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Interstitial Murine Cytomegalovirus Pneumonia After Irradiation: Characterization of Cells That Limit Viral Replication During Established Infection of the Lungs

MATTHIAS J. REDDEHASE, FRANK WEILAND, KONRAD MÜNCH, STIPAN JONJIC,† ANKE LÜSKE, AND ULRICH H. KOSZINOWSKI*

Federal Research Centre for Virus Diseases of Animals, D-7400 Tübingen, Federal Republic of Germany

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Interstitial pneumonia associated with viral replication in lung tissue was observed after cytomegalovirus infection of total-body γ-irradiated mice, whereas in noncompromised hosts the lungs were not affected and virus multiplication was restricted to the salivary glands. The radiation damage could either predispose normally nonpermissive cell types for productive infection or abrogate an immune control of the tissue manifestation of infection by elimination of lymphocytes. Adoptive transfer of lymphoid cells into irradiated, infected recipients supported the second alternative. Even when infection was established in the lungs, as manifested by the presence of infected lung tissue cells in the alveolar septa, an antiviral effect could be assigned to the Lyt-2$^+$, L3T4$^-$ subset of T lymphocytes specifically sensitized in the immunocompetent donor. These cells did not require in vitro propagation to perform effector cell functions in vivo and were operative under physiological conditions in comparatively low numbers. Hence, there is reason to assume that T lymphocytes are responsible for the tissue distribution of cytomegalovirus replication during infection.

In the therapeutic treatment of leukemia patients by irradiation and bone marrow transplantation, a major complication results from interstitial pneumonia associated with reactivation of latent herpesviruses. In most cases viral pneumonia with a very high incidence of mortality is due to cytomegalovirus (CMV), whereas pneumonia associated with herpes simplex virus or with varicella zoster is less frequent and also less severe (30).

The experimental infection of mice with murine CMV (MCMV) has proven an appropriate model to study CMV pathogenesis since this infection of a natural host is also linked with interstitial pneumonia (5, 19, 39). Like in the infection of humans with human CMV, immunosuppression is a prerequisite for MCMV reactivation from latency (13, 20, 29) and for infection of the lungs (5). These findings indicate that the establishment of productive infection in the tissues of the host is not only dependent on the virulence of the virus and the presence of permissive cells but is also controlled by the immune system.

During acute intraplantar MCMV infection of immunocompetent BALB/c mice, the presence of activated interleukin (IL)-receptive cytolytic T-lymphocyte precursors (IL-CTL$_p$) has been demonstrated in the draining popliteal lymph node (LN) by measuring the lytic potential of the IL-CTL$_p$-derived effector CTLs after in vitro propagation mediated by IL only (34). In this combined protocol of in vivo sensitization and in vitro propagation, Lyt-2$^+$ CTLs are generated that are able to recognize and destroy embryonic fibroblast target cells at a stage of viral replication when only immediate-early proteins are expressed (35, 36). It has been proposed that CTLs with such a specificity are candidates to limit viral replication in infected tissues before the production of progeny virions occurs (36).

Adoptive transfer experiments have been applied to demonstrate the in vivo potential of immune lymphoid populations (1, 17, 24, 26, 31, 41, 45, 47), as well as of CTL lines or clones (8, 27, 28, 38), in various virus infections, including the infection with MCMV (17, 41). Collectively, these studies have confirmed that mature, self-restricted Lyt-2$^+$ CTLs can exert an antiviral effect in vivo.

Provided that CTLs are also responsible for the prevention of MCMV replication in the lungs of the immunocompetent host during persistent infection, it follows that sensitized CTL$_p$ do not require in vitro propagation to mature to antiviral effector cells. This prediction can be tested by direct adoptive transfer by using the infected immunocompetent animal as donor of activated lymphocytes and the irradiated infected animal as recipient.

MATERIALS AND METHODS

Donors and recipients for adoptive transfer. BALB/c mice (H-2^d haplotype) were bred in our own colony and controlled for the absence of murine hepatitis virus. To serve as donors for sensitized lymphocytes, 8-week-old females were infected in the left hind footpad with 10^5 PFU of MCMV (strain Smith, ATCC VR-194) grown in BALB/c mouse embryo fibroblast cell culture and partially purified as described (34). The stock used for priming contained 10^9 PFU of MCMV per ml in Ca- and Mg-free phosphate-buffered saline and was controlled for absence of mycoplasma. To serve as recipients, female BALB/c mice at the age of 10 weeks were subjected to total-body γ irradiation in a single dose of 6 Gy (1 Gy = 1 J/kg = 100 rads) by using a cesium-137 source, delivering a dose rate of 1.325 Gy/min. Infection was performed as it was in the immunocompetent donors. Thereafter, mice were kept under laminar flow.

Activated lymphocytes derived from the draining popliteal LN of the donors 8 days after intraplantar infection were suspended in 0.5 ml of 0.15 M NaCl supplemented with 1%

* Corresponding author.
† Present address: Faculty of Medicine, University of Rijeka, 51000 Rijeka, Yugoslavia.

BALB/c normal serum and injected into the tail vein of the recipient. The time schedule for transfer depended on the protocol intended. For the prophylactic transfer cells were infused after irradiation and 2 h before infection, whereas for the therapeutic transfer cells were infused 6 days postinfection (p.i.) when MCMV was already replicating in the lungs.

Treatment of donor cells for selection of T-lymphocyte subsets. Complement-dependent antibody-mediated cytolysis was applied to eliminate certain lymphocyte subpopulations. The cells were treated with monoclonal antibody (mAB) for 10 min at 4°C and low-toxicity rabbit complement (diluted 1:15) for 45 min at 37°C in two cycles. All mABs were used at their plateau activity as undiluted hybridoma supernatants. Anti-Lyt-2.2 mAB (immunoglobulin G2a) was produced by hybridoma 19/178 (16). To deplete for $L3T4^+$ lymphocytes, mAB GK1.5 (immunoglobulin G2b) was used (10).

To prove the efficiency of depletion of $L3T4^+$ lymphocytes, normal splenocytes were treated either with mAB GK1.5 and complement or, for control, with complement alone. Thereafter, 5×10^6 cells per ml of minimal essential medium α were incubated for 20 h in the presence of 10 μg of the T-cell mitogenic lectin concanavalin A (Pharmacia, Uppsala, Sweden). Minimal essential medium α without nucleosides (GIBCO Laboratories, Grand Island, N.Y.) was supplemented with 5% fetal calf serum, 2 mM L-glutamine, and 5×10^{-5} M 2-mercaptoethanol. The IL-2 content of the culture supernatant was then assessed with a nonhelper subline (21) of the IL-2-dependent BALB/c-derived T-cell line HT-2 (44) and was found to be 10% of the control value.

Bone marrow cell populations obtained by flushing medium through the shafts of the femori and tibiae of normal BALB/c mice were depleted for cells of the T-cell lineage by using a mixture of the mABs anti-Lyt-2.2 and GK1.5. Before intravenous infusion, all cell suspensions were passed through nylon mesh to remove clumps.

Determination of tissue virus titers and statistical evaluation. Organs were frozen, thawed, and passed for homogenization through stainless steel mesh in minimal essential medium with Earle salts (Serva) supplemented with 5% fetal calf serum, 2 mM L-glutamine, 10 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer (pH 7.2), 100 U of penicillin per ml, 0.1 mg of streptomycin sulphate per ml, 0.2 mg of gentamycin per ml, and 2.5 μg of amphotericin B per ml. The homogenates were titrated in $\log_{10}$ dilutions on subconfluent second-passage mouse embryo fibroblasts in 48-well culture plates (Costar, Cambridge, Mass.), starting with an 0.5% sample of the homogenate of the lungs, salivary glands, and spleen and an 0.05% sample of the liver. Usually, each dilution was represented by two replicate cultures. Only when titers were expected to be low was the highest concentration tested in four replicates, thereby defining the detection level as 500 PFU for the liver and 50 PFU for the other organs. The pooled parotid, greater sublingual, and mandibular glands are collectively referred to as salivary glands.

To enhance the sensitivity of the assay, infection was performed under the influence of a centrifugal field of 800 × *g* for 30 min (18). Thereafter, monolayers were washed and covered with methylcellulose, and plaques were counted 4 days later.

The distribution-free exact rank sum test (Wilcoxon-Mann-Whitney test) was applied to compare independent sets of data (2). This test is indicated when a normal distribution of data can not be considered as proven. The null hypothesis of similar location is rejected, and the alternative hypothesis of dissimilar location is accepted for $P < \alpha = 0.05$, where P denotes the observed probability value and α denotes the selected significance level.

Light and electron microscopy. For light microscopy lungs derived from irradiated, infected mice were fixed with Bouin fixative. Tissue samples were embedded in paraffin, and thin sections (5 μm) were stained with hematoxylin and eosin. For electron microscopy fixation was performed with 2.5% glutaraldehyde buffered at pH 7.2 with 0.1 M sodium cacodylate. After postfixation in 1% osmium tetroxide, tissue blocks were embedded in araldite. Thin sections (1 μm) were stained with toluidine blue to select sites of interest by light microscopy. Ultrathin sections from selected regions were stained with uranyl acetate and lead citrate and then examined with a Siemens 101 electron microscope.

In situ hybridization of lung tissue sections with cloned MCMV DNA fragments. Microtome thin sections (5 to 10 μm) of frozen lung tissue were spread on pretreated slides (3) and processed for hybridization by published procedures (4). The hybridization solution (15) contained 0.5 ng of plasmid cloned MCMV DNA *Hin*dIII fragments A, B, D, and C per μl (11, 22) tritiated by nick translation (37) to a specific activity of 4×10^7 dpm/μg. After hybridization at 37°C for 24 h the specimens were washed at 37°C for 5 min three times in 50% formamide–3× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) and for 60 min three times in 3× SSC, dehydrated, coated for autoradiography with NTB 3 photoemulsion (Eastman Kodak Co., Rochester, N.Y.) and exposed for 3 days at 4°C.

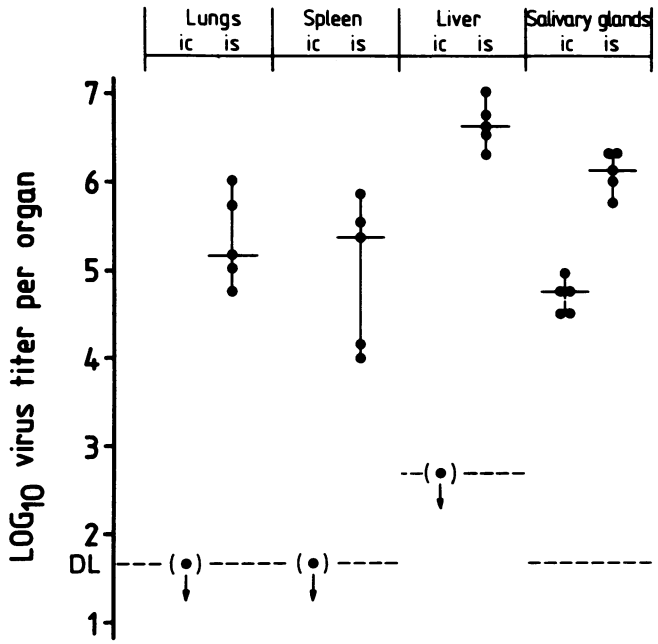
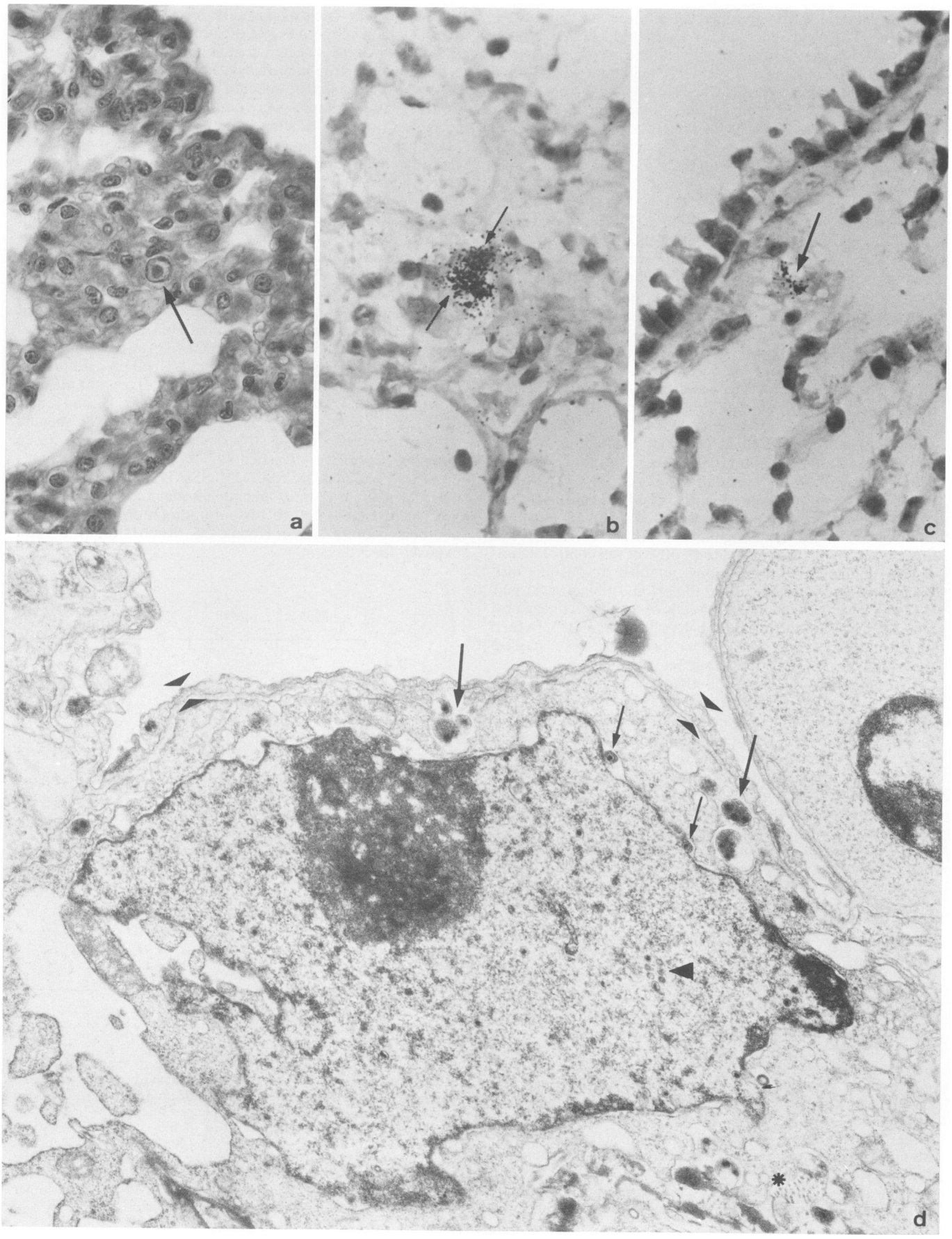


FIG. 1. Effect of irradiation on the tissue manifestation of MCMV infection. Virus titers were measured in the indicated tissues of five immunocompetent (ic) and five immunosuppressed (is) BALB/c mice 14 days after intraplantar infection with 10^5 PFU of MCMV. Immunosuppression was achieved by total-body γ irradiation with a single dose of 6 Gy. The median values of the individual titer determinations are marked by horizontal bars. The dashed line represents the experimental detection level (DL) that was defined as 500 PFU for the liver and 50 PFU for the other organs. The arrows indicate that titers were below DL.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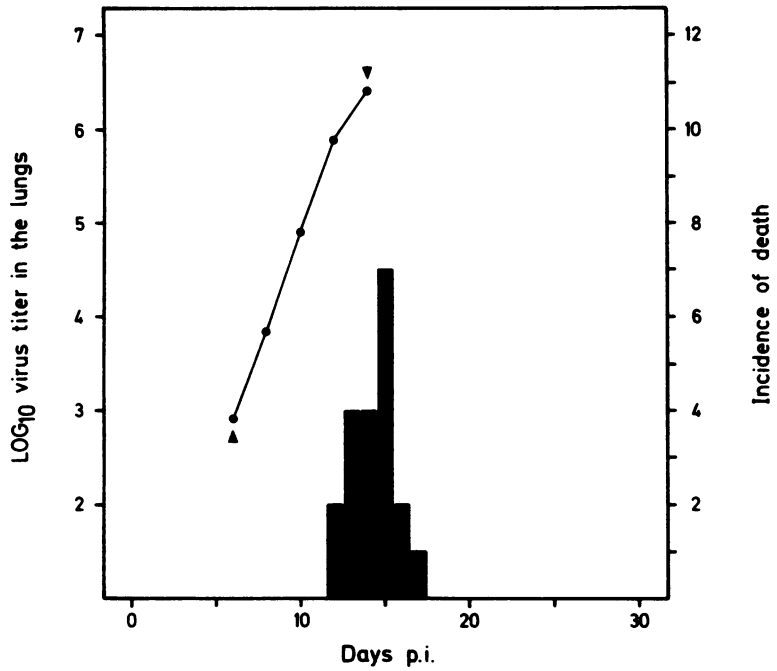

FIG. 3. Coincidence of mortality and increase in lung tissue virus titers. Forty 10-week-old female BALB/c mice were subjected at day zero to γ irradiation with a single dose of 6 Gy (equal to a lethal dose causing 50% of the mice to die within 30 days), followed by intraplantar infection with 10^5 PFU of MCMV. Thereafter, two groups were formed by distributing the mice randomly. The kinetics of virus multiplication in the lungs was measured in one group (the mean titer of two random samples is depicted), and the incidence of death was monitored in the second group (the bars represent numbers of deceased animals for each day). In infected but not irradiated controls 0 of 20 mice died, whereas in irradiated but not infected controls 11 of 20 mice died within the observation period of 30 days. The arrowheads mark the virus titers at day 6 and day 14, which are important for the subsequent experimental schedules.

RESULTS

Effect of γ irradiation on the tissue manifestation of infection. In immunocompetent donors, footpad infection elicited a marked cellular immune response in the draining popliteal LN, as indicated by an increase in cell numbers from 10^6 to 2×10^7 within 8 days and a frequency of activated MCMV-specific CTL_p in the order of 10^{-3} to 10^{-4} depending on the in vitro propagation protocol applied (34). When 10^5 PFU of cell culture-grown MCMV was used for infection, no signs of inflammation were detectable at the site of infection, nor could infectious virus be recovered at day 14 p.i. from organs such as lungs, spleen, or liver, although infectious virus was recovered from the salivary glands, which are a privileged site for MCMV replication during persistency (Fig. 1). Accordingly, MCMV, formerly named salivary gland virus of the mouse, has originally been isolated from persistently infected salivary glands (40).

On the contrary, in 6-Gy γ-irradiated recipients, immunosuppression was manifested by a decline in the cell numbers of the popliteal LN from 10^6 to 10^4 within 8 days and the absence of activated MCMV-specific CTL_p after infection (data not shown). In the footpad, a highly exudative process with extensive hemorrhages and local necrosis was seen histologically at day 14 p.i. (F. Weiland, manuscript in preparation), and high virus titers were observed not only in the salivary glands but also in liver, spleen, and lungs (Fig. 1).

Evidence for interstitial pneumonia. To establish a model for viral pneumonia, it had to be verified that virus detected in the lungs of the immunosuppressed host is autochtonously replicating in lung tissue and is not just disseminated from the salivary glands. Histological studies demonstrated symptoms of a moderate focal interstitial pneumonia: cell swelling, exudation of fluid into interstitial areas, and cells located in the widened alveolar septa containing intranuclear inclusions (Fig. 2a). The etiology of this infection was confirmed by in situ hybridization (Fig. 2b) by using plasmid cloned MCMV DNA fragments (11, 22). With this highly specific and sensitive technique, infected cells could be detected in lung tissue at day 6 p.i., and thereby it was proved that infection was established at that time (Fig. 2c). Electron

FIG. 2. Histological evidence for established infection of lung tissue. Lung tissue was derived at day 14 p.i. (panels a, b, and d) or at day 6 p.i. (panel c) from BALB/c mice infected in the footpad with 10^5 PFU of MCMV after immunosuppression by total-body γ irradiation with a single dose of 6 Gy. (a) Thin section of lung tissue stained with hematoxylin and eosin and examined by light microscopy (magnification, ×160), demonstrating the widening of the alveolar septa and a cell bearing a typical intranuclear inclusion (arrow). (b and c) Infected cells in cryostat thin sections of frozen lung tissue were detected at day 14 p.i. (b) and at day 6 p.i. (c) by in situ hybridization of viral nucleic acid with a mixture of plasmid cloned MCMV DNA *Hin*dIII fragments as specific probe. Autoradiographs were exposed for 3 days and examined by light microscopy (magnification, ×160). Infected cells were located in the alveolar septa (arrows). (d) Electron micrograph (magnification, ×15,000) showing an infected interstitial fibroblastic cell producing collagen (asterisk). This cell is covered with a rim of thin flattened cytoplasm extruding from a membraneous pneumocyte (opposed arrowheads), Nucleocapsids can be detected in the nucleus (arrowhead), monocapsid virions in the perinuclear cisterna (small arrows), and multicapsid virions in the extracellular space (large arrows).

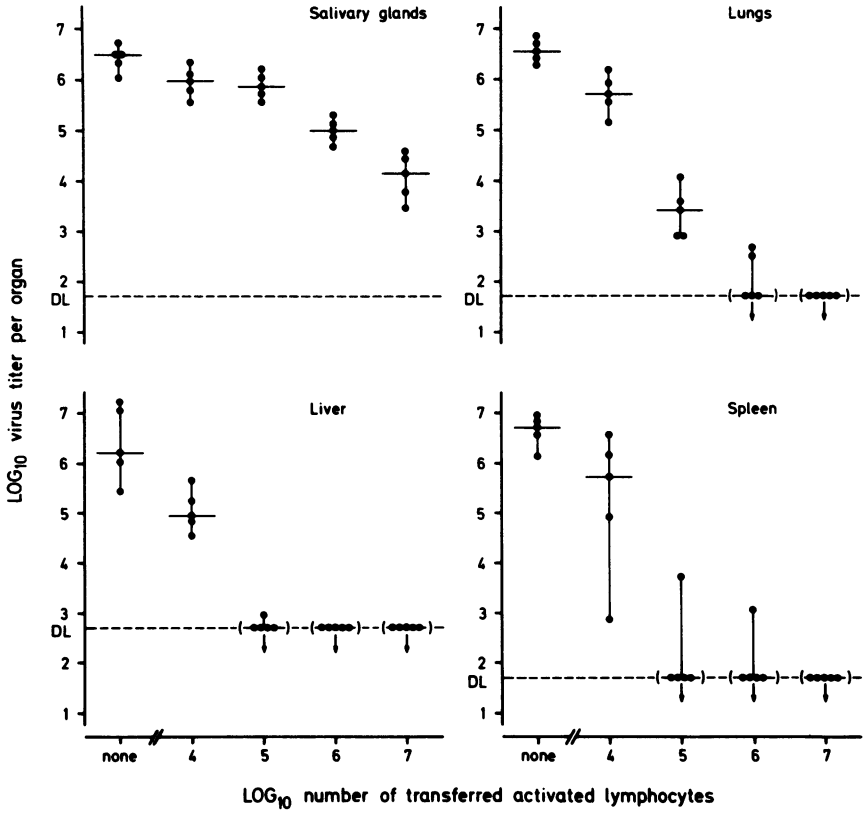

FIG. 4. Differential antiviral efficiency of activated donor lymphocytes in the tissues of cell transfer recipients. Irradiated animals received specifically activated donor lymphocytes by intravenous infusion before intraplantar infection. At day 14 p.i. MCMV titers were measured in the organs of five recipients for each dose of transferred lymphocytes. The median values of the individual titer determinations are shown by horizontal bars. The dashed lines represent the detection levels (DL).

microscopic analysis identified interstitial cells (Fig. 2d), pneumocytes, and endothelial cells (data not shown) as the target cells for MCMV replication in lung tissue after irradiation when cellular infiltration was virtually absent. After immunosuppression of mice by treatment with antilymphocyte antiserum, MCMV has been shown to replicate also in pulmonary macrophages (5).

The dying of the irradiated, infected animals coincided with a log-linear increase in tissue virus titers, as exemplified for the lungs (Fig. 3). The virus titer observed in the liver (Fig. 1 and 4) was due to infection of hepatocytes causing focal necrosis (data not shown), and in addition other essential organs may be affected.

Prophylactic adoptive transfer of specifically activated lymphocytes. This type of transfer protocol was applied for probing the capacity of in vivo-sensitized lymphocytes to prevent the tissue manifestation of the MCMV infection. The lymphocytes were taken from the draining popliteal LN of immunocompetent donors (BALB/c mice, H-2^d haplotype) infected in the footpad 8 days previously and were transferred intravenously into irradiated syngeneic recipients that were infected in the footpad 2 h after cell transfer. The read-out time for the virus titers in the organs was defined as 14 days p.i. at the peak of mortality in the nonreconstituted control group (Fig. 3). From the results depicted in Fig. 4, the following information can be deduced. First, a significant antiviral effect in the lungs ($P = 0.005$; one-sided distribution-free Wilcoxon-Mann-Whitney test), liver ($P = 0.01$), and spleen ($P = 0.025$) can be established after transfer of only 10^4 activated lymphocytes (i.e., <0.1% of the lymphocyte content of a single donor LN). This number of activated lymphocytes contains ca. 10^3 Lyt-2^+ lymphocytes, as revealed by fluorescent-activated cell sorter analysis (data not shown) and within this subset ca. 10 sensitized MCMV-specific CTL_p (34). Second, prevention of detectable MCMV replication in the tissues is mediated by 10^5 activated lymphocytes in the case of the liver and spleen (in four of five recipients the virus titer did not exceed the detection level) and by 10^6 activated lymphocytes in the case of the lungs. Third, although lymphocytes proved to be effective in preventing viral replication in certain tissues that are relevant for pathogenesis, even 10^7 transferred activated lymphocytes were not sufficient to clear the MCMV infection since viral replication persisted in the salivary glands—again underlining the exceptional role of the salivary glands in CMV infections. The tissue distribution of viral replication after transfer of 10^7 activated lymphocytes closely resembled that displayed by the infected immunocompetent host (compare Fig. 1 and 4), and accordingly all recipients survived.

Clinical trials in the therapy of leukemia involve reconstitution with type-matched bone marrow after total-body or total-lymphoid irradiation (30). In this context it is important to note that transfer of up to 10^7 syngeneic bone marrow cells which were depleted for Lyt-2^+ and L3T4^+ cells of the T-cell lineage had no significant effect on the virus titers in the lungs ($P > 0.2$ when virus titers in the lungs of control mice that did not receive bone marrow cells were

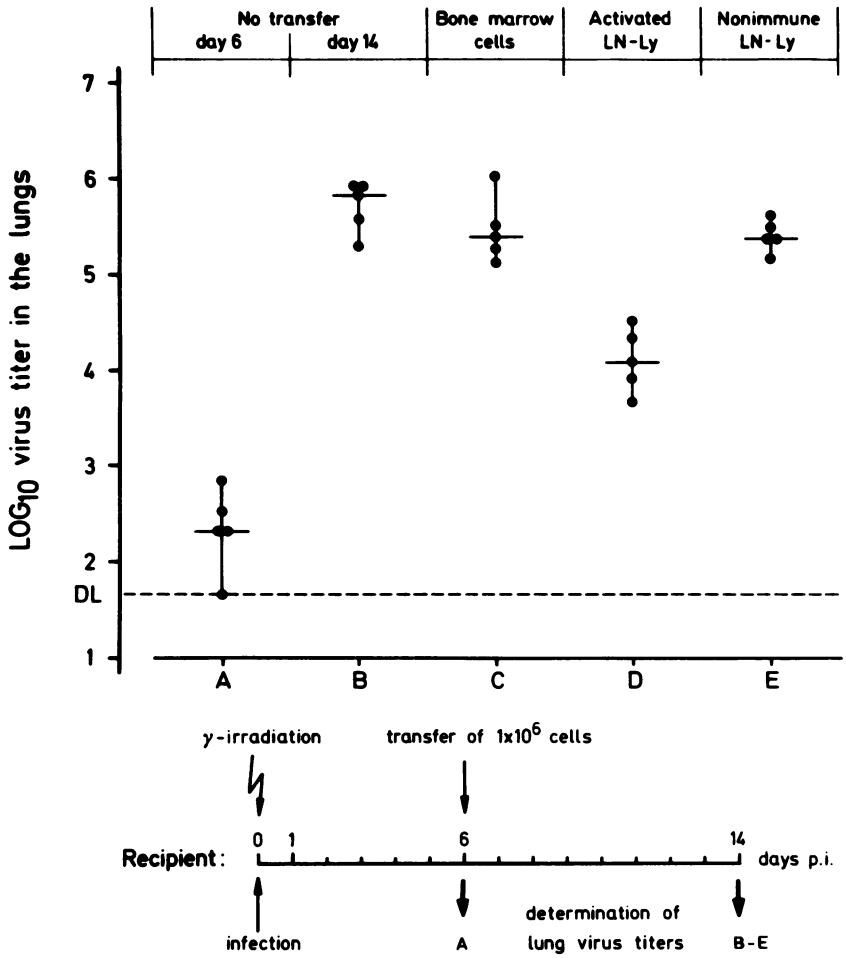

FIG. 5. Specifically activated lymphocytes limit virus multiplication during established infection of the lungs. The experimental schedule for therapeutic adoptive transfer is delineated. For the control, virus titers were determined in nonreconstituted hosts at day 6 p.i., the time of transfer (column A), and at day 14 p.i. (column B). Bone marrow cells were depleted for cells carrying the T-cell markers Lyt-2.2 and L3T4 by treatment with the corresponding antibodies (anti-Lyt-2.2 and GK1.5) and complement (column C). Activated lymph node lymphocytes (LN-Ly) were derived from popliteal LN of donors infected in the footpad with 10^5 PFU of MCMV 8 days previously (column D), and noninfected mice served as donors for nonimmune popliteal lymph node lymphocytes (column E). The virus titers in the lungs of five recipients for each group are depicted, and the median values are indicated by horizontal bars.

compared at day 14 p.i. with titers measured in lungs of reconstituted mice; two-sided Wilcoxon-Mann-Whitney test). In the same period of time the spleen was repopulated by the transferred bone marrow cells, as manifested by enlargement of the spleen, increase in leucocyte content from 1.2×10^6 to 50.1×10^6 (median values for five transfer recipients each), and the typical development of reconstitution nodules.

Therapeutic adoptive transfer of specifically activated lymphocytes. The therapeutic adoptive transfer is a means to investigate the question of whether activated lymphocytes can limit viral replication in the lungs even during established infection of the tissue. MCMV-infected cells were detectable in the lungs at day 6 p.i. (Fig. 2c), when four of five animals were virus positive, showing MCMV titers of only 10^2 to 10^3 PFU (Fig. 3; resolved to greater detail in Fig. 5, column A). Taking into account that ca. 50 PFU per cell can be recovered from productively infected fibroblasts in vitro (data not shown) and assuming a similar burst size in vivo, an observed virus titer of 10^3 PFU corresponds with ca. 20 producing cells in the lungs. Interference with viral replication at such a germinal stage of lung tissue infection should retard the log-linear increase in virus titers. Accordingly, transfers were performed at day 6 p.i., and as was the case for the prophylactic transfer, the virus titers were determined at day 14 p.i. (Fig. 5). Under these conditions, syngeneic bone marrow transplantation was again inefficient with respect to the control of lung tissue infection ($P > 0.1$; compare columns B and C), whereas transfer of 10^6 activated lymphocytes (column D) confined the increase in virus titers (significant [$P = 0.005$]) to <5% of the positive control values (columns B, C, and E), resulting in an absolute difference in virus load of $>10^5$ PFU. This antiviral effect was evidently mediated by lymphocytes sensitized in the infected donors, since transfer of the same amount of resting lymphocytes derived from popliteal LN of nonimmune donors was not effective ($P = 0.1$; compare columns B and E).

Absence of specific antibody in transfer recipients. To evaluate a possible contribution of antiviral antibody in the control of tissue manifestation of MCMV infection after therapeutic transfer, the antibody titer in the serum of the recipients (the same animals as shown in Fig. 5, column D)

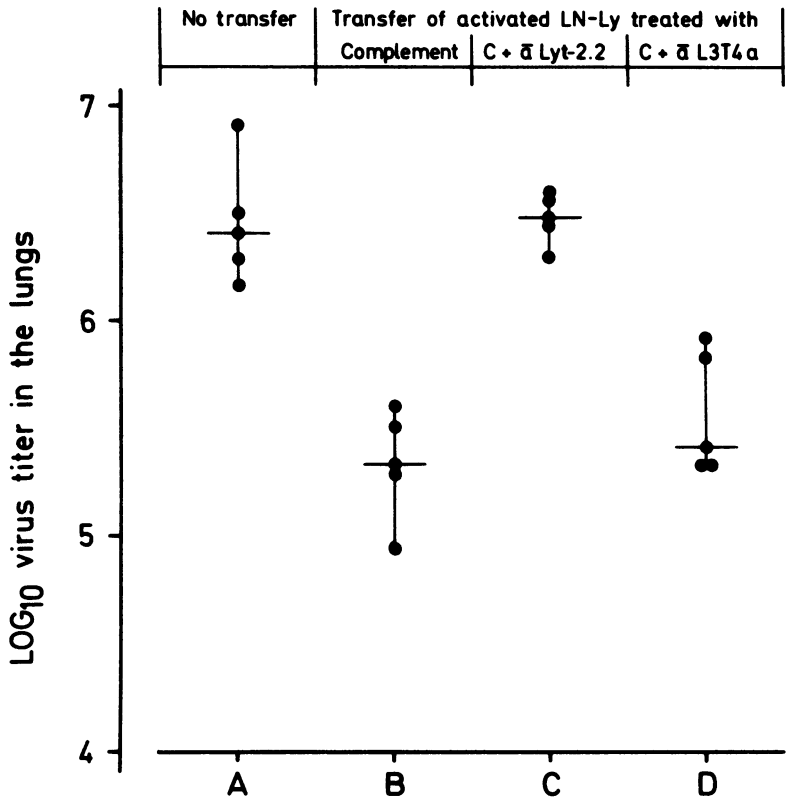

FIG. 6. T lymphocytes of the Lyt-2$^+$, L3T4$^-$ subset limit virus multiplication during established pulmonary MCMV infection. Therapeutic adoptive transfer of 10^6 subset-selected specifically activated lymph node lymphocytes (LN-Ly) was performed according to the schedule outlined in Fig. 5. Donor lymphocyte populations were depleted for distinct T-lymphocyte subsets by two cycles of treatment with mAB anti-Lyt-2.2 and complement (column C) or with mAB GK1.5 (anti-L3T4a) and complement (column D). The median values of the virus titers determined in the lungs of five recipients for each experimental group are marked by horizontal bars. The distribution-free Wilcoxon-Mann-Whitney test (rank sum test, one-sided) was applied to evaluate the significance of differences between independent sets of virus titers: P (columns A and B) = 0.005; P (columns B and C) = 0.005; P (columns C and D) = 0.005; P (columns A and D) = 0.005; P (columns A and C) > 0.1; and P (columns B and D) > 0.1.

was monitored by an enzyme-linked immunosorbent assay at day 8 posttransfer. There was no indication of a role for sensitized B lymphocytes at the early stages of reconstitution, since MCMV-specific antibody could not be detected in the recipients, whereas latently infected mice, inoculated with MCMV as newborns, proved to be seropositive (data not shown).

Serological characterization of cells that limit viral replication in the lungs. Before therapeutic adoptive transfer into irradiated and infected recipients, the activated donor lymphocytes were preselected for distinct subsets by complement-dependent cytolysis with mABs to the cell surface antigens Lyt-2.2, preferentially expressed by class I major histocompatibility complex antigen-restricted T lymphocytes (42), or L3T4, specific for most class II major histocompatibility complex antigen-restricted T lymphocytes (10, 33) (Fig. 6). These markers appear not to be coexpressed on mature T lymphocytes (10, 33), whereas both are present on immature thymocytes (9, 10). Treatment with mAB GK1.5, specific for the L3T4a determinant (10), did not significantly affect the ability of the population to limit viral replication (P > 0.1; compare columns B and D), whereas in a control experiment the lectin-induced production of IL-2 by splenocytes was reduced to 10%, indicating that IL-2-producing helper T lymphocytes can be eliminated by the protocol applied (data not shown; for details see above). On the other hand, depletion of the transferred population for Lyt-2$^+$ lymphocytes, predominantly representing CTL and their precursors, abrogated the antiviral effect in lung tissue (significant [P = 0.005]; compare columns B and C). By screening a series of thin sections from lungs of transfer recipients, it was found that histopathological alterations and the frequency of infected cells that bear the characteristic intranuclear inclusion were reduced only when Lyt-2$^+$ lymphocytes were present in the transferred population. In conclusion, the Lyt-2$^+$, L3T4$^-$ subset of sensitized donor T lymphocytes can control MCMV replication in the lungs.

DISCUSSION

Infection of the lungs with MCMV is not observed in the noncompromised host but occurs after total-body γ irradiation. Radiation could either predispose lung tissue for productive MCMV infection by altering the permissivity of cells or abrogate an immune control of virus replication by eliminating antiviral lymphocytes. A therapeutic effect of transferred lymphocytes directly derived from infected, immunocompetent donors would indicate that the second of the proposed mechanisms is a relevant one.

The present study allows the following conclusions. First, lymphocytes sensitized in vivo during infection of an immunocompetent host have an antiviral effect when transferred into irradiated and thereby immunosuppressed, in-

fected recipients. This finding implies either that the donor LN already contain effector cells or that sensitized precursors mature to effector cells in the recipient due to secondary encounter with antigen.

Second, activated lymphocytes are effective in a therapeutic adoptive transfer when infection of the lungs is already established, suggesting that they can operate in the affected tissue. Under the conditions tested neither bone marrow cells nor nonactivated lymphocytes were efficient with respect to the control of virus replication in the lungs, demonstrating that a property acquired in the infected donor is measured.

Third, the therapeutic antiviral effect of sensitized lymphocytes during established infection of lung tissue can be assigned to the Lyt-2^+, L3T4^- subset of T lymphocytes. This result implies that natural killer cells which do contribute to the defense against MCMV early in acute infection (6, 7) were either not contained in sufficient numbers in the transferred populations or not operative under the conditions of the therapeutic transfer protocol applied. Recent studies on the Lyt phenotype of T lymphocytes that affect virus multiplication during acute infections have demonstrated that cloned Lyt-1^-2^+ CTLs can operate in adoptive transfer experiments (8, 27, 28). In herpes simplex virus infection, the antiviral effect of transferred acutely sensitized lymphocyte populations has been assigned to the Lyt-1^+2^- subset (24). Besides cloned Lyt-1^-2^+ CTLs, cloned Lyt-1^+2^+ CTLs have been shown to be active in allogeneic tumor rejection (12), and a role of Lyt-1^+2^+ lymphocytes in the control of viral infection has also been suggested (43). Sensitized Lyt-1^+2^+ lymphocytes represent the majority of the IL-receptive IL-CTL_p present in draining LN during acute MCMV infection (34) and hence are contained in the donor lymphocyte population tested in our adoptive transfer experiments. Since the differences in Lyt-1 antigen expression between subsets of T lymphocytes are only quantitative (25), use of the mutually exclusive and hence discriminative markers Lyt-2 and L3T4 (10, 33, 42) was indicated to characterize the T-lymphocyte subset that is operative in vivo in a particular experimental situation. Clearly, interference with MCMV replication in an established infection of the lungs is a function of Lyt-2^+, L3T4^- T lymphocytes.

By using the limiting dilution assay, the minimum estimate for the frequency of in vivo-activated MCMV-specific CTL_p was found to be in the order of 10^3 per 10^6 LN lymphocytes (34) or 10^3 per 10^5 Lyt-2^+ LN lymphocytes. After prophylactic transfer, 10^4 lymphocytes containing an estimated number of only ca. 10 specifically sensitized CTL_p were able to diminish virus titers in several organs. As demonstrated for the lungs, infected cells were first detectable in low numbers in target tissue at day 6 p.i., indicating that even in the immunosuppressed host the spread of virus from the local site of intraplantar infection to target organs occurs with low efficiency and is likely to be very sensitive to early-operating adopted immune surveillance. Therefore, in the prophylactic transfer protocol, effector cells most probably act by affecting the spread of virus to target organs rather than by eliminating infected cells in various target tissues. Accordingly, when at day 6 p.i. the infection was established in target tissue, 100-fold more cells had to be transferred to cope with virus multiplication.

Besides mediating direct cytolysis of infected cells, CTLs could also operate by producing lymphokines (14, 46). Evidence in favor of direct in vivo cytolysis has been presented recently for influenza A virus infection (28), and arguments against direct in vivo cytolysis have been discussed for infection with lymphocytic choriomeningitis virus (26). Our data do not disfavor either of these theories.

According to a recent report, the spread of established pulmonary sarcoma metastases could be inhibited only when high numbers of in vitro lymphokine-activated killer cells and high doses of recombinant IL-2 were administered in combination (32). Compared with this tumor model, the established pulmonary MCMV infection appears to be more accessible to immune surveillance since significant interference with viral replication was achieved with comparatively low numbers of in vivo-sensitized Lyt-2^+, L3T4^- T lymphocytes without in vitro restimulation and without application of IL-2. Experiments are in progress to test whether the therapeutic effect of transferred MCMV-specific lymphocytes can be enhanced by IL-2.

CMV specify a multitude of polypeptides, and it would be of interest to know against which viral or virus-induced antigens the cellular immune response is directed. It has been shown that MCMV replication stage-specific CTLs can be distinguished that recognize target cells carrying viral structural proteins or expressing a lymphocyte-detected immediate-early antigen (35). Although only one major immediate-early protein is synthesized in infected cells (23), a high number of activated CTL_p present in the draining LN after infection were found to be specific for lymphocyte-detected immediate-early antigen (36), suggesting that this antigen is immunodominant.

Virus replication in the lungs of irradiated hosts after therapeutic transfer of CTL lines or clones specific for lymphocyte-detected immediate-early antigen or other antigens specified by MCMV provides a relevant parameter to test the functional competence of CTLs with different specificities and to evaluate the implication of particular antigens in immune surveillance during acute, persistent, and latent CMV infection.

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