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Suppression of Rat Cytomegalovirus Replication by Antibodies against Gamma Interferon

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The role of gamma interferon (IFN-γ) in the resolution of rat cytomegalovirus (RCMV) infection was investigated. In the spleen, IFN-γ-producing cells reached maximum numbers on day 7 after infection. Prophylactic treatment with high doses of recombinant rat IFN-γ exerted antiviral activity in fibroblasts and protected immunosuppressed rats against a lethal RCMV challenge. Remarkably, in immunocompetent rats, neutralization of endogenous IFN-γ activity significantly reduced the numbers of RCMV antigen-expressing cells in the spleen, the predominant site of viral replication. Moreover, protection of radiation-immunosuppressed infected rats by transferred immune T cells was enhanced by coinjection of IFN-γ neutralizing antibodies. The observations were paralleled by in vitro findings: low concentrations of IFN-γ enhanced viral replication in both macrophages and fibroblasts. These data suggest that IFN-γ can play different and even opposite roles in the regulation of RCMV replication in vivo; T lymphocytes may contribute to the progression of RCMV infection by secreting IFN-γ.

Cytotoxic T lymphocytes (CTLs) play a crucial role in the host defense against viral infections (31). CTLs focused on the virus-infected target cell may deliver a lethal hit by the pore-forming protein perforin (45). However, often only a few specific T cells can control a viral infection in the absence of any tissue destruction (21). This may be explained by a second mechanism; CTLs also produce lymphokines, which exert antiviral activity by inducing other immune responses. Thus, Morris et al. (26) demonstrated that T-cell receptor triggering via antigen and major histocompatibility complex (MHC) stimulation induces gamma interferon (IFN-γ) production. Moreover, Fong and Mosmann (8) recently showed that $CD8^+$ T-cell clones secrete a cytokine pattern similar to that of T helper 1 clones including IFN-γ and tumor necrosis factor (TNF).

IFN-γ possesses both immunomodulatory and antimicrobial effects (for a review, see reference 27). Mice pretreated with neutralizing monoclonal antibodies (MAbs) to IFN-γ no longer resist sublethal doses of infections with *Listeria monocytogenes*, *Toxoplasma gondii*, and *Leishmania major* (2, 27). A protective role of IFN-γ has also been demonstrated in viral infections. Administration of anti-IFN-γ serum enhanced the mortality of mice infected with herpes simplex virus (37) and raised organ titers of lymphocytic choriomeningitis virus (LCMV) and vaccinia virus (15, 23). Wille et al. suggested that IFN-γ may act as an autocrine maturation signal for antiviral CTL (43). Moreover, IFN-γ also enhances B-cell proliferative responses, upregulates MHC class I and II expression, and increases the cytotoxic activity of natural killer cells (22, 27, 38). Other studies in the LCMV model system suggested that IFN-γ produced by splenic T cells limits viral spread by protecting cells surrounding the focus of infection (18, 23). In a pseudorabies virus infection model, we also could provide evidence for a direct antiviral effect of IFN-γ in vivo (32) that was cell type dependent (34). In view of these findings, the mode of IFN-γ action in vivo is still subject to controversy.

In this study, we analyzed the production, cellular localization, and function of IFN-γ during a rat cytomegalovirus (RCMV) infection. In contrast to immunosuppressed rats, immunocompetent animals are highly resistant to RCMV infection (4, 5), suggesting that immune-mediated mechanisms and IFN-γ are involved in the control of viral replication. We provide evidence that besides exerting immunostimulatory effects and direct inhibition of viral replication, IFN-γ also enhances virus replication in vivo.

MATERIALS AND METHODS

Virus. Stocks of RCMV were prepared as 10% (wt/vol) homogenates of salivary gland tissue taken from brown Norway (BN) rats that had been inoculated 3 weeks previously with 10^4 PFU of RCMV via the intraperitoneal (i.p.) route. RCMV was passaged in rat embryo fibroblast (REF) cells. At maximal cytopathic effect, supernatants were harvested and centrifuged at 900 × *g* for 10 min. Virus was stored in aliquots at −70°C until use.

Infectivity titration. Infectivity titers of RCMV were determined by using a plaque assay on REF cells as described previously (5). Plaques were counted 6 days postinfection (p.i.) after fixation of the monolayers with 10% formalin in phosphate-buffered saline (PBS) and staining with 1% aqueous methylene blue. Titers are expressed as PFU per gram of tissue.

Cells. REF cells were prepared from 17-day-old rat embryos and grown in Dulbecco's modified Eagle's medium containing 2% fetal calf serum (DMEM-FCS), penicillin (100 IU/ml),

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streptomycin (100 μg/ml), and amphotericin B (5 μg/ml). Cells were grown in plastic culture flasks (Nunc, Breda, The Netherlands) at 37°C in a humidified CO_2 incubator and used at the third passage. For isolation of spleen macrophages (spleen adherent cells), rats were sacrificed, their spleens were minced, the erythrocytes were lysed, and cells were seeded into 16-mm-diameter dishes of 24-well plates. After 1 h, nonadherent cells were removed and the remaining cells were cultured for indicated times in DMEM-FCS. Adherent cells were identified as macrophages from their morphology and their nonspecific esterase activity (>95% positive).

Antiviral assay. REF cells and spleen macrophages were treated with recombinant rat IFN-γ (rrIFN-γ) and recombinant mouse TNF-α (specific activity of 75×10^6 U/mg of protein, as determined in L929 cells) for 24 h and infected with RCMV at a multiplicity of infection as indicated. After 1 h, the inoculum was removed, and cells were washed and cultured in DMEM-FCS. Plaques and viral antigen-positive cells were counted at 3 days p.i. Cells were fixed with 70% ethanol (5 min at room temperature) and washed with phosphate-buffered saline (PBS). Staining for RCMV antigen was performed as described for histology.

IFN-γ assays. IFN-γ serum levels were determined by using a rat IFN-γ-specific enzyme-linked immunosorbent assay (ELISA) (41). Briefly, wells of a 96-well microtiter plate (Flow) were coated for 16 h at 4°C with 100 μl of a PBS solution containing MAb DB-1 at 10 μg/ml. The wells were washed 10 times with PBS containing 0.05% Tween 20 (wash buffer). After blocking with 200 μl of a 2% bovine serum albumin (BSA) solution in PBS, the plates were emptied and incubated with the samples. As a positive control, rrIFN-γ was used. Thereafter, the wells were extensively washed and refilled with 100 μl of biotinylated antibody DB-12 at 2 μg/ml in PBS and incubated. After 10 wash cycles, 100 μl of antibiotin-peroxidase conjugate (1 μg/ml diluted in 1% BSA) was added to each well. Again the plate was incubated and subsequently washed 10 times. Then 100 μl of a solution of the substrate tetramethyl benzidine (Sigma) was added to each well. The substrate conversion was stopped by the addition of 30 μl of 3 N H_2SO_4, and the A_{450} was read. All incubations were for 1 h at 37°C.

Alternatively, IFN-γ bioactivity was determined by its ability to mediate MHC class II expression on peritoneal cells. These were obtained after lavage of the peritoneal cavity of BN rats and cultured for 48 h in the presence of different serum dilutions. Thereafter, cells were fixed with 3% paraformaldehyde and incubated with mouse anti-rat MHC class II antibody OX6. Subsequently they were stained with fluorescein isothiocyanate-conjugated goat anti-mouse antibodies (Sigma). As a positive control, cultures were incubated with rrIFN-γ (100 U/ml). The detection limit of the assay was 0.1 U/ml.

Antibodies to RCMV. REF cells were seeded in 96-well plates and subsequently infected with RCMV (multiplicity of infection of 3). After 4 days, plates were fixed with 4% paraformaldehyde in PBS and permeabilized with 70% ethanol. The wells were incubated with 3% H_2O_2 in methanol for 15 min to block endogenous peroxidase activity and preincubated for 20 min at room temperature with normal goat serum (diluted 1:40) to reduce nonspecific binding. Twofold dilutions of heat-inactivated sera were added to the wells, incubated for 1 h at room temperature, and then reacted with goat anti-rat peroxidase conjugate for 30 min. Peroxidase activity was visualized by using 0.003% H_2O_2 and 0.5% 3,3′-diaminobenzidine in 0.05 M Tris-HCl buffer (pH 8.3). Titers were expressed as the highest dilution with detectable staining of RCMV-infected cells.

Animals. Inbred specific-pathogen-free male BN rats were obtained from Harlan (Austerlitz, The Netherlands). The animals were kept in filter top cages. The experimental protocols had been approved by the institutional Animal Welfare Committee.

Experimental design. Rats were infected via the i.p. route with 10^6 PFU of RCMV. Orbital blood samples were allowed to clot at 4°C for 1 h, centrifuged, and kept at −20°C until use. Organs were removed on different days after infection and homogenized (10%, wt/vol) in DMEM-FCS; supernatants after low-speed centrifugation were titrated for infectivity. Mouse anti-rat CD8 MAb OX8 (500 μg) was administered 24 h before infection by the i.p. route. At 1 h p.i., a group of animals was given an i.p. injection of IFN-γ neutralizing MAb DB-1 (42), which recognizes rat IFN-γ (10^4 neutralizing units per rat), while control rats received a similar amount of isotype-matched control MAb directed against chloramphenicol (UD-15; all injections at 200 μl per rat).

To study the effect of exogenous IFN-γ, rats were daily injected i.p. with rrIFN-γ (1,000 or 2,000 U/g of body weight in 200 μl of PBS containing 1% BSA; specific activity of rrIFN-γ, 4×10^6 U/mg) for 5 consecutive days. The animals were monitored twice daily for survival.

T-cell transfer studies. For isolation of immune T lymphocytes, 8-week-old male rats were immunized by i.p. injection of 5×10^4 PFU of RCMV followed by a booster of similar dose 5 weeks later via the intravenous route. Seven days later, the animals were sacrificed, and their spleens removed, minced, and pressed through a filter (NPBI, Emmer-Compascuum, The Netherlands). Erythrocytes were lysed, the cell suspension was enriched for T lymphocytes by passage through nylon wool columns, and the viability of nonadherent cells was determined by trypan blue staining. Nonimmune spleen T lymphocytes were isolated from age- and sex-matched untreated or PBS-treated animals. Donor T cells (5×10^7 or 5×10^6) suspended in 0.5 ml of DMEM-FCS were injected into the tail veins of 5-week-old recipients that had been subjected to whole-body irradiation (500 rad at 350 cGy/min) 24 h previously. Two hours after the cell transfer, the recipient rats were challenged with 5×10^4 PFU of RCMV via the i.p. route. At 1 h p.i., groups of rats were given an i.p. injection of DB-1 (10^4 neutralizing units per rat) or UD-15 (specific for chloramphenicol).

Histology. Organ samples were fixed immediately after removal in liquid nitrogen and kept at −70°C until use. Cryostat sections (8 μm, cut at −30°C) were fixed for 10 min in acetone containing 0.02% H_2O_2. Sections were examined for the presence of viral antigen or IFN-γ as described previously (36, 40). Briefly, the slides were incubated overnight at 4°C with a MAb against IFN-γ (DB-1) or a mixture of MAbs 8 and 35, directed against nuclear and cytoplasmic RCMV antigens, respectively. Double-staining experiments were performed with rabbit anti-asialo glycoprotein (Wako Chemicals, Neuss, Germany). All reagents were diluted in PBS containing 0.1% BSA and titrated to obtain optimal results. Subsequently the slides were washed three times for 5 min and incubated for 30 min at room temperature with the diluted secondary antibody conjugates (rabbit anti-mouse peroxidase or goat anti-rabbit alkaline phosphatase conjugates; Dakopatts, Glostrup, Denmark) containing 1% normal rat serum. Thereafter, slides were washed and histochemical reactions were performed as previously described (40). All washes were in PBS. The preparations were counterstained with hematoxylin and mounted.

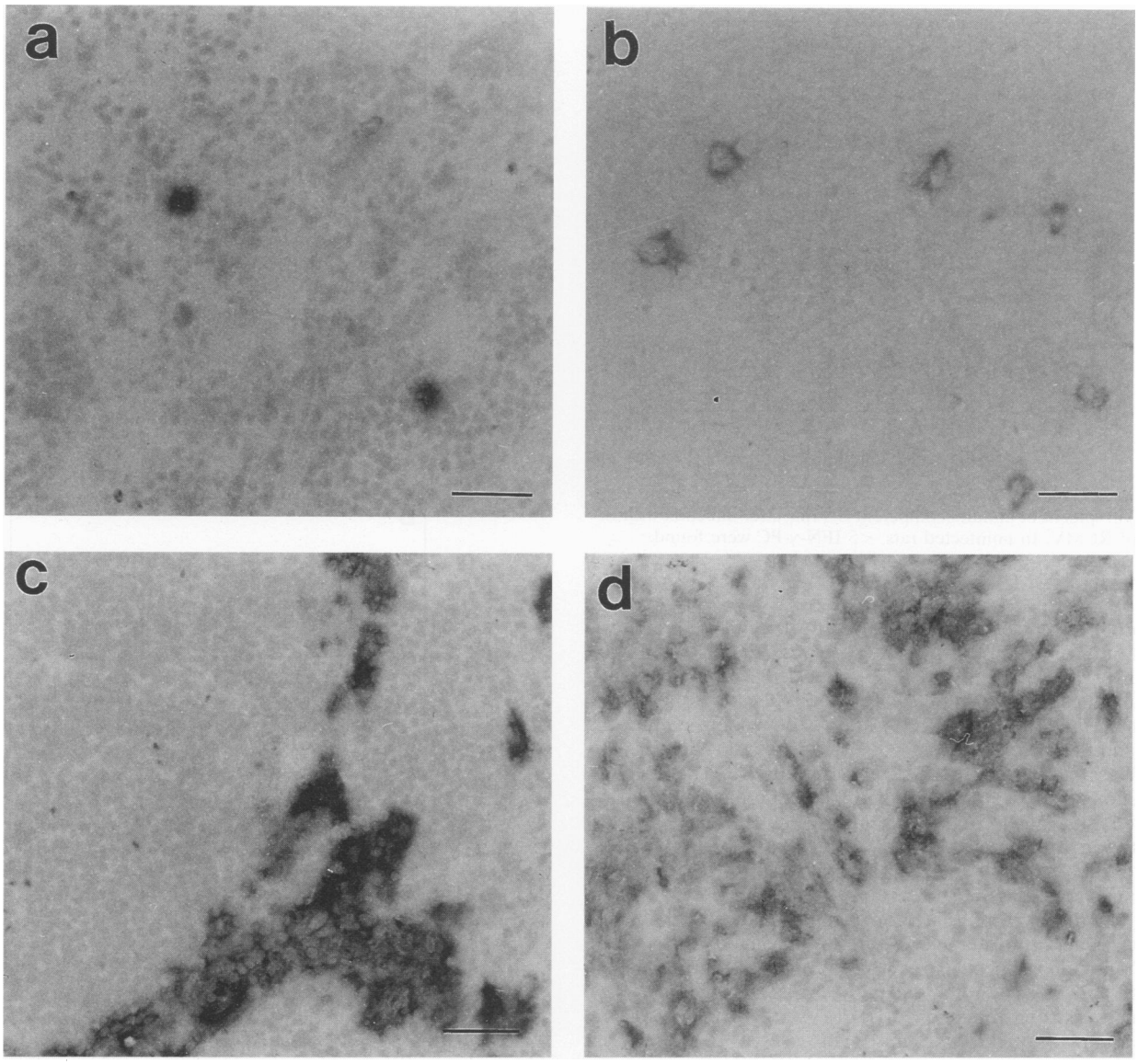

FIG. 1. Immunohistological detection of virus-infected and IFN-γ-PC in spleens of RCMV-infected rats at day 5 p.i. Sections had been stained for RCMV antigen (a, c, and d) or IFN-γ (b) in control (a and b), CD8 antibody-treated (c), and whole-body radiation-immunosuppressed (d) rats. Bars, 83.5 μm (a, c, and d) and 25 μm (b).

RESULTS

Detection of IFN-γ in RCMV-infected rats. In rats infected with 10^6 PFU of RCMV via the i.p. route, virus could be recovered only from the spleen (500 to 1,000 PFU per spleen). Immunoperoxidase staining revealed only few antigen-containing cells at day 3 and high numbers at day 5 p.i. (Fig. 1a and 2). Peritoneal serosa cells lining visceral organs and scattered macrophages in the liver, lungs, and kidneys were also positive (not shown). At day 9 p.i., antigen-containing cells were no longer detectable (Fig. 2). Low serum IFN-γ activity was found on days 5 and 7 p.i. in RCMV-infected immunocompetent rats (3.6 and 2.8 U/ml, respectively, as measured by ELISA). Sera obtained at other time points were devoid of activity. Moreover, IFN-γ-producing cells (IFN-γ-PC) were detected in frozen spleen sections stained with MAb DB-1 (Fig. 1b and 2). The IFN-γ staining was weak compared with that of a positive control (mice immunized with trinitrophenol-Ficoll [40]; not shown). The spleens of uninfected rats contained only low numbers of IFN-γ-PC (<5 per section). The specificity of the staining was verified by omission of the first antibody and inhibition assays as described earlier (40). Double-staining techniques revealed that most IFN-γ-PC did not react with anti-asialo glycoprotein, suggesting that IFN-γ is T cell derived (not shown).

Effect of rrIFN-γ on RCMV infection in vitro. The effect of IFN-γ on RCMV replication was determined in cultures of primary fibroblasts and macrophages, which are representative target cell types in vivo. In contrast to fibroblasts, freshly isolated macrophages replicate RCMV to only low titers. Remarkably, pretreatment with low concentrations of IFN-γ (0.1 to 10 U/ml) resulted in an increased number of antigen-positive cells (Fig. 3A) and infectivity titers (not shown), most notably in macrophages; similar results were found with use of TNF-α (not shown). The enhancing effect was dose dependent.

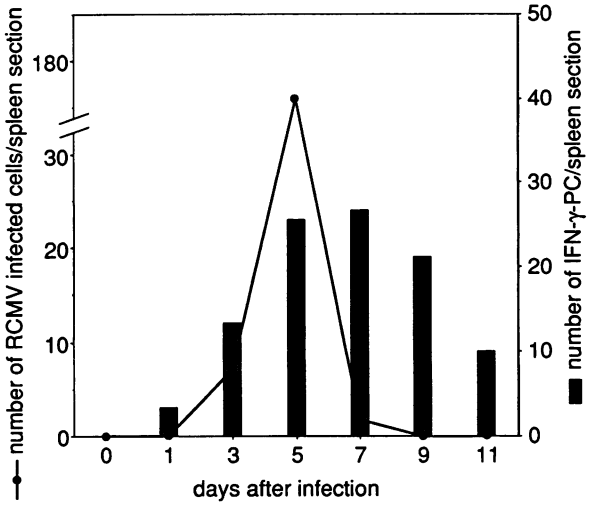

FIG. 2. Numbers of IFN-γ-PC (bars) and virus-infected cells (line graph) in spleens of immunocompetent BN rats after infection with 10^6 PFU of RCMV. In uninfected rats, <5 IFN-γ-PC were found.

In fibroblasts pretreated with doses of >10 U/ml, an inhibitory effect on RCMV replication was observed (Fig. 3B). Preincubation of IFN-γ with MAb DB-1 reduced its activity, while DB-1 alone did not influence RCMV replication. Thus, the effect of IFN-γ on RCMV replication is dependent on the cell type and dosage.

Effect of rrIFN-γ on RCMV infection in vivo. The effect of exogenous rrIFN-γ was studied in radiation-immunosuppressed rats infected with 5×10^5 PFU of RCMV, which resulted in 100% mortality (Fig. 4). Daily treatment with 10^3 U/g of body weight starting 3 days before infection and continued for 5 days was necessary to confer complete protection. Regimens starting later or using lower doses prolonged survival times but resulted in no or only partial protection (Fig. 4). Treatment reduced RCMV replication 10-fold in spleen, kidney, and liver but not in the lungs (Fig. 5). Immunohistology revealed a strong reduction of RCMV antigen expression in these organs (not shown). Thus, high doses of IFN-γ protect immunosuppressed rats against lethal RCMV disease only when applied before the infection.

Antiviral effect of T lymphocytes. To determine the in vivo role of T lymphocytes (the major source of IFN-γ) in RCMV clearance, we suppressed the lymphoid cell population by injection of anti-CD8 antibodies at 1 day before RCMV infection. As illustrated in Fig. 1c and 6, this treatment resulted in an increase of infected cells in the spleen, indicating that $CD8^+$ lymphocytes control RCMV replication. Whole-body irradiation resulted in a more severe infection (Fig. 1d). The antiviral activity of T cells was further examined in adoptive transfer experiments. Splenic T lymphocytes obtained from RCMV-sensitized donor rats were injected intravenously into sublethally irradiated recipients which were given a lethal virus dose i.p. 2 h later. A transfer of 5×10^7 T cells conferred complete protection to recipients; protection was only partial when 10-fold fewer cells were given (Table 1). Protection was reflected by a significant RCMV titer reduction in all organs tested (Fig. 7). Spleen cells obtained from nonimmunized donor rats did not protect against a lethal challenge (not shown). Thus, T cells protect against RCMV infection in both immunocompetent and adoptively immunized rats.

Effects of anti-IFN-γ antibodies on RCMV infection. To

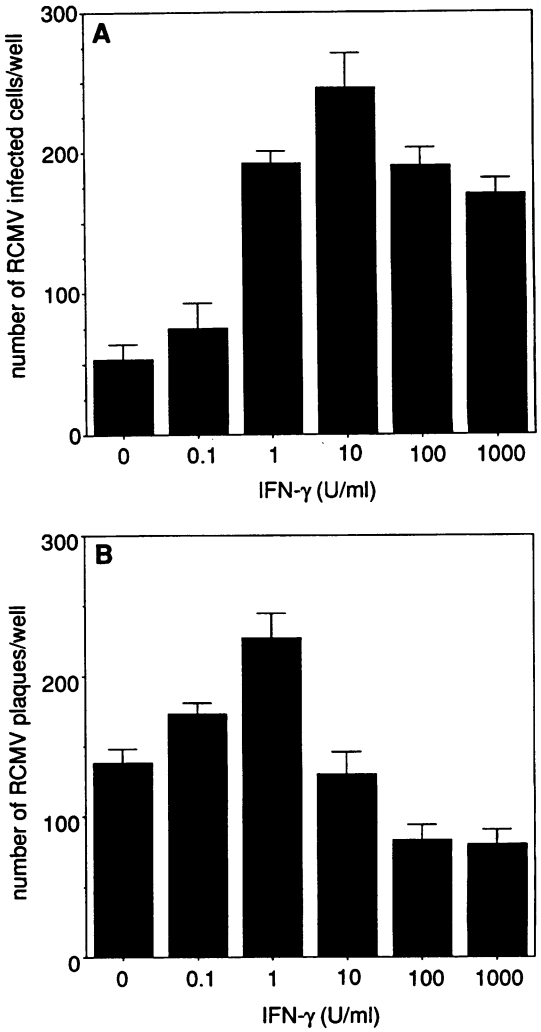

FIG. 3. In vitro effect of IFN-γ on RCMV replication. Macrophages (A) and REF cells (B) were treated for 24 h with IFN-γ doses as indicated and infected with RCMV at a multiplicity of infection of 3 (A) or 0.01 (B). At 72 h p.i., the numbers of RCMV-infected cells (A) or plaques (B) were determined.

determine whether T-cell-derived IFN-γ contributes to the resistance against RCMV infection, rats were treated with a single dose of MAb DB-1. This treatment reduced serum IFN-γ to nondetectable levels when assayed on day 7 p.i. Moreover, the sera were unable to induce class II expression on peritoneal macrophages, while those from control rats did. The neutralizing activity from the MAb could be demonstrated in the rat sera for >7 days.

Administration of IFN-γ neutralizing MAbs did not abrogate resistance against RCMV infection. The animals remained healthy and even showed diminished viral replication in the spleen, as determined on day 4 p.i. (Fig. 6). On day 7 p.i., neither infectious virus nor antigen-containing cells were detected. No evidence was obtained for a redistribution to extrasplenic sites; we could not detect viral antigen in the lungs, kidneys, liver, and salivary glands. The anti-IFN-γ treatment did not influence antibody responses to RCMV despite a significant decrease of the number of plasma cells in the germinal centers of the spleen (data not shown).

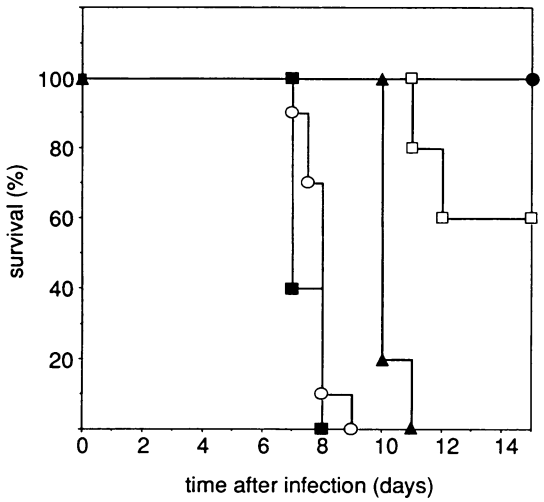

FIG. 4. Effect of recombinant IFN-γ on survival of infected rats. Rats were immunosuppressed by whole-body irradiation (day −1), infected with 5×10^5 PFU of RCMV (day 0), and treated with 10^3 U of IFN-γ per g of body weight (●, days −3 to +1, $n = 10$; □, days −2 to +1, $n = 5$; ▲, days −3 to −1, $n = 5$), 2×10^3 U of IFN-γ per g of body weight (■, days +4 to +6, $n = 5$), and PBS (○, days −3 to +7, $n = 30$).

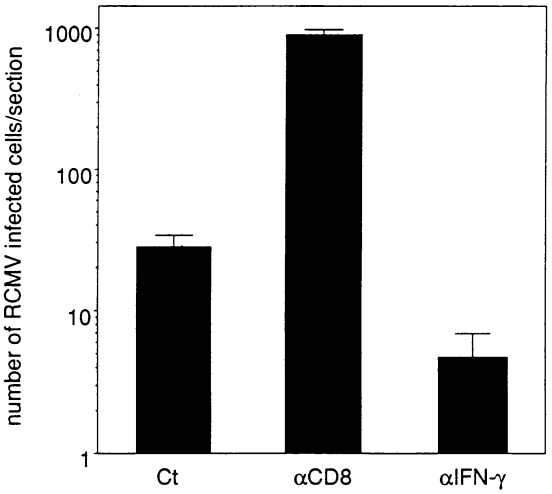

FIG. 6. Effect of anti-IFN-γ antibody treatment on RCMV replication in vivo. BN rats were infected with 10^6 PFU of RCMV and treated via the i.p. route with CD8 antibody (αCD8), IFN-γ MAb (αIFN-γ), or a control antibody (Ct). Viral replication in the spleen at day 4 p.i. is given as the mean number (± standard error) of RCMV-infected cells per spleen section of four rats per group.

Coinjection of IFN-γ neutralizing MAbs with a protective number of T cells in immunosuppressed RCMV-infected recipients further reduced the virus titers. This effect was most pronounced in the lungs: 62 ± 2 PFU/g of tissue in the DB-1-treated rats versus 472 ± 173 PFU/g of tissue in the control animals (Fig. 7); in neither group were symptoms noted. IFN-γ neutralization in rats that had received a nonprotective number of T cells (5×10^6) resulted in complete protection (Table 1). This treatment affected neither survival, virus titers, nor antigen expression in immunosuppressed animals to which T cells had not been transferred. These results demonstrate that T cells may contribute to the enhancement of RCMV replication by the secretion of IFN-γ.

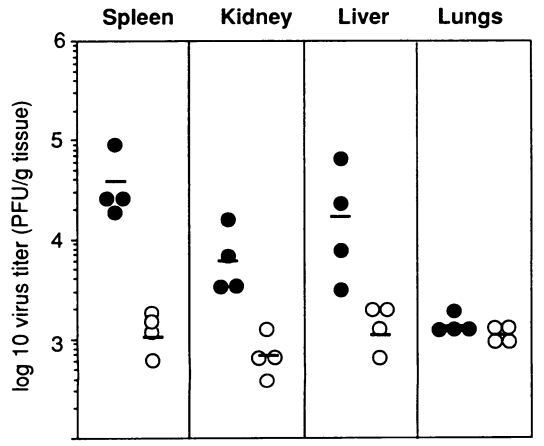

FIG. 5. Effect of recombinant IFN-γ on RCMV replication in radiation-immunosuppressed RCMV-infected rats. Rats were immunosuppressed by total-body irradiation (day −1) and infected with 5×10^5 PFU of RCMV (day 0). Individual virus titers were determined at 7 days p.i. in IFN-γ-treated rats (○, 10^3 U/g of body weight, days −3 to +1); PBS-treated animals (●) served as a control. Horizontal bars represent mean values.

DISCUSSION

IFN-γ plays an important role in the host defense against infectious agents. It is one of the cytokines responsible for activating or otherwise regulating the differentiation and function of mononuclear phagocytes. Normally this will lead to enhanced killing of intracellular pathogens and to inhibition of viral replication (27). In this report, we present evidence that endogenously produced IFN-γ enhances RCMV replication, which implies that T cells may play different and even opposite roles in viral pathogenesis.

Once T cells recognize virus-infected cells, they are activated by a unique set of stimuli and release cytokines, e.g., IFN-γ. Natural killer cells and certain subsets of $CD4^+$ and $CD8^+$ cells can produce IFN-γ (27). However, IFN-γ is rarely found in the circulation of humans or rodents after immune stimulation, likely because of its rapid removal and inactivation. We were able to detect IFN-γ activity in the sera and IFN-γ-PC in the spleens of RCMV-infected rats, using a highly sensitive rat IFN-γ-specific ELISA and immunohistology, respectively. Our results are consistent with the findings of Gessner et al. (10), who showed similar kinetics of IFN-γ production during

TABLE 1. Effect of anti IFN-γ treatment on survival of immunosuppressed RCMV-infected rats that had received immune T cells[a]

Treatment	% survival after T-cell transfer (no. of survivors/no. of animals)		
	0[b]	5×10^6	5×10^7
Irrelevant antibody	0 (0/7)	20 (1/5)	100 (3/3)
Anti-IFN-γ antibody	0 (0/4)	100 (5/5)	100 (3/3)

[a] Whole-body-irradiated rats received T cells 2 h prior to infection and antibodies 1 hour after infection.
[b] Number of cells transferred.

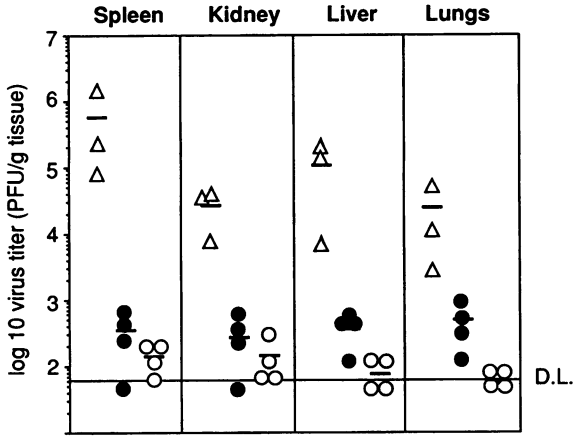

FIG. 7. Effect of anti IFN-γ antibody treatment on RCMV replication in radiation-immunosuppressed rats reconstituted with immune T cells. On day 7 p.i., individual virus titers were determined in recipients of 5×10^7 splenic T lymphocytes (●) and recipients that had been injected with IFN-γ MAb (○); PBS-treated rats (△) served as a control. Horizontal bars represent mean values. D.L., detection limit.

LCMV infection and the presence of IFN-γ-PC in noninfected mice. The weak IFN-γ staining is likely due to the low stimulatory or even immunosuppressive actions of cytomegalovirus (CMV) (30).

Although IFN-γ was first defined by its antiviral effect in vitro, it is primarily an immunomodulator. Thus, high doses of IFN-γ were needed to protect rats against a lethal RCMV infection. In most organs of IFN-γ-treated rats, virus titers were reduced approximately 10-fold. A reduction in RCMV inoculum dose by this factor also reduces mortality in immunosuppressed rats (36). However, this effect was evident only when IFN-γ was applied before the infection. Moreover, TNF-α induced after whole-body irradiation (13) can enhance the antiviral activity of IFN-γ (44). Besides direct antiviral activity, IFN-γ immunostimulatory effects and induced cytokines like TNF and interleukin-1, which exert radioprotective effects (28), may contribute to the observed protection. Similar findings have been made in the mouse CMV infection model; IFN-γ administered p.i. also failed to exert protection, whereas prophylactic treatment did (7, 25).

Surprisingly, IFN-γ produced during the RCMV infection did not exert antiviral activity. On the contrary, we found that neutralization of IFN-γ by MAbs suppressed RCMV replication in immunocompetent rats. Adoptive transfer experiments also demonstrated that the antiviral efficacy of T cells was enhanced by coinjection of the well-characterized IFN-γ neutralizing MAb (2, 17). Sera of anti-IFN-γ-treated rats did not contain detectable IFN-γ activity, as determined in an MHC class II expression test, confirming the efficient neutralization of the lymphokine. We therefore conclude that T cells, besides eliminating virus-infected cells, may also contribute to enhancement of a viral infection by the release of the cytokine.

Thus far, little evidence has been obtained for a direct replication inhibitory role of IFN-γ in viral infections (15, 18). In mice lacking the IFN-γ receptor, vesicular stomitits virus and pseudorabies virus infection took a normal course, despite the sensitivity of these viruses to exogenously administered IFN-γ (15, 32–34, 39). Moreover, recent studies have demonstrated that IFN-γ produced locally by T cells was not effective in controlling the replication of another coinjected virus and in the clearance of mouse CMV, except from the brain and salivary glands, respectively (20, 25). Thus, the fact that a recombinant cytokine possesses antiviral activity does not necessarily imply a role during the infection.

How can the reduced RCMV replication by the anti-IFN-γ treatment be explained? The neutralization of IFN-γ may have interfered with the specific immune response resulting in enhanced T-cell reactivity (6, 24). However, anti-IFN-γ-treated rats generated RCMV-specific antibodies to the same extent as animals receiving control antibodies. A more likely explanation can be drawn from our in vitro findings: pretreatment of macrophages with IFN-γ upregulated RCMV replication. Cultured fibroblasts are the only fully susceptible cell type, while macrophages, the major target cell in vivo (36), do not support RCMV replication efficiently (4). Cellular activation or differentiation may be responsible for the observed enhanced viral replication; thus, human CMV productively infects differentiated macrophages (16). Moreover, we and others have observed that other cytokines, such as TNF-α and transforming growth factor β, activate the immediate-early promoter of human CMV (1, 11). The former may be involved in the IFN-γ-mediated enhancement of RCMV replication; using neutralizing antibodies to TNF-α, we found a suppression of RCMV replication (12). IFN-γ has been shown to upregulate the expression of TNF and its receptors on a variety of cell types (35). Upregulation of viral replication by IFN-γ has also been demonstrated in human immunodeficiency virus-infected monocytes and promonocytic cells (3, 19).

To our knowledge, this report represents the first experimental evidence that IFN-γ may enhance the replication of a virus in vivo. This cytokine has been used as a therapeutic agent in the treatment of both human immunodeficiency virus infection and Kaposi's sarcoma with conflicting results. Reduction of plasma levels of p24 antigen and improvement of immune function and of the patient's condition have been reported, whereas exacerbation and disease progression have been reported by others (9, 14). Recently, an enhancing effect of IFN-γ on the growth of *Trypanosoma brucei* was reported (29). Thus, although T cells are indispensable in the clearance of RCMV-infected cells, they also contribute to the progression of RCMV infection by lymphokine release. The balance between upregulation of viral replication and other T-cell mechanisms such as lysis of the infected cells may determine the outcome of the infection.

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