Protection against murine cytomegalovirus infection by passive transfer of neutralizing and non-neutralizing monoclonal antibodies

Helen E. Farrell*† and Geoffrey R. Shellam

Department of Microbiology, University of Western Australia, Queen Elizabeth II Medical Centre, Nedlands 6009, Australia

The ability of eight neutralizing monoclonal antibodies (MAbs) specific for structural proteins of murine cytomegalovirus (MCMV) to protect mice passively against MCMV infection was examined to determine firstly whether a correlation existed between the neutralization titres of the MAbs in vitro and the protection afforded by the MAbs in vivo and, secondly, the contribution of the host towards neutralization by the MAbs in vivo. The reduction in MCMV titre in the livers of BALB/c and C57BL/10 mice by the MAbs closely correlated with their neutralization titres in vitro. However, in the spleens of BALB/c mice, in which MCMV replicates to high titre, almost all of the MAbs tested were ineffective in reducing MCMV replication. Indeed, a significant increase in splenic MCMV replication was observed in mice treated 24 h prior to MCMV replication with either neutralizing MAbs or polyclonal Ig. Each of six MAbs prophylactically protected between 66 and 100% of mice from an intraperitoneal challenge with 4 LD₅₀ MCMV regardless of their neutralization titre in vitro. The persistence of MCMV replication in the salivary gland was not prevented by either polyclonal Ig or MAbs. Despite the absolute requirement for complement for the neutralization of MCMV in vitro, both polyclonal Ig and MAb 4F9 protected A/J mice, which are deficient in the fifth component of complement, as efficiently as they did complement competent BALB/c mice. These results demonstrate that MAbs specific for single MCMV polypeptides are protective in vivo. In addition, the extent to which the MAbs protected against MCMV could not be predicted from their immunoreactive or neutralizing titres in vitro or by their effect on splenic MCMV replication in vivo. Furthermore, these studies suggest that the mechanism(s) of neutralization of MCMV in vitro are different to those which act in vivo.

Introduction

Previous studies have shown that humoral immunity to cytomegalovirus (CMV) is important in modifying the clinical manifestations associated with subsequent CMV infections, particularly in the neonate (Yeager et al., 1981; Stagno et al., 1982) and the immunocompromised host (Neiman et al., 1977; Suwansirikul et al., 1977; Medearis & Prokay, 1978; Stagno et al., 1982). The infection of mice with murine CMV (MCMV) provides a model to study the parameters that influence the protection afforded by adoptive humoral immunity. Previous studies in this laboratory have described viral and host genetic factors which influence the kinetics of the total and neutralizing antibody response following MCMV infection (Lawson et al., 1988; Farrell & Shellam, 1989). Administration of either early [< day 7 post-infection (p.i.)] or late (> day 47 p.i.) immune sera 24 h prior to MCMV infection reduces virus replication and dissemination below detectable levels in both immunocompetent (Araullo-Cruz et al., 1978; Shanley et al., 1981; Lawson et al., 1988) and immunosuppressed mice, although the establishment of a latent infection was not prevented (Shanley et al., 1981). However, protection against a lethal MCMV infection in BALB/c mice was not observed if antibody was administered 24 h after MCMV infection (Starr & Allison, 1977), suggesting that specific antibody may only be effective if administered prophylactically.

We have previously reported the immunoreactive and neutralizing activity in vitro of MAbs directed against structural MCMV proteins (Farrell & Shellam, 1990). To investigate further the specificity of protective antibodies in vivo, the ability of MAbs to reduce virus titres in BALB/c and C57BL/10 mice and protect against a lethal MCMV challenge was determined. As virus titres and the pathogenesis of MCMV infection are markedly different in these mouse strains, the role of the host...
genetic status in the efficacy of immune prophylaxis was investigated. Finally, we examined whether a correlation existed between the ability of MAbs to protect mice in vivo and the immunoreactive and neutralizing titres of MAbs in vitro.

Methods

Mice. Inbred, specific pathogen-free BALB/c, C57BL/10 and A/J mice were obtained from Animal Resources Centre (Murdoch, Western Australia) and housed under minimal disease conditions. All mice were seronegative for MCMV prior to study. These strains were chosen because the levels of MCMV replication in the spleen and liver and the pathogenesis of MCMV disease are different (Allan & Shellam, 1984), a phenomenon which is influenced by the non-H-2 gene Cmv-1 (Scalzo et al., 1990). Male mice (10 weeks old) were used in this study.

Virus. MCMV (Smith strain) was passaged in weanling female BALB/c mice as described elsewhere (Allan & Shellam, 1984). The infectivity of virus stocks was determined by LD50 using five mice per virus dilution and calculated from a modification of the Kärber equation: \[ \log_{10} LD_{50} = 0.5 + \log_{2} (\text{highest dilution of virus}) - \left[ \frac{\text{sum} \text{ percent dead mice/100}}{\text{sum}} \right] \]. One LD50 of the virus stock used in these experiments corresponded to 10^4.5 pfu for BALB/c mice and 10^5 pfu for C57BL/10 mice. Mice were inoculated intraperitoneally (i.p.) with 0-1 ml MCMV diluted in phosphate-buffered saline (PBS) having the osmolarity of mouse serum (330 mOsm).

Reference sera. For the preparation of hyperimmune anti-MCMV Ig, BALB/c and C57BL/10 mice were inoculated i.p. with 10^6 pfu MCMV and rechallenged with 10^5 pfu. MCMV 4 weeks later. Sera were collected 14 days after the final challenge. Normal mouse sera (NMS) were collected from uninfected BALB/c and C57BL/10 mice.

Monoclonal antibody (MAb) production. The production and in vitro characterization of MAbs to MCMV have been described (Farrell & Shellam, 1990). The Ig subclass, ELISA and neutralization titres, and the MCMV proteins recognized by the MAbs used in this study are summarized in Table 1. All ascites and polyclonal Igs were treated with 30% (v/v) ammonium sulphate at 4 °C to precipitate contaminating proteins; Igs were subsequently precipitated with 50% (v/v) ammonium sulphate and dialysed extensively against PBS. Protein concentrations were determined spectrophotometrically by absorbance at 280 nm.

Neutralization assay. Details of the neutralization assay and the requirement for complement of the MAbs used in this study to neutralize MCMV have been previously described (Farrell & Shellam, 1990). The neutralizing titre is defined as \(\mu\)g MAb required to neutralize 50% of the virus inoculum.

ELISA. The ELISA used to detect antibodies reactive with MCMV antigen has been previously described (Lawson et al., 1988). Briefly, serial dilutions of antibodies were detected with a goat anti-mouse (IgG + IgM) antibody conjugated with horseradish peroxidase, using o-phenylenediamine dihydrochloride (Sigma) as the substrate. The absorbance was read at 492 nm using a Titertek Multiskan spectrophotometer (Flow Laboratories). Antibody titres are expressed as the reciprocal of the highest antibody dilution which gave an \(A_{492}\) reading higher than the mean \((+3 \text{ s.e.})\) of NMS. The goat anti-mouse peroxidase conjugate could detect all subclasses of murine IgG (not shown).

Western blotting and immunoprecipitation analysis. The reactivity of MAbs with structural MCMV polypeptides is described elsewhere (Farrell & Shellam, 1990).

Prophylactic inoculation of antibodies. Antibodies were diluted in PBS to either 1, 10, 100 or 250\(\mu\)g/0.2 ml (see Tables). For prophylactic inoculation, antibodies were administered i.p. to mice 24 h prior to MCMV infection.

Preparation of organ homogenates. Spleen, liver and salivary glands from MCMV-infected mice were aseptically collected and homogenized (20% w/v) in \(2\%\) Eagle's EMEM (EMEM-2) at 4 °C. The homogenates were clarified at 2000 g at 4°C and stored at -70°C until use. All organs were processed individually.

Determination of virus titres in organ homogenates by plaque assay. Details of the plaque assay have been previously described (Allan & Shellam, 1984). Briefly, mouse embryonic fibroblast (MEF) cultures were infected for 1 h at 37°C with organ homogenates which had been serially diluted in EMEM-2. The inoculum was removed, replaced with EMEM-2 containing 1-75% (w/v) methyl cellulose and cultures were incubated for a further 5 days. All samples were titrated in duplicate. The lower limits of virus titres which could be reliably detected were 400 p.f.u./liver, 100 p.f.u./spleen and 200 p.f.u./salivary gland.

Protection against lethal MCMV infection by MAbs. BALB/c mice were inoculated i.p. with 500 \(\mu\)g MAb 24 h prior to a 4 LD50 MCMV i.p. infection and were observed for 21 days. Control mice received 500 \(\mu\)g NMS.

Statistical analysis. Statistical analysis was performed by the \(\chi^2\) test with Yate's correction, and by Student's t-test.

Results

Lack of correlation between ELISA levels and neutralization titres of MAbs to MCMV

A comparison between the level of immunoreactive antibody by ELISA and the titre of functional antibody by neutralization assay was made for all MAb preparations. No correlation was found between antibody isotype and either the titre of immunoreactive antibody or neutralization in vitro (Table 1). Indeed, highly neutralizing MAbs AC1 and 4F9 possessed relatively low titres by ELISA, whereas the non-neutralizing MAb 1B4 possessed levels of immunoreactive antibody by ELISA comparable with hyperimmune Ig. In addition, no correlation was observed between the titres of functional antibody and the recognition of 60K, 68K, 92K and 98K MCMV proteins, which were recognized by more than one MAb.

Effect of prophylactic transfer of polyclonal and MAbs on MCMV titres in spleens and livers of BALB/c and C57BL/10 mice

Previous studies in this laboratory have demonstrated differences between C57BL/10 and BALB/c mice in the dissemination of MCMV during acute infection (Allan & Shellam, 1984). Indeed, recent studies have shown that the restriction of MCMV replication in the spleens of C57BL/10 mice is controlled by a single gene, Cmv-1.
Table 1. **MAbs to MCMV**: comparison of neutralization titres, ELISA titres, MCMV protein specificity and protection against lethal MCMV infection

<table>
<thead>
<tr>
<th>MAb</th>
<th>Isotype</th>
<th>Neutralization titre*</th>
<th>ELISA titre†</th>
<th>MCMV protein specificity (Mr x 10⁻³)‡</th>
<th>Protection against lethal MCMV infection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Number survived/number tested§</td>
</tr>
<tr>
<td>1E8</td>
<td>IgG2b</td>
<td>60</td>
<td>25600</td>
<td>56, 60¶</td>
<td>10/12</td>
</tr>
<tr>
<td>2F7</td>
<td>IgM</td>
<td>3</td>
<td>16400</td>
<td>98</td>
<td>12/12</td>
</tr>
<tr>
<td>3B2</td>
<td>IgG2b</td>
<td>0.6</td>
<td>&gt;102400</td>
<td>50</td>
<td>12/12</td>
</tr>
<tr>
<td>4F9</td>
<td>IgG2b</td>
<td>1.0</td>
<td>3200</td>
<td>28, 34, 36, 48-50, 60, 68</td>
<td>10/12</td>
</tr>
<tr>
<td>AC1</td>
<td>IgM</td>
<td>3</td>
<td>3200</td>
<td>92, 98</td>
<td>10/12</td>
</tr>
<tr>
<td>1B4</td>
<td>IgM</td>
<td>100</td>
<td>&gt;102400</td>
<td>87, 92, 98, 102, 110</td>
<td>9/12</td>
</tr>
<tr>
<td>1G4</td>
<td>IgM</td>
<td>150</td>
<td>&lt;100</td>
<td>68, 123</td>
<td>2/20</td>
</tr>
<tr>
<td>EA4</td>
<td>IgM</td>
<td>1.5</td>
<td>25600</td>
<td>ND</td>
<td>8/12</td>
</tr>
<tr>
<td>Anti-MCMV Ig</td>
<td>All isotypes</td>
<td>0.6</td>
<td>&gt;102400</td>
<td>Multiple</td>
<td>12/12</td>
</tr>
<tr>
<td>NMS</td>
<td>All isotypes</td>
<td>&gt;500</td>
<td>&lt;20</td>
<td>None</td>
<td>3/16</td>
</tr>
</tbody>
</table>

* Neutralization titres are shown as the concentration of antibody (in μg/ml) required to reduce the number of MCMV plaques by 50%.
† The determination of ELISA titres is described in Methods.
‡ The reactivity of MAbs and polyclonal Ig with MCMV proteins was detected by Western blotting or immunoprecipitation (Farrell & Shellam, 1990).
§ Groups of between 12 and 20 BALB/c mice were inoculated i.p. with 500 μg antibody 24 h prior to a 4 LD₅₀ i.p. MCMV challenge.
¶ Values underlined indicate the MCMV protein with which the MAbs exhibited greatest reactivity. ND, Not determined.
* The χ² test was performed with Yate's correction.

Table 2. **Effect of a single inoculation of MAbs on MCMV titre in spleens and livers of BALB/c and C57BL/10 mice during acute MCMV infection**

<table>
<thead>
<tr>
<th>Antibody (μg) required to reduce MCMV titres by 50% (days 2 and 4 p.i.)* (% virus reduction following 250 μg antibody)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse strain</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>BALB/c</td>
</tr>
<tr>
<td>BALB/c</td>
</tr>
<tr>
<td>C57BL/10</td>
</tr>
</tbody>
</table>

* Mice were inoculated i.p. with either 1, 10, 100 or 250 μg of antibodies 24 h prior to an i.p. MCMV challenge. BALB/c mice received 10⁴ p.f.u. MCMV; C57BL/10 mice received 10⁵ p.f.u. p.f.u. MCMV. MCMV titres of organs taken at days 2 and 4 p.i. were determined by plaque assay as described in Methods. Spleen titres in p.f.u./organ (±SD) of control BALB/c mice were 1306 ± 184 and 163221 ± 9575 on days 2 and 4 p.i. respectively. Spleen titres in C57BL/10 mice were less than 400 p.f.u./organ and were not considered for analysis. Liver titres of control BALB/c mice were 60088 ± 5058 and 21527 ± 2726 on days 2 and 4 p.i. respectively; for control C57BL/10 mice, titres were 220147 ± 18435 and 72505 ± 7066 on days 2 and 4 p.i. respectively. Control mice received PBS instead of antibodies prior to MCMV infection.
† Determined on days p.i. when peak titres in control mice were detected, being day 2 p.i. for the liver and day 4 p.i. for the spleen.
‡ NR, No reduction in MCMV titre.
(Scalzo et al., 1990). In the light of these observations, the ability of either passively administered MAbs or polyclonal Ig to restrict MCMV dissemination in the spleens and livers of BALB/c and C57BL/10 mice was assessed. The efficacy of protection was determined by measuring the minimum dose of each antibody required to reduce MCMV titres by 50% in these organs relative to control mice (Table 2). A 50% reduction in MCMV titre was shown to be significant compared with control mice (P < 0.05). However, the effect of MAbs on spleen titres in C57BL/10 mice could not be determined because virus titres were below the detection limits of the assay in both control and antibody-treated mice. In addition, the maximum reduction in MCMV titre detected in antibody-treated mice during days of peak virus titre was determined.

Firstly, with respect to the liver, a single 10 μg i.p. inoculation of either MAb 2F7 or 3B2, or polyclonal Ig was required to reduce virus titres by 50% in C57BL/10 and BALB/c mice on days 2 and 4 p.i., whereas 100 μg of either MAb 4F9 or AC1 was required to achieve this reduction. Furthermore, each of the four MAbs and polyclonal Ig effected an 18 to 100% reduction in peak MCMV titres in the liver at day 2 p.i. in both mouse strains. Although 250 μg of MAb 1E8 was required to reduce the MCMV titre by 50% in the livers of C57BL/10 mice, significant protection was not afforded by this MAb in BALB/c mice at the doses tested. Similarly, MAb 1E8 produced a 77% reduction in peak liver titres in C57BL/10 mice but the protection of BALB/c mice by this MAb was markedly lower. Finally, MAbs 1G4 and 1B4 failed to reduce virus titres significantly in the livers of either mouse strain.

For the spleen, a single dose of 10 μg of MAb 3B2 or polyclonal Ig was effective in reducing virus titres by 50% in BALB/c mice. In addition, only MAb 3B2 and polyclonal Ig gave a maximum reduction in excess of 90% of titres detected in control mice. Of interest was the observation that MAbs 2F7, 4F9 and AC1 were ineffective in reducing titres in the spleen of BALB/c mice, in contrast to the protection observed in the liver, suggesting that the level of protection afforded by these MAbs may be dependent on the organ in which MCMV replication is assayed. Furthermore, the administration of MAbs 1E8, 2F7 and AC1 and polyclonal Ig caused a modest, although significant, increase (P < 0.05) in splenic virus titres at all doses tested (Table 3). Thus, enhanced splenic MCMV replication was detected despite a concomitant reduction in virus titre in the liver. Enhanced splenic MCMV replication was observed in all BALB/c mice pretreated with either 1, 10, 100 or 250 μg of MAb 1E8, whereas the titres of mice pretreated with MAbs 2F7 and AC1 and polyclonal Ig were enhanced at doses of 1 or 10 μg only, indicating that this phenomenon was dose-dependent. Indeed, previous experiments showed that 250 μg of MAb AC1 or polyclonal Ig reduced splenic virus titres by 33% and 100% respectively (Table 2). Finally, the prophylactic inoculation of BALB/c mice with non-neutralizing MAbs 1B4 or 1G4 had no significant effect on splenic MCMV titres.

### Table 3. Maximum enhancement of splenic MCMV titres in BALB/c mice following prophylactic inoculation with either MAbs 1E8, 2F7 or AC1 or polyclonal Ig

<table>
<thead>
<tr>
<th>Day p.i.</th>
<th>1E8</th>
<th>2F7</th>
<th>AC1</th>
<th>Anti-MCMV polyclonal Ig</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>3.43 (250)†</td>
<td>3.21 (10)</td>
<td>3.03 (10)</td>
<td>1.54 (10)</td>
</tr>
<tr>
<td></td>
<td>P &lt; 0.005</td>
<td>P &lt; 0.005</td>
<td>P &lt; 0.005</td>
<td>P &lt; 0.005</td>
</tr>
<tr>
<td>4</td>
<td>1.23 (250)</td>
<td>1.36 (10)</td>
<td>1.76 (1)</td>
<td>&lt;1.00†</td>
</tr>
<tr>
<td></td>
<td>P &lt; 0.05</td>
<td>P &lt; 0.05</td>
<td>P &lt; 0.05</td>
<td>P &lt; 0.05</td>
</tr>
</tbody>
</table>

† Values in parentheses indicate the antibody dose (μg) at which the maximum ratio of virus titres between antibody-treated and control mice were detected.

* Mice were inoculated with a single dose of either 1, 10, 100 or 250 μg of MAbs or polyclonal Ig 24 h prior to an i.p. inoculation of 10⁴ p.f.u. MCMV. Control mice received PBS instead of antibody. MCMV titres of control mice are presented in Table 2. Values >1:00 indicate that an increased level of MCMV replication was detected in antibody-treated mice compared with control mice. The maximum ratio observed between MCMV titres of antibody-treated and control mice are shown.

Effect of neutralizing and non-neutralizing MAbs on MCMV replication in the salivary gland

The effect of passive immunization with MAbs before MCMV challenge was also studied to determine whether the persistence of MCMV infection in the salivary gland could be prevented or significantly reduced in titre. Accordingly, 100 μg of each MAb was inoculated i.p. to groups of five BALB/c mice 24 h prior to a 10⁴ p.f.u. MCMV challenge and virus titres on days 7, 14 and 21 p.i. were compared to those from mice which received 100 μg NMS. Titres in mice from all groups were determined individually.

Although virus titres in the salivary glands of all groups of mice on day 7 p.i. did not differ significantly (P > 0.05), the titres in mice inoculated with either neutralizing MAbs 3B2, 4F9 or EA4, or the non-neutralizing MAb 1G4, were lower than those in controls at day 14 p.i. (P < 0.05; data not shown). Nevertheless, equivalent virus titres were detected in the salivary glands from all groups of mice on day 21 p.i. Thus, a
single inoculation of neutralizing or non-neutralizing MAbs did not protect against the establishment of persistent MCMV infection.

Ability of MAbs to protect mice against a lethal MCMV infection

With the exception of MAb 1G4, the i.p. administration of 500 μg MAb 24 h prior to a 4 LD$_{50}$ (10$^{6.1}$ p.f.u.) MCMV challenge in BALB/c mice resulted in a significantly reduced mortality rate compared to that of control mice (Table 1). No difference was observed in the time to death between antibody-treated and control mice, with >75% of deaths occurring on day 5 p.i. Although highly neutralizing MAbs 2F7 and 3B2 afforded greater protection than the non-neutralizing MAbs 1B4 and 1G4, no direct correlation was observed between the level of protection in vivo and either neutralization titres in vitro or reduction in virus titres in the spleen and liver in vivo. Indeed, comparable protection was produced by MAbs 1E8, 1B4 and EA4 despite their having different neutralizing titres in vitro.

Effect of passive transfer of MAbs in A/J mice

Since complement was required by the MAbs for neutralization in vitro, the possible role of the lytic pathway of complement in protection in vivo was studied using A/J mice which are deficient in the fifth component of complement (Nilsson & Müller-Eberhard, 1967). The ability of a single prophylactic i.p. inoculation of 100 μg of either MAb 2F7 or 4F9 to reduce virus titres in the spleen or liver following a 10$^5$ p.f.u. i.p. MCMV infection was determined. These MAbs were chosen as they were highly neutralizing in vitro and reduced virus titres in the livers of complement-sufficient BALB/c and C57BL/10 strains. In addition, given that both A/J and BALB/c mice sustain high levels of splenic MCMV replication (Allan & Shellam, 1984) and that previous experiments have shown an enhancement in MCMV replication in BALB/c mice pretreated with these MAbs, it was of interest to determine the effect of these MAbs on splenic MCMV titres in A/J mice. The use of these MAbs also allowed a comparison to be made between the requirement for complement by an IgM and an IgG2b MAb.

The MAbs were ineffective in reducing virus titres in either spleens or livers of the A/J strain on day 2 p.i., whereas reduced MCMV replication was observed in both organs of antibody-treated A/J mice on day 4 p.i. and in the livers of BALB/c mice on days 2 and 4 p.i. (Table 4). These results suggest that the C5 component of complement was not required for the reduction of MCMV titres mediated by the MAbs in vivo. Indeed, subsequent studies showed that sera from BALB/c were as equally ineffective as A/J sera as a source of complement for the neutralization of MCMV by the MAbs in vitro (not shown). Whereas the MAbs were effective in reducing titres in the livers of BALB/c mice at both time points, a significant reduction in splenic virus titre was not detected, in agreement with previous results (Table 2). These results indicate that the genetic constitution of the host influences the efficacy of protection afforded by the MAbs. Alternatively, in the absence of specific antibody, the possession of the full complement cascade may promote the dissemination of splenic MCMV replication.

### Table 4. The effect of passive immunization on MCMV titre in spleens and livers of BALB/c mice and complement-deficient A/J mice

<table>
<thead>
<tr>
<th>Mouse strain</th>
<th>Organ</th>
<th>Day p.i.</th>
<th>Passive antibody transfer*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Control†</td>
</tr>
<tr>
<td>BALB/c</td>
<td>Spleen</td>
<td>2</td>
<td>3.91‡</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>2</td>
<td>4.81‡</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>4</td>
<td>4.39§</td>
</tr>
<tr>
<td>A/J</td>
<td>Spleen</td>
<td>2</td>
<td>3.84</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>4</td>
<td>4.48</td>
</tr>
</tbody>
</table>

* Groups of three mice received 250 μg MAb i.p. 24 h prior to being infected with either 10$^4$ p.f.u. (BALB/c) or 10$^3.7$ p.f.u. (A/J) MCMV. † Control mice received 250 μg NMS. ‡ Virus titres of organ homogenates were determined by plaque assay and are expressed as log$_{10}$. Standard errors were less than 10% of the mean and are not shown. § Values underlined denote titres which were significantly lower than titres from control mice (P < 0.05).

Discussion

The mechanisms by which antibodies neutralize virus infectivity in vivo are not clearly understood, although it appears that in addition to antibody specificity, antibody isotype as well as cellular and host factors contribute to the neutralization process (reviewed by Dimmock, 1984, 1987). Taking these parameters in turn, we have identified MAbs specific for the structural proteins of MCMV which significantly reduced the level of hepatic MCMV replication in mice during acute MCMV infection and protected against a lethal MCMV challenge. In particular, MCMV proteins of 50K, 60K, 92K and 98K were each recognized by at least one MAb which neutralized MCMV in vitro and afforded protection in vivo. In a previous study (Farrell & Shellam, 1989),
MCMV proteins of 50K, 92K and 98K were shown to be highly immunogenic in mice, with polyclonal Ig to these proteins being detected in sera by Western blotting within 7 to 14 days p.i. Thus, it is possible that antibodies specific for these MCMV proteins contribute to the reduction of MCMV titres early in infection. Indeed, previous studies have shown that immune sera derived from mice in the first week p.i. were effective in passively protecting naive mice from MCMV challenge (Araullo-Cruz et al., 1978; Lawson et al., 1988). However, in contrast to studies by Araullo-Cruz et al. (1978), which attributed protection against lethal infection to the IgG rather than the IgM fraction of polyclonal Ig, the data presented here demonstrated that both IgM and IgG2b antibody isotypes were protective.

Neutralizing MAbs were more effective in restricting MCMV replication in the livers of BALB/c and C57BL/10 mice than non-neutralizing MAbs but no correlation was observed between the ability of MAbs to neutralize MCMV in vitro and the protection of mice against a lethal MCMV challenge. A lack of correlation between in vitro and in vivo functions of antibodies to herpes simplex virus (HSV; Dix et al., 1981; Balachandran et al., 1982), Sindbis virus (Schmaljohn et al., 1982), vesicular stomatitis virus (Lefrancois, 1984), mumps virus (Löve et al., 1986) and Semliki Forest virus (Boere et al., 1985) has also been described. It is possible that neutralizing epitopes for non-neutralizing MAbs are masked on the intact virion in vitro. Antigenic sites may become accessible on the surface of the infected cell, thus facilitating interaction with non-neutralizing MAbs in vivo.

Previous studies have identified differences between mouse strains in the protective effect of MAbs to HSV (Balachandran et al., 1982). Evidence is presented in this study which demonstrates the contribution of the host towards the efficacy of immunoprophylaxis. In vivo, MAb 1E8 was more effective in reducing MCMV titres in the livers of C57BL/10 mice than in BALB/c mice. In addition, high titres of splenic MCMV replication in genetically susceptible BALB/c mice were not reduced by the MAbs, with the exception of MAb 3B2. Significantly, mice inoculated i.p. with neutralizing MAbs 1E8, 2F7 and AC1, and polyclonal Ig, exhibited splenic MCMV titres which were enhanced compared to those in control mice. The mechanism of this enhancement has not been determined, although previous studies have indicated that MCMV infection of peritoneal cells is enhanced by the inoculation of either sub-neutralizing or non-neutralizing doses of polyclonal Ig (Inada et al., 1985; Inada & Mims, 1985), a phenomenon which has been described for dengue, Sindbis and West Nile viruses (Halstead & O'Rourke, 1977; Chanas et al., 1982; Peiris et al., 1982). Studies not reported here have confirmed that MCMV infection of peritoneal cells is enhanced in the presence of sub-neutralizing concentrations of polyclonal Ig. However, no such enhancement has been observed with peritoneal cells pretreated with the MAbs.

The complement-dependent lysis of infected cells bound with both neutralizing and non-neutralizing antibodies as a possible mechanism of antibody protection has been described for other viruses (Balachandran et al., 1982; Schmaljohn et al., 1982; Boere et al., 1985). However, in the present study, A/J mice deficient in the C5 component of complement (Nilsson & Müller-Eberhard, 1967) were protected by either of two neutralizing MAbs as effectively as were C5-sufficient BALB/c mice, which suggests that the lytic pathway of complement is not critical for protection in the A/J strain. Indeed, data not presented here have shown that sera from BALB/c mice are as equally ineffective as A/J sera as a source of complement for the neutralization process in vitro. Conversely, the lysis of virus-infected cells by specific antibody and complement may contribute to the pathology of the infection (Perrin et al., 1976). Complement-mediated inflammation and the deposition of immune complexes in renal glomeruli during acute and latent MCMV infections have been reported (Olding et al., 1976; Chong & Mims, 1983).

The lysis of virus-infected cells by antibody-dependent cellular cytotoxicity (ADCC) may be another mechanism by which IgG MAbs afford protection, particularly for poorly neutralizing antibodies (Lefrancois, 1984). Indeed, this mechanism has been described for HSV (Shore et al., 1974; Kohl et al., 1979). Using a panel of IgG MAbs specific for MCMV, the contribution of ADCC towards the lysis of MCMV-infected MEF and the ability of F(ab')2 fragments of MAbs to protect in vivo is currently being addressed.

The results presented here demonstrate the protective effect against MCMV of passively administered MAbs in immunocompetent mice. However, as host cellular factors of recipient mice appear to participate in the protection afforded by the MAbs, we are currently examining the ability of the MAbs to protect X-irradiated and immunosuppressed mice. Previous studies have shown that either X-irradiation or treatments which ablate thymus-dependent lymphocyte reactivity reduce the protective effect of MAbs to HSV-1 and HSV-2 (Oakes et al., 1980). Nevertheless, studies by Shanley et al. (1981) demonstrated that administration of polyclonal Ig prior to subcutaneous MCMV challenge effectively reduced virus dissemination in both immunosuppressed and immunocompetent mice. An evaluation of combinations of MAbs would also be of interest because it has been shown previously that MAb 1E8 inhibits the neutralizing activity of several other MAbs (Farrell &
Shellam, 1990). In addition, it has been shown that antibodies may enhance each other's neutralizing effects (Dimmock, 1984).

Studies of the murine model of CMV infection provide the opportunity to define antigenic determinants on viral proteins which are important recognition sites for MAbs in functional assays in vitro or of biological relevance in vivo. This study shows that the ability of MAbs to protect mice against MCMV cannot be determined by in vitro measurements of biological function, such as antigen binding or neutralization. These results confirm the reports of others (reviewed by Dimmock, 1987) and suggest interesting implications for viral immunity in general and for vaccine strategy. Elucidation of the mechanism(s) of neutralization of virus by antibodies in target tissues in vivo is required to achieve effective immunotherapy. Studies of these mechanisms in the murine model are being investigated. Finally, the criteria for the selection of hyperimmune Ig for MAbs for passive immunotherapy against CMV infection in man have been based on the titres of immunoreactive and neutralizing antibodies specific for CMV in vitro, but recent studies have indicated that these criteria may not be appropriate (Emanuel et al., 1988). By defining these criteria using MAbs to MCMV, the murine model should continue to provide very useful insights into the management of CMV infections in man.

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References


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