Effects of L3T4+ Lymphocyte Depletion on Acute Murine Cytomegalovirus Infection

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SUMMARY

We examined the role of T lymphocytes bearing the L3T4 phenotype in acute murine cytomegalovirus (MCMV) infection. In vivo administration of rat IgG2b monoclonal antibody (MAb) GK 1.5 was used to deplete mice of L3T4+ lymphocytes during acute MCMV infection. Unlike the saline-treated controls that resolved their infections, mice receiving the MAb developed persistent and high levels of virus in the salivary gland, lung and spleen. The production of antibody to MCMV was delayed and the titres achieved were markedly less than in the controls. Despite the higher levels of virus replication, there was no increase in mortality seen in animals treated with the MAb. Following intraperitoneal challenge with MCMV, depletion of L3T4+ lymphocytes was protective, increasing the dose of MCMV required to produce death. These data indicate that T lymphocytes of the L3T4 phenotype influence the degree of MCMV replication during acute infection and may contribute to mortality following intraperitoneal virus challenge.

INTRODUCTION

Cytomegalovirus (CMV) infection is common in immunocompromised hosts, particularly in patients with AIDS. Many immunocompromised patients shed CMV asymptomatically following primary or reactivated infection, whereas others develop tissue invasion resulting in retinitis, colitis, hepatitis or adrenalitis (Jacobson & Mills, 1988). The immune mechanisms which control CMV infection are not completely understood. T and B lymphocytes, natural killer cells and macrophages have been shown to be important components of the host response to CMV infection (Ho, 1982; Quinnan, 1985). Virus-specific cell-mediated and humoral immune responses develop during acute CMV infection, but the relative importance of these immune responses in the host recovery from CMV infection is unknown.

Because of similarities in its biology, murine CMV (MCMV) has provided useful models for acute, chronic and latent human CMV (HCMV) infections (Plummer, 1973). The cell-mediated and humoral immune responses which occur in mice following MCMV infection appear to be similar to those which occur in CMV-infected humans. Additionally, mice with congenital or acquired defects in cell-mediated immunity have decreased resistance to MCMV infection (Brody & Craighead, 1974; Jordan et al., 1977; Mayo et al., 1977; Mercer & Spector, 1986; Selgrade et al., 1982; Shanley & Pesanti, 1986). As with humans, however, the relative importance of specific immune responses in the host control of MCMV infection is only partially understood.

The recent availability of monoclonal antibodies (MAbs) that recognize murine lymphocyte surface antigens has provided a powerful tool for the identification and selective depletion in vivo of individual lymphocyte subsets (Benjamin et al., 1986; Cobbold et al., 1984; Wofsy et al., 1985; Wofsy & Seaman, 1986). The murine L3T4+ surface antigen identifies a subset of T lymphocytes which are homologous to human CD4+ lymphocytes and which perform predominantly 'helper-
inducer' immune functions (Dialynas et al., 1983; Wilde et al., 1983). By administration of a MAb directed against the L3T4+ surface antigen, we depleted mice selectively of L3T4+ lymphocytes and demonstrated the importance of this cell population in the control of acute MCMV infection.

METHODS

Mice. Four-week-old female BALB/cAnN mice, obtained from Simonsen Laboratories were used in all experiments. These mice were housed in groups of four or five in individual unit isolators and fed Laboratory Chow 500 (Ralston Purina) and water ad libitum. Late term pregnant CD-1 mice were obtained from Charles River Breeding Laboratories.

Viruses. The Smith strain of MCMV (originally obtained from M. C. Jordan, University of Minnesota, Minneapolis, Minn., U.S.A.) was used in all experiments. The virus was maintained by serial passage in BALB/c mice and prepared as a 10% (w/v) homogenate of salivary gland tissue from mice infected subcutaneously with 1 × 10^5 p.f.u. 17 days earlier. Virus stocks were stored with 10% DMSO at -70 °C (Shanley & Pesanti, 1985). The virus pool for these experiments contained 2.97 × 10^7 p.f.u./ml.

Cell culture methods. Mouse embryo cells (MEC) were prepared from embryos of late term pregnant CD-1 mice by trypsin-EDTA disaggregation as previously described (Shanley & Pesanti, 1985), propagated as monolayers in Eagle’s minimum essential medium (MEM) containing 10% fetal calf serum (FCS), 1% L-glutamine, 100 units/ml penicillin and 50 μg/ml gentamicin. Virus quantification was performed by plaque assay in MEC with an overlay containing MEM, 1% methylcellulose, 10% FCS, 0.02 M-HEPES, 1% glutamine and antibiotics.

Preparation and administration of MAbs. A rat MAb used to deplete L3T4+ lymphocytes was produced by hybridoma GK 1.5-producing rat IgG2b (Dialynas et al., 1983). Antibody was prepared as ascitic fluid in sublethally irradiated BALB/c mice, partially purified by ammonium sulphate precipitation and dialysed against phosphate-buffered saline (PBS). Quantification of immunoglobulin was performed by radial immunodiffusion using plates containing antibody to rat IgG2b in agar (ICN Immunobiologics). The antibody in the ascitic fluid was determined using a standard curve derived from samples with known concentrations of rat IgG2b.

Selective depletion of L3T4+ lymphocytes was achieved by intraperitoneal (i.p.) administration of 400 μg of antibody to L3T4 24 h prior to MCMV infection. To maintain depletion, 200 μg of antibody was administered every 7 days thereafter (Wofsy & Seaman, 1986; K. S. Erlich, unpublished method). The MAb was diluted to a final volume of 0.5 ml in normal saline. Control mice received identical volumes of normal saline only.

Flow cytometric analysis of lymphocyte subpopulations. Single-cell suspensions of peripheral blood were prepared as previously described (Wofsy & Seaman, 1986). Ficoll-Hypaque-purified mononuclear cell suspensions were incubated for 30 min at room temperature with saturating concentrations of MAbs to lymphocyte surface antigens in PBS containing 0.1% bovine serum albumin and 0.02 M-sodium azide. Following washing, the cells were stained for 30 min at room temperature with fluorescein isothiocyanate (FITC)-conjugated goat antibody to rat IgG2b (Tago Laboratories). The stained lymphocytes were counted on an Ortho 2S cytofluorograph using an argon laser for FITC excitation (488 nm). Fluorescence data were collected using logarithmic amplification on 50000 viable cells as determined by forward light scatter and propidium iodide gating. Monochromatic fluorescence data were displayed as cell frequency histograms or immunofluorescence profiles in which log10 immunofluorescence was plotted on the x axis and the cell number on the y axis. The MAbs used to identify lymphocytes included antibody to L3T4, 'helper-inducer' T cells (hybridoma GK 1.5, rat IgG2b) (Dialynas et al., 1983), antibody to LYT2, 'suppressor-cytotoxic' T cells (hybridoma 30-H12, rat IgG2b) (Ledbetter et al., 1980) and antibody to Thy 1.2, total T lymphocytes (hybridoma 30-H12, rat IgG2b) (Ledbetter et al., 1980).

MCMV antibody measurement. Serum samples were collected by retroorbital venipuncture. Serum antibody to MCMV was determined by ELSA as previously described (Classen et al., 1987). Serum samples were screened for antibody against MCMV antigens at a dilution of 1:10. Control sera with and without antibody to MCMV were used in all tests. Quantification of antibody to MCMV was determined by testing serial twofold dilutions of serum; the highest dilution that resulted in a specific absorbance value of >0.1 was recorded as the reciprocal endpoint dilution.

Virus infection. Mice were infected by diluting stock virus in PBS and administering 1 × 10^4 p.f.u. of MCMV in 0.2 ml aliquots subcutaneously. At various times after infection, four L3T4+ lymphocyte-depleted mice and saline-treated controls were sacrificed, serum was collected and tissue samples were prepared for virus quantification. Organ virus titres were determined by preparation of a 10% (w/v) homogenate in Eagle’s MEM with 10% FCS and 10% DMSO. The tissue homogenates were centrifuged at 1500 r.p.m. for 20 min and frozen at -70 °C until plaque titration was carried out (Shanley, 1987).

To determine the dose of MCMV producing 50% mortality (LD50), weaning mice were inoculated i.p. with 0.2 ml of stock virus in serial 10-fold dilutions. The number of deaths in each group over a 14 day period was recorded, and the LD50 was determined by the Reed–Muench method (Reed & Muench, 1938).

Statistical analysis. Where appropriate, comparisons of treatment groups were performed by the Spearman–Kärber method (Sachs, 1984).
L3T4+ depletion during murine CMV infection

RESULTS

Effects of anti-L3T4+ on lymphocyte subset analysis

A single i.p. injection of 400 µg of MAb against the L3T4 lymphocyte marker depleted > 95% of circulating L3T4+ lymphocytes within 24 h. This level of depletion of L3T4+ lymphocytes could be maintained by the weekly administration of 200 µg of antibody to L3T4 every 7 days. The numbers of peripheral Lyt2+ lymphocytes were not affected by repeated anti-L3T4 therapy (data not shown).

Effects of L3T4+ lymphocyte depletion on MCMV infection

The extent of MCMV replication in mice, given either 400 µg anti-L3T4 or normal saline 1 day prior to subcutaneous MCMV infection, was assessed on days 4, 7, 14, 21, 28, 35 and 42 after infection (Table 1). Saline-treated mice had maximal MCMV titres in salivary gland, lung and spleen by day 14 of infection followed by rapid clearance of virus from these organs. By comparison, salivary gland, lung and spleen virus titres were markedly elevated in L3T4+ lymphocyte-depleted mice, and virus persisted in these organs through to day 42 of infection, the last time point that was studied. During the 42 day observation period there were no deaths in either study group.

Antibody response to MCMV

The effect of L3T4+ lymphocyte depletion on the production of antibody to MCMV was also assessed during acute infection (Table 2). Control mice developed antibody against MCMV which was detectable as early as day 7 after infection. In contrast, antibody production in L3T4+ lymphocyte-depleted mice was not detected until 21 days after infection. In addition, the antibody titres achieved in such mice were 10- to 20-fold lower than those found in the saline-treated controls.

Effects of L3T4+ lymphocyte depletion on mortality due to MCMV

We examined the effect of L3T4+ depletion on mortality following i.p. virus challenge with various doses of MCMV (Table 3). Unexpectedly, L3T4+ lymphocyte depletion exerted a significant protective effect against the lethal effect of virus challenge. As shown in Table 3, the LD50 of MCMV was increased to 3.4 x 10^4 p.f.u./mouse following depletion of L3T4+ lymphocytes compared to 3.5 x 10^3 p.f.u./mouse for non-depleted saline controls (p < 0.01). This was not a non-specific effect due to the administration of rat immunoglobulin, because studies examining the susceptibility to MCMV of mice depleted of T lymphocytes using antibody to Thy 1.2 revealed an increased mortality compared to saline-treated controls (data not shown).

Table 1. MCMV replication in organs of BALB/c mice receiving either saline or MAb GK 1.5 during acute MCMV infection

<table>
<thead>
<tr>
<th>Time after infection (days)</th>
<th>MCMV titre†</th>
<th>MCMV titre†</th>
<th>MCMV titre†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Salivary gland</td>
<td>Lung</td>
<td>Spleen</td>
</tr>
<tr>
<td></td>
<td>Saline</td>
<td>MAb‡</td>
<td>Saline</td>
</tr>
<tr>
<td>4</td>
<td>4.82</td>
<td>5.54</td>
<td>2.18</td>
</tr>
<tr>
<td>7</td>
<td>7.97</td>
<td>12.8</td>
<td>3.48</td>
</tr>
<tr>
<td>14</td>
<td>5.41</td>
<td>12.1</td>
<td>1.70</td>
</tr>
<tr>
<td>21</td>
<td>8.75</td>
<td>12.9</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>28</td>
<td>8.75</td>
<td>12.8</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>35</td>
<td>&lt;0.5</td>
<td>13.0</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>42</td>
<td>&lt;0.5</td>
<td>11.3</td>
<td>&lt;0.5</td>
</tr>
</tbody>
</table>

* Mice were infected with 10^4 p.f.u. of MCMV given subcutaneously on day 0.
† MCMV titres are expressed as log_{10} p.f.u./g of 10% (w/v) tissue homogenates of organs pooled from four mice per group.
‡ Mice were given 400 µg of MAb GK 1.5 i.p. 24 h prior to virus inoculation. Additional 200 µg doses were given every 7 days to maintain lymphocyte depletion.
Table 2. Effect of L3T4+ lymphocyte depletion* on the production of antibody to MCMV during acute viral infection†

<table>
<thead>
<tr>
<th>Time after infection (days)</th>
<th>Antibody titre to MCMV‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saline</td>
</tr>
<tr>
<td>0</td>
<td>&lt;10</td>
</tr>
<tr>
<td>4</td>
<td>&lt;10</td>
</tr>
<tr>
<td>7</td>
<td>20</td>
</tr>
<tr>
<td>14</td>
<td>80</td>
</tr>
<tr>
<td>21</td>
<td>80</td>
</tr>
<tr>
<td>28</td>
<td>640</td>
</tr>
<tr>
<td>35</td>
<td>320</td>
</tr>
</tbody>
</table>

* As described in footnote ‡ of Table 1.
† Mice were given 10⁴ p.f.u. of MCMV in 0.2 ml PBS administered subcutaneously.
‡ Antibody titres were determined by an ELISA and expressed as the reciprocal value. All values represent pooled samples derived from four mice in each group.

Table 3. Effects of L3T4+ lymphocyte depletion* on the dose of MCMV required to kill BALB/c mice following i.p. inoculation†

<table>
<thead>
<tr>
<th>Group</th>
<th>MCMV dose (p.f.u./0.2 ml)</th>
<th>Mortality‡ in indicated group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saline</td>
<td>MAb</td>
</tr>
<tr>
<td>1</td>
<td>10²</td>
<td>0/18 (0%)</td>
</tr>
<tr>
<td>2</td>
<td>10³</td>
<td>5/17 (29.4%)</td>
</tr>
<tr>
<td>3</td>
<td>10⁴</td>
<td>12/18 (66.7%)</td>
</tr>
<tr>
<td>4</td>
<td>10⁵</td>
<td>9/9 (100%)</td>
</tr>
</tbody>
</table>

Calculated LD₅₀§

3.5 x 10³ 3.2 x 10⁴

* Mice were given 400 µg of Mab GK 1-5 or an identical volume of PBS 24 h prior to virus challenge.
† Stock MCMV was diluted in PBS and the indicated dose (p.f.u.) was administered i.p. in 0.2 ml aliquots.
‡ Number of mice dead/total at risk, 2 weeks after virus challenge. The data represent numbers pooled from two separate experiments.
§ The virus dose (p.f.u./0.2 ml) required to kill 50% of the mice in 14 days. The difference between the values is significant by the Spearman–Kärber method at P < 0.01.

DISCUSSION

Although T lymphocyte function has been shown to be an important determinant in the outcome of acute MCMV infection, the roles of T cell subsets in host immunity to MCMV are poorly understood. We have used a MAb which recognizes and depletes the L3T4+ ‘helper-inducer’ lymphocyte subset in vivo in order to examine the role of these cells in host immunity to acute MCMV infection. L3T4+ lymphocyte depletion markedly increased and prolonged virus replication in several tissues following subcutaneous infection and also specific production of antibody to MCMV was delayed and depressed. Despite these effects, no increased mortality was observed in the L3T4+ lymphocyte-depleted group. In the LD₅₀ assays L3T4+ depletion exerted a protective effect following lethal virus challenge. These data indicate that L3T4+ lymphocytes play a critical role in eradication of replicating virus by the host. Paradoxically, these lymphocytes may also contribute to mortality following acute virus challenge. The mechanisms responsible for these observations are unknown.

Previous attempts to determine the relative importance of individual immune effector mechanisms in the host response to MCMV infection have provided conflicting results. Studies in nude mice and studies examining acquired immunosuppression have demonstrated that in the absence of T lymphocytes, MCMV replication is unrelenting and invariably fatal (Brody & Craighead, 1974; Jordan et al., 1977; Mayo et al., 1977; Selgrade et al., 1982; Starr & Allison,
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1977; Shanley & Pesanti, 1985, 1986). Passive transfer of splenic T lymphocytes from MCMV-immunized mice protects infected mice against a fatal outcome, emphasizing the importance of cell-mediated immunity in the host response to MCMV infection (Ho, 1980; Shanley, 1987). However, the specific T lymphocyte subset that confers this protection remains controversial. Experiments conducted by Reddehase, Koszinowski and their collaborators (Reddehase et al., 1985, 1986, 1987, 1988) have demonstrated the importance of L3T4-negative, Lyt2.2-positive (homologous to CD4- and CD8+) lymphocytes for the control of murine CMV infection. In their model, lethally irradiated mice were infected by intraplantar injection, and the effect of specific lymphocyte subsets from immunologically intact, MCMV-immune mice were assessed by the passive transfer of cells. Numerous studies with this model failed to demonstrate antiviral activity against MCMV in the L3T4+ lymphocyte subset, whereas the Lyt2.2 lymphocytes exert a protective effect (Reddehase et al., 1986, 1987, 1988). Our experiments differ substantially from these previous studies, and show that in otherwise normal mice, the elimination of the L3T4-bearing lymphocyte subset has a profound effect on the course of subsequent acute infection. In a nude mouse model, Shanley (1987) demonstrated that passively transferred L3T4+ lymphocytes also had a definite antiviral effect. Taken together, these latter results do not necessarily conflict with the findings of Reddehase. One possible explanation is that L3T4+ lymphocytes are important in the generation of the immune response, but once MCMV-immune Lyt2.2 lymphocytes have been generated by infection, the L3T4+ subset is no longer necessary for control of infection.

L3T4+ lymphocytes are critical in antigen processing and generation of both cell-mediated and humoral immune responses (Wofsy et al., 1985; Wofsy & Seaman, 1986). Selective L3T4+ lymphocyte depletion has profound effects on the host immune response to virus infection (Leist et al., 1987). In our studies, production of antibody to MCMV was both delayed and depressed in mice depleted of L3T4+ lymphocytes compared to saline-treated controls. The absence of a normal antibody response or an optimal cell-mediated immune response in these mice may contribute to the increased virus replication observed in these animals (Manischewitz & Quinnan, 1980; Shanley et al., 1981). Despite the absence of L3T4+ lymphocytes, however, cytotoxic T lymphocyte activity is not completely absent in L3T4+ lymphocyte-depleted mice (Buller et al., 1987; Leist et al., 1987) and baseline natural killer cell activity is increased following the administration of anti-L3T4 (K. S. Erlich, unpublished observations). It has been suggested that these retained immune responses are capable of protecting mice against death following MCMV infection (Bukowski et al., 1984).

Of the numerous immune abnormalities which occur in patients with AIDS, numerical depletion and functional deficiency of CD4+-expressing T lymphocytes is the most characteristic finding (Seligmann et al., 1984). Depletion of CD4+ lymphocytes has been suggested as the primary cause of the severe immunodeficiency characteristic of patients with AIDS, but this hypothesis has not been tested directly in humans. Prolonged CMV replication and shedding is common in patients with AIDS, yet only occasional patients suffer from the invasive disease (Jacobson & Mills, 1988). Although there are apparent similarities between the pathogenesis of CMV infection in AIDS patients and MCMV infection in L3T4+ lymphocyte-deficient mice, further studies are required to explore the basis for these similarities.

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