Effect of host genotype in determining the relative roles of natural killer cells and T cells in mediating protection against murine cytomegalovirus infection

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The influence of host genotype on the relative importance of T cell subsets and natural killer (NK) cells in controlling murine cytomegalovirus (MCMV) replication has been investigated. Genetically susceptible BALB/c and A/J, moderately resistant C57BL/10, and resistant CBA/CaH mouse strains were treated with monoclonal antibodies (MAb) to the CD4 and CD8 markers and the extent of MCMV replication in major target tissues was determined. Both mouse strain-specific and tissue-specific effects were observed. CBA/CaH and C57BL/10 mice were found not to require CD4⁺ or CD8⁺ T cells for control of MCMV replication in the spleen or liver. In contrast, in A/J mice, as well as BALB/c mice, the CD8⁺ T cell population was primarily responsible for the clearance of virus from these tissues. However, in all strains of mice, CD4⁺ T cells were required for delayed type hypersensitivity and antibody responses, and for virus clearance in the salivary glands. The dependence of mice with the BALB genetic background on CD8⁺ T cells for limitation of acute MCMV infection was found to be negated in the BALB.B6-Cmv1⁺ congenic strain, in which an effective NK cell response has been generated through the introduction of the resistant Cmv1⁺ allele from C57BL/6 mice. Depletion of NK cells in the BALB.B6-Cmv1⁺ strain using anti-NK1.1 MAb restored the role of CD8⁺ T cells in mediating viral clearance. These analyses demonstrate that some, but not all, strains of mice use CD8⁺ T cells to control MCMV replication and that even when CD8⁺ T cell-dependence exists, this can be circumvented by an appropriate NK cell response.

Introduction

The genetic makeup of the host is known to influence the response to infection with a number of pathogens including the herpesvirus murine cytomegalovirus (MCMV) (reviewed by Malo & Skamene, 1995). The resistance of inbred strains of mice to lethal infection caused by this virus is mediated by genes that are located both within and outside the MHC [Grundy (Chalmer) et al., 1981]. Mice with the H-2k haplotype for example, are 10-fold more resistant to lethal MCMV infection than congenic mice with either the H-2d or H-2k haplotypes. In addition, genes located outside the MHC confer increased resistance in the C57BL, C3H/HeJ and CBA/CaH strains of mice.

The mechanisms by which the host genotype alters the progression and outcome of MCMV infection are not fully understood. Both susceptibility of cells to infection (Price et al., 1987, 1990), and the effectiveness of the host response appear to be influenced by host genotype [Allan & Shellam, 1985; Grundy (Chalmer) et al., 1982; Lawson et al., 1989]. Previous studies have shown that the relative importance of natural killer (NK) cell activity, interferon (IFN) production and virus-specific T cell responses in the control of MCMV infection correlate with genotype in certain cases. Thus, in the resistant CBA/CaH and moderately resistant C57BL/6 and C57BL/10 strains early protective mechanisms such as IFN production [Allan & Shellam, 1985; Grundy (Chalmer) et al., 1982] and NK cell activity (Bancroft et al., 1981; Bukowski et al., 1984; Shellam et al., 1985) control infection within 48 h of infection, before T cell responses reach maximum activity. In contrast, the more susceptible BALB/c mice rely on CD8⁺ T cell-mediated immunity for the control of MCMV infection (Reddehase et al., 1985, 1987).

Earlier studies demonstrating roles for CD4⁺ and CD8⁺ T cells in the control of MCMV infection have generally used the
BALB/c mouse strain (Erlich et al., 1989; Joniec et al., 1989, 1990; Reddehase et al., 1985, 1987). However, it appears that the importance of the T cell response may differ between strains of mice. A comparative study of the susceptibility of athymic mice to MCMV suggested that host genotype influences the importance of T cell-mediated immunity (Grundy & Melief, 1982). Furthermore, cyclosporin A treatment, which inhibits T cell function, has been shown to markedly increase the susceptibility to infection of BALB/c and A/J mice, but not that of more resistant mouse strains (Lawson et al., 1989). Thus, our first aim was to examine the importance of immunity mediated by T cell subsets in the control of MCMV infection in a panel of inbred strains of mice, including a second susceptible mouse strain (A/J), and two relatively resistant mouse strains (CBA/CaH and C57BL/10). This represents the first analysis of the role of T cell subset-mediated immunity in controlling MCMV infection in inbred strains of mice differing in resistance to MCMV infection.

NK cells also provide protection against MCMV infection in some relatively resistant strains of mice (C57BL/6J, C57BL/10, CBA/CaH, C3H) (Shellam et al., 1981; Bancroft et al., 1981; Bukowski et al., 1984), in contrast to the BALB/c strain, in which MCMV induces a less effective, though strongly activated NK cell response (Scalzo et al., 1992). Thus, it was of interest to determine whether superimposition of a more potent NK cell response in BALB/c mice would alter the course of infection and the role of T cells. NK cell control of MCMV replication in the spleens of C57BL/6J mice has been shown to be regulated by the Cmv1 gene (Scalzo et al., 1992). C57BL/6J mice, which have the Cmv1T resistance allele at this locus, have increased resistance to MCMV infection when compared to BALB/c or A/J mice, which have the susceptibility allele (Cmv1b) (Scalzo et al., 1990). Mice of the BALB.B6-Cmv1T congenic strain possess the portion of chromosome 6 containing the C57BL/6J alleles for Cmv1T and genes of the NK cell gene complex (NKC), but otherwise have the BALB/c genotype (Scalzo et al., 1995). NK cells from this mouse strain express the NK1.1 antigen, and the depletion of these cells by in vivo antibody treatment leads to high levels of MCMV replication in the spleens of infected animals. Thus, the development of the BALB.B6-Cmv1T congenic mouse strain provided a unique opportunity to investigate the effect of a strong NK-cell response on the generation of a virus-specific CD8+ T cell response. In this study, mice were depleted of NK1.1+ NK cells and/or CD4+ and CD8+ T cell subsets, and the level of replication of MCMV was assessed. The results demonstrate that in the presence of an effective NK cell response, a CD8+ T cell response is not essential.

**Methods**

**Animals.** Specific pathogen-free inbred BALB/c, A/J, CBA/CaH, C57BL/10 and BALB.B6-Cmv1T mice and outbred Swiss/ARC mice were obtained from the Animal Resources Centre (Murdoch, Western Australia), and housed under minimal disease conditions. All mice used were between 8 and 14 weeks of age. The production of the BALB.B6-Cmv1T congenic mouse strain has been described previously (Scalzo et al., 1995a).

**Virus.** The K181 strain of MCMV was prepared as a salivary gland homogenate from infected 3-week-old female BALB/c mice, and stored in the gaseous phase of liquid nitrogen. Mice were inoculated via the intraperitoneal (i.p.) route with MCMV diluted in PBS supplemented with 1% fetal calf serum (FCS), using a dose adjusted to 0.25 LD50 for each inbred strain (Grundy et al., 1981). Accordingly, A/J mice received 1.7 x 10^6 p.f.u., CBA/CaH mice 1.2 x 10^6 p.f.u., C57BL/10 mice 2.7 x 10^6 p.f.u., BALB/c mice 5 x 10^6 p.f.u. and BALB.B6-Cmv1T congenic mice 2 x 10^6 p.f.u.

**In vivo depletion of T cells or NK cells.** The monoclonal antibodies (MAbs) used for in vivo depletion of cells were PK.136 (murine IgG2b, anti-NK1.1) (Koo & Peppard, 1984), YTS-169.4 (rat IgG2a, anti-CD8) (Cobbold et al., 1984), and YTS-191.1 (rat IgG2a, anti-CD4) (Cobbold et al., 1984). Ascites of the PK.136 hybridoma was prepared in pristane-primed female (C3H/HeJ x BALB/c)f1 mice. The YTS-169.4 and YTS-191.1 ascites were prepared in pristane-primed (Lou/M x DA/F) rats. For the in vivo depletion of both T cells and NK cells, mice were injected with 100 ml of (NH4)2SO4-precipitated antibody (protein concentration 25 mg/ml), diluted 1:2 with PBS supplemented with 1% FCS, by either the i.p. or intravenous route. The route by which antibody was administered did not affect either the success of the T cell subset depletion or the patterns of virus replication obtained within the different treatment groups. Antibody was administered 2 days prior to infection with MCMV, on the day of infection, 2 days after infection, and subsequently every fourth day to maintain depletion of the appropriate cell population, a regime optimized in preliminary experiments.

**Fluorescence staining and flow cytometry.** The MAbs in tissue culture supernatants used for phenotypic analyses were 30F11 (rat IgG2a, anti-CD8) (Sarmiento et al., 1980) and RL-172 (rat IgM, anti-CD4) (Ceredig et al., 1985). Binding of the anti-T cell subset MAbs 3.168.8 and RL-172 was not inhibited by the presence of YTS-169.4 or YTS-191.1, respectively.

Single cell suspensions were prepared from the pooled mesenteric and inguinal lymph nodes of mice from each treatment group. Adherent cells were removed by incubation on plastic at 37 °C for 45 min. Non-adherent lymph node cells (2 x 10^5) were incubated with antibody for 20 min on ice, washed twice with PBS supplemented with 5% newborn calf serum (NCS) and 0.1% NaN3, and resuspended in mouse-adSORbed, FITC-conjugated, goat anti-rat immunoglobulin (Silenus). Following incubation for 20 min on ice, the cells were washed and resuspended in 50 µl of 10 µg/ml ethidium monoazide (Molecular Probes) to identify and exclude dead cells from the analysis. After a minimum of 15 min in the dark on ice, the cells were exposed to fluorescent light in a laminar flow hood for 15 min, washed three times, and fixed with 1% paraformaldehyde. A FACScan (Becton Dickinson) flow cytometer was used to collect 6000 live events for each sample, and the data were analysed using the LYSYS software.

**MCMV plaque assay.** The plaque assay performed on secondary mouse embryo fibroblast (MEF) cultures to quantify titres of MCMV (Shellam et al., 1989). Thus, our first aim was to examine the role of T cell subset-mediated immunity in controlling MCMV infection in inbred strains of mice differing in resistance to MCMV infection.

**Cell lines.** Primary MEF were prepared from 14- to 15-day-old embryos of Swiss/ARC mice and maintained in Minimal Essential
Medium (MEM) supplemented with 10% NCS and 80 μg/ml gentamicin. The YAC-1 and P815 target cell lines were cultured in RPMI 1640 supplemented with 10% NCS and 80 μg/ml gentamicin.

- **NK cell cytotoxicity assay.** Single cell suspensions were prepared from spleens by gentle homogenization in cold RPMI supplemented with 5% FCS and 10 mM-HEPES. Effector spleen cells were added to $1 \times 10^5$ $^{51}$Cr-labelled YAC-1 target cells in a volume of 200 μl of RPMI supplemented with 10% FCS and 10 mM-HEPES, at effector-to-target ratios of 100:1 to 12:1, in triplicate. Following incubation for 4 h at 37 °C, 100 μl of supernatant from each well was removed and $^{51}$Cr release measured in a gamma counter (Packard). Total release was determined by addition of 1% Triton X-100. Data are presented as percentage specific lysis = ([(experimental c.p.m. – spontaneous c.p.m.) / (total c.p.m. – spontaneous c.p.m.)] × 100). The spontaneous release values were generally less than 15% of the total release values.

- **T cell cytotoxicity assay.** Erythrocytes present in single cell spleen suspensions were lysed by incubation with Tris-buffered ammonium chloride (0·14 m-NaHCl and 0·017 m-Tris, pH 7·2) at 37 °C for 5 min. Cells were then resuspended at a concentration of $2 \times 10^6$/ml in RPMI supplemented with 10% FCS, 10 mM-HEPES, 2 mM-L-glutamine and 10^{-2} M-mercaptoethanol. For the *in vitro* restimulation of virus-specific CTL, the peptide YPHFMPTNL from the pp89 immediate early protein of MCMV (Del Val et al., 1988) was used. P815 (H-2^d) stimulator cells were coated with peptide by incubation with a peptide concentration of 50 μM in RPMI 1640 for 30 min at 37 °C. After two washes, the peptide-coated P815 cells were X-irradiated with 80 Gy, and added to spleen cell suspensions at a ratio of 1:50. After 5 days incubation at 37 °C, the cultured spleen cells were tested for cytotoxicity against $^{51}$Cr-labelled, peptide-pulsed, P815 cells.

- **MCMV antibody measurement.** Blood samples were collected by cardiac puncture from anaesthetized mice, and sera stored at −70 °C until assay. The ELISA method used to determine combined IgG + IgM levels has been described previously (Lawson et al., 1988). Serial 2-fold dilutions of pooled sera from three mice per group were tested, and the highest dilution resulting in a specific absorbance value of $>0·1$ (when compared to normal mouse serum), was recorded as the reciprocal endpoint dilution.

- **Measurement of delayed type hypersensitivity (DTH) response.** Ear swelling was used as a measure of DTH to MCMV, as described previously (Lawson et al., 1987). The DTH response was measured 24 h after ear challenge with a pre-determined amount of UV-inactivated, tissue-culture passaged MCMV. The data are presented as mean percentage increase in ear thickness from three mice per group, in comparison to that of the control ear, according to the formula (mean thickness of right ear − mean thickness of left ear) x100/mean thickness of left ear.

- **Statistical analysis.** Statistical significance was established using Student's *t*-test.

### Results

**Effect of depletion of T cell subsets on the course of MCMV infection in A/J, CBA/CaH and C57BL/10 mouse strains**

The role of T cell subsets in the control of MCMV infection has been described primarily in studies of the BALB/c strain (Ehrlich et al., 1989; Jonjic et al., 1989, 1990; Reddehase et al., 1985, 1987), and our studies of this strain (see below) are in accordance with those observations. To examine whether our understanding of the data generated has been biased by the choice of mouse strain, the effect of T cell subset depletion on the course of MCMV infection in A/J, CBA/CaH and C57BL/10 mice was investigated. Virus titres in major target tissues (the spleen, liver and salivary glands) of mice treated with MAbs to CD4 and/or CD8 were determined at days 2, 5, 9, and 16 following i.p. infection with MCMV. The amount of MCMV given to each mouse strain was adjusted for the relative susceptibility of mice to lethal disease with MCMV.

The amount of MCMV present in the spleens of A/J mice depleted of either CD8^+ T cells or of both T cell subsets was found to be substantially higher than in the intact control group (Fig. 1a) from day 5 to day 10 after infection. Tissue-specific differences were apparent, as in contrast to the spleen, T cell subset depletion did not have any marked effect on the amount of MCMV present in the livers of infected mice (Fig.1d) during the period of study. All A/J mice exhibited signs of disease, and those treated with either anti-CD8 MAb alone or with both anti-T cell subset MAbs remained clinically affected for longer than mice treated with anti-CD4 MAb or those receiving virus alone.

In contrast to the A/J strain, T cell subset depletion in C57BL/10 and CBA/CaH mice did not result in any substantial increases in the overall replication of MCMV in the spleens (Fig. 1b, e) or livers (Fig. 1c, f). The amount of MCMV present in the spleens and livers of CBA mice was higher than that present in the C57BL/10 mice at day 2 p.i., an observation that is in agreement with other published data (Allan & Shellam, 1984). At no stage during infection were signs of disease observed in the CBA/CaH or C57BL/10 mice. There was increased replication of virus within the salivary glands of all three mouse strains when depleted of either CD4^+ T cells alone, or of both T cell subsets (data not shown). The depletion of the CD4^+ T cell subset in all three mouse strains also resulted in a substantial decrease in the production of anti-MCMV antibodies, and in a significant reduction in the DTH response to MCMV in CBA/CaH and C57BL/10 mice (Table 1).

The results from these investigations show that A/J mice, as previously described for BALB/c mice (Reddehase et al., 1985; see below also for data), require CD8^+ T cells for the control of MCMV replication in the spleen. However, with the exception of the salivary glands, MCMV replication in CBA/CaH and C57BL/10 mice is effectively controlled in the absence of T cells.

**Comparison of the effect of T cell subset and NK cell depletion on the course of MCMV infection in the BALB/c and BALB.B6-Cmv1^c strains of mice**

BALB.B6-Cmv1^c congenic mice have the BALB/c background, but their NK cells express the NK1.1 alloantigen due to the presence of the alleles for the NK cell gene complex
Table 1. Effect of T cell subset depletion on MCMV-specific antibody production and DTH responses

<table>
<thead>
<tr>
<th>Strain</th>
<th>MCMV-infected</th>
<th>in vivo treatment</th>
<th>DTH reading*</th>
<th>Anti-MCMV antibody titre†</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL</td>
<td>--</td>
<td>None</td>
<td>0±0.6</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>PBS</td>
<td>18.9±1.4</td>
<td>25,600</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>Anti-CD4</td>
<td>1.0±0.4</td>
<td>200</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>Anti-CD8</td>
<td>13.8±0.3</td>
<td>6,400</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>Anti-CD4 + anti-CD8</td>
<td>2.1±0.4</td>
<td>3,200</td>
</tr>
<tr>
<td>CBA</td>
<td>--</td>
<td>None</td>
<td>1.9±0.6</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>PBS</td>
<td>10.4±1.6</td>
<td>25,600</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>Anti-CD4</td>
<td>1.5±0.2</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>Anti-CD8</td>
<td>11.0±0.7</td>
<td>25,600</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>Anti-CD4 + anti-CD8</td>
<td>2.1±1.2</td>
<td>3,200</td>
</tr>
<tr>
<td>A/J</td>
<td>--</td>
<td>None</td>
<td>2.4±0.6</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>PBS</td>
<td>9.3±1.2</td>
<td>12,800</td>
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<td></td>
<td>+</td>
<td>Anti-CD4</td>
<td>4.3±1.5</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>Anti-CD8</td>
<td>3.5±1.0</td>
<td>12,800</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>Anti-CD4 + anti-CD8</td>
<td>0.8±0.4</td>
<td>100</td>
</tr>
</tbody>
</table>

* Percentage increase in ear thickness measured at day 9 p.i., expressed as the mean of three mice ± SEM.
† Determined in pooled sera from three mice per group at day 16 p.i.
‡ P < 0.05 when compared to MCMV-infected, PBS control group.
ND, Not determined.

inherited from C57BL/6j mice (Scalzo et al., 1995a). BALB.B6-Cmr1' mice show restricted splenic MCMV replication which can be abrogated by pretreatment with anti-NK1.1 MAb. It was therefore of considerable interest to determine if T cell-mediated immunity played any role in controlling MCMV infection in the presence of such a strong MCMV-controlling NK cell response. The effect of depletion of CD8⁺ T cells, NK1.1⁺ NK cells or of both T cell subsets on the course of
Table 2. Effect of T cell and NK1.1+ cell depletion on in vitro cytotoxic activity by spleen cells from MCMV-infected BALB/c and BALB.B6-CmvI+ mice

<table>
<thead>
<tr>
<th>Strain</th>
<th>in vitro treatment</th>
<th>NK cell activity*</th>
<th>CD8+ CTL activity†</th>
</tr>
</thead>
<tbody>
<tr>
<td>BALB/c</td>
<td>PBS</td>
<td>85</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>Anti-NK1.1</td>
<td>69</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Anti-CD8</td>
<td>91</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Anti-CD4 + anti-CD8</td>
<td>77</td>
<td>ND</td>
</tr>
<tr>
<td>BALB.B6-CmvI+</td>
<td>PBS</td>
<td>86</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>Anti-NK1.1</td>
<td>1</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>Anti-CD8</td>
<td>76</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Anti-CD4 + anti-CD8</td>
<td>86</td>
<td>1</td>
</tr>
</tbody>
</table>

* Percentage lysis at an E:T ratio of 50:1 at day 3 p.i.
† Percentage lysis at an E:T ratio of 40:1 following in vitro stimulation of spleen cells taken at day 9 p.i.
ND, Not done (no live spleen cells were obtained).

MCMV infection in BALB/c and BALB.B6-CmvI+ mice was compared. The success of the in vitro depletion protocol was confirmed by flow cytometry (data not shown), and by in vitro assay of NK cell and CTL functions (Table 2). The CTL response was assessed by measuring cytolytic activity specific for the peptide YPHPMPTNL, a sequence from the MCMV pp89 immediate early protein that has previously been shown to represent the immunodominant response in BALB/c mice (Del Val et al., 1988). It should be noted that the level of MCMV-induced NK cell activity from the control groups of both strains of mice was equally high, demonstrating that the in vitro activity of both NK1.1+ and NK1.1− NK cells against YAC-1 target cells is similar. Similarly, a strong pp89 peptide-specific CTL response was obtained for the control groups of both mouse strains. The extent of tissue damage in the spleens from BALB/c mice that had been treated with anti-T cell MAbs was such that no live cells remained at day 9 p.i.

Fig. 2 summarizes the titres of MCMV present in organs taken from MCMV-infected, antibody-treated BALB/c and BALB.B6-CmvI+ mice. For BALB/c mice, the depletion of CD8+ T cells resulted in significantly higher titres of MCMV in the spleen at both days 9 and 16 p.i. (Fig. 2a). However, an even greater increase in splenic virus titres was observed for the group depleted of both T cell subsets, with the disease in these mice becoming so severe that they had to be sacrificed at day 11 rather than at day 16. In a preliminary experiment the depletion of CD4+ T cells alone did not alter MCMV replication in either the spleens or livers of BALB/c mice (data not shown) during the acute phase of infection.

High titres of MCMV were present at day 3 p.i. in the spleens of BALB.B6-CmvI+ mice that had been treated with anti-NK1.1 MAb (Fig. 2b). In contrast, the amount of MCMV present in the spleens of BALB.B6-CmvI+ mice that had been depleted of either CD8+ T cells or of both T cell subsets was never significantly higher than PBS control levels. Thus, the dependence of mice with a BALB/c genetic background on T cells for the control of MCMV replication in the spleen during the first 9 days of infection was completely negated by the altered NK cell phenotype of the BALB.B6-CmvI+ mice. At day 9 p.i., the titre of MCMV in the spleens of anti-NK1.1 treated mice had returned to PBS control levels, suggesting that in the absence of NK1.1+ NK cells, T cells ultimately mediate clearance of MCMV from the spleens of BALB.B6-CmvI+ mice.

The depletion of both T cell subsets had a marked effect on the replication of MCMV in the livers of BALB/c mice (Fig. 2c). In this mouse strain, virus was not detected in the livers of the PBS control group and anti-NK1.1 MAb treated group other than at day 3, whereas the replication of MCMV in the livers of mice treated with both anti-CD4 and anti-CD8 MAbs continued to increase for the duration of the experiment. Titres of MCMV present in the livers of BALB.B6-CmvI+ mice depleted of NK1.1+ cells at day 3 p.i. were significantly higher than those present in the PBS control group (Fig. 2d). These data confirm that NK1.1+ NK cells, as regulated by the CmvI locus, also play a role in controlling the replication of MCMV in this organ during the early stages of infection (Scalzo et al., 1992). As observed for the spleen, by day 9 p.i., virus replication in the livers of anti-NK1.1 MAb-treated BALB.B6-CmvI+ mice was barely detectable. Although the depletion of both T cell subsets delayed the complete clearance of MCMV from the livers of BALB.B6-CmvI+ mice, this effect was not consistent between experiments.

Within the salivary glands of BALB/c mice, no significant difference was observed in the titres of MCMV present in any of the treatment groups in this experiment (Fig. 2e). Organs were harvested from the group of mice treated with both anti-CD4 and anti-CD8 MAbs at day 11 p.i., since from a
MCMV clearance from the spleens and livers of BALB.B6-anti-NK1.1 MAb, suggesting that in this mouse strain, NKI.1+ BALB/c mice (Erlich et al., 1989; Jonjic et al., 1989) were in accordance with published data showing that CD4+ T cells alone were depleted, probably underestimated. Results from an earlier experiment (data not shown), in which CD4+ T cells alone were depleted, confirmed the conclusion based on the study of nude BALB/c mice that had been treated with anti-NK1.1 MAb, and infected with 2 x 10^4 p.f.u. of MCMV. While mice of all other groups had recovered or cleared virus by day 8, mice of this treatment group died at this time-point, showing that MCMV replication and resulting disease could not be controlled in the absence of both T cells and NK cells.

![Diagram of virus titres](image_url)

**Fig. 2.** Effect of T cell subset and NK cell depletion on MCMV replication in the spleens (a, b), livers (c, d) and salivary glands (e, f) of BALB/c (a, c, e) and BALB.B6-Cmv1r (b, d, f) mouse strains. The values represent the mean of three mice per group at each time-point. * Values significantly different (P < 0.01) from the PBS control group. † P value obtained by comparison with the day 16 PBS control. ○, PBS control; ●, anti-NK1.1; □, anti-CD8; ■, anti-CD4 + anti-CD8. Salivary gland virus titres were determined on day 9 and day 16 only. The detection limit for the salivary glands was 1 x 10^1 log_10 p.f.u./g. † Harvested on day 11, not day 16. ■, PBS control; ●, anti-NK1.1; ■, anti-CD8; □, anti-CD4 + anti-CD8.

Discussion

We have demonstrated that host genotype directly influences the relative importance of T cell-mediated immunity in the control of MCMV infection. In both the highly resistant CBA/CaH, and moderately resistant C57BL/10 mouse strains, *in vivo* depletion of T cells did not affect the replication of MCMV in either the spleen or liver. The replication of MCMV within the visceral organs of CBA/CaH mice is thought to be controlled before the T cell response is fully activated, by mechanisms such as the level of susceptibility of individual target cells to infection by MCMV (Price et al., 1987, 1990); the production of IFN-α/β (Allan & Shellam, 1985) and the action of NK cells (Bancroft et al., 1981). Similarly, studies using either NK cell deficient C57BL/6 beige mice (Shellam et al., 1981), or C57BL/6J mice selectively depleted of NK cells (Bukowski et al., 1983; Scalzo et al., 1992; Stanley, 1990; Welsh et al., 1990), have demonstrated that NK cells are primarily responsible for the control of MCMV replication in the spleens and livers of C57BL/6J mice during the early stages of infection.

In contrast to the results obtained with the CBA/CaH and C57BL/10 mice, T cells were essential for the control of MCMV replication in the spleens and livers of the susceptible BALB/c and A/J mouse strains. This implies that the IFN and NK cell responses that are induced early in infection are inadequate or ineffective in controlling MCMV replication in these mouse strains. Depletion of both T cell subsets resulted in persistently high titres of MCMV in both the livers and spleens of A/J and BALB/c mice, and in severe disease, confirming the conclusion based on the study of nude BALB/c mice that T cells are necessary for the control of lethal infection (Grundy & Melief, 1982). Using a different experimental approach in which virus-specific T cells were adoptively transferred to irradiated recipients, it has been demonstrated that CD8+ T cells in BALB/c mice mediate MCMV clearance from visceral target organs without any requirement for CD4+ T cells (Reddehase et al., 1985; Jonjic et al., 1989), although CD4+ T cells can clear MCMV in CD8+ T cell-deficient mice (Jonjic et al., 1990; Polic et al., 1996).

The control of MCMV replication in the salivary glands of all five strains of mice studied was dependent on CD4+ T cells, an observation previously described for BALB/c and CBA/CaH mice (Erlich et al., 1989; Jonjic et al., 1989; Lucin et al., 1992). Thus, it appears that CD4+ T cells are generally required to control active infection of the salivary glands regardless of host genotype. In addition to mediating MCMV clearance...
from the salivary glands of infected mice and providing help for anti-MCMV antibody production, CD4+ T cells were also responsible for the MCMV-specific DTH response. This response was ablated in C57BL/10 and CBA/CaH mice that had been depleted of these T cells, providing the first direct evidence that CD4+ T cells are the primary effectors of this response in MCMV-infected mice. Previous studies using cyclosporin A have indirectly implicated T cells as the effector mechanism in the DTH response to MCMV (Lawson et al., 1989).

Our results raise the possibility that NK cells also exert control of MCMV replication within the salivary glands of BALB.B6-Cmvir congenic mice. The effect of the CD4+ T cells in the salivary glands of BALB/c mice is at least in part mediated by the cytokines IFN-γ and TNF-α (Lucin et al., 1992; Pavic et al., 1993), and NK cells can produce both of these cytokines (Anegon et al., 1988; Cuturi et al., 1989; Orange et al., 1995), suggesting a common effector mechanism. At this stage, however, it cannot be excluded that the NK11+ CD4+ TCRint T cell (reviewed by MacDonald, 1995) is responsible, and this possibility is currently under investigation. In an earlier study, a significant increase in the amount of MCMV in the salivary glands was also observed when NK11+ cells were depleted from C57BL/10 mice by treatment with PK136 MAb (Shanley, 1990). In β2m-deficient mice which have an impaired CD8+ T cell response, a similar increase in virus replication in the salivary glands resulted from the depletion of NK11+ cells (Polic et al., 1996).

The results obtained from our experiments and others previously published using both genetically resistant and susceptible mouse strains imply that the presence of an efficient NK cell response early in infection is a major factor in determining whether T cells are required for the ultimate control of acute MCMV replication. This was confirmed by study of the BALB.B6-Cmvir congenic mouse strain. Unlike the parental BALB/c strain, T cells were not required for the control of MCMV replication in either the spleens or the livers of untreated BALB.B6-Cmvir mice. Instead, as observed for C57BL mice, NK11+ Cmvir NK cells were responsible for the early control of virus replication in these target organs of this mouse strain. In the absence of NK11+ cells, T cells were responsible for virus clearance from the liver and spleen.

Thus, the activity of the NK11+ Cmvir NK cells present in the BALB.B6-Cmvir mice alleviates the dependence of BALB/c mice on CD8+ T cells for the control of MCMV replication in the spleen and liver. At present it is not known why C57BL-like, NK11+, Cmvir NK cells are more effective in vivo at controlling MCMV infection than BALB/c-like, Cmvir, NK cells. It will be interesting to see which effector mechanism is dominant in the reciprocal congenic strain – that is, in C57BL/6 mice with BALB/c NK alleles and the Cmvir phenotype. CBA mice are neither NK11+ or Cmvir in genotype (Koo & Peppard, 1984; Scalzo et al., 1990, 1995b), raising the possibility that there are other genetic effects such as the level of IFN-α/β production (as discussed earlier) which may modulate NK cell control of virus replication.

NK cells can be induced to produce a variety of cytokines such as IFN-γ, TNF-α, GM-CSF, IL-3 and M-CSF (Anegon et al., 1988; Cuturi et al., 1989; reviewed by Biron, 1994), and it has recently been established that NK cells are responsible for IFN-γ production early in MCMV infection of C57BL/6 mice, and that IFN-γ contributes to the antiviral effect of NK cells in vivo (Orange et al., 1995). However, in preliminary experiments we have not observed any difference in either the amount of IFN-γ or the kinetics of its production between BALB/c mice and either C57BL/6 or BALB.B6-Cmvir mice.

The Cmvir gene product, which has not yet been characterized, may be directly involved in the recognition of virus-infected target cells, either as a receptor, or as a regulatory protein that influences lysis of target cells. There are precedents for this, as other molecules that are encoded by genes within the NK cell complex on mouse chromosome 6, such as Ly49 and mNKR-P1, are known to act as receptors capable of delivering either positive or negative signals to NK cells (reviewed by Yokoyama, 1995). If Cmvir is a regulatory protein, then an NK cell subset expressing a possible human homologue could exert a similar effect on the requirement for a CD8+ CTL response to HCMV.

MCMV is not the only infectious agent against which NK cells may be the dominant defence mechanism, as defined by host genotype. The resistance of C57BL/6 mice to lethal infection with ectromelia virus is controlled by at least three autosomal dominant genes (Brownstein et al., 1991). One of these, Rnp1, is located on mouse chromosome 6, and it appears to be linked to the NK cell complex with its effect being mediated by NK cells (Delano & Brownstein, 1995), as found for Cmvir. In BALB/c mice, NK cells are unable to control the early dissemination of Leishmania major from the site of infection, resulting in severe disease (Laskay et al., 1995). In contrast, in resistant C57BL/6 mice, NK11+ cells restrict the spread of the parasite within the first 24 h of infection, and severe disease does not develop. The relative importance of this restriction in parasite dissemination, and the precise role of NK cells in the subsequent development of protective T cell responses in resistant mice is unknown.

As a positive correlation has been observed between the development of significant levels of nonspecific cytotoxic activity and the recovery of bone marrow and renal transplant patients from HCMV disease, the importance of NK cells in controlling HCMV infection should not be underestimated (Quinnan et al., 1982; Bowden et al., 1987; Venema et al., 1994). If there is heterogeneity in the effectiveness of the NK cell response against HCMV, then it may be possible to control HCMV disease in some instances by the adoptive transfer of NK cell clones, or the use of NK cell stimulatory factors.

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References


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