IN VIVO APPLICATION OF RECOMBINANT INTERLEUKIN 2 IN THE IMMUNOTHERAPY OF ESTABLISHED CYTOMEGALOVIRUS INFECTION

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As with other members of the herpesvirus group, cytomegaloviruses (CMV)[1] do not seriously harm the immunocompetent host, but establish latent infection. Congenital CMV infection, however, is responsible for severe neurological disorders in immunologically immature newborns (1), and, by causing interstitial pneumonia, opportunistic primary or recurrent CMV infection is an often fatal complication in bone marrow transplant recipients after iatrogenic immunosuppression (2). More recently, CMV disease has been diagnosed as one of the leading immediate causes of death in acquired immunodeficiency syndrome (AIDS) victims immunocompromised by infection with human immunodeficiency virus (HIV) (3). Experimental infection with murine CMV (MCMV) of immunodeficient in comparison with immunocompetent or reconstituted mice has proven a valid model system for studying CMV pathogenesis (4–6), and has indicated a role for the cytolytic, CD8 (Lyt-2)-positive subset of T lymphocytes (CTL) in controlling CMV infection (6, 7).

In a previous communication (6) we have described an adoptive transfer model for the immunotherapy of established CMV infection, characterized by the following conditions: (*a*) in the immunocompetent BALB/c mouse, replication of MCMV is restricted to glandular epithelial cells in the acini of the salivary glands; (*b*) after immunodepletion by total-body γ irradiation, MCMV replicates in various tissues, including lung tissue; (*c*) viral genome can be detected in cells of the alveolar septa 6 d after intraplantar infection; (*d*) an antiviral effect in immunodepleted transfer recipients is mediated by sensitized CD8 (Lyt-2)-positive T lymphocytes derived directly from draining popliteal donor lymph nodes, while CD4 (L3T4)-positive lymphocytes are neither efficient nor necessary; (*e*) the CD8 effector cells can be applied therapeutically after MCMV has colonized lung tissue.

In recent studies and clinical trials interleukin 2 (IL-2) has proven an effective means to enhance the efficacy of antitumor immunotherapy (for review see 8–10). The prospects for an IL-2-supported immunotherapy of an established viral

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[1] *Abbreviations used in this paper:* AIDS, acquired immunodeficiency syndrome; CMV, cytomegalovirus; MCMV, murine CMV; rhIL-2, recombinant human IL-2.

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infection depend on the pathobiology of the agent with particular respect to target tissues, cytopathogenicity, and speed of virus spreading.

For the immunotherapy of an established MCMV infection in the immunocompromised natural host, our data demonstrate that application of IL-2 in vivo can significantly improve the control of virus multiplication.

Materials and Methods

Adoptive Transfer. Lymphocyte transfer was performed essentially as described previously (6) with the modification that cells were given intraperitoneally (i.p.). In brief, sensitized lymphocytes derived from draining popliteal lymph nodes of immunocompetent BALB/c donors 8 d after intraplantar infection with 10^5 PFU of MCMV (strain Smith, VR-194; American Type Culture Collection, Rockville, MD) were transferred into the peritoneal cavity of BALB/c recipients infected in the footpad with the same dose of MCMV after immunodepletion by total-body γ irradiation with 6 Gy. Depletion of donor lymphocytes for $CD8^+$ and $CD4^+$ cells was achieved by treatment with monoclonal antibodies anti-Lyt-2.2 (hybridoma 19/178) and anti-L3T4 (GK 1.5), respectively, and complement (6).

Tissue Virus Titers. Infectious MCMV in the indicated tissues was quantitated 14 d postinfection, as described previously (6). The detection limit of the plaque assay was 50 PFU of MCMV per organ homogenate. The pooled parotid, greater sublingual, and mandibular glands are collectively referred to as salivary glands. Sets of virus titers are regarded as significantly different for $p < \alpha = 0.05$ (one-sided), where p denotes the observed probability value, and α denotes the selected significance level (Wilcoxon-Mann-Whitney exact rank sum test).

Recombinant Human IL-2 (rhIL-2). rhIL-2 was generously supplied by the Sandoz Forschungsinstitut, Vienna, Austria. Lot 89050/84802 is >99% pure IL-2 (1.04 ± 0.35 ng of LPS per milligram of protein) with a specific activity of 7.3×10^6 U/mg of protein normalized to a reference standard (lot LSP-841) supplied by the Biological Resources Branch at the National Cancer Institute, Frederick, MD. Bioavailability of rhIL-2 was controlled with an IL-2-dependent subline of the BALB/c-derived T helper line HT-2. Cells were recovered from the peritoneal cavity of immunodepleted recipients 4 d after i.p. transfer of 10^7 HT-2, and the number of viable clone-forming HT-2 was determined in a limiting-dilution assay. With no IL-2 added in vivo, only 1420 (95% confidence interval: 930–2150) HT-2 could be recovered (0.014%), while i.p. administrations of 10^4 U (1.37 μg, 880 pmol) of rhIL-2 in 12-h intervals for 4 d yielded $3.3\ (2.4 - 4.6) \times 10^7$ HT-2, indicating proliferation in vivo. Lower doses of IL-2 or longer intervals between the IL-2 applications were less effective, and a gelatin depot did not improve the recovery. Therefore, the experiments were performed with 10^4 U of rhIL-2 in 0.5 ml of 0.15 M NaCl administered i.p. in 12-h intervals.

Results and Discussion

Application of rhIL-2 In Vivo Improves Immunoprophylaxis and Immunotherapy of MCMV Disease. In an immunocompromised but not completely immunodeficient host, a limited number of sensitized lymphocytes may still be present, though not sufficient in quantity to cope with CMV infection. In the murine model system, adoptive transfer allows study of the antiviral potential of a defined, limited number of lymphocytes, and allows evaluation of the prospects for enhancing this potential. Prophylactic (Fig. 1) and therapeutic (Fig. 2) transfer models (6) mimic two different clinical conditions in the immunocompromised host. In the first case, virus has not yet colonized tissue, and protective immunity may operate by preventing this. In the second case, virus is already present in tissues and protective immunity has to counteract the progression of established

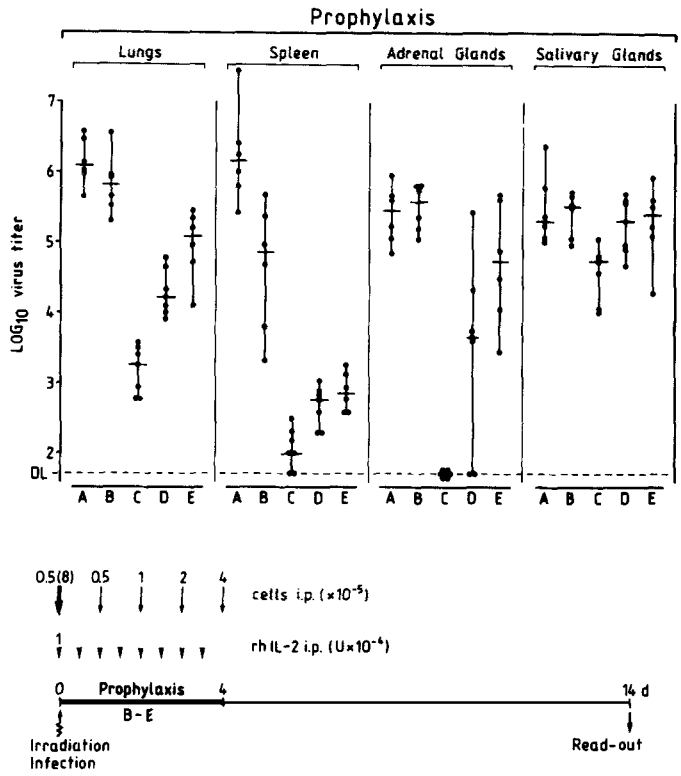

FIGURE 1. Preventive treatment for CMV disease by prophylactic adoptive transfer. The treatment period was started 2 h after intraplantar infection of eight immunodepleted BALB/c mice per experimental group (prophylaxis). Closed circles represent the virus titers in the indicated organs of individual recipients determined at day 14 postinfection. The median values of the virus titers are marked by horizontal bars. The dashed line indicates the detection limit. (*A*) No transfer of lymphocytes. (*B*) Transfer of 50,000 sensitized donor lymphocytes (defined as one unit dose) at day 0. (*C*) Transfer of 16 unit doses at day 0. (*D*) Repetitive transfer of one, one, two, four, and eight unit doses in 24 h intervals at days 0, 1, 2, 3, and 4, respectively (*arrows*). (*E*) Transfer of one unit dose of sensitized donor lymphocytes at day 0 supported by eight subsequent applications of rhIL-2 (10^4 U each) in 12-h intervals (*arrowheads*), the first of which was given along with the lymphocytes.

infection. During acute MCMV infection of immunodepleted BALB/c mice, virus has colonized target tissues by day 6 postinfection, as has been demonstrated by detection of the viral genome in various cell types of the lungs (6), and likewise also in reticular cells of the spleen, hepatocytes of the liver, parenchymal cells in the reticular and fascicular zones of the adrenal gland cortex, and secretory as well as connective tissue cells of the salivary glands (K. Münch and F. Weiland, personal communication). Transfer of lymphocytes at that stage of infection can therefore be regarded as a therapeutic regimen.

The idea in our experiments (Figs. 1 and 2) was to augment the function of a defined, insufficient number of lymphocytes (compare columns B and B′ with the respective columns A, Figs. 1 and 2) by in vivo application of rhIL-2 under conditions that exclude recruitment of recipient lymphocytes. With the assumption that IL-2 in vivo maintains growth and functional activity of transferred

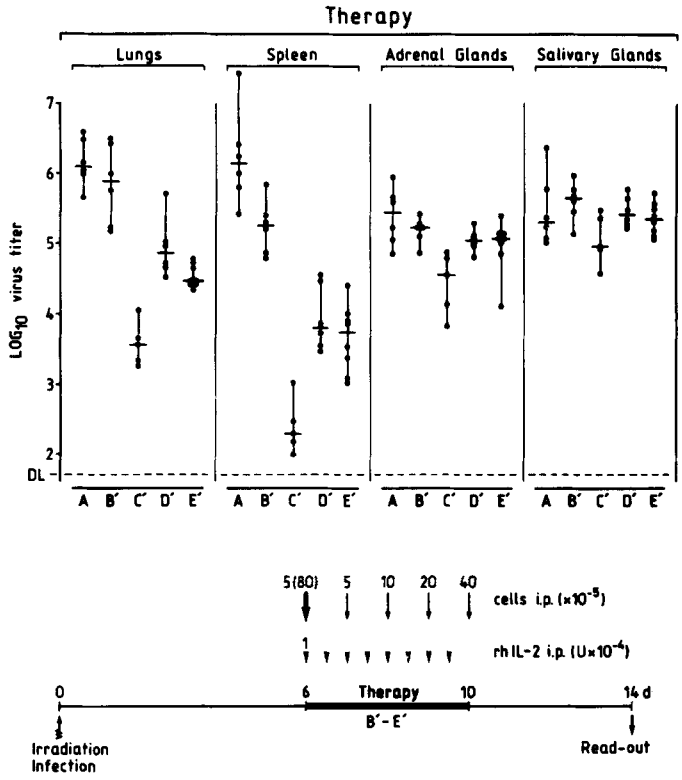

FIGURE 2. Treatment of established tissue infection by therapeutic adoptive transfer. The treatment period was started 6 d after intraplantar infection of eight immunodepleted BALB/c mice per experimental group at a time when MCMV has colonized target tissue (6). Controls that have not received lymphocytes were the same individuals as in Fig. 1 (*A*). The experimental schedules *B'–E'* correspond to that of the prophylactic regimen (Fig. 1; *B–E*), except that the unit dose of transferred cells was 10-fold.

donor lymphocytes, and that the lymphokine thereby supports one effector population doubling per day, the total number of cells generated during a 4-d period would be 16-fold. This amount of effector cells is effective in controlling virus multiplication when given in a single dose on the first day of the treatment period, depending in efficacy on the time of transfer and the site of virus multiplication (columns C and C', Figs. 1 and 2). During IL-2-mediated augmentation, however, this high amount of effector cells is not available at once, but is generated, with delay, at a speed determined by the generation time of the lymphocytes, with the consequence that increasing cell numbers have to cope with infection in progressing stages. Such a condition was simulated by repetitive transfer of sensitized lymphocytes in daily intervals and resulted in intermediate tissue virus titers (columns D and D'). Repeated application of rhIL-2 (columns E and E') indeed significantly enhanced the antiviral effect of the low initial number of lymphocytes in several tissues in prophylaxis and, except for the adrenal glands, also in therapy [lungs: $p(\mathrm{BE}) = 0.005$, $p(\mathrm{B'E'}) < 0.001$; spleen: $p(\mathrm{BE}) < 0.005$, $p(\mathrm{B'E'}) < 0.001$; adrenal glands: $p(\mathrm{BE}) < 0.005$, $p(\mathrm{B'E'}) > 0.1$]. This result is consistent with the assumption of one population doubling per day,

as approximately the same antiviral effects were observed when lymphocyte proliferation was simulated by repetitive lymphocyte transfer (compare columns D and D′ with E and E′, respectively, Figs. 1 and 2). Regardless of whether support of cell proliferation was the only effect of IL-2, the data demonstrate that the net effect of IL-2 was equivalent to one effector population doubling per day.

Site-specific Differences in Antiviral Efficacy of Prophylactic and Therapeutic Lymphocyte Transfer. Antiviral lymphocytes proved to be effective in the prophylactic control of infection in the lungs and the spleen and, provided higher cell numbers were transferred, treatment was effective also during established infection of those organs (Figs. 1 and 2). Consistent with the fact that salivary gland tissue is a preferred site of MCMV replication in the immunocompetent host (6), virus multiplication in salivary glands was only moderately affected [Fig. 1, $p(AC) < 0.005$; Fig. 2, $p(AC') = 0.1$]. Because MCMV always colonized the salivary glands, the protection observed for other tissues was not caused by prevention of virus spreading from the plantar site of infection. Transferred lymphocytes confined MCMV replication in the salivary glands to glandular epithelial cells, while in the immunodepleted host, infection expands to connective tissue fibroblasts (F. Weiland, manuscript in preparation), indicating cell-type–specific rather than organ-specific reasons for the preferred role of the salivary glands in CMV infection. The property of adoptively transferred donor-type T lymphocytes to distribute widely and persist long-term in the recipient has been demonstrated in another model system (11).

MCMV adrenalitis has been described recently for T cell–deficient *nu/nu* mice (12), and adrenal necrosis is one symptom of AIDS (13). Considering the life-threatening consequences of adrenal cortical dysfunction, it is worth noting that infection of that gland could be prevented only when high numbers of lymphocytes were available at an early stage of infection (Fig. 1, column C), while repetitive transfer as well as IL-2-supported prophylactic treatment failed to protect from adrenal infection in five of seven (Fig. 1, column D) and in six of six (Fig. 1, column E) recipients, respectively. Accordingly, MCMV multiplication could only moderately be inhibited during established adrenalitis (Fig. 2, column C′). Whether in the adrenal gland cortex the hormonal environment suppresses protective immunity remains to be investigated.

Identification of IL-2-responsive Antiviral Effector Cells. The control of MCMV infection has previously been assigned to the CD8 (Lyt-2)-positive T lymphocyte subset (6). This, together with the known function of IL-2 to mediate proliferation of that subset, strongly suggested that $CD8^+$ antiviral T lymphocytes are the targets of the IL-2 effect observed. To confirm this, alternatives had to be excluded (Fig. 3). Application of rhIL-2 alone did not limit virus multiplication (Fig. 3*A*; $p > 0.1$). Thus, IL-2 by itself had no antiviral effect, nor did it recruit putative residual lymphocytes in the irradiated host. IL-2 also failed to elicit antiviral effectors from transferred nonprimed lymphocytes (*B*; $p > 0.1$). This result excluded a significant participation of in vivo–generated lymphokine-activated killer (LAK) cells (8). Sensitized lymphocytes depleted of CD4 (L3T4)-positive lymphocytes contained the IL-2-responsive antiviral effectors (*C*; $p =$

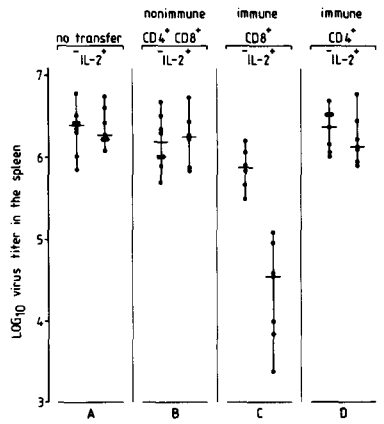

FIGURE 3. IL-2 enhances the antiviral effect of CD8+ lymphocytes in vivo. Virus titers in the spleen were determined at day 14 postinfection after therapeutic transfer at day 6 postinfection of 5×10^5 lymphocytes with no rhIL-2 (−IL-2) or with rhIL-2 administered according to the schedule shown in Fig. 2 (IL-2+). (*A*) No transfer of lymphocytes. (*B*) Transfer of lymphocytes derived from popliteal lymph nodes of noninfected donors. (*C*) Transfer of sensitized lymphocytes depleted of CD4 (L3T4)-positive lymphocytes. (*D*) Transfer of sensitized lymphocytes depleted of CD8 (Lyt-2.2)-positive lymphocytes.

0.001), while CD8+ populations made up of CD4+ (T helper) lymphocytes and accessory cells were not effective (Fig. 3*D*; $p > 0.1$).

Predictions Deduced from the Model System. IL-2 has been demonstrated previously to enhance in vitro the CMV-specific cytolytic activity of lymphocytes isolated from AIDS patients (14), indicating the existence of a residual, IL-2-responsive antiviral CD8+ population. Our results in a model system predict that the effect of a limited number of antiviral effectors can be enhanced by application of IL-2 also in vivo. While an immunotherapy of interstitial CMV pneumonia might have some chance, the prospects for controlling established CMV infection of the adrenal glands appear to be less promising.

Summary

We have shown in a murine model system for cytomegalovirus (CMV) disease in the immunocompromised host that in vivo application of recombinant human IL-2 (rhIL-2) can enhance the antiviral effect of a limited number of CD8+ T lymphocytes, not only in prophylaxis, but also in therapy, when virus has already colonized host tissues. The observed net effect of IL-2 was consistent with the assumption of daily effector population doublings. The prospects for IL-2-supported immunotherapy of established CMV infection depend upon the tissues involved in disease. It appears that the prospects for controlling established CMV adrenalitis are less promising than for a therapy of interstitial CMV pneumonia.

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References

1. Hanshaw, J. B. 1971. Congenital cytomegalovirus infection: a fifteen year perspective. *J. Infect. Dis.* 123:555.
2. Meyers, J. D., N. Flournoy, and E. D. Thomas. 1980. Cytomegalovirus infection and specific cell-mediated immunity after marrow transplant. *J. Infect. Dis.* 142:816.
3. Niedt, G. W., A. Roger, and A. Schinella. 1985. Acquired immunodeficiency syndrome. Clinicopathologic study of 56 autopsies. *Arch. Pathol. Lab. Med.* 109:727.
4. Brody, A. R., and J. E. Craighead. 1974. Pathogenesis of pulmonary cytomegalovirus infection in immunosuppressed mice. *J. Infect. Dis.* 129:677.
5. Bukowski, J. F., B. A. Woda, and R. M. Welsh. 1984. Pathogenesis of murine cytomegalovirus infection in natural killer cell-depleted mice. *J. Virol.* 52:119.
6. Reddehase, M. J., F. Weiland, K. Münch, S. Jonjić, A. Lüske, and U. H. Koszinowski. 1985. Interstitial murine cytomegalovirus pneumonia after irradiation: characterization of cells that limit viral replication during established infection of the lungs. *J. Virol.* 55:264.
7. Reddehase, M. J., and U. H. Koszinowski. 1984. Significance of herpesvirus immediate early gene expression in cellular immunity to cytomegalovirus infection. *Nature (Lond.).* 312:369.
8. Ettinghausen, S. E., and S. A. Rosenberg. 1986. The adoptive immunotherapy of cancer using lymphokine activated killer cells and recombinant interleukin-2. *Springer Semin. Immunopathol.* 9:51.
9. Cheever, M. A., J. A. Thompson, D. J. Peace, and P. D. Greenberg. 1986. Potential uses of interleukin 2 in cancer therapy. *Immunobiol.* 172:365.
10. Lotze, M. T., and S. A. Rosenberg. 1986. Results of clinical trials with the administration of interleukin 2 and adoptive immunotherapy with activated cells in patients with cancer. *Immunobiol.* 172:420.
11. Cheever, M. A., D. Britzmann Thompson, J. P. Klarnet, and P. D. Greenberg. 1986. Antigen-driven long term-cultured T cells proliferate in vivo, distribute widely, mediate specific tumor therapy, and persist long-term as functional memory T cells. *J. Exp. Med.* 163:1100.
12. Shanley, J. D., and E. L. Pesanti. 1986. Murine cytomegalovirus adrenalitis in athymic nude mice. *Arch. Virol.* 88:27.
13. Tapper, M. L., H. Z. Rotterdam, C. W. Lerner, K. Al'Khafaji, and P. A. Seitzman. 1984. Adrenal cortical function in the acquired immunodeficiency syndrome. *Ann. Intern. Med.* 100:239.
14. Rook, A. H., H. Masur, H. C. Lane, W. Frederick, T. Kasahara, A. B. Macher, J. Y. Djeu, J. F. Manischewitz, L. Jackson, A. S. Fauci, and G. V. Quinnan, Jr. 1983. Interleukin-2 enhances the depressed natural killer and cytomegalovirus-specific cytotoxic activities of lymphocytes from patients with the acquired immune deficiency syndrome. *J. Clin. Invest.* 72:398.