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Interferon-γ is required for lupus nephritis in mice treated with the hydrocarbon oil pristane

Hanno B. Richards, Minoru Satoh, J. Charles Jennette, Byron P. Croker, Hideo Yoshida, and Westley H. Reeves

Department of Medicine, Division of Rheumatology & Clinical Immunology and, Department of Pathology, University of Florida, Gainesville, Florida, and Department of Pathology, University of North Carolina, Chapel Hill, North Carolina, USA

Interferon-γ is required for lupus nephritis in mice treated with the hydrocarbon oil pristane.

Background. Although the precise mechanisms leading to lupus nephritis remain obscure, both T_H1 and T_H2 cytokines have been implicated. The present study examined the roles of interleukin (IL)-4 and interferon-γ (IFN-γ) in a novel inducible form of lupus that develops in non-autoimmune mice treated with the hydrocarbon oil pristane.

Methods. BALB/c IL-4 or IFN-γ deficient mice (IL-4 −/−, IFNγ −/−) and wild type controls (+/+) received either pristane or phosphate-buffered saline (PBS) IP. Serial sera were analyzed for anti-DNA/chromatin, anti-RNP/Sm, and total immunoglobulin levels. Proteinuria was measured and kidneys were examined by direct immunofluorescence and light microscopy.

Results. Renal disease did not develop in pristane-treated IFN-γ −/− mice, as assessed by the absence of capillary immune deposits, glomerular pathology and proteinuria whereas IL-4 −/− mice developed renal disease similar to +/+ mice. Production of IgG anti-single stranded DNA and anti-chromatin antibodies was abrogated in IFN-γ −/− mice. In contrast, these autoantibodies were produced at similar or higher frequencies and levels by IL-4 −/− versus wild-type mice. The frequency of anti-nRNP/Sm was markedly reduced in IFN-γ −/− mice. IL-4 deficiency had little effect on the production of anti-DNA/chromatin and anti-nRNP/Sm.

Conclusions. IFN-γ is essential for the induction of nephritis and anti-DNA/chromatin following pristane exposure in BALB/c mice, suggesting that genetic or environmental factors influencing T_H1-T_H2 balance could be an important determinant of renal disease in lupus.

Lupus nephritis is the prototype human immune complex disease [1]. It is associated with disease-specific autoantibodies, such as anti-double-stranded (ds) DNA and anti-Sm, which are enriched in glomerular eluates [2] and can have a direct pathogenic role in glomerulonephritis [3, 4]. Nephritis and autoantibodies characteristic of systemic lupus erythematosus (SLE) also occur spontaneously in certain strains of mice, such as NZB/W (F1) and MRL/*lpr* [4]. Murine lupus models facilitate studies of the relationship between autoantibody formation and end organ damage [4–6]. Like SLE, murine lupus has a strong genetic component [7]. However, there are differences between murine and human lupus. Although glomerular immune complex deposits can be found in virtually every lupus patient [8], only ~30 to 60% develop overt nephritis [9]. In contrast, nearly all NZB/W (F1) and MRL/*lpr* mice have advanced nephritis by six to eight months of age [10]. Moreover, the spectrum of autoantibodies produced by NZB/W mice and to a lesser degree MRL/*lpr* differs substantially from that of SLE.

Lupus also can be induced using the hydrocarbon oil pristane in non-autoimmune prone strains of mice, including BALB/c, SJL, C57BL/6 (B6), and others [11, 12]. The syndrome induced by pristane shares many of the characteristics of human lupus including both clinical features (arthritis, serositis, glomerular hypercellularity, glomerular IC deposition, proteinuria) and the autoantibody profile (anti-dsDNA, -nRNP/Sm, -Su, -chromatin, -histone) [13]. Following pristane treatment, 30 to 40% of BALB/c mice develop glomerular immunoglobulin and complement deposition, marked mesangial hypercellularity and proteinuria closely resembling lupus nephritis [14]. This frequency of overt nephritis is akin to human SLE. Although the precise mechanisms involved in the development of lupus-like disease in this model remain to be defined the profound imbalance in cytokine homeostasis induced by pristane [15] may be integral to the pathogenesis of pristane-induced lupus. Indeed, there is evidence that alteration in cytokine balance alone is sufficient to induce autoimmunity [16].

Many studies have shown that cytokines are involved in the pathogenesis of lupus in humans [17, 18] and mice

Key words: glomerulonephritis, antinuclear antibodies, pristane, immune complex disease, systemic lupus erythematosus.

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Table 1. Renal lesions in BALB/cJ mice 7.5 months after treatment

			% of mice positive								
Strain	Rx	*N*	IgG1[a]	IgG2a[a]	IgG2b[a]	IgG3[a]	IgM[a]	C3[a]	Cap[b]	LM[c]	Prot.[d]
+/+	Prist.	15	40	46	20	33	26	53	13	33	33
+/+	PBS	8	0	0	0	0	0	0	0	0	0
IL-4 −/−	Prist.	18	39[f]	73[f]	11	56[f]	73[f]	73[f]	28	56[f]	11
IL-4 −/−	PBS	6	0	0	0	0	0	0	0	0	0
IFN-γ −/−	Prist.	21	0[e]	10[e]	5	0[e]	24	24	0	5	0[e]
IFN-γ −/−	PBS	5	0	0	0	0	0	0	0	0	0

Abbreviations are: IL-4, interleukin-4; IFN-γ, interferon-gamma; Rx, treatment; Prist., pristane; PBS, phosphate-buffered saline; IgG, immunoglobulin G.
[a] Positive renal mesangial and/or capillary pattern immunofluorescence on frozen sections for IgG1, IgG2a, IgG2b, IgG3, IgM, or C3
[b] Presence of staining in a capillary pattern
[c] Presence of light microscopic (LM) changes by hematoxylin and eosin staining (H&E)
[d] Proteinuria (≥3+ or 300 mg/dL) 7.5 months after pristane treatment
[e] $P < 0.05$ +/+ vs. IFN-γ −/− pristane treated mice (Fisher exact test)
[f] $P < 0.05$ IFN-γ −/− vs. IL-4 −/− pristane treated mice (Fisher exact test)

[19–22]. But the importance of T_H1 versus T_H2 cytokines in lupus is controversial, perhaps reflecting heterogeneity of the disease. A more complete picture of lupus can be achieved by defining how cytokines shape the phenotype of lupus in several models. The goal of this study was to evaluate the effects of T_H1 versus T_H2 cytokines in pristane-induced lupus. We show that the proinflammatory cytokine IFN-γ plays a key role in the development of both autoantibodies and nephritis in pristane-treated mice.

METHODS

Mice

Female BALB/cJ IFN-γ–deficient (IFNγ −/−) or IL-4–deficient (IL-4 −/−) mice and age/sex matched controls (+/+) (Jackson Laboratory, Bar Harbor, ME, USA), age 10 to 12 weeks were housed in specific pathogen free conditions. They received 0.5 mL of pristane (2, 6, 10, 14-tetramethylpentadecane; Sigma Chemical Co., St. Louis, MO, USA) or an equal volume of PBS IP [14]. Serum samples were collected from the tail vein before treatment, two weeks later, and then at one-month intervals. At 7.5 months, kidneys were processed for light and immunofluorescence microscopy. Proteinuria was measured with Albustix (Miles Laboratories, Elkhart, IN, USA). Proteinuria ≥3+ (300 mg/dL) was considered significantly elevated.

Renal pathology

Renal lesions were graded in a blinded manner [23]. For light microscopy (LM), tissue was fixed in 4% paraformaldehyde and 3 μm paraffin sections were stained with periodic acid Schiff (PAS) and hematoxylin and eosin (H&E). Sections were graded as follows: 1+ = mild focal mesangial hypercellularity alone; 2+ = moderate mesangial hypercellularity; 3+ = complex endocapillary hypercellularity sometimes with mild sclerosis or necrosis; 4+ = severe endocapillary proliferative glomerulonephritis with necrosis or crescent formation. Scores ≥1+ were positive.

For immunofluorescence, tissue was embedded in OCT Compound (Miles) and 4 μm unfixed frozen sections were stained with 1:20 goat anti-mouse IgG1, -2a, -2b, or -3, or IgM (Southern Biotechnology, Birmingham, AL, USA) or with rabbit anti-mouse C3 (Cappel Laboratories, Durham, NC, USA). Glomerular staining was graded by intensity (0 = no staining, 4+ = maximum intensity staining) and pattern (predominantly mesangial vs. capillary plus mesangial). Background was defined as the strongest staining observed in PBS treated control mice (IFN-γ −/−, IL-4 −/− and +/+) and only staining above background was considered as positive. The Fisher exact test with Bonferoni's correction for multiple comparisons was used for statistical analysis.

Immunoprecipitation

Immunoprecipitation of [^{35}S] methionine labeled cell extract from K562 (human erythroleukemia) cells was performed as described [19].

Anti-nRNP/Sm ELISA

Anti-nRNP/Sm antigen capture ELISA was performed as described [24]. Briefly, the wells of microtiter plates were coated with monospecific purified human IgG anti-nRNP/Sm. Wells were washed and K562 cell lysate was added to half of the wells and buffer alone to the other half. After washing, mouse sera diluted 1:500 were added to the wells. Alkaline phosphatase-conjugated goat anti-mouse IgG (γ chain-specific; Southern Biotechnology) was used as second antibody. The plates were developed with p-nitrophenyl phosphate substrate and OD was read at 405 nm. To correct for non-specific binding, OD values obtained from the control wells (no antigen) were subtracted from the values from antigen-coated wells. Using Y2 (anti-Sm-B mAb) culture supernatant as a standard anti-nRNP/Sm activity (arbitrary units) for each sample was determined.

Anti-ssDNA and anti-chromatin ELISA

Anti-ssDNA, and anti-chromatin autoantibody levels were determined by enzyme-linked immunosorbent assay

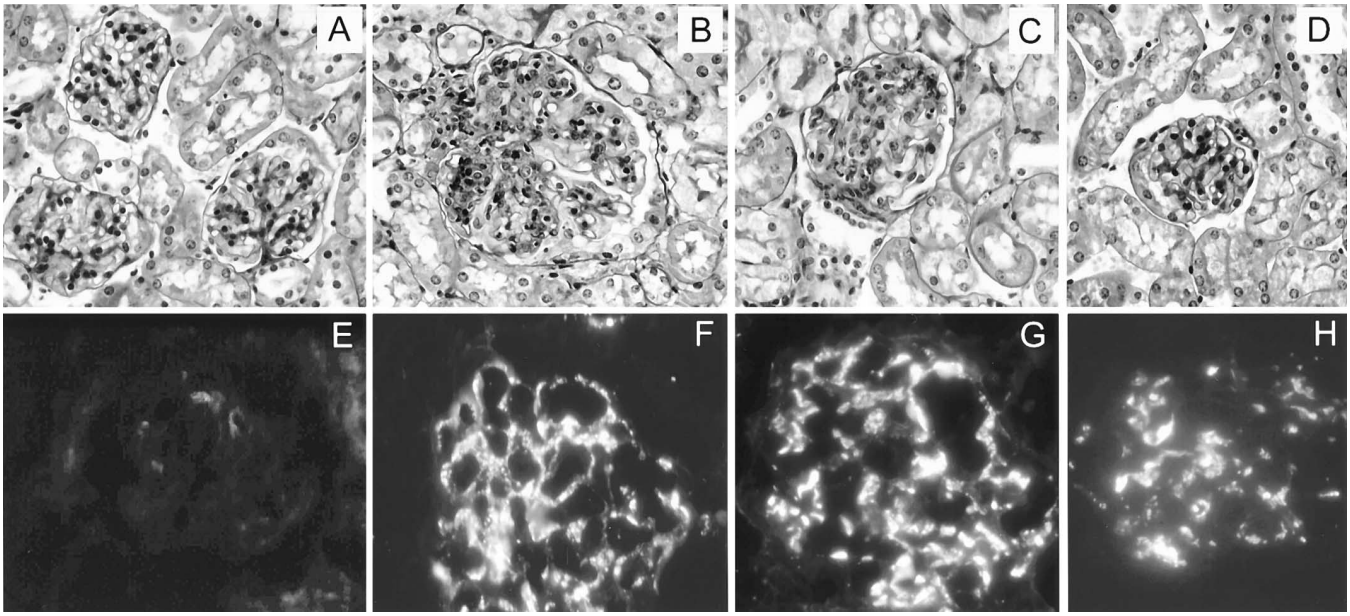

Fig. 1. Renal disease. (*A–D*) Light microscopy. Renal tissue from mice 7.5 months after treatment was fixed in 4% paraformaldehyde and 3 μm paraffin sections were stained with PAS. (A) PBS-treated +/+ with normal glomeruli. (B) Pristane-treated +/+ with extensive endocapillary hypercellularity, thickening of the capillary walls, obliteration of capillary lumens and segmental sclerosis (note glomerular enlargement). (C) Pristane-treated IL-4 −/− with well defined mesangial expansion. (D) pristane-treated IFN-γ −/− with a normal glomerulus. (*E–H*) Immunofluorescence. Four micrometer unfixed frozen sections 7.5 months after treatment were stained with 1:20 goat anti-mouse IgG2a. (E) PBS-treated +/+ with no significant staining for IgG2a. (F) Pristane-treated +/+ with marked capillary and mesangial IgG2a. (G) Pristane-treated IL-4 −/− with capillary and granular mesangial IgG2a. (H) Pristane-treated IFN-γ −/− with granular mesangial IgG2a.

(ELISA) using sera diluted 1:500 [19]. Secondary antibodies were alkaline phosphatase-conjugated goat anti-mouse IgG (1:1000 dilution; Southern Biotechnology). For the anti-chromatin ELISA, a high titer positive MRL/*lpr* serum served as a standard and the cutoff was the mean + 3 SD of 11 PBS-treated wild-type control mice for each time point.

Total IgG levels

Total levels of each isotype were determined by ELISA as described [19]. Microtiter plate wells were coated with goat anti-mouse κ/λ light chain antibodies (Southern Biotechnology). Sera were diluted 1:200,000 and added to the wells. After washing, the wells were incubated with alkaline phosphatase-labeled goat anti-mouse antibodies specific for IgG1 and 2a (1:1000 dilution, Southern Biotechnology), substrate was added, and OD determined at 405 nm as above. Isotype standards were used for standard curve fitting and Ig concentrations were calculated using Softmax software.

Anti-dsDNA assay

The *Crithidia luciliae* assay (The Binding Site, Birmingham, UK) was used to analyze sera (1:20 dilution) for anti-dsDNA antibodies [19]. Secondary antibody was FITC conjugated goat anti-mouse IgG (Southern Biotechnology).

RESULTS

T cells are central to the pathogenesis of lupus autoantibodies in pristane-treated mice [23]. To further define their role, we asked whether cytokines produced by helper T cell subsets are required for the induction of glomerulonephritis. For this purpose lupus-like disease was induced in IL-4– or IFN-γ–deficient BALB/c mice. No spontaneous nephritis or lupus-related autoantibody production have been reported in either of these strains [25, 26].

Pristane-induced lupus nephritis is IFN-γ dependent

The induction of renal disease was examined in mice that were alive at 7.5 months after pristane treatment. As in SLE patients and in marked contrast to the nearly 100% frequency of renal disease in NZB/W and MRL/*lpr* mice [10], pristane induced proteinuria, immune complex deposition, and histological changes in about one-third of BALB/c +/+ mice (Table 1 and Fig. 1 A, B, E, F). When compared with +/+ controls and IL-4 −/− mice, pristane-treated IFN-γ −/− mice had a markedly reduced frequency of immune deposits and capillary involvement was absent (Table 1 and Fig. 1 D, H). Only 1/21 pristane-treated IFN-γ −/− mice had mild focal mesangial hypercellularity. The remainder had no LM changes (Fig. 1D). IgG2a (Fig. 1H) deposits in a

predominantly mesangial pattern were found in only 2 of 21 pristane-treated IFN-γ −/− mice. None developed significant proteinuria (Table 1). Given their low levels of total IgG2a (Fig. 4), the presence of moderate glomerular IgG2a deposits in ~10% of IFN-γ −/−mice was unexpected. Specificity of the labeled antibody for IgG2a was verified using myeloma proteins (not shown).

Following pristane treatment, +/+ and IL-4 −/− mice had similar immune complex deposits, with the exception of IgM (Table 1 and Fig. 1 F, G). LM changes were most advanced in some of the pristane-treated +/+ mice in which diffuse proliferative glomerulonephritis with segmental necrosis was seen (Fig. 1B). LM changes in pristane-treated IL-4 −/− mice were somewhat milder with focal or widespread moderate mesangial hypercellularity (Fig. 1C). However, pathological findings were more common than in +/+ controls. There was a trend toward less frequent proteinuria in pristane-treated IL-4−/− mice that may reflect a decrease in glomerular filtration rate in this group. However, this trend was statistically not significant. Immune deposits and LM changes were not seen in PBS-treated mice (Table 1, Fig. 1 A, E). In contrast to MRL mice, tubulointerstitial changes were absent in pristane-treated mice as well as PBS controls.

Autoantibody production is IFN-γ dependent

Anti-DNA autoantibodies are associated with lupus nephritis in humans [4]. As shown in Figure 2A, some pristane-treated +/+ and IL-4 −/− mice had markedly elevated levels of IgG anti-ssDNA (ELISA) at 7.5 months. In contrast, IFN-γ −/− mice had very low levels, suggesting that IgG anti-ssDNA antibodies are IFN-γ dependent. More importantly, the frequency of IgG anti-dsDNA was much lower in pristane-treated IFN-γ −/− versus +/+ mice, whereas the absence of IL-4 had little or no effect (Table 2). Thus, IFN-γ, but not IL-4, deficiency markedly attenuated the production of autoantibodies implicated in the pathogenesis of lupus nephritis.

IgG anti-chromatin autoantibodies in pristane-induced lupus are highly IL-6 dependent [19] and correlate closely with nephritis (abstract; Richards, *Arthritis Rheum* 42: S361, 1999). Pristane induced IgG anti-chromatin antibodies at levels comparable to those in MRL/*lpr* mice in some of the +/+ and IL-4 −/− mice (Fig. 2B). Production of IgG anti-chromatin antibodies was dramatically accelerated by pristane treatment in IL-4 −/− mice (onset 2 to 3 months) vs. +/+ controls (onset ≥6 months; Fig. 2B, top). The frequency of anti-chromatin was also higher in the IL-4 −/− versus +/+ mice ($P < 0.05$, Fisher exact test; Table 2). Strikingly none of the pristane-treated IFN-γ −/− mice produced IgG anti-chromatin antibodies (Fig. 2B and Table 2). Some wild-type and IL-4 −/− mice showed low levels of spontaneous anti-chromatin activity (Fig. 2B, +/+ PBS and IL-4 −/− PBS). This also was absent in IFN-γ−/− mice, strongly suggesting that autoimmunity to chromatin requires IFN-γ as well as IL-6 [19]. There was a trend toward an association between nephritis and anti-chromatin in +/+ and IL-4 −/− mice, but this did not achieve statistical significance. No other autoantibody specificities were associated with renal disease.

Although not implicated in renal disease, anti-nRNP/Sm are highly characteristic of SLE [27]. Pristane induced anti-nRNP/Sm in 78% of +/+ mice (Table 2 and Fig. 2C, left). The frequencies in IL-4 −/− and +/+ mice were comparable, but levels were generally lower in the IL-4 −/− group (Fig. 2C, right). In contrast, the frequency of anti-nRNP/Sm in IFN-γ −/− mice was only 22% (5 of 22 mice, $P < 0.05$ vs. +/+ and IL-4 −/− groups, Fisher exact test). Remarkably, the positive mice had anti-nRNP/Sm levels comparable to those in +/+ controls (Fig. 2C, right), and they were readily detectable by immunoprecipitation (Fig. 3). Anti-nRNP/Sm antibodies also were readily detectable in IL-4 −/− mice (Fig. 3), suggesting that their avidity was high. Moreover, since the immunoprecipitates were absorbed onto protein A-Sepharose beads, the strong signal suggested that IgG2a and/or IgG2b anti-nRNP/Sm autoantibodies were produced in view of the poor binding of mouse IgG1 and IgG3 to protein A. Unexpectedly, given the strong dependence of this isotype on IFN-γ [28] and relative lack of IgG2a in IFN-γ −/− mice [26], anti-nRNP/Sm were predominantly IgG2a in IFN-γ −/− mice (not shown). Anti-Su autoantibodies were greatly decreased in IFN-γ −/− versus +/+ or IL-4 −/− mice, as well (Fig. 3 and Table 2). Spontaneous production of anti-nRNP/Sm or Su was not observed in PBS-treated mice by either immunoprecipitation or ELISA (not shown).

Pristane unexpectedly stimulates IgG2a production in IFN-γ −/− mice

Although IgG2a is strongly IFN-γ dependent [28], it was the predominant isotype of anti-nRNP/Sm autoantibodies and was enriched in the glomerular immune deposits of IFN-γ −/− mice. We therefore examined the effect of IFN-γ or IL-4 deficiency on total immunoglobulin levels. As expected, pristane treated +/+ mice markedly increased their IgG1 and IgG2a levels versus PBS-treated controls (Fig. 4). IgG2a showed the same pattern in IL-4 −/− mice, but IgG1 was not induced by pristane, consistent with its IL-4 dependence [28]. However, even though IgG2a was barely detectable in control IFN-γ −/− mice (Fig. 4, IFN-γ −/−, PBS), its production was enhanced by pristane.

DISCUSSION

Altered cytokine homeostasis is a feature common to human and experimental lupus. It has been proposed that SLE is mediated by T_H2 cytokines such as IL-10 and IL-4 [18, 29]. The situation is less clear in murine lupus. IL-4 transgenic B6 mice develop spontaneous im-

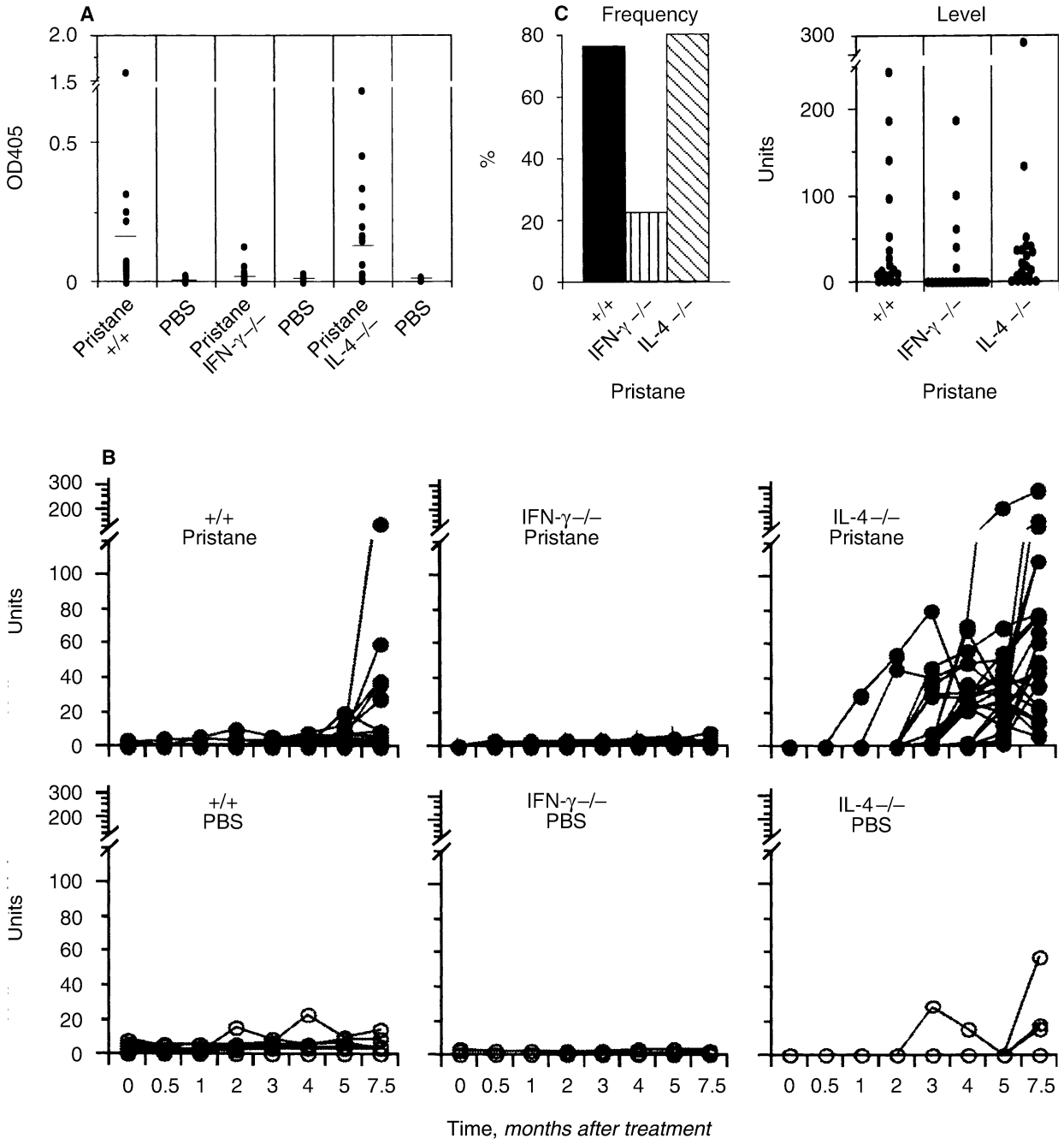

Fig. 2. Effect of pristane on autoantibody production in BALB/c mice. Sera were tested for autoantibodies (ELISA, 1:500 dilution) from baseline (0) to 7.5 months after pristane or PBS treatment. (*A*) Levels of serum IgG anti-ssDNA antibodies at 7.5 months. (*B*) Levels of anti-chromatin autoantibodies from 0 to 7.5 months. (*C*) IgG anti-nRNP/Sm activity. PBS-treated mice did not produce IgG anti-nRNP/Sm and are not shown; +/+ pristane ($N = 18$), +/+ PBS ($N = 11$); IFN-γ −/− pristane ($N = 22$); IFN-γ −/− PBS ($N = 6$); IL-4 −/− pristane ($N = 22$); IL-4 −/− PBS ($N = 6$).

mune complex nephritis [30], but IL-4 transgenic NZW/C57Bl/6Yaa mice are protected [31]. T_H1 cytokines have been linked to both the production of nephritogenic autoantibodies and renal disease in murine lupus nephritis [20–22, 32]. Thus, the precise role of cytokines in lupus nephritis is incompletely understood. The present study demonstrates that the T_H1 cytokine IFN-γ is critical for the development of nephritis as well as autoantibodies implicated in its pathogenesis in an inducible form of lupus caused by exposure to the hydrocarbon oil pristane.

Table 2. Frequency of autoantibodies in BALB/c mice

	+/+		IL-4 −/−		IFN-γ −/−	
	Pristane	PBS	Pristane	PBS	Pristane	PBS
Number of mice	18	11	22	6	22	6
α-chromatin	39%	0%	82%[b]	16%	0%[a]	0%
α-dsDNA	39%	0%	41%	0%	9%	0%
α-nRNP/Sm	78%	0%	82%	0%	22%[a]	0%
α-Su	56%	0%	68%	0%	17%[a]	0%

Sera were obtained 7.5 months after treatment were analyzed for IgG anti-chromatin (ELISA), anti-nRNP/Sm and anti-Su (ELISA and immunoprecipitation), and anti-dsDNA (Crithidia assay).

[a] $P < 0.05$ vs. pristane treated +/+ and IL-4 −/− groups, Fisher exact test

[b] $P < 0.05$ vs. pristane treated +/+ group, Fisher exact test

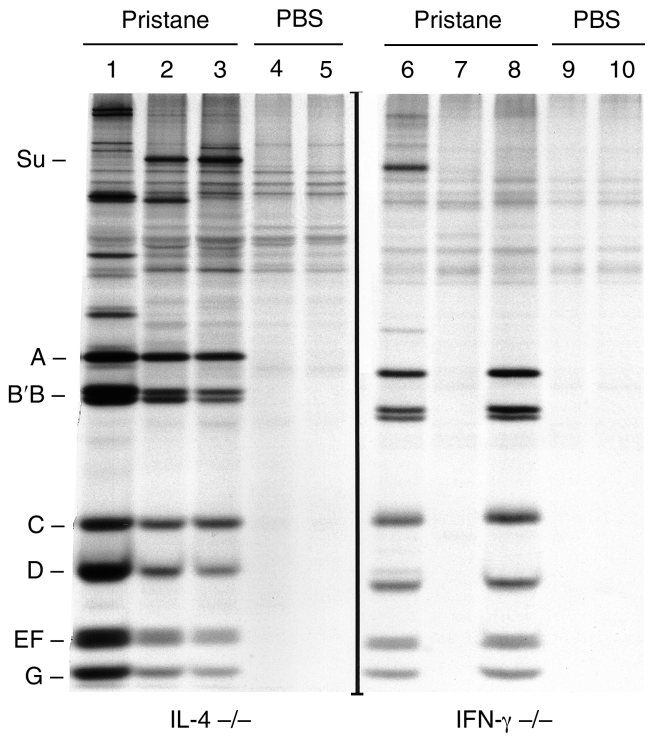

Fig. 3. Immunoprecipitation. Sera were analyzed by immunoprecipitation 7.5 months after treatment with pristane or PBS. Anti-nRNP/Sm (proteins A, B/B′, C, D, E/F and G) activity is apparent in lanes 1, 2, 3, 6 and 8 and anti-Su in lanes 2, 3 and 6. Lanes 1, 2, and 3 show representative samples from pristane-treated IL-4 −/− mice. Lanes 6 and 8 show 2 samples representative of the 5 IFN-γ −/− mice positive for anti-nRNP/Sm showing that these autoantibodies were readily detectable by immunoprecipitation. The increased intensity of non-specific bands in pristane-treated IL-4 −/− is due to higher levels of IgG2a in this group. All sera were tested and only representative samples are shown.

Pristane-induced lupus nephritis is attenuated by IFN-γ deficiency

Lupus nephritis induced by pristane resembles spontaneous lupus nephritis in MRL/*lpr* and NZB/W mice in its IFN-γ dependence. Histological changes and proteinuria were nearly absent in IFN-γ −/− mice following pristane treatment, and immune complexes were markedly reduced. Moreover, glomerular immune deposits in IFN-γ −/− mice were predominantly mesangial compared with the capillary deposits in +/+ and IL-4 −/− mice (Table 1 and Fig. 1 E-H). Our previous studies on pristane-induced nephritis confirmed that a mesangial immunofluorescence pattern correlates with mesangial electron dense deposits, whereas capillary staining correlates with subepithelial and subendothelial electron dense deposits [23]. Thus, IFN-γ has effects on the renal disease in pristane-induced lupus akin to those in MRL/*lpr* [20, 22] and NZB/W [32] mice. Whether this is due to reduced production of nephritogenic antibodies or other effects such as altered clearance of immune complexes remains to be determined. There are several types of lupus nephritis, consistent with different pathogenic mechanisms. It is clear that some forms of glomerulonephritis can develop in the absence of IFN-γ [33, 34], but further studies will be needed to determine if the pathogenesis of nephritis in pristane-treated mice differs in IFN-γ deficient versus wild type mice. In view of the subepithelial localization of immune complexes in BALB/c mice and the suggested Th2-mediated pathogenesis of membranous nephropathy [35], it is tempting to speculate that IFN-γ may promote capillary wall deposits with proliferative nephritis, whereas IFN-γ deficiency might tend to favor the development of membranous lesions. It is likely that the pathogenesis of the renal lesions in both pristane-induced and spontaneous lupus is multifactorial and the relative role of autoantibodies and local factors are yet to be defined. There may also be a role for the potential secondary effects of IFN-γ or IL-4 deficiency on the disease pathogenesis such as the increased susceptibility to certain pathogens and the relative shift in immunoglobulin levels.

It is noteworthy that newly emerging evidence points toward a critical role of Th1 cytokines in human lupus nephritis [36] and there is some data to suggest that IFN-γ may be involved specifically in proliferative nephritis [37]. Related interferons also may be involved in the pathogenesis of lupus as evidenced by the finding of lupus-like features in patients treated with interferon [38, 39].

Finally, although the relationship of pristane-induced lupus to SLE remains uncertain, it may be relevant that human hydrocarbon exposure has been reported to promote glomerulonephritis, but not other features of SLE [40].

Autoantibody production is IFN-γ dependent

Glomerular autoantibody deposition is thought to play an important role in the pathogenesis of lupus nephritis [1–3]. Anti-DNA, chromatin, nRNP/Sm, and Su autoantibody production was strongly regulated by IFN-γ in pristane-treated mice, suggesting that the development of nephritis may be modulated through cytokine effects

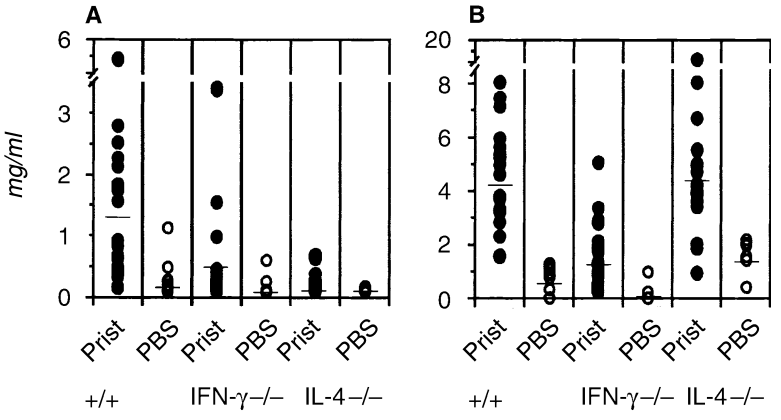

Fig. 4. Total immunoglobulin levels in IgG1 (*A*) and IgG2a (*B*). Sera obtained 7.5 months after treatment were analyzed for total IgG1and IgG2a (ELISA, 1:200,000 dilution). Levels are in mg/mL.

on the production of pathogenic autoantibodies. Interestingly, at least in the case of anti-nRNP/Sm, the primary effect was on the probability of autoantibody formation rather than on autoantibody levels (Fig. 2C).

Although IL-4 transgenic mice produce antinuclear antibodies [41] and mice with chronic graft-versus-host disease develop a T_H2 cytokine mediated lupus-like syndrome [42], IL-4 exerted a mild protective effect on autoimmunity in pristane-induced lupus. IL-4 −/− mice showed a trend toward higher autoantibody levels and more severe renal disease following pristane treatment than wild type controls. The effect was modest on a BALB/c background, but autoantibody levels were increased ~40-fold in IL-4 −/− B6 mice versus wild-type B6 controls (not shown). B6 mice are relatively resistant to the induction of nephritis by pristane, and nephritis could not be induced even in the absence of IL-4. Although suggesting that IL-4 protects against pristane-induced lupus, our data do not exclude the possibility that it might promote autoimmunity under other conditions.

The profound effect of IFN-γ deficiency on the induction of IgG anti-DNA/chromatin by pristane is consistent with the findings in spontaneous lupus [20, 21]. Anti-DNA levels are very low in IFN-γ −/− MRL/*lpr* [20, 21, 43] and NZB/W mice [44]. There is only one study addressing the role of IFN-γ and IL-4 on anti-nRNP/Sm [20]. Perhaps not surprisingly, the effects of IFN-γ on anti-nRNP/Sm autoantibody formation differ somewhat in pristane-induced lupus vs. the MRL/*lpr* model [20]. In pristane-treated mice, the frequency of anti-nRNP/Sm was reduced but the time of onset was similar in +/+ versus IFN-γ −/− mice. In contrast, in MRL/*lpr* mice, the onset was later and titers were reduced in IFN-γ −/− mice. This disparity may reflect different pathways of autoantibody formation in pristane-induced lupus versus *lpr* or *gld* disease [45]. These differences do not, however, exclude a unifying hypothesis for the role of IFN-γ for anti-nRNP/Sm production. Rather, they suggest that IFN-γ promotes the initiation of anti-nRNP/Sm responses whereas additional factors modulate the response. IL-6, which influences the level of anti-nRNP/Sm but not the frequency, is a likely candidate [19].

In summary, glomerulonephritis and the probability of autoantibody formation were IFN-γ dependent in pristane-induced lupus. In the case of anti-chromatin autoantibodies, the requirement for IFN-γ is more stringent than for anti-nRNP/Sm or Su. Together with recent findings in MRL/*lpr* and NZB/W mice, the present data support the importance of T_H1 cytokines in a subset of murine lupus. The development of anti-Sm and anti-DNA antibodies by IFN-γ transgenic mice lends further support to that idea [16]. The importance of IFN-γ in human SLE remains controversial. However, the therapeutic use of IFN-γ, especially in combination with IFN-α-2b, can induce lupus autoantibodies as well as disease [46], suggesting that IFN-γ promotes the development of lupus in a subset of humans as well as in mice.

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Reprint requests to Hanno B. Richards, M.D., Division of Rheumatology and Clinical Immunology, P.O. Box 100221, 1600 SW Archer Rd, University of Florida, Gainesville, Florida 32610-0221, USA. E-mail: richahb@medicine.ufl.edu

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