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T-lymphocyte Effects on Murine Cytomegalovirus Pulmonary Infection

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Cytomegalovirus (CMV) infections were induced in male BALB/c mice treated with rat monoclonal antibodies (MAb) to deplete selectively CD8 and CD4 cell populations in vivo. ***The animals were then inoculated intraperitoneally with murine CMV and the infection was monitored virologically and histologically. High concentrations of virus were found in the lungs of mice depleted of CD4 or both CD4 and CD8 cells. These animals developed pulmonary infections that persisted for at least 49 days after inoculation. In contrast, immunologically intact mice and those administered anti-CD8 MAb experienced only a transient infection of the lungs. Focal interstitial infiltrates of mononuclear cells were demonstrated in pulmonary tissues of CD4 MAb-treated animals, but not in normal mice and those receiving the CD8 MAb. Adoptive transfer of CD4 cells to animals (rendered immune-incompetent by thymectomy and irradiation) protected against pulmonary infection and the development of interstitial pneumonia. Mice treated with CD4 MAb failed to produce specific CMV antibody, whereas the depletion of CD8 cells had no effect on antibody elaboration. Administration of anti-CD4 and CD8 MAb did not affect virus replication in the salivary glands, the preferential site for CMV infection in the mouse. Induction of pulmonary infection and interstitial pneumonia by CMV in BALB/c mice is mediated by CD4 T cells. (Am J Pathol 1990, 137:907–912)***

Latent infections with cytomegalovirus (CMV) are common in immunologically competent humans.[1] They often become disseminated in persons treated with immunosuppressive agents after organ transplantation and in those with the acquired immune deficiency syndrome (AIDS).[2–5] In addition, immunosuppressed individuals are unusually susceptible to disseminated infections caused by newly acquired virus.[5] Although a variety of organs are involved, the lung is a major site of CMV replication.[6] Interstitial pneumonia often, but not invariably, accompanies these pulmonary infections, and the resulting pulmonary insufficiency is life-threatening.[7]

To understand the pathogenesis of CMV pneumonia, mice infected with murine CMV have been studied in an attempt to develop a model of human disease. In a previous investigation, Brody and Craighead[8] produced an interstitial exudative pneumonia in mice immunosuppressed by the administration of antiserum raised against a mixed population of murine lymphocytes. A number of additional studies have been conducted by others using irradiation,[9] cyclophosphamide,[10] cyclosporine, and corticosteroids[11,12] to modify the immunologic state of adult mice during CMV infection. Immunosuppression by these interventions results in a disseminated infection with lesions in various organs, but CMV pneumonia has been a variable feature. Because these treatments are nonspecific, they have not defined the role of the various cellular elements of the immune system in the control of infection, and the pathogenetic mechanisms involved.

The development of MAb reactive with specific lymphocyte cell surface antigens makes it possible to selectively deplete lymphocyte subsets *in vivo*.[13,14] Thus, the investigator can explore the role of individual cell types in the infectious process. We describe here the effects of long-term depletion of CD4 and CD8 cells on the replication of murine CMV in various organs, with specific attention focused on the lung.

Methods

Viral Studies

The Smith strain of CMV (provided by J. Shanley, University of Connecticut, Farmington, CT) was maintained by serial passage in newborn mice. It was prepared and stored at −70°C as a 10% homogenate of suckling

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mouse lung tissue in Dulbecco's Modified Eagle Medium containing 10% fetal calf serum (GIBCO, Grand Island, NY), 10% dimethyl sulfoxide, and gentamicin. Infectivity was assessed in secondary cell cultures of mouse embryo fibroblasts using cytopathic effects to establish end points.[15] Mouse serum was heated at 56°C for 30 minutes and the neutralization antibody concentrations determined against 100 tissue culture infectious doses, 50% effective ($TCID_{50}$) of virus. The beginning serum dilution was 1:10, and serial twofold dilutions were tested.

Mice

BALB/c mice (originally purchased from Cumberland Farms, Clinton, TN) were bred and maintained at the University of Vermont. They were given water and fed Purina mouse chow 5015 (Ralston Purina Company, St. Louis, MO) *ad libitum*. Three- to four-week-old male mice were inoculated intraperitoneally with $10^{4.5}$ $TCID_{50}$ of virus in 0.5 ml phosphate-buffered saline (PBS).

Monoclonal Antibody Production and Administration

Anti-CD4 (GK 1.5) and anti-CD8 (2.43) hybridomas (American Type Culture Collection, Bethesda, MD) were used to prepare MAb in female pristane-treated, irradiated BALB/c mice using standard techniques. Ascitic fluid was stored at −20°C, purified by ammonium sulfate precipitation, and chromatographed in G-25 Sephadex (Pharmacia, Upsalla, Sweden) before determining immunoglobulin concentrations. To deplete a specific T-cell population, mice were injected intraperitoneally with 1 mg MAb 48 hours before and 48 hours after inoculation of the virus. Animals were administered 1 mg MAb at weekly intervals thereafter. Cells teased from the mesenteric lymph nodes of these mice were assayed 4 days after the second MAb administration by fluorescence-activated cell sorting to assure that more than 95% of cells of a specific subset were depleted. The kinetics of T-cell depletion in animals treated with the MAb used in this study have been described.[16,17]

Tissue Preparation

The thoracic cavity of Pentothal (Abbott Hospital Products, North Chicago, IL)-anesthetized animals was exposed and the right ventricle cannulated. Buffered formalin then was instilled into the left lung for 1 minute after the great vessels and bronchi of the right lung had been clamped and the right lung removed for virologic study.[18] The formalin-perfused left lung then was excised and fixed for 24 hours, after which it was cut in the horizontal plane from apex to base for pathologic study. Tissue sections were prepared by standard techniques and stained with hematoxylin and eosin (H&E).

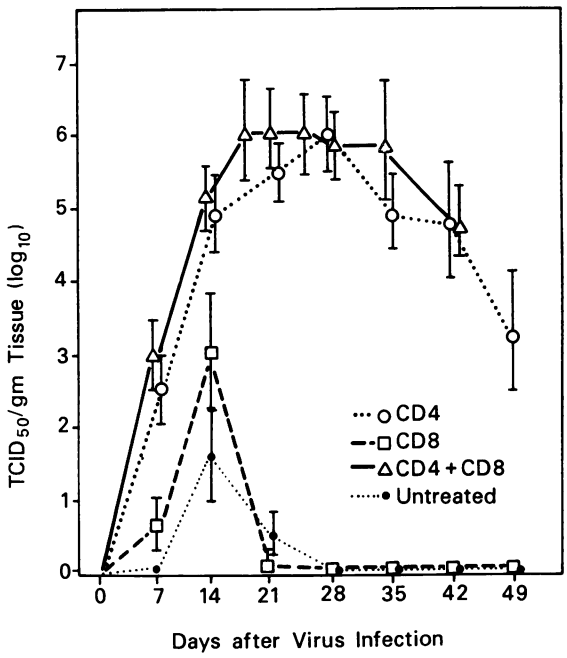

Figure 1. *CMV concentrations in lung tissue of normal and immunosuppressed mice on indicated days after intraperitoneal injection of $10^{4.5}$ $TCID_{50}$ of CMV. The different groups of animals were treated with CD4, CD8, or both MAb before the infection.*

Adoptive Transfer of CD4 and CD8 Cell Populations

At 3 weeks of age, male BALB/c mice were thymectomized and x-ray irradiated (600 rad, Theratron Jr. gamma cobalt 60, Anatomic Energy of Canada, Ltd., Ontario, Canada) and intravenously reconstituted with approximately 10^7 syngeneic age- and sex-matched bone marrow cells that had been pretreated with both anti-CD8 or anti-CD4 and complement. Three weeks later, either CD4 or CD8 cells teased from periaortic lymph nodes of uninfected syngenic mice were administered intravenously to these animals. The cells were prepared by incubating crude cell suspension from the lymph nodes with complement and either CD4 or CD8 MAb. After a 3-week interval, the animals were infected with $10^{4.5}$ $TCID_{50}$ CMV.

Results

Cytomegalovirus was detected in the lungs of normal adult mice 7 to 21 days after inoculation (Figure 1). In animals treated with CD4 MAb, the virus was present in pul-

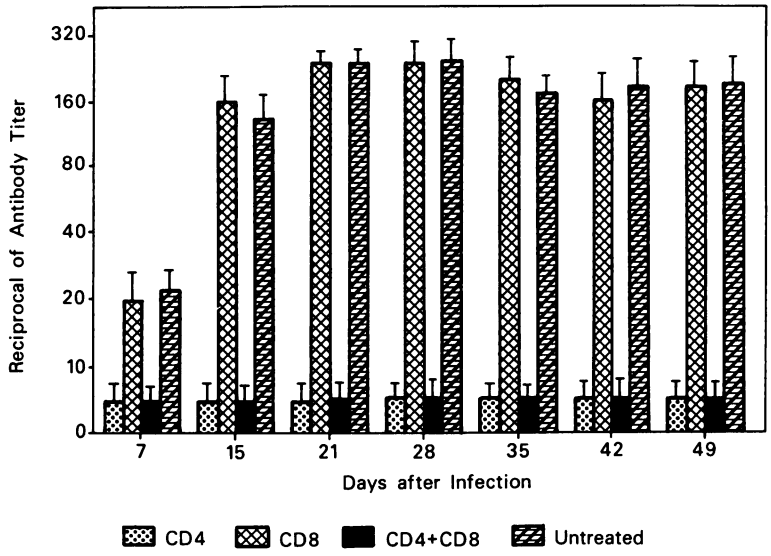

Figure 2. *CMV-specific serum antibody in MAb-treated and normal mice. Animals were injected intraperitoneally with $10^{4.5}$ $TCID_{50}$ of CMV and the neutralization antibody response was determined at intervals after infection. The titer represents the mean of four or more animals.*

monary tissue by day 7 and relatively large amounts were found in the lungs by day 14. Virus was recovered from the lung tissue of CD4 MAb-treated mice for extended periods of time thereafter, although MAb administration was discontinued on day 28 of the experiment. CD8 MAb treatment had only a slight, transient effect on virus proliferation in the lungs. Administration of both MAbs simultaneously yielded results comparable to those attained with CD4 MAb treatment alone. The observations in Figure 1 were confirmed in two repeat experiments.

Cytomegalovirus-specific neutralizing antibody was elaborated by infected normal animals, but was not detected in the blood serum of mice treated with CD4 MAb over the duration of the experiment (Figure 2). CD8 MAb treatment had no effect on antibody elaboration.

The lungs of CD4 MAb-treated mice exhibited focal interstitial mononuclear cell infiltration, but no inclusion-bearing cells were observed microscopically, and evidence of an exudative process in the airspaces was not seen (Figure 3). These animals exhibited no evidence of respiratory insufficiency and there was no weight loss or increase in mortality.

In vivo lymphocyte depletion with MAb individually or in combination did not appear to affect virus replication in the salivary glands. Virus was recovered from the spleen for a 3- to 4-week period after inoculation. The virus concentrations in this organ were higher in animals treated with CD4 MAb (Figure 4).

Thymectomized and irradiated animals that had been administered lymphocyte-depleted bone marrow developed chronic infections of the lung. Adoptive transfer of 10^7 of mixed CD4 and CD8 lymphocytes (Figure 4) or 10^7 of CD4 cells reduced the concentrations of virus detected in lung 3 weeks after virus inoculation. CD8 cells had no effect (Figure 5).

Discussion

Cytomegalovirus interstitial pneumonia is an important clinical problem in immunosuppressed allograft recipients and among those with AIDS.[2,3] Its unique occurrence in these patient groups strongly suggests that an imbalance between CD4 and CD8 T cells plays a key role in controlling a latent CMV infection. Murine models of CMV pneumonia have been developed in several laboratories using different approaches to immunologically modify the animals.[8–12] To date, however, these studies have failed to define the mechanism of immune regulation of the virus and the specific factors that permit it to replicate with seeming abandon. The studies recorded in this report were undertaken to address this question.

Immune regulation of murine CMV infection is complex but incompletely defined. Macrophages are intimately involved in supporting the replication of the virus *in vivo* and may be a site where latent infections smolder.[19,20] While serum antibody is protective, its role in an established active or latent infection is problematic.[21–23] Cytomegalovirus infection alters the immune responsivity of the mouse and its capacity to elaborate interferon during the early stages of the primary infection.[24] Natural killer cells are a critical element in determining the outcome of a primary infection early in its course.[25] And, shortly after virus inoculation, specific cytolytic CD8 cells are elaborated. Once sensitized to the virus, these cells exhibit a prophylactic effect when adoptively transferred into recipients before infection and reduce the virus concentrations of various organs in animals with an established infection.[26,27] A lethal outcome can be aborted by relatively few sensitized CD8 T cells.

Recently Erlich et al[28] showed that the depletion of CD4 cells by the administration of MAb increased virus

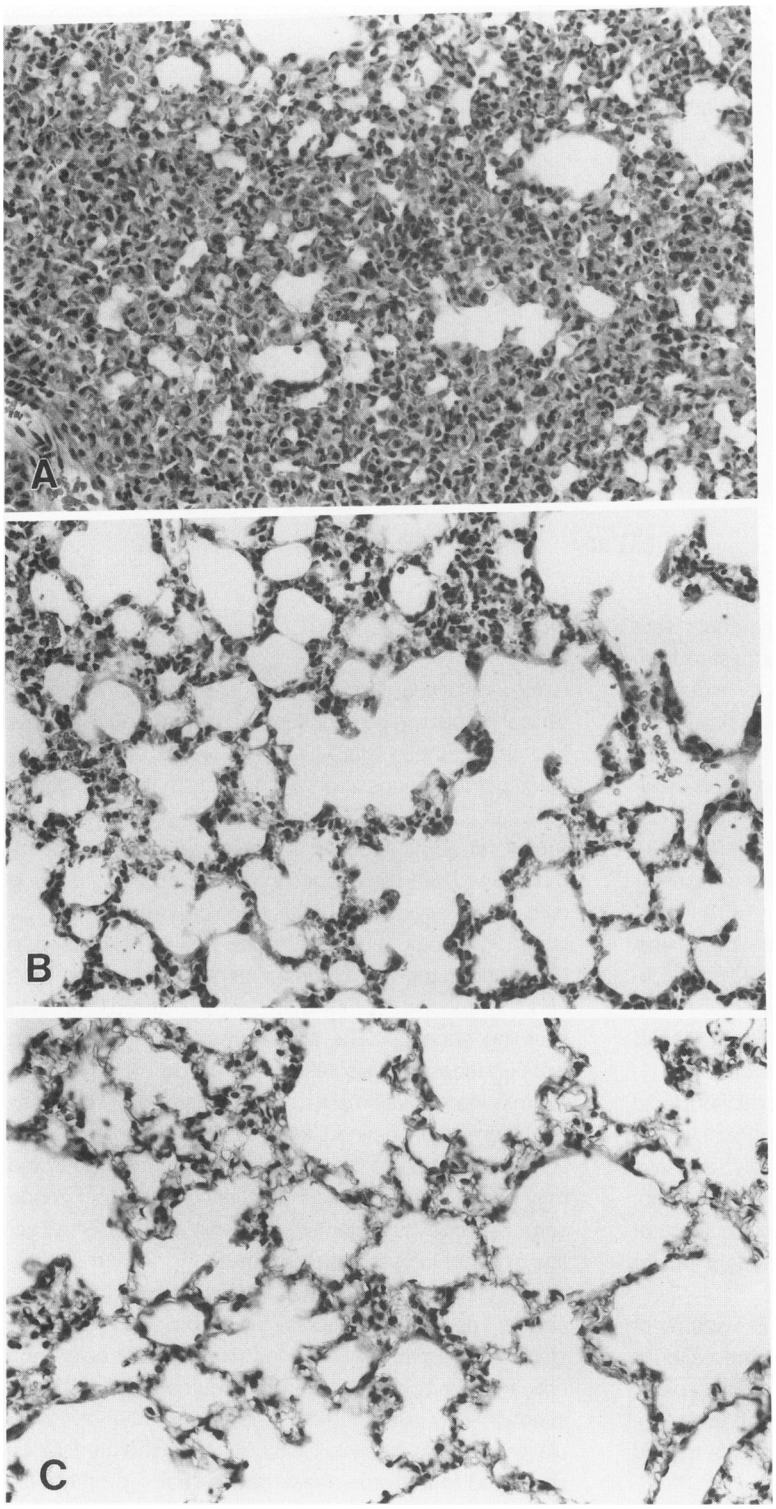

Figure 3. *Lungs of CMV-infected mice administered CD4 MAb* (A), *CD8 MAb* (B), *and PBS* (C). *The animals were killed 21 days after virus infection. Note the interstitial accumulations of lymphocytes in the lung of the mouse deficient in CD4 cells.*

replication in the salivary glands, lung, and spleen of CMV-infected mice. In our studies, anti-CD4 MAb treatment dramatically increased virus accumulation in the lung, but had no effect on salivary gland virus replication. Conversely, Reddehase et al[27] found CD4 cells from virus-sensitized donors to be ineffective in altering the course of a lethal CMV infection in irradiated animals lacking CD8 cells. It seems plausible to propose tentatively that 'helper' CD4 cells facilitate sensitization of CD4 'cytolytic' T cells, but do not have a direct viricidal effect. In

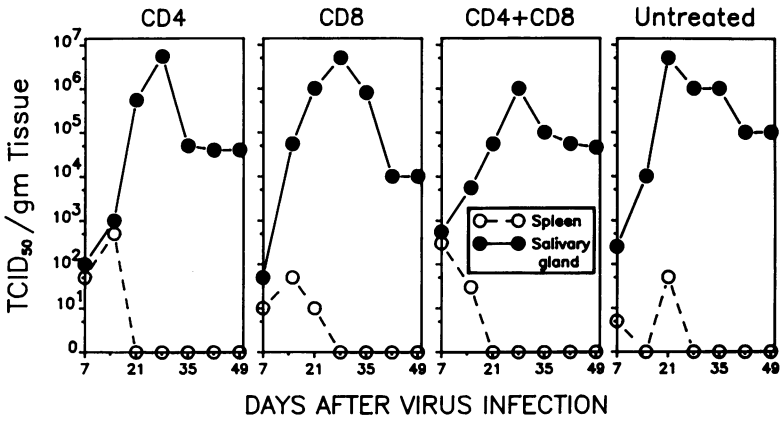

Figure 4. *CMV in salivary glands and spleen of MAb-treated and normal mice. Each point is geometrical mean of value for five or more animals.*

preliminary experiments, however, we failed to find evidence for the elaboration of T cells *in vivo* that alter the course of infection after adoptive transfer into infected animals. In addition, lymphocytes cytolytic for infected cultured cells are not demonstrable *in vitro*.

The pathogenetic mechanism of the interstitial pulmonary disease in the CMV-infected mouse remains to be defined. In an earlier study, a prominent inflammatory infiltrate with intra-airspace fluid exudation developed in CMV-infected mice administered antilymphocytic serum.[8] In this model, the pulmonary interstitial and alveolar macrophages exhibited intranuclear inclusion and were found to be the major, if not the exclusive, carriers of the virus.[29] Similar lesions are observed in thymectomized animals that have been irradiated and replenished with lymphocyte-depleted bone marrow. These findings contrast with the observations reported here in which lymphocytes predominated in the interstitium and inclusion-bearing cells were not found. The complex morphologic features of the murine pulmonary CMV disease remain to be evaluated. It seems plausible to suggest that the exudative pneumonia dominated by inclusion-bearing infected macrophages reflects a profound degree of immunosuppression in which lymphocytes of all types are depleted. Conversely, in the model described here, the immune control of virus replication is targeted, but exudative disease does not occur. In immunosuppressed humans, interstitial pneumonia is a variable morphologic feature even in lungs containing large amounts of virus, and the number of cells with inclusions only roughly correlate with the lung virus titer.[4,5,30,31]

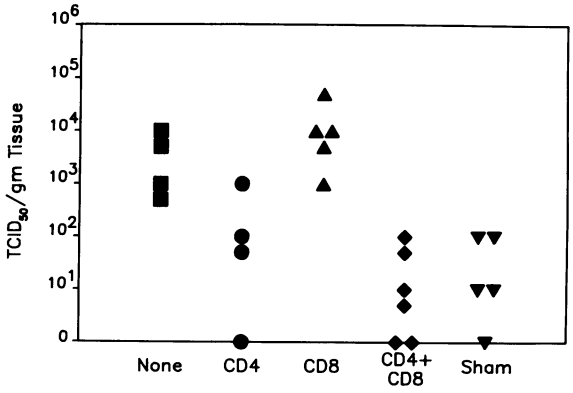

Figure 5. *CMV concentration in lung tissue of mice after thymectomy, irradiation, and bone marrow transplantation. The animals were reconstituted by the specific subsets, intravenously, 10^7 lymphocytes of infusion.*

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