Kidney International, Vol. 64 (2003), pp. 897–905

Nephritogenic autoantibodies but absence of nephritis in Il-12p35–deficient mice with pristane-induced lupus

Nicola Calvani, Minoru Satoh, Byron P. Croker, Westley H. Reeves, and Hanno B. Richards

Department of Medicine, Division of Rheumatology & Clinical Immunology; Department of Pathology, Immunology and Laboratory Medicine, University of Florida; and Malcolm Randall VA Medical Center, Gainesville, Florida

Nephritogenic autoantibodies but absence of nephritis in Il-12p35–deficient mice with pristane-induced lupus.

Background. There is strong evidence that Th1 cytokines are essential for disease in murine models of lupus. Interleukin-12 (IL-12) is essential for Th1 cell differentiation and induces interferon-γ (IFN-γ) production. Paradoxically, it has been suggested that an IL-12 defect drives the pathogenesis of lupus, although its precise role remains unclear. We investigated the role of IL-12 for lupus-like disease induced by pristane. IL-12p35–deficient (−/−) and control (+/+) BALB/c mice were treated with pristane or phosphate-buffered saline (PBS).

Methods. Proteinuria was measured and renal pathology evaluated 10 months after treatment. Sera were analyzed for autoantibodies and total immunoglobulin levels. Cytokine expression and production was analyzed.

Results. Pristane induced nephritogenic autoantibodies and renal immunoglobulin and complement deposition in both IL-12 −/− and +/+ mice. However, proliferative pathology and proteinuria were absent in IL-12−/− mice, whereas pristane induced severe nephritis in one third of the +/+ mice. As expected, cytokine balance was skewed toward a Th2 response in pristane-treated IL-12 −/− mice.

Conclusion. These data indicate that renal immune complex deposition can occur in the absence of IL-12p35, but that structural renal damage requires the presence of IL-12p35 or mediators induced by this molecule, such as IFN-γ. In contrast to the abrogation of nephritogenic autoantibodies by the lack of IFN-γ, such antibodies are induced by pristane in IL-12p35–deficient mice. Absence of structural renal disease, despite the presence of nephritogenic autoantibodies in pristane-treated IL-12−/− mice, indicates that antibody deposition alone is not sufficient for the development of lupus nephritis in this model.

Lupus nephritis (LN) is a major contributor to morbidity and mortality in systemic lupus erythematosus (SLE). It is generally considered to be mediated by the glomerular deposition of immune complexes, initiating a cascade of inflammatory events and ultimately leading to tissue damage. Certain autoantibodies, in particular, anti-DNA/chromatin, are believed to be directly involved in the pathogenesis of LN [1]. Nevertheless, even very high levels of these autoantibodies do not obligatorily result in the development of LN, and other events, such as those mediated by Fc receptor signaling [2], play an essential role for the induction of nephritis. Although there is a substantial body of evidence linking the development of disease in SLE to Th1 cytokines [3–7], the prevailing paradigm remains that SLE is a Th2 cytokine–driven disease. In particular, it has been suggested that an interleukin-12 (IL-12) defect and relative abundance of Th2 cytokines is integral to the pathogenesis of both murine [8] and human lupus [9, 10].

Interleukin-12 is a 70 kD (p70) heterodimer composed of two disulfide-linked chains of 35 kD (p35) and 40 kD (p40), both of which are required for biological activity [11]. In conjunction with IL-18, IL-12 strongly promotes differentiation of naïve T cells into Th1 cells. IL-12 is produced mainly by antigen-presenting cells (APC), such as macrophages and dendritic cells. Mice lacking IL-12 have a relative defect but are not entirely devoid of Th1 responses [12]. In the absence of IL-12, interferon-γ (IFN-γ) production can be induced through IL-18 signaling [13], although this process is thought to require the presence of other cytokines, such as IL-2 [14].

To further define the role of IL-12 in SLE we induced lupus-like disease in IL-12p35–deficient (−/−) mice with the hydrocarbon oil pristane [6]. Lupus-like nephritis is induced by pristane in about one third of BALB/c mice after intraperitoneal administration, akin to the frequency of nephritis in humans with SLE [15]. We show here that IL-12p35 is essential for the development of structural renal damage induced by pristane. However, immune complex deposits and nephritogenic autoantibodies were induced in both IL-12–deficient mice and control mice. These results further support the idea that LN is Th1 mediated and argue that nephritogenic antibodies alone are insufficient to generate nephritis in pristane-induced lupus.

Key words: SLE, IL-12, nephritis, autoantibodies, pristane.

Received for publication January 31, 2003
and in revised form March 13, 2003, and April 9, 2003
Accepted for publication May 6, 2003

METHODS

Mice

Female BALB/c J IL-12p35 −/− mice and age-/sex-matched control mice (+/+) (Jackson Laboratory, Bar Harbor, ME, USA), aged 10 to 12 weeks, housed under specific pathogen-free (SPF) conditions, were injected once intraperitoneally with either 0.5 mL of pristane (2, 6, 10, 14-tetramethylpentadecane, Sigma Chemical Co., St. Louis, MO, USA) or an equal volume of phosphate-buffered saline (PBS) [6]. Serum samples were collected from a limited number of animals from the tail vein before treatment, 2 weeks later, and then at 1-month intervals. At 10 months, mice were euthanized and kidneys and spleens were removed. Proteinuria was measured at 10 months with Albustix (Bayer, Elkhart, IN, USA). Only values ≥3+ (300 mg/dL) were graded as abnormal.

Renal pathology

All samples were evaluated by a single renal pathologist (B.P.C.) in a blinded manner as previously reported [16]. For light microscopy (LM), tissue was fixed in 4% paraformaldehyde and 3 μm paraffin sections were stained with hematoxylin and eosin (H&E) and periodic acid-Schiff (PAS). Mice were scored as having glomerulonephritis only when >50% of their glomeruli showed a qualitative (segmental and/or global mesangial, or proliferative) lesion on multiple sections.

For immunofluorescence (IF), tissue was embedded in OCT compound (Sakura, Japan) and 4 μm unfixed frozen sections were stained with 1:20 fluoroscein isothiocyanate (FITC)-conjugated goat anti-mouse immunoglobulin (Ig) G1, 2a, 2b, or 3, or IgM (Southern Biotechnology, Birmingham, AL, USA), or with rabbit anti-mouse C3 (Cappel, Durham, NC, USA). Glomerular staining was graded according to intensity on a 0 to 4+ scale (0, no staining, 4+, maximum intensity staining) and the pattern of staining (predominantly mesangial vs. capillary) was recorded. Background was defined as the strongest level of staining observed in PBS-treated +/+ mice and only staining above background was considered positive.

Immunoprecipitation

Autoantibodies to cellular proteins were analyzed by immunoprecipitation of [^{35}S] methionine-labeled cell extract from K562 (human erythroleukemia) cells, as previously described [6]. Specificity of autoantibodies was confirmed using murine prototype sera for nRNP/Sm and Su.

ELISA

Sera were tested for anti-nRNP/Sm and anti-chromatin autoantibodies as previously described. For the anti-nRNP/Sm enzyme-linked immunosorbent assay (ELISA) [6], sera were tested at 1:500 dilution and Y2 culture supernatant served as standard (IgG2a anti-Sm-B + D mAb). Alkaline phosphatase–conjugated goat anti-mouse IgG (1:1000 dilution, Southern Biotechnology) was used as second antibody.

For the anti-chromatin ELISA [17] sera were diluted 1:500 and high titer–positive MRL/*lpr* serum served as standard. Binding was detected using alkaline phosphatase–conjugated goat anti-mouse IgG (Southern Biotechnology). The cutoff for positive was the mean + 3 standard deviation (SD) of PBS-treated +/+ mice for each time point.

For the anti-dsDNA ELISA, wells of microtiter plates (Nunc Maxisorp; Nunc, Naperville, IL, USA) were coated with 50 μL of 20 μg/mL plasmid DNA and incubated with sera at a dilution of 1:200. Alkaline phosphatase–labeled goat anti-mouse IgG (Southern Biotechnology) served as second antibody. A high titer–positive MRL/*lpr* serum served as standard.

Total levels of IgG1 and IgG2a were measured by sandwich ELISA as previously described [6].

ELISAs for cytokines were performed on culture supernatants of splenocytes using rat mAb pairs for mouse IFN-γ and IL-4 (PharMingen, San Diego, CA, USA). Samples were added undiluted and purified mouse cytokines served as standards (PharMingen). After incubation with biotinylated rat anti-mouse cytokine-specific antibodies, 100 μL/well of 1:1000 streptavidin-alkaline phosphatase (Southern Biotechnology) was added, and the reaction was developed.

Cytokine RT-PCR

Total RNA from freshly isolated splenocytes was prepared by TRIzol™ Reagent (Invitrogen, Carlsbad, CA, USA) and mRNA expression for various cytokines was detected using the ThermoScript™ reverse transcription-polymerase chain reaction (RT-PCR) System (Invitrogen). For each sample, 1 μg of total RNA was reverse transcribed and control reactions lacking reverse transcriptase were carried out to exclude contamination with genomic DNA. Amplification of cDNA was carried out in a thermocycler with 0.5 units of Taq DNA polymerase in a 20 μL reaction. Amplification conditions were 94°C for 1 minute, 60°C for 1 minute, 72°C for 1.5 minutes, and 72°C for 10 minutes as the final extension. Cycle numbers were optimized for each primer pair. PCR products were analyzed on 3% agarose gels and visualized by ethidium bromide staining. Primer sequences were as follows: mouse IL-12p35 sense (5′-GCAAGAGACA CAGTCCTGGG-3′), antisense (5′-TGCATCAGCTC ATCGATGGC-3′), yielding a PCR product of 617 bp; mouse IL-12p40 sense (5′-GAGGTGGACTGGACTC CCGA-3′), antisense (5′-CAAGTTCTTGGGCGGGT CTG-3′), yielding a product of 617 bp; mouse IL-4 sense (5′-CGAAGAACACCACAGAGAGTGAGCT-3′), anti-

Table 1. Renal lesions in BALB/cJ mice 10 months after treatment (% of mice positive)

	N	IgG1[a]	IgG2a[a]	IgG2b[a]	IgG3[a]	IgM[a]	C3[a]	Cap[b]	LM[c]	Proteinuria[d]
+/+ Pristane	28	50	64	21	46	25	68	25	29	29
+/+ PBS	13	0	0	0	0	0	0	0	0	0
IL-12 −/− Pristane	20	45	45	35	70	10	40	5	0[e]	0[e]
IL-12 −/− PBS	5	0	0	0	0	0	0	0	0	0

PBS is phosphate-buffered saline.
[a] Positive glomerular immunofluorescence on frozen sections for IgG1, IgG2a, IgG2b, IgG3, IgM, or C3
[b] Presence of staining in a capillary pattern
[c] Presence of light microscopy proliferative changes by periodic acid-Schiff
[d] Only values ≥3+ (300 mg/dL) at 10 months were considered elevated
[e] $P < 0.05$ vs. +/+ pristane-treated mice (Fisher exact test)

sense (5′-GACTCATTCATGGTGCAGCTTATCG-3′), yielding a 180 bp product; mouse IFN-γ sense (5′-AGC GGCTGACTGAACTCAGATTGTAG-3′) antisense (5′-GTCACAGTTTTCAGCTGTATAGGG-3′), yield ing a PCR product of 246 bp; mouse IL-1 8 sense (5′-ACTGTACAACCGCAGTAATACGG-3′) antisense (5′-TCCATCTTGTTGTGTCCTGG-3′), yielding a 319 bp product. Each reverse transcribed mRNA was internally controlled with β-actin using the primers mouse sense (5′-TGGAATCCTGTGGCATCCATGAAAC-3′) and antisense (5′-TAAAACGCAGCTCAGTAACAG TCCG-3′), yielding a PCR fragment of 348 bp.

Indirect IF

The Crithidia luciliae assay (The Binding Site, Birmingham, UK) was used to analyze sera (1:20 dilution) for anti-ds DNA. The second antibody was FITC-conjugated goat anti-mouse IgG (1:60 dilution; Southern Biotechnology).

Statistical analysis

Frequencies of renal lesions and autoantibodies were compared by Fisher exact test. Mann-Whitney test was used to compare levels of autoantibodies and cytokines.

RESULTS

Pristane treatment induces nephritis and production of autoantibodies associated with SLE in non-autoimmune mice [18]. Development of nephritis and production of certain autoantibodies is IFN-γ dependent not only in pristane-induced lupus [6] but also in MRL/*lpr* [4, 5, 19] and NZB/W mice [20], suggesting that lupus-like disease is Th1 driven. IL-12 is one of the most powerful inducers of a Th1 immune response. In this study, we investigated the role of this cytokine for the pathogenesis of lupus-like disease induced by the hydrocarbon oil pristane.

IL-12 is essential for the development of pristane-induced nephritis

The effect of IL-12 on pristane-induced nephritis was examined 10 months after treatment. This time point was chosen to ensure that all mice originally entered into the study were still alive. In previous experiments we found that some BALB/c +/+ mice can die of nephritis after 10 months. As expected, pristane induced diffuse proliferative glomerulonephritis in about one third of +/+ mice, all of which also had proteinuria (Table 1, Fig. 1). In contrast to this, none of pristane-treated IL-12 −/− mice had LM changes (Table 1, Fig. 1), and none of these mice developed significant proteinuria (Table 1). Surprisingly, immune complex deposition was similar in both +/+ and IL-12 −/− mice and, although capillary staining was more common in +/+ mice (Table 1, Fig. 1), this difference was not statistically significant. Mice with staining for IgG commonly were positive for all IgG subclasses and had C3 deposits. No LM changes or immune deposits were seen in any of the PBS-treated mice (Table 1, Fig. 1). Tubulo-interstitial pathology was not present in either pristane- or PBS-treated mice in any group.

IL-12 is not required for the induction of nephritogenic autoantibodies

The absence of IL-12 did not affect the production of nephritogenic autoantibodies, and there was no statistically significant difference in frequency of both IgG anti-dsDNA and anti-chromatin between pristane-treated IL-12 −/− and +/+ mice after 6 months (Table 2). Unexpectedly, anti-dsDNA was found more commonly in IL-12 −/− mice (Fig. 2). To confirm this result we compared the measurement of anti-dsDNA by ELISA with the Crithidia luciliae assay for all samples (Table 2, Fig. 2). Anti-dsDNA ELISA is generally less specific and tends to produce more false-positive results, whereas the Crithidia assay has higher specificity [21]. We found that by either method there was no significant difference in pristane-induced anti-dsDNA between IL-12 −/− and +/+ mice (Fig. 2). Although the frequency of anti-chromatin antibodies was similar in both groups of mice, the onset of anti-chromatin production may have been somewhat delayed in pristane-treated IL-12−/− mice (Fig. 3).

In contrast to the effect on anti-dsDNA and chromatin, pristane induced production of IgG anti-nRNP auto-

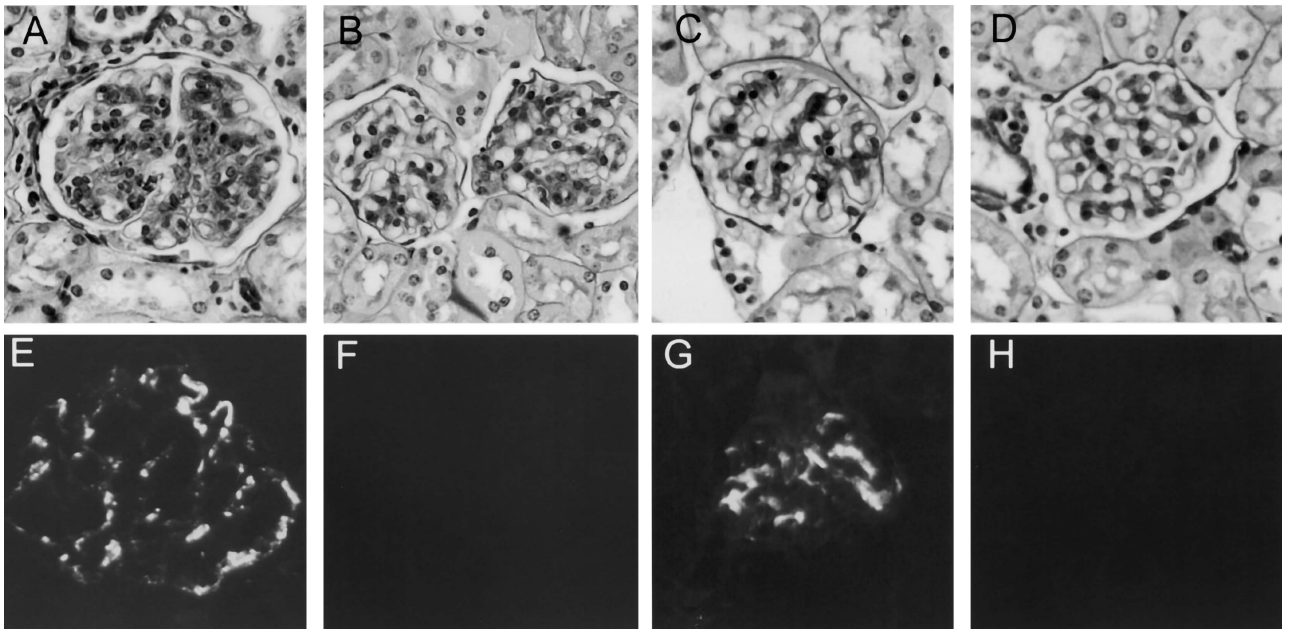

Fig. 1. Light microscopy (LM) and direct immunofluorescence (IF). Renal tissue was obtained from mice 10 months after treatment and fixed in 4% paraformaldehyde. Sections (3 μm) were stained with periodic acid-Schiff (PAS) for LM. Unfixed frozen sections were stained by direct IF with fluoroscein isothiocyanate (FITC)-conjugated goat anti-mouse immunoglobulin (Ig) G2a. Photomicrographs were taken using a 40× lens for all samples. (*A*) Pristane-treated +/+ mice with extensive endocapillary and mesangial hypercellularity, leukocyte infiltration, and thickening of the capillary walls. (*B*) Phosphate-buffered saline (PBS)-treated +/+ mice with normal glomeruli. (*C*) pristane-treated IL-12 −/− mice with normal glomerulus, (*D*) PBS-treated IL-12 −/− mice with normal glomerulus, (*E*) pristane-treated +/+ mice with marked capillary IgG2a deposits, (*F*) PBS-treated +/+ mice without staining for IgG2a, (*G*) pristane-treated IL-12 −/− mice with mesangial IgG2a deposits, (*H*) PBS-treated IL-12 −/− mice with no significant staining for IgG2a.

antibodies in 80% of +/+ mice at 6 months versus 21% in IL-12 −/− mice ($P < 0.05$, Fisher exact test) (Table 2). Moreover, levels of IgG anti-nRNP/Sm were dramatically lower in pristane-treated IL-12 −/− mice compared to pristane-treated +/+ mice ($P < 0.05$, Mann-Whitney test) (Fig. 4, left). Frequencies of IgG anti-Su (immunoprecipitation assay) were similar in pristane-treated IL-12 −/− and +/+ mice (Table 2). Spontaneous production of IgG anti-nRNP/Sm was not observed by ELISA (data not shown) or immunoprecipitation (Fig. 4, right) in PBS-treated mice.

Table 2. Frequencies of autoantibodies in BALB/cJ mice 6 months after treatment

	+/+		IL-12 −/−	
	Pristane ($N = 15$)	PBS ($N = 6$)	Pristane ($N = 14$)	PBS ($N = 5$)
Anti-chromatin	80%	0%	57%	0%
Anti-dsDNA[a]	14%	0%	43%	0%
Anti-nRNP/Sm	80%	0%	21%[b]	0%
Anti-Su	26%	0%	21%	0%

PBS is phosphate-buffered saline. Note: serum was not available for all mice.
[a] Measured by crithidia assay
[b] $P < 0.05$ vs. pristane treated +/+ mice (Fisher exact test)

Pristane-induced hypergammaglobulinemia is reduced in IL-12 −/− mice

Given the association of pristane-induced lupus with marked hypergammaglobulinemia [22], it was of interest to examine the influence of IL-12 deficiency on total IgG production. As expected, pristane-treated +/+ mice had elevated IgG1 and IgG2a levels by 6 months after treatment when compared with PBS-treated +/+ mice (Fig. 5). Pristane-induced hypergammaglobulinemia was significantly reduced in IL-12 −/− mice with lower levels of both total IgG1 and IgG2a versus +/+ mice ($P < 0.05$, Mann-Whitney test) (Fig. 5).

Cytokine production

Levels of IFN-γ and IL-4 were examined in splenocyte culture supernatants after 10 months of treatment (Fig. 6A). IFN-γ production was similar in PBS-treated IL-12 −/− mice and PBS-treated +/+ mice, confirming that IL-12 −/− mice are not devoid of IFN-γ production [12]. Pristane treatment enhanced IFN-γ expression in +/+ mice, whereas IFN-γ levels remained unchanged in IL-12 −/− mice. In contrast, pristane markedly enhanced IL-4 production in IL-12 −/− mice compared to PBS-treated IL-12 −/− ($P < 0.05$, Mann-Whitney test) and pristane-treated +/+ mice. We also examined expression of key cytokines of interest by RT-PCR on RNA from splenocytes (Fig. 6B). Pristane preferentially induced IL-4 expression in IL-12−/− mice, whereas both IFN-γ and IL-18 expression were similar in all groups. IL-12p40 appeared to be up-regulated by pristane in both groups.

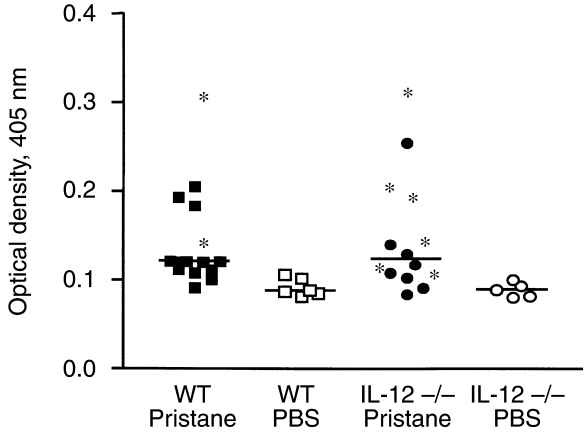

Fig. 2. Immunoglobulin G (IgG) anti-dsDNA. Sera from mice obtained 6 months after treatment were analyzed for IgG anti-dsDNA by enzyme-linked immunosorbent assay (ELISA). Results are shown in optical density (OD). Horizontal lines indicate the mean levels in each group. Levels of anti-dsDNA are not different in pristane-treated IL-12 −/− mice and +/+ mice after 6 months of treatment. Samples that were also positive for anti-dsDNA by Crithidia luciliae assay are denoted by an asterisk.

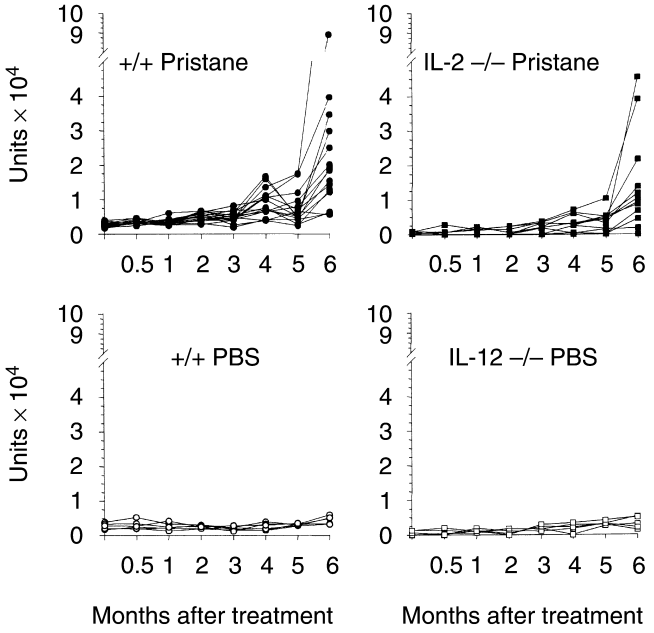

Fig. 3. Immunoglobulin G (IgG) anti-chromatin autoantibodies. Sera from mice obtained before treatment, at 0.5 months, and monthly thereafter were analyzed for anti-chromatin by enzyme-linked immunosorbent assay (ELISA). Results are shown in units.

Absence of IL-12p35 message in the targeted mutants was also demonstrated (Fig. 6B). These data confirm that cytokine balance is fundamentally skewed toward a Th2 response in pristane-treated IL-12 −/− mice.

DISCUSSION

Study of the pathogenesis of SLE has led to growing appreciation of the complex role of cytokines in the regulation of autoimmune renal injury in SLE. It has become evident that alteration in cytokine milieu is a common feature, both in human and experimental lupus. Although LN has been thought to be Th2 mediated [23, 24], there is now strong evidence linking Th1 cytokine production to induction of nephritogenic autoantibodies and renal disease [3, 5, 19, 20]. Adding to this evidence we recently reported that IFN-γ −/− mice are resistant to LN after pristane treatment, whereas the absence of IL-4 has no influence on the development of renal disease [6].

IL-12 directs the differentiation of naïve T lymphocytes toward Th1 cells by inducing IFN-γ [25] and inhibiting the development of IL-4–producing Th2 cells [26]. The role of IL-12 in the pathogenesis of SLE remains unclear. It has been proposed that a defect in IL-12 production by macrophages is essential for disease in lupus-prone MRL and NZB/W mice [8]. Down-regulation of IL-12 is thought to reflect increased disease activity of SLE in humans [9] and is believed to be related to the development of LN [10]. In contrast to these findings others have reported significant elevations of serum IL-12 levels in MRL/*lpr* mice and found that recombinant IL-12 injection leads to accelerated glomerulonephritis [27]. Moreover, it has been suggested that IL-12 plays a key role in the pathogenesis of autoimmune disease in MRL/*lpr* mice by promoting the differentiation of CD4+ T cells into Th1 cells [28].

Pristane-induced LN is IL-12 dependent

Our results indicate that IL-12p35 is essential for the development of structural renal damage in pristane-induced LN. None of the 20 pristane-treated IL-12 −/− animals showed changes in glomerular morphology, and proteinuria was absent (Table 1). This was the case despite a similar degree of immune complex deposition in both IL-12 +/+ and −/− mice. However, glomerular immune deposits in IL-12 −/− mice were mostly in a mesangial pattern (Fig. 1), whereas capillary deposits were seen more frequently in the +/+ group (Fig. 1), suggesting the presence of subepithelial and/or subendothelial deposits as shown previously [18]. Particularly, the subendothelial immune deposits may be a prerequisite for the development of proliferative glomerular pathology.

Our data further underscore the importance of Th1 responses for pristane-induced LN [6], as pristane treatment enhanced IFN-γ expression in +/+ mice (Fig. 6). Interestingly, IL-12 −/− mice were not devoid of IFN-γ production (Fig. 6, PBS-treated IL-12 −/− group), probably due to IL-18 production [29]. IFN-γ levels remained unchanged after pristane treatment. It is perhaps noteworthy that despite significant levels of IFN-γ RNA at

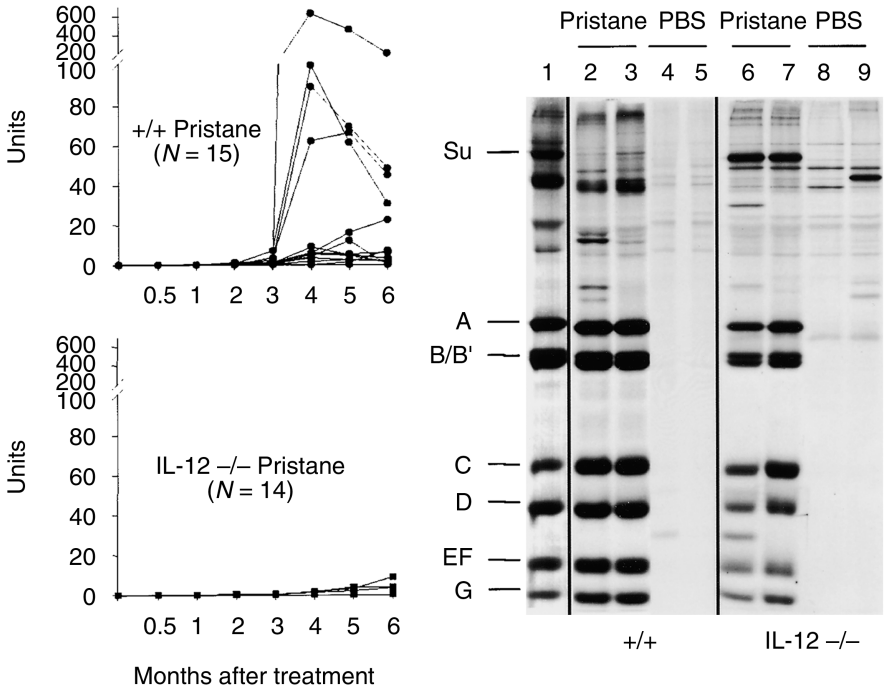

Fig. 4. Immunoglobulin G (IgG) anti-nRNP/Sm autoantibodies. Sera from mice obtained before treatment, at 0.5 months, and monthly thereafter were analyzed for IgG anti-nRNP/Sm by enzyme-linked immunosorbent assay (ELISA) (left). Results are shown in units. Phosphate-buffered saline (PBS)-treated mice did not produce anti-nRNP/Sm and are not shown. Sera obtained 6 months after treatment were analyzed by immunoprecipitation. Representative samples are shown (right). Lane 1 shows a reference serum immunoprecipitating the characteristic bands for anti-nRNP/Sm (A, B/B′, C, D, EF, and G) and the characteristic 110 kD band for anti-Su. Lanes 2, 3, 6, and 7 show bands for anti-nRNP/Sm, and lanes 6 and 7 are positive for anti-Su.

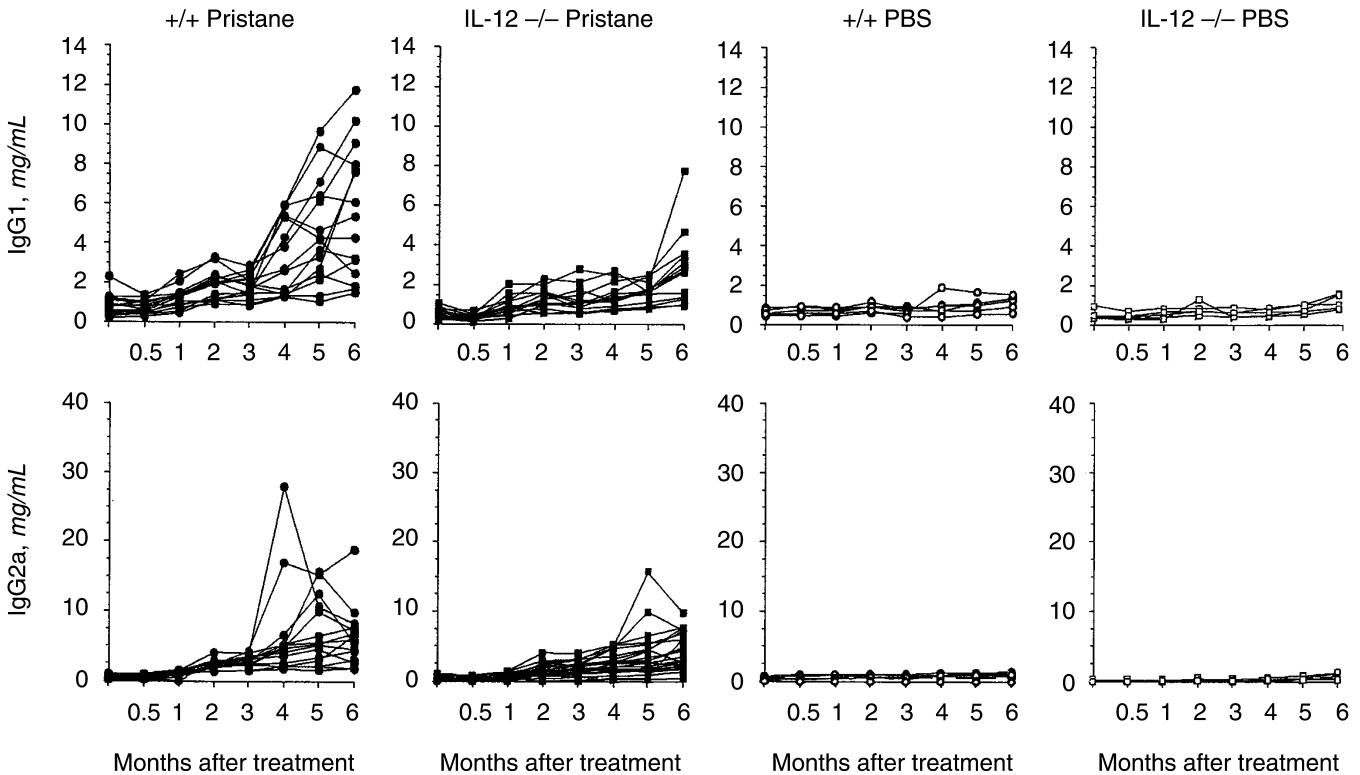

Fig. 5. Total immunoglobulin (Ig) levels. Sera from mice obtained before treatment, at 0.5 months, and monthly thereafter were analyzed for IgG1 and IgG2a by enzyme-linked immunosorbent assay (ELISA). Results are shown in mg/mL.

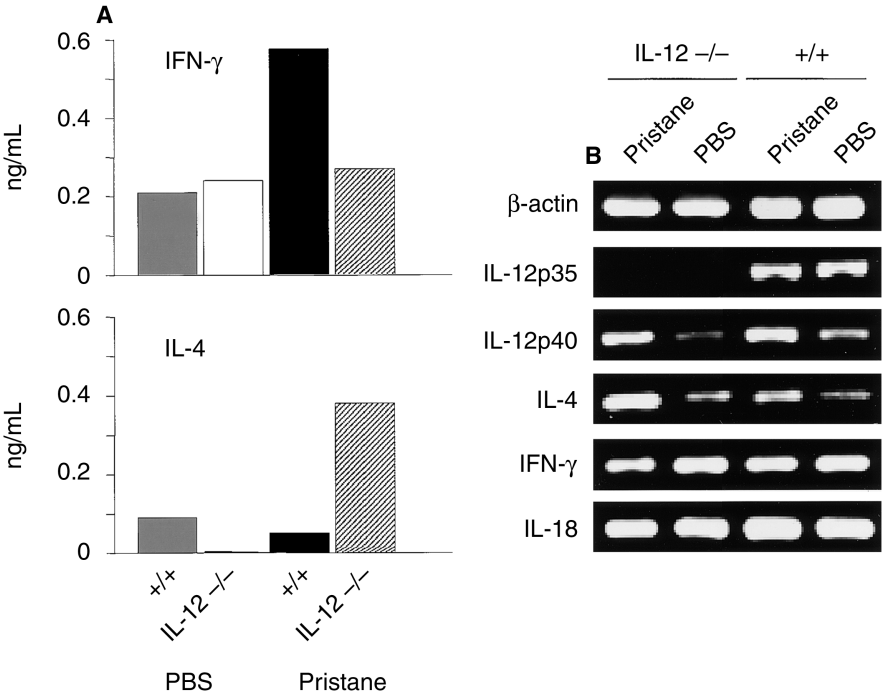

Fig. 6. Cytokine production. (*A*) Splenocytes from 10-month-old mice were cultured for 48 hours in the presence of 2 μg/mL anti-CD3. Supernatants were removed and activity of interferon-γ (IFN-γ) and interleukin-4 (IL-4) was analyzed by enzyme-linked immunosorbent assay (ELISA). Median values are shown in ng/mL. (*B*) Total RNA was extracted from freshly isolated splenocytes and cytokine expression was analyzed by reverse transcription-polymerase chain reaction (RT-PCR). β-actin served as control.

baseline (Fig. 6B), production of the protein was only minimally enhanced following anti-CD3 stimulation (Fig. 6A). This points toward the requirement for both IL-12 and IL-18 to synergistically promote IFN-γ production. Also of interest was the increased level of IL-12p40 RNA following pristine treatment in both and IL-12p35 +/+ and −/− mice. This suggests that pristane may have an effect on either the transcriptional rate of p40 mRNA or on its stability. In contrast, pristane enhanced IL-4 production in IL-12 −/− mice (Fig. 6), suggesting that a Th2-skewed cytokine profile in the absence of IL-12 may possibly prevent the onset of LN. This amelioration of nephritis may have therapeutic implications.

In favor of an involvement of IL-12 in murine lupus–related renal disease, it has been reported that intrarenal IL-12 production is up-regulated in MRL/*lpr* mice, leading to enhanced IFN-γ expression and promoting the development of a renal-specific Th1 immune response [30]. In addition, local provision of IL-12 using an ex vivo retroviral gene transfer strategy enhances renal damage by fostering the expansion of IFN-γ–secreting CD4+ T cells within the kidney in MRL/*lpr* mice [31]. The role of IL-12 in human SLE remains controversial. Decreased production of this cytokine was reported in stimulated lymphocytes from 10 patients with recent-onset active lupus, 9 of who had renal involvement [9]. Another group reported decreased IL-12 and IFN-γ but increased IL-4 and IL-10 in stimulated peripheral blood mononuclear cells [10]. There were no differences in serum IL-12 levels between normal patients and patients with SLE. In contrast to this, others have found high serum levels of IL-12 and IFN-γ in SLE patients, but no significant correlation with the presence of nephritis [32]. These discrepancies may reflect the heterogeneity of the disease or the differences in racial composition of the study population. Further confounding the interpretation of these studies, stimulatory effects of peripheral blood mononuclear cells (PBMC) separation on lymphocytes [33], and the presence of rheumatoid factors in the sera of patients with SLE [34] may directly interfere with the cytokine assays used in the above studies. Future studies exploring the roles of IL-12 and IFN-γ in human LN seem warranted. Until such studies become available it will remain unclear whether human LN, like murine LN, is mediated by Th1 cytokines or whether the pathogenesis of the disease is different in humans and mice.

Lack of relationship between nephritogenic antibodies, immune complex deposition, and structural renal damage

Elevated anti-dsDNA levels with a corresponding depression of total hemolytic complement mark active nephritis [35, 36]. Similarly anti-chromatin antibodies have been linked to LN [1]. However, the pathogenic relevance of these antibodies is much debated and it has been suggested that the presence of such antibodies alone is not sufficient for the development of nephritis. Indeed, nephritis in the absence of anti-DNA autoantibodies is seen in both murine and human lupus [37–39]. Glomerular deposits of immunoglobulins and complement com-

ponents are a hallmark of LN. Nevertheless, there is evidence that the presence of certain mediators, such as Fc receptors, is required for the pathogenic effects of such deposits.

Our results support the idea that the presence of immune deposits and nephritogenic antibodies is insufficient to lead to nephritis. We speculate that either local effects of IL-12 itself, or effects mediated through IFN-γ, are required for the development of renal tissue damage. The latter is known to enhance cell adhesion molecule expression on glomerular cells [40], which in turn may facilitate influx of inflammatory cells. Alternatively, the slightly altered isotype distribution of the glomerular immune deposits brought about by lack of IL-12 (Table 1), leading to decreased Fc receptor triggering, may explain the lack of proliferative disease in IL-12 −/− mice. It is further possible that the relative predominance of Th2 cytokines and/or of Th2 T cells in IL-12 −/− mice downmodulates the inflammatory response in the kidney.

CONCLUSION

We have established that IL-12 is essential for the development of pristane-induced nephritis, whereas immune complex deposits and nephritogenic autoantibodies are independent of IL-12. Pristane-induced anti-nRNP/Sm is, in turn, IL-12–dependent. Future studies are needed to determine the relevance of these observations to human LN. Presently available information implicates Th1 cytokines in murine disease and Th2 cytokines in human disease. It will be important to know if this represents a fundamental difference between the pathogenesis of human and murine lupus, or only an apparent dichotomy.

ACKNOWLEDGMENTS

This work was supported by the National Institutes of Health (grant NIH NIDDK K08 DK02890-02) and the Florida Chapter of the Arthritis Foundation. The authors would like to thank Dr. Franco Silvestris and Dr. Franco Dammacco (University of Bari, Italy) for facilitating Dr. Calvani's research efforts at the University of Florida.

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

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