Interferon-γ is Required for Lupus-like Disease and Lymphoaccumulation in MRL-*lpr* Mice

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Abstract

Congenic MRL-*lpr* mice homozygous and heterozygous for the IFN-γ gene disruption were created to assess the role of this pleotropic cytokine on the lymphoaccumulation and lupus-like disease of Fas-defective mice. Early death was prevented, and glomerulonephritis severely reduced in IFN-$\gamma^{-/-}$ mice. Hypergammaglobulinemia was maintained with a switch from IgG2a to IgG1 predominance, but the dramatic decrease in levels of the dominant IgG2a anti-dsDNA autoantibodies was not associated with a compensatory increase in T_H2-associated IgG subclasses. Remarkably, early death and glomerulonephritis were also prevented in IFN-$\gamma^{+/-}$ mice, although autoantibody levels and glomerular immune deposits were equivalent to IFN-$\gamma^{+/+}$ *lpr* mice, indicating the importance of additional locally-exerted disease-promoting effects of IFN-γ. IFN-$\gamma^{-/-}$ mice exhibited reduced lymphadenopathy concomitant to a decrease in DN B220$^+$ T cells. In vivo BrdU labeling showed reduced proliferation of DN B220$^+$ cells in IFN-$\gamma^{-/-}$ vs. IFN-$\gamma^{+/+}$ *lpr* mice, while enhanced proliferation of all other T cell subsets was unaffected. Macrophages of IFN-$\gamma^{-/-}$*lpr* mice expressed markedly decreased levels of MHC class I and II molecules compared with controls. Moreover, the heightened expression of MHC class II molecules on proximal tubules of IFN-$\gamma^{+/+}$ *lpr* mice was significantly reduced in both IFN-$\gamma^{-/-}$ and IFN-$\gamma^{+/-}$ mice. The data indicate that IFN-γ hyperproduction is required for lupus development, presumably by increasing MHC expression and autoantigen presentation to otherwise quiescent nontolerant anti-self T cells, and also by promoting local immune and inflammatory processes. (*J. Clin. Invest.* 1998. 101:364–371.) Key words: lupus • IFN-γ • MRL-*lpr*

Introduction

Cytokines play a critical role in regulating the quantitative and qualitative responses of T cells, B cells, macrophages, and other cell types. As such, these molecules would be expected to exert inhibitory or promoting effects on the initiation and perpetuation of autoimmune diseases, including lupus (1, 2). Cytokine disturbances have been extensively analyzed in mice and humans with lupus, and a broad spectrum of abnormalities have been identified (for review see references 3 and 4). It is unclear, however, whether these abnormalities constitute a primary or secondary event in the disease process. Nevertheless, among the notable cytokine changes that occur in mouse lupus models, that of IFN-γ hyperproduction is a consistent finding (5, 6). The possible importance of this cytokine in lupus pathogenesis has been suggested by the demonstration that New Zealand lupus mice treated with IFN-γ (7) showed accelerated disease manifestations while, conversely, treatment with anti-IFN-γ antibody (7, 8) or soluble IFN-γR (8) early in life caused significant delay in disease progression. In contrast to the uniformity of data on the beneficial effects of IFN-γ agonists in the New Zealand lupus model, those with MRL-*lpr* mice are conflicting. A study by Takahashi et al. (9) on a long-lived substrain of MRL-*lpr* mice (MRL-*lpr/lpr* $\ell\ell$) showed reduced IFN-γ levels compared with the parental strain, concomitant with a shift of IgG isotypes from the C-fixing IgG2a and the cryogenic IgG3 to the apparently less pathogenic IgG1 isotype. Moreover, administration of IL-12, a powerful IFN-γ inducer (10), accelerated glomerulonephritis (GN)[1] development in the MRL-*lpr* mice (11). In contrast, administration of anti-IFN-γ mAb was reported not to affect GN or survival of these mice (12).

To further define the extent of IFN-γ participation in lupus pathogenesis, we created congenic MRL-*lpr* mice in which one or both copies of the IFN-γ gene had been inactivated, respectively designated as IFN-$\gamma^{+/-}$ and IFN-$\gamma^{-/-}$. Our data conclusively indicate that IFN-γ is required for the antinuclear autoantibody response, GN development, accumulation of the apoptosis-defective double-negative (DN) B220$^+$ TCRαβ$^+$ T cells, and early mortality. These effects appear to be mediated by both immunologic and nonimmunologic means. These and previous results indicate that approaches that reduce and/or block the action of IFN-γ may be an important means to inhibit the development and progression of lupus.

Methods

Animals. Mice were obtained from The Scripps Research Institute and were kept under specific pathogen-free conditions. MRL-*lpr* IFN-$\gamma^{-/-}$ mice were produced by backcrossing IFN-γ-deficient C57BL/6 mice (13) to the MRL *lpr* background followed by a final intercrossing of F_7 IFN-$\gamma^{+/-}$ mice. IFN-$\gamma^{+/+}$, IFN-$\gamma^{-/-}$ and IFN-$\gamma^{+/-}$ mice were identified by genomic DNA PCR (35 cycles at 94°C/20 s, 55°C/30 s, and 72°C/90 s) using two pairs of primers, one detecting the neomycin gene insertion (sense TTGAACAAGATGGATTGCACGCAGG and antisense GGCTGGCGCGAGCCCCTGATGCTCT) and the other the IFN-γ second exon (sense AGAAGTAAGTG-

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1. *Abbreviations used in this paper:* DN, double negative; GN, glomerulonephritis; HPRT, hypoxanthine-guanine phosphoribosyltransferase; NO, nitric oxide; RT, reverse transcription.

GAAGGGCCCAGAAG and antisense AGGGAAACTGGGAG-AGGAGAAATAT).

Histologic and serologic analysis. Histologic examination of sectioned kidneys and severity of GN were determined in a blind manner using a scale from 0 to 4+ (14). Blood urea nitrogen (BUN) levels at autopsy were determined using AZOSTIX strips (Bayer Corp., Elkhart, IN). Serum IgG subclasses were captured with a $(Fab')_2$ goat anti–mouse $(Fab')_2$ antibody (Jackson Labs, San Jose, CA), and levels were determined by ELISA, as described (15). The reactivity of anti-IgG1, -IgG2a, -IgG2b (Caltag Labs, San Francisco, CA), and -IgG3 (PharMingen, San Diego, CA) antibodies against the corresponding myeloma subclasses was normalized for equivalent OD by ELISA (15). Levels of subclass-specific anti-dsDNA, anti-ssDNA, and antichromatin autoantibodies were measured as described (15), and expressed as U/ml in reference to standard curves obtained with a serum pool from 16-wk-old MRL-*lpr* mice.

Immunohistochemistry. Kidneys of mice were snap-frozen, and 4-μm sections were cut. Air-dried sections were fixed in chilled acetone for 10 min. Samples were stained either with an FITC-conjugated goat anti–mouse IgG (Jackson ImmunoResearch Labs, Inc., West Grove, PA) or with a rat anti–mouse class II antibody (Biosource International, Camarillo, CA) followed by peroxidase-conjugated anti–rat antibody (Jackson ImmunoResearch Labs, Inc.), and developed with aminoethyl carbazole as substrate.

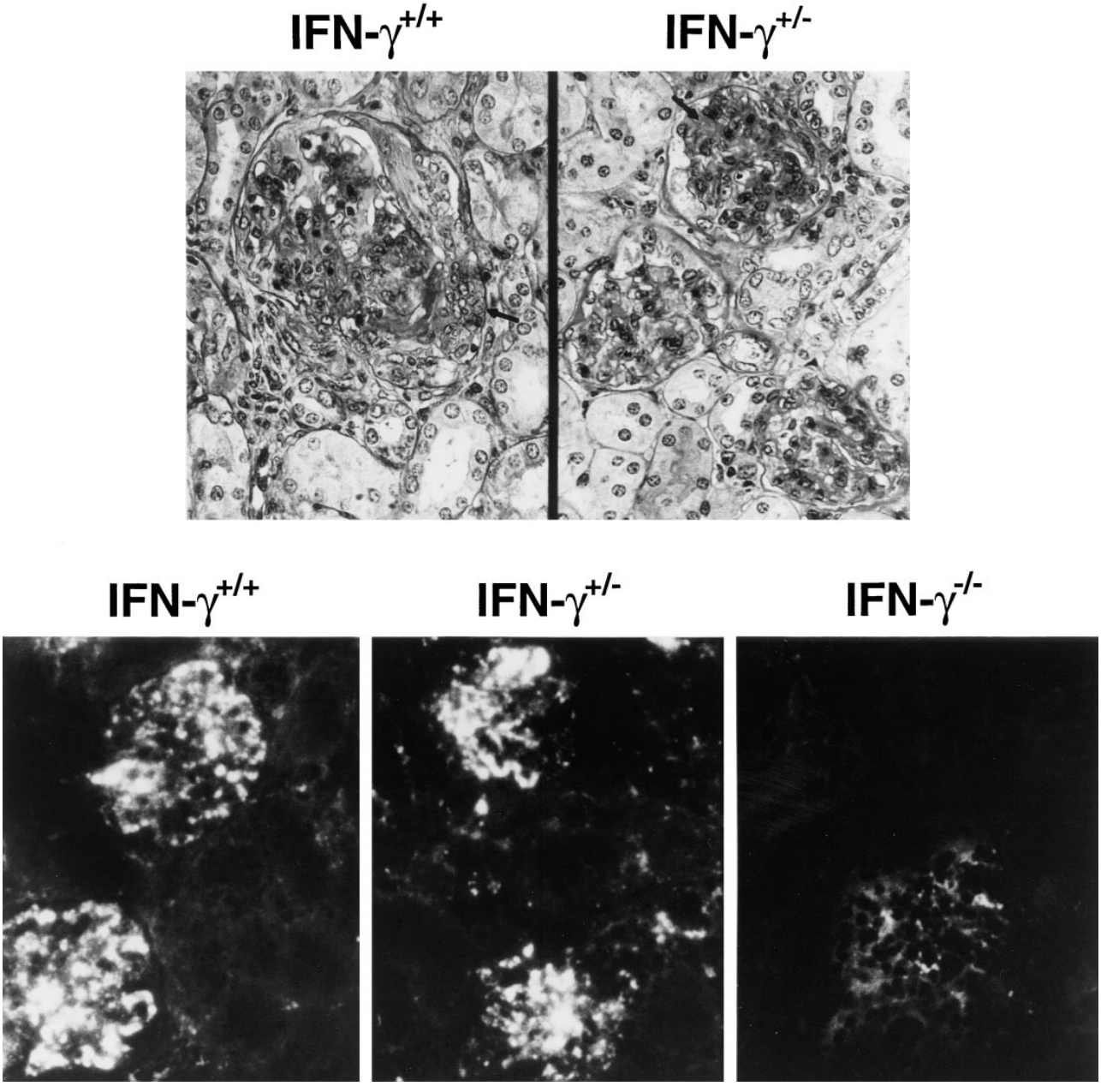

Figure 1. Effect of IFN-γ on GN development in MRL-*lpr* mice. (*Top*) A typical glomerulonephritic lesion of an IFN-$\gamma^{+/+}$ MRL-*lpr* mouse is shown, and includes an enlarged glomerulus, proliferation of glomerular cells, infiltrating inflammatory cells, increased mesangial matrix, and crescent formation (*arrow*). The lesions seen in IFN-$\gamma^{+/-}$ mice show a lesser degree of GN changes and varying degrees of focal glomerular cell proliferation, occasional inflammatory cells, and small increases in mesangial matrix (*arrow*; 500×). (*Bottom*) IgG deposits in kidneys of IFN-$\gamma^{+/+}$, IFN-$\gamma^{+/-}$, and IFN-$\gamma^{-/-}$ mice (40×).

Table I. Evaluation of Glomerular Disease and Survival of IFN-$\gamma^{+/+}$, IFN-$\gamma^{+/-}$, and IFN-$\gamma^{-/-}$ MRL-lpr Mice

	Renal Disease*	BUN	Survival‡
			%
IFN-$\gamma^{+/+}$	3.4±0.2	55±16	50
IFN-$\gamma^{+/-}$	2.0±0.4	25±8	100
IFN-$\gamma^{-/-}$	1.4±0.5	21±9	100

*Glomerular damage was scored on a scale of 0–4+ at 28 wk of age or earlier if euthanasia was required. Values represent mean±SD of six mice/group. ‡Percent survival at 5 mo of age for IFN-$\gamma^{+/+}$ mice and at 7 mo of age for IFN-$\gamma^{+/-}$ and IFN-$\gamma^{-/-}$ mice (10 mice per group).

Cytokine levels. IFN-γ, IL-4, IL-10, IL-2, IL-1β, and TNFα mRNA lymph node levels were determined by a semiquantitative reverse transcription (RT)-PCR in the presence of a control plasmid containing sense and antisense primer sequences of the tested cytokines as well as of hypoxanthine–guanine phosphoribosyltransferase (HPRT, housekeeping gene) as we have previously described (6). In brief, serial dilutions of the control plasmid were added to the cDNA equivalent of 40 ng of total RNA (extracted from the cervical lymph nodes of 16-wk-old mice) and amplified by PCR using the same primers for the target and control templates. The PCR products were quantitated after ethidium bromide staining on an Eagle Eye Video Imaging System (Stratagene Inc., La Jolla, CA). The results were normalized for HPRT levels and expressed as a percent of cytokine levels in RNA from pooled lymph nodes of conventional MRL-*lpr* mice.

BrdU labeling. For BrdU labeling, mice were thymectomized at 5 wk of age, and at 8 wk of age received drinking water containing 0.8 mg/ml BrdU (Sigma Chemical Co., St. Louis, MO) prepared fresh every 2 d for a period of 9 d (16). For assessing BrdU decay, mice were then switched to regular water for 7 d (16).

Flow cytometry analysis. The following Mab were purchased from PharMingen (San Diego, CA): anti-CD4, anti-CD8, anti-CD11b (MAC-1) conjugated to FITC; anti-CD4, anti-CD8, anti-TCRαβ, anti-I-A^k, anti-H-$2K^k$ conjugated to PE and anti-CD4, anti-CD8, and anti-B220 conjugated to Cy-Chrome. Staining with a rat anti-F4/80 mAb (Biosource), a maturation marker for macrophages, was followed by an anti–rat antibody conjugated to biotin (Jackson ImmunoResearch) and Cy-Chrome-conjugated streptavidin (PharMingen). Combinations of these antibodies were used for surface staining of spleen and lymph node cells. To avoid nonspecific staining, cells were preincubated with a mixture of unconjugated rat anti–mouse CD32/CD16 antibody (PharMingen). BrdU-labeled cells were stained with an FITC-conjugated anti-BrdU mAb (Becton Dickinson, San Jose, CA) as described (16), in combination with one or more of the above mAb to surface markers. Stained cells were analyzed on a FACScan® flow cytometer (Becton Dickinson).

Statistics. The Student's *t* test was used to evaluate statistical significance.

Results

Survival and histologic characteristics. As previously established (14), the 50% mortality rate for the control mice was at 5 mo. In contrast, 100% of both the IFN-$\gamma^{-/-}$ and IFN-$\gamma^{+/-}$ mice were alive at 7 mo (Table I), the latest point of observation reported in this manuscript. These mice continue to be followed.

The GN grade in control mice at the time of death was 3.4±0.2. In contrast, that of IFN-$\gamma^{-/-}$ was 1.4±0.5 ($P < 0.0003$), and that of IFN-$\gamma^{+/-}$ was 2.0±0.4 ($P < 0.0003$) in separate groups of mice killed at 5 mo of age (Table I, Fig. 1, *top*). Moreover, BUN levels correlated with GN grade (Table I). As expected, glomerular IgG deposits were dramatically reduced in IFN-$\gamma^{-/-}$ compared with wild-type mice. However, levels of such deposits were similar in IFN-$\gamma^{+/-}$ and IFN-$\gamma^{+/+}$ mice (Fig. 1, *bottom*).

Serologic characteristics. As previously shown (9, 17), MRL-*lpr* mice exhibited marked hypergammaglobulinemia, with IgG2a being the dominant subclass (Fig. 2). IFN-$\gamma^{-/-}$ mice showed a shift to IgG1 predominance concomitant to severe decreases in IgG2a and IgG3, although total IgG levels remained equivalent to controls. Significant but less pronounced

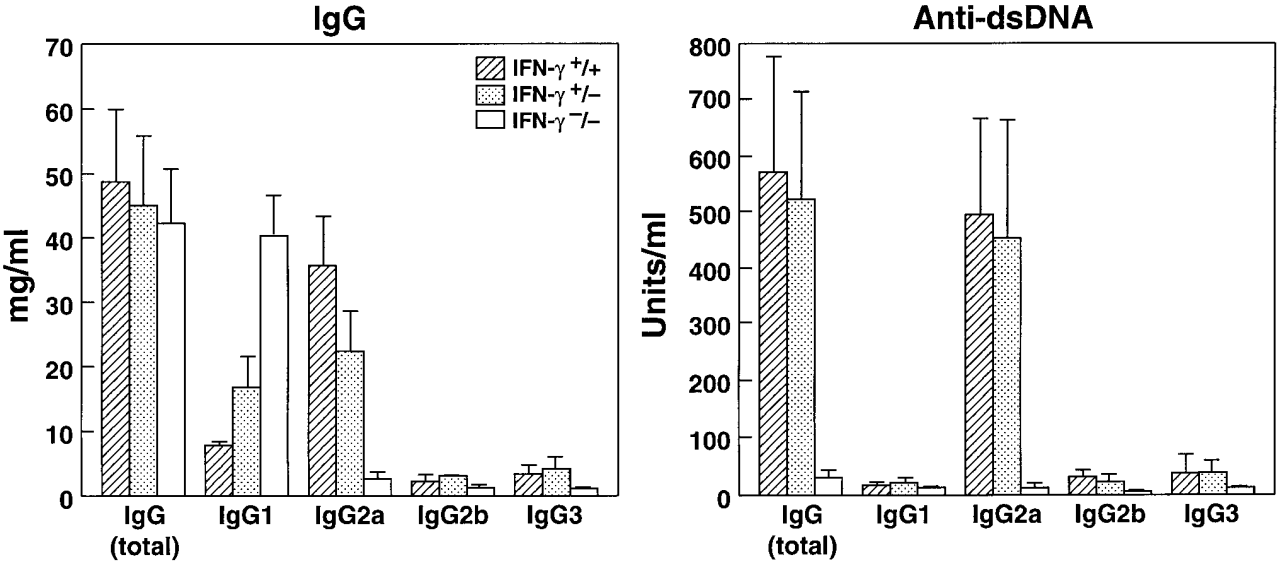

Figure 2. Total IgG and IgG anti-dsDNA levels in IFN-$\gamma^{+/+}$, IFN-$\gamma^{+/-}$, and IFN-$\gamma^{-/-}$ MRL-*lpr* mice. (*Left*) Serum levels of IgG isotypes. Total IgG is derived from the addition of all IgG subclasses. (*Right*) Serum levels of IgG anti-dsDNA antibody isotypes. Data represent mean±SD of 6–8 mice/group at 16 wk of age.

increases in IgG1 and decreases in IgG2a were also noted in the IFN-$\gamma^{+/-}$ mice, but levels of IgG3 remained similar to controls.

The anti-dsDNA autoantibody response in control mice was almost exclusively of the IgG2a subclass, with very small contributions by the other subclasses (Fig. 2). IFN-$\gamma^{-/-}$ mice had severely reduced IgG2a and IgG3 anti-dsDNA antibodies, but the contribution of IgG1 to these autoantibodies remained very low. In contrast, IFN-$\gamma^{+/-}$ mice had levels and IgG subclass distribution of anti-dsDNA autoantibodies similar to the control mice. Antichromatin and anti-ssDNA levels were also dramatically diminished in IFN-$\gamma^{-/-}$ mice, but remained elevated in IFN-$\gamma^{+/-}$ (not shown).

Cytokine levels. Expression of lymph node mRNA IFN-γ was below detectable levels in 16-wk-old IFN-$\gamma^{-/-}$ mice, while IFN-$\gamma^{+/-}$ mice expressed ~ 55% the levels of the wild-type mice (Fig. 3). Because T_H1 and T_H2 cells cross-regulate each other via their cytokines, we examined whether elimination (IFN-$\gamma^{-/-}$) or reduction (IFN-$\gamma^{+/-}$) of IFN-γ levels resulted in altered expression for other cytokines. As defined by a semiquantitative RT-PCR using lymph node mRNA from 16-wk-old mice, levels of T_H2 cytokines such as IL-4 and IL-10 were unaffected (Fig. 3). Other cytokines, such as IL-2, IL-1β, and TNFα in both groups of IFN-γ–modified mice were also equivalent to those in the control IFN-$\gamma^{+/+}$ MRL-*lpr* mice (not shown).

Cellular characteristics. To determine the effects of IFN-γ deletion/depletion in MRL-*lpr* lymphadenopathy, the total weight of cervical, axillary, inguinal, and mesenteric lymph nodes was determined in control, IFN-$\gamma^{-/-}$, and IFN-$\gamma^{+/-}$ mice at 16 wk. A significant reduction in total LN weight was detected in IFN-$\gamma^{-/-}$ mice (2.8-fold reduction; $P < 0.0002$), while reduction in IFN-$\gamma^{+/-}$ mice was insignificant (Table II). FACS analysis showed that the diminished lymphadenopathy in IFN-$\gamma^{-/-}$ mice was accounted for by a threefold reduction in the frequency of DN $B220^+$ T cells compared with controls (76±7% vs. 24±6%, $P < 0.0001$). In IFN-$\gamma^{-/-}$ *lpr* mice, there was also a proportional increase in $CD4^+$ and $CD8^+$ T cells, while the proportion of $CD4^+B220^+$ was unchanged.

T cell activation/division. Using long-term in vivo BrdU-labeling, we recently showed that $CD4^+$ and $CD8^+$ T cells of 8-wk-old MRL-*lpr* mice divide at increased rates (~ 15%) compared with corresponding MRL-+/+ subsets (~ 5%; 18). Even more impressive was the finding that ~ 70% of the DN $B220^+$ cells and 55% of the $CD4^+B220^+$ T cells in these younger *lpr* mice were in cycle, as indicated by their $BrdU^{hi}$ phenotype. To determine the role of IFN-γ in MRL-*lpr* T cell proliferation, we performed long-term in vivo BrdU-labeling and -chase experiments with 8-wk-old IFN-$\gamma^{-/-}$ *lpr* mice and compared the results with those in the IFN-$\gamma^{+/+}$ counterparts. Absence of IFN-γ had no effect on proliferation of $CD4^+$, $CD8^+$, or $CD4^+$ $B220^+$ cells, as shown by the equal proportion of $BrdU^{hi}$ cells to those in the controls (Fig. 4). Contrastingly, the frequency of $BrdU^{hi}$ DN $B220^+$ T cells was reduced from 71% in the IFN-$\gamma^{+/+}$ mice to 46% in the IFN-$\gamma^{-/-}$ mice (Fig. 4). More importantly, the division rates of the proliferating DN $B220^+$ cells was significantly lower in IFN-$\gamma^{-/-}$ than IFN-$\gamma^{+/+}$ mice, as defined by their conversion from $BrdU^{hi}$ to $BrdU^{lo}$ phenotype upon BrdU discontinuation and analysis 7 d thereafter (Fig. 5, *top*). Thus, the $BrdU^{lo}/BrdU^{hi}$ ratio was ~ 3.0 for IFN-$\gamma^{+/+}$ mice, while that of IFN-$\gamma^{-/-}$ mice was ~ 1. Forward scatter analysis of $BrdU^{lo}$ DN $B220^+$ cells showed that a higher proportion of these cells exhibited larger size in IFN-$\gamma^{+/+}$ compared with IFN-$\gamma^{-/-}$ mice (Fig. 5, *bottom*), indicative of their potential to undergo another round of division.

MHC expression levels. IFN-γ exerts profound enhancing effects on MHC class I and class II expression (13, 19), and MRL-*lpr* mice were reported previously (20–22) to display a dramatic increase in IA^+ resident peritoneal macrophages. Therefore, we examined the effect of IFN-γ gene deletion on MHC class I and class II expression by splenic $CD11b^+$ and $F4/80^+$ macrophages. As shown in Fig. 6 (*top*), the mean intensity

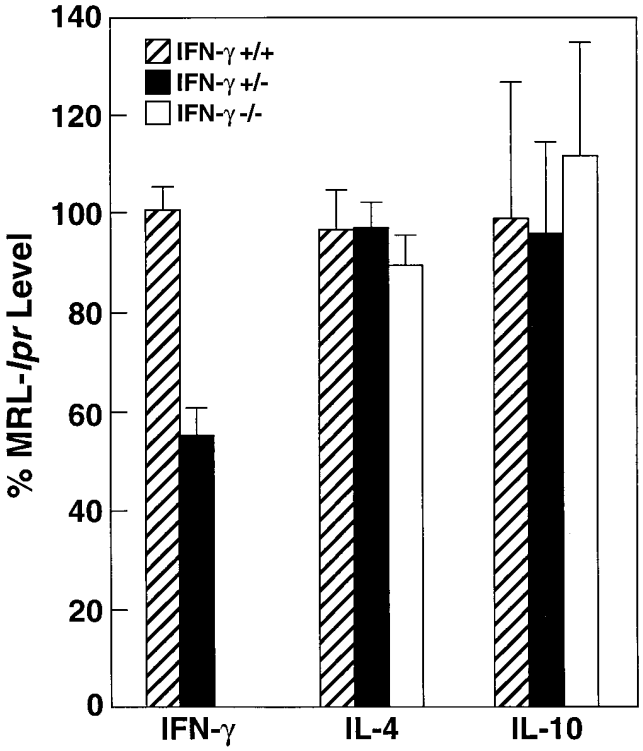

Figure 3. Cytokine levels (IFN-γ, IL-4, IL-10) in IFN-γ gene-deleted homozygous and heterozygous mice. Levels in control MRL-*lpr* mice were assigned a 100% value. Results are the mean±SD of three mice per group at 16 wk of age.

Table II. Lympadenopathy and Lymphocyte Distribution in IFN-$\gamma^{+/+}$, IFN-$\gamma^{+/-}$, and IFN-$\gamma^{-/-}$ MRL-lpr Mice

	Lymph node weight*	$CD4^+$	$CD4^+B220^+$	$CD8^+$	DN
	g	%	%	%	%
IFN-$\gamma^{+/+}$	2.2±0.4	5.5±0.4	6.0±0.2	3.8±0.8	76±7
IFN-$\gamma^{+/-}$	2.0±0.5	5.8±0.6	6.4±0.5	4.5±1.0	70±8
IFN-$\gamma^{-/-}$	0.8±0.05	26.0±0.8	6.0±1.5	32.5±3.5	24±6

Data represent mean±SD from four mice/group at 16 wk of age. *Lymph node cells were stained using the following antibody combinations: (*a*) anti-CD4 and anti-B220 antibodies to identify $CD4^+$ and $CD4^+B220^+$ cells; (*b*) anti-CD8 to identify $CD8^+$ cells; or (*c*) anti-TCRαβ and a combination of anti-CD4 and anti-CD8 to identify DN T cells. The weights (g) are of pooled cervical, axillary, inguinal, and mesenteric lymph nodes.

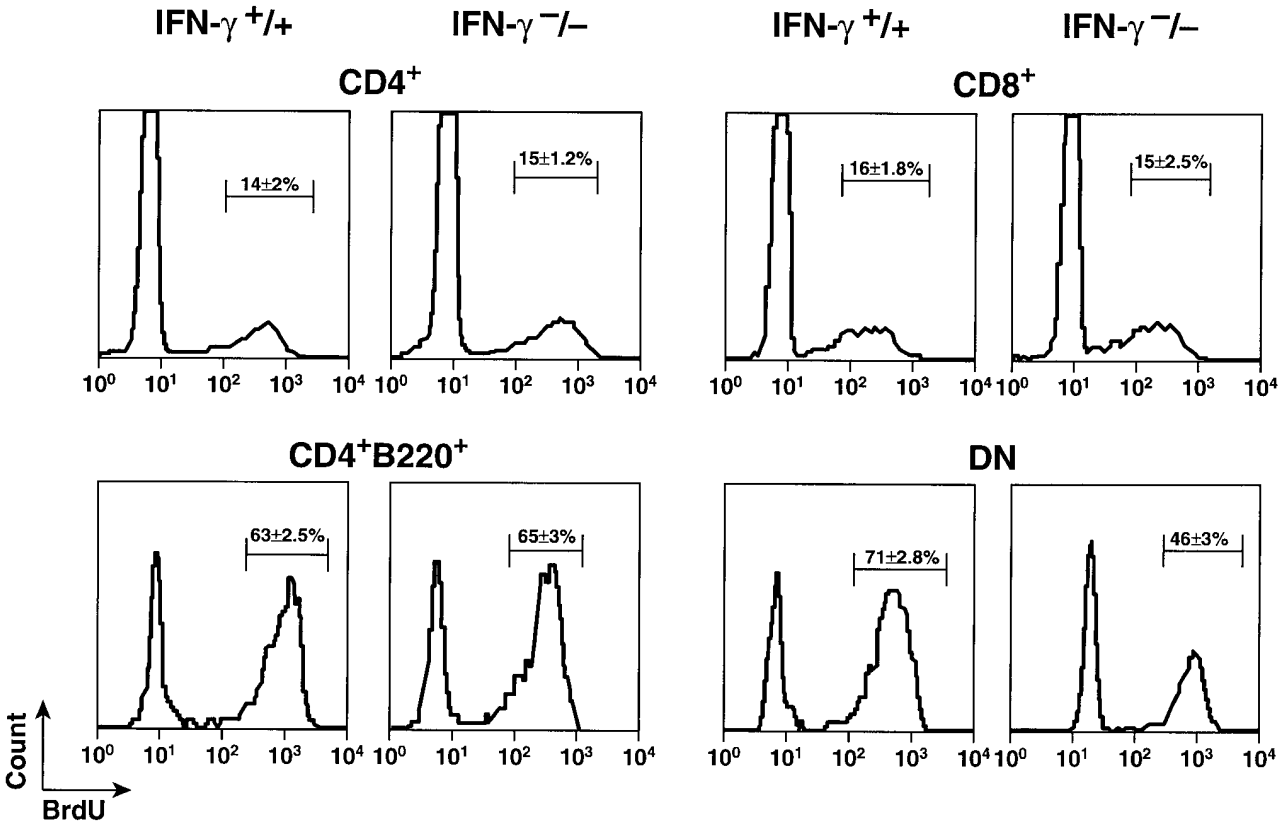

Figure 4. In vivo BrdU labeling of IFN-$\gamma^{+/+}$ and IFN-$\gamma^{-/-}$ MRL-*lpr* T cells. 8-wk-old thymectomized mice received BrdU in drinking water for 9 d. $CD4^+$, $CD8^+$, $CD4^+B220^+$, and DN cells were identified using combinations of antibodies. FACS profiles are representative of one mouse and mean±SD of $BrdU^{hi}$ cells are derived from three mice/group.

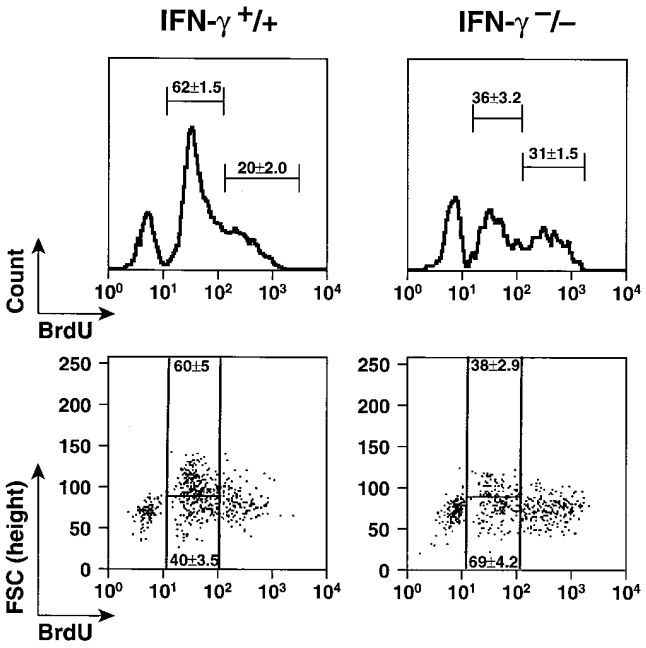

Figure 5. Division rates of BrdU-labeled DN cells in IFN-$\gamma^{+/+}$ and IFN-$\gamma^{-/-}$ MRL-*lpr* mice. (*Top*) BrdU decay (conversion from $BrdU^{hi}$ to $BrdU^{lo}$) at 7 d after BrdU discontinuation. (*Bottom*) Size distribution of $BrdU^{lo}$ DN cells. FACS profiles are representative of one mouse, and mean±SD for the indicated values are derived from three mice/group at 8 wk of age.

of MHC class I and class II molecules on macrophages from 10-wk-old IFN-$\gamma^{+/+}$ mice was 2.5-fold and 2.2-fold, respectively, higher than that of MRL-+/+ mice. More importantly, *lpr* mice homozygous for the IFN-γ gene deletion exhibited a 5.6-fold reduction in MHC class I and a fourfold reduction in MHC class II expression compared with IFN-$\gamma^{+/+}$ *lpr* mice, expression levels that are even lower than those in the late-life, mild disease–developing MRL-+/+ mice. Expression levels for MHC class II on splenic macrophages were, however, unaltered by the one-half reduction in IFN-γ levels in the heterozygous mice.

Renal proximal tubules from older MRL-*lpr* mice have been reported to display elevated MHC class II expression, suggesting a role of local immune responses in GN development (23). Absence or even partial reduction of IFN-γ resulted in a dramatic decline in MHC class II expression by tubular cells (Fig. 6, *bottom*). In contrast, expression of MHC class II in glomerular perivascular mononuclear cell infiltrates of heterozygous mice was similar to that of wild-type mice, while the intensity of staining was lower in IFN-$\gamma^{-/-}$ mice (not shown). The data indicate that MHC class II expression appears to be dependent on IFN-γ more in tubular than in mononuclear cells.

Discussion

Using MRL-*lpr* mice genetically deficient in IFN-γ production, we demonstrated the critical role of this cytokine in the

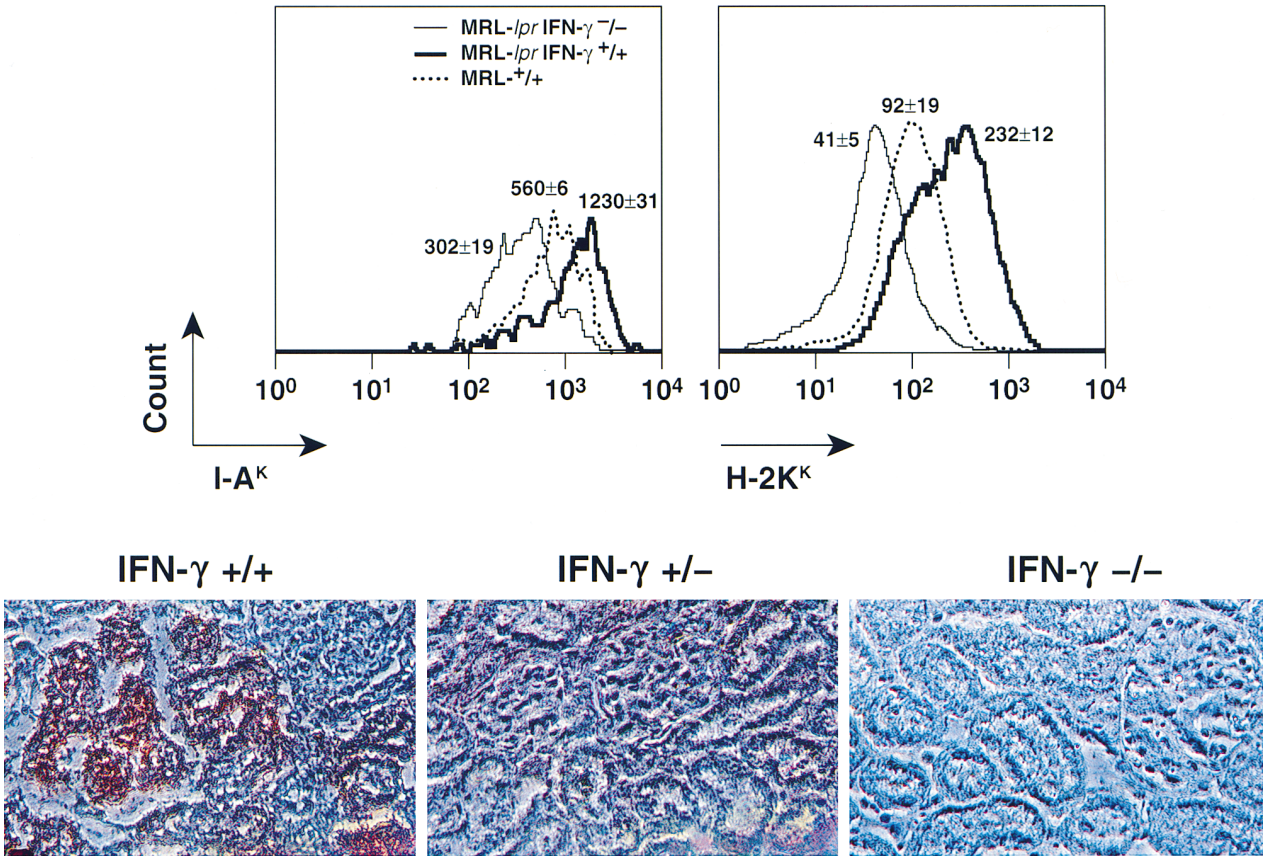

Figure 6. Relationship of IFN-γ levels and MHC expression in MRL-*lpr* mice. (*Top*) MHC class I (*right*) and class II (*left*) expression by splenic macrophages. Histograms are representative of one mouse and values are mean±SD of three mice/group at 10 wk of age. (*Bottom*) Immunostaining for I-A^k of kidney cryostat sections (40×).

pathogenesis of the lupus-like disease and lymphoaccumulation in these Fas-defective mice. Specifically, we have established the following: (*a*) IFN-γ is essential for production of the dominant IgG2a anti-dsDNA autoantibodies of these mice; (*b*) absence of IFN-γ leads to a switch of total IgG from the IgG2a to IgG1 dominancy, but no such compensatory switch of anti-dsDNA autoantibody occurs; (*c*) complete absence (IFN-$\gamma^{-/-}$) and even partial reduction (IFN-$\gamma^{+/-}$) of IFN-γ leads to the absence of, or minimal GN, respectively, and prolonged survival that occurs despite the fact that autoantibody levels and kidney immune deposits are unaffected in the IFN-$\gamma^{+/-}$ mice; (*d*) in vivo proliferation and accumulation of the Fas-defective DN $B220^+$ cells is significantly reduced in IFN-$\gamma^{-/-}$ mice; and (*e*) a major effect of IFN-γ gene deletion is severe reduction in MHC class I and class II expression in splenic macrophages and renal tubular cells, which are elevated in conventional IFN-$\gamma^{+/+}$ *lpr* mice.

Because immune responses are highly regulated by cytokines, expression levels of these molecules have been extensively investigated in lupus strains of mice (3, 4). With regard to MRL-*lpr* mice, earlier assessments with biologic assays showed an age-dependent decrease in production and response to IL-2 (24, 25), and elaboration of undefined B cell differentiation factors (26). Subsequent extensive studies by RNase protection (27) and PCR (5, 6) assays encompassing most known cytokines indicated additional abnormalities, of which the most consistent finding was upregulation of IFN-γ. High levels of IFN-γ corresponded well with the IgG2a predominance in sera of *lpr* mice that display exceedingly high total IgG levels (≥ 40 mg/ml; 3) due to defective T and B cell apoptosis (28). The dominance of T_H1 over T_H2 IgG isotypes is also apparent in the anti-dsDNA autoantibody profile, which is almost exclusively of the IgG2a isotype, with a far smaller contribution by the IgG3 subclass, and very little by the IgG1 isotype (this study and 9).

Based on earlier accounts of the detrimental effects of IFN-γ in the NZBxW lupus disease (7), efforts to determine the role of this cytokine in *lpr* disease have also been made. Nicoletti et al. (12) reported that in vivo treatment with a mAb to IFN-γ affected neither survival nor incidence of GN in MRL-*lpr* mice. Negative results with antibody treatment are not conclusive, however, since the amounts given might be insufficient, or more likely the antibody may not reach the lymphoid and afflicted organ microenvironment. In contrast, a comparative study by Takahashi et al. (9) between a long-lived MRL-*lpr* substrain (MRL-*lpr*,ℓℓ) and the short-lived conventional MRL-*lpr* mice suggested that hyperproduction of IFN-γ is a contributor to the early-life severe disease.

Careful comparison of the data of Takahashi et al. (9) with the present findings reveals an interesting difference: while the low IFN-γ–producing MRL-*lpr* ℓℓ had switched both non-anti-dsDNA and anti-dsDNA autoantibody IgG subclasses to the

T_H2 profile (IgG1 > IgG2a), our IFN-$\gamma^{-/-}$ mice switched only with respect to the non-anti-dsDNA IgG subclasses, but not with respect to the anti-dsDNA subclasses, which remained at very low levels. Perhaps this difference may be explained on the basis of complete absence of IFN-γ in the gene-deleted homozygous mice, but presence of IFN-γ (albeit at one-third the levels of conventional *lpr* mice) in the MRL-*lpr* $\ell\ell$ substrain. Such low levels of IFN-γ may be necessary to induce the IgG1 switch of anti-dsDNA autoantibodies. In our study, absence of IFN-γ did not affect the levels of T_H2 cytokines (IL-4, IL-10), indicating that disease inhibition was directly mediated by T_H1 cytokine change, and not by downregulation of the T_H2 component. The data overall indicate that the anti-dsDNA response is highly dependent on the presence of IFN-γ, suggesting that this cytokine is essential for the break of peripheral tolerance toward nuclear autoantigens.

The mechanisms for ablation of the autoimmune process and the failure of lupus occurrence in the absence of IFN-γ may include both immunologic and nonimmunologic pathways. A major immunologic mechanism appears to be the insufficient upregulation of MHC class I and class II molecules. Such a mechanism has also been implicated by von Herrath and Oldstone (29) for the absence of diabetes in IFN-$\gamma^{-/-}$ mice transgenic for β cell–expressed lymphocytic choriomeningitis virus (LCMV) proteins after LCMV infection. As noted earlier, previous studies (20–22) have shown elevated numbers of IA^+ peritoneal macrophages in MRL-*lpr* mice. In this study, FACS analysis showed increased expression of MHC class I and class II molecules in splenic macrophages of MRL-*lpr* mice compared with MRL-+/+ mice. Significantly, deletion of the IFN-γ gene in MRL-*lpr* mice caused a decrease in MHC class II and class I expression below that of MRL-+/+ mice. Insufficient upregulation of MHC class II molecules may compromise the activation of anti-self $CD4^+$ cells. In this case, the immunizing antigen for anti-dsDNA autoantibodies (perhaps chromatin or nucleosomes; 15, 30, 31) may be presented in low amounts and/or exhibit low MHC affinity so that MHC upregulation may be a prerequisite for adequate presentation and engagement of peripheral, low-affinity nondeleted $CD4^+$ T cells. In this regard, it has been reported that a T_H1 or T_H2 response correlates with the dose and MHC affinity of a model immunizing peptide, low dose/affinity being dependent on, and deviating primarily to, T_H1 responses (32). It is of interest, however, that BrdU in vivo labeling studies did not show reduced overall proliferation of $CD4^+$ cells in IFN-$\gamma^{-/-}$ *vs.* IFN-$\gamma^{+/+}$ *lpr* mice. This finding may indicate that helper responses to most antigens other than nuclear antigens may not be dependent on IFN-γ hyperproduction and MHC upregulation, an argument supported by the severe reduction of anti-DNA autoantibody levels in IFN-$\gamma^{-/-}$ *lpr* mice without a concomitant decrease in hypergammaglobulinemia.

A similar argument can be invoked for the equal $CD8^+$ T cell proliferation in IFN-$\gamma^{-/-}$and IFN-$\gamma^{+/+}$ *lpr* mice despite the severely reduced expression of MHC class I in the former instance. It is of interest, however, that proliferation of the DN $B220^+$ cells, the likely derivatives of $CD8^+$ cells (33, 34), was reduced in IFN-$\gamma^{-/-}$ mice. It is reasonable to suggest that because these cells lack coreceptor molecules, they probably require MHC class I upregulation in order to be optimally engaged, regardless of the nature of the antigen they recognize. Unlike DN cells, however, $CD4^+B220^+$ cells proliferated similarly in IFN-$\gamma^{+/+}$ and IFN-$\gamma^{-/-}$ mice. This subpopulation remained numerically low, however, indicating that their generation from $CD4^+$ precursors is compromised in the absence of IFN-γ.

Absence or reduction of IFN-γ may also affect local immune responses within the afflicted tissue. Thus, it has been suggested that increased MHC class II expression by renal proximal tubules in MRL-*lpr* mice could result in local presentation of autoantigens to circulating T cells which, in turn, could cause kidney damage either by cytotoxicity or by stimulation of B cells to produce autoantibodies (23). Assuming such a mechanism, the reduced tubular cell expression of MHC class II in IFN-$\gamma^{-/-}$ and even IFN-$\gamma^{+/-}$ MRL-*lpr* mice may play a role in disease amelioration.

Finally, the possibility that IFN-γ gene deletion affects autoimmunity by inefficient antigen processing/presentation or development of T and B cells may be considered. Modifications in these processes, however, appear unlikely since (*a*) most immune responses to exogenous antigens as well as T and B cell development were found to proceed normally in both IFN-$\gamma^{-/-}$ (13, 35) and IFN-$\gamma R^{-/-}$ (36) normal background mice, and (*b*) the degree of hypergammaglobulinemia was similar in IFN-$\gamma^{-/-}$ and wild-type MRL-*lpr* mice (present study).

Among the nonimmunologic mechanisms, enhanced monocyte activation and phagocytosis, oxidative stress and nitric oxide (NO) production are likely candidates. With regard to NO, it has been shown that IFN-γ is required for NO generation by monocytes (13). Previous studies have also shown increased production of NO in MRL-*lpr* mice (11, 37), and prevention of GN upon oral administration of a nitric oxide synthase (NOS) inhibitor (37).

Peng et al. (38) also reported findings with intercrosses of MRL-*lpr* × DBA-IFN-$\gamma^{-/-}$ wherein the crucial role of IFN-γ in autoantibody production and lymphadenopathy was evident. While the present study was under review, Haas et al. (39) also reported a dramatic reduction of GN in MRL-*lpr* mice homozygous for deletion of the IFN-γR. Unlike the present study wherein GN was reduced in IFN-$\gamma^{+/-}$ mice, however, GN in IFN-$\gamma R^{+/-}$ was equal to controls. It could be argued that in this latter case, enough receptors were available for effective IFN-γ–mediated signaling.

In conclusion, this study in conjunction with previous investigations (7–9, 38, 39) strongly points to the significant contribution of heightened IFN-γ production in the pathogenesis of murine lupus, and suggests that efforts to reduce its levels would be an important means to halt development and progression of this disease. As documented here, reduction in IFN-γ may selectively affect pathogenic autoimmune responses without significantly compromising the immune status of the host. Other cytokines such as TNFα (40), IL-10 (41), IL-4 (42, 43), and IL-12 (11) have also been experimentally manipulated to affect serologic and histologic manifestations of murine lupus. In this regard, the overall findings question the widely-held view that T_H2 cytokines play the primary role in this antibody-mediated autoimmune disease. This view has also been challenged in studies with other models of antibody-mediated autoimmune diseases such as experimental autoimmune myasthenia gravis, wherein IFN-γ was shown to be required (35). Further investigations into the effects of cytokines in lupus and other autoimmune diseases will have a strong impact on defining the mechanisms leading to these diseases and on devising new therapeutic strategies.

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