Interleukin-4 Induces Mouse Cytomegalovirus Interstitial Pneumonia in a Latent Infection Model

JUNKO YONEMITSU

Department of Medicine, Kurume University School of Medicine, Kurume 830-0011, Japan

Summary: To better understand immune mechanisms involved in onset of cytomegalovirus pneumonia, we initially examined the replication of a low virulence strain of mouse cytomegalovirus (MCMV) in nude and BALB/c mice infected by intranasal inoculation. MCMV was detected by plaque assay in the salivary glands of nude mice from days 3 to 16, and in those of BALB/c mice from days 7 to 11. Nude mice became infected with MCMV earlier than BALB/c mice. Moreover, MCMV-DNA was detected in the salivary glands until day 16 after MCMV inoculation in nude and BALB/c mice. However, we did not find evidence of interstitial pneumonia at day 16 in either BALB/c or nude mice. These results suggest that this system represents a latent infection model in BALB/c mice and a persistent infection model in nude mice. We treated latently infected BALB/c mice with methylprednisolone or IL-4 every other day. The mice treated with IL-4 developed interstitial pneumonia, whereas those treated with m-PSL did not. In the present study, we constructed a model of MCMV latent infection that could be used to induce development of interstitial pneumonia. IL-4 appears to be a key cytokine for onset of interstitial pneumonia in mice with latent MCMV infection.

Key words mouse cytomegalovirus, interleukin 4, interstitial pneumonia

INTRODUCTION

Human cytomegalovirus (HCMV) is one of potent pathogens for patients with immune abnormalities, especially in those with alterations of T cell immunity [1-3]. Recently, HCMV infection was determined to be a frequent cause of morbidity and mortality in recipients of renal and bone marrow transplants, and in patients with acquired immunodeficiency syndrome (AIDS) [4]. Serious CMV infection in the immunocompromised host often induces interstitial pneumonia, which occasionally leads to death of the patient. Once CMV infects host, CMV is alive as long as the host is alive (latent term). The latent term is operationally defined as a period of persistent infection in which the viral genome is present but gene expression is limited and infectious virus is not produced [5]. It has been reported that CMV can be reactivated by various factors in, for example, AIDS patients and transplantation patients [3-4,6-7]. However, the detailed mechanisms of CMV reactivity have not been clarified. It was reported that the mechanism for immune evasion is the down-regulation of major histocompatibility complex (MHC) class I molecules, which are required for target recognition by cytotoxic T lymphocytes (CTL) [8], and it was reported that NK cells help to restrict the viral replication in some organs during the early phase [9-11].

Murine models of CMV infection have provided important insights into the pathogenesis of acute, chronic, and latent CMV infections in humans [12-16]. Although many experiments using MCMV have been conducted in the past, a virus stock with powerful pathogenicity was recently propagated from the salivary glands of MCMV-infected BALB/c mice [17-21]. In the present study, we report a model of MCMV latent infection with interstitial pneumonia.
due to a low pathogenicity MCMV.

MATERIALS AND METHODS

MCMV infection model

Male BALB/c mice 6 weeks old and nude mice 6 weeks old were used in all experiments. The Smith strain of MCMV was obtained from the American Type Culture Collection (Manassas, VA). The virus was passaged once in tissue culture using mouse embryo cells (MEC), and aliquots were prepared from clarified tissue culture supernatant [21]. Inocula for sham-infected controls were prepared from supernatants of uninfected cells. Infectious virus in the supernatant was quantified by plaque-forming assay. Virus and control stocks were stored at -80°C. Intranasal infection of MCMV was initiated by instilling 100 µl of stock virus (10⁴ PFU) into the nose under ethereal anesthesia.

Cell culture

Mouse C-127 lung fibroblasts were obtained from the Riken Cell Bank (Saitama, Japan). C-127 cells were maintained in Dulbecco’s modified Eagle’s medium (D-MEM) (Gibco/BRL, Life Technologies, Inc., Rockville, MD) supplemented with 10% fetal calf serum (FCS), 1% MEM Non-Essential Amino acids solution (Gibco/BRL), penicillinG (100 units/ml), and streptomycin (100 µg/ml).

Plaque assay

At various times after virus inoculation, mice were killed under ethereal anesthesia. For virus quantitation, tissues (salivary glands, lung, liver, spleen) were prepared as 10% (wt/vol) homogenates with D-MEM containing 10% newborn calf serum and stored at -80°C until the virus assay was performed. Prior to experiments, the stock supernatants were thawed. Supernatants were diluted ten-fold, and 0.2 ml of diluted supernatant was overlaid on 80 to 90% confluent C-127 mouse fibroblast cultures. Cultures were incubated for 60 min at 37°C with agitation for 10 min. At the end of this adsorption period, a mixture containing 25 ml of 2×MEM, 1 ml of 100% FCS, and 0.5 ml MEM Non-Essential Amino Acids solution was added. After 6 to 7 days, the monolayers were stained with 0.1 ml neutral red and infectious viral plaques were counted under light microscopy.

Detection of MCMV DNA by PCR

Organs to be assayed were removed, minced, transferred into 5 ml of TNE buffer [10 mM Tris-HCl (pH 8.0), 100 mM NaCl, and 1 mM EDTA], then 2% SDS and 0.5 mg/ml of proteinase K (Roche Diagnostics, Mannheim, Germany) were added. Samples were incubated for 90 min at 56°C in an agitating water bath. DNA was isolated by standard procedures, including phenol/chloroform extraction and ethanol precipitation. The amount of purified DNA was determined from the optical density at 260 nm. For the detection of cytomegalovirus DNA, primers for amplification of the 289 bases in the region coding the immediate early antigen, IE1 (5'-TCC-AGG-CTT-AAT-AGC-AGG-CG-3') and IE2 (5'-ACG-AAA-GAT-CCG-ATC-GAG-GC-3'), were synthesized with a DNA synthesizer (Gliner Japan Co., Tokyo, Japan). DNA amplification for capillary PCR was performed in 50 mM Tris (pH 8.5), 3 mM MgCl₂, 20 mM KCl, 500 µg/ml bovine serum albumin, 0.5 µM of each primer, 0.5 mM of each deoxynucleotide triphosphate, 2 µl of DNA sample, and 2.5 units of Thermus aquaticus DNA polymerase (Amersham Life Sciences Inc., Piscataway, NJ). The reaction mixture (20 µl) was drawn into the center of 10.8 cm long capillary tubes (Idaho Technology, Idaho Falls, ID) by capillary action, then the ends were sealed. Thirty-five cycles of DNA amplification were performed in a hot air thermal cycler (Idaho Technology) as follows: denaturation, 94°C, 10 s; annealing, 62°C, 10 s; and extension, 72°C, 20s. PCR-amplified samples were subjected to electrophoresis in 2% agarose gels, and bands were visualized with UV following ethidium bromide staining.

Model of MCMV pneumonia onset

BALB/c mice were intranasally inoculated with 100 µl MCMV (10⁴ PFU/ml) and infected mice were divided into two groups 16 days after inoculation, and treated with either intra-peritoneal methylprednisolone (m-PSL 0.5 mg; Pharmacia & Upjohn, Tokyo, Japan) every other day or intra-peritoneal IL-4 (500U; R & D Systems, Inc., Minneapolis, MN) every other day.

Histologic evaluation

For qualitative histologic evaluation, tissues were fixed with 10% buffered formalin. Paraffin-embedded sections were then stained with hematoxylin-eosin and examined by light microscopy.
RESULTS

Results of plaque assay

We compared MCMV replication in nude mice and BALB/c mice after intranasal viral challenge (Fig. 1). MCMV replication was detected only in the salivary glands. In the nude mice, MCMV was detected continuously from days 3 to 16 after inoculation. In the BALB/c mice, MCMV was detected from days 7 to 11 after inoculation.

Detection of MCMV-DNA

MCMV-DNA in nude mice was detected by PCR from days 3 to 16 in the salivary glands; from days 7 to 16 in the lung and liver; from days 11 to 16 in peripheral blood. In BALB/c mice, MCMV-DNA was detected from days 7 to 16; from days 7 to 11; and in peripheral blood only on day 7.

TABLE 1.
PCR detection of MCMV-DNA in tissues (peripheral blood, lung, liver, salivary gland) of BALB/c mice and nude mice

<table>
<thead>
<tr>
<th>days after inoculation</th>
<th>peripheral blood</th>
<th>lung</th>
<th>liver</th>
<th>salivary gland</th>
</tr>
</thead>
<tbody>
<tr>
<td>day 3</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>day 7</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>day 11</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>day 16</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

2. BALB/c mice

<table>
<thead>
<tr>
<th>days after inoculation</th>
<th>peripheral blood</th>
<th>lung</th>
<th>liver</th>
<th>salivary gland</th>
</tr>
</thead>
<tbody>
<tr>
<td>day 3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>day 7</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>day 11</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>day 16</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
</tbody>
</table>

TABLE 2.
PCR detection of MCMV-DNA in tissues (lung, salivary gland) of MCMV-infected BALB/c mice treated intraperitoneally with m-PSL or IL-4 every other day

<table>
<thead>
<tr>
<th>days after i.p. m-PSL or IL-4</th>
<th>m-PSL</th>
<th>IL-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>salivary gland</td>
<td>lung</td>
<td>salivary gland</td>
</tr>
<tr>
<td>day 4</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>day 8</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>day 11</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>day 26</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>day 33</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Fig. 1. MCMV replication in salivary glands of nude mice and BALB/c mice at various times after intranasal inoculation with $10^4$ PFU of MCMV. Nude mice (●); BALB/c mice (■). Sham-infected mice (▲). Results are expressed as the mean MCMV titer (log PFU/ml)±SD (n=5). Each point is the mean for samples from at least five mice.
Onset of CMV pneumonia

BALB/c mice inoculated with 100 μl MCMV (10^4 PFU/ml) intranasally were killed at day 16 after inoculation. Interstitial pneumonia was not found at this time point (Fig. 2). Therefore, these mice were presumed to have latent MCMV infection. These latently infected mice had been treated with m-PSL or IL-4. MCMV-DNA was detected by PCR in the salivary glands and lung of all m-PSL treated mice and IL-4 treated mice until day 33 after treatment with m-PSL or IL-4 (Table 2). However, in histologic sections, evidence of interstitial pneumonia was found in the mice treated with IL-4, but not in those treated with m-PSL (Fig. 3).

DISCUSSION

Cellular immunity plays a critical role in the course and outcome of CMV infection [3,7]. It has been reported that NK cells help to restrict CMV replication during the early phase of infection, whereas cytotoxic T cells are involved in controlling CMV replication during the late phase [22,23]. In the present study, MCMV infection occurred earlier in nude mice lacking T cells than in BALB/c mice (Fig. 1). These results indicate that T cells are involved in controlling primary MCMV infection. Mycobacterium tuberculosis, like CMV, which is an intracellular pathogen, is controlled by γδ the primary infection in the T cells [24-26]. It has recently been reported that γδ T cells are involved in the immune response to primary CMV infection [27,28]. This would explain why, in our study the nude mice deficient in γδ T cells were infected with MCMV earlier than were the BALB/c mice. MCMV-DNA

Fig. 2. Lung sections stained with hematoxylin eosin (H & E) from BALB/c mice 16 days after intranasal MCMV infection (×200).

Fig. 3. Lung sections stained with hematoxylin eosin (H & E) from BALB/c mice 33 days after intraperitoneal inoculation with m-PSL or IL-4. A: Lung section from mice treated with m-PSL (H & E ×200). B: Lung section from mice treated with IL-4 (H & E ×200).
was detected by PCR in all tissues assayed (peripheral blood, lung, liver, salivary glands) in both BALB/c and nude mice (Table 1). However, we could only detect CMV by plaque assay in the salivary glands (Fig. 1). These results indicate that CMV was present only at low titers in organs other than the salivary glands, and suggest that salivary glands are a preferred location for establishment of MCMV latency.

In the usual MCMV infection model, MCMV is maintained by serial passages in mice and harvested as a 10% (wt/vol) homogenate of salivary gland tissue. Salivary gland homogenate is toxic to the murine respiratory epithelium [29]. In the present study, we used a low virulence strain of MCMV derived from tissue culture to construct a latent MCMV infection model. BALB/c and nude mice that were inoculated intranasally with this strain did not develop interstitial pneumonia. Thus, this strain allowed study of latent infection in BALB/c mice and persistent infection in nude mice.

Many studies on interstitial pneumonia due to MCMV have been conducted [17,19-21,30], though none examined MCMV interstitial pneumonia arising from reactivation of latent infection. Therefore, we studied interstitial pneumonia in our MCMV latent infection model. We treated latently infected BALB/c mice with m-PSL or IL-4 every other day, and examined them for evidence of development of interstitial pneumonia. The mice treated with IL-4 every other day developed interstitial pneumonia, whereas those treated with m-PSL every other day did not (Fig. 3). However, MCMV-DNA was detected consistently in lung and salivary glands of BALB/c mice (Table 2). Glucocorticoids (GC) can affect multiple aspects of the primary anti-viral immune response, including cytokine production by T-helper cells. Specifically, corticosterone leads to suppression of the CD4+Th 1 responses (secretion of IL-2 and IFN-γ) involved in cellular immunity and resolution of viral infection [31-33]. In contrast, CD4+Th 2 cell responses, which promote B-cell activation, have been shown to be augmented by exogenous GC treatment [34-38]. However, some investigators have demonstrated that corticosteroids inhibit IL-4 production in lymphocytes [38]. Thus, the influence of m-PSL on cellular immunity is as yet unclear. However, our results indicated that IL-4 is required for induction of CMV interstitial pneumonia. Mice inoculated with Th 2 cytokine products develop severe herpes simplex virus infection while mice treated with a mixture of monoclonal antibodies directed against Th 2 cytokines do not [40]. Therefore, IL-4 appears to be a key cytokine for induction of interstitial pneumonia in our latent MCMV infection mouse model.

In conclusion, we constructed a latent infection model by using a low virulence strain of MCMV. Our results show that T cells are involved in control of primary MCMV infection, and that IL-4 is required for development of interstitial pneumonia in the setting of latent MCMV infection.

ACKNOWLEDGMENTS: The author thanks Professor Kotaro Oizumi, Assistant Professor Junichi Honda and Dr. Yasumitsu Okubo (Department of Medicine, Kurume University School of Medicine) for their valuable advice and technical assistance.

REFERENCES


