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Pivotal Role of Stat4 and Stat6 in the Pathogenesis of the Lupus-Like Disease in the New Zealand Mixed 2328 Mice

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We have developed novel genetically lupus-prone (NZB × NZW)F1-derived congenic New Zealand mixed (NZM) 2328 lines, which are either Stat4- or Stat6-deficient. Our studies show that the deficiency of Stat4 and Stat6 significantly alters the phenotype of the lupus-like disease in NZM 2328 congenic mice. Specifically, Stat4-deficient NZM mice develop accelerated nephritis and increased charges. This article must therefore be hereby marked...


The pathogenesis of systemic lupus erythematosus (SLE), a prevalent but highly heterogeneous systemic autoimmune disorder, is still unknown. The profound heterogeneity of the human disease suggests that SLE might not be a single disease. There are several mouse models of SLE with striking similarities to human SLE. However, none of the animal models reproduce human SLE perfectly (1). What makes these animal models particularly interesting to study is that the differences may reflect different forms of the human disease. In addition, the availability of efficient transgenic and knockout (KO) technologies to produce genetic modifications provides considerable advantage using these mouse models. Genetic manipulations of the mouse immune system have revealed a complex regulatory network previously unappreciated in the study of autoimmunity. Nevertheless, these animal studies remain largely incomplete with regard to the complexity of SLE. At least partly, this may be related to the fact that the majority of the genetic manipulations in lupus-prone mice used the MRL-lpr/lpr strain, which lacks functional activity of the CD95 (Fas) apoptosis gene, an important regulator of activated T and B lymphocytes. Consequently, extrapolation of conclusions derived from these mice to human SLE in general must bear in mind that the vast majority of SLE patients possess functionally intact CD95 (2) and CD95 ligand (3). Therefore, we postulate that greater correlation to human SLE can be obtained by performing such experiments in non-lpr strains. The (NZB × NZW)F1 mice constitute one of the best-studied animal models of spontaneous systemic autoimmunity, and these mice have intact CD95 and CD95 ligand (4, 5). There are several (NZB × NZW)F1-derived inbred mouse strains (6) of which New Zealand mixed (NZM) 2410 is the most widely used congenic strain. However, NZM 2410 differs significantly from the classical (NZB × NZW)F1 model and human SLE, in that both males and females develop the disease. Although the NZM 2328 strain has been much less used so far (5), it is probably the closest congenic strain to the original (NZB × NZW)F1 model. In this study, we have used NZM 2328 mice to produce genetic alterations and study spontaneous autoimmunity in the setting of a precise predetermined genetic modification.

Glomerulonephritis (GN; including glomerular hypercellularity, glomerular immune complex deposition, and proteinuria) is one of the major clinical hallmarks of SLE. In addition, there are characteristic serological abnormalities, some highly specific for the diagnosis of SLE (e.g., anti-dsDNA Abs, anti-Sm Abs), and other lupus-associated but not pathognomonic, of SLE (e.g., antinuclear Abs, anti-nRNP, anti-chromatin). The deposition of pathogenic autoantibodies in target organs such as kidneys implies the participation of autoantibody producing B lymphocytes (1). In addition, various cytokines, including IFN-γ and IL-4, have been suggested to be involved in the pathogenesis of the autoimmune disease in human and mouse models of SLE (7–11).

Stat proteins are transcription factors that provide a direct link between the cytokine receptors and cytokine-induced gene transcription (12). Stat4 and Stat6 are members of this family that are essential in mediating responses to IL-12 and IL-4, respectively (12, 13).

In the present study, we have produced NZM 2328 deficient in Stat4 or Stat6 and have used these new genetic models to address questions related to mechanisms important in the induction and perpetuation of autoimmune disease in SLE.

Materials and Methods

Generation of mice

The original Stat4 and Stat6 null mice on a 129, C57BL/6 mixed background were kindly provided to us by Dr. M. Grusby (Harvard University School of Public Health, Boston, MA). The NZM2328 are maintained at...
the University of Southern California Laboratory Animal Resource Center (Los Angeles, CA). The Stat4- and Stat6-deficient mice were crossed onto the NZM2328 background, using a marker-assisted selection strategy. Each backcross generation was screened with polymorphic microsatellite markers at a density of ~25 cM. In each generation 25–50 males were screened, and the progeny carrying the target gene in combination with the lowest content of donor genes (129/C57BL/6) was chosen for breeding with NZM 2328 females to obtain the next backcross generation. For identification of Stat4 and Stat6 null mutations, PCR-based assays were used. One assay amplified the linked neomycin resistance (neo) gene using the primers 5'-GTT GAG ATG ATA GGA GAT C-3' and 5'-GAC TGG GCC CA GAA CAA TCC-3'. The other assay amplified a segment of the respective Stat gene spanning the null-generating mutation using the following primers: 5'-GTA CAT GAG GTA GCC TG-3' and 5'-CTG CCA GAC AGT CTT TTC C-3' (for Stat4) and 5'-CTC AGA GTC GCT AAA GGC G-3' and 5'-GGG AGT GGT TGT ATG GTG-3' (for Stat6).

**In vitro culture**

Pooled spleen cells from control and Stat4- or Stat6-deficient mice were activated with 1 μg/ml plate-bound anti-CD3 (145-2C11). After 1 wk in culture, cells were washed and restimulated with anti-CD3 for 24 h. Where indicated, IL-12 was added at 200 U/ml. Supernatants were harvested, and IFN-γ and IL-4 production was quantified using commercial ELISA kits per the manufacturer’s instructions.

For measuring NK cell cytolytic activity, splenocytes were enriched for NK cells (stained with biotin-anti-CD3 and rat anti mouse Ig and depleted of T cells by magnetic beads), and incubated with IFN-γ and 1/100 mouse sera for 1 h at room temperature, washed, and incubated with alkaline phosphatase-conjugated goat anti-mouse IgG subclass-specific Ab (Southern Biotechnology Associates, Birmingham, AL). Bound IgG subclasses were measured using a scale of 0 to 3 (maximum staining). The other assay amplified a segment of the lupus nephritis was classified and graded histopathologically according to the World Health Organization classification and scored according to the morphologic index of glomerular activity, the method widely used in evaluation of kidney biopsies in humans with SLE.

**Flow cytometry**

Splenocytes were obtained from control and Stat4- and Stat6-deficient mice and enriched for small resting B cells by treatment of splenocytes with anti-Thy-1.2 and complement followed by Percoll gradient centrifugation. Resting B cells were cultured with IL-4 (30 U/ml). After 48 h, cells were stained with FITC-anti-CD23 and biotinylated 10.2.16 anti-I-α-β Ab (kindly provided by Dr. B. Kotzin, University of Colorado, Denver, CO). Flow cytometry was performed on a FACScan (BD Biosciences, San Jose, CA).

**Serologic analysis**

Serum Ig profile. Total IgG and IgG subclasses (IgG1, 2a, 2b, 3) were determined by ELISA as described (14). Microtiter plate wells were coated with Fc-specific F(ab')2 goat anti-mouse IgG (Southern Biotechnology Associates, Birmingham, AL). Bound IgG subclasses were measured using alkaline phosphatase-conjugated goat anti-mouse IgG subclass-specific Ab (Caltag Laboratories, San Francisco, CA). Isotype standards were used for standard curve fitting and Ig concentrations were calculated using Software (Molecular Devices, Menlo Park, CA).

Anti-chromatin Abs were measured by ELISA as described (15) with minor modifications. Wells were coated with 100 μl of 1 mg/ml chicken chromatin. Washed wells were incubated in 100 μl of 1/100 mouse sera for 1 h at room temperature, washed, and incubated with alkaline phosphate-labeled goat-anti-IgG. Plates were developed with p-nitrophenyl phosphate (pNPP) substrate (Sigma-Aldrich, St. Louis, MO). OD_{405} was converted to units based on the standard curve produced by serial dilutions of pooled sera from MRL-lpr/lpr mice.

Anti-dsDNA Abs were assayed in two steps. First, a semiquantitative, but highly specific Cribbidia helicae assay was used at a serum dilution of 1/20 as described (14). Secondary Ab was FITC-conjugated goat anti-mouse IgG (Southern Biotechnology Associates). Second, positive sera were further assayed for IgG and IgG subclass-specific anti-dsDNA Ab by a less specific but much more quantitative ELISA as previously described (16). Samples were screened at a 1/50 dilution and the results were expressed in arbitrary units relative to a standard positive sample derived from MRL-lpr/lpr mice.

**Assessment of nephritis**

The development of proteinuria was assessed weekly using Albus test assay strips (Bayer, Elkhart, IN) using a scale of 0 to +4. Severe proteinuria was defined as >300 mg/dl (+3 or more) on two consecutive examination. For the assessment of renal histology, half of each kidney was fixed in 4% paraformaldehyde. Paraffin sections (3 μm) were stained with H&E, periodic acid Schiff (PAS), and Masson’s trichrome, and scored in a blinded fashion for the following features (using a 0 to 3 scale): glomerular hypercellularity, neocapillary formation, 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 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). In addition, lupus nephritis was classified and graded histopathologically according to the World Health Organization classification and scored according to the morphologic index of glomerular activity, the method widely used in evaluation of kidney biopsies in humans with SLE.

**Statistical analyses**

The data are presented as mean ± SEM. The Mann-Whitney U test was used to analyze nonparametric data for statistical significance. Values of p ≤ 0.05 were considered significant.

**Results**

**Establishment of Stat4 and Stat6 null NZM2328 strains**

We have transferred the Stat4 and Stat6 null mutations onto the lupus background of NZB × NZW. However, rather then using the original parental strains, we used the (NZB × NZWF1)-derived inbred mouse strains NZM 2328 which most closely resembled the (NZB × NZWF1)F1. This eliminated the need to backcross the null mice separately to NZB and NZW backgrounds.

A marker-assisted selection protocol was used, which accelerates the introgression of genes by using genetic markers to facilitate this process (17–19). By specifically selecting progeny at each backcross generation that carry the target gene and have the lowest content of heterozygosity throughout the genome, the rate of donor genomic segment elimination is significantly accelerated. We screened each generation with microsatellite markers at a density of ~25 cM. At least 25 males were screened each generation. In all, six backcrosses were completed in each of the lines. The final backcross mice carried 76 of 76 (100%) NZM 2328-specific markers but none (0%) of the C57BL/6 or 129 strain-specific marker alleles. These mice were intercrossed to produce homozygous null mice used for all the experiments described below. To follow the Stat4 and Stat6 null mutations in the mice, we used PCR-based assays: one that amplified the neomycin-resistance (neo) gene and the other that amplified a part of the respective Stat gene containing the eliminated portion. The defect in the resulting mice was confirmed by the absence of these Stat proteins in Western blot analysis using polyclonal anti-Stat4 and anti-Stat6 (data not shown).

**Characterization of the mice lines produced**

The first step in characterizing these newly created mice models was to verify the effect of Stat4 and Stat6 null mutations on the production and regulation of Th1 and Th2 cytokines.

Spleen cells from “wild-type” NZM 2328 (control) and mutant NZM.Stat4−/− and NZM.Stat6−/− mice were activated in vitro with plate-bound anti-CD3 in the absence of exogenous cytokines to determine their developmental potential into Th1 or Th2, as described (20, 21). IFN-γ and IL-4 production was quantified by ELISA. Representative results are shown in Fig. 1. Under neutral...
null mice, there was a significant reduction in the level of IgG1 in NZM2328 mice compared with Stat4 wild-type controls (Fig. 4). Similarly to the original 129/B6 Stat-4-deficient mice, the NZM2328.Stat4 null mice secreted high levels of IFN-γ but very little IL-4 (p < 0.05). We have also tested the ability of splenocytes from Stat4−/− and Stat6−/− NZM mice to produce IFN-γ in response to IL-12. Similarly to the original 129/B6 Stat−4−/− mice, the NZM2328.Stat4−/− mice do not show enhanced IFN-γ secretion following IL-12 stimulation. In contrast, NZM2328.Stat6 null mice do demonstrate enhanced IL-12-induced IFN-γ secretion (Fig. 2).

Next, IL-12-induced NK cell cytotoxicity was tested in NZM Stat4 null mice. Splenocytes were enriched for NK cells, and incubated with IL-12. NK cytolytic activity against 51Cr-labeled YAC-1 targets was assayed. As shown in Fig. 3, NZM.Stat4 null mice do not show IL-12-induced NK cell cytotoxicity (p < 0.05). In control experiments, NK-enriched splenocytes from NZM.Stat4 null mice, stimulated with 250 μg/ml poly I:C (to evaluate non-IL-12-mediated NK activity), had readily detectable cytolytic activity against YAC-1 cells (data not shown).

To monitor total Ig and various Ig isotypes, we measured autoantibody levels in NZM Stat4 null mice. Stat4−/− mice showed a significant decrease in KBS at 5.5 mo of age (Fig. 7). NZM2328.Stat6-deficient female mice showed a significant delay in development of kidney disease (p < 0.001). Interestingly, the NZM2328.Stat4−/− mice with accelerated kidney disease displayed much lower levels of anti-dsDNA Ab and anti-chromatin Abs compared with the wild-type NZM2328 mice. Fig. 7 shows results for anti-chromatin and anti-dsDNA Abs tested in 4- to 7-mo-old mice of the different genotypes. Stat4−/− animals had significantly lower levels of total IgG autoantibodies compared with Stat4+/− NZM2328 control mice (p < 0.05). In contrast, Stat6-deficient mice produced as high (or somewhat higher) levels of IgG autoantibodies as the wild-type NZM2328 animals (differences are not statistically significant).

The NZM2328.Stat4−/− mice show a panisotype elevation of autoantibody levels. In the Stat4-deficient animals the autoantibodies were predominantly of the IgG1 subclass, with relative paucity of IgG2a (p < 0.01); Fig. 7C shown for anti-dsDNA Abs), whereas autoantibodies in the Stat6-deficient cohort were predominantly IgG2a with relative scarcity of IgG1 (p < 0.01).

For assessment of renal pathology, paraffin sections were stained with H&E, PAS, and Masson’s trichrome, and scored in a blinded fashion. The severity of kidney pathology was graded according to the World Health Organization classification followed by computing a composite KBS for each kidney evaluated. By the age of 5.5 mo, most Stat4-deficient female mice showed diffuse proliferative GN World Health Organization class IV or diffuse proliferative and sclerosing GN class IV (Fig. 8). Stat 6-deficient female mice showed very little pathology at 5–5.5 mo of age. By 10–12 mo of age, they showed mostly mesangio proliferative GN class II or focal proliferative GN class II. As shown in Fig. 8F, by 5.5 mo of age Stat4−/− NZM2328 showed significant increase in the composite KBS compared with wild-type NZM2328 mice (p < 0.05). In contrast, Stat6-deficient NZM2328 mice had a significant decrease in KBS at 5.5 mo of age (p < 0.01), and even...
at 10–12 mo of age \((p/0.05)\), an age when all Stat4-deficient mice and most control NZM 2328 mice were already dead. The Stat4 null mice could not be differentiated from Stat6 null mice by the extent of involvement of interstitium and blood vessels or on the level of evidence for glomerulosclerosis vs glomerular infiltration (data not shown).

A trivial explanation for why severe disease is seen in the absence of high levels of circulating autoantibodies might be that the Abs are bound up as immune complexes in the kidneys and the kidneys are serving as a sink for formation of immune complexes. To determine whether these Abs actually deposit in the glomeruli in vivo, we have performed immunohistochemistry analysis of kidneys of the different congenic mice. For immunofluorescence, kidneys were embedded in OCT and \(3\)-\(\mu\)m frozen sections were stained with 1/20 goat anti-mouse IgM, IgG1, 2a, 2b, 3 or with rabbit anti-mouse C3. Glomerular staining was graded by intensity. NZM 2328 \(+/+\) mice show high levels of panisotype deposition of Ig and complement (Fig. 9G) compared with the NZM 2328 mice. The Stat6 mice show also reduced immune deposits. However, although in the Stat4 \(+/−\) mice the deposits shown are mostly IgM and IgG1 and no IgG2a or IgG2b, the Stat6 \(+/−\) mice deposits are mainly IgM, IgG2a and IgG2b and almost no IgG1 (Fig. 9H).

**Discussion**

In this study, we explore the development and progression of mouse SLE as a consequence of deficiency in Stat genes (Stat4 and Stat6). The generation of these new mice has provided useful experimental models to explore cytokine regulation, as signaling by a specific cytokine using a specific Stat pathway has been abolished. Among Stat family members, Stat4 and Stat6 are key regulators of T cell differentiation involved in immune responses (12).

The Stat4 and Stat6 genes encode transcription factors that, when phosphorylated by Janus kinases, are activated and transported to the nucleus where they regulate cytokine-induced gene expression (22). Until very recently, production of IL-4, an essential cytokine for differentiation of Th2-responsive cells, was thought to be completely dependent on signaling via Stat6. Indeed, original studies with Stat6 \(+/−\) mice suggested that these KO mice were completely deficient in Th2 production (20, 21, 23). However, more recent studies indicate there is an alternative pathway for differentiation of Th2 cells that is independent of Stat6 (24–27). Despite this alternative pathway, CD4 \(+/+\) T cell differentiation in Stat6 \(+/−\) mice defaults to a Th1-like response and a deficiency of functional Th2 cells (26, 28, 29).

Similarly, Stat4 was originally thought to be critical for development of Th1 cells (30) because it is a transcriptional regulatory molecule for IL-12, which is a potent differentiation agent for Th1 cells (31). However, recent studies demonstrate that there are both Stat4-dependent and Stat4-independent pathways for the development of Th1 cells. Although the Stat4-dependent pathway involves induction of IL-12, the Stat4-independent pathway does not, and thereby represents a default pathway that occurs in the absence of Stat4 signaling (32). Even though Stat4 \(+/−\) mice contain an alternative pathway for generating Th1 cells, the CD4\(+\) T cell response...
in these mice is strongly skewed toward a Th2 response, and Stat4−/− mice are deficient in Th1-type cells.

There is great interest in the possibility that a skewing toward a Th2-type response plays a major role in murine lupus. Such skewing has been suggested because CD4+ T lymphocytes from (NZB × NZW)F1 mice secrete less IL-2 as the mice age and the disease becomes more clinically evident (33). Further, IL-4 mRNA expression increases with age and disease development in (NZB × NZW)F1, while IL-12 mRNA expression decreases (34). Cytokine profiles in mitogen-stimulated T cells in these mice showed high Th2-type cytokine production (IL-4, IL-5, and IL-10), while Th1 cytokine (IL-2, IFN-γ) production appears to be low (10). Moreover, the administration of anti-IL-10 to (NZB × NZW)F1 mice delays disease onset (35), while treatment with IL-4 antagonists decreases anti-DNA Ab and associated renal disease (36). Also, transgenic expression of IL-4 under a MHC class I promoter induces autoimmunity in a nonautoimmune background (37).

Although the above studies suggest a pivotal role for Th2 cytokines, other findings suggest an important role for Th1 responses, as well. For example, the major isotype eluted from glomerular lesions of lupus mice is IgG2a (38), which depend on IFN-γ for its synthesis. Furthermore, lupus mice are protected from disease development by inhibition of IFN-γ signaling. Thus, it has been shown that neutralization of IFN-γ by mAb (8) or soluble IFN-γ receptor (9) prevent GN in (NZB × NZW)F1 mice, while administration of exogenous IFN-γ accelerates renal disease. Interestingly, deficiency in IFN-γ receptor crossed onto NZB and NZW mice causes a marked reduction in anti-DNA Ab, including IgM and IgG1 as well as IgG2a (39). Further, IFN-γ receptor null (NZB × NZW)F1 mice show significant reduction in kidney disease development (39).

Our results presented in this study suggest that the validity of the Th1/Th2 dichotomy in explaining the pathogenesis of lupus appears too simplistic. Some recent studies have raised doubts about the validity of the Th1/Th2 paradigm in other systems as well (40, 41).

The results raise questions regarding the accepted paradigm that autoantibodies (especially anti-dsDNA Ab) play a major, direct, and culpable role in inducing some of the disease manifestations of SLE (especially nephritis). It is commonly believed that if Ab-Ag interaction occurs and complement is activated, tissue damage will result. The mechanism by which anti-DNA Ab is believed to fix to tissue and cause damage is either by forming immune complexes

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**FIGURE 5.** Diminished IL-4-induced CD23 and I-A expression in Stat6−/−.NZM2328, but not in Stat4−/−.NZM2328, mice. Small resting B cells, obtained as described in Results, were cultured with IL-4 (30 U/ml). After 48 h, cells were stained with FITC-anti-CD23 and biotinylated 10.2.16 anti-I-A Ab. CD23 and I-A expression was evaluated by flow cytometry.

**FIGURE 6.** Cumulative proteinuria (A) and mortality (B) of NZM 2328 lines with age. A total of 15–25 female mice for each experimental group were monitored up to 12 mo of age. Data are presented as the percent cumulative prevalence of severe (≥300 mg/dl) proteinuria and as percent cumulative survival at the ages shown. *, p < 0.001 vs control mice.
of the correct size and charge to be trapped in the basement membranes of glomeruli, or by binding directly to DNA, DNA-histone, or to non-DNA Ag present in the basement membranes. Our results suggest that the association between the presence of autoantibody and kidney disease is not a simple and direct cause-effect relationship–any explanation reduced to such a simple cause-effect relationship is precarious, at best.

Recently, Shlomchik and colleagues (42) have demonstrated an Ab-independent mechanism for renal and vascular disease in the MRL-1pr/lpr mouse model using a mutant transgene encoding membrane Ig, but which did not permit the secretion of circulating Ab. Thus, a possible explanation of the accelerated nephritis obtained in Stat4-deficient lupus mice might be that autoantibodies are not critical for the development of lupus nephritis. However, while the membrane only Ig transgenic MRL-1pr/lpr mice showed preferential involvement of the interstitium and blood vessels rather than proliferative GN, there seems to be no such preferential involvement in the Stat4-deficient NZM2328 mice. In contrast, the genetic background of the mice developing the Ab-independent lupus nephritis is very different from the background of the Stat 4 null mice. Thus, the differences in the nature of the nephritis between these mice might be a consequence of their different genetic background. An alternative mechanistic explanation might be that for developing lupus nephritis a certain threshold of insult to the kidney is necessary to initiate the process. Presumably, such an insult can be delivered by autoantibody complement complexes. If the threshold of the insult is achieved, the additional excess of autoantibody might make no difference in terms of the overall outcome of the kidney disease. We are currently testing these possibilities.

Our results in Stat4-deficient NZM 2328 mice also substantiate the importance of Th1-type cytokines in autoantibody production. These results also raise questions regarding the claimed crucial role of IFN-γ in the development of lupus pathology because we found that the nephritis in Stat4 null mice is worse than in wild-type NZM 2328 mice despite reduced production of IFN-γ. Furthermore, the data might suggest a protective role for IFN-γ in disease initiation, possibly by a mechanism similar to what has been shown in transplantation studies (43, 44) and in some cases of autoimmunity (45–47). Further studies will attempt to verify these possibilities.

It is also possible that the observed effects of the Stat4 or Stat6 KO reflect the removal of lupus susceptibility or resistance genes during backcrossing of the mutated locus from the 129/C57BL6 background on to the NZM2328 genetic background. Stat4 and

FIGURE 7. Comparison of serum IgG, anti-chromatin, and anti-dsDNA Ab levels in NZM2328 mice with the indicated genotypes: ●, NZM 2328+/+; ○, Stat4+/−; △, Stat6+/−. The mean Ab level of 8–12 mice per group at the indicated ages is shown. A, Total IgG anti-chromatin Ab levels shown in the different groups. B, Total IgG anti-dsDNA Ab levels in the mice groups and (C) serum IgG1 and IgG2a levels of anti-dsDNA Ab in the NZM2328 mice with the indicated genotypes.

FIGURE 8. Assessment of renal pathology in Stat4- and Stat6-deficient NZM 2328 mice. A–E, Representative kidney histopathologies of individual mice are shown. A, H&E-stained section, ×200, of kidney showing diffuse proliferative GN in a 5-mo-old Stat4-deficient NZM2328 mouse; B, PAS-stained section of kidney, ×200, from a 5.5-mo-old Stat4-deficient mouse showing a glomerulus with segmental scaring crescent, a second glomerulus with fibrocellular crescents, and a third glomerulus with proliferative GN; C, Masson’s trichrome-stained glomerulus section, ×400, showing fibrocellular crescent in a 5.5-mo-old Stat4-deficient mouse; D, PAS-staining, ×400, of a glomerulus showing hypercellularity and early crescent in a 12-mo-old Stat6-deficient NZM2328 mouse; E, H&E staining, ×400, showing mesangial deposits, but otherwise a normal glomerulus in a 11.5-mo-old Stat6-deficient mouse. F, Composite kidney biopsy scores (KBS) of Stat-mutated and control NZM 2328 mice at the ages indicated. The mean score of each group (n = 10–15 mice) is marked.
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 2328 mice (5). 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.

More importantly, these findings may be relevant to human SLE because there is abundant clinical experience demonstrating that...

**FIGURE 9.** Relative distribution of Ig isotype and complement deposits in glomeruli of Stat-deficient and control NZM 2328 mice. The severity of GN in the kidneys was first graded according to the World Health Organization classification before the immunofluorescence studies. A–F, Representative Ig isotype deposits in the various lines using immunofluorescence. A, Granular IgG1 deposits in wild-type NZM2328 mouse with diffuse proliferative GN class IV; B, diffuse IgG2a deposits in wild-type NZM 2328 mouse with diffuse proliferative and sclerosing GN; C, IgG2a focal glomerular deposits in a Stat6−/− mouse with focal proliferative GN class II; D, IgM deposits in Stat4-deficient mouse with diffuse proliferative GN class IV; E, minimal IgG1 deposits in a Stat4−/− mouse with diffuse proliferative GN class IV; F, segmental IgG1 deposits in Stat4-deficient mouse with diffuse proliferative and sclerosing GN class IV; G, comparison of relative deposition of complement C3 in renal glomeruli of the congenic mouse strains tested at 4–4.5 and 5.5–6 mo of age, respectively. Values are the mean of seven to nine mice per group. *, p < 0.05 compared with the control NZM2328 mice. H, Comparison of distribution of Ig isotype deposits in glomeruli of individual Stat-deficient and control mice tested at 5–5.5 mo of age.
severe GN can arise even in the absence of elevated anti-dsDNA Ab. In addition, in our lupus clinic we are following several patients that have stable high titers of anti-dsDNA Ab in their serum, without any evidence of kidney disease and a very mild SLE, an occurrence that is reminiscent of our Stat6-deficient NZM 2328 mice.

Finally, our results suggest that development of drugs that specifically regulate Stat4 and Stat6 activity may pave the way for novel therapeutic intervention in SLE.

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References


