cytotoxicity. In contrast to the MAbs against 130K or 55K antigens, none of the four MAbs against the 64K antigen had neutralizing activity. Recently, Redmond et al. (1986) reported that human MAbs against an antigen similar to our 64K antigen neutralized CMV. This study suggests that these human MAbs capable of neutralizing viral infectivity may provide a new means of passive immunization against CMV infection in immunocompromised patients. Large-scale production of these MAbs is now in progress.

REFERENCES


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