JOURNAL OF VIROLOGY, Mar. 1996, p. 1521–1526 Vol. 70, No. 3
0022-538X/96/$04.00+0

Modulation by Gamma Interferon of Antiviral Cell-Mediated Immune Responses In Vivo

OLAF UTERMÖHLEN,[1] ASTRID DANGEL,[1] ATTILA TÁRNOK,[2]† AND FRITZ LEHMANN-GRUBE[1]*

Heinrich-Pette-Institut für Experimentelle Virologie und Immunologie an der Universität Hamburg[1] and Institut für Entwicklungsneurobiologie, Zentrum für Molekulare Neurobiologie,[2] Hamburg, Germany

Received 18 August 1995/Accepted 29 November 1995

Mice were infected with lymphocytic choriomeningitis virus and injected once 24 h later with a monoclonal antibody directed against gamma interferon. In comparison with controls, the increase of numbers of $CD8^+$ T cells and the generation of virus-specific cytotoxic T lymphocytes in spleens and virus clearance from organs were diminished, as was the ability of spleen cells to transmit adoptive immunity to infected recipients. The same treatment slightly but consistently lessened rather than augmented the virus titers early in infection, which was also observed in thymusless *nu*/*nu* mice. Injection into infected mice of the lymphokine itself in quantities probably higher than are produced endogenously resulted in lower virus titers in spleens but higher titers in livers. The adoptive immunity in infected mice achieved by infusion of immune spleen cells was not altered by treating the recipients with gamma interferon monoclonal antibody. Such treatment did not measurably affect the production of antiviral serum antibodies. We conclude that in lymphocytic choriomeningitis virus-infected mice, gamma interferon is needed for the generation of antivirally active $CD8^+$ T lymphocytes, and furthermore that in this experimental model, direct antiviral effects of the lymphokine elude detection.

In a previous study, we had monitored lymphocytic choriomeningitis (LCM) virus infection in mice treated with a monoclonal antibody (MAb) that neutralizes gamma interferon (IFN-γ). Injection during the antiviral effector phase did not measurably alter the infectious course, but early treatment, i.e., during the immunological induction phase, impaired virus elimination and generation of cytotoxic T lymphocytes (CTL). We concluded that IFN-γ promotes the generation of antiviral effector cells (51). At about the same time, Leist and colleagues (29) published similar observations, which they, however, interpreted to mean that ordinarily virus replication is reduced by IFN-γ, the neutralization of which allows higher titers to be attained. This, in turn, leads to high-dose immune paralysis, well known in mice experimentally infected with LCM virus. Initially this finding was taken to mean that the proportion of mice that die following intracerebral infection decreases with increasing virus doses (3, 24), but later it was found that other cell-mediated immune responses follow a similar pattern, although the ability to control the infection is only slightly affected by a high infectious dose (26).

Since a distinction between these two opposing interpretations has consequences for understanding the development of antiviral immunity, this work was continued. We have come to the conclusion that in LCM virus-infected mice, IFN-γ functions as an immunomodulator, whereas this lymphokine's direct antiviral effects elude detection.

MATERIALS AND METHODS

Mice. BALB/cAnNCrlBR mice (Charles River, Sulzfeld, Germany) and BALB/cABom-*nu*/*nu* and BALB/cABom +/+ mice (Bomholdgård, Ry, Denmark) were obtained specific pathogen free and kept by us under strict barrier conditions; they were used when 8 to 12 weeks old.

Virus. The WE strain LCM virus (39) was produced in L cells and titrated in mice as mouse-infectious units (mouse IU) or in L cells as PFU, which was converted to mouse IU (25).

IFN-γ. Two types of recombinant murine IFN-γ were used. One was produced by transformed Chinese hamster ovary cells (8) and used either in the form of cell culture fluid (kindly supplied by R. Dijkmans and A. Billiau, Leuven, Belgium) or purified (Holland Biotechnology BV, Leiden, The Netherlands). The other, an *Escherichia coli*-derived preparation, was a generous gift of R. G. Adolf from Bender & Co. GmbH, Wien, Austria. Biological activities were determined as ability to block the cytopathic effect of vesicular stomatitis virus in L cells (40). IFN-γ in mouse sera was assessed by enzyme-linked immunosorbent assay (ELISA) with a commercial kit (Genzyme, Cambridge, Mass.).

MAb. Rat MAb R4-6A2, directed against murine IFN-γ (47), was purified by affinity chromatography from hybridoma culture fluids (2) and quantitated by its ability to block the antiviral effect of IFN-γ against vesicular stomatitis virus in L cells (18). On the basis of this determination, we have calculated that the standard dose injected by us into mice had the capacity to neutralize 2×10^5 U of IFN-γ. Rat MAb YTH 89.1, which is directed against human glycophorin A (34), served as a control. Initially MAb was injected intravenously (i.v.), but when it was found that the intraperitoneal route was as effective, the latter was used. For flow cytometry, the fluorescein isothiocyanate-tagged CD4 MAb H129.19 (38) and the fluorescein isothiocyanate-tagged CD8 MAb 53-6.7 (23) were purchased (Boehringer, Mannheim, Germany).

Adoptive immunization. The immunization protocol has been described elsewhere (25). Virus doses and time intervals are detailed in the table footnotes.

Flow cytometry. Dispersed spleen cells were counted, isolated by Ficoll density centrifugation, and stained with MAb and propidium iodide. Aliquots of 5,000 propidium iodide-excluding cells were run through an EPICS 751 cell sorter (Coulter, Hialeah, Fla.), using standard two-color cytometry parameters for fluorescein isothiocyanate-propidium iodide discrimination. Data were analyzed with a data analysis system supplied by W. Beisker, Neuherberg, Germany.

Measurement of CTL activity. The 4-h chromium-release assay has been described previously (51).

Measurement of antiviral serum antibodies. Immunoglobulin M (IgM), IgG, and IgG2a were determined by solid-phase ELISA, using purified LCM virus as the antigen (35).

RESULTS

Effect of treatment of LCM virus-infected mice with IFN-γ MAb on generation of virus-specific CTL and on ability to eliminate the virus from organs. In i.v.-infected mice, the WE strain of LCM virus replicates in essentially all tissues, most

* Corresponding author. Mailing address: Heinrich-Pette-Institut, Martinistrasse 52, D-20251 Hamburg, Germany. Phone: 40/480510. Fax: 40/48051-180.

† Present address: Klinik für Kinderkardiologie, Herzzentrum Leipzig Universitätsklinikum, D-04289 Leipzig, Germany.

TABLE 1. Inability of LCM virus-infected IFN-γ MAb-treated mice to generate antiviral effector cells as revealed by adoptive immunization

No. of immune cells transferred[a]	Donor mice treated with IFN-γ MAb[b]			Untreated donor mice		
	Titer[c] (mean ± SEM)	% Residual virus	*P*[d]	Titer (mean ± SEM)	% Residual virus	*P*
0[e]	NA[f]			$(9.43 \pm 1.43) \times 10^7$	100	
1.0×10^8	$(1.12 \pm 0.39) \times 10^8$	>100	>0.05	ND[g]		
2.5×10^7	$(6.34 \pm 1.00) \times 10^8$	>100	<0.01	ND		
6.3×10^6	$(1.09 \pm 0.13) \times 10^9$	>100	<0.01	$(1.33 \pm 0.53) \times 10^5$	<1	<0.01
1.6×10^6	ND			$(3.39 \pm 0.64) \times 10^6$	4	<0.01
3.9×10^5	ND			$(3.17 \pm 0.43) \times 10^7$	34	<0.01

[a] On day 8 of infection, the spleen cells of donor mice were counted on the basis of trypan blue exclusion, and indicated numbers were injected i.v. into syngeneic recipients that had been infected with 10^3 mouse IU 16 h previously.
[b] BALB/c donor mice were infected by i.v. injection of 10^5 mouse IU and treated 24 h later with 250 μg of IFN-γ MAb or left untreated.
[c] Mouse IU per gram of spleen in five mice determined 40 h after cell transfer (56 h after virus injection). The group of control mice (no immune cells transferred) consisted of eight mice.
[d] In comparison with the titers in spleens of control mice; calculated with Student's *t* test.
[e] Each control mouse received i.v. 2.5×10^7 splenocytes from uninfected untreated donors.
[f] NA, not applicable.
[g] ND, not done.

rapidly and to highest titers in spleens and lymph nodes. The rate depends on the infectious dose, and after injection of 10^5 mouse IU, spleen titers as high as 10^9 mouse IU/g are attained within 1 to 2 days. During the subsequent virus elimination, LCM virus CTL appear with highest activities on days 7 to 9. IFN-γ MAb was given 1 day after virus, which resulted in greatly diminished ability to generate CTL and to control the infection. This effect was less obvious with lower infectious doses (e.g., 10^3 mouse IU) and essentially absent when the time interval between injection of virus and injection of antibody was extended. Since they confirm what has previously been reported (51), data are not shown.

Effect of treatment of infected mice with IFN-γ MAb on generation of cells capable of adoptively immunizing infected recipients. The efficacy of antivirally active $CD8^+$ T lymphocytes can be assessed by determining the numbers of lymphoid cells that have to be infused in order to reduce to a defined extent multiplication of the virus in recipients (25). As many as 10^8 splenocytes from mice that had been given LCM virus 8 days previously and treated 1 day later with IFN-γ MAb failed to transfer antiviral immunity, whereas ca. 4×10^5 splenocytes from untreated donors were effective (Table 1). In a repeat experiment, even 2×10^8 spleen cells from infected and MAb-treated mice did not confer antiviral immunity (data not shown). On the contrary, more infectivity was always detected in the spleens of recipients of immune cells obtained from treated donors than in controls, and there was an inverse relationship between numbers of injected cells and virus concentrations. One explanation for this paradoxical dose response is opposite effects of the infused splenocytes by exerting residual antiviral activity on the one hand and stimulating virus replication in the recipients' spleens on the other, perhaps by activation of mononuclear phagocytes (27). This explanation is speculative, but the fact remains that the treatment with IFN-γ MAb of LCM virus-infected mice all but abolished the ability of the mice to generate cells capable of adoptively immunizing recipients.

Effect of treatment of infected mice with IFN-γ MAb on early virus multiplication in spleens. Increased virus replication resulting from treatment with IFN-γ MAb should be evident early in infection. As the data in Table 2 show, on days 2, 3, and 4, the infectious titers were lower rather than higher in antibody-treated mice. In contrast, on day 5, when antiviral immunity sets in, there was more virus in treated mice, evidence of the basic phenomenon that injection of IFN-γ MAb 1 day after virus impairs the animals' ability to control the infection (51).

Effect of treatment of infected athymic mice with IFN-γ MAb on virus replication in spleens. Just as in ordinary mice, in thymusless *nu/nu* mice there was slightly less virus in the spleens of MAb-treated mice than in the spleens of mice treated with control antibody (Table 3), which, again, is contrary to the notion that neutralization of endogenously produced IFN-γ results in higher infectious titers. In the +/+

TABLE 2. Effect of treatment of mice with IFN-γ MAb on multiplication of LCM virus in spleens early in infection[a]

Day of infection	Mean ± SEM mouse IU/g of spleen in 5 mice		*P*[b]
	IFN-γ MAb	Human glycophorin A MAb (control)	
2	$(1.57 \pm 0.21) \times 10^9$	$(1.65 \pm 0.16) \times 10^9$	>0.05
3	$(4.73 \pm 0.52) \times 10^8$	$(6.05 \pm 0.47) \times 10^8$	>0.05
4	$(1.28 \pm 0.22) \times 10^8$	$(1.33 \pm 0.62) \times 10^8$	>0.05
5	$(1.15 \pm 0.13) \times 10^8$	$(0.51 \pm 0.07) \times 10^8$	<0.01

[a] BALB/c mice were infected by i.v. injection of 10^5 mouse IU. One day later a single dose of the indicated MAb was injected i.v.
[b] Calculated with Student's *t* test.

TABLE 3. Effect of treatment of thymusless *nu/nu* mice with IFN-γ MAb on multiplication of LCM virus in spleens[a]

Mouse group	Day of infection	Mean ± SEM mouse IU/g of spleen in 4 mice		*P*[b]
		IFN-γ MAb	Human glycophorin A MAb (control)	
nu/nu	2	$(1.30 \pm 0.20) \times 10^9$	$(1.32 \pm 0.40) \times 10^9$	>0.05
	3	$(4.98 \pm 1.66) \times 10^8$	$(8.68 \pm 0.71) \times 10^8$	>0.05
	4	$(1.84 \pm 0.16) \times 10^8$	$(5.30 \pm 3.93) \times 10^8$	>0.05
	8	$(1.25 \pm 0.62) \times 10^8$	$(2.06 \pm 0.55) \times 10^8$	>0.05
+/+	2	ND[c]	ND	
	3	ND	ND	
	4	$(7.37 \pm 0.52) \times 10^7$	$(8.04 \pm 1.29) \times 10^7$	>0.05
	8	$(4.88 \pm 3.49) \times 10^6$	$(3.58 \pm 2.28) \times 10^4$	<0.01

[a] BALB/c *nu/nu* or +/+ control mice were infected by i.v. injection of 10^5 mouse IU. One day later, a single dose of the indicated MAb was injected i.v.
[b] Calculated with Student's *t* test.
[c] ND, not determined.

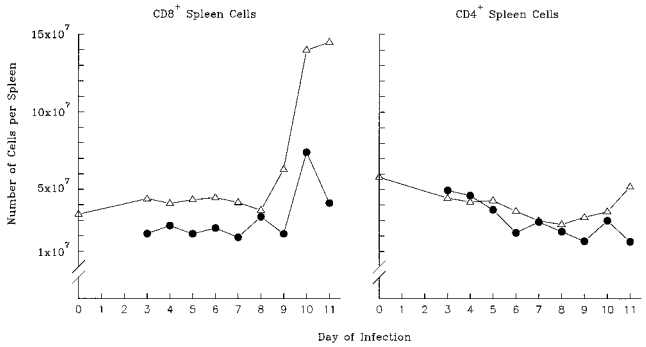

FIG. 1. Effect of treatment with IFN-γ MAb on numbers of $CD4^+$ and $CD8^+$ T lymphocytes in spleens of LCM virus-infected mice. BALB/c mice were infected by i.v. inoculation of 10^5 mouse IU and 1 day later injected with IFN-γ MAb (closed circles) or left untreated (open triangles). On the indicated days, splenocytes were analyzed by flow cytometry, and total numbers of $CD4^+$ and $CD8^+$ cells per organ were calculated from the percentages of fluorescein isothiocyanate-labeled cells above background and from total mononuclear cell counts per spleen.

mice, on day 8 the virus in IFN-γ MAb-treated animals exceeded the virus in controls by more than 2 $\log_{10}$, which once more conforms to the basic phenomenon and at the same time demonstrates the efficacy of the antibody. A repeat experiment confirmed these results.

Effect of treatment of infected mice with IFN-γ MAb on numerical expansion of subset T lymphocytes. During infection of mice with LCM virus, the $CD8^+$ T-lymphocyte subset in peripheral lymphatic tissues enlarges while numbers of $CD4^+$ T cells remain relatively constant (20, 49). This increase of $CD8^+$ T lymphocytes was suppressed by injection of IFN-γ MAb (Fig. 1), which was consistently seen in several experiments; the numbers of $CD4^+$ cells were not measurably altered.

IFN-γ in the circulation of infected mice. Increased production of IFN-γ by lymphoid cells during murine infection with LCM virus has been documented (13, 49), but we and others had been unable to detect it in the serum (33; our unpublished observations). As Fig. 2 shows, this situation has changed through use of an ELISA, which we had previously found to be very sensitive (49). From below detectability, during infection IFN-γ rose to a first height on day 2, fell slightly, and rose again to concentrations as high as 65 ng/ml of serum on day 5. Similar results were obtained with C57BL/6J mice, for which the two-peak pattern was even more pronounced (data not shown).

Effect of treatment of infected mice with IFN-γ on virus multiplication in spleens and livers. The finding that neutralization of endogenously produced IFN-γ did not affect viral replication early in infection led to the question of whether the lymphokine is antivirally active in vivo. The answer was sought by determining the effect that treatment of infected mice with recombinant murine IFN-γ had on the kinetics of virus increases in organs. When 10^5 mouse IU of LCM virus was injected, highest titers were attained so rapidly as to prevent evaluation in the early phase of infection (Tables 2 and 3). Therefore, the inoculum was lowered to 10^3 mouse IU, which led to reduced virus multiplication in spleens when large quantities of IFN-γ were repeatedly injected (Table 4). However, the infectious titers were increased in the livers of the same animals (Table 4). This experiment was repeated with C57BL/6J mice and IFN-γ of eukaryotic origin with essentially identical results.

Effect of treating recipients of immune cells with IFN-γ MAb on adoptive immunity. We had previously reported that the accelerated ability of infected mice to eliminate the virus as a result of infusion of immune cells was not affected by concomitant injection of IFN-γ MAb (51). The protocol was altered by injecting the antibody before the immune cells. The results (Table 5) show that the rapid elimination of LCM virus from recipients of day 8-immune splenocytes was not altered even by prior injection of IFN-γ MAb.

Effect of treatment of infected mice with IFN-γ MAb on production of LCM virus antibodies. Injection of IFN-γ MAb once on day 1 into LCM virus-infected mice did not measurably alter the serum concentrations of antiviral IgM, IgG, and IgG2a (Table 6).

DISCUSSION

IFN-γ has frequently been implicated in the generation of $CD8^+$ CTL both in vitro and in vivo (6, 10, 14, 16, 31, 37, 42, 43, 48, 52), but other observations have been interpreted to mean that CTL develop independent of this lymphokine (4, 7, 19, 22). Thus, the relevance of IFN-γ for the generation of CTL effectors is uncertain, which was the main reason for investigating whether the impaired antiviral cell-mediated immune responses in LCM virus-infected mice in which IFN-γ was neutralized by antibody has a virological (29) or an immunological (51) basis.

If the reduced ability to generate CTL and to control the infection in MAb-treated mice were indeed the result of increased virus multiplication leading to high-dose immune paralysis (29), higher virus titers resulting from neutralization of antivirally active IFN-γ should have been obvious early in infection, which was not seen by us. Even in immunoincompetent thymusless mice, virus multiplication was not affected, which contrasts with findings of Leist et al. (29) and, more recently, of Moskophidis et al. (33), who observed increased titers in organs of infected *nu/nu* as well as ordinary mice treated with antiserum against IFN-γ. One explanation is differences of the experimental protocols. While we injected purified MAb once 24 h after virus, Leist and colleagues injected polyclonal sheep antiserum at the time of infection and repeat-

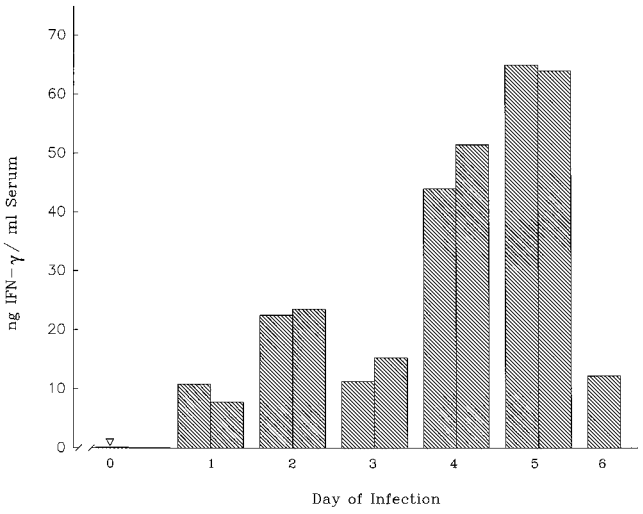

FIG. 2. IFN-γ in sera of mice during the course of LCM virus infection. BALB/c mice were injected i.v. with 10^5 mouse IU and bled on the indicated days. IFN-γ was determined by ELISA; each column represents the serum concentration in one mouse.

TABLE 4. Effect of treatment of mice with IFN-γ on multiplication of LCM virus early in infection

Tissue	Day of infection[a]	Mean ± SEM mouse IU/g of tissue in 5 mice		% Residual virus[b]	P^c
		Treated mice	Untreated mice		
Spleen	1	$(5.22 \pm 0.98) \times 10^5$	$(22.20 \pm 4.28) \times 10^5$	24	<0.01
	2	$(3.93 \pm 0.54) \times 10^7$	$(11.22 \pm 1.74) \times 10^7$	35	<0.01
	3	$(1.73 \pm 0.12) \times 10^8$	$(3.89 \pm 0.51) \times 10^8$	44	<0.01
	4	$(1.21 \pm 0.19) \times 10^8$	$(1.51 \pm 0.17) \times 10^8$	80	>0.05
Liver	1	$(3.87 \pm 0.97) \times 10^4$	$(1.04 \pm 0.32) \times 10^4$	372	<0.05
	2	$(53.32 \pm 14.93) \times 10^4$	$(2.84 \pm 0.45) \times 10^4$	1,877	<0.01
	3	$(14.89 \pm 2.13) \times 10^5$	$(0.88 \pm 0.21) \times 10^5$	1,692	<0.01
	4	$(13.03 \pm 3.13) \times 10^5$	$(0.92 \pm 0.21) \times 10^5$	1,416	<0.01

[a] BALB/c mice were infected by i.v. injection of 10^3 mouse IU. Treated mice received recombinant *E. coli*-derived IFN-γ in doses of 5×10^4 U injected subcutaneusly 24 h before virus and every 12 h thereafter until 12 h before determination of infectious titers.
[b] Percentage of infectious virus in organs after treatment.
[c] Calculated with Student's *t* test.

edly again with 24-h intervals, and Moskophidis and coworkers administered the same immune reagent 4 h before virus and every second day thereafter.

Perhaps, had we chosen a protocol similar to the one of Leist, Moskophidis, and their colleagues, we might have seen similar effects, although our data raise the question of whether IFN-γ does indeed interfere with LCM virus replication in mouse tissues. It is produced during infection (12) and appears in the serum with two peaks (this report), which coincide with activation of natural killer cells (50) and T lymphocytes (25, 49), respectively. While the latter produce IFN-γ during murine LCM virus infection (13, 49), similar information does not seem to exist for natural killer cells, which, however, have been shown to make this lymphokine under other conditions (9).

We do not know the distribution of IFN-γ throughout the mouse body nor the mode of its transfer from synthesizing to potentially virus-producing cells, but two facts are certain: in a mouse acutely infected with LCM virus, high infectious titers are attained in lymphatic tissues in which the producing cells are predominantly localized, indicating that saturating amounts of IFN-γ, or any IFN for that matter, are not set free; and, as shown in this report, multiple injections of 50,000 U of recombinant IFN-γ of eukaryotic or prokaryotic origin, beginning 24 h before virus and at 12-h intervals thereafter, resulted in decreased virus replication in spleens but increased virus replication in livers, which invalidates any conclusion as to direct antiviral effects.

It appears that the main function of endogenously synthesized IFN-γ in LCM virus-infected mice is that of an immunomodulator and that it is involved in the generation of antivirally active $CD8^+$ T lymphocytes. IFN-γ upregulates major histocompatibility complex-encoded molecules on cells in vivo (32, 44) and could thus improve antigen presentation by antigen-presenting cells; it could also act directly on $CD8^+$ T-lymphocyte precursors. Our data are not compatible with the interpretation that an augmented expression of class I molecules on LCM virus-infected cells makes them better targets for antiviral $CD8^+$ T cells, which, under other experimental conditions, has been shown to be the case (5). The findings reported previously (51) and confirmed here, that injection of IFN-γ MAb on day 1 of infection virtually abolished the generation of antiviral $CD8^+$ effector cells, while later treatment (when cell-mediated antiviral immunity begins operating) was ineffective, militates against such a possibility.

The same conclusion may be drawn from our failure to compromise the adoptive immunity in LCM virus-infected recipients by their treatment before or at the time of transfer of immune cells with IFN-γ MAb. Our results differ in this regard from what has been reported by Klavinskis and colleagues, who observed diminished antiviral efficacy of the infused immune cells by treatment of the recipients with IFN-γ MAb (21). The infectious titers that these authors measured were surprisingly low, but they used the Armstrong strain of LCM virus, which renders comparative evaluation of the data difficult.

TABLE 5. Effect of treatment of infected mice with IFN-γ MAb on the accelerated elimination of LCM virus from spleens due to infusion of day 8-immune splenocytes

Treatment[a]		Mean ± SEM mouse IU/g of spleen in 4 mice		
MAb	Cells	4 h[b]	12 h	24 h
−	−	$(6.06 \pm 0.27) \times 10^6$	$(2.47 \pm 0.27) \times 10^7$	$(3.93 \pm 0.67) \times 10^7$
−	+	$(1.04 \pm 0.14) \times 10^7$	$(3.48 \pm 1.83) \times 10^6$	$<9 \times 10^4$
+	−	$(1.37 \pm 0.18) \times 10^7$	$(1.33 \pm 0.09) \times 10^7$	$(4.55 \pm 0.48) \times 10^7$
+	+	$(5.54 \pm 1.47) \times 10^6$	$(1.62 \pm 0.73) \times 10^6$	$<9 \times 10^4$

[a] BALB/c mice were infected by i.v. injection of 10^3 mouse IU and treated 20 h later with IFN-γ MAb or left untreated. Four hours thereafter (24 h after virus infection), they were infused with 5×10^7 trypan blue-excluding splenocytes from donor mice that had been infected by i.v. inoculation of 10^3 mouse IU 8 days previously; control mice received no cells.
[b] Time after transfer at which infectious titers of spleens were determined.

TABLE 6. Effect of treatment of LCM virus-infected mice with IFN-γ MAb on antiviral serum antibodies

Day of infection[a]	MAb[b]	ELISA titer[c]		
		IgM	IgG	IgG2a
5	−	200	100	<50
	+	200	100	<50
8	−	400	800	200
	−	400	1,600	400
	+	400	1,600	200
	+	400	1,600	400
14	−	ND[d]	12,150	4,050
	−	ND	12,150	4,050
	+	ND	12,150	4,050
	+	ND	36,450	4,050

[a] BALB/c mice were infected by i.v. injection of 10^5 mouse IU.
[b] One injection of IFN-γ MAb 24 h after virus.
[c] Reciprocal of serum dilution at which optical density was half-maximal.
[d] ND, not determined.

Similar experiments with other viruses have led to variable results. The high degree of immunity against vaccinia virus in mice injected with spleen cells from immune donors was abrogated by treating the recipients with IFN-γ MAb (41), which also abolished the accelerated ability of mice to control the infection with herpes simplex virus as a result of infusion of immune $CD4^+$ or $CD8^+$ T lymphocytes (45), and the elimination of murine cytomegalovirus (MCMV) from salivary glands because of the infusion of MCMV-primed $CD4^+$ T lymphocytes (30). On the other hand, the clearing potential of transferred MCMV-primed $CD8^+$ T lymphocytes in other mouse tissues was not affected by treatment of the MCMV-infected recipients with IFN-γ MAb (30) and in immunosuppressed cytomegalovirus-infected rats, the antiviral immunity due to transfer of syngeneic immune cells was improved by treating the recipients with IFN-γ MAb (17).

Immune responses have been studied in mice with disrupted genes for IFN-γ (7) or its receptor (19). Our data are not directly comparable with data obtained with IFN-γ knockout mice, but they differ considerably from what has been reported for mice lacking the IFN-γ receptor. In the latter case, the generation of LCM virus-specific $CD8^+$ T lymphocytes was much less affected than has been seen by us (19), although one might have expected the opposite; total absence of the receptor should have been more efficacious than neutralization of the ligand. One explanation is that inactivity of IFN-γ throughout the animals' ontogeny promotes the development of compensatory mechanisms, which, perhaps for lack of time, cannot be activated when the lymphokine is needed in an emergency. The same consideration may explain why cell-mediated immune responses against influenza A virus were essentially unaltered in mice with a targeted disruption of the IFN-γ gene (15).

In a comparative study, mice lacking receptors for either IFN-γ or IFN-α/β had reduced ability to control the infection with LCM virus (36). In the absence of the IFN-α/β receptor, this coincides with lack of a measurable CTL response, but in the case of IFN-γ receptor-deficient mice, which respond to the infection with the generation of relatively potent $CD8^+$ T effector cells (19), the prolonged infectious process is not easily explained.

There remains the question of why for suppressing cell-mediated immunity against the LCM virus it is critical that the MAb be injected early and why the effect is more obvious after the injection of 10^5 than of 10^3 mouse IU; it may also be asked why responses to other immunogens are not similarly affected (29, 30). Our hypothetical answer has been given previously (51) and will be briefly repeated. In adult mice, the WE strain of LCM virus characteristically localizes and multiplies in lymphatic tissues. While this statement is generally true, it applies in particular to the protocol used here, namely, i.v. injection of 10^5 mouse IU; under these conditions, very high virus concentrations are attained as rapidly as 1 to 2 days later. Lacking direct cytopathogenicity for murine cells (reviewed in reference 28), much virus is tantamount to much immunogen, more than is likely to be present after injection of most other immunogenic substances, be they replicating or not. The high concentration of antigen is bound to activate instantaneously the majority of those T-lymphocyte precursors that are destined to respond. It is apparently at this stage that IFN-γ is needed, its functional absence preventing the generation of antivirally active T effector cells.

Most of the experiments performed and discussed above involved $CD8^+$ T lymphocytes. It appears that under similar conditions, $CD4^+$ T lymphocytes retain their activity. These cells are stringently required for the production of LCM virus antibodies (1, 34), which was not reduced by treatment of infected mice with IFN-γ-neutralizing MAb. Activation by antigen of the $CD4^+$ T helper cells may be less dependent on IFN-γ. Alternatively, autocrine effects on these cells, which probably are the lymphokine's main producers, may be less affected by the antibody. Nor was the IgG2a level affected by MAb treatment, which is noteworthy because IFN-γ is assumed to be a potent inductor for Ig subclass switching of B cells to produce IgG2a (11, 46). Similarly, in infection of IFN-γ knockout mice with influenza virus, the virus-specific IgG2a attained levels similar to those in wild-type control mice, although IgG1 was increased (15). We are aware, however, that measuring serum antibody is a crude procedure and that subtle effects might have been revealed by more sensitive methods.

ACKNOWLEDGMENTS

The excellent technical help of Heike Dralle is sincerely appreciated.

This work was financially aided by a research grant from the Deutsche Forschungsgemeinschaft. The Heinrich-Pette-Institut is supported by Freie und Hansestadt Hamburg and Bundesministerium für Gesundheit.

REFERENCES

1. **Ahmed, R., L. D. Butler, and L. Bhatti.** 1988. $T4^+$ T-helper cell function in vivo: differential requirements for induction of antiviral cytotoxic T-cell and antibody responses. J. Virol. **62:**2102–2106.
2. **Bazin, H., L.-M. Xhurdebise, G. Burtonboy, A.-M. Lebacq, L. de Clercq, and F. Cormont.** 1984. Rat monoclonal antibodies. I. Rapid purification from in vitro culture supernatants. J. Immunol. Methods **66:**261–269.
3. **Bengtson, I. A., and J. G. Wooley.** 1936. Cultivation of the virus of lymphocytic choriomeningitis in the developing chick embryo. Publ. Health Rep. **51:**29–41.
4. **Bucy, R. P., D. W. Hanto, E. Berens, and R. D. Schreiber.** 1988. Lack of an obligate role for IFN-γ in the primary in vitro mixed lymphocyte response. J. Immunol. **140:**1148–1152.
5. **Bukowski, J. F., and R. M. Welsh.** 1986. Enhanced susceptibility to cytotoxic T lymphocytes of target cells isolated from virus-infected or interferon-treated mice. J. Virol. **59:**735–739.
6. **Chen, L., B. Tourvieille, G. F. Burns, F. H. Bach, D. Mathieu-Mahul, M. Sasportes, and A. Bensussan.** 1986. Interferon: a cytotoxic T lymphocyte differentiation signal. Eur. J. Immunol. **16:**767–770.
7. **Dalton, D. K., S. Pitts-Meek, S. Keshav, I. S. Figari, A. Bradley, and T. A. Stewart.** 1993. Multiple defects of immune cell function in mice with disrupted interferon-γ genes. Science **259:**1739–1742.
8. **Dijkmans, R., H. Heremans, and A. Billiau.** 1987. Heterogeneity of Chinese hamster ovary cell-produced recombinant murine interferon-γ. J. Biol. Chem. **262:**2528–2535.
9. **Dunn, P. L., and R. J. North.** 1991. Early gamma interferon production by natural killer cells is important in defense against murine listeriosis. Infect. Immun. **59:**2892–2900.
10. **Farrar, W. L., H. M. Johnson, and J. J. Farrar.** 1981. Regulation of the production of immune interferon and cytotoxic T lymphocytes by interleukin 2. J. Immunol. **126:**1120–1125.
11. **Finkelman, F. D., I. M. Katona, T. R. Mosmann, and R. L. Coffman.** 1988. IFN-γ regulates the isotypes of Ig secreted during in vivo humoral immune responses. J. Immunol. **140:**1022–1027.
12. **Gessner, A., R. Drjupin, J. Löhler, H. Lother, and F. Lehmann-Grube.** 1990. IFN-γ production in tissues of mice during acute infection with lymphocytic choriomeningitis virus. J. Immunol. **144:**3160–3165.
13. **Gessner, A., D. Moskophidis, and F. Lehmann-Grube.** 1989. Enumeration of single IFN-γ-producing cells in mice during viral and bacterial infection. J. Immunol. **142:**1293–1298.
14. **Giovarelli, M., A. Santoni, C. Jemma, T. Musso, A. M. Giuffrida, G. Cavallo, S. Landolfo, and G. Forni.** 1988. Obligatory role of IFN-γ in induction of lymphokine-activated and T lymphocyte killer activity, but not in boosting of natural cytotoxicity. J. Immunol. **141:**2831–2836.
15. **Graham, M. B., D. K. Dalton, D. Giltinan, V. L. Braciale, T. A. Stewart, and T. J. Braciale.** 1993. Response to influenza infection in mice with a targeted disruption in the interferon γ gene. J. Exp. Med. **178:**1725–1732.
16. **Gromo, G., R. L. Geller, L. Inverardi, and F. H. Bach.** 1987. Signal requirements in the step-wise functional maturation of cytotoxic T lymphocytes. Nature (London) **327:**424–426.
17. **Haagmans, B. L., P. H. van der Meide, F. S. Stals, A. J. M. van den Eertwegh, E. Claassen, C. A. Bruggeman, M. C. Horzinek, and V. E. C. J. Schijns.** 1994. Suppression of rat cytomegalovirus replication by antibodies against gamma

interferon. J. Virol. **68:**2305–2312.
18. **Havell, E. A., and G. L. Spitalny.** 1983. Production and characterization of anti-murine interferon-gamma sera. J. Interferon Res. **3:**191–198.
19. **Huang, S., W. Hendriks, A. Althage, S. Hemmi, H. Bluethmann, R. Kamijo, J. Vilček, R. M. Zinkernagel, and M. Aguet.** 1993. Immune response in mice that lack the interferon-γ receptor. Science **259:**1742–1745.
20. **Kasaian, M. T., K. A. Leite-Morris, and C. A. Biron.** 1991. The role of $CD4^+$ cells in sustaining lymphocyte proliferation during lymphocytic choriomeningitis virus infection. J. Immunol. **146:**1955–1963.
21. **Klavinskis, L. S., R. Geckeler, and M. B. A. Oldstone.** 1989. Cytotoxic T lymphocyte control of acute lymphocytic choriomeningitis virus infection: interferon γ, but not tumour necrosis factor α, displays antiviral activity *in vivo*. J. Gen. Virol. **70:**3317–3325.
22. **Klein, J. R., and M. J. Bevan.** 1983. Secretion of immune interferon and generation of cytotoxic T cell activity in nude mice are dependent on interleukin 2: age-associated endogenous production of interleukin 2 in nude mice. J. Immunol. **130:**1780–1783.
23. **Ledbetter, J. A., and L. A. Herzenberg.** 1979. Xenogeneic monoclonal antibodies to mouse lymphoid differentiation antigens. Immunol. Rev. **47:**63–90.
24. **Lehmann-Grube, F.** 1971. Lymphocytic choriomeningitis virus. Virol. Monogr. **10:**57–59.
25. **Lehmann-Grube, F., U. Assmann, C. Löliger, D. Moskophidis, and J. Löhler.** 1985. Mechanism of recovery from acute virus infection. I. Role of T lymphocytes in the clearance of lymphocytic choriomeningitis virus from spleens of mice. J. Immunol. **134:**608–615.
26. **Lehmann-Grube, F., J. Cihak, M. Varho, and R. Tijerina.** 1982. The immune response of the mouse to lymphocytic choriomeningitis virus. II. Active suppression of cell-mediated immunity by infection with high virus doses. J. Gen. Virol. **58:**223–235.
27. **Lehmann-Grube, F., I. Krenz, T. Krahnert, R. Schwachenwald, D. Moskophidis, J. Löhler, and C. J. Villeda Posada.** 1987. Mechanism of recovery from acute virus infection. IV. Questionable role of mononuclear phagocytes in the clearance of lymphocytic choriomeningitis virus from spleens of mice. J. Immunol. **138:**2282–2289.
28. **Lehmann-Grube, F., L. Martínez Peralta, M. Bruns, and J. Löhler.** 1983. Persistent infection of mice with the lymphocytic choriomeningitis virus. Compr. Virol. **10:**43–103.
29. **Leist, T. P., M. Eppler, and R. M. Zinkernagel.** 1989. Enhanced virus replication and inhibition of lymphocytic choriomeningitis virus disease in anti-gamma interferon-treated mice. J. Virol. **63:**2813–2819.
30. **Lučin, P., I. Pavić, B. Polić, S. Jonjić, and U. H. Koszinowski.** 1992. Gamma interferon-dependent clearance of cytomegalovirus infection in salivary glands. J. Virol. **66:**1977–1984.
31. **Maraskovsky, E., W.-F. Chen, and K. Shortman.** 1989. IL-2 and IFN-γ are two necessary lymphokines in the development of cytolytic T cells. J. Immunol. **143:**1210–1214.
32. **Momburg, F., N. Koch, P. Möller, G. Moldenhauer, and G. J. Hämmerling.** 1986. *In vivo* induction of H-2K/D antigens by recombinant interferon-γ. Eur. J. Immunol. **16:**551–557.
33. **Moskophidis, D., M. Battegay, M.-A. Bruendler, E. Laine, I. Gresser, and R. M. Zinkernagel.** 1994. Resistance of lymphocytic choriomeningitis virus to alpha/beta interferon and to gamma interferon. J. Virol. **68:**1951–1955.
34. **Moskophidis, D., S. P. Cobbold, H. Waldmann, and F. Lehmann-Grube.** 1987. Mechanism of recovery from acute virus infection: treatment of lymphocytic choriomeningitis virus-infected mice with monoclonal antibodies reveals that Lyt-2^+ T lymphocytes mediate clearance of virus and regulate the antiviral antibody response. J. Virol. **61:**1867–1874.
35. **Moskophidis, D., and F. Lehmann-Grube.** 1984. The immune response of the mouse to lymphocytic choriomeningitis virus. IV. Enumeration of antibody-producing cells in spleens during acute and persistent infection. J. Immunol. **133:**3366–3370.
36. **Müller, U., U. Steinhoff, L. F. L. Reis, S. Hemmi, J. Pavlovic, R. M. Zinkernagel, and M. Aguet.** 1994. Functional role of type I and type II interferons in antiviral defense. Science **264:**1918–1921.
37. **Novelli, F., M. Giovarelli, R. Reber-Liske, G. Virgallita, G. Garotta, and G. Forni.** 1991. Blockade of physiologically secreted IFN-γ inhibits human T lymphocyte and natural killer cell activation. J. Immunol. **147:**1445–1452.
38. **Pierres, A., P. Naquet, A. van Agthoven, F. Bekkhoucha, F. Denizot, Z. Mishal, A.-M. Schmitt-Verhulst, and M. Pierres.** 1984. A rat anti-mouse T4 monoclonal antibody (H129.19) inhibits the proliferation of Ia-reactive T cell clones and delineates two phenotypically distinct ($T4^+$, Lyt-$2,3^-$, and $T4^-$, Lyt-$2,3^+$) subsets among anti-Ia cytolytic T cell clones. J. Immunol. **132:**2775–2782.
39. **Rivers, T. M., and T. F. M. Scott.** 1935. Meningitis in man caused by a filterable virus. Science **81:**439–440.
40. **Rubinstein, S., P. C. Familletti, and S. Pestka.** 1981. Convenient assay for interferons. J. Virol. **37:**755–758.
41. **Ruby, J., and I. Ramshaw.** 1991. The antiviral activity of immune $CD8^+$ T cells is dependent on interferon-γ. Lymphokine Cytokine Res. **10:**353–358.
42. **Siegel, J. P.** 1988. Effects of interferon-γ on the activation of human T lymphocytes. Cell. Immunol. **111:**461–472.
43. **Simon, M. M., S. Landolfo, T. Diamantstein, and U. Hochgeschwender.** 1986. Antigen- and lectin-sensitized murine cytolytic T lymphocyte-precursors require both interleukin 2 and endogenously produced immune (γ) interferon for their growth and differentiation into effector cells. Curr. Top. Microbiol. Immunol. **126:**173–185.
44. **Skoskiewicz, M. J., R. B. Colvin, E. E. Schneeberger, and P. S. Russel.** 1985. Widespread and selective induction of major histocompatibility complex-determined antigens in vivo by γ interferon. J. Exp. Med. **162:**1645–1664.
45. **Smith, P. M., R. M. Wolcott, R. Chervenak, and S. R. Jennings.** 1994. Control of acute cutaneous herpes simplex virus infection: T cell-mediated viral clearance is dependent upon interferon-γ (IFN-γ). Virology **202:**76–88.
46. **Snapper, C. M., and W. E. Paul.** 1987. Interferon-γ and B cell stimulatory factor-1 reciprocially regulate Ig isotype production. Science **236:**944–947.
47. **Spitalny, G. L., and E. A. Havell.** 1984. Monoclonal antibody to murine gamma interferon inhibits lymphokine-induced antiviral and macrophage tumoricidal activities. J. Exp. Med. **159:**1560–1565.
48. **Stuhler, G., and P. Walden.** 1993. Collaboration of helper and cytotoxic T lymphocytes. Eur. J. Immunol. **23:**2279–2286.
49. **Utermöhlen, O., A. Tárnok, L. Bönig, and F. Lehmann-Grube.** 1994. T lymphocyte-mediated antiviral immune responses in mice are diminished by treatment with monoclonal antibody directed against the interleukin-2 receptor. Eur. J. Immunol. **24:**3093–3099.
50. **Welsh, R. M.** 1987. Regulation and role of large granular lymphocytes in arenavirus infections. Curr. Top. Microbiol. Immunol. **134:**185–209.
51. **Wille, A., A. Gessner, H. Lother, and F. Lehmann-Grube.** 1989. Mechanism of recovery from acute virus infection. VIII. Treatment of lymphocytic choriomeningitis virus-infected mice with anti-interferon-γ monoclonal antibody blocks generation of virus-specific cytotoxic T lymphocytes and virus elimination. Eur. J. Immunol. **19:**1283–1288.
52. **Zanovello, P., E. Vallerani, G. Biasi, S. Landolfo, and D. Collavo.** 1988. Monoclonal antibody against IFN-γ inhibits Moloney murine sarcoma virus-specific cytotoxic T lymphocyte differentiation. J. Immunol. **140:**1341–1344.