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Gamma Interferon Is Critical for Resistance to Theiler's Virus-Induced Demyelination

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Administration of neutralizing monoclonal antibody to gamma interferon increased Theiler's virus-induced demyelination and virus antigen persistence in the spinal cord in susceptible SJL/J mice and completely abrogated resistance such that all C57BL/10SNJ mice developed demyelination. These experiments support the hypothesis that gamma interferon is critically important for resistance to Theiler's virus-induced disease but is not required for myelin destruction.

Gamma interferon (IFN-γ) plays a critical role in the protective immune response to viral and protozoan infections (30), which is mediated primarily by $CD8^+$ T cells (29). In contrast, IFN-γ enhances immunopathology in autoimmune diseases by upregulating major histocompatibility complex antigens (34) and activating macrophages (31). One environmental trigger for autoimmune diseases is virus infection (20, 32). Because IFN-γ could be protective or a detriment, we investigated the function of IFN-γ in an experimental murine model of multiple sclerosis triggered by virus infection. Intracerebral injection of Theiler's murine encephalomyelitis virus (TMEV), a picornavirus, into susceptible SJL/J mice results in virus persistence and immune system-mediated primary demyelination (26, 35, 37). In contrast, infection of resistant C57BL/10SnJ mice results in acute encephalitis followed by virus clearance without demyelination. Because IFN-γ may paradoxically be important in clearing virus but inducing immunopathology, we treated susceptible and resistant strains of mice with a neutralizing monoclonal antibody (MAb) to IFN-γ prior to TMEV infection and during the initial stages of chronic demyelination.

SJL/J and C57BL/10SnJ female mice (4 to 8 weeks of age) were injected intracerebrally with 2×10^5 PFU of the Daniel's (DA) strain of TMEV in a 10-μl volume. XMG1.2, a rat immunoglobulin (IgG1) MAb specific for mouse IFN-γ (2), and GL113, a rat IgG1 MAb specific for anti-β-galactosidase (used as an isotype control) were purified from tissue culture supernatants by gel filtration and ion-exchange chromatography. Both preparations were >98% pure and contained <2 EU of endotoxin per mg of antibody. Antibodies were given intraperitoneally at an initial dose of 2 mg 1 day prior to virus infection (day −1) and then at 1 mg on days 7, 14, 21, and 28 following virus infection. Some animals received 2 mg on day 15 following infection and then 1 mg on days 21 and 28. Thirty-five days following infection, mice were perfused by intracardiac puncture with Trump's fixative. Detailed morphological analysis, without knowledge of treatment group, was performed by examining for the presence of gray matter inflammation, meningeal inflammation, and demyelination in each quadrant from 12 to 18 spinal cord coronal sections embedded in plastic (27). Brains were cut into three coronal sections, embedded in paraffin, and stained with hematoxylin and eosin. The cerebellum, brain stem, hippocampus, thalamus, cerebral cortex, meninges, and corpus callosum were graded independently on a four-point scale for the presence of inflammation and necrosis (25).

To detect virus antigen, spinal cord coronal sections from perfused animals were stored in 0.1 M phosphate buffer, rinsed

TABLE 1. Enhancement of Theiler's virus-induced demyelination by anti-IFN-γ treatment of susceptible SJL/J and resistant B10 mice

Mice and treatment[a] (*n*)	No. of spinal cord quadrants examined	% Quadrants [mean ± SEM]		
		Inflammation		Demyelination (P^b)
		Gray matter	Meninges	
SJL/J				
Anti-β-Gal (17)	1,135	0.1 ± 0.1	22.4 ± 3.5	21.5 ± 3.7
Anti-IFN-γ				
Day −1 (13)	682	1.9 ± 1.5	37.1 ± 6.8	38.8 ± 6.3 (0.028)
Day +15 (15)	815	2.4 ± 1.5	36.6 ± 6.6	37.9 ± 6.3 (0.03)
B10				
Anti-β-Gal (15)	832	0.1 ± 0.1	0.8 ± 0.7	0.7 ± 0.6
Anti-IFN-γ (8)	452	0.3 ± 0.3	4.5 ± 1.2	11.2 ± 2.3 (0.0001)

[a] Unless indicated otherwise, treatment commenced 1 day before (day −1) virus infection. β-Gal, β-galactosidase.
[b] Versus the value for the anti-β-galactosidase antibody (Student's *t* test).

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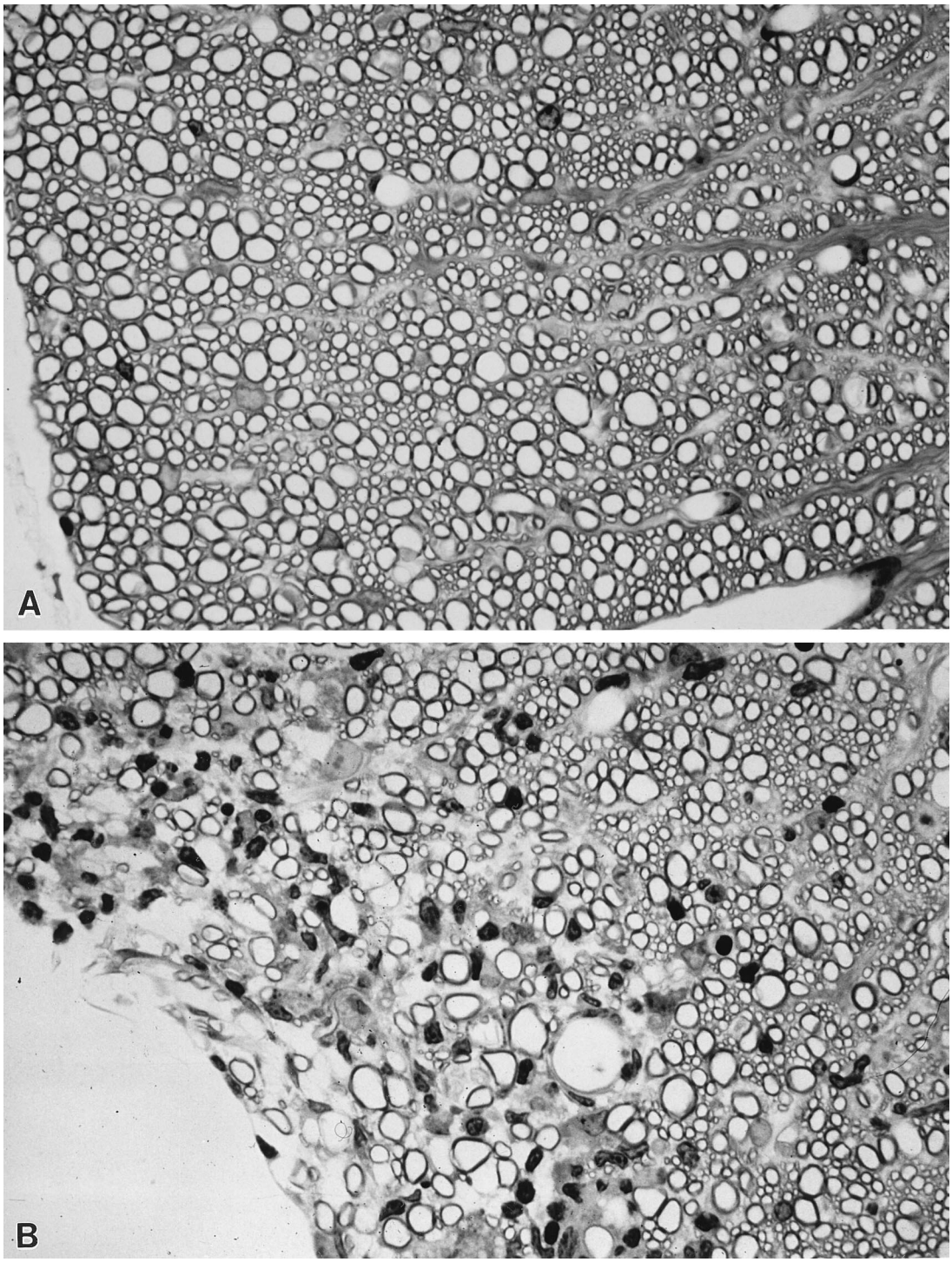

FIG. 1. (A) Absence of demyelination and inflammation in the spinal cord of a B10 mouse treated with control anti-β-galactosidase antibody starting 1 day prior to intracerebral infection with TMEV. (B) Multiple demyelinated axons and inflammatory cells in the spinal cord of a B10 mouse treated with anti-IFN-γ MAb starting 1 day prior to infection with TMEV. Mice were sacrificed on day 35 following infection. Spinal cord sections were embedded in glycol methacrylate plastic and stained with a modified erichrome cresyl violet stain. Magnification, ×630.

in 0.1 M Tris buffer with 25 mM hydroxylamine (pH 7.4), treated with 10% dimethyl sulfoxide for 1 h, and quick-frozen in isopentane chilled in liquid nitrogen. Avidin-biotin immunoperoxidase staining was performed on 10-μm cryostat sections by using polyclonal rabbit antiserum to DA virions (23). The number of virus antigen-positive cells per square millimeter in five or six spinal cord coronal sections from each mouse was determined with an image analysis system. Serum anti-TMEV antibodies were measured by an enzyme-linked immunosorbent assay (ELISA) that used purified TMEV antigen

(22). For isotype-specific ELISA, rabbit antibodies specific for IgG1 and IgG2a were used as described elsewhere (4).

Treatment with anti-IFN-γ enhanced the severity of demyelination in the spinal cord in SJL/J mice (Table 1). All TMEV-infected SJL/J mice, whether treated with anti-IFN-γ or control antibody, showed demyelination. However, there were more spinal cord quadrants showing demyelination in anti-IFN-γ-treated mice than in control-treated mice ($P = 0.03$). In anti-IFN-γ-treated mice, demyelinating lesions were large and encompassed most of the spinal cord white matter. An increase in meningeal inflammation but not neuronal disease was observed in anti-IFN-γ-treated mice (Table 1). To test the contribution of IFN-γ to immunopathology, we began treatment on day +15, when there was inflammation but minimal demyelination. More demyelination was detected in mice treated with anti-IFN-γ beginning on day +15 than in control-treated mice ($P = 0.03$). We asked whether neutralization of IFN-γ would convert normally resistant C57BL/10SnJ mice to susceptibility. Only 2 of 15 infected C57BL/10SnJ mice treated with control MAb showed demyelination in the spinal cord, but these demyelinated lesions were small. In marked contrast, all eight C57BL/10SnJ mice treated with anti-IFN-γ showed severe demyelination (Fig. 1) ($P = 0.0001$ compared with control-treated mice).

Total brain scores for control antibody-treated mice were increased in infected SJL/J mice (3.6 ± 0.9 [range, 0 to 10]) compared with infected B10 mice (0.9 ± 0.3 [range, 0 to 2]). No increase in total scores was observed in anti-IFN-γ-treated (SJL/J or B10) mice (day −1 or day +15). However, more brain stem disease was observed for anti-IFN-γ-treated SJL/J mice (day −1 or day +15) than for control-treated SJL/J mice. Severe (+3) pathology (massive inflammation, necrosis, and neuronal injury) was observed in the brain stem in 5 of 5 SJL/J mice treated with anti-IFN-γ (day −1), 5 of 7 SJL mice treated with anti-IFN-γ (day +15), but only 2 of 10 SJL/J mice treated with control antibody ($P = 0.01$ [chi-square test using Yate's correction]).

Fewer virus antigen-positive cells were observed in the spinal cord in infected B10 mice than for infected SJL/J mice (Table 2). More virus antigen-positive cells per spinal cord area were observed in anti-IFN-γ-treated mice than for control-treated SJL/J ($P < 0.008$) or B10 ($P < 0.03$) mice. All virus antigen-positive cells were in the white matter. An excellent correlation ($r = 0.64$; $P = 0.00003$) was obtained between the number of virus antigen-positive cells per square millimeter of spinal cord with the demyelination score (Fig. 2). Increased

TABLE 2. Virus antigen expression in the spinal cord in TMEV-infected mice[a]

Mice and treatment[b]	No. of Ag-positive cells	Spinal cord area (mm^2)	No. of cells/mm^2 (P^c)
SJL			
Anti-β-Gal	17.6 ± 4.2	4.2 ± 1.1	4.7 ± 0.8
Anti-IFN-γ			
Day −1	37.0 ± 4.7	5.9 ± 0.9	6.6 ± 0.7 (0.05)
Day +15	37.4 ± 5.2	5.4 ± 0.6	7.2 ± 0.4 (0.008)
B10			
Anti-β-Gal	7.2 ± 1.7	6.6 ± 1.5	1.1 ± 0.2
Anti-IFN-γ	13.0 ± 1.4	6.6 ± 0.7	2.0 ± 0.3 (0.03)

[a] The data are means ± standard errors of the means.
[b] Unless indicated otherwise, treatment commenced 1 day before (day −1) virus infection. $n = 5$ except for the B10 anti-β-galactosidase (anti-β-Gal) group ($n = 4$).
[c] Versus the value for the anti-β-galactosidase antibody (Student's t test).

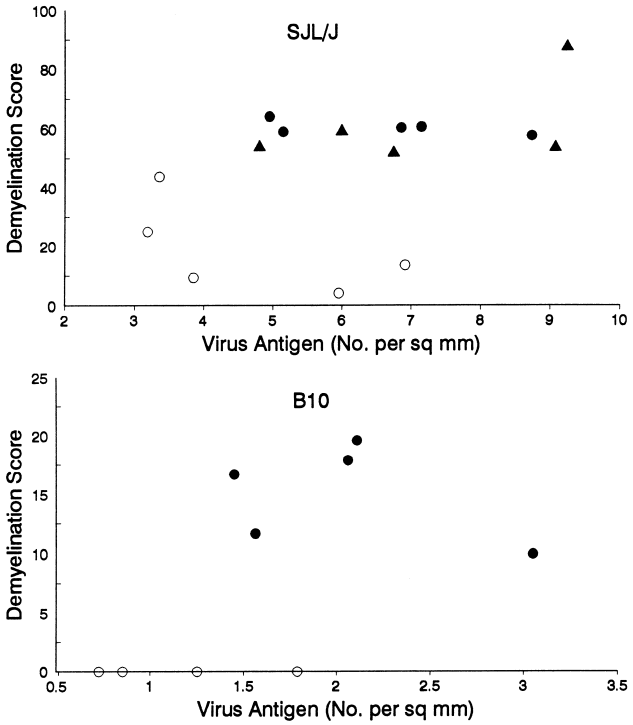

FIG. 2. Correlation between the number of virus antigen-positive cells per square millimeter of spinal cord (immunoperoxidase staining on frozen sections) and demyelination scores (erichrome cresyl violet stain of plastic-embedded sections) for SJL/J and B10 mice on day 35 post-TMEV infection. Note the difference in the y-axis scales. ●, anti-IFN-γ on day −1; ▲, anti-IFN-γ on day +15; ○, anti-β-galactosidase on day −1.

levels of virus-specific total antibody were observed in infected SJL/J mice compared with infected B10 mice (Fig. 3A and B). However, no difference in the levels of virus-specific total Ig's (IgG and IgM) between anti-IFN-γ-treated and control-treated SJL/J or B10 mice was observed (Fig. 3A and B). Treatment with anti-IFN-γ increased the ratio of virus-specific IgG1 to IgG2a, on the basis of a fivefold increase in IgG1 without a change in IgG2a (Fig. 3C and 3D).

Our studies and those of others (10, 19) demonstrate convincingly the critical role for IFN-γ in resistance to Theiler's virus demyelinating disease. One of the genes controlling viral persistence maps close to the IFN-γ locus on chromosome 10 (1). In addition, infection of inbred 129SV mice lacking an IFN-γ receptor results in extensive primary demyelination (7). Treatment with MAbs to IFN-γ resulted in widespread demyelination and virus antigen-positive cells in spinal cord white matter but did not produce the overwhelming neuronal central nervous system TMEV infection observed in immunosuppressed (12, 25), T-cell-deficient athymic nude (28) or neonatal (24) mice. No deaths were observed following treatment with anti-IFN-γ, nor was there clinical or pathological evidence of encephalitis. Therefore IFN-γ contributes primarily to resistance to chronic demyelination and virus persistence in the spinal cord.

Most studies suggest that the antiviral activity dependent on IFN-γ is mediated by $CD8^+$ T cells (29, 36). However, neutralization of endogenous IFN-γ abrogates the activity of $CD4^+$ (TH1) cells following cytomegalovirus (13) and *Schistosoma mansoni* (33) infection. IFN-γ may function via natural killer (NK) cells, since a MAb to IFN-γ prevents recovery from a vaccinia virus infection in nude mice (9). T cells but not NK

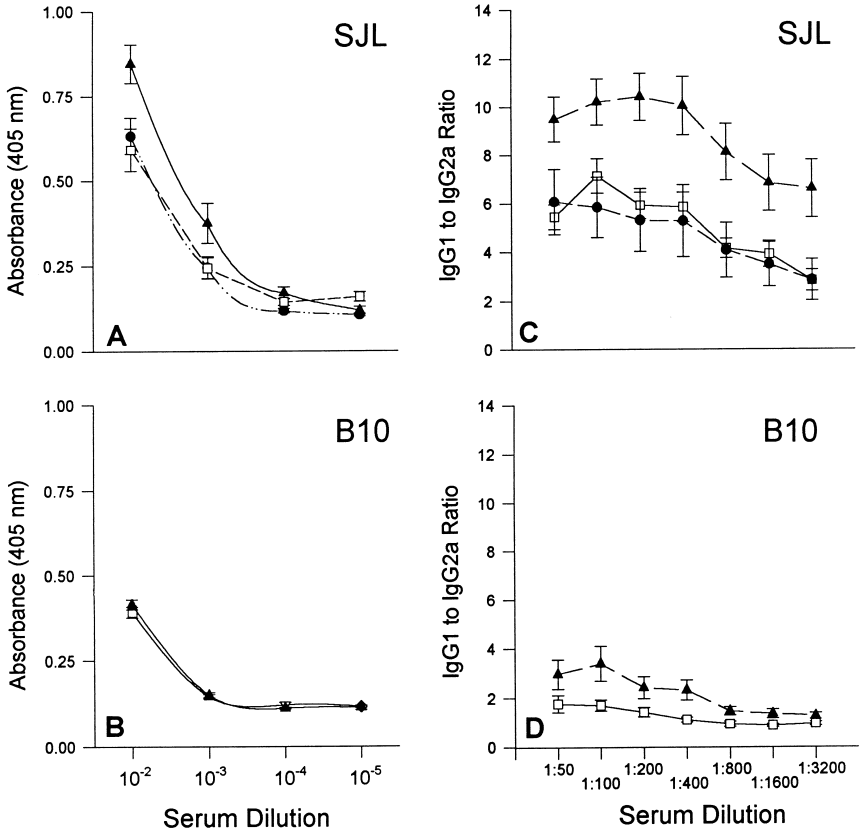

FIG. 3. Virus-specific total Ab (IgG and IgM) as detected by antigen-specific ELISA in SJL/J (A) and B10 (B) mice. (C and D) Virus-specific IgG1-to-IgG2a ratios in SJL/J and B10 mice. □, anti-β-galactosidase on day −1; ▲, anti-IFN-γ on day −1; ●, anti-IFN-γ on day +15.

cells play the major role in resistance to TMEV-induced demyelination. Resistant mice generate virus-specific H-2-restricted $CD8^+$ cytotoxic lymphocytes in the central nervous system (11). Resistant mice depleted of $CD4^+$ or $CD8^+$ T cells (25) and mice deficient in β2-microglobulin develop demyelination (6, 18, 21). In contrast, depletion of NK cells enhances encephalitis but not demyelination (16). Therefore, antibody to IFN-γ probably inhibited the effector function of protective $CD8^+$ or $CD4^+$ T cells, resulting in more demyelination in SJL/J mice and the abrogation of resistance in B10 mice.

The pathogenesis of chronic TMEV-induced demyelination has been proposed to be mediated by TH1 cells, which secrete IFN-γ, interleukin 2, tumor necrosis factor, and lymphotoxin and participate in delayed-type hypersensitivity responses (3, 8, 14, 17). IFN-γ promotes the differentiation of TH1 cells but inhibits TH2 cells (15). In *Leishmania* infection, in which the TH1 response is protective but the TH2 response is fatal (5), IFN-γ stimulates clearance of the protozoa and is required for the development of a TH1 response in healer strains of mice. Treatment with MAbs to IFN-γ should have diminished demyelination if disease was mediated primarily by TH1 cells. In contrast, TMEV-infected mice treated with anti-IFN-γ showed more demyelination and less virus clearance, similar to the results in *Leishmania* infection.

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