Delayed-type Hypersensitivity Responses to Murine Cytomegalovirus in Genetically Resistant and Susceptible Strains of Mice

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SUMMARY

The delayed-type hypersensitivity (DTH) response in mice infected with murine cytomegalovirus (MCMV) was measured by ear swelling following a challenge with heat-treated MCMV. DTH was dose-dependent and could be detected as early as 3 days post-infection with peak responses occurring between days 15 and 21 post-infection. The DTH response was found to be specific for MCMV since it could not be elicited by either herpes simplex virus type 1 or influenza A virus in MCMV-primed mice. The elicited DTH response was greater in mice primed with attenuated compared with virulent MCMV. The DTH response was shown to correlate positively with the genetically determined resistance status of mouse strains to this virus. Previous research has shown that resistance to lethal infection with MCMV is controlled by H-2-linked genes since mice having the k haplotype are more resistant than mice having the b or d haplotype of the H-2 complex. Also, non-H-2-linked genes in CBA, C3H, C57BL/10 and probably other strains confer resistance. Resistant strains (C3H [H-2k], CBA [H-2k]) developed greater DTH responses than those of susceptible strains (BALB/c [H-2d], C57BL/10 [H-2b]) inoculated with the same dose of virus. In addition, the genetically resistant mouse strains B10.BR [H-2k] and BALB.K [H-2k] gave a significantly greater DTH response than that of the corresponding congenic strains C57BL/10 [H-2b], BALB/c [H-2d] and BALB.B [H-2b] which are genetically susceptible to the virus. Also, the DTH response of C57BL/10 [H-2b] was significantly higher than that of BALB.B [H-2b] which correlates with their relative genetic resistance to MCMV, indicating the importance of non-H-2-linked genes. Furthermore, in addition to the response of greater magnitude, resistant strains (CBA, C3H, B10.BR) produced DTH responses to MCMV by day 3 compared with day 5 post-infection for susceptible BALB/c mice. These findings indicate that the magnitude and the time of appearance of the DTH response correlates positively with the genetically determined resistance status, although the role of DTH responses in controlling MCMV infections remains to be determined.

INTRODUCTION

The infection of mice with murine cytomegalovirus (MCMV) elicits the production of T cells which proliferate (Howard et al., 1978) or produce lymphokines (Sinickas et al., 1985c) following re-exposure to MCMV in vitro; they lyse virus-infected target cells in vitro (Ho, 1980; Reddehase & Koszinowski, 1984; Sinickas et al., 1985a, b) or mount delayed-type hypersensitivity (DTH) responses in vivo (Chong & Mims, 1982). Although the relative importance of T cell-mediated responses compared with other host protective mechanisms remains to be determined, T cells are important in regulating the course of primary MCMV infection since nude mice show
increased susceptibility to MCMV compared with their heterozygous littersmates [Grundy (Chalmer) & Melief, 1982], and T cells from MCMV-immune mice reduce the titre of MCMV in recipients in an H-2-restricted manner (Ho, 1980). However, other host factors have been shown to influence the course of MCMV infection and among these the effect of host genotype has been shown to be important.

Variation in susceptibility to MCMV among strains of mice was first noted in 1936 (McCordock & Smith, 1936) and recent studies have established that genes within the H-2 complex as well as non-H-2 genes regulate resistance to infection [Chalmer et al., 1977; Grundy (Chalmer) et al., 1981]. Host resistance genes influence the growth of MCMV in target cells in vitro (Harnett & Shellam, 1982) and the effectiveness of the interferon response (Harnett & Shellam, 1985), although their effect is thought to be largely on early mechanisms which are non-immunological (Shellam et al., 1983). There is no direct evidence that host resistance genes influence immunological responses to MCMV. Accordingly the effect of the genetic resistance status on the DTH response to MCMV has been investigated and evidence that the degree of DTH responsiveness to MCMV appears to be associated with the genetic resistance status of the mouse strain to MCMV is presented.

METHODS

Mice. Highly inbred specific pathogen-free mice were obtained from the Animal Resources Centre (Murdoch, Western Australia, Australia) and housed under minimal disease conditions. The strains used, with their respective haplotypes and relative LD50 values given in parenthesis [Grundy (Chalmer) et al., 1981; Bancroft et al., 1981; Grundy (Chalmer) & Melief, 1982; Allan & Shellam, 1984] were: BALB/c (H-2d, 1 LD50), CBA/CaH (CBA; H-2k, 24 to 28 LD50), C3H/HeJ (C3H; H-2k, 24 to 28 LD50), C57BL/10SnJ (B10; H-2b, 2 to 4 LD50), BALB.K (H-2k, 8 to 12 LD50). BALB.B (H-2b, 1 LD50). C57BL/10BR (B10 BR; H-2k, 32 to 36 LD50) and (B10 x B10 BR) F1 hybrid. The relative LD50 refers to the LD50 of MCMV in a particular mouse strain compared with that in BALB/c mice which is arbitrarily assigned the value of 1.0. Thus a relative LD50 of 10 indicates that the LD50 in that particular strain is 10 times the LD50 in the BALB/c strain. Age-matched female mice aged 8 weeks were used unless otherwise stated. Mice were inoculated by the intraperitoneal (i.p.) route with 0.1 ml of a suspension of live salivary gland-derived MCMV in phosphate-buffered saline with an osmolarity adjusted to be equivalent to that of mouse serum (MOBS, 330 mosmol), unless otherwise stated.

Cell cultures. Mouse embryonic fibroblast (MEF) cultures were prepared by trypsin dispersion of 14 to 15 day old embryos from outbred CD1 mice. The single cell suspension was seeded at 10^5.3 cells/ml in Eagle’s MEM (Gibco) and 10% foetal calf serum in 75 cm² tissue culture flasks (Lux Scientific Corp., Newbury Park, Ca., U.S.A.). The cells were incubated at 37 °C in 10% CO2 and after 24 h the medium was renewed.

Virus. MCMV (Smith strain) was originally obtained from Dr D. J. Lang, Duke University, Durham, N.C., U.S.A. The virus was maintained by passage in vitro as described elsewhere (Allan & Shellam, 1984) except that virus stocks were prepared as 20% salivary gland homogenates and were stored in the gas phase of liquid nitrogen. Normal salivary gland homogenate was prepared as a 20% homogenate in the same manner from uninoculated mice. Two virus stocks were used; separate stocks were employed for the experiments described in Fig. 1, 2, 3, and Fig. 4, 5, 6 and Table 1, respectively. Attenuated MCMV was prepared by six serial passages in MEF (Osborn & Walker, 1970). Herpes simplex virus type 1 (HSV-1) was obtained from Dr C. Lopez, Memorial Sloan-Kettering Institute for Cancer Research, New York, U.S.A. and passaged in Vero cells. Influenza virus (A/WSN) was passaged in eggs and inactivated by exposure to u.v. light (320 µW/cm²) for 7 min according to the method of Leung & Ada (1980).

Plaque assay. Secondary cultures of MEF (CD1) were seeded at 10^5.3 cells/well in 24-well plates (Costar). After 24 h, the confluent cells were washed with MOBS. Virus (100 µl/well in triplicate) which was diluted in EMEM and 2% foetal calf serum was allowed to adsorb at 37 °C for 1 h with rocking (12 cycles/min). The inocula were then removed and replaced with EMEM containing 2% foetal calf serum and 2% methyl cellulose (Fisher Scientific, Fairlawn, N.J., U.S.A.). Trays were incubated for 5 days at 37 °C in 10% CO2 and then stained with 1% methylene blue containing 10% formalin. A standard virus of known titre was processed in parallel with the test virus of unknown titre.

DTH. Ear swelling was used as a measure of DTH. At various days after primary i.p. immunization with live MCMV, anaesthetized mice were challenged intradermally in the right ear pinna with 10 µl of an eliciting dose of heat-treated (56 °C, 30 min) virus which gave no infectivity in a plaque assay, and in the left ear pinna with 10 µl of similarly heat-treated normal salivary gland homogenate. As a further control, normal unimmunized mice were similarly challenged with heat-treated virus and the heat-treated normal salivary gland homogenate.

In this study, virus used for the elicitation of DTH responses was derived from one of two separate virus batches.
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For each batch, the eliciting dose used was the optimum selected from experiments in which a wide range (10^2 to 10^5 p.f.u.) of eliciting doses was tested. Heat-treated normal salivary gland homogenate elicited no detectable increase in ear swelling in either immune or uninfected mice of any strain used.

The ear swelling was routinely measured with a micrometer 24 h following virus challenge. The data are presented as mean values of percentage increase in ear thickness from groups of five mice in comparison to that of the control ear according to the formula [(mean thickness of right ear - mean thickness of left ear) × 100]/mean thickness of left ear. Statistical significance was established using the Student t-test.

RESULTS

Comparison of DTH in mice immunized with virulent or attenuated MCMV

We examined the DTH response to attenuated and virulent MCMV in inbred B10 mice. An equivalent dose of virus (10^3 p.f.u.) that was either virulent (derived from salivary gland) or attenuated by passage in cell culture was used to immunize male B10 mice. Both groups of mice were then challenged in the pinnae with 10^3 p.f.u. of heat-treated salivary gland virus at various times thereafter. Although the DTH response elicited by attenuated virus declined after day 5 post-infection (p.i.) the response was significantly greater (P < 0.005) at all days tested than that for virulent virus (Fig. 1). Furthermore, to investigate whether MCMV antigen alone can prime for a DTH response, mice were primed with u.v.-inactivated or infectious virus that was either attenuated or salivary gland-derived. Mice primed with u.v.-inactivated attenuated virus gave a DTH response at day 6 p.i. of 27% increase in ear thickness which was significantly lower (P < 0.0005) than the response of mice primed with untreated attenuated virus which was a 55% increase. Similarly, mice primed with u.v.-inactivated salivary gland virus gave a DTH response of 13% which was significantly lower (P < 0.025) than the response of mice primed with untreated salivary gland virus which was 27%. However, virulent virus was used to elicit the DTH response in all further experiments because it is the form of MCMV associated with natural infections in mice, and genetically determined resistance to MCMV in mice has been established using only virulent virus.

Virus specificity of the DTH response

HSV-1 and influenza A virus were used to study the specificity of the DTH response induced or elicited by MCMV in CBA mice (Table 1). Mice infected with HSV-1 by the i.p. route and challenged in the pinnae with heat-treated HSV-1 gave a peak response of 29% increase in ear thickness at day 8 p.i. However, mice primed with HSV-1 and challenged in the pinnae with MCMV did not give a DTH response at days 8, 10 and 15 p.i. that was greater than that in unimmunized mice which were challenged with heat-treated MCMV (0.1 > P > 0.05).

Fig. 1. Effect of attenuation of virus on the DTH response. B10 mice (13 to 17 weeks old) were primed with the same dose (10^3 p.f.u.) of either attenuated (●) or virulent (▲) MCMV of the same batch and were challenged in the pinnae at various times thereafter with 10^3 p.f.u. of heat-treated salivary gland virus. Five mice were used in each group and the mean of individual percentage increases in ear thickness ± s.e.m. is shown.
Table 1. Virus specificity of DTH response in MCMV-infected CBA mice

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<tr>
<th>Virus used</th>
<th>Ear challenge</th>
<th>DTH response*</th>
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<td>Day 8‡</td>
<td>Day 10</td>
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<tr>
<td>HSV-1 (10⁵ p.f.u.)</td>
<td>HSV-1 (10⁵.⁹ p.f.u.)</td>
<td>29.0 ± 2.0</td>
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<td>HSV-1 (10⁵ p.f.u.)</td>
<td>MCMV (10³ p.f.u.)</td>
<td>8.8 ± 1.1</td>
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<tr>
<td>MCMV (10³ p.f.u.)</td>
<td>HSV-1 (10⁴ p.f.u.)</td>
<td>9.0 ± 1.3</td>
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<td>MCMV (10³ p.f.u.)</td>
<td>30.4 ± 1.4</td>
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<td>B</td>
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<tr>
<td>A/WSN (10³ HAU)</td>
<td>A/WSN (10².⁹ HAU§)</td>
<td>38.8 ± 5.3</td>
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<tr>
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<td>A/WSN (10².⁹ HAU)</td>
<td>8.5 ± 1.0</td>
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<tr>
<td>MCMV (10³ p.f.u.)</td>
<td>MCMV (10³ p.f.u.)</td>
<td>30.4 ± 1.4</td>
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* Ear thickness ±S.E.M. measured after challenge with virus in 10 μl.
§ HAU, Haemagglutinating units.
‡ Immunized mice received a challenge with the viruses indicated at the days specified after immunization. The DTH response was measured 24 h later.
† Immunization was by the i.p. route.

Similarly, no significant DTH response (0.1 > P > 0.05) was obtained for mice primed with MCMV and challenged in the pinnae with HSV-1. Mice immunized with A/WSN and challenged with heat-treated A/WSN gave a peak DTH response of 39% increase in ear thickness at day 6 p.i., whereas mice primed with A/WSN and challenged with MCMV or vice versa, did not give a significant DTH response at days 6, 8 or 14 p.i. (0.1 > P > 0.05) compared with unimmunized mice challenged with heat-treated MCMV.

Comparison of DTH in resistant and susceptible strains of mice

Mice of resistant (CBA, C3H and BALB.K) and susceptible (BALB/c) strains were immunized i.p. with 10³ p.f.u. and were challenged in the pinnae with 10³ p.f.u. on days 3, 5, 7, 9, 15 or 21 p.i. in the same experiment. Heat-treated normal salivary gland homogenate produced no detectable ear swelling in either immune or uninfected mice of these strains.

The earliest time for a significant DTH response compared with the ear swelling of unimmunized mice challenged with heat-treated virus was at day 3 p.i. (P < 0.005) for both C3H and CBA mice (Fig. 2a). However, susceptible BALB/c mice did not produce a significant response until day 7 p.i. (P < 0.005) compared with control mice. In addition, the DTH response of resistant CBA mice was significantly higher than that of susceptible BALB/c mice at days 3, 5 and 7 (P < 0.005), day 15 (P < 0.025) and day 21 (P < 0.01) p.i. Similarly resistant C3H mice produced significantly greater DTH responses than BALB/c at days 3, 5 and 7 (P < 0.005), day 9 (P < 0.025), day 15 (P < 0.005) and day 21 (P < 0.01) p.i., and also produced a greater DTH response than mice of the CBA strain at days 5 and 7 (P < 0.005), days 9 and 15 (P < 0.05) and at day 21 (P < 0.005) p.i.

The effect of H-2-linked genes on the DTH response can be seen by comparing the response of BALB/c and congenic BALB.K mice which are resistant to MCMV (Fig. 2b). The response of BALB.K was significantly higher than that of BALB/c at days 5 and 7 (P < 0.005), day 9 (P < 0.025) and at days 15 and 21 p.i. (P < 0.01). Furthermore, DTH in BALB.K mice was comparable to that of C3H and CBA mice, except at day 3 p.i.

Comparison of DTH in B10, B10.BR and (B10 × B10.BR) F₁ mice

The DTH response of susceptible B10, highly resistant B10.BR and (B10 × B10. BR) F₁ mice whose resistance to MCMV resembles that of the B10.BR strain (G. R. Shellam, unpublished observation) is shown in Fig. 3. Mice were immunized and challenged as described above. The
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Fig. 2. DTH responses to MCMV in resistant and susceptible strains of mice. Mice of the resistant C3H (○), CBA (●), BALB.K (■) and susceptible BALB/c (□) strains (8 weeks old) received 10⁵ p.f.u. of MCMV i.p. of the same virus batch on the same day and were challenged with 10⁵ p.f.u. of heat-treated MCMV. There were five mice/group and the mean of individual percentage increases in ear thickness ± s.e.m. is shown. Non-immune mice given the same dose of heat-treated virus gave a response of 6.7 ± 3.2%, 11.8 ± 1.1%, 1.9 ± 1.1% and 3.8 ± 1.8% increase in ear thickness for CBA, C3H, BALB/c and BALB.K mice, respectively. For comparative purposes the DTH response for BALB/c is shown with that of CBA and C3H mice (a) and with that of BALB.K (b).

earliest significant increase in ear thickness compared with unimmunized controls was seen at day 3 p.i. (P < 0.005) for B10.BR and F₁ hybrid mice but not until day 5 p.i. (P < 0.025) for B10 mice. In addition, the DTH response of resistant B10.BR and F₁ hybrid mice was significantly higher than that of susceptible B10 mice at days 3, 5, 7, 9, 15 and 21 p.i. (P < 0.01). No significant difference was found between the DTH response of the B10.BR strain and F₁ hybrids at days 3, 5, 7 and 21 (0.375 > P > 0.1) p.i. Although the data suggest that there are intrinsic differences in the DTH response to MCMV in resistant and susceptible strains, there remains the possibility that the differences reflect a greater degree of immunosuppression induced by MCMV in the susceptible strains. To investigate this, the DTH responses to ovalbumin in normal or MCMV-infected mice of the susceptible B10 and the resistant B10.BR strains were studied and were found to be comparable regardless of virus infection. For example, the DTH responses to ovalbumin at day 10 p.i. for MCMV-infected B10 and B10.BR mice were 47% and 52%, respectively, compared with responses of 44% and 48% in mice primed with ovalbumin alone. Thus, MCMV does not induce a greater generalized immunosuppression of the DTH response in the susceptible B10 than in the resistant B10.BR strain.

Effect of dose of virus on kinetics of DTH responses in various mouse strains

The effect of different priming doses of virus (10², 10³, 10³.5, 10⁴, 10⁴.5, 10⁵ and 10⁵.5 p.f.u.) on the DTH response of CBA and BALB/c mice ear-challenged with 10⁵ p.f.u. was investigated (Fig. 4, 5). CBA mice produced a significant DTH response compared with controls as early as day 3 p.i. (P < 0.005) for doses between 10² and 10⁵ p.f.u. In contrast, the earliest significant DTH response of BALB/c compared to unimmunized mice was seen at day 5 (P < 0.005) for the virus dose of 10⁵ p.f.u., day 7 (P < 0.005) for 10³ p.f.u. and day 9 (P < 0.005) for 10³.5 p.f.u. With sublethal doses of virus, the DTH response elicited by BALB/c mice began lower but surpassed the response of CBA after day 15. When a lethal dose of virus (10⁴ p.f.u.) was used to prime BALB/c mice no DTH response was seen at days 3, 5 or 7 p.i., and mice died from day 3 with 100% mortality at day 8. However, CBA mice given a uniformly lethal dose (10⁵.5 p.f.u.) were able to produce a significant DTH response by day 5 (P < 0.005) as shown in Fig. 5 even though mice began to die at day 5.
Fig. 3. DTH responses to MCMV in B10, B10.BR and F1 mice. B10 (△), B10.BR (▲) and (B10 × B10.BR) F1 hybrid (◇) mice were 19, 19 and 17 weeks old, respectively, and received 10^3 p.f.u. of MCMV i.p. with the same virus batch on the same day and were challenged in the pinnae with 10^5 p.f.u. of heat-treated MCMV. Five mice were used in each group and the mean of individual percentage increases in ear thickness ± S.E.M. is shown. Non-immune mice gave a response of 9.1 ± 1.5%, 9.1 ± 2.0% and 9 ± 1.1% increase in ear thickness for B10, B10.BR and F1 mice, respectively.

Fig. 4. Effect of various doses of virus on the DTH response in CBA and BALB/c mice. Mice (8 weeks old) of the CBA (●) and BALB/c (□) strain were immunized with doses of 10^2 (a), 10^3 (b), 10^3.5 (c) and 10^4 (d) p.f.u. on the same day with the same virus batch and were challenged in the pinnae with 10^5 p.f.u. of heat-treated salivary gland virus at various times later. Five mice were used in each group and the mean of individual percentage increases in ear thickness ± S.E.M. is shown.

Fig. 5. Effect of various doses of virus on the DTH response in CBA mice. Mice were immunized with doses of 10^4.5 (a), 10^5 (b) and 10^5.5 (c) p.f.u. on the same day using the same protocol as described in Fig. 4.

It was decided to study the effect of various priming doses for B10 and BALB.B mice which differ only in non-H-2-linked genes. Although these strains are susceptible to MCMV, B10 are two- to fourfold more resistant than BALB.B. The resistant B10 mice produced a DTH response that was significantly higher than controls as early as day 3 (P < 0.01) for the virus doses 10^2 and 10^3 p.f.u. and at day 5 (P < 0.05) for 10^3.5 p.f.u., whereas the BALB.B response became
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Fig. 6. Effect of virus dose on the DTH response in BALB.B and B10 mice. Mice (8 weeks old) of the BALB.B (▼) and B10 (▲) strains were immunized with doses of 10^2 (a), 10^3 (b), 10^3.5 (c) and 10^4 (d) p.f.u. of MCMV i.p. and were challenged in the pinnae at various times later. Five mice were used in each group and the mean of individual percentage increases in ear thickness ± S.E.M. is shown. Non-immune mice gave a response of 9.4 ± 2.0% and 10.8 ± 1.2% increase in ear thickness for BALB.B and B10 respectively.

DISCUSSION

In this study, various strains of mice which differ in their resistance to MCMV have been shown to produce a virus-specific DTH response during MCMV infection. The response was detected from 3 to 21 days p.i. The degree of ear swelling was found to vary depending on the strain of mouse. Consistent findings were that resistant strains gave higher DTH responses over the first 10 days than those of the susceptible strains. Our results of DTH responses in C3H and B10 mice differ from those of Chong & Mims (1982), which may reflect the strain of virus since Chong & Mims used the Osborn strain whereas we have used the more virulent Smith strain of virus (Chong & Mims, 1982). Both major histocompatibility complex (MHC)-linked and background genes that determine resistance were shown to influence the DTH response. Resistant strains of mice having the H-2^k haplotype [CBA, C3H, B10.BR; Chalmer et al., 1977; Grundy (Chalmer) et al., 1981] produced a greater DTH response which was initiated earlier after infection than the DTH response of the susceptible mouse strains possessing the H-2^b or H-2^d haplotypes [BALB/c [H-2^d], BALB.B [H-2^b], B10 [H-2^d]; Chalmer et al., 1977; Grundy (Chalmer) et al., 1981]. In addition, the resistant (B10 × B10.BR) F_1 hybrid gave a DTH response similar to the resistant B10.BR parent (Fig. 3). Thus the DTH response and the resistance status of mice to MCMV may be associated. When the MHC genes were matched, mice of the BALB background gave lower DTH responses than the B10 background (Fig. 6). This also correlated with genetically determined resistance of mice to MCMV [Grundy (Chalmer) et al., 1981]. Thus DTH responses to MCMV correlate with genetically determined resistance of mice to the virus in vivo.

As the dose of virus given to BALB/c mice was increased from 10^2 to 10^3.5 p.f.u. (Fig. 4), the appearance of the earliest significant DTH response was delayed from day 5 to day 9 p.i. However, the earliest DTH response of CBA mice was observed at day 3 p.i. for all the virus doses between 10^2 to 10^5 p.f.u. It was not until the high dose of 10^5.5 p.f.u. was used that a delayed onset of the response was seen. Indeed, the peak response of CBA mice was 15 days earlier than that for BALB/c mice, although the BALB/c DTH response eventually reached equivalent levels (Fig. 4). Clearly, this more rapid T cell response seen in CBA mice could be important in determining the level of virus replication in the animal. BALB/c mice given 10^4 p.f.u. began to die by day 3 and showed no detectable DTH response whereas CBA mice
produced a strong DTH response at this dose. Higher doses of virus elicited a DTH response in CBA mice that persisted to day 15 to 21 p.i. However, over the course of infection with lower virus doses, the DTH response in CBA mice declined. This result suggests that there was insufficient virus to elicit a response due to resolution of the virus in this resistant strain.

Evidence is presented that the DTH response to attenuated virus was greater than that to virulent MCMV when the same number of p.f.u. was inoculated. This confirms the result of Chong & Mims (1982). Indeed, the DTH response induced by the attenuated virus was greater than any DTH response seen in any mouse strain inoculated with virulent MCMV. The DTH response in mice primed with u.v.-inactivated virus was much less than the response seen in mice primed with infectious virus, regardless of whether attenuated or virulent virus preparations were used. Thus, the virus must be capable of replication in the animal in order to induce a good DTH response. However, since salivary gland virus is capable of replicating to higher titres in mice than attenuated virus (Osborn & Walker, 1970), the data suggest that the lower DTH responses induced by salivary gland virus reflect events subsequent to virus replication, such as the induction of immunosuppression. Since infection with virulent MCMV depresses antibody formation (Osborn & Medearis, 1967; Howard & Najarian, 1974), suppresses interferon induction by an unrelated virus (Osborn & Medearis, 1966, 1967), suppresses the lymphocyte response to non-specific mitogens (Allan et al., 1982) and suppresses the generation of cytotoxic T cells against heterologous viruses (Ho, 1980), it is possible that the immunosuppressive properties of virulent MCMV infection may be acting to limit the DTH response in both resistant and susceptible mouse strains.

Grundy & Shearer (1984) have found that the cell-mediated response to allogeneic or hapten-modified syngeneic histocompatibility antigens during MCMV infection is characterized by an early phase of suppression followed by an enhancement phase. Interestingly, the degree of suppression is greatest in mice genetically susceptible to MCMV than in resistant strains. The extent of suppression is also comparable with mitogen responses (Allan et al., 1982) during MCMV infection and is greatest in strains of mice with susceptible H-2 haplotypes. Thus in addition to our findings of DTH responses, other cell-mediated responses during MCMV infection including alloreactivity and mitogen responses appear to be under genetic control.

What role do T cells play in controlling MCMV infection? T cells have been shown to contribute to the protection of the host in MCMV infection since nude mice show greater susceptibility to lethal infection than controls [Grundy (Chalmer) & Melief, 1982]. Furthermore, in an adoptive transfer model, T cells protect against MCMV infection (Starr & Allison, 1977; Ho, 1980) and are restricted by the K and D region of the H-2 complex (Ho, 1980). In addition, Lyt2+ L3T4- T cells confer protection against MCMV infection in the lung (Reddehase et al., 1985). Cytotoxic T cells generated in vivo during MCMV infection are specific for cells that express immediate early proteins in assays in vitro (Reddehase & Koszinowski, 1984; Reddehase et al., 1984a, b). However, the primary cytotoxic response in the animal cannot be easily detected by assays in vitro and requires a further step of incubation of lymphocytes from infected mice with IL-2 in vitro (Reddehase & Koszinowski, 1984; Sinickas et al., 1985a, b). This observation raises uncertainty concerning the usefulness of assays in vitro for the detection of cytotoxic T cells involved in immunity against herpesviruses. In this study, DTH was chosen as an assay of cell-mediated immunity in different strains of mice infected with MCMV because it could readily be measured and did not require such an assay.

DTH responses have been well documented in HSV-1 infection (Nash et al., 1980, 1981; Schrier et al., 1982). Immune protection against HSV-1 was found to be attributable to cells possessing either I or K, D compatibility at the H-2 complex, but long lasting protection was achieved only by cells requiring I region compatibility and were characterized as being Lyt1+2- cells capable of inducing DTH responses (Howes et al., 1979; Nash et al., 1980; Nash & Gell, 1983). It appears that Lyt1-2+ cells have no antiviral role in adoptively transferred suspensions (Nash & Gell, 1983).

Cells responsible for DTH responses have been shown to be important in protection of mice from leishmanial infection and genetic differences underlying DTH responses to this parasite have been found (for review, see Mitchell et al., 1982). BALB/c mice were shown both to be more
susceptible to disease and to exhibit lower DTH responses to the antigen than CBA or C57BL/6 mice.

Although there is evidence of MHC class I-restricted T cells being important in vivo during MCMV infection (Ho, 1980; Reddehase et al., 1985), the present data suggest that a comparison of the effect of MHC class I- and class II-restricted T cells in mouse strains of various resistance status rather than in one strain may be instructive in studying virus infection. The role of L3T4+ cells in controlling MCMV infection remains unresolved.

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