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Gamma Interferon Is a Major Mediator of Antiviral Defense in Experimental Measles Virus-Induced Encephalitis

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Measles virus infection of the central nervous system in the murine model of experimental measles virus-induced encephalitis is successfully controlled by virus-specific T-helper lymphocytes. T cells from BALB/c mice that are resistant to measles virus encephalitis proliferate well against measles virus in vitro, and bulk cultures recognize viral nucleocapsid and hemagglutinin as well as fusion proteins. The measles virus-specific T cells secrete large amounts of interleukin 2 (IL-2), gamma interferon (IFN-γ), and tumor necrosis factor alpha (TNF-α) but no IL-4, IL-6, or IL-10, and hence the cytokine pattern is consistent with that of subtype 1 T-helper lymphocytes. In contrast, cells obtained from measles virus-infected susceptible C3H mice recognize measles virus proteins only weakly and secrete little IFN-γ and TNF-α. Treatment of infected mice with anti-TNF-α antibodies has no effect on survival or virus clearance from the brain. Upon neutralization of IFN-γ in vivo, the phenotype of measles virus-specific T-helper cells isolatable from BALB/c mice is reversed from subtype 1 to subtype 2-like. Anti-IFN-γ antibody-treated BALB/c mice are susceptible to measles virus encephalitis, and viral clearance from the central nervous system is impaired. These results indicate that IFN-γ plays a significant role in the control of measles virus infection of the central nervous system.

Measles is usually a relatively harmless disease which is overcome without problems or severe sequelae in most cases. However, when the virus gains access to the central nervous system (CNS), dreaded complications in the form of measles inclusion body encephalitis and subacute sclerosing panencephalitis may develop as a result of persistent measles virus (MV) infection of brain cells. Clinical and experimental observations indicate that T-cell-mediated immunity is critical in determining the outcome of MV infections in both humans and experimentally infected rodents. Individuals with depressed T-cell function are prone to severe complications like giant-cell pneumonia and otitis media (28), whereas patients with hypogammaglobulinemia control MV infections normally (7, 10). Both $CD4^+$ and $CD8^+$ T-cell clones that recognize MV in the context of class II or class I major histocompatibility complex (MHC) molecules, respectively, have been isolated from several patients recovering from acute measles (41, 42). Interindividual differences in the major viral antigens recognized were found, and even within the same individual, when tested at different time points, the reactivity to viral antigens was highly variable (32). Moreover, which cell type, $CD4^+$ or $CD8^+$, is the prime effector cell responsible for virus elimination after natural infection still remains unclear.

To determine the protective role of the cell-mediated immune response in the CNS we have analyzed the effect of virus-specific T cells in the rodent model of experimental measles virus-induced encephalitis. After intracerebral infection with the neurotropic MV strain CAM/RB, C3H (*H-2*^{k}) mice develop a lethal acute encephalitis, while BALB/c (*H-2*^{d}) mice are resistant. In the latter, the acute infection can be overcome in the absence of $CD8^+$ T lymphocytes. $CD4^+$ T lymphocytes are, however, necessary for recovery, and following their depletion BALB/c mice become susceptible to MV infection with high mortality rates (9, 29).

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In the present study, the functional characteristics of murine MV-specific $CD4^+$ T cells with regard to virus protein specificity and cytokine secretion pattern were analyzed, and the role of cytokines in protection from experimental MV infection of mice was studied in vivo.

MATERIALS AND METHODS

Animals, virus, and infection. Inbred BALB/cJ (*H-2*^{d}) and C3H/He (*H-2*^{k}) mice were obtained from the Zentralinstitut für Versuchstierzucht, Hannover, Germany. For induction of encephalitis, 6- to 7-week-old mice were infected intracerebrally with the rodent-adapted neurotropic MV strain CAM/RB as previously described (standard dose, 10^4 50% tissue culture infective doses [$TCID_{50}$] in 25 μl) (19, 20). In separate experiments, mice were immunized intraperitoneally with the Edmonston strain of MV (10^7 PFU) (9). To determine the distribution of $CD4^+$ and $CD8^+$ T-cell subsets in mice, splenic T lymphocytes were incubated simultaneously with fluorescein isothiocyanate-conjugated anti-L3T4 monoclonal antibodies (MAb; CD4 specific), phycoerythrin-conjugated anti-Lyt-2 MAb (CD8 specific), and biotinylated anti-murine Thy-1.2 MAb (Pharmingen); this was followed by incubation with streptavidin-cytochrome (Dianova, Hamburg, Germany). The number of cells detected was determined by flow cytofluorometry (FACScan; Becton Dickinson) as previously described (9). For analysis of the neuropathological correlate to clinical disease, the animals were killed at variable intervals postinfection (p.i.). Brains and spinal cords were fixed in buffered paraformaldehyde. Sections cut from paraffin blocks were stained with hematoxylin and eosin. For the detection of infectious virus from brain tissue, Vero cells were cocultivated with fresh brain material or incubated with diluted brain homogenate as described previously (20).

$CD4^+$ T lymphocytes. MV-specific $CD4^+$ T lymphocytes were generated from animals 10 to 14 days after infection with MV or vaccinia virus recombinants as described recently (9). Six to nine days after two in vitro stimulations with UV-inactivated sucrose gradient-purified MV (UV-MV) (33), the phenotype of the cell cultures was predominantly $CD4^+$. The rate of lymphocyte proliferation (LPR) was determined by incorporation of [^{3}H]thymidine (0.5 μCi; Amersham) into cellular DNA and expressed as a stimulation index (SI), which was calculated from the ratio of [^{3}H]thymidine incorporation in the presence of MV or control proteins and in their absence. Values greater than 2 indicate specific antigen recognition. The following antigen preparations were used in concentrations of 1 to 10 μg/ml/2×10^5 cells: UV-MV; nucleocapsid (N), phosphoprotein (P), and matrix (M) proteins expressed as bacterial fusion proteins, using the pBD2-type expression vector; hemagglutinin (H) and fusion (F) protein purified from measles virus virions by affinity chromatography (33). In some LPR experiments, N and H proteins bound to nitrocellulose (NC) were added in a microparticulate form in different dilutions (50, 5, and 0.5 μl/ml) to T-cell cultures. The material was prepared from sodium dodecyl sulfate-polyacrylamide gel electrophoresis-separated MV proteins which were transferred to NC membranes as previously described (1). NC sections containing appropriate individual MV

proteins were cut out and dissolved in dimethyl sulfoxide (1 h, 37°C). After treatment with bicarbonate buffer (5.6%), the resultant precipitate was diluted in 3 ml of RPMI medium containing 1% fetal calf serum, aliquoted, and stored at −20°C. As a negative control in LPR assays, NC particles without antigen, protein extracted from the pBD2-type expression vector-transfected bacteria, Vero cell lysate, or medium alone was always tested in parallel. For assaying NK cell-mediated cytotoxicity, spleen cells from MV-immunized mice were tested in a 5-h chromium release assay, with YAC cells as targets.

Cytokine assays. Supernatants of $CD4^+$ T-cell cultures were harvested 24 to 72 h after the second in vitro stimulation with UV-MV. Interleukin 2 (IL-2) was assayed by its ability to promote the growth of the IL-2-dependent murine T-cell line CTLL-2 in the presence and in the absence of anti-IL-4 antibody (clone 11B11, kindly provided by A. Schimpl, Würzburg, Germany). Diluted T-cell culture supernatant was added to CTLL (5×10^4/ml) in a final volume of 200 μl. After incubation for 24 h, [^{3}H]thymidine incorporation into CTLL-2 was determined. IL-4, IL-6, and IL-10, as well as gamma interferon (IFN-γ), were determined by enzyme-linked immunosorbent assay (ELISA) according to instructions and with standards provided by the manufacturers (Cell Systems, Remagen, Germany; Dianova; and PerSeptive Diagnostics, Cambridge, Mass.). A highly sensitive bioassay, in which T-cell culture supernatant was added in geometric dilutions to cultured L929 fibroblasts preincubated in 2 μg of actinomycin D per ml, was used to quantitate tumor necrosis factor (TNF) activity (36). Cytotoxicity was determined colorimetrically by the uptake of crystal violet. A standard curve was established, using recombinant murine TNF-α.

Treatment of mice with MAb in vivo. Six- to seven-week-old mice were depleted of $CD4^+$ T cells by intraperitoneal inoculation of MAb as described previously (9). In some experiments, mice were treated with MAb by intraperitoneal injection of 1×10^4 neutralizing units of either anti-murine IFN-γ antibody (rat immunoglobulin G, subclass 1 [IgG1]; hybridoma R4.6A2 or AN18) or anti-murine TNF-α antibody (clone V1Q, ammonium sulfate precipitates of ascites, kindly provided by S. Jonjic, Rijeka, Croatia). Administration of MAb was started 12 h before intracerebral CAM/RB infection and repeated every 2 days thereafter. Control mice were injected with irrelevant rat immunoglobulin (Dianova). The neutralization titer of anti-murine IFN-γ antibodies was assessed by a plaque reduction assay as previously described (13). Briefly, equal volumes of recombinant murine IFN-γ (5 IU/ml) were incubated with serially diluted R4.6A2 IgG or AN18 IgG (24 h, 37°C) before L929B cells (1.8×10^5/ml) and vesicular stomatitis virus (multiplicity of infection of 0.1) were added. At 24 h, the cultures were examined for neutralization of vesicular stomatitis virus infectivity, i.e., prevention of cell destruction. The neutralization titer was defined as the reciprocal of the highest antibody dilution that reduced the protective effect of 5 IU of recombinant IFN-γ per ml by more than 50%. The neutralizing activity of anti-TNF-α antibody was determined by its ability to prevent the cytopathic effect of recombinant TNF-α on L929 cells (43). Following incubation of TNF-α with various dilutions of anti-TNF-α antibody (1 h, 4°C), 100 μl of each sample was added to L929 cells in the presence of 4 μg of actinomycin D per ml. After overnight incubation at 37°C, the TNF-mediated cytopathic effect on L929 cells was visualized by crystal violet staining. The neutralization capacity of anti-TNF-α antibody was defined as the reciprocal of the highest dilution that neutralizes 50% of the cytotoxic activity of 5-IU/ml recombinant TNF-α.

RESULTS

MV protein specificity and cytokine secretion. Splenic T-cell lines obtained from MV-infected mice were phenotyped by fluorescence-activated cell sorter analysis after two in vitro restimulation cycles with inactivated MV antigen. In a typical experiment, more than 92% of cells were $CD4^+/CD8^-$ and ≤2% were $CD4^-/CD8^+$. In both resistant BALB/c as well as susceptible C3H mice, a virus-specific lymphoproliferative response was found (Fig. 1). The major $CD4^+$ T-cell reactivity in BALB/c mice was directed to H, F, and N proteins (SIs, 6.8, 5.2, and 3.7, respectively). Additionally, P and M proteins were recognized specifically with low SIs of 2.6 and 2.5, respectively. T cells from the susceptible C3H strain clearly proliferated less well in the presence of MV antigens. Only F (SI, 2.3) and M (SI, 2.2) proteins were weakly recognized. No increase in LPR response was observed after subsequent further antigen-specific restimulations, and there was no LPR response in the presence of non-MV control proteins. The pattern of cytokines released from the $CD4^+$ T cells into tissue culture supernatant was determined (Fig. 2). While BALB/c-derived T cells produced large amounts of IL-2, IFN-γ, and TNF, these cytokines were present in small quantities in the supernatant of C3H T cells. Interestingly, the levels of IL-6 and IL-10 detected in C3H T cells were much higher than in BALB/c. No clear evidence for production of IL-4 in either strain was found by ELISA.

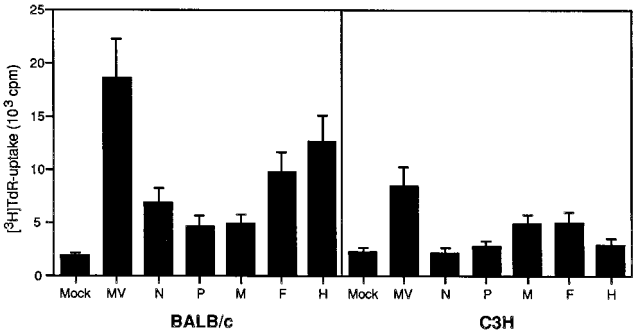

FIG. 1. Protein specificity of $CD4^+$ T cells derived from spleens of MV-infected mice. MV-primed T cells from BALB/c (left) or C3H (right) mice were stimulated twice in vitro with UV-MV. The protein specificity was determined by using MV; recombinant N, P, or M; or virion-purified F or H protein as previously described (33). Antigen preparation from uninfected Vero cells or lysate from *E. coli* expressing β-galactosidase but no MV sequences served as negative controls (mock). The columns represent the mean values from experiments carried out with 10 different mice of either strain. [^{3}H]TdR, [^{3}H]thymidine.

Effect of antibodies against IFN-γ and TNF-α. The influence of IFN-γ or TNF-α on the course of MV encephalitis in BALB/c mice, which are resistant to acute encephalitis and develop a high virus-specific $CD4^+$ T-cell immune response, was analyzed. For this purpose, IFN-γ- or TNF-α-neutralizing antibodies were intraperitoneally injected into MV-infected mice. The effect of this treatment on the yield of splenic nucleated cells as well as the frequency of T-lymphocyte subpopulations was determined after 6 days by flow cytofluorometry. No difference in the total number or in the proportion of $CD4^+$ and $CD8^+$ T-cell subsets was seen when mice treated with anti-IFN-γ antibody or with an irrelevant rat antibody were compared (Table 1). Similar results were obtained following treatment with anti-TNF-α antibody. Groups of 10 to 14 BALB/c mice were treated with antibodies to either IFN-γ, TNF-α, or CD4 prior to intracerebral infection with CAM/RB. Mortality and morbidity were monitored for a period of 4 weeks. In contrast to anti-TNF-α antibody treatment, which had no effect, both depletion of $CD4^+$ T cells and neutralization of endogenous IFN-γ had a profound influence on morbidity and mortality (Fig. 3). Beginning at day 5 p.i., anti-IFN-γ antibody-treated mice developed signs of disease, and 93% were dead by day 14. Depletion of $CD4^+$ T cells resulted ultimately in a similar mortality rate, but onset of disease was delayed on average by 2 days. BALB/c mice treated with the control antibody showed no clinical or histological signs of encephalitis, and infectious MV, which is already eliminated within 4 to 5 days p.i., could not be isolated from the CNS (9c). In contrast, infectious virus was readily isolatable from the brains of anti-IFN-γ antibody-treated mice. A neuropathological investigation revealed disseminated encephalitis, with inflammatory and degenerative lesions involving the entire brain. Injection of anti-TNF-α antibodies did not abrogate the resistance of BALB/c mice to MV encephalitis (Table 2). In this group, only 2 of 10 animals exhibited temporarily mild symptoms indicative of CNS infection which resolved within 2 days. No inflammatory changes or destruction of brain parenchyma except gliotic scar formation in the immediate vicinity of the injection site were detected histopathologically. Attempts at virus isolation were uniformly negative in anti-TNF-α antibody-treated mice.

In order to investigate if weak IFN-γ production can be

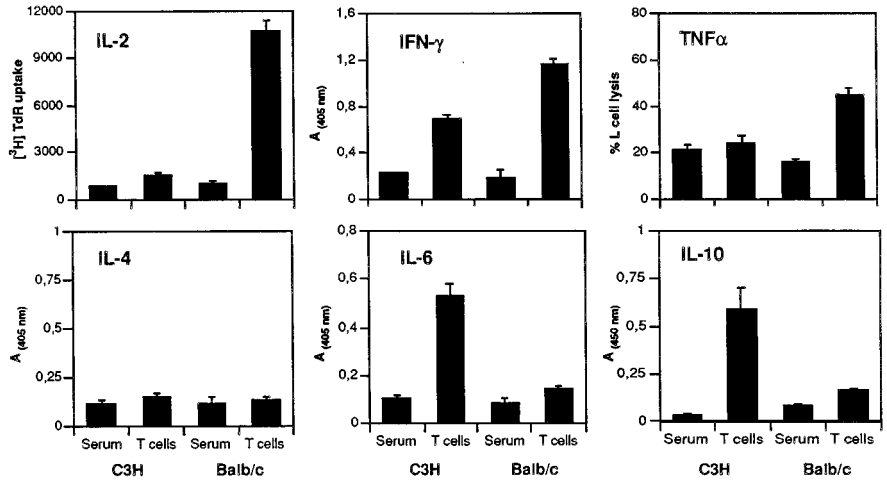

FIG. 2. Profiles of cytokines secreted from MV-specific $CD4^+$ T cells. Cytokine activities were determined by ELISA (IFN-γ, IL-6, IL-4, and IL-10) or bioassays (TNF-α and IL-2) from supernatants of T cells stimulated with UV-MV. Pooled sera from uninfected mice served as controls. [^{3}H]TdR, [^{3}H]thymidine.

directly implicated as the basis for susceptibility, C3H mice were infected with 10^4 $TCID_{50}$ (standard dose) or 10 $TCID_{50}$ (low dose) of CAM/RB MV with or without anti-IFN-γ antibody treatment (Table 3). While all mice infected with the standard dose died within 8 days regardless of the treatment, mice infected with 100-fold-less virus were largely resistant. Treatment with anti-IFN-γ or anti-CD4 antibodies, however, resulted in the usual susceptible phenotype. Onset of disease and mean survival were similar when the groups of anti-IFN-γ antibody-treated and anti-CD4 antibody-treated mice were compared. Additionally, the question of how long after infection administration of antibodies which neutralize IFN-γ is able to change the phenotype of mice from resistance to susceptibility was addressed. For this purpose, BALB/c mice were infected with the standard dose of CAM/RB, and at several time points before and after infection, treatment with anti-IFN-γ antibody was started. The results clearly demonstrate that a full effect of the antibody administration was seen only when anti-IFN-γ antibody was given before infection. At already 24 h after infection, susceptibility was reduced to 70%, and no effect could be detected when anti-IFN-γ antibody treatment was started 5 days after infection (Table 4). Consistently, incubation periods increased and attempts to isolate infectious virus from the CNS failed.

Effect of in vivo anti-IFN-γ antibody treatment on antibody response, NK cell activity, and cytokine secretion. The humoral MV-specific immune response was tested by ELISA in sera from mice treated with either control or anti-IFN-γ rat antibody 14 days after infection. Consistently higher levels of antiviral antibody were found in anti-IFN-γ antibody-treated mice than in untreated control mice (mean change in optical density at identical serum dilution, 0.3), probably indicating that in vivo neutralization of IFN-γ amplifies antibody secretion (data not shown). The influence of IFN-γ deficiency at early stages of MV infection on NK cell activity was determined, using YAC cells as targets (Fig. 4). While no NK cell-mediated lysis was detected in uninfected mice, a peak activity was seen 3 days after MV infection. No difference in kinetics or in quantity of cytolysis was seen in IFN-γ-deficient and normal mice (maximum cytolysis, 24 and 19%, respectively). The cytokine profile in $CD4^+$ T-cell cultures obtained from anti-IFN-γ antibody-treated BALB/c mice was determined (Fig. 5). Seven days after MV infection, T cells produced IL-4, IL-6, and IL-10 but produced only very little IL-2 and no IFN-γ when stimulated in vitro with inactivated MV. In contrast, T cells obtained from non-IFN-γ-deficient BALB/c mice released, under identical conditions, large quantities of IFN-γ and IL-2 but showed no detectable IL-10, IL-4, or IL-6 activity. Control $CD4^+$ T cells from uninfected mice revealed a similar cytokine pattern in the presence of mitogen, with large or moderate amounts of IL-4 and IL-6, respectively, in IFN-γ-deficient mice and high levels of IL-2 activity in normal mice. Attempts to generate T cells from noninfected mice, using UV-MV for in vitro stimulation, were unsuccessful.

TABLE 1. Spleen cell composition after MV infection[a]

Treatment of mice	No. of spleen cells obtained (10^7)	% Cells positive for:		
		CD3	CD4	CD8
Control rat IgG	6.4	29.1	11.9	42.4
Anti-IFN-γ antibody	6.1	28.8	11.4	43.6

[a] All mice were infected with MV intracerebrally. Cell count and frequency of phenotypic lymphocyte subtypes were determined 6 days after single dose injection of anti-IFN-γ antibody or irrelevant rat IgG.

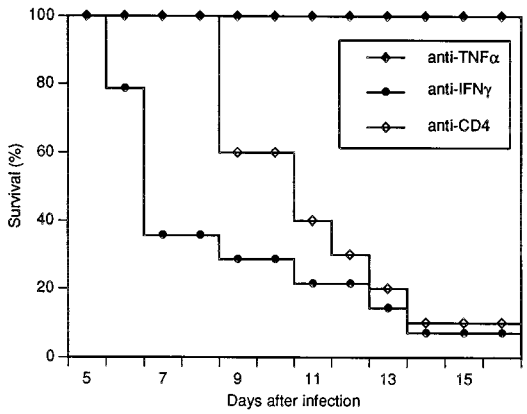

FIG. 3. Comparative survival of MV-infected BALB/c mice. Mice were either depleted of $CD4^+$ T cells by treatment with anti-CD4 antibodies (n = 10), treated with anti-IFN-γ antibodies (n = 14), or treated with anti-TNF-α antibodies (n = 10).

TABLE 2. Effect of anti-TNF-α or anti-IFN-γ antibody treatment on MV encephalitis[a]

MAb treatment	No. of surviving mice/total	No. of mice with histopathological lesions/total	Virus isolation (no. of mice positive/total)[b]
None	9/10	1/10	1/10
Anti-CD4	1/10	10/10	10/10
Anti-TNF-α	10/10	0/10	0/10
Anti-IFN-γ	1/14	13/14	13/14

[a] Histological examination and attempts at virus isolation were done either when CAM/RB-infected BALB/c mice were moribund and died or at 14 days p.i.
[b] From CNS material.

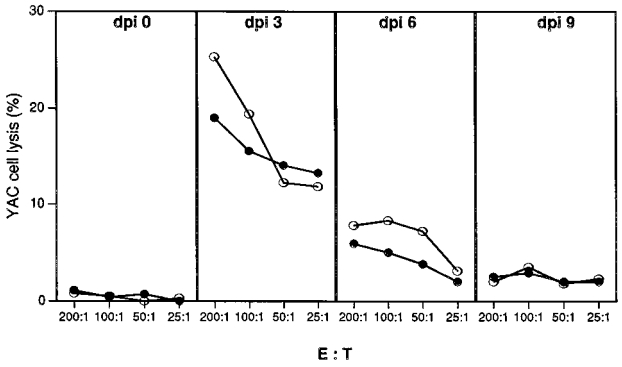

FIG. 4. NK cell activity in infected mice following treatment with anti-IFN-γ antibodies. YAC cells labelled with ^{51}Cr were incubated for 5 h with spleen cells from normal or IFN-γ-deficient BALB/c mice at different ratios from 25:1 to 200:1. The relative specific lysis was determined at different intervals after MV infection. —●—, infected, mock treated; —○—, infected, anti-IFN-γ treated. E:T, effector-to-target cell ratio.

DISCUSSION

Investigations using diverse animal infection models, including lymphocytic choriomeningitis virus, Friend virus, and respiratory syncytial virus models, have documented clearly that the capacity to generate an antiviral cellular immune response is MHC linked and genetically controlled (24, 30). Additionally, the recognition of particular dominant proteins or peptides is essential for protective immune responses. For example, the M2 protein of respiratory syncytial virus is a protective antigen in *H-2*^{d} mice but not in *H-2*^{b} or *H-2*^{k} mice (17). In experimental MV infection of mice, priming of $CD8^+$ T cells with high levels of N-specific L^d-restricted cytotoxic-T-lymphocyte (CTL) activity was detected in resistant BALB/c mice, but only very minor CTL activity was detected in the susceptible C3H strain (29). The poor response is virus specific, as in other infection models, for example, poliovirus infections, C3H mice are able to generate better in vitro T-cell responses than BALB/c mice (18). Despite the observed correlation between the ability to generate a high MV-specific CTL response in vitro and resistance against intracerebral MV infection, $CD8^+$ CTLs are dispensable and the crucial role in the murine defense against MV is played by $CD4^+$ T cells (9). In the present paper we show that $CD4^+$ T cells derived from resistant BALB/c mice proliferate well when stimulated in vitro with MV N, H, or F protein, while in MV-infected C3H/He mice only poor $CD4^+$ T-cell proliferation with weak recognition of M and P proteins was identified. The same MV proteins that are recognized in BALB/c mice induce a protective immune response in susceptible Lewis rats, where priming with vaccinia virus recombinants expressing N, H, or F protein prevented the occurrence of disease (3, 6). Similar results were obtained with C3H mice (9b). Our data suggest that recognition of MV N, H, and F proteins by $CD4^+$ T cells is important for an efficient immune response against MV.

Activated $CD4^+$ T cells are key regulators of both antibody- and cell-mediated immune responses. There is growing evidence that cytokines delivered locally by $CD4^+$ T cells can contribute to selective virus elimination from the CNS either by direct interaction with infected brain cells; by activation of nonspecific effector cells, e.g., phagocytes; or by regulation of T-cell differentiation (5, 21, 38, 45). In the present study, T cells from resistant BALB/c mice were shown to produce IL-2, IFN-γ, and TNF and no IL-4, IL-6, or IL-10. This cytokine pattern corresponds to the profile attributed to helper cells of type 1 (TH1) (26). In contrast, MV-specific $CD4^+$ T cells from susceptible C3H mice are consistent with the TH2-like subtype. To investigate the hypothesis that cytokines secreted locally from T cells are involved in resistance of mice against MV encephalitis (45), endogenous TNF-α was neutralized by injection of specific antibodies. Although in other virus systems TNF-α is an integral part of the protective mechanism against infection (31, 35), in MV encephalitis, deficiency of TNF-α had no influence on MV clearance from the brain. Ablation of IFN-γ, however, completely suppressed the inherent protective effect of a functioning cellular immune system and converted the resistance of BALB/c mice into high susceptibility. Interestingly, elimination of IFN-γ is even more damaging than elimination of TH1 cells, and the mice died several days earlier than the CD4-depleted mice. Although $CD4^+$ T cells are of prime importance because ablation of $CD4^+$ T cells renders resistant animals susceptible, the observation indicates that the anti-IFN-γ antibody is more efficient. In order to shed some light on the question of whether only the action of IFN-γ is required rather than the induction of TH1 cells, intrathecal administration of IFN-γ into C3H mice has to be carried out.

Our observation also suggests a role for additional IFN-γ

TABLE 3. Effect of treatment with anti-CD4 or anti-IFN-γ antibodies in MV-infected C3H mice[a]

MV infection (dose)	MAb treatment	No. of sick mice/total	Survival period (days) (mean)	Virus isolation (no. of mice positive/total)[b]
10^4 $TCID_{50}$	None	5/5	6, 7, 7, 7, 8 (7.0)	5/5
	Anti-IFN-γ	5/5	5, 5, 5, 6, 8 (5.8)	5/5
	Anti-CD4	4/4	6, 6, 6, 7 (6.3)	ND
10 $TCID_{50}$	None	1/10	9 (9.0)	2/10
	Anti-IFN-γ	7/8	6, 6, 7, 7, 7, 8, 9 (7.1)	7/8
	Anti-CD4	5/5	6, 7, 8, 8, 9 (7.6)	ND

[a] C3H mice were infected with either 10 or 10^4 $TCID_{50}$ with or without anti-IFN-γ antibody treatment. The experiment was terminated 14 days p.i.
[b] By cocultivation. ND, not determined.

TABLE 4. Interdependence of anti-IFN-γ antibody treatment and susceptibility of BALB/c mice to MV encephalitis

Initiation of treatment[a]	No. of susceptible mice/total (%)	Survival time (days) (mean)	Virus isolation (no. of mice positive/total)[b]
−24	6/6 (100)	6, 6, 8, 9, 10, 13 (8.7)	6/6
+24	7/10 (70)	6, 7, 9, 11, 11, 12, 13 (9.9)	8/10[c]
+72	2/10 (20)	9, 16 (12.5)	2/10
+120	0/10 (0)	NA[d]	0/10
Untreated	0/6 (0)	NA	0/6

[a] In hours; anti-IFN-γ antibody treatment was initiated before (−) or after (+) MV infection.
[b] Cocultivation was done at time of death. In survivors, attempts to isolate virus from brain tissue were carried out on day 18 p.i.
[c] A positive result was obtained for only one survivor.
[d] NA, not available (experiment was terminated 18 days after infection).

producers that contribute to the antiviral effector functions. Besides $CD8^+$ T cells that have been shown, at least if cloned, to be able to produce IFN-γ, a possible source for IFN-γ during the early stages of infection is NK cells that were shown to be responsible in certain circumstances for host resistance against bacterial as well as some viral infections (8, 14). In IFN-γ-deficient mice, however, the cytotoxic NK cell function is unchanged. Despite this, the possibility that NK cells and early IFN-γ production could be involved in the murine MV model cannot be excluded.

The importance of IFN-γ in virus clearance from infected tissue has also been demonstrated in infection models with pox virus, herpesvirus, and myxovirus (15, 16, 22, 40). Conceivably, the mechanism of IFN-γ action in viral clearance involves upregulation of MHC class II expression on astrocytes and amplification of their ability to act as antigen-presenting cells in the neighborhood of infected neurons (46). Moreover, IFN-γ enhances the expression of VCAM-1 on brain endothelial cells to which stimulated T cells bind before entering the brain, where they encounter viral antigen and secrete cytokines (4). In this context, the multiple immunoregulatory functions of IFN-γ, particularly in activation and differentiation of cytotoxic helper T cells, have to be considered (for a review, see reference 25).

While the effect of IFN-γ on $CD8^+$ T cells is obviously unimportant, because they play only a minor part or no part at all in the recovery from experimental MV infection (9), the preferential induction of the protective helper lymphocytes with a TH1 cytokine profile constitutes an important parameter in the pathogenesis of murine MV encephalitis. The generation of a TH1 response is essential in protection against viruses, fungi, and intracellular parasites (34, 37, 39). For example, transfer of specific TH1 cells can protect mice infected with influenza virus against lethal pneumonia, whereas transfer of TH2 cells fails to exert a protective effect (11). It has been suggested that, in human immunodeficiency virus type 1 infection, the switch of cytolytic T cells with a TH1-like cytokine secretion profile to cells that make TH2-type cytokines may contribute to the reduced defense against viral infections and intracellular parasites (23). Moreover, the lack of virus-specific TH1 cells and predominant generation of TH2 cells, which are seen after both natural MV infection and vaccination against measles in men, may contribute to MV-induced immunosuppression (12, 44).

In murine respiratory syncytial virus infection, the transfer of surface glycoprotein-specific TH2-like cells induces a severe illness, while F protein-specific TH1-like cells cause only minimal enhancement of pulmonary pathology (2). In the murine model, simultaneous with the change in the profile of cytokines released from MV-specific T-helper cells, IFN-γ-deficient BALB/c mice become susceptible to MV infection and the susceptible C3H mice generate predominantly TH2-like cells. Consequently, anti-MV antibody levels were higher in C3H than in BALB/c mice, and the $CD4^+$ T cells from BALB/c mice lysed 56% of antigen presenters in an MHC class II-restricted fashion, while helper T cells from C3H exerted no CTL activity (9a). Since proliferation, protein specificity, and cytokine secretion of $CD4^+$ T cells after infection differ between inbred mouse strains, it is concluded that MV infection is not followed by a uniform type of T-cell response and that this response depends on MHC haplotype, antigen affinity to certain MHC molecules, and the type of effector response. For example, human collagen type IV primes either TH1 or TH2 cells, depending on the MHC class II genotype of the responding mice (27).

The studies with the murine model of MV encephalitis presented here suggest that helper T cells with the TH2-type cytokine profile are unable to clear MV from the brain. The question of whether induction of TH1 cells with specificity for individual MV proteins is necessary or only the action of IFN-γ is required for MV clearance of the brain arises. One approach implies

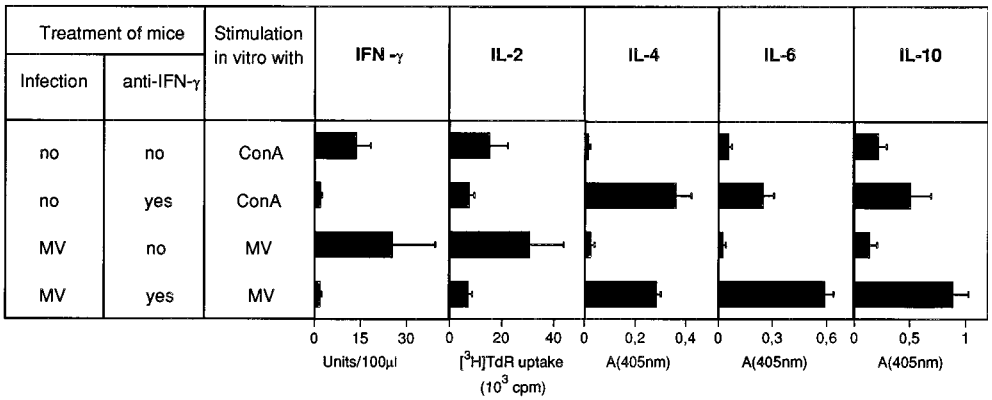

FIG. 5. Cytokine pattern of $CD4^+$ T cells isolated from anti-IFN-γ-treated BALB/c mice. $CD4^+$ T-cell cultures derived from uninfected and MV-infected BALB/c mice treated either with anti-IFN-γ antibodies or with control rat Ig were stimulated in vitro with UV-MV (10 μg/ml) or concanavalin A (ConA) (2.5 μg/ml). Twenty-four to seventy-two hours later, supernatants from four to eight animals of each group were harvested and tested by ELISA (IFN-γ, IL-4, IL-6, and IL-10) or biological assays (IL-2) for the respective cytokine. [^{3}H]TdR, [^{3}H]thymidine.

substitution of susceptible mice with IFN-γ delivered continuously by osmotic pumps. Additionally, adoptive transfer of MV-specific helper T cells that either secrete IFN-γ or do not will help to elucidate whether the immunoregulatory effect of IFN-γ plays the key role in virus clearance from the brain.

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