Key words: cytomegalovirus, murine/cyclosporin/MHC

The Effect of Cyclosporin on Major Histocompatibility Complex-linked Resistance to Murine Cytomegalovirus

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SUMMARY

The ability of mice to survive infection with murine cytomegalovirus (MCMV) is known to be influenced by genes of the major histocompatibility complex (MHC). One hypothesis to account for this association is that MHC-linked resistance to MCMV is an ‘immune response’ gene effect, caused by differences in the strength of the MHC-restricted T cell response of mouse strains with different MHC haplotypes. Therefore, removal of T cell responses in mouse strains differing only at the MHC should render them equally susceptible to the virus infection. To test this hypothesis, the immunosuppressive drug cyclosporin (CsA) was used to reduce T cell responses in inbred congenic mouse strains carrying either a resistant or susceptible MHC haplotype. CsA reduced the delayed-type hypersensitivity (DTH) response to MCMV in both resistant and susceptible mouse strains to background levels, equivalent to control uninfected mice. CsA treatment had little effect on the susceptibility of C57BL/10 and B10.BR mice to the virus and the differences in susceptibility between these strains remained. In contrast, CsA increased the susceptibility of the genetically susceptible BALB/c mice (H-2<sup>d</sup>) by 100-fold and increased the susceptibility of resistant BALB.K mice (H-2<sup>k</sup>) by 15-fold. Thus the H-2-determined difference in susceptibility between these strains was increased after CsA treatment. The results obtained with congenic strains show that MHC-linked resistance patterns to MCMV are not eliminated by CsA and suggest therefore that T cells are not responsible for this phenomenon. Interestingly, the mean time to death was delayed for CsA-treated BALB/c mice compared with untreated mice given equivalent virus doses. In addition, although CsA prevented DTH responses in both genetically susceptible A/J (H-2<sup>a</sup>) and resistant CBA (H-2<sup>k</sup>) mice, CsA treatment markedly increased the susceptibility of A/J mice (32-fold) but had little effect on the susceptibility of CBA mice to the virus.

The role of the major histocompatibility complex (MHC) genes in determining the level of resistance of mice to lethal infection with murine cytomegalovirus (MCMV) has been established [Chalmer et al., 1977; Grundy (Chalmer) et al., 1981]. Possession of the H-2<sup>k</sup> haplotype is associated with a 20- to 30-fold increase in resistance compared to otherwise identical strains with H-2<sup>d</sup> or H-2<sup>b</sup> haplotypes [Grundy (Chalmer) et al., 1981]. In addition, non-H-2-linked genes of the C57BL/10 (B10) background increase resistance to MCMV [Grundy (Chalmer) et al., 1981]. Resistance to certain other virus infections is also influenced by the H-2 haplotype including Friend mouse leukaemia virus (Chesebro & Wehrly, 1978), lymphocytic choriomeningitis virus (Oldstone et al., 1973; Zinkernagel et al., 1985), mouse mammary tumour

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virus (Dux, 1983), Moloney leukaemia virus (Debre et al., 1980) and Theiler's virus (Rodriguez & David, 1985).

The importance of MHC-restricted T cell responses in recovery from virus infections (reviewed by Doherty, 1985) suggests that MHC-linked resistance may reflect differences in the ability of T lymphocytes to recognize viral antigens in association with certain MHC alleles (Klein & Nagy, 1982). Thus, the MHC may behave as an immune response (Ir) gene. A variation of this hypothesis is suggested by the recent findings that human CMV (HCMV) contains a gene homologous to the MHC genes (Beck & Barrell, 1988). Although a similar gene has not yet been reported for MCMV, the observation suggests the possibility that a virus-encoded class I MHC-like molecule on the surface of infected cells may be recognized by cytotoxic T cells (Wiley, 1988), and therefore the number of T cell precursors capable of recognizing this molecule could be affected by the host MHC genotype.

The contribution of T cells to resistance to MCMV has been shown by passive transfer of immune spleen cells (anti-theta sensitive) which assist recovery from an otherwise lethal MCMV infection (Starr & Allison, 1977). Also, athymic nude mice display less resistance to MCMV than their heterozygous littermates [Grundy (Chalmer) & Melief, 1982] and treatment of wild mice with anti-theta serum may reactivate a latent MCMV infection into a fatal illness (Gardner et al., 1974). A major proportion of cytotoxic T cells produced following MCMV infection of BALB/c mice are specific for immediate early antigens of MCMV (Reddehase et al., 1986) and cytotoxic T cell clones (Lyt2+, L3T4+) specific for these antigens limit MCMV replication in the lung and adrenal gland (Reddehase et al., 1985, 1987; Reddehase et al., 1988). In addition, the delayed-type hypersensitivity (DTH) response to MCMV has been shown to be greater and more rapid in resistant mice carrying the H-2^k haplotype than in congenic strains bearing the H-2^b or H-2^d haplotypes (Lawson et al., 1987). Furthermore, studies using cyclosporin (CsA), which specifically inhibits T cell receptor (TCR)-mediated activation of T cells (Jenkins et al., 1988), demonstrated marked increases in CMV titres and greater mortality rates in infected mice (Kurtz & Homan, 1982) and guinea-pigs (Bia et al., 1985). The above evidence suggests that the T cell response contributes to genetically determined resistance to MCMV infection. This conclusion implies that alterations to the strength of the T cell response, by an Ir gene mechanism, may affect the levels of resistance of mice to MCMV. Therefore, lack of the T cell immune response in mice, differing only by possession of a resistant or susceptible MHC haplotype, would render them equally susceptible to MCMV infection. However, the results presented in this paper suggest that the MHC-linked resistance patterns to MCMV infection are not T cell-mediated.

The effectiveness of CsA treatment in reducing T cell responses following MCMV infection was tested by measurement of DTH. The ear swelling assay was used as a measure of DTH to MCMV (Smith strain) at 24 h after ear-challenge with 10^5 p.f.u. of heat-treated (56 °C, 30 min) salivary gland-derived MCMV as previously described (Lawson et al., 1987). Heat-treated normal salivary gland homogenate elicited no detectable increase in ear thickness in either immune or uninfected mice of any mouse strain tested. Resistant 8-week-old B10.BR (H-2^k), CBA (H-2^k), moderately susceptible B10 (H-2^b) and susceptible A/J (H-2^a) female mice obtained from the Animal Resources Centre (Murdock, Western Australia) were inoculated with 10^3 p.f.u. of virulent MCMV by the intraperitoneal route (i.p.). The DTH response elicited to heat-treated MCMV antigen was determined at days 0, 4 and 10 post-infection (p.i.; Table 1). As observed previously (Lawson et al., 1987), the DTH response was greater in the resistant B10.BR than the moderately susceptible congenic B10 strain (P < 0.0025). Mice treated with CsA received inoculations of 75 mg/kg/day of fresh CsA dissolved in olive oil (70 μl) by the subcutaneous (s.c.) route beginning 24 h before virus infection and continuing for 25 days, unless otherwise specified. Control mice received similar daily treatments of olive oil. CsA treatment at 75 mg/kg/day, a dose shown to be immunosuppressive (Denham et al., 1980), reduced the DTH response in all strains at both day 4 and 10 p.i. to background levels comparable with responses of control uninfected mice. Thus, the CsA treatment suppressed the induction of a DTH response to MCMV in both resistant and susceptible mouse strains.

To study the effect of CsA on resistance to lethal MCMV infection, the murine congenic pairs
### Table 1. Effect of CsA on the DTH response to MCMV

<table>
<thead>
<tr>
<th>Day p.i.</th>
<th>CsA treatment</th>
<th>B10</th>
<th>B10.BR</th>
<th>CBA</th>
<th>A/J</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mean ± S.E.M.</td>
<td>mean ± S.E.M.</td>
<td>mean ± S.E.M.</td>
<td>mean ± S.E.M.</td>
</tr>
<tr>
<td>0</td>
<td>-</td>
<td>8.1 ± 1.5</td>
<td>9.0 ± 2.0</td>
<td>6.7 ± 3.2</td>
<td>1.9 ± 1.0</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>11.2 ± 1.8</td>
<td>26.0 ± 1.9</td>
<td>22.9 ± 2.7</td>
<td>11.9 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>7.0 ± 2.0</td>
<td>2.9 ± 0.8</td>
<td>6.3 ± 1.0</td>
<td>3.9 ± 1.7</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>15.6 ± 2.3</td>
<td>41.8 ± 1.0</td>
<td>30.7 ± 3.1</td>
<td>12.4 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>7.7 ± 2.9</td>
<td>2.6 ± 1.5</td>
<td>10.5 ± 1.0</td>
<td>1.3 ± 1.0</td>
</tr>
</tbody>
</table>

* Mice were inoculated with $10^3$ p.f.u. of MCMV i.p. and ear-challenged with heat-inactivated (56 °C, 30 min) MCMV antigen, equivalent to $10^5$ p.f.u.
† Mice were inoculated with or without CsA dissolved in olive oil at 75 mg/kg/day s.c. beginning 24 h before virus infection and treatment continued for 11 days p.i.
‡ Mean percentage increase in ear thickness of five mice ± S.E.M.

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B10, BR and B10, BALB.K and BALB/c in addition to A/J, and CBA strains were inoculated with twofold serial dilutions of MCMV to determine the minimum dose which killed 50% of the animals inoculated [LD$_{50}$ value; Grundy (Chalmers) et al., 1981] and were treated daily with or without CsA for 25 days beginning the day before MCMV infection (Table 2). All strains of mice treated with CsA were less resistant to MCMV than untreated control groups.

Although CsA treatment resulted in a slight increase in susceptibility (approx. twofold) for both B10 and B10, BR mice despite the loss of T cell function as assessed by DTH, B10, BR mice remained substantially more resistant (approx. 30-fold) than B10. This result indicates that MHC-linked resistance to MCMV is not mediated by a CsA-sensitive mechanism. In contrast, CsA-treated BALB.K mice showed a sixfold increase in susceptibility whereas CsA-treated BALB/c mice became nearly 100-fold more susceptible to MCMV compared to untreated control mice. These results indicate that a CsA-sensitive response is more important in protecting mice of the BALB/c background than mice of the B10 background. Thus survival of BALB/c mice is particularly dependent on a CsA-sensitive response (presumably T cells) whereas B10 and B10, BR mice are not dependent on such a mechanism. Furthermore, the difference in resistance between BALB/c and BALB.K mice was sixfold for untreated and 15-fold for CsA-treated mice, again indicating that MHC-linked resistance to MCMV is not eliminated by CsA. A dramatic increase in susceptibility to MCMV infection was also observed for CsA-treated A/J mice (30-fold increase) but little effect was seen for CsA-treated CBA mice. Thus removal of T cell responses by treatment of congenic mice, differing only in MHC haplotype, with CsA did not eliminate genetically determined resistance to MCMV infection. Therefore, these results suggest that the differences in strength of the T cell response cannot explain MHC-linked patterns of resistance to MCMV.

The mortality studies revealed different effects of CsA treatment on the time to death for the different mouse strains. Plotting the mean survival time for CsA-treated BALB/c mice against the dose of virus inoculum ($\log_{10}$) yielded a straight line of best fit (correlation coefficient, $r = 0.94$) consistent with exponential growth of the virus to a lethal threshold (Fig. 1). This relationship may also occur for CsA-treated BALB.K mice although longer periods may be required for the low dose challenge of virus to reach lethal levels. This is consistent with the possibility that the virus replicates more slowly in CsA-treated BALB.K than in CsA-treated BALB/c mice. In contrast to BALB/c mice, untreated BALB/c, BALB.K, B10 and B10, BR in addition to CsA-treated B10 and B10, BR mice displayed abrupt transitions from 100% to 0% mortality, usually occurring after a twofold dilution of inoculum (Fig. 2). This behaviour would be expected in animals developing an adaptive immune response, where a race develops between virus replication and the ability of the response to cope with the level of infection as it develops. Reducing virus levels increases the time that the immune response has to develop. However, the protective mechanism in CsA-treated B10 and B10, BR mice remains to be determined.

Interestingly, the mean times to death for CsA-treated and non-treated BALB/c mice given
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Fig. 1. Dose-response curve for CsA-treated BALB/c mice. BALB/c mice were treated with CsA (75 mg/kg/day), infected with various doses of MCMV (log_{10} p.f.u.) by i.p. inoculation and observed for time to death. Mean time to death of five mice/group is plotted against each virus dose. The bold line indicates the line of best fit (correlation coefficient r = 0.94).

Fig. 2. The effect of CsA (75 mg/kg/day for 25 days) on mortality rates of MCMV-infected mice. Mice were infected with various twofold dilutions of MCMV (i.p.) and the mean percentage mortality after 25 days was calculated from five mice per group. (a) B10 mice treated with (□) or without (O) CsA, (b) B10. BR mice treated with (□) or without (O) CsA, (c) BALB/c mice treated with (△) or without (△) CsA, (d) BALB. K mice treated with (▼) or without (▼) CsA.

Table 2. Effect of CsA on resistance to lethal MCMV infection

<table>
<thead>
<tr>
<th>Strain</th>
<th>H-2</th>
<th>Relative LD_{50}^* (–CsA)$</th>
<th>Relative LD_{50}^* (+CsA)$</th>
<th>Increase in susceptibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/J</td>
<td>a</td>
<td>1.3</td>
<td>0.04</td>
<td>32.0</td>
</tr>
<tr>
<td>BALB/c</td>
<td>d</td>
<td>1.0</td>
<td>0.01</td>
<td>92.6</td>
</tr>
<tr>
<td>BALB. K</td>
<td>k</td>
<td>6.2</td>
<td>1.0</td>
<td>6.2</td>
</tr>
<tr>
<td>B10</td>
<td>b</td>
<td>2.0</td>
<td>1.5</td>
<td>1.3</td>
</tr>
<tr>
<td>B10. BR</td>
<td>k</td>
<td>55.7</td>
<td>46.4</td>
<td>1.2</td>
</tr>
<tr>
<td>CBA</td>
<td>k</td>
<td>7.0</td>
<td>6.4</td>
<td>1.1</td>
</tr>
</tbody>
</table>

*Relative LD_{50} of mice expressed as the relative LD_{50} value compared to the LD_{50} value of non-CsA-treated BALB/c mice arbitrarily set the value of 1.0 (3 × 10^4 p.f.u.). In the case of CsA-treated BALB/c mice the value (0.00926) was rounded off. Comparison of the dose response of CsA-treated and untreated mice of each strain by chi-square analysis: A/J (P < 0.001), BALB/c (P < 0.001), BALB. K (P < 0.001), B10 (P > 0.70), B10. BR (P < 0.05) and CBA (P > 0.70).

†Mice were inoculated with CsA at 75 mg/kg/day s.c. beginning 24 h before virus infection and continued for 25 days.

equivalent virus doses (Table 3) were significantly delayed for mice treated with CsA (P < 0.025). Similar results were obtained for MCMV-infected homozygous nude BALB/c and CBA mice compared to heterozygous lirrentes (G. R. Shellam, unpublished observations). Elevated levels of natural killer (NK) cells have been implicated [Grundy (Chalmer) & Melief,
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Table 3. Effect of CsA on mean time to death in BALB/c MCMV-infected mice

<table>
<thead>
<tr>
<th>Virus dose (p.f.u.)</th>
<th>Treatment†</th>
<th>Days at which deaths occurred‡</th>
<th>Mean time to death (days ± S.E.M.)§</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 × 10⁴</td>
<td>+CsA</td>
<td>6, 7, 7, 8, 8</td>
<td>7.2 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>−CsA</td>
<td>4, 5, 5, 6, 6</td>
<td>5.2 ± 0.4</td>
</tr>
<tr>
<td>4 × 10⁴</td>
<td>+CsA</td>
<td>8, 8, 9, 9, 12</td>
<td>9.4 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>−CsA</td>
<td>4, 5, 8</td>
<td>5.7 ± 0.9</td>
</tr>
</tbody>
</table>

* BALB/c mice were inoculated with MCMV by the i.p. route.
† Mice were inoculated with or without CsA at 75 mg/kg/day s.c. beginning 24 h before virus infection and continued for 25 days. Control mice received daily s.c. injections of olive oil for 25 days.
‡ One death occurred at each day p.i. indicated.
§ Mean time to death ± S.E.M., calculated at each virus dose. Significant differences in the mean time to death were observed between CsA-treated and non-CsA-treated mice given the virus doses 8 × 10⁴ p.f.u. (P < 0.005) and 4 × 10⁴ p.f.u. (P < 0.025).

1982] as the reason for the delayed deaths in the homozygous nude mice since early resistance to MCMV infection has been shown to be mediated by the activity of such cells (Bancroft et al., 1981; Shellam et al., 1985). However, no enhanced levels of NK cell activity have been found in CsA-treated and MCMV-infected BALB/c mice (Gui et al., 1982). Furthermore, CsA does not affect the ability of MCMV to infect and form plaques in the fibroblast monolayers (data not shown) which confirms the findings of Gui et al. (1982). Similarly, CsA does not inhibit the penetration of herpes simplex virus into cells (McKenzie et al., 1987). Therefore, it appears that CsA does not interfere with virus replication. However, activated T cells may either directly or indirectly, through the action of released lymphokines, enhance virus replication, or alternatively, may induce earlier deaths from the effects of an autoaggressive T cell response.

This study indicated two distinct effects of CsA on murine resistance to MCMV infection. BALB/c, BALB.K and A/J mice became considerably more susceptible to MCMV infection after CsA treatment whereas B10, B10. BR and CBA mice were much less affected. However, after CsA treatment, all mouse strains reflected MHC-linked patterns of resistance to the virus. These findings clearly show that CsA immunosuppression does not eliminate the genetically determined, MHC-linked resistance to MCMV infection. In addition, comparison of B10, BR, BALB.K and CBA strains which have identical MHC haplotypes but different non-MHC background genes revealed that mice with the BALB background were affected to a greater extent by CsA treatment than mice having the B10 or CBA background. Therefore resistance mechanisms influenced by non-H-2-linked genes in B10 and CBA mice are probably not T cell-mediated. However, individual T cell clones may vary in their sensitivity to CsA as suggested by Kabellitz et al. (1987). It has recently been reported by Jenkins et al. (1988) that CsA interferes with the development of mature TCR γδ single positive cells without affecting the development of γδ T cells in the mouse thymus. Clearly the effects of CsA on the activation signals and release of cytokines by different immune cell types is far from being completely explored.

The data presented in this paper suggest that T cells, including those responsible for DTH reactions, do not mediate MHC-linked resistance to MCMV. However, we previously reported higher DTH responses to MCMV in mouse strains exhibiting MHC-linked resistance to this virus (Lawson et al., 1987). These results may reflect an immunosuppressive effect of the virus load on the immune response since mice given higher doses of virus produced DTH responses of lower magnitude compared to the response of mice inoculated with a lower dose of virus. The effect of the host genotype on the outcome of CsA immunosuppression during MCMV infection suggests that T cells are of greater importance in the susceptible A/J (H-2a) and BALB/c (H-2b) than in the moderately susceptible B10 (H-2b) and resistant BALB.K (H-2k), CBA (H-2k), and B10. BR (H-2k) mouse strains. T cell responses appear to be critically important for recovery in genetically susceptible strains of mice since, without these responses (CsA-treated mice), they are rendered markedly more susceptible to MCMV. These findings have clinical importance since CsA treatment of certain individuals, who may be genetically more susceptible to HCMV, may result in an increased incidence and severity of HCMV infection.
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REFERENCES


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