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Differential Contribution of IL-4 and STAT6 vs STAT4 to the Development of Lupus Nephritis

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Mechanisms that initiate lupus nephritis and cause progression to end-stage renal disease remain poorly understood. In this study, we show that lupus-prone New Zealand Mixed 2410 mice that develop a severe glomerulosclerosis and rapidly progressive renal disease overexpress IL-4 in vivo. In these mice, STAT6 deficiency or anti-IL-4 Ab treatment decreases type 2 cytokine responses and ameliorates kidney disease, particularly glomerulosclerosis, despite the presence of high levels of IgG anti-dsDNA Abs. STAT4 deficiency, however, decreases type 1 and increases type 2 cytokine responses, and accelerates nephritis, in the absence of high levels of IgG anti-dsDNA Abs. Thus, STAT6 and IL-4 may selectively contribute to the development of glomerulosclerosis, whereas STAT4 may play a role in autoantibody production.

such as IL-4 and IL-13. STAT6-deficient mice have reduced type II cytokine production, reduced IgE levels, and IL-4-induced B cell proliferation (24–26). In contrast, STAT4 protein, which drives type I immune responses, is activated after the interaction between IL-12 and the IL-12R. This in turn induces the transcription of IFN-γ (23). STAT4-deficient mice lack IL-12-induced IFN-γ production and display a predominant type II cytokine phenotype (27).

In this work, we demonstrate that STAT6 deficiency or anti-IL-4 mAb treatment markedly inhibits the progression of lupus nephritis, despite the unaffected or even increased IgG anti-dsDNA Ab levels in NZM.2410 mice. STAT4 deficiency in these mice, however, increases renal disease, despite a decrease in IgG anti-dsDNA Ab levels.

Materials and Methods

Mice

Four strains of lupus-prone mice, NZM.2410, (New Zealand Black (NZB) × New Zealand White (NZW))F1 (BWF1), MRL-lpr, and NZB, and five nonautoimmune strains, NZW, BALB/c, (BALB/c × NZW)F1 (CWF1), B10.Pi, and (NZB × B10.Pi)F1 (BPF1), were used in this study. Breeding pairs of NZM.2410, NZB, NZW, MRL-lpr, BALB/c, and B10.Pi were purchased from The Jackson Laboratory (Bar Harbor, ME) and were bred locally. Nonautoimmune NZW and B10.Pi mice express MHC class II haplotype identical with that in NZB mice. All experiments were performed in accordance with the institutional animal research committee guidelines.

Generation of STAT4 and STAT6 null NZM.2410 mice

STAT4 and STAT6 null mutations (24, 27) on a 129, C57BL/6 mixed background were crossed onto the NZM.2410 background, using a marker-assisted selection strategy to accelerate the development of desired congenic mice. Each backcross generation was screened with microsatellite DNA markers at a density of 25 cm. At least 25 males were screened at each generation. At each backcross generation, we specifically selected progeny that carried the target gene and had the lowest content of donor (129, C57BL/6) genes throughout the genome. To follow the STAT4 and STAT6 null mutations, we used PCR-based assays: one assay amplified the linked neomycin-resistance (neo) gene, and the other amplified the segment of the respective STAT gene spanning the null-generating deletion.

Abs and cytokines

The anti-IL-4 and anti-IFN-γ mAbs used for our studies have been previously described (22). Neutralizing anti-IL-4 mAb (1B11) was kindly provided by C. Reynolds (National Cancer Institute, Frederick, MD). Purified mouse rIL-4 and rIFN-γ were purchased from BD PharMingen (San Diego, CA).

Treatment protocol

Animals were injected i.p. with 250 µg of an anti-IL-4 mAb (1B11) or an isotype-matched rat IgG1 mAb (GL113) three times per week for 12 wk.

In vivo cytokine capture assay

In vivo IL-4 and IFN-γ levels were measured by an in vivo capture assay (22) with some modifications. In brief, mice were injected i.v. with 10 µg of biotinylated anti-mouse IL-4 (BVD4-1D11) or anti-mouse IFN-γ (R4-6A2) mAb in PBS containing 1% normal mouse serum, and were bled within 2–4 h (for IL-4) or 12–24 h (for IFN-γ). Sera (containing cytokine/anticytokine complexes) were collected. Flat-bottom white high-binding ELISA plates (ISC Bioexpress, Kaysville, UT) were coated with 100 µl of anti-IL-4 (2G12.3) or anti-IFN-γ (AN-18) mAbs at a concentration of 10 µg/ml in 0.1 M Tris-HCl, pH 8.3, for 2 h at room temperature (RT). Plates were then filled with 150 µl super block solution (Pierce, Rockford, IL) and emptied immediately by inversion. This process was repeated three times. Plates were then dried and washed once with distilled water using a MicroWell II microtiter plate washer (Skatron, Sterling, VA). Positive controls were prepared by mixing 10 µl of 10 µg/ml cytokine with the appropriate biotinylated anti-cytokine mAb (5 µl of 2 mg/ml, BVD4-1D11.2 for IL-4 and R4-6A2 for IFN-γ) for 3 min at RT, and adding washing buffer to obtain a final IL-4 or IFN-γ concentration of 100 ng/ml. A total of 25 µl of positive control or serum samples diluted 2× (or 10× if the cytokine concentration was predicted to be high) was added to the plate for 1 h at 37°C. Plates were then rinsed twice with washing buffer (blocking buffer contained 1% Tween 20 solution and TBS), and then 10 times with distilled water. Streptavidin-HRP (1:20,000 in washing buffer; Pierce) was then added for 1 h at RT. After rinsing with washing buffer 5 times, and with water 20 times, plates were filled with BupH Tris saline (Pierce) and emptied by inversion. A total of 150 µl of freshly prepared Fermo maximum sensitivity substrate (1 part enhancer, 1 part peroxidase, 18 part of BupH Tris saline; Pierce) was added, and plates were read immediately at 425 nm using a luminometer (Labsystems, Helsinki, Finland).

In vitro culture

Splenocytes were activated with 1 µg/ml plate-bound anti-CD3. After 1 wk in culture, cells were washed and restimulated with plate-bound anti-CD3 for 24 h. In some experiments, splenocytes were stimulated with Con A (2 µg/ml) for 72 h. Supernatants were harvested, and IL-4 and IFN-γ levels were quantified by ELISA, as described previously (28).

Measurement of serum IgE levels

Serum IgE levels were measured using flat-bottom white high-binding ELISA plates (ISC Bioexpress), which were coated with 50 µl of anti-mouse IgE mAb (EM-95; 10 µg/ml in 0.1 M Tris-HCl, pH 8.3) overnight at 4°C. Plates were blocked three times with 150 µl superblocking solution (Pierce). A total of 25 µl of a standard or test sera (diluted 2× to 10× in 10% blocking buffer in Tris saline/0.01% Tween) was added to the plates for 30 min at RT, followed by washing and incubation with 25 µl biotinylated anti-IgE (R1E4; 1/10,000 dilution) for 30 min at RT. Plates were then washed six times, and with 4819The Journal of Immunology

Measurement of IgG1 and IgG2a in serum and kidney extracts

High-binding ELISA plates (Costar, Cambridge, MA) were first coated with 10 µg/ml goat anti-mouse IgG1 or IgG2a. After blocking with 10% FCS in PBS, standard, sera, or kidney extracts were added onto the plates for 1 h at RT. After washing, alkaline phosphatase-conjugated anti-mouse IgG1 or IgG2a (1/1000 dilution) was added for 1 h at RT. The plates were then developed with pNPP and read at 405 nm on a Multiskan MS ELISA reader. Total protein in kidney extracts was determined using bichinchoninic acid protein assay kit (Pierce). Renal Ig levels are represented as picograms Ig per milligram of kidney protein.

Detection of anti-dsDNA Ab

Anti-DNA Ab were detected, as described previously (29), using purified calf thymus DNA (Sigma-Aldrich, St. Louis, MO) to coat enhanced protein assay plates (NPP). The mean scores for individual features were summed to obtain a mean score for each of the individual readers were averaged to obtain a mean score for each of the individual features. The raw scores assigned by various readers were averaged to obtain a mean score for each of the individual features. The mean scores for individual features were summed to obtain the three main scores (glomerular activity score, tubulointerstitial activity score, and chronic lesion score) and then the three main scores were summed to obtain a composite kidney biopsy score (KBS).

Assessment of nephritis

Proteinuria was estimated utilizing Albumin assay strips (Bayer, Elkhart, IN), using a scale of 0–4+. Severe proteinuria was defined as ≥300 mg/dl (3+ or more) on two consecutive examinations, as described previously (30). For the assessment of renal histology, one-half of each kidney was fixed in 4% paraformaldehyde. Paraffin sections were subsequently stained with H&E, Jones silver stain, periodic acid Schiff, and Masson’s trichrome, and scored in a blind fashion for the following features (using a 0–3 scale): glomerular hypercellularity, necrotizing lesions, karyorrhexis, cellular crescents, and hyaline deposits (these features indicate glomerular activity score); interstitial inflammation, tubular cell necrosis, and epithelial cells or macrophages in tubular lumens (tubulointerstitial activity score); glomerulosclerosis, glomerular scars, fibrous crescents, tubular atrophy, and interstitial fibrosis (chronic lesion score). The raw scores assigned by various readers were averaged to obtain a mean score for each of the individual features. The mean scores for individual features were summed to obtain the three main scores (glomerular activity score, tubulointerstitial activity score, and chronic lesion score), and then the three main scores were summed to obtain a composite kidney biopsy score (KBS).
Statistical analysis

Mann-Whitney U and Student’s t tests were used to compare cytokine, Ig, and anti-DNA Ab levels in different groups of mice. The cumulative prevalence of proteinuria was compared between the test and control groups of mice using a log-rank test.

Results

NZM.2410 mice develop severe glomerulosclerosis

Glomerulonephritis in patients with SLE is a heterogeneous disorder: some patients experience severe glomerular and interstitial inflammation, while others develop rapidly progressive glomerulosclerosis and end-stage renal disease. Different lupus-prone mouse strains develop different forms of renal disease, which may be analogous to different subsets of human lupus nephritis. To test this idea, we compared components of renal histology in NZM.2410 and MRL-lpr mice at 3–4, 5–7, and 7–8 mo of age. Results from 5- to 7-mo-old mice are shown in Fig. 1. At each age tested, glomerulosclerosis was markedly increased in NZM.2410 mice as compared with MRL-lpr mice (p < 0.01; Mann-Whitney U test; Fig. 1), whereas inflammatory lesions, such as interstitial and perivascular inflammation (p < 0.01; Fig. 1) and glomerular infiltration (p = borderline (<0.1>0.05); data not shown), were more severe in MRL-lpr mice. Thus, different lupus-prone mouse strains exhibit different patterns of nephritis: NZM.2410 mice have more severe glomerulosclerosis, whereas MRL-lpr mice develop more severe glomerular and interstitial inflammation.

Spontaneous IL-4 production is increased in the NZM.2410 mice

Because type 2 cytokines have been implicated in the development of tissue fibrosis (10, 31, 32), we examined whether expression of type 2 cytokines differs between lupus-prone strains that develop severe glomerulosclerosis vs those that develop predominantly inflammatory disease. Using a sensitive in vivo cytokine capture assay, we found that in vivo IL-4 production was significantly increased in the NZM.2410 strain when compared with other lupus-prone strains, MRL-lpr, BWF1, and NZB mice, and several nonautoimmune strains including BALB/c, B10.Pl, CF1, and BPF1 mice (p = 0.04–0.00005, Student’s t test). Results from lupus-prone mice at the early disease stage are shown in Fig. 2A. Similar results were obtained at a very young age (5–9 wk old), prenephritic age (12–16 wk old), and advanced nephritic age (>25 wk old).

IFN-γ levels, as determined by the in vivo capture assay, were not elevated in NZM.2410 mice as compared with other lupus-prone and most nonautoimmune strains tested, except when compared with CWF1 and BPF1 mice (p < 0.01; Fig. 2B). In contrast, MRL-lpr mice had higher IFN-γ levels than any other strain tested (p < 0.01; Fig. 2B).

Consistent with the increased IL-4 levels observed in NZM.2410 mice, serum IgE and IgG1 levels that are dependent on IL-4 (33) increased selectively in diseased NZM.2410 mice (p < 0.05–0.0001; data not shown). In contrast, serum IgG2a

FIGURE 1. NZM.2410 mice develop more severe glomerulosclerosis than MRL-lpr mice. A, H&E-stained kidney sections show increased inflammatory cell infiltrates in the interstitium (arrows, INT INF) and glomeruli (HG (hypercellular glomerulus)) of MRL-lpr mice, and increased glomerulosclerosis (GS) and tubular atrophy (TA) in NZM.2410 mice. B, Nephritis scores in kidneys harvested from 5- to 7-mo-old mice (n = 15 NZM.2410 and 10 MRL-lpr). Interstitial inflammatory infiltrate (p < 0.01); glomerulosclerosis (p < 0.01).
levels, which usually are dependent on IFN-γ (S), were not elevated in NZM.2410 mice as compared with other strains.

Neutralization of endogenous IL-4 in NZM.2410 mice reduces renal disease, but not serum IgG anti-dsDNA Ab level

To assess the contribution of IL-4 to disease expression in lupus-prone mice, we treated 20-wk-old NZM.2410 mice that already had circulating autoantibodies and early nephritic changes with a neutralizing anti-IL-4 mAb (11B11), an isotype-matched rat IgG1 mAb (GL113), or saline (PBS) for 12 wk. As expected, anti-IL-4 mAb treatment decreased IL-4 and increased IFN-γ production from cultured spleen cells (p < 0.05–0.01; Fig. 3A). The efficacy of this treatment was further shown by decreased serum total IgG1/ IgG2a and IgE levels in anti-IL-4 mAb-treated mice (p < 0.05; Fig. 3, B and C).

The proportion of mice developing severe proteinuria (≥300 mg/dl) was reduced in 11B11-treated vs GL113- or PBS-treated mice (p < 0.01, log-rank test; Fig. 4A). Consistent with the decreased proteinuria, a composite KBS was decreased in the 11B11-treated mice as compared with PBS- or GL113-treated mice (p < 0.01) (Fig. 4B).

Kidney lesions in lupus glomerulonephritis include acute glomerular infiltration and chronic scarring (glomerulosclerosis). To assess the effect of 11B11 treatment on these lesions, we scored H&E-, Jones silver-, periodic acid Schiff-, and Masson’s trichrome-stained renal sections, as described in Materials and Methods. Although glomerular cellularity was not significantly different between the treated and control mice, glomerulosclerosis, both global and segmental, was decreased in the 11B11-treated mice (p < 0.01; Fig. 4, C and D).

Surprisingly, 11B11 treatment did not decrease IgG anti-dsDNA Ab production (Fig. 4E). In fact, serum IgG anti-DNA Ab levels were increased in 11B11-treated mice as compared with GL113- or PBS-treated controls (p < 0.05 at 5 mo of age). Anti-DNA Ab levels of both IgG1 and IgG2a isotypes were slightly higher in 11B11-treated mice than in control animals (p = NS; data not shown). The beneficial effects of 11B11 treatment on kidney disease did not appear to be due to changes in the degree of IgG deposits in kidneys (Fig. 4F). In kidney extracts, IgG1 levels were lower and IgG2a levels were higher in 11B11-treated than in GL113-injected control animals, although the differences were not statistically significant.

These observations suggest that elevated IL-4 levels in NZM.2410 mice may contribute to the development of glomerulosclerosis and chronic renal lesions, but may not be required for, or may even inhibit, autoantibody production.

Characterization of STAT4 and STAT6 knockout NZM.2410 mice

To further address the role of type 1/2 cytokines, we transferred the STAT4 and STAT6 null mutations onto the NZM.2410 background using a marker-assisted selection protocol, which accelerates the introgression of genes by using genetic markers distributed over the whole mouse genome. The final N6 backcross mice that were used in our experiments carried 79 of 79 (100%) NZM.2410 markers. Presence of the STAT4 and STAT6 null mutations was followed by PCR-based assays, and the absence of the respective STAT protein products was confirmed by Western blot analysis (not shown). To verify the effects of STAT4 or STAT6 null mutations on the production of type 1 and 2 cytokines, spleen cells from wild-type and mutant NZM.2410 mice were isolated and activated in vitro with plate-bound anti-CD3 Ab in the absence of exogenous cytokines. This allowed determination of the absence of STAT4 or STAT6 function on the developmental potential of spleen cells into Th1 or Th2 cytokine-secreting cells. After 1 wk in culture, the activated spleen cells were restimulated with anti-CD3

FIGURE 2. Intrinsic in vivo levels of IL-4 and IFN-γ in lupus-prone and nonautoimmune mice. Lupus-prone mice, assayed at an age when they begin to develop disease (16- to 20-wk-old NZM.2410 and BWF1; 12- to 16-wk-old MRL-lpr; and 24- to 32-wk-old NZB mice), and 16- to 20-wk-old nonautoimmune control mice were injected with 10 μg of biotinylated anti-IL-4 or anti-IFN-γ; their sera were obtained and tested for levels of Ab-captured IL-4 (A) or IFN-γ (B), as described in Materials and Methods. Values in pg/ml from individual mice are shown (n = 10–12 mice per group). Horizontal bars represent the arithmetic mean for each group.

FIGURE 3. Treatment of NZM.2410 mice with anti-IL-4 mAb (11B11) inhibits IL-4 production, and reduces IgG1 and IgE levels. Twenty-week-old female NZM.2410 mice were treated with 11B11, GL113 (control Ig), or PBS. A, IL-4 and IFN-γ levels in supernatants of spleen cells cultured with Con A for 48–72 h. B and C, Total serum IgG1, IgG2a, and IgE levels were determined in 28- to 30-wk-old mice (p < 0.05–0.01 in 11B11-treated vs control groups; n = 5–8 mice per group). Results are expressed as the mean ± SE values from a representative of two independent experiments.
rum IgE levels were increased in STAT4

*p*(significantly reduction in the development of kidney disease. Thus, NZM.2410 mice (*p* for 24 h, and IL-4 and IFN-γ production was quantified by ELISA. As shown in Fig. 5A, under neutral activation conditions, splenocytes from NZM.2410 secreted both IL-4 and IFN-γ. The STAT4−/− NZM.2410 mice secreted less IFN-γ (*p* < 0.05), but more IL-4, whereas STAT6−/− NZM.2410 mice secreted less IL-4 (*p* < 0.05), but more IFN-γ (*p* < 0.05).

Because IgE and IgG1 production is dependent on IL-4 (33), while IgG2a production is usually dependent on IFN-γ (5), IgG isotypes and IgE were evaluated in serial serum samples from the mutant and wild-type NZM.2410 mice. As shown in Fig. 5B, there was a significant isotype switch toward IgG1 in STAT4−/− NZM.2410 mice (*p* = 0.05), while STAT6−/− NZM.2410 mice showed a clear IgG2a predominance (*p* < 0.05). In addition, serum IgE levels were increased in STAT4−/− mice (*p* < 0.05), while IgE levels were decreased in STAT6−/− NZM.2410 mice compared with wild-type littermates (*p* = 0.02; Fig. 5C).

Targeted disruption of STAT6 reduces kidney disease in NZM.2410 mice

Long-term follow-up of STAT6−/− NZM.2410 mice showed a significant reduction in the development of kidney disease. Thus, the proportion of mice developing severe proteinuria (>300 mg/dl) was markedly reduced in STAT6 null mice as compared with control mice (*p* = 0.005; Fig. 6A). Consistent with the decreased proteinuria, a composite KBS was decreased in these STAT6 null mice (*p* = 0.005; Fig. 6B). Furthermore, as with anti-IL-4 mAb treatment (Fig. 4, C and D), the decrease in glomerulosclerosis was the most consistent effect of STAT6 null mutation on kidney histology (Fig. 6C). Intriguingly, while we observed a significant effect on the development of kidney disease in STAT6−/− NZM.2410 mice, there was no decrease in IgG anti-dsDNA Ab levels in these mice when compared with their wild-type littermates (Fig. 6D).

Targeted disruption of STAT4 exacerbates kidney disease without increasing IgG anti-dsDNA Ab production in NZM.2410 mice

STAT4−/− NZM.2410 mice showed increased development of kidney disease, as evident by the accelerated development of severe proteinuria (>300 mg/dl) compared with wild-type littermates (*p* = 0.02; Fig. 6A), and by increase in the composite KBS (*p* = 0.05; Fig. 6B). There was no significant difference in the
effect of STAT4 null mutation on glomerular infiltration vs glomerulosclerosis (Fig. 6C). Other renal lesions, including tubular atrophy, crescent formation, and interstitial fibrosis, were increased in STAT4−/− NZM.2410, although the differences were not statistically significant (data not shown). Most interestingly, the exacerbated kidney disease in the STAT4 null mice was not associated with an increase in IgG anti-dsDNA autoantibodies. In fact, as shown in Fig. 6D, STAT4−/− NZM.2410 mice have significantly lower levels of IgG anti-dsDNA Abs compared with the STAT6−/− and control NZM.2410 mice (p = 0.05). Most autoantibodies were of IgG1 isotype in STAT4−/− mice, whereas STAT6−/− mice showed a slight increase in IgG2a compared with wild-type mice, but this difference was not statistically significant.

Discussion
In this study, we explore the abnormalities of cytokine production in lupus, and examine their role in the development and progression of lupus nephritis. Previous attempts to identify cytokine abnormalities in lupus and other diseases have generally required stimulation of immune cells, which may not reflect the intrinsic abnormality of a cytokine. We therefore used an in vivo cytokine capture assay, which does not require any exogenous stimulation, and allows detection of physiological and quite small amounts of cytokines. Using this assay, we identified two strains of lupus-prone mice, one (NZM.2410) that overexpresses IL-4 and the other (MRL-lpr) that overexpresses IFN-γ (Fig. 2). The use of these mice should help to define the roles of these cytokines in the development and progression of lupus.

IL-4 can rescue B cells from apoptosis and enhance their survival (6, 34). The increased expression of IL-4, therefore, may result in the expansion and activation of autoreactive B cells, and thus may contribute to the development or aggravation of autoantibody-mediated disease. Indeed, IL-4 transgenic C3H mice develop an autoimmune-type disorder that resembles lupus (17).
Thus, IL-4 may exacerbate B cell-mediated autoreactivity. IL-4 can also inhibit T cell activation in some in vivo systems (35). Consistent with this role, NZM.2410 mice that overexpress IL-4 have less renal inflammation compared with MRL-lpr mice that overexpress IFN-γ (Fig. 1). NZM.2410 mice, however, have more glomerulosclerosis than MRL-lpr mice (Fig. 1). Strikingly, the in vivo depletion of IL-4 or STAT6 gene deletion markedly inhibits chronic renal lesions and glomerulosclerosis (Figs. 4C, 4D, and 6C). These observations suggest a correlation between increased IL-4 levels and the development of glomerulosclerosis, and elevated IFN-γ levels with inflammatory cell infiltration. Thus, two different subsets or stages of disease may characterize lupus nephritis; one subset or stage of nephritis may be exemplified by MRL-lpr-type nephritis with marked renal inflammation, and the other subset or stage may be exemplified by the NZM.2410-type nephritis, which presents with glomerulosclerosis and less marked renal inflammation (Fig. 1).

Type 1 cytokines have been implicated in the development of anti-DNA Ab and lupus in MRL-lpr, BWF1, and BXSB mouse models of lupus (4, 18, 20, 36). Treatment of young, prenephritic BWF1 mice with an anti-IL-12 mAb decreases IgG anti-dsDNA Ab levels (36). Our results in STAT4-deficient NZM.2410 mice substantiate the importance of type 1 cytokines in autoantibody production (Fig. 6D). The STAT4-deficient mice, however, experienced accelerated nephritis in our study. Studies are underway to examine the following possibilities to explain this finding: 1) increased type 2 cytokine production in STAT4-deficient mice aggravates glomerulosclerosis in lupus-prone mice; 2) autoantibodies are not critical for the development of lupus nephritis; and 3) autoantibodies other than anti-dsDNA Ab may cause nephritis in NZM.2410 mice.

Our results show that STAT6 deficiency or anti-IL-4 mAb treatment markedly inhibits the progression of lupus nephritis in NZM.2410 mice (Figs. 4B, 4C, 6A, and 6B), even though IgG anti-dsDNA Ab levels are unchanged or slightly increased (Figs. 4E and 6D). STAT4-deficient NZM.2410 mice, by contrast, exhibited increased renal disease, despite a decrease in IgG anti-dsDNA Ab levels. These results appear to contradict a direct cause-effect relationship between the presence of autoantibodies and nephritis in NZM.2410 mice. One possibility is that some genes control the production and renal deposition of autoantibodies, while others contribute to the development and progression of renal disease (37). Consistent with this idea, mouse chromosome 11, which harbors the genes for IL-4, IL-5, and IL-13, also contains two loci, D11 Mit23 and D11 Mit164, which are linked to glomerulonephritis, but not to IgG anti-dsDNA Ab production in (NZM.2410 × C57BL/6)F2 progeny (38). Another possibility is that renal deposition of autoantibodies in SLE may represent an early event, which later triggers renal cells to secrete extra cytokines and growth factors, such as IL-4 and TGF-β, which may perpetuate glomerulosclerosis and chronic renal fibrosis. Finally, it is also possible that observed effects of the STAT4 or STAT6 knockout reflect the removal of lupus susceptibility or resistance genes during backcross of the mutated locus from the Sv129 onto the NZM.2410 genetic background. STAT4 and STAT6 genes, however, are on mouse chromosome 1 (25.9 cM) and mouse chromosome 10 (70.0 cM), respectively; thus, both genes are clearly outside any known lupus susceptibility or resistance region in NZM.2410 mice. Therefore, it is unlikely that the phenotypes presented by these mice are due to any interference of the mutated locus with potential lupus susceptibility genes.

Our results raise the possibility that IL-4 and STAT6 may be directly involved in the development of lupus nephritis, particularly glomerulosclerosis and chronic renal fibrosis. IL-4 is known to promote fibroblast proliferation, collagen gene expression, and collagen synthesis in mouse models of pulmonary fibrosis (39–41). Another type 2 cytokine, IL-13, which is also significantly increased in NZM.2410 mice as compared with BALB/c mice (our unpublished data), can increase type 1 procollagen synthesis in vitro (42). Furthermore, IL-4 serves as a growth factor for cells that secrete TGF-β (7), a cytokine known to cause tissue fibrosis (43). IL-4 transgenic mice exhibit increased renal TGF-β expression and develop glomerulosclerosis, which is independent of Ig deposition (44). Studies are underway to examine these possibilities.

Finally, the cause of dysregulated IL-4 expression in NZM.2410 mice is not known. NZM.2410 mice have significantly increased numbers of IL-4-secreting CD4+ T cells (our unpublished data), which may be committed to produce IL-4 through a genetic regulation (45). Also, increased IL-4 expression may be regulated at the level of CD1d-restricted T cells (46), as CD1d null NZM.2410 mice have decreased IL-4 production (our unpublished data). Thus, CD1d-regulated events may contribute, at least in part, to the elevated IL-4 levels in NZM.2410 mice.

In summary, there may be several distinct immune pathways that can lead to the development of renal disease in mice (or probably humans) who have SLE. In the MRL-lpr mice, autoantibody and immune complex deposition probably have an important, although not exclusive role in the development of glomerulonephritis, proteinuria, and loss of renal function. In the NZM.2410, it is not at all clear that autoantibody and immune complex deposition is important; IL-4 and perhaps other cytokines induced by IL-4, such as IL-13, may be acting directly on glomerular cells to induce glomerulosclerosis. Thus, the immune system would be important in both strains for the induction of autoantibody production and cytokine production, but the autoantibodies would contribute importantly to disease in the MRL-lpr, while the cytokines would contribute more directly to disease in the NZM.2410. If these differences can be observed in mouse models of SLE, they may also subset human SLE patients, with the implication that different subsets of patients might have different prognoses and benefit from different therapies.

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