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A Proinflammatory Role of IL-18 in the Development of Spontaneous Autoimmune Disease[1]

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Serum from patients with systemic lupus erythematosus (SLE) contained significantly higher concentrations of IL-18 than normal individuals. MRL/*lpr* mice, which develop spontaneous lupus-like autoimmune disease, also had higher serum levels of IL-18 than wild-type MRL/++ mice. Daily injections of IL-18 or IL-18 plus IL-12 resulted in accelerated proteinuria, glomerulonephritis, vasculitis, and raised levels of proinflammatory cytokines in MRL/*lpr* mice. IL-18-treated MRL/*lpr* mice also developed a "butterfly" facial rash resembling clinical SLE. In contrast, MRL/*lpr* mice treated with IL-18 plus IL-12 did not develop a facial rash. The facial lesion in the IL-18-treated mice showed epidermal thickening with intense chronic inflammation accompanied by increased apoptosis, Ig deposition, and early systemic Th2 response compared with control or IL-12 plus IL-18-treated mice. These data therefore show that IL-18 is an important mediator of lupus-like disease and may thus be a novel target for therapeutic intervention of spontaneous autoimmune diseases. *The Journal of Immunology,* **2001, 167: 5338–5347.**

Systemic lupus erythematosus (SLE),[5] the prototypic spontaneous systemic autoimmune disease, damages multiple organ systems and causes diverse and variable clinical manifestations (1). In some patients, skin rash and joint pain predominate, while in others glomerulonephritis is the main lesion; however, the common denominator is elevated serum Abs to nuclear constituents. Studies of animal models of SLE have contributed greatly to the elucidation of its pathogenesis. MRL/MP-*lpr/lpr* (MRL/*lpr*) mice develop a similar spontaneous autoimmune disease which has been used extensively as a model for clinical SLE. The mice develop lymphadenopathy and inflammatory manifestations such as nephritis, vasculitis, and arthritis associated with autoantibody production (2, 3). The etiology is multifactorial, but an important factor is the effect of a single gene mutation (*lpr*) of the *fas* apoptosis gene on mouse chromosome 19 (4, 5) on a background gene from the MRL strain (3, 5).

An impressive range of clinical and experimental evidence supports a critical role of T cells in the manifestation of SLE. T cell-deficient MRL/*lpr* mice do not produce autoantibodies and do not develop glomerulonephritis (6–8). Similarly, disruption of T cell activation by blocking CD28-B7 (9, 10) or CD40-CD40 ligand (11, 12) interactions prevents SLE. Furthermore, $CD4^+$ T cells appear to be of paramount importance as CD4 deficiency (13) and anti-MHC class II-TCR Ab (14, 15) blocked autoantibody production and ameliorated disease progression in mice. However, despite the proven importance of T cell function in the pathogenesis of SLE, the relative role of Th1 and Th2 cells remains controversial. Although IL-4 has been implicated in SLE pathogenesis (16–19), elevated levels of IFN-γ occur consistently in SLE (20, 21). Also, IFN-γ and IFN-γR knockout mice develop a milder disease with a delayed onset (22, 23); conversely, administration of rIFN-γ accelerates the disease progression (24). NO (25, 26) and IL-12 (26) also play a pathogenetic role in murine SLE. Thus, IL-12 induces the differentiation of Th1 cells which produce IFN-γ that stimulates macrophages to produce high levels of NO which, at least in part, causes the tissue damage in SLE (25, 26). Recent evidence clearly demonstrates that several factors are required for optimal induction of Th1 activity: chief among them are IL-12 and IL-18.

IL-18 is a member of the IL-1 cytokine family (27). Pro-IL-18 is cleaved by IL-1β-converting enzyme (caspase-1) to yield an active 18-kDa glycoprotein (28) that recognizes a heterodimeric receptor, consisting of unique α (IL-1Rrp) and nonbinding β (AcPL) signaling chains (29, 30) that are widely expressed on cells that mediate both innate and adoptive immunity. IL-18 is expressed by various cell types, including macrophages, dendritic cells, keratinocytes, osteoblasts, pituitary gland cells, adrenal cortical cells, intestinal epithelial cells, skin cells, and brain cells (31–35). IL-18 promotes proliferation and IFN-γ production by Th1, $CD8^+$, and NK cells in mice and in humans (31). It shares some biological activities with IL-12, but without significant structural homology, and serves as a costimulatory factor in the activation of Th1 cells (27). It does not drive Th1 cell development but induces IL-12R expression (36) and thus synergizes with IL-12 for IFN-γ production (37). In the absence of IL-12, IL-18-mediated effects on T cells may extend beyond Th1 differentiation to include type 2 cytokine production (38–40).

IL-18 is expressed in several human diseases including rheumatoid arthritis (41) and inflammatory bowel disease (42, 43). However, the functional role of IL-18 in SLE is unknown. We report here that MRL/*lpr* have significantly elevated serum IL-18 concentration compared with MRL/+/+ controls. MRL/*lpr* mice

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[5] Abbreviation used in this paper: SLE, systemic lupus erythematosus.

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treated with IL-18 or a combination of IL-18 and IL-12 developed accelerated proteinuria, glomerulonephritis and vasculitis compared with controls. These effects were accompanied by enhanced production of anti-dsDNA Abs and proinflammatory cytokines. Furthermore, IL-18-treated mice, but not mice treated with IL-12 plus IL-18, developed a "butterfly" facial rash with inflammation and increased apoptosis and Ig deposition in the skin lesion. This was correlated with the initial elevation of type 2 cytokines. These results suggest that IL-18 is an important mediator of lupus disease and therefore a potential target for therapeutic intervention in this and other related diseases. Furthermore, the results also show that IL-18 can induce a type 2 response in the relative absence of IL-12 and that both type 1 and type 2 responses play an important role in lupus.

Materials and Methods

SLE patients and control

Serum samples were collected from 32 patients at the Connective Tissue Diseases Clinic at Glasgow Royal Infirmary; all fulfilled at least four of the American Rheumatism Association criteria for the classification of SLE (44). Patients were all female with a mean ± SD age of 36 ± 11 years (range, 17–52). Special attention was paid to disease activity, selected organ involvement, presence of infection, and therapy. The SLE Disease Activity Index (45) was applied to all patients. Serum samples from 20 healthy blood donors of comparable age and gender were also studied.

Mice

Homozygous female MRL/MP-*lpr/lpr* and age- and sex-matched control MRL/MP-+/+ mice were purchased from Harlan Olac (Bicester, U.K.). Some of these pairs were bred and maintained in a conventional animal facility at the University Of Glasgow.

Cytokines and reagents

rIL-18 was produced by *Escherichia coli* M15 strain (Qiagen, Dorking, U.K.) transfected with a pQE-30 expression vector (Qiagen) carrying an insert encoding IL-18. Protein was extracted under native conditions following induction with isopropyl-D-thiogalactoside (Bioline, London, U.K.) and purified as a 6x histidine-tagged fusion protein using a nickel agarose purification system (Qiagen) according to the manufacturer's recommendation with some modifications. Purity was assessed by SDS-PAGE and Coomassie blue staining, which showed a single band at 19 kDa. IL-18 activity was analyzed by IFN-γ production from murine spleen cells cultured in 96-well plates coated with anti-CD3 Ab (2 μg/ml). Cytokines used for in vivo studies were endotoxin free as showed by the *Limulus* amebocyte assay (Sigma, Poole, U.K.). In some experiments, murine IL-18 purchased from PeproTech EC (London, U.K.) was used with similar results. Murine IL-12 was kindly provided by Genetics Institutes (Cambridge, MA). LPS (*Salmonella enteritidis*) were obtained from Sigma.

Cytokines treatment

Female MRL/*lpr* or control MRL/+/+ mice (4 wk old) were injected daily i.p. with rIL-18 (500 ng/mouse), a combination of rIL-18 (500 ng/mouse) and rIL-12 (100 ng/mouse), or the PBS diluent for up to 8 wk. The doses of IL-12 and IL-18 used were found to be effective in previous experiments in MRL/*lpr* mice (26) and in collagen-induced arthritis models (46). Mice were monitored daily for disease progression and then sacrificed at various time intervals to assess cytokine profile, anti-DNA Abs, and organ involvement.

Assessment of renal disease

Proteinuria and hematuria were assessed using a commercially available kit (Multistix; Bayer, Cambridge, U.K.) and graded according to the manufacturer's instructions. Kidneys were bisected, fixed in neutral buffered Formalin, and embedded in paraffin wax. H&E-stained sections from both halves were coded and assessed by an experienced pathologist without knowledge of the experimental group to which the animals belonged. The severity of glomerulonephritis was graded on an arbitrary five-point scale as previously described and the groups were compared with the Mann-Whitney *U* test (26). The reproducibility of the grading system was assessed by grading 50 sections blind on two separate occasions and calculating a correlation coefficient. The sections were also screened for the presence or absence of vasculitis. Ig deposition was detected by a standard direct immunofluorescence technique using FITC-labeled Abs recognizing mouse Igs in frozen sections and in paraffin sections using a standard immunoperoxidase technique.

Skin histology

Sections were stained with H&E and periodic acid-Schiff and then analyzed independently by two experienced pathologists unaware of the treatment. Nuclear DNA fragmentation was examined by standard TUNEL method using a commercial kit (TdT-FragEL; Oncogene Research Products, San Diego, CA) according to the manufacturer's instruction. For quantitation, samples were examined with a Leitz DRMB microscope linked to a Panasonic F15 video camera; images were first transferred to an IBM-compatible computer by means of Neotech Image Grabber software (version 1.21; Neotech, Eastleigh, U.K.). Cell counts were made with computer image analysis software (Count Gem; ME Electronics, Reading, U.K.). Sections were also assessed for Ig deposition by immunohistochemistry. Formalin-fixed paraffin section (5 μm) were passaged through graded alcohol to PBS and endogenous peroxidase activity was blocked by incubation with 0.5% (v/v) H_2O_2 in 50% (v/v) methanol. The sections were incubated with 0.1% (w/v) trypsin solution for 10 min at 37°C and, after washing, then incubated for 30 min with 5% (v/v) goat serum to reduce nonspecific background binding. The sections were then incubated with primary Ab (goat anti-mouse IgG-HRP; DAKO, Glostrup, Denmark) in a 1/50 dilution. Ab binding was revealed by exposure to diaminobenzidine (DAKO) and then sections were counterstained with hematoxylin. Quantitation was conducted as above for apoptosis.

Mouse peritoneal and spleen cell preparation and culture

Peritoneal cells were collected by injecting 5 ml of ice-cold PBS into the peritoneal cavity before harvesting. Spleen cells were prepared by gently forcing the spleen through a sterile tea strainer into RPMI 1640 (Life Technologies, Paisley, U.K.) containing 1% FCS. The cells were then washed in serum-free RPMI 1640 and viability was determined by trypan blue exclusion. Spleen cells were pooled and cultured as single-cell suspensions (2×10^6 viable cells/ml) in 24-well plates coated with anti-CD3 Ab (2 μg/ml) in full medium at 37°C and 5% CO_2. Peritoneal cells were pooled and cultured at 3×10^6 viable cells/ml in 96-well plates (Nunc, Roskilde, Denmark) in the presence or absence of IFN-γ (50 U/ml) and LPS (50 ng/ml). Culture supernatants were collected at the times indicated.

Cytokine and NO and IgG measurements

All cytokine levels in serum and culture supernatants were measured by ELISA as described previously (26). The Ab pairs for murine cytokines were: IL-4, IL-10, IL-5, IL-6, IFN-γ (BD PharMingen, San Diego, CA), and TNF-α (Genzyme, Cambridge, MA). Detection limits were 10 pg/ml for TNF-α, IL-4, IL-5, IL-6, and IFN-γ and 40 pg/ml for IL-10. Human IL-18 and murine IL-18 were assayed with paired Abs (R&D Systems, Oxon, U.K.) with overnight coating in Costar plates at room temperature for human IL-18 and in Immulon 4 (Dynatech Laboratories, Chantilly, VA) plates for murine IL-18. Assays were performed according to the manufacturer's instructions. The lower detection limit for IL-18 was 30 pg/ml for both human and murine IL-18. Total nitrate and nitrite concentrations in serum were determined by the conversion of nitrate into nitrite following deproteinization as described previously (47). Total nitrite content in the serum and the level of nitrite in the supernatant were then measured by the Griess method (48) using sodium nitrite as standard. The detection limit was 1 μM. Anti-dsDNA Abs were measured as previously described (26). Briefly, poly-L-lysine-treated Immulon 2 flat-bottom plates (Dynatech Laboratories) were coated with 10 μg/ml calf thymus DNA (Sigma) and blocked with 1% BSA/PBS solution. Serum samples (100 μl, start at 1/100 dilution) were serially diluted and added to the plates. Total IgG bound was measured by adding HRP-conjugated goat anti-mouse Ig (DAKO). IgG, IgG1, and IgG2a isotypes were determined by HRP-conjugated goat anti-mouse isotype- specific Abs (BD PharMingen). Results are expressed in units per milliliter in reference to a standard curve obtained with human dsDNA and a reference standard of pooled sera from 20-wk-old MRL/*lpr* mice. Although the method detected predominantly anti-dsDNA Abs, it may also detect low levels of ssDNA.

Statistical analysis

This was performed using Minitab software (Minitab software program; State College, PA) and analyzed by Student's *t* test or Mann-Whitney *U* test.

Results

SLE patients have higher serum concentrations of IL-18 than normal controls

Serum from patients with active SLE ($n = 32$) were collected and analyzed for IL-18 by ELISA and compared with those of healthy individuals ($n = 20$). Fig. 1*a* shows that SLE patients have higher serum IL-18 levels than controls ($p < 0.02$). This is in contrast to the serum from patients with rheumatoid arthritis where only a low level of serum IL-18 was detectable in a small number of patients (data not shown). Although in our study the number of patient samples did not permit a power calculation on the correlation between IL-18 levels and disease severity, the data obtained are consistent with recent reports that patients with active SLE have elevated levels of IL-18 that correspond to disease activity (49–51). Together they suggest that production of IL-18 may be associated with the pathogenesis of SLE.

*MRL/*lpr *mice produce more IL-18 than normal mice*

To determine the potential pathogenic role of IL-18 in SLE, we investigated IL-18 production in MRL/*lpr* mice that develop spontaneous lupus-like autoimmune disease. Serum of MRL/*lpr* mice contained significantly more IL-18 than that of age- and sex-matched control wild-type MRL/++ mice (Fig. 1*b*). Mouse spleen and peritoneal cells were cultured in vitro and supernatants were analyzed for IL-18 content by ELISA. Cells from MRL/*lpr* mice again produced markedly more IL-18 than the cells from control MRL/++ mice (Fig. 1*c*). These results are consistent with the findings in human SLE and support a pathogenetic role for IL-18 in spontaneous SLE.

*rIL-18 accelerates autoimmune disease in MRL/*lpr *mice*

To investigate directly the role of IL-18 in the development of autoimmune disease, young (4-wk-old) MRL/*lpr* mice ($n = 10$)

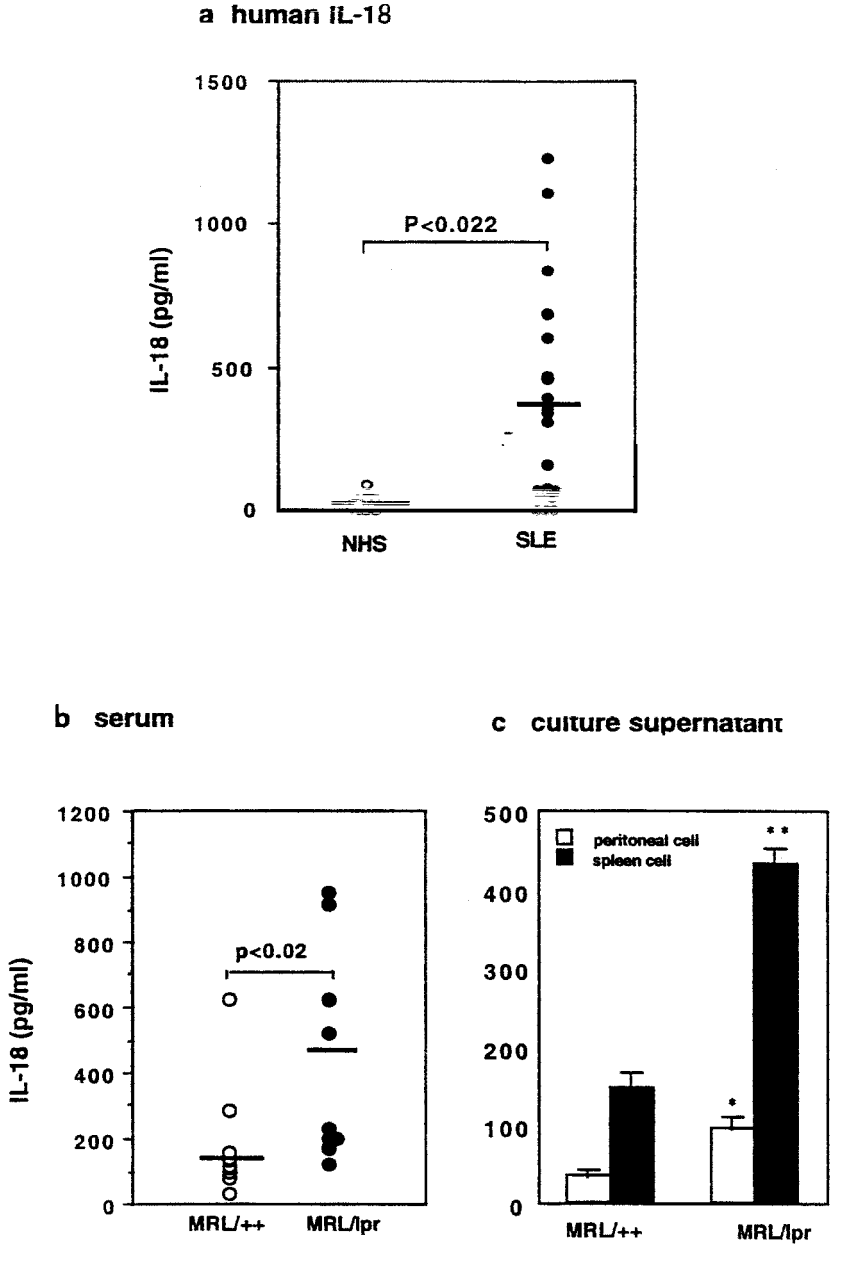

FIGURE 1. Elevated IL-18 production by SLE patients and MRL/*lpr* lupus strain mice. *a,* Serum samples from SLE patients ($n = 32$) and healthy controls ($n = 20$) were assayed for IL-18 by ELISA. Serum IL-18 levels were significantly higher in SLE patients than in controls ($p < 0.02$, Mann-Whitney *U* test). *b,* MRL/*lpr* lupus mice ($n = 10$) at the ages of 4–6 mo (with obvious proteinuria and kidney involvement) had serum IL-18 levels significantly higher than age- and sex-matched MRL/++ mice ($n = 9$) ($p < 0.028$, Mann-Whitney *U* test). *c,* Pooled splenic or peritoneal cells from MRL/*lpr* and MRL/++ (14 wk old, $n = 5$) were cultured with culture medium alone. Culture supernatants were collected after 48 h and IL-18 levels were measured by ELISA. IL-18 concentrations were expressed as mean ± SEM of triplicate cultures. Spleen cells and peritoneal cells from MRL/*lpr* lupus strain spontaneously produced more IL-18 than that of MRL/++ controls (*, $p < 0.05$; **, $p < 0.001$).

were given daily i.p. injections of rIL-18 (500 ng/mouse/day) for 56 days. Control mice ($n = 10$) were injected with the same volume of diluent (PBS). We have previously reported that IL-12 has a proinflammatory role in SLE and that administration of rIL-12 exacerbated disease in MRL/*lpr* mice. Moreover, IL-18 synergizes with IL-12 in the induction of Th1 response. We therefore also injected mice ($n = 10$) with a combination of IL-12 and IL-18. As additional control, wild-type MRL/++ mice ($n = 10$) were similarly treated. Hematuria and proteinuria were monitored daily. Mice were then sacrificed and the severity of renal damage was graded by microscopy. Control MRL/*lpr* mice developed the expected spontaneous disease with hematuria/proteinuria appearing after 35 days and progressing steadily throughout the study period. MRL/*lpr* mice treated with IL-18 developed accelerated hematuria/proteinuria compared with the PBS group. Significant divergence occurred as early as 20 days after treatment (Fig. 2*a*). MRL/*lpr* mice treated with a combination of IL-12 and IL-18 had hematuria/proteinuria levels indistinguishable from that of the PBS group until the 40th day of treatment. Thereafter, the hematuria/proteinuria levels increased rapidly and overtook that of the group treated with IL-18 alone (Fig. 2*a*). Neither treatment affected the wild-type MRL/++ mice (Fig. 2*a*), indicating a major influence of the *lpr* gene on the susceptibility to the effect of IL-18.

Histological examination

All MRL/*lpr* mice showed features typical of lupus nephritis, which have been well documented in this model. These consisted of segmental and global mesangial hypercellularity, increased mesangial matrix, and some capillary wall thickening. More severely affected glomeruli often contained inflammatory cells, apoptotic bodies, and tuft-to-capsule adhesions; the most severely damaged glomeruli contained fibrin deposits, focal and segmental necrosis, and crescents. Igs and complement were detected in the glomerular tufts by immunostaining, suggesting immune complex deposition (data not shown). Vasculitis predominantly affected the origins of the radial arteries in the deep cortex. There was fibrinoid necrosis in the arterial media and prominent periarteritis (Fig. 2*d*). These changes occurred on a background of focal chronic pyelonephritis and lymphoproliferative disease, which occurs in this model. The wild-type MRL/+/+ mice did not show any of the above changes. MLR/*lpr* mice treated with IL-18 and IL-18 plus IL-12 had significantly increased glomerulonephritis scores (Fig. 2*b*). Again, the treatment had no effect on the MRL/+/+ mice (Fig. 2*b*). MRL/*lpr* mice treated with IL-18 showed a modest increase in vasculitis (from 40 to 60%). In contrast, all MRL/*lpr* mice treated with IL-18 plus IL-12 developed vasculitis (Fig. 2*c*).

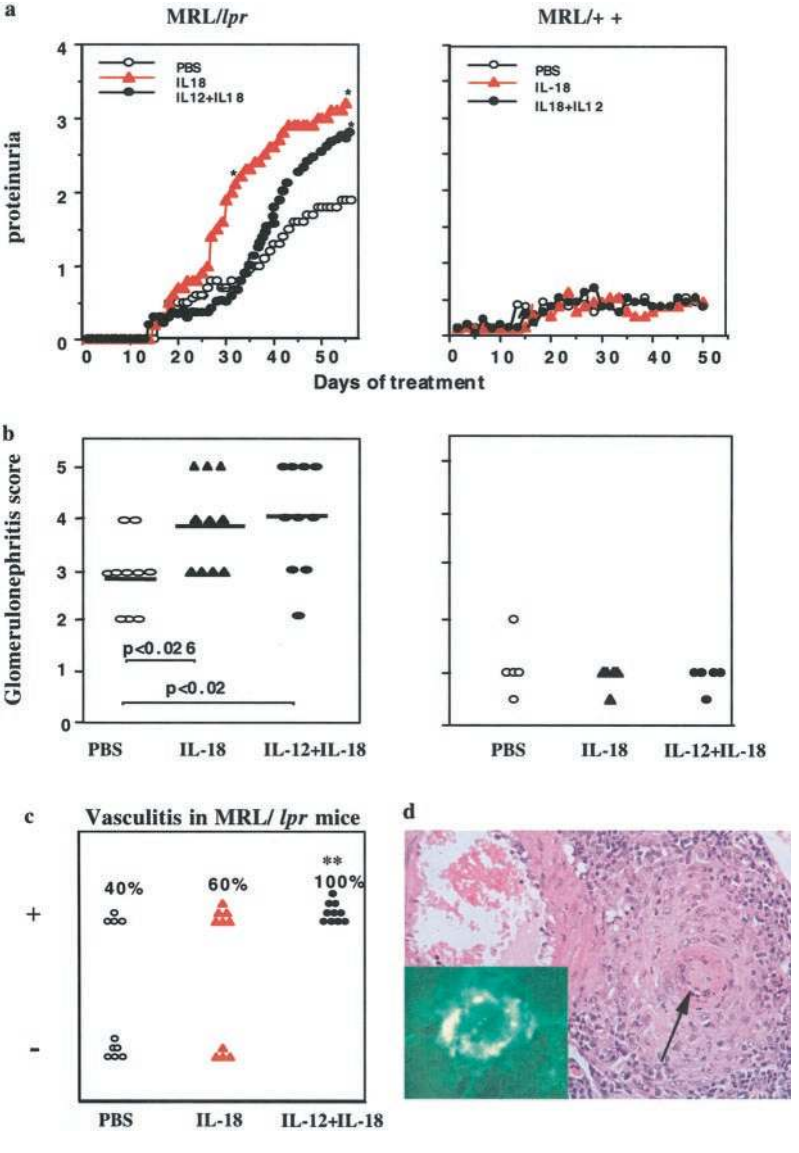

FIGURE 2. Effects of IL-18 treatment of MRL/*lpr* mice on renal damage. MRL/*lpr* and MRL/++ mice (female, 4 wk old) were injected i.p. daily with IL-18, IL-12 plus IL-18, or PBS alone for 56 days. *a,* Kidney involvement was monitored by daily hematuria/proteinuria measurement. Data for proteinuria only are shown. Similar data were obtained for hematuria. *b,* Kidney sections from different treated groups were scored as described in *Materials and Methods*. IL-18-treated MRL/*lpr* mice had significantly more severe glomerulonephritis than the control PBS group ($p < 0.026$, Mann-Whitney U test); IL-12 plus IL-18-treated mice also had more severe glomerulonephritis than the controls ($p < 0.02$). There were no significant differences between the IL-18 and the IL-12 plus IL-18 groups. *c,* Vasculitis in cytokine-treated MRL/*lpr* mice. All mice treated with IL-18 plus IL-12 had vasculitis. IL-12 plus IL-18 treatment induced significantly more renal vasculitis in MRL/*lpr* mice than in the PBS group ($p < 0.005$, Fisher test). There was no significant difference between the IL-18 group and controls. *d,* Vasculitis predominantly affected the origins of the radial arteries in the deep cortex (arrow). There was fibrinoid necrosis in the arterial media and prominent periarteritis. *Inset,* Positive immunofluorescent staining for IgG in the same kidney (IL-12 plus IL-18 treatment).

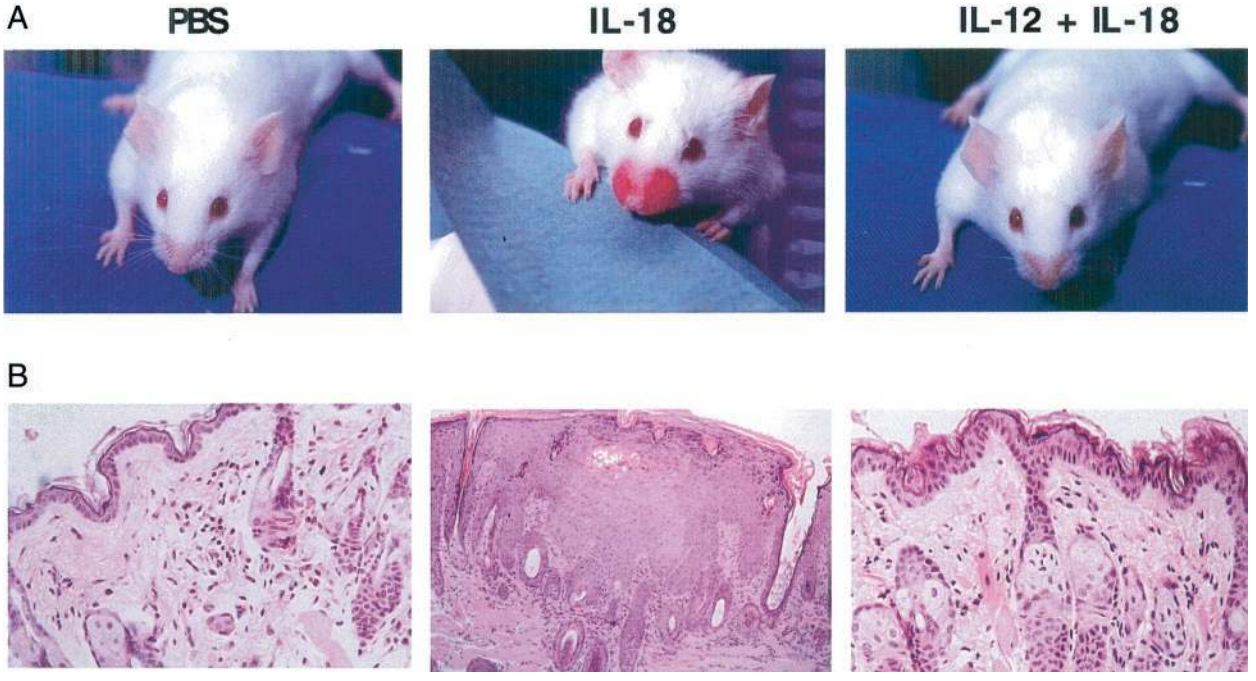

FIGURE 3. Recombinant IL-18 induced a facial rash in all MRL/*lpr* mice ($n = 10$). *a,* The characteristic red "butterfly" symmetrical skin rash in mice treated with IL-18 (*middle*) but not in the controls (*left*) or those treated with IL-12 plus IL-18 (*right*). *b,* Representative histological sections from the facial skin stained with H&E. The IL-18-treated mice showed thickened epidermis with acanthosis and hyperkeratosis. The dermis contained a dense population of mainly lympho-histocytic chronic inflammatory cells. The PBS-injected control mice had a minimal increase in chronic inflammatory cells, mainly small lymphocytes (*left*). The IL-12 plus IL-18 group showed a slight thickening of epidermis and minimal chronic inflammatory cells (*right*). Results are pooled from two independent experiments.

IL-18-treated mice developed facial rash

In about one-third of the patients, SLE involves the skin; the erythematous rash is often photosensitive with a malar butterfly distribution. MRL/*lpr* mice kept in a conventional environment did not develop skin lesions (Fig. 3*a, left*). In contrast, all mice injected with IL-18 developed a symmetrical skin rash on the malar

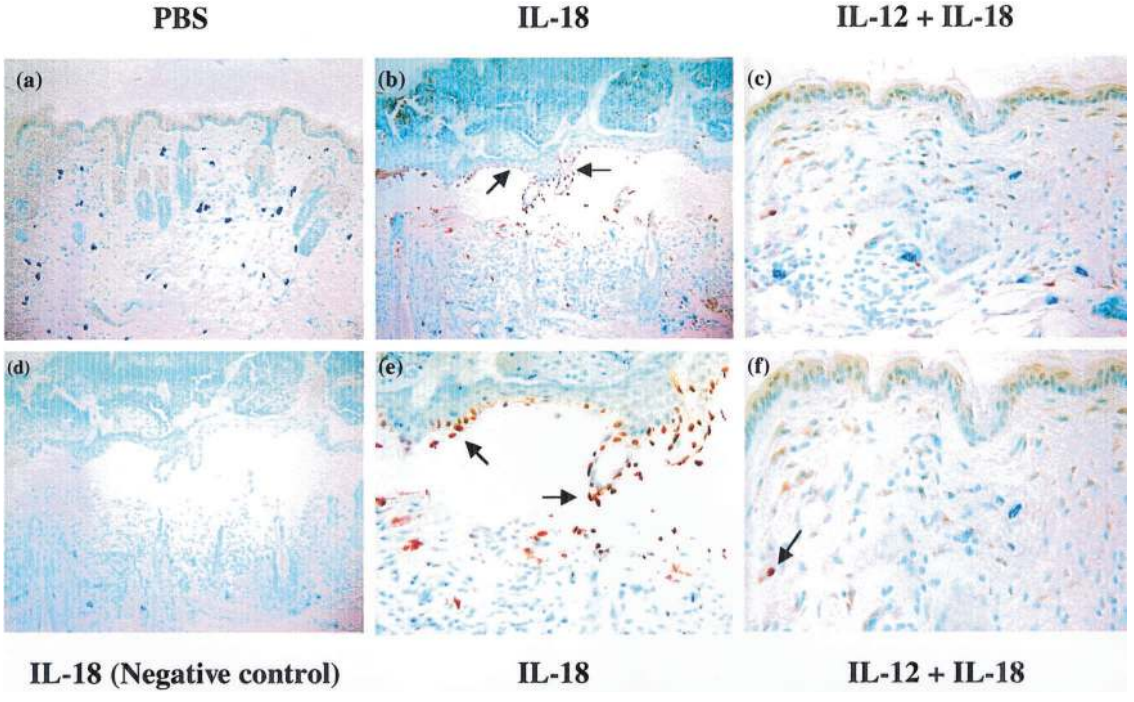

FIGURE 4. TUNEL staining of apoptosis in the facial skin. Skin lesions from IL-18-treated MRL/*lpr* mice revealed apoptotic cell death in the epidermis, dermis, and epidermo-dermal junction, shown as brown nuclear staining (*b* and *e*). The negative control section (*d*) from an IL-18-treated skin lesion was incubated with reaction buffer containing no protein. DNA fragmentation was not detectable in the PBS-treated mice (*a*). Skin from the IL-12 plus IL-18-treated group showed (*c* and *f*) significantly fewer apoptotic cells in the epidermis and dermis than IL-18-treated skin (arrows). Staining was also conducted with skin before the onset of lesion (4 wk into the treatment). Apoptosis was clearly evident in the IL-18-treated group but appeared less intensive than that when the lesions appeared. No apoptosis was detected in the skin of the other two groups (data not shown).

region (Fig. 3*a, middle*) at around 36 days after treatment. The rash persisted throughout the study period, up to 12 wk, when the mice were sacrificed as required by the Animal Experimentation Guideline, U.K. Unexpectedly, MRL/*lpr* mice treated with both IL-18 plus IL-12 showed no skin rash (Fig. 3*a, right*) despite developing more severe glomerulonephritis and vasculitis (Fig. 2). None of the treated wild-type MRL/++ mice developed skin lesions (data not shown). Histological examination of the rash in the IL-18-treated mice showed epidermal thickening with acanthosis, hyperkeratosis, and parakeratosis. In occasional animals the skin was excoriated. However, apart from these areas the basal layer of the epidermis was intact. The epidermal basement membrane was normal. The dermis contained an intense chronic inflammatory cell infiltrate consisting mainly of lymphocytes and histiocytes (Fig. 3*b, middle*). Polymorphs were also present, even when the epidermis was intact. By contrast, untreated mice had a minimal increase in chronic inflammatory cells, consisting mainly of small lymphocytes (Fig. 3*b, left*). In the IL-12 plus IL-18-treated group, there were slightly more lymphocytes and these tended to form loose aggregates associated with the skin appendages (Fig. 3*b, right*). Polymorphs were not detected in the PBS and IL-12 plus IL-18-treated groups.

The facial lesions were also analyzed for evidence of apoptosis by the TUNEL method (Fig. 4). Sections from the control PBS-treated MRL/*lpr* mice, that developed glomerulonephritis and vasculitis but without facial lesion, showed no evidence of apoptosis (Fig. 4*a*). In marked contrast, sections of facial lesions from MRL/*lpr* mice treated with IL-18 showed clear evidence of linear TUNEL staining in the parakeratotic layer. More dramatically, the basal layer of the epidermis of these sections showed strong nuclear staining. There were also positively stained nuclei within the inflamed dermis (Fig. 4, *b* and *e*). Interestingly, the IL-12 plus IL-18 group showed only scanty positive nuclei in the epidermis (Fig. 4, *c* and *f*). Scanning photometry show that the numbers of apoptotic cells per 10-nm^2 field are (mean ± SD, $n = 4$): PBS group, 1.8 ± 0.8; IL-18 group, 27 ± 6 ($p < 0.001$ compared with the PBS control); IL-12 plus IL-18 group, 4.6 ± 1 (not significantly different from control).

Ig deposition in the facial skin of different groups of mice ($n = 4$) after 4-wk treatment (just before the appearance of the gross skin lesions) were examined by immunohistochemistry. Stainable Ig was increased in the whole skin sections from the IL-18 and IL-18 plus IL-12-treated mice ($p < 0.001$ and $p < 0.05$, respectively) compared with the PBS control mice (Fig. 5). However, granular subepidermal Ig deposition was only observed in the IL-18-treated group and not in the IL-12 plus IL-18-treated or control mice.

Cytokine and Ab analysis

The cytokine production profiles of MRL/*lpr* mice treated with IL-18 or IL-18 plus IL-12 were analyzed by ELISA. After 2 wk of treatment, serum from MRL/*lpr* mice injected with IL-18 contained a significant level of IL-5. This was undetectable in the serum of the PBS or IL-12 plus IL-18-treated groups (Fig. 6). In contrast, mice treated with IL-12 plus IL-18 produced high levels of IFN-γ, which was undetectable in the PBS or IL-18-treated mice. Similar results were obtained in the culture supernatant of spleen cells cultured with medium alone or with anti-CD3 Ab in vitro (data not shown). IL-18-treated mice also produced higher levels of IgG1 and IgG2a anti-DNA Abs than the other two groups (Fig. 6). IL-4, IL-10, and TNF-α were undetectable in the serum. Thus, at the early stage of treatment, IL-18 administration augmented the Th2 response, whereas, the combination of IL-12 and

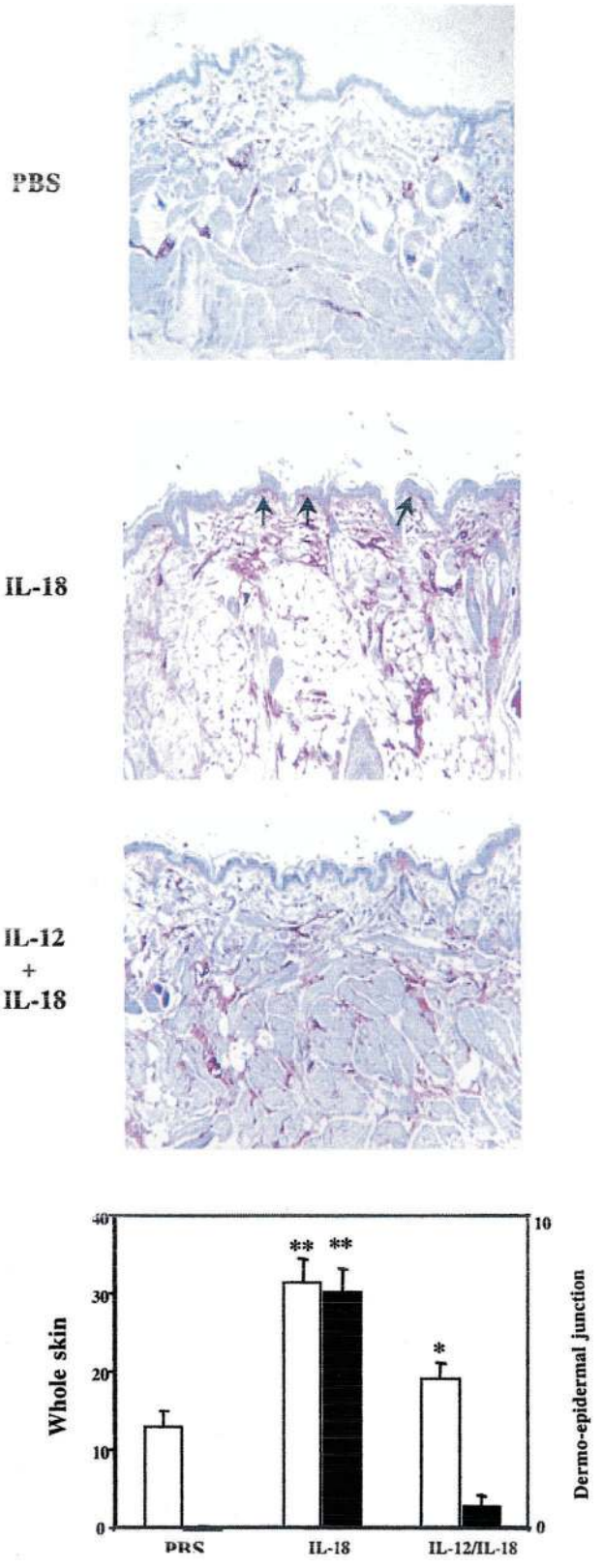

FIGURE 5. Ig deposition in the facial skin after 4-wk treatment. Ig deposition in the skin was assessed by immunohistochemistry of 5-μm Formalin-fixed section of skin. Arrows, Ig deposition in the dermo-epidermal junction of mice treated with IL-18. This is largely absent in the other two groups. Ig deposition was quantified by image analysis and presented as number of granular IgG deposition in 10 nm^2 of tissue. □, Whole skin and ■, dermo-epidermal junction. Vertical bars are SD of mean, $n = 4$, **, $p < 0.01$; *, $p < 0.05$.

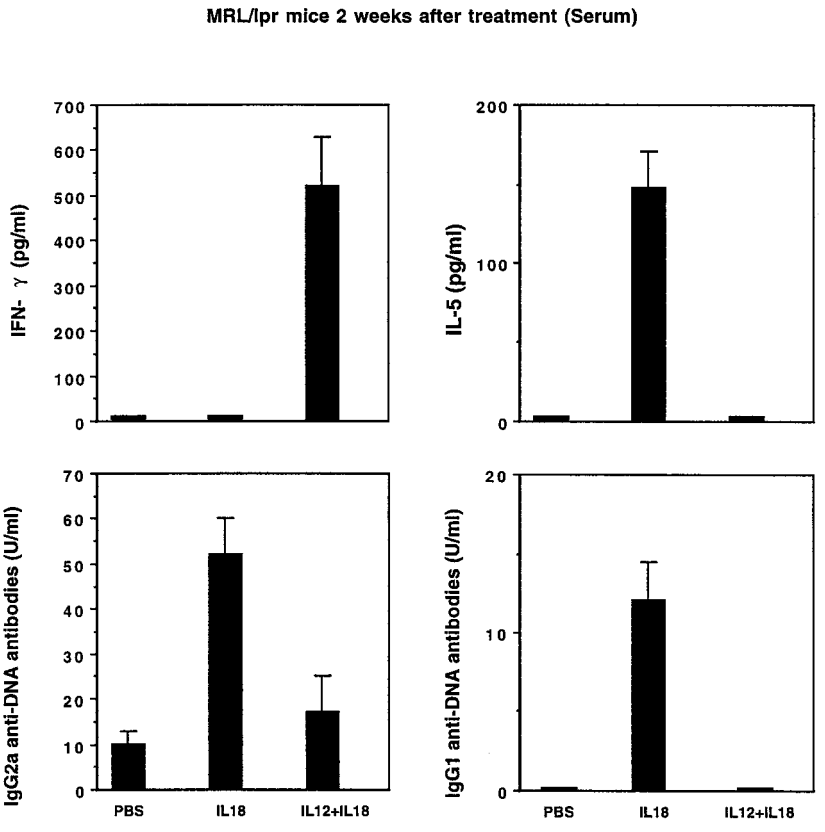

FIGURE 6. Serum cytokine and anti-DNA Ab levels in MRL/*lpr* mice after 2-wk cytokine treatment. IFN-γ was detected at high levels only in the IL-12 plus IL-18 group. In contrast, IL-5 was detected only in the group treated with IL-18. IL-4 was undetectable in the any of the mice. The levels of IgG2a anti-dsDNA Abs in the IL-18 group were significantly higher than those in both the PBS and IL-12 plus IL-18 groups ($p < 0.001$, $n = 5$). IgG1 anti-DNA Abs were detected only in the serum of IL-18-treated mice. The total anti-dsDNA IgG levels were similar to those of IgG2a.

IL-18 induced a predominant Th1 activity. After 8 wk of treatment, the cytokine profile changed; both IFN-γ and TNF-α levels were elevated in the IL-18-treated and especially in the IL-12 plus IL-18-treated mice compared with the PBS control group (Fig. 7). IL-4 and IL-5 were not detected in all three groups of mice (data not shown). In contrast, IL-10 was significantly suppressed in the IL-18-treated and the IL-12 plus IL-18-treated groups compared with the PBS control mice. Serum nitrite/nitrate levels were elevated in mice treated with IL-12 plus IL-18, but not with IL-18 alone. IgG2a anti-DNA Ab was increased in both the cytokine-treated groups compared with the control PBS group. IgG1 anti-DNA levels remained low and variable. Thus, at the end of treatment, mice treated with IL-18 alone or a combination of IL-12 and IL-18 developed an elevated Th1 type of response and produced more proinflammatory cytokines than control mice.

Discussion

Data presented in this report show that IL-18 accelerates spontaneous autoimmune lupus disease with characteristic glomerulonephritis and vasculitis. This is further exacerbated by the synergistic action of IL-12 and IL-18, a combination known to promote Th1 cell development (37). The mechanism of such synergy in vivo is unknown at present, but may include reciprocal enhancement of their receptor expression (52, 53). Earlier studies demonstrated critical roles of IL-12 and NO in the manifestation of lupus pathology in the MRL/*lpr* mice. Thus, IL-18 in combination with IL-12 promotes Th1 cell development which augments type 1 cytokine (IFN-γ) production, leading to the enhanced expression of inducible NO synthase and the production of large amounts of NO that mediate glomerulonephritis and vasculitis (25, 26). It is also important to note that IL-18 and IL-12 plus IL-18 treatments led to a profound suppression of the production of IL-10, a cytokine closely associated with the Th2 response in the mouse and anti-inflammatory in numerous systems (54). IL-18 plays an important role in the host defense against infections. IL-18-deficient mice exhibited impaired Th1 response to intracellular bacteria, including *Propionibacterium acnes*, *Mycobacterium bovis*, and *Staphylococcus aureus,* and parasites such as *Leishmania major* (55, 56). Our results show that SLE is associated with excessive endogenous production of IL-18 and the exogenous administration of IL-18 can cause autoimmune disease in susceptible mice, demonstrating the negative side of the therapeutic use of IL-18. This is consistent with a number of reports showing that IL-18 mRNA is up-regulated in nonobese diabetic mice and the murine IL-18 gene maps to the *Idd2* susceptibility locus, suggesting a predisposition to autoimmunity (57). IL-18 has also been detected in inflammatory bowel disease (42, 43) and we have recently shown that administration of IL-18 induced collagen-induced arthritis in the mouse (41). However, the mechanisms by which IL-18 promotes SLE in the present study were unlikely to be fully explained by synergy with endogenous expression of IL-12. In earlier studies, we demonstrated that IL-18 could induce TNF-α production by macrophages independent of IL-12 (41). Moreover, as IL-18, but not IL-12, activates the IFN-γ promoter directly through an AP-1 site, independent of TCR signaling (58), it is possible that IL-18, like IL-15, IL-1β, and IL-6, can contribute to Ag-independent T cell activation in chronic inflammatory responses (59, 60). This is in agreement with a recent observation that the induction of lethal toxic effect by daily injection of large doses of IL-18 plus IL-12 occurred similarly in athymic or euthymic mice (61).

Autoantibodies have long been associated with the pathology of SLE (62). Anti-DNA Abs form immune complexes the deposition of which results in nephritis and arteritis. MRL/*lpr* mice treated with IL-12 plus IL-18 produced more IgG2a anti-DNA Ab but less IgG1 Ab than the PBS control mice. This is consistent with the notion that IL-12 plus IL-18 is a powerful inducer of Th1 response. In contrast, mice treated with IL-18 alone developed Th2 response at the early stage of treatment. They produced mostly IL-5 and

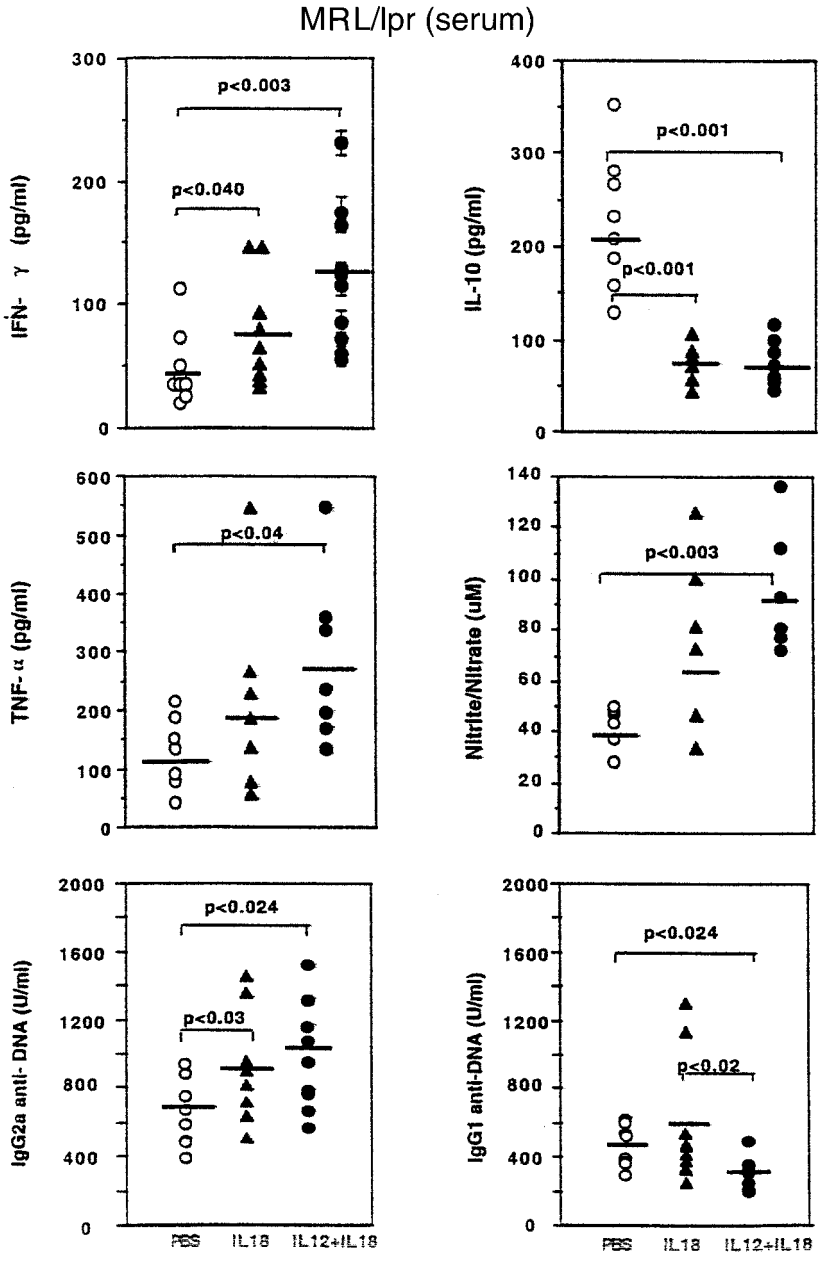

FIGURE 7. Serum cytokine and autoantibody concentrations in cytokine-treated MRL/*lpr* mice at the end of 8 wk of treatment. IFN-γ, TNF-α, and nitrite/nitrate concentrations were significantly increased in the IL-12 plus IL-18 and IL-18 groups compared with the PBS control group. In contrast, significant reduction of IL-10 was observed in the IL-18 plus IL-2 group and the IL-18 group compared with the PBS group. IL-4 and IL-5 were undetectable in all groups. IgG2a anti-dsDNA was significantly elevated in the IL-18 and IL-12 plus IL-18 groups compared with the PBS group. However, IgG1 anti-DNA Abs in the IL-12 plus IL-18 group were significantly lower than those in control and IL-18 groups. Results are the mean ± SEM.

little or no IFN-γ. This is also reflected in the isotype of anti-DNA Ab produced. These mice produced high levels of IgG1 and IgG2a Abs, whereas mice treated with IL-12 plus IL-18 produced no detectable IgG1 anti-DNA Ab. It should also be noted that although IgG2a is a good correlate of Th1 response, the case for IgG1 has often been controversial. This is probably due to the fact that IgG1 can be divided into two distinct subtypes: one has anaphylactic activity and its synthesis is IL-4 dependent, being inhibited by IL-12 and IFN-γ; the other lacks this activity and its synthesis is stimulated by IL-12 and IFN-γ (63). Nevertheless, the enhanced IgG1 synthesis as a result of early Th2 responses in the IL-18-treated mice may contribute to the extensive Ig deposition in the facial lesion. Alternatively, the Ig deposition may be the result of increased apoptosis in the lesion. The increased amount of nuclear materials from the apoptotic cells may induce Ab synthesis, which is associated with the local lesions.

The most striking feature of the present study was the development of symmetrical malar skin rash in MRL/*lpr* mice treated with IL-18. This was clinically reminiscent of, but not identical to, that which develops in one-third of SLE patients. Histological study of the rash in these mice showed epidermis thickening with acanthosis, hyperkeratosis, and parakeratosis and an association with chronic inflammatory cell infiltration and local apoptosis and Ig deposition. Impressively, this rash was completely suppressed by the coadministration of IL-12. Furthermore, IL-18 had no effect on the control MRL/++ mice. Although the precise mechanism(s) for this facial pathology is at present unknown, a number of conclusions may be drawn at this stage of investigation. (1) The facial rash and the renal pathology in SLE could be caused by separate mechanisms. Mice treated with IL-12 plus IL-18 developed the most severe glomerulonephritis and vasculitis compared with the IL-18-treated and the PBS control groups, yet IL-12 plus IL-18-treated mice did not develop the facial rash (2). The facial rash is associated with the *lpr* gene mutation since the disease was not induced in the wild-type MRL/++ mice during the period of investigation. It should be noted that the MRL/+/+ mice also developed spontaneous autoimmune disease albeit at a much later time point. It may be that the *lpr* gene defect merely accelerates the

response to IL-18 and that prolonged treatment with IL-18 may also result in such lesions in the MRL/+/+ mice. This notion is consistent with a recent report that lymphocytes from MRL/*lpr* mice are hyperresponsive to IL-18 (64). Thus, the induction of facial rash may not be entirely dependent on the defect of the *lpr* gene since SLE patients are not known to have the mutated *lpr* gene (3). The rash is associated with the appearance of local apoptosis, which is independent of the Fas/Fas ligand pathway. The *fas* gene is deficient in the MRL/*lpr* mice but remains intact in the MRL/++ mice. This notion is consistent with a recent report showing that a strain of skin-specific caspase-1-transgenic mice showed cutaneous apoptosis which is mediated by IL-18 but is not affected by anti-Fas ligand Ab treatment (65).

Cutaneous apoptosis has been closely linked to the aberrant presentation of nuclear proteins and subsequent development of antinuclear Ab (66). IL-18 could promote this process at several levels including enhancement of apoptosis, which may operate directly through Fas-dependent and -independent pathways or through enhanced activation of local NK cells. IL-18 can also modulate dendritic cell function and supports autoreactive T cell expansion as recently demonstrated in an experimental allergic encephalitis model (67). IL-18 could thereby promote expansion of antinuclear T cell responses with maturation initially to Th2 (38–40) and thereafter, in synergy with endogenous IL-12, to Th1 polarity (27, 37). Our data demonstrate early Th2 polarization in IL-18-treated MRL/*lpr* mice commensurate with this hypothesis. Importantly, the rash was not observed with simultaneous administration of IL-12 and IL-18. In such mice a dominant Th1 response associated with high levels of IFN-γ production was detected early after treatment. Although IFN-γ can induce apoptosis, it does so through a Fas-dependent pathway that does not operate in the MRL/*lpr* strain. We observed markedly reduced numbers of cutaneous apoptotic cells in IL-12 plus IL-18-treated mice. In such circumstances, high levels of local IFN-γ concentration may subvert other IL-18-mediated apoptotic pathways. IFN-γ inhibits TNFR-associated factor 6-mediated osteoclasts maturation (68) and we are currently investigating whether a similar effect may operate upon TNFR-associated factor 6-dependent IL-18 signaling.

In conclusion, we have provided evidence that IL-18 can accelerate spontaneous autoimmune lupus disease in the MRL/*lpr* mice. The disease was characterized by glomerulonephritis, vasculitis, and symmetrical malar facial rash. Importantly, coadministration of IL-18 with IL-12 led to more severe systemic pathology but failed to induce the facial rash. The rash was associated with local apoptosis and Ig deposition. IL-18 treatment also resulted in induction of early Th2 polarization followed by a dominant Th1 response, whereas IL-12 plus IL-18 treatment enhanced and sustained only a strong Th1 activity throughout the disease. These results demonstrate that both Th1 and Th2 cells are involved in the pathogenesis of SLE. Th1 cells are more important for the systemic nephritis, whereas Th2 cells are associated with facial rash. Our data therefore go some way in explaining the frequently observed controversial roles of Th1 and Th2 cells in SLE. Furthermore, this finding suggests that IL-18 is a potential important target of therapeutic intervention in spontaneous autoimmunity.

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