Enhanced Virus Replication and Inhibition of Lymphocytic Choriomeningitis Virus Disease in Anti-Gamma Interferon-Treated Mice

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The role of gamma interferon (IFN-γ) induced during a viral infection in the ability of the host to acquire antiviral immunity was studied in mice. They were injected subcutaneously daily with an ammonium sulfate-precipitated sheep anti-IFN-γ antibody preparation able to neutralize 10^4 U of IFN-γ. Specificity of the anti-IFN-γ antiserum was demonstrated by absence of detectable activity against natural IFN-α and -β. Controls were treated with a similarly prepared normal sheep serum. Treatment with the IFN-γ-specific antibody preparation had no influence on the ability of mice to generate anti-vaccinia virus- or anti-vesicular stomatitis virus (VSV)-specific cytotoxic T-cell (CTL) responses or T helper-dependent immunoglobulin G responses to VSV. In contrast, treatment of mice with sheep anti-IFN-γ impaired CTL responses against lymphocytic choriomeningitis (LCM) virus (LCMV, Aggressive isolate); in addition, under the experimental conditions used, it prevented lethal LCM. Cytotoxic T-cell activity measured in the spleens of anti-IFN-γ-treated mice was comparable to that found in mice initially infected with a 100-fold-larger dose of LCMV. Evaluation of the effects of treatment on the kinetics of virus replication revealed that in both euthymic and athymic nude C57BL/6 mice, anti-IFN-γ treatment led to an increase of virus titers up to 100-fold compared with control mice. Therefore, IFN-γ may play a role in controlling viruses with tropism for lymphocytes and monocytes/macrophages, such as LCMV.

Gamma interferon (IFN-γ), a product of activated lymphocytes, was first defined by its antiviral effect in vitro (34). It has been shown that IFN-γ is predominantly secreted by T cells which bear the interleukin-2 (IL-2) receptor (25) and that its release is at least partially triggered by helper T-cell-derived IL-2 (10). However, T cells seem not to be the only possible source of IFN-γ; e.g., natural killer cells (15, 25, 35) produce IFN-γ, and mice with severe combined immunodeficiency, which do not express detectable T- and B-cell function, and athymic nude mice can also produce this lymphokine (3, 33). IFN-γ has been shown to induce expression of class II (Ia) major histocompatibility (MHC) antigens, to modulate macrophage differentiation and activation, and to influence antibody response and cell-mediated immunity (9, 30). Therefore, IFN-γ is thought to be a key lymphokine involved in immune regulation, and its antiviral effect is not considered its major function. Most of the summarized properties have been attributed to IFN-γ on the basis of in vitro experiments. Possible roles of IFN-γ in vivo have been evaluated by using recombinant IFN-γ and specific antibodies; these studies established that IFN-γ stimulates macrophages to express increased bactericidal activity against facultative intracellular bacteria (18, 29), modulates natural killer cell activity (31), and induces class II MHC antigens (27, 28); nevertheless, its role in vivo is still poorly understood.

It is well established that control of virus replication plays a crucial role in limiting disease caused by cytopathic viruses (22). Because this is the most commonly considered situation, it may be pointed out here that effects on virus replication may appear to be paradoxical in infections of mice with noncytopathic viruses, such as lymphocytic choriomeningitis virus (LCMV). Disease caused by LCMV is T-cell mediated (19) and depends on a delicate balance between the kinetics and tropisms of the virus and the kinetics of the immunopathological T-cell response. Small doses of most LCMV isolates injected intracerebrally (i.c.) cause lymphocytic choriomeningitis (LCM) in T-cell-competent but not T-cell-deficient mice. However, large doses of some LCMV isolates fail to induce lethal disease. This phenomenon is poorly understood (16). It may indicate that limited numbers of effector T cells are diluted out in heavily LCMV-infected mice; therefore, only small numbers of effector T cells that are apparently insufficient to cause lethal meningitis are recruited to the infected meninges (36). Alternatively, large doses of virus may induce LCMV-specific or nonspecific immune suppression (20), which may limit immunopathological disease. Whether in vivo selection of appropriate mutant virus plays a major role remains to be evaluated (2).

In the present study, we investigated the role of IFN-γ in antiviral host defense against LCMV, vesicular stomatitis virus (VSV), and vaccinia virus infections by assessing the effects in vivo of a sheep antiserum against mouse IFN-γ (32).

MATERIALS AND METHODS

Mice. Six- to 8-week-old inbred C57BL/6 (H-2^b), CBA/J (H-2^k), and outbred ICR mice of either sex were obtained from the breeding colony of the Institut für Zuchthygiene, Tierspital Zürich, Zurich, Switzerland. C57BL/6 *nu/nu* mice were purchased from Bomholtgard, Ry, Denmark.

Viruses. LCM Aggressive, a gift from C. Pfau, Troy, N.Y., was isolated from the blood of a mouse persistently infected with the UBC strain of LCMV (17, 24). The virus

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was cloned on the basis of distinctive plaque morphologies on Madin Darby canine kidney (MDCK) cells. The viruses were titrated either in vivo in footpads of ICR mice as infectious doses (ID) for 50% of the mice (16) or in vitro on MDCK cells. LCMV strain WE was originally obtained from F. Lehmann-Grube, Hamburg, Federal Republic of Germany (20), and subsequently propagated in L929 cells. VSV Indiana (Mudd-Summer isolate) was originally obtained from D. Kolakofsy, Geneva, Switzerland; stocks were prepared in BHK-21 cells (14). Lyophilized vaccinia virus was purchased from the Schweizerisches Serum- und Impfinstitut, Bern, Switzerland. Virus dilutions were prepared in minimal essential medium containing 2% heat-inactivated fetal calf serum.

Antisera and treatment of animals. For the production of anti-IFN-γ antisera, two sheep were initially immunized intramuscularly with 100 mg of recombinant murine IFN-γ at a titer of 1×10^7 to 2×10^7 U/mg (Genentech, South San Francisco, Calif., and Ernst Boehringer Institut für Arzneimittelforschung, Vienna, Austria) in Freund complete adjuvant. The sheep were boosted 11 times in the course of 14 months with the same amount of recombinant IFN-γ in Freund incomplete adjuvant. With this immunization procedure, neutralizing titers of 5×10^4 neutralizing unit (NU)/ml were obtained. Pooled sera from the two sheep were partially purified by ammonium sulfate precipitation. Mice were treated daily subcutaneously (s.c.) with 2×10^4 NU of anti-IFN-γ in 0.2 ml of balanced salt solution for the period of time indicated for the individual experiments. Control mice were injected with preimmunization serum.

Determination of IFN neutralization titers. The IFN neutralization titers of the sheep antisera were measured in a cell protection assay on L929 monolayers grown in 96-well flat-bottomed plates (13). Duplicates of twofold serial dilutions of the sera were preincubated for 1 h at 37°C with 32 U of IFN-α, IFN-β, or IFN-γ. Subsequently, 10 50% tissue culture-infectious doses of VSV Indiana were added per well in an equal volume. After overnight incubation, the plates were screened for cytopathic effect caused by VSV. One unit of IFN per milliliter is defined as the concentration which resulted in 50% protection from cytopathic effect; one NU per milliliter corresponds to the serum concentration which neutralized 1 U/ml.

Antibody response to VSV Indiana. Neutralizing activities of the sera of anti-IFN-γ-treated mice infected intravenously (i.v.) with 10^6 PFU of VSV Indiana were determined as described in detail elsewhere (24). Briefly, serial twofold dilutions of heat-inactivated sera were preincubated with 30 PFU of virus for 1 h at 37°C. The mixture was then transferred to Vero cell monolayers in 96-well plates for absorption before an overlay was added. To determine the immunoglobulin G (IgG) titer alone, the sera were first treated with 0.1 M β-mercaptoethanol (14).

LCM in anti-IFN-γ-treated mice. Anti-IFN-γ-treated C57BL/6 mice were infected i.c. with 1,000 PFU of LCMV Aggressive, and the incidence of death due to the induced T-cell immune response was monitored (16, 20, 38).

Measurement of primary footpad swelling reaction. A dose of 40 PFU of LCMV WE was injected in 30 μl into the footpads of C57BL/6 mice. Swelling of the footpads was measured from days 6 to 13 after injection (16, 20, 38) with a spring-loaded Caliper (Kröplein, Schluchtern, Hessen, Federal Republic of Germany).

Cytotoxicity assay. Activity of cytotoxic T cells was tested in a ^{51}Cr release assay (38) with MC57G (H-2^b), FS9 (H-2^k), and natural-killer-cell-sensitive YAC (H-2^a) mice as target

TABLE 1. IFN-α/β titers in serum of LCMV-infected mice treated with anti-IFN-γ[a]

Treatment	IFN-α/β titers (U/ml) on day postinfection:		
	1	3	5
Anti-IFN-γ	<40	320	160
Normal serum	<40	320	160
None	<40	160	160

[a] C57BL/6 mice infected i.v. with 10^3 PFU of LCMV Aggressive were treated with daily doses of anti-IFN-γ and bled at the time points indicated. IFN-α/β titers were determined as described in Materials and Methods. Values represent averages of six serum samples. Values measured for the individual samples did not differ by more than one titration step from the average shown.

cells. Groups of three to five adult mice were usually infected with 1,000 PFU of LCMV Aggressive or as stated in the respective experiments at the indicated time points prior to the measurement of cytotoxicity. Briefly, single-cell suspensions of spleen cells and of meningal infiltrate cells were assayed in duplicates. Effector and target cells (10^4) were each added in 0.1 ml to round-bottomed 96-well plates to yield effector-to-target cell ratios of 50:1, 25:1, and 13:1 for the spleen lymphocytes and 10:1, 5:1, and 3:1 for the meningal infiltrate cells. After mixing, the plates were spun at $1,500 \times g$ for 6 min and incubated at 37°C in air containing 5% CO_2. The test duration was 4 h for YAC and 5 h for the other target cells. The percentage of the specific ^{51}Cr release was calculated as [(experimental cpm − spontaneous cpm)/(maximal cpm − spontaneous cpm)] × 100. Spontaneous release was determined in supernatants of targets plus 0.1 ml of medium, and maximal release was measured with target cells plus 0.1 ml of 1 N HCl.

To measure the cytotoxic T-lymphocyte (CTL) response induced in animals infected with either 10^6 PFU of VSV Indiana or 5×10^6 PFU of vaccinia virus and treated with anti-IFN-α, anti-IFN-β, or anti-IFN-γ, the following protocols were used for the infection of the target cells. Target cells were incubated with VSV at 37°C with a 30-fold excess of virus. After 2 h, ^{51}Cr and medium were added, and the mixture was incubated for an additional hour. Vaccinia virus was added to the targets at the time of labeling at a virus multiplicity of 10 for 1 to 2 h. One lytic unit (LU) is defined as the number of CTL needed to lyse 30% of the target cells in a standard 5-h assay; it represents a semiquantitative measure for CTL activity (8).

Meningeal infiltrate cells. The procedure to obtain meningeal infiltrate cells has been described previously (7, 36). Briefly, mice were ether anesthetized and exsanguinated. The cerebrospinal fluid (CSF) was tapped through the tectum of the fourth ventricle after careful preparation of the area. The skull cap was cut off, and the brain and the skull were rinsed with medium; when fewer than 4×10^5 cells per animal were harvested, meningeal infiltrate cells from two to three animals were pooled.

RESULTS

Characterization of the sheep anti-IFN-γ antibody preparation. The anti-IFN-γ antiserum did not cross-react with IFN-α or IFN-β when tested in vivo and in vitro (Tables 1 and 2). IFN-α and -β titers in serum, assessed in acutely LCMV-infected C57BL/6 mice treated with daily doses of anti-IFN-γ antiserum or normal serum, were comparable and amounted to equal or slightly higher values than those measured in infected but otherwise untreated controls (Table

TABLE 2. In vitro IFN-α/β titers in the presence of anti-IFN-γ antiserum dilutions[a]

Serum prepn	IFN-α/β titer (U/well) at serum dilution:			
	1:20	1:40	1:80	1:160
Anti-IFN-γ	16	8	16	16
Normal serum	16	16	8	8
Anti-IFN-α/β	<2	<2	<2	<2

[a] Sixteen units of naturally induced IFN-α/β was titrated as described in Materials and Methods after incubation with the respective serum dilution. Values represent averages of triplicate samples.

TABLE 3. Comparison of effects of anti-IFN-γ and dose of LCMV Aggressive on levels of H-2-restricted CTL activity and on virus titers in spleens of 8-day-old immune C57BL/6 mice[a]

Treatment	LCMV dose (PFU)	Avg no. of lymphocytes/ spleen	LU	LU/ spleen	Virus titer (log_{10} ID) per spleen ± SEM
Anti-IFN-γ	10^3	0.9×10^8	56×10^{-4}	160	4.1 ± 0.4
Normal serum	10^3	1.1×10^8	8×10^{-4}	2,380	2.3 ± 0.4
None	10^3	1.2×10^8	6×10^{-4}	2,410	1.8 ± 0.7
	10^4	1.3×10^8	26×10^{-4}	520	3.4 ± 0.4
	10^5	1.4×10^8	42×10^{-4}	320	4.6 ± 0.7

[a] For experimental details, see the legend to Fig. 1.

1). Similarly, antiviral titers of IFN-α and -β measured in vitro remained unaffected by the presence of dilutions of either anti-IFN antiserum or normal serum, while they were reduced beyond detectability by anti-IFN-α/β antiserum (Table 2).

Effect of anti-IFN-γ treatment on maturation of CTL. The effect of anti-IFN-γ on the maturation of cytotoxic T cells was assessed in mice infected with LCMV Aggressive, vaccinia virus, and VSV Indiana. Mice were treated with daily s.c. doses of anti-IFN-γ from the day of infection until the day before the ^{51}Cr release assay. Cytotoxicity was measured on infected and uninfected MC57G ($H-2^b$) and FS9 ($H-2^k$) target cells.

The specific cytotoxicity induced in C57BL/6 ($H-2^b$) mice infected with 10^3 PFU of LCMV Aggressive and treated with either anti-IFN-γ or normal serum was compared with that in untreated mice infected i.v. with 10^3, 10^4, or 10^5 PFU of virus. The cytotoxicity assayed in the spleens of anti-IFN-γ-treated animals infected with 10^3 PFU of virus was lower than that measured in untreated animals injected with the same virus dose (Fig. 1; Table 3). This result suggests that anti-IFN-γ reduced generation of CTL responses. The more likely explanation, however, as will be shown later, is that IFN-γ treatment caused increased LCMV titers in mice injected with the antiserum. High infectious doses of LCMV have been shown to lead to lower CTL activities in the spleen than lower doses (19, 36). CTL activity measured in the spleens of anti-IFN-γ-treated animals was comparable to that found in mice infected with a 100-fold-greater initial virus dose (10^5 PFU). When cytotoxic activities measured in

the spleen were compared on the basis of LU per spleen (Table 3), their values were 2,410 LU (100%) for mice infected with 10^3 PFU of LCMV Aggressive, 2,380 LU (99%) for normal-serum-treated mice infected with the same dose, and 160 LU (7%) for anti-IFN-γ-treated mice. The values measured for mice infected with 10 or 100 times greater virus doses amounted to 520 LU (21%) and 320 LU (13%), respectively. Killing measured on uninfected MC57G ($H-2^b$) or infected H-2-incompatible FS9 ($H-2^k$) target cells was less than 100 LU per spleen. The influence of anti-IFN-γ treatment on LCMV titers was evaluated in spleens (Table 3). In the organs of anti-IFN-γ-treated animals, LCMV Aggressive reached titers comparable to those found in animals originally infected with a 100-fold greater virus dose, i.e., the measured values were about 30 times higher than those found in normal-serum-treated or untreated mice (Table 3).

Similarly, in mice infected i.c. with 10^3 PFU of LCMV Aggressive and treated with anti-IFN-γ, lower levels of cytotoxicity were detected in spleens and meningeal infiltrates 7, 8, and 10 days after inoculation of virus (Fig. 2). The reduction of cytotoxicity found in the CSF was considerable in animals inoculated with 10^3 PFU of LCMV Aggressive. No such effect could be observed on day 10 in animals treated with anti-IFN-γ for only part of the time, i.e., days 5 to 9 (data not shown).

The induction of specific cytotoxicity under the influence of anti-IFN-γ treatment in day 6 vaccinia virus- or VSV

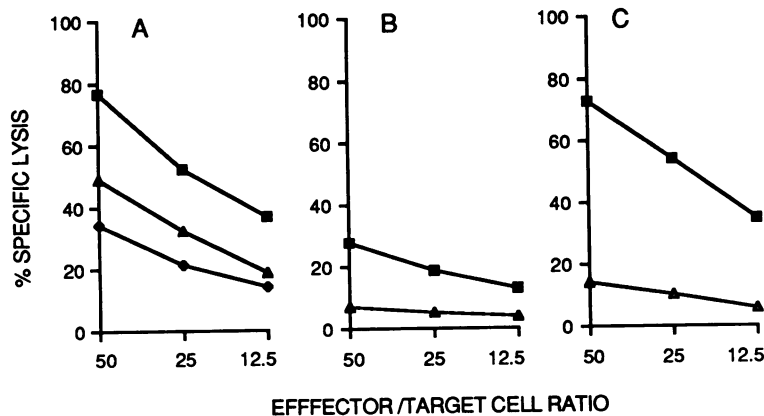FIG. 1. Spleen cells of C57BL/6 mice infected with 10^3 (■), 10^4 (▲), or 10^5 (◆) PFU of LCMV Aggressive were tested on either infected (solid symbols) or uninfected (▲ in panels B and C) MC57G ($H-2^b$) target cells for 5 h. Spontaneous release was less than 18%. Nonrestricted killing on infected FS9 ($H-2^k$) target cells (data not shown) was less than 15%. Values are the means of the cytotoxic activities of three individual spleen cell populations; SEM was less than 10%. (A) Infected mice not receiving any treatment; (B) mice treated with daily doses of anti-IFN-γ from day 0 to day 7; (C) mice injected with normal serum for the same period of time.
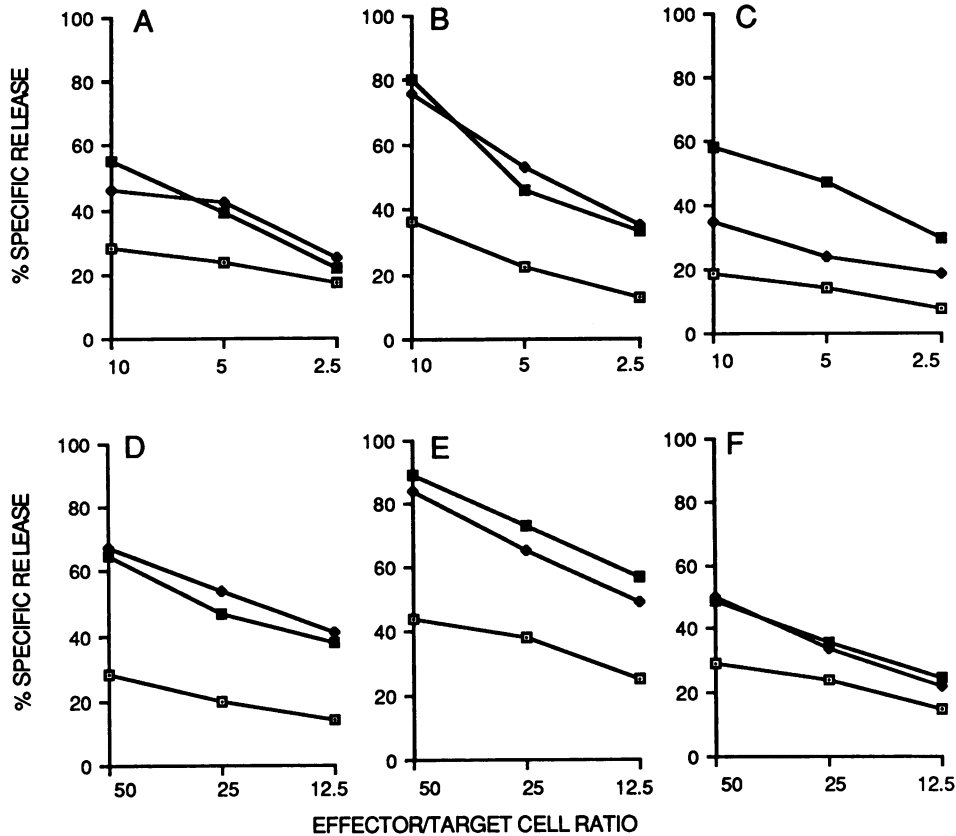
FIG. 2. Meningeal infiltrate cells (A to C) and spleen cells (D to F) of C57BL/6 mice infected with 10^3 PFU of LCMV Aggressive. Mice were injected with daily doses of anti-IFN-γ (□) or normal serum (◆) from day 0 to the day prior to the assay or did not receive any additional treatment (■). Effector cells were tested on day 7 (A and D), day 8 (B and E), or day 10 (C and F) after infection on infected and uninfected MC57G ($H-2^b$) for 5 h. Spontaneous release was less than 19%, and nonrestricted killing on infected FS9 ($H-2^k$) target cells amounted to less than 14%. Values are the means of the cytotoxic activities of spleen cells or meningeal cells of two to three mice per group; SEM was less than 12%.

Indiana-immune C57BL/6 mice remained unchanged compared with normal-serum-treated or infected but otherwise untreated mice (Table 4); also, a fivefold increase in the daily anti-IFN-γ dose had no detectable effect (data not shown).

Effect of anti-IFN-γ treatment on susceptibility to LCM. The influence of daily anti-IFN-γ treatment on the course of an LCMV infection was tested in C57BL/6 mice inoculated i.c. with 10^3 PFU of LCMV Aggressive. Lethality due to LCM was monitored up to day 15 after infection. A high

percentage of i.c. infected animals injected with anti-IFN-γ survived, while untreated or normal-serum-treated mice died from LCM. Table 5 shows the results of one of four experiments with similar results. In the blood of surviving, anti-IFN-γ-treated mice which were originally infected with 10^3 PFU of LCMV, virus could still be detected 22 days after inoculation, suggesting that this treatment facilitated virus growth or persistence in mice.

Footpad swelling reaction under the influence of anti-IFN-γ. C57BL/6 mice were injected in the foot with 40 PFU of LCMV WE to measure primary footpad swelling reaction. Mice treated with anti-IFN-γ developed swelling earlier, from day 6 onwards, compared with day 8 in controls, and maximal swelling of the footpads was measured on day 8, i.e., a day earlier than for normal-serum-treated mice or

TABLE 4. Effect of anti-IFN-γ treatment on generation of CTL against VSV Indiana and vaccinia virus[a]

Treatment	% Specific ^{51}Cr release at indicated E:T[b] ratio					
	VSV Indiana			Vaccinia virus		
	50:1	25:1	13:1	50:1	25:1	13:1
Anti-IFN-γ	41	26	18	71	55	42
Normal serum	45	33	21	64	53	43
None	46	30	17	68	49	37

[a] C57BL/6 mice were infected with 2 × 10^6 PFU of either VSV Indiana or vaccinia virus 6 days prior to testing. The cytotoxicity of the spleen lymphocytes was tested on infected and uninfected MC57G ($H-2^b$) target cells in a ^{51}Cr release assay. Test duration was 5 h. Spontaneous release was less than 17%. On uninfected MC57G target cells, killing was less than 6%. Animals were treated from day 0 to day 5.
[b] E:T, Effector to target cell.

TABLE 5. Effect of anti-IFN-γ treatment in C57BL/6 mice on susceptibility to LCMV Aggressive-induced disease[a]

Treatment	% Mortality (mean time to death in days)
Anti-IFN-γ	10 (7.5)
Normal serum	80 (10.7)
None	100 (11)

[a] Groups of 10 mice infected with 10^3 PFU of LCMV Aggressive were treated with daily s.c. doses of anti-IFN-γ from day 0 to day 12, and survival was monitored up to day 15.
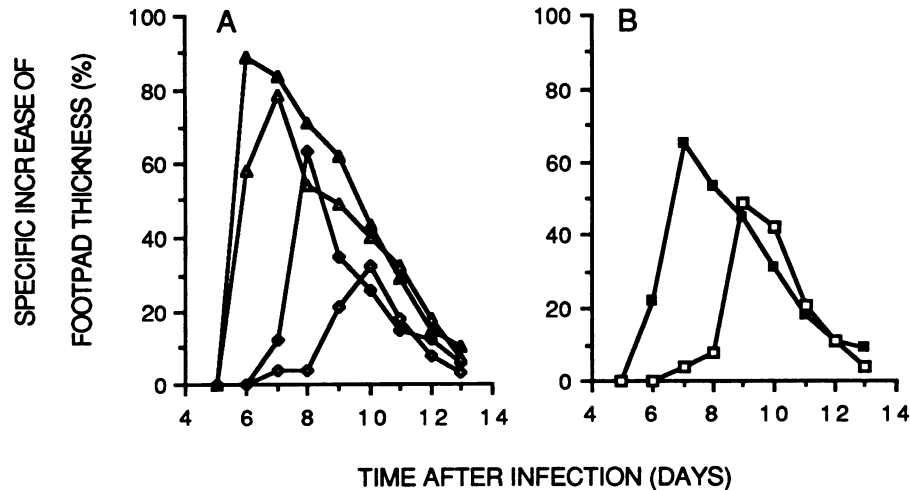
TIME AFTER INFECTION (DAYS)

FIG. 3. (A) Control C57BL/6 mice infected with either 4×10^3 PFU (▲), 4×10^2 PFU (△), 40 PFU (◆), or 4 PFU (◇) of LCMV WE did not receive any further treatment. (B) Experimental mice were locally infected with 40 PFU of LCMV WE in the footpad and injected from day 0 to day 7 with anti-IFN-γ (■) or normal serum (□). Footpad thickness was monitored daily thereafter and compared with preinfection values. Values are the means of measurement of four footpads: SEM was less than 8%.

infected but otherwise untreated animals (Fig. 3A). This shift to earlier responses paralleled what was seen for footpad responses to higher doses of LCMV in comparison to what was found with smaller virus doses (Fig. 3B). All mice shown in Fig. 3B were bled on day 10 after infection, and the presence of virus was monitored; only the anti-IFN-γ-treated mice were virus positive (data not shown).

Effect of anti-IFN-γ treatment on virus replication in immunocompetent and athymic mice infected with LCMV. To evaluate the extent of the enhancing effect of anti-IFN-γ on LCMV titers in organs and to establish whether this effect was dependent on mature T cells, the following experiments were performed. Normal and athymic C57BL/6 mice inoculated i.v. with 30 PFU of LCMV Aggressive were treated with anti-IFN-γ or normal serum, and virus titers in liver and spleen were determined 4 days (normal mice only) and 8 days after infection. C57BL/6 +/+ and C57BL/6 nu/nu mice treated with anti-IFN-γ both exhibited 70- to 100-fold-higher virus titers than similar animals treated with normal serum (Table 6).

Effect of anti-IFN-γ treatment on generation of neutralizing antibodies. C57BL/6 mice injected with 10^6 PFU of VSV

TABLE 6. Effect of anti-IFN-γ treatment on virus titers in immunocompetent and athymic C57BL/6 mice[a]

Mouse strain	Treatment	Infectious virus ($\log_{10}$ ID) per organ ± SEM			
		Day 4		Day 8	
		Spleen	Liver	Spleen	Liver
+/+	Anti-IFN-γ	4.9 ± 0.4	2.8 ± 0.4	3.4 ± 0.4	2.1 ± 0.7
	Normal serum	2.8 ± 0.3	2.1 ± 0.3	<1.8	<1.8
	None	2.1 ± 0.7	1.9 ± 0.7	<1.8	<1.8
nu/nu	Anti-IFN-γ			6.6 ± 0.4	3.4 ± 0.4
	Normal serum			5.1 ± 0.4	1.9 ± 0.7
	None			4.6 ± 0.7	1.8 ± 0.7

[a] C57BL/6 +/+ and C57BL/6 nu/nu mice were infected i.v. with 30 PFU of LCMV Aggressive and killed 4 or 8 days later. Virus titers were determined by the footpad assay. Lower detection limit in spleen and liver was $1.8 \log_{10}$ of infectious virus per organ. Values represent means of three to four organs assayed individually. Animals were treated from day 0 until the day before they were sacrificed.

Indiana were treated with daily anti-IFN-γ doses up to day 11. They were bled 4, 8, and 12 days after infection, and neutralizing anti-VSV IgM and IgG titers in the serum were determined. No significant difference in neutralizing IgM or IgG responses could be observed (data not shown).

DISCUSSION

Neutralization of IFN-γ induced after infection with LCMV Aggressive by sheep anti-IFN-γ antiserum enhanced replication of LCMV (Tables 3 and 6). This enhancement of LCMV replication was not dependent on the presence of functional T cells, since similar effects were seen in athymic C57BL/6 nu/nu mice (Table 6). Anti-IFN-γ treatment protected mice from lethal disease. This correlated with the finding that the cytotoxicity measured in the spleens and CSF of C57BL/6 mice infected with LCMV Aggressive and treated with anti-IFN-γ was lower than in animals which were injected with normal serum or which did not receive any additional treatment (Fig. 2). The possibility that enhanced LCMV replication during anti-IFN-γ treatment induced a form of high-dose immune paralysis, as postulated by Hotchin (16) was supported by control experiments. Relative cytotoxic T-cell activity was similar to that found for otherwise untreated mice initially infected with a 100 times higher virus dose (Fig. 1; Table 3). It has been shown in adoptive transfer experiments (12) that impairment of cytotoxic T cell generation by high virus doses is unlikely to be the only possible cause for this paradoxical phenomenon. Nevertheless, there exists an inverse relationship between infectious doses of LCMV and T-cell cytotoxicity in spleens (37). Therefore, high-dose immune paralysis may at least in part be explained by abundance of infected target cells and the relative dilution of cytotoxic T cells (14). The possibility that LCMV-induced dose-dependent immunosuppression may contribute to the high-dose immune paralysis phenomenon remains to be evaluated (1). It is noteworthy that a protective effect of the anti-IFN-γ antiserum could only be observed in animals receiving daily doses of 10^4 NU; this is in contrast to the finding (26) that a single injection on day 0 of 2×10^4 NU of anti-IFN-α/β could spare mice from LCM. Anti-IFN-γ antiserum did not interfere obviously with the

generation of specific T cells (Table 2). These findings are supported by studies of the specific footpad thickness increase after local infection with LCMV WE (19); this strictly T-cell-dependent response exhibited an earlier onset in mice treated with anti-IFN-γ. This result supports the notion that anti-IFN-γ enhances LCMV replication, since greater doses of LCMV also cause an earlier local swelling reaction than lower doses (Fig. 3).

To avoid the possible complication of mutual influences of LCMV titer increase on detectable cytotoxic T-cell activities, effects of anti-IFN-γ treatment on cytotoxic T cell responses against vaccinia virus and VSV were measured; neither virus replicated measurably in adult mice except when injected i.c. (vaccinia virus Lancy isolate, our unpublished observations; VSV, reference 5). Mice treated with anti-IFN-γ antibodies generated VSV- and vaccinia virus-specific cytotoxic T cells with activities comparable to those from normal-serum-treated or untreated mice. Also, anti-IFN-γ-treated mice and animals receiving normal serum exhibited no difference in their capacity to generate neutralizing anti-VSV IgM and IgG antibodies.

IFN-γ was originally recognized for its ability to protect cells from viral infections (34); however, it has recently received more attention as a lymphokine because of its immunoregulatory action. Treatment of virus-infected mice with anti-IFN-α/β is known to induce dramatically increased virus titers by neutralization of the antiviral effect of IFN-α/β. We report here on a similar enhancing effect of polyclonal sheep anti-IFN-γ antiserum on the replication of LCMV Aggressive in the absence of a major influence of anti-IFN-γ treatment on either the induction of cell-mediated immunity, as assayed by specific T-cell cytotoxicity, or on generation of T helper-cell-dependent neutralizing anti-VSV IgG titers. Since in both immunocompetent and athymic mice treatment with anti-IFN-γ led to an up to 100-fold increase in LCMV titers, dependence of the phenomenon on mature T cells seems to be excluded; this finding is interesting because production of IFN-γ was assumed to be a specialized function of T cells (25). Recently, however, it has been reported that mice with severe combined immunodeficiency are able to produce this lymphokine (33); there is also evidence that natural killer cells produce IFN-γ (15, 35). The titers of IFN-α/β are usually high during the first 4 to 6 days of infection (21) and have been shown to inhibit LCMV replication (26). It appears from our results that the usually very low serum titers of IFN-γ (11) apparently influence LCMV titers considerably. In fact, the present results indicate that the prominent effect of anti-IFN-γ in vivo in an LCMV infection seems to be the neutralization of the antiviral properties of IFN-γ; this may suggest that IFN-γ plays a major role in controlling viruses with tropisms for lymphocytes and possibly monocytes/macrophages, such as LCMV (6, 14, 23), herpesviruses (34), and human immunodeficiency virus (4).

ACKNOWLEDGMENTS

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