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Membranous Glomerulonephritis Development with Th2-Type Immune Deviations in MRL/lpr Mice Deficient for IL-27 Receptor (WSX-1)¹

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MRL/lpr mice develop spontaneous glomerulonephritis that is essentially identical with diffuse proliferative glomerulonephritis (World Health Organization class IV) in human lupus nephritis. Lupus nephritis is one of the most serious complications of systemic lupus erythematosus. Diffuse proliferative glomerulonephritis is associated with autoimmune responses dominated by Th1 cells producing high levels of IFN- γ . The initial mounting of Th1 responses depends on the function of the WSX-1 gene, which encodes a subunit of the IL-27R with homology to IL-12R. In mice deficient for the WSX-1 gene, proper Th1 differentiation was impaired and abnormal Th2 skewing was observed during infection with some intracellular pathogens. Disruption of the WSX-1 gene dramatically changed the pathophysiology of glomerulonephritis developing in MRL/lpr mice. WSX-1^{-/-} MRL/lpr mice developed disease resembling human membranous glomerulonephritis (World Health Organization class V) with a predominance of IgG1 in glomerular deposits, accompanied by increased IgG1 and IgE in the sera. T cells in WSX-1^{-/-} MRL/lpr mice displayed significantly reduced IFN- γ production along with elevated IL-4 expression. Loss of WSX-1 thus favors Th2-type autoimmune responses, suggesting that the Th1/Th2 balance may be a pivotal determinant of human lupus nephritis development. *The Journal of Immunology*, 2005, 175: 7185–7192.

Systemic lupus erythematosus (SLE)⁴ is characterized by multiorgan inflammation and the production of autoantibodies by activated B lymphocytes. These autoantibodies lead to immune complex (IC) formation that can cause a renal disorder called lupus nephritis, one of the most serious complications of SLE (1, 2). Lupus nephritis is manifested with considerable phenotypic and histological heterogeneity. In particular, diffuse proliferative glomerulonephritis (DPGN; World Health

Organization (WHO) class IV) and membranous glomerulonephritis (MGN; WHO class V) represent two morphologic forms that are polar opposites (3, 4). The pathogenesis of DPGN is associated with a predominance of Th1 cytokines, indicating that DPGN occurs in a Th1-dominant immune milieu. The renal tissue of DPGN patients shows increased levels of Th1 cytokines, including IFN- γ (5, 6). Furthermore, the ratio of IFN- γ vs IL-4 production correlates positively with the histological activity index of the nephritis (5, 6). In contrast, PBLs from MGN patients show a decreased IFN- γ vs IL-4 production ratio (5), suggesting that a Th2-dominant cytokine response is associated with the pathogenesis of MGN. This hypothesis is supported by the phenotype of serum cryoglobulins isolated from DPGN and MGN patients, with mostly Th1-related IgG3 autoantibodies (murine equivalent IgG3) being present in DPGN cases, and mostly Th2-related IgG4 (murine IgG1) autoantibodies dominating in MGN cases (7). These observations indicate that the differential pathogenesis of DPGN and MGN may be governed by the Th1/Th2 cytokine balance.

MRL/MpJ-lpr/lpr (MRL/lpr) mice have proven particularly valuable for the investigation of SLE pathogenesis (2). MRL/lpr mice develop a lupus-like autoimmune disease characterized by severe pan-isotypic hypergammaglobulinemia, autoantibody production, lymphadenopathy, and immune complex (IC)-associated nephritis. Because MRL/lpr nephritis involves the glomerulus, interstitium, and vascular components, and shows infiltration of macrophages and T cells, it is considered a suitable histological model for human DPGN. Moreover, like human DPGN, MRL/lpr nephritis is associated with Th1 responses (8). The induction of a Th1 response in MRL/lpr mice accelerates the disease (9), while significantly reduced lymphadenopathy and nephritis develop in MRL/lpr mice deficient for IL-12, IFN- γ , or IFN- γ R (10–12). Thus, the contribution of Th1 responses to the development of

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⁴ Abbreviations used in this paper: SLE, systemic lupus erythematosus; DPGN, diffuse proliferative glomerulonephritis; IC, immune complex; MGN, membranous glomerulonephritis; PAM, periodic acid-methenamine-silver.

DPGN has been well established in animal models. However, to date, no suitable spontaneous animal model for human MGN has been identified, precluding detailed studies of the pathophysiology of this form of lupus nephritis. Accordingly, the effects of Th1 vs Th2 responses on MGN development remain unclear, and evidence-based drug development for human MGN has been stymied.

Differentiation of Th1 and Th2 cells is largely mediated by signaling initiated by the binding of cytokines to their cognate receptors. WSX-1 is a class I cytokine receptor expressed mainly on T lymphocytes and was originally cloned as a homologue of gp130 (13). *WSX-1*^{-/-} C57BL/6 mice show increased susceptibility to intracellular pathogens such as *Listeria monocytogenes* and *Leishmania major* due to impaired IFN- γ production early in the infection (14, 15). In addition, the production of Th2 cytokines, including IL-4 and IL-13, is enhanced in *WSX-1*^{-/-} mice infected with *L. major*, indicating an abnormal skewing of the Th1/Th2 balance toward Th2. WSX-1 thus plays a critical role in the initiation of Th1 responses to infection by some intracellular pathogens. However, the function of WSX-1 in the induction of autoimmune diseases, either organ specific or systemic, has yet to be clarified.

In this study, we disrupted the *WSX-1* gene in MRL/*lpr* mice and characterized the effects of this mutation on mouse survival, the pathology of glomerulonephritis, and various immunological parameters. *WSX-1*^{-/-} MRL/*lpr* mice developed disease essentially identical with human MGN, accompanied by marked impairment of Th1-type immune responses with Th2-type immune deviation. These data strongly support the hypothesis that alterations in the Th1/Th2 balance strongly influence the pathogenesis of autoimmune glomerulonephritis.

Materials and Methods

Generation of *WSX-1*^{-/-} MRL/*lpr* mice

The generation of WSX-1-deficient mice in the C57BL/6 background has been described previously (15). MRL/*lpr* mice were purchased from The Jackson Laboratory. *WSX-1*^{+/-} mice in MRL/*lpr* background were produced by crossing *WSX-1*^{+/-} mice into the MRL/*lpr* background six times (continual backcrossing: 98.44% in MRL/*lpr* background). All of the mice used were examined for homozygosity of *lpr* allele by PCR. Genotyping for *lpr* alleles was performed by PCR amplification of genomic DNA. Primers used for PCR amplification were: 5'-AGC AGA CTT GCT TCT GTT TA-3' (sense for both wild-type and *lpr* allele), 5'-AGC TAG GGT CAG TGA GTA-3' (antisense for wild-type allele), and 5'-CTG TCC GTT GCT CCG ATG T-3' (antisense for *lpr* allele). Genotypes for *WSX-1* gene were likewise performed by PCR amplification of genomic tail DNA. Primers used for PCR amplification were: 5'-AAC AGG TGT CTT GCC ATC ATT-3' (sense for both wild-type and mutated *WSX-1* genes), 5'-CCA GGT GTC TCA GGG TCT AAC-3' (antisense for wild type *WSX-1* gene), and 5'-CGA AGG GGC CAC CAA AGA ACG-3' (antisense for mutated *WSX-1* gene). Female homozygous mutant mice (*WSX-1*^{-/-}) and heterozygous littermate mice (*WSX-1*^{+/-}) born to *WSX-1* heterozygous intercrosses were used for all experiments. Mice were maintained in the Laboratory of Animal Experiments of Kyushu University. All experiments were approved by the Institutional Animal Research Committee of Kyushu University and conformed to the animal care guidelines of the American Physiologic Society.

Serologic analysis and urinalysis

For serum Ig determinations, ELISA were performed using the following Abs: rat anti-mouse IgG1 (Zymed Laboratories; 04 6100), rat anti-mouse IgG1-HRP (BioSource International; AMI2311), goat anti-mouse IgG2a (Bethyl Laboratories), and rabbit anti-mouse IgG2a-HRP (Cappel Lab.; 50283). The level of anti-dsDNA Ab was measured using a commercially available ELISA kit (Shibayagi). Determination of serum cytokine levels was performed using Cytometric Bead Array Kit (BD Pharmingen), according to the manufacturer's direction. For urinalysis, ~200 μ l of urine was taken from each mouse ad libitum and was analyzed for protein and creatinine levels at SRL. For brief quasi-quantification of 24-h protein in the urine, urinary protein:creatinine ratio was calculated for each sample.

Histopathological and immunohistopathological studies of kidneys

Mouse kidneys were fixed in 10% Formalin for 24 h at 4°C. Paraffin sections (4 μ m) were stained either with H&E, periodic acid Schiff stain, or periodic acid-methenamine silver (PAM). For glomerular proliferative activity, 50 glomerular cross sections per kidney were examined and scored according to the following criteria: grade 0, no recognizable lesion in glomeruli; grade 1, mild cell proliferation and/or cell infiltration; grade 2, the same as grade 1 with mesangial proliferation, lobulation, and hyaline droplet, associated with macrophage infiltration; grade 3, the same as grade 2 with crescent and granuloma formation and/or hyalinosis. The grade score for individual mice was an average of 50 glomerular cross sections from the respective mice. For membranous changes, 30 glomerular cross sections per mouse of PAM-stained specimens were examined for spikelike alterations of basement membranes with diffuse thickening of peripheral capillary walls in the paucity of cellular proliferation. For immunohistochemical staining, kidneys were snap frozen in optimal cutting temperature compound (Sakura). To detect IC deposits, cryostat sections (2 μ m) were fixed in chilled acetone and stained with FITC-conjugated goat polyclonal anti-mouse IgG Abs (Organon Teknika), a FITC-conjugated goat anti-mouse IgG1 Ab (Southern Biotechnology Associates), and FITC-conjugated goat anti-mouse IgG2a Ab (Southern Biotechnology Associates). For negative controls, sections were treated with normal goat IgG (Santa Cruz Biotechnology). For electron microscopy, tissues from 36-wk-old *WSX-1*^{-/-} MRL/*lpr* mice were fixed in 3% glutaraldehyde, postfixed in osmium tetroxide, dehydrated in a graded alcohol series, and embedded in Epon 812 before serial cutting. Ultrathin sections (60–80 nm) were double stained with uranyl acetate and Reynold's lead citrate (16).

Real-time quantitative PCR and TaqMan primers and probes

Expression levels of IFN- γ , IL-4, IL-5, and IL-13 in CD4⁺ T cells were determined relative to that of GAPDH. Cytokine levels were measured using TaqMan-PCR and an ABI prism 7700 sequence detection system (PerkinElmer), according to the manufacturer's instructions (Applied Biosystems). Oligonucleotide primers and probes were designed using the Primer Express program (Applied Biosystems). The relative expression of each mRNA was determined and normalized to the expression of the internal housekeeping gene GAPDH. Primer and probe sequences (5' to 3') are as follows: IFN- γ , PCR primers 5'-TGA ACG CTA CAC ACT GCA TCT TG-3' and 5'-TCT TCC ACA TCT ATG CCA CTT GA-3'. Probe sequence (135-bp amplicon): 5'-TCA TGG CTG TTT CTG GCT GTT ACT GCC-3'. IL-4: PCR primers 5'-TCT CAT GGA GCT GCA GAG ACT CT-3' and 5'-TCC AGG AAG TCT TTC AGT GAT GTG-3'. Probe sequence (184-bp amplicon): 5'-TCG ATA AGC TGC ACC ATG AAT GAG TCC A-3'. GAPDH: PCR primers 5'-GCA GTG GCA AAG TGG AGA TTG-3' and 5'-ATT TGC CGT GAG TGG AGT CAT-3'. Probe sequence (95-bp amplicon): 5'-CCA TCA ACG ACC CCT TCA TTG ACC TC-3'.

In vitro induction of cytokine production by CD4⁺ T cells

CD4⁺ T cells were purified from splenic extracts using magnetic beads (Miltenyi Biotec). Purified CD4⁺ T cells (2.5 \times 10⁵/200 μ l) were cultured in the presence of irradiated (30 Gy) syngeneic spleen cells (1 \times 10⁶/200

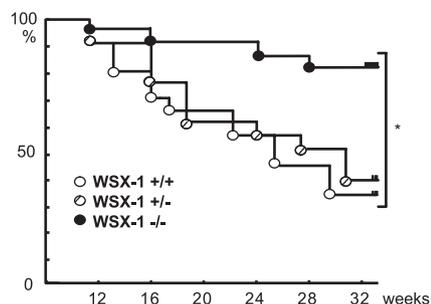


FIGURE 1. Prolonged survival of MRL/*lpr* mice in the absence of WSX-1. The cumulative survival of female *WSX-1*^{+/+}, *WSX-1*^{+/-}, and *WSX-1*^{-/-} mice in the MRL/*lpr* background was monitored weekly ($n = 20$ per group). *, $p < 0.05$ by the Kaplan-Meier method. There was no difference in the survival rate of *WSX-1*^{+/+} MRL/*lpr* and *WSX-1*^{+/-} MRL/*lpr* mice.

μl) and activated by treatment with Con A (30 $\mu\text{g}/200 \mu\text{l}$) for 24, 48, or 72 h. Culture supernatants were analyzed for the production of IFN- γ or IL-4 using ELISA development kits (Genzyme), according to the manufacturer's directions. For flow cytometric analysis of intracellular IFN- γ or IL-4 production, splenic CD4⁺ T cells ($1 \times 10^5/\text{ml}$) were cultured for 6 h with PMA (10 ng/ml) plus Ca²⁺ ionophore (500 ng/ml) in the presence of brefeldin A and GolgiStop (BD Pharmingen). Cells were then stained with allophycocyanin-conjugated anti-CD4 Ab (BD Pharmingen) and fixed and permeabilized with the Cytofix/Cytoperm Plus kit (BD Pharmingen), according to the manufacturer's directions. Cells were stained with FITC-conjugated anti-IFN- γ Ab (BD Pharmingen) or FITC-conjugated anti-IL-4 Ab (BD Pharmingen) and analyzed for the percentage of IFN- γ - or IL-4-positive cells.

Statistical analyses

Data are expressed as the mean \pm SD. Statistical differences among mouse survival rates were determined using either the Mann-Whitney *U* test or the unpaired *t* test. A level of $p < 0.05$ was considered statistically significant.

Results

Prolonged survival and alteration of renal pathology in *WSX-1*^{-/-} *MRL/lpr* mice

MRL/lpr mice spontaneously develop lethal glomerular disease accompanied by various immunological abnormalities (17). To examine the impact of *WSX-1* deficiency on the pathophysiology of *MRL/lpr* mice, we generated *WSX-1*^{-/-} mice in the *MRL/lpr* background (*WSX-1*^{-/-} *MRL/lpr* mice) and monitored their survival (Fig. 1). Although *WSX-1*^{+/-} *MRL/lpr* and *WSX-1*^{+/+} *MRL/lpr* mice died after birth at a similar rate ($\sim 50\%$ dead by 24 wk after birth in both cases), *WSX-1*^{-/-} *MRL/lpr* mice showed significantly better survival ($\sim 10\%$ dead at week 24). In accordance with this striking difference in survival, analyses of clinical features and serum chemistry parameters of *WSX-1*^{+/-} *MRL/lpr* and *WSX-1*^{-/-} *MRL/lpr* mice at week 24 showed that deterioration of renal functions including blood urea nitrogen and serum creatinine in *MRL/lpr* mice was significantly suppressed in *WSX-1*^{-/-} group over *WSX-1*^{+/-} group, while the degree of proteinuria was worse in *WSX-1*^{-/-} group than in *WSX-1*^{+/-} group (Table I).

We then performed histopathological examination of kidneys from *WSX-1*^{+/-} *MRL/lpr* and *WSX-1*^{-/-} *MRL/lpr* mice, because glomerular changes are the primary cause of death of *MRL/lpr* mice. Canonical histological features of DPGN were observed in 24-wk-old *WSX-1*^{+/-} *MRL/lpr* mice, including inflammatory cell infiltration, glomerular sclerosis, mesangial proliferation, and crescent formation (Fig. 2, A and B, left). In striking contrast, neither inflammatory cell infiltration nor proliferative lesions were found

in the glomeruli of *WSX-1*^{-/-} *MRL/lpr* mice of the same age (data not shown), or even at 36 wk of age (Fig. 2, A and B, right). Glomerular activity score determined as described in *Materials and Methods* was 2.03 ± 0.321 vs 0.04 ± 0.045 for 24-wk-old *WSX-1*^{+/-} and 36-wk-old *WSX-1*^{-/-} mice ($n = 10$, $p < 0.01$). Perivascular infiltration by inflammatory cells was also attenuated in the kidneys of *WSX-1*^{-/-} *MRL/lpr* mice as compared with those of *WSX-1*^{+/-} *MRL/lpr* mice (Fig. 2A insets). The decrease in glomerular activity score did not mean lack of pathological changes in the kidney. Virtually all of the glomeruli examined showed diffuse thickening of the basement membrane in the glomerulus (Fig. 2, A and B, right), indicating membranous changes occurring in the kidneys of *WSX-1*-deficient mice. Staining of *WSX-1*^{-/-} *MRL/lpr* glomeruli with PAM clearly showed a generalized diffuse thickening of peripheral capillary walls, with spikelike alterations of basement membranes (Fig. 2C). Six of six *WSX-1*^{-/-} *MRL/lpr* mice examined showed generalized (in virtually all glomeruli) spikelike alterations of basement membranes, confirming membranous changes in the kidneys of *WSX-1*-deficient mice, while such alterations were observed in none of six *WSX-1*^{+/-} *MRL/lpr* mice examined. Electron microscopic examination revealed deposition of electron-dense materials in the subepithelial layer of the basement membrane (Fig. 2D). Because deposition of Ig is one of the hallmarks of glomerulonephritis, we performed immunofluorescent staining to detect Ig in kidneys of *WSX-1*^{+/-} *MRL/lpr* and *WSX-1*^{-/-} *MRL/lpr* mice. Although Ig deposition was observed in mice of both genotypes, the pattern of deposition was markedly different (Fig. 2E). In *WSX-1*^{+/-} *MRL/lpr* mice, IgG deposition was detected in mesangial lesions and along the capillary walls of glomeruli. In contrast, widespread discrete, granular deposition localized to the glomerular capillary walls was observed in *WSX-1*^{-/-} *MRL/lpr* mice. The isotype of the deposited Ig was also different. Although mainly IgG2a was detected in *WSX-1*^{+/-} *MRL/lpr* glomeruli, *WSX-1*^{-/-} *MRL/lpr* glomeruli showed predominance of IgG1 deposition, but almost no IgG2a (Fig. 2E). Taken together, these data suggest that, in the absence of *WSX-1*, *MRL/lpr* mice develop autoimmune nephritis with the features of human MGN rather than DPGN.

Elevation of Th2-associated Ig in the sera of *WSX-1*^{-/-} *MRL/lpr* mice

To further examine the immunological changes in *WSX-1*^{-/-} *MRL/lpr* mice, the level and nature of serum Igs and autoantibodies were evaluated. Production of anti-dsDNA Abs was significantly lower in *WSX-1*^{-/-} *MRL/lpr* mice compared with *WSX-1*^{+/-}

Table I. Clinical manifestations and serum chemistry in female *WSX-1*^{+/-} *MRL/lpr* and *WSX-1*^{-/-} *MRL/lpr* mice^a

	16 wk		24 wk	
	<i>WSX-1</i> ^{+/-}	<i>WSX-1</i> ^{-/-}	<i>WSX-1</i> ^{+/-}	<i>WSX-1</i> ^{-/-}
Body weight (g)	38.6 \pm 2.9	41.5 \pm 4.2	39.8 \pm 5.5	43.3 \pm 7.3
Spleen weight (g)	0.63 \pm 0.28	0.61 \pm 0.54	1.00 \pm 0.48	1.02 \pm 0.35
Total lymphnode weight (g)	2.16 \pm 1.14	2.40 \pm 1.40	7.10 \pm 1.72	7.07 \pm 2.62
Urinary protein:				
Creatinine ratio	5.85 \pm 9.5	9.45 \pm 10.2	10.18 \pm 6.15 ^b	22.3 \pm 10.5 ^b
Serum protein (g/dl)	6.67 \pm 0.98	6.54 \pm 1.42	6.70 \pm 0.89	7.45 \pm 0.54
Serum albumin (g/dl)	3.00 \pm 0.91	3.02 \pm 0.71	3.15 \pm 0.07	3.02 \pm 0.27
BUN ^c (mg/dl)	36.2 \pm 11.8	26.4 \pm 11.2	56.5 \pm 9.3 ^d	41.0 \pm 6.4 ^d
Serum creatinine (mg/dl)	0.17 \pm 0.04	0.21 \pm 0.06	0.52 \pm 0.07 ^d	0.28 \pm 0.08 ^d

^a $n = 8$ for both genotypes.

^b $p < 0.05$.

^c BUN, blood urea nitrogen.

^d $p < 0.01$.

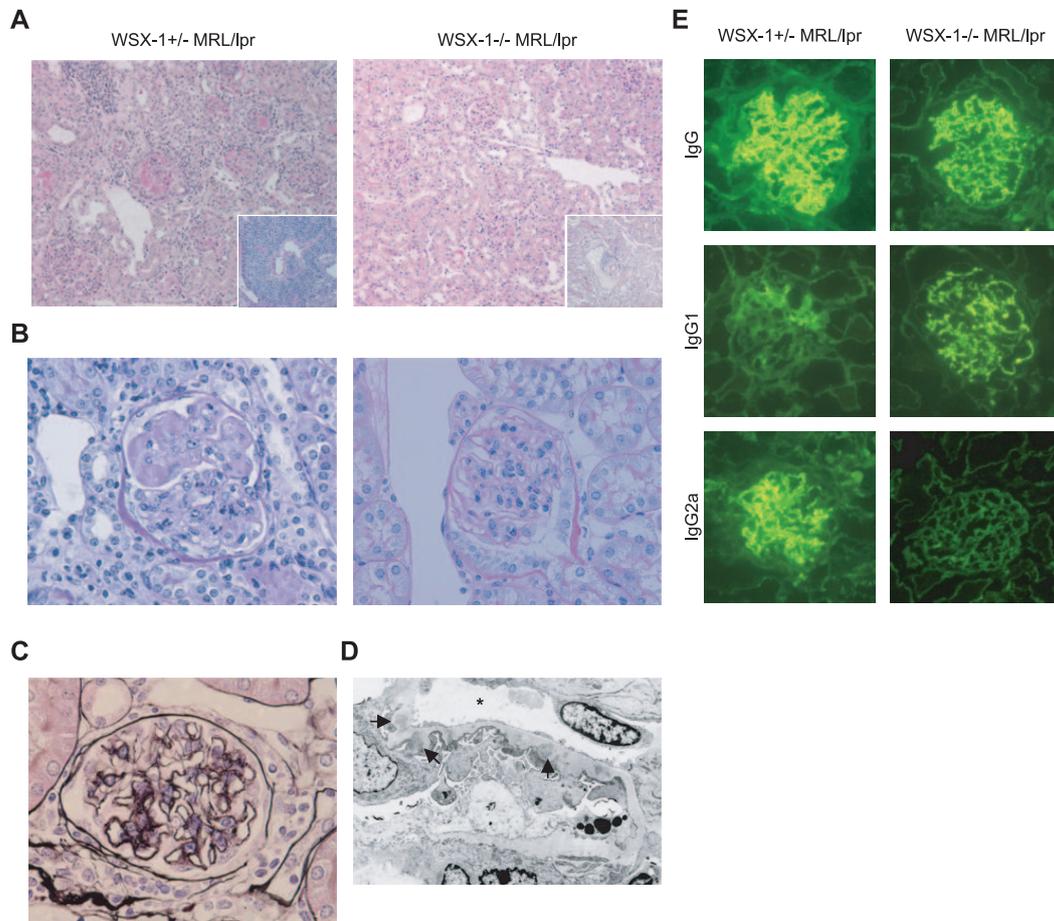


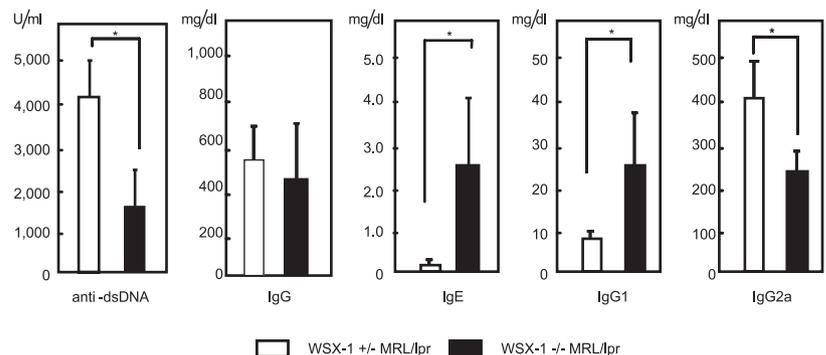
FIGURE 2. Altered histopathology of glomerulonephritis in *WSX-1^{-/-}* *MRL/lpr* mice. *A* and *B*, Light microscopic examination of the kidney (*A*) and a representative glomerulus (*B*) of a 24-wk-old *WSX-1^{+/-}* *MRL/lpr* mouse and a 36-wk-old *WSX-1^{-/-}* *MRL/lpr* mouse (periodic acid Schiff staining; original magnification, $\times 100$ (*A*) and $\times 400$ (*B*)). *Insets* in *A*, Perivascular lesions. *C*, PAM staining of the glomerulus of a 36-wk-old *WSX-1^{-/-}* *MRL/lpr* mouse ($\times 400$). *D*, Electron micrograph ($\times 4000$) of the glomerular capillary of the mouse in *C*, showing numerous subepithelial electron-dense deposits (arrows) in the basement membrane. *, Capillary lumen. *E*, Altered Ig deposition. IgG, IgG1, and IgG2a deposits in the glomeruli of a 24-wk-old *WSX-1^{+/-}* *MRL/lpr* mouse and a 36-wk-old *WSX-1^{-/-}* *MRL/lpr* mouse were visualized using immunofluorescent anti-Ig staining ($\times 400$). Figures in *E* are representative data obtained from 30 glomerular cross sections per kidney (six mice per group) with similar staining patterns. Note membranous changes in *WSX-1^{-/-}* *MRL/lpr* mice were less apparent at 24 wk of age than at 36 wk.

MRL/lpr mice (Fig. 3). Although there were no significant differences in the levels of total IgG, serum IgG1 and IgE were significantly higher in *WSX-1^{-/-}* *MRL/lpr* mice, while serum IgG2a was significantly lower in *WSX-1^{-/-}* mice over *WSX-1^{+/-}* mice. These results strongly suggest that a deficiency for *WSX-1* biases the autoimmune response in *MRL/lpr* mice toward Th2.

Unaltered lymphocyte compartment in *WSX-1*-deficient *MRL/lpr* mice

MRL/lpr mice show massive lymphoid hyperplasia due mainly to an accumulation of abnormal $B220^{+}CD3^{+}$ lymphocytes that cannot undergo apoptosis (18). To our surprise, despite the significantly improved survival rate of *WSX-1^{-/-}* *MRL/lpr* mice, the relative numbers of $B220^{+}CD3^{+}$ cells in these animals (Fig. 4A),

FIGURE 3. Altered production of serum Igs and autoantibodies in *WSX-1^{-/-}* *MRL/lpr* mice. Sera were collected from 20-wk-old *WSX-1^{+/-}* *MRL/lpr* and *WSX-1^{-/-}* *MRL/lpr* mice, and levels of anti-dsDNA Ab and serum Igs were measured, as described in *Materials and Methods*. Data shown are mean concentration \pm SD of five mice per group. Experiments were repeated three times with similar results. *, $p < 0.05$ by unpaired *t* test.



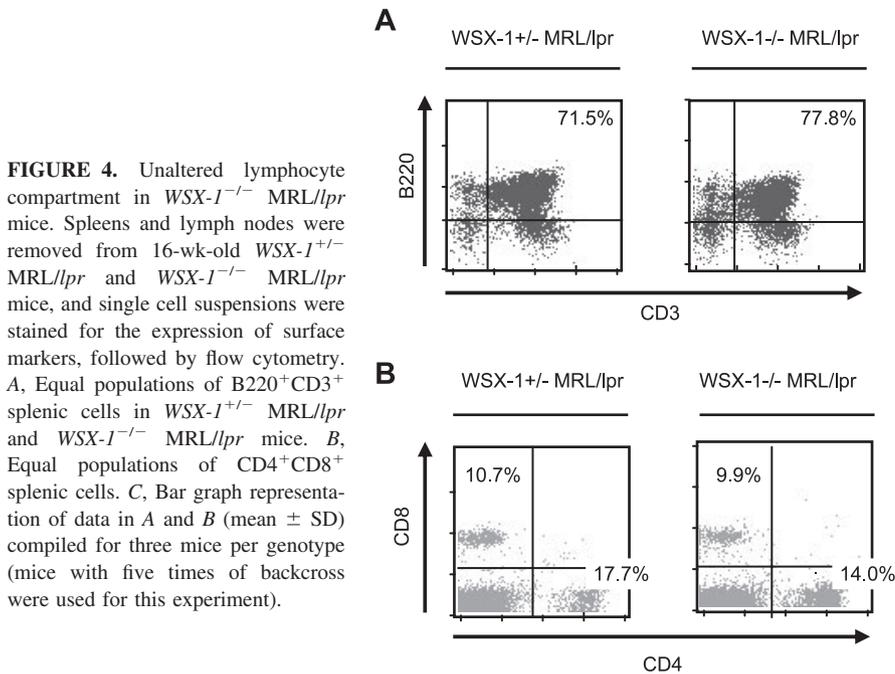


FIGURE 4. Unaltered lymphocyte compartment in *WSX-1*^{-/-} MRL/lpr mice. Spleens and lymph nodes were removed from 16-wk-old *WSX-1*^{+/-} MRL/lpr and *WSX-1*^{-/-} MRL/lpr mice, and single cell suspensions were stained for the expression of surface markers, followed by flow cytometry. **A**, Equal populations of B220⁺CD3⁺ splenic cells in *WSX-1*^{+/-} MRL/lpr and *WSX-1*^{-/-} MRL/lpr mice. **B**, Equal populations of CD4⁺CD8⁺ splenic cells. **C**, Bar graph representation of data in **A** and **B** (mean ± SD) compiled for three mice per genotype (mice with five times of backcross were used for this experiment).

as well as numbers of CD4⁺ and CD8⁺ T cells (Fig. 4, *B* and *C*), were not affected by *WSX-1* deficiency. There were also no significant differences in the degree of splenomegaly and lymph adenopathy, as well as in the total numbers of lymphocytes in the spleen and lymph nodes of *WSX-1*^{+/-} MRL/lpr and *WSX-1*^{-/-} MRL/lpr mice (Table I and data not shown).

Impaired IFN- γ production by *WSX-1*-deficient CD4⁺ T cells

To further characterize the altered immune responses in *WSX-1*^{-/-} MRL/lpr mice, we examined cytokine production by CD4⁺ T cells isolated from pararenal lymph nodes. As expected, *WSX-1*^{-/-} MRL/lpr CD4⁺ T cells stimulated as described in *Materials and Methods* produced 10-fold less IFN- γ than similarly treated *WSX-1*^{+/-} MRL/lpr CD4⁺ T cells (Fig. 5*A*). IL-4 production was not detected in CD4⁺ T cells from mice of either genotype (data not shown). Flow cytometric analysis of intracellular cytokine production by splenic CD4⁺ T cells confirmed that the percentage of IFN- γ -producing cells was significantly lower in *WSX-1*^{-/-} MRL/lpr mice than in *WSX-1*^{+/-} MRL/lpr mice (Fig. 5*B*). Again, CD4⁺ T cells producing IL-4 were not detectable in either group (data not shown). However, quantitative real-time RT-PCR analysis revealed that splenic CD4⁺ T cells from *WSX-1*^{-/-} MRL/lpr mice expressed higher amounts of IL-4 mRNA than those from *WSX-1*^{+/-} MRL/lpr mice (Fig. 5*C*). Concomitantly, the expression of IFN- γ mRNA appeared to be lower in *WSX-1*^{-/-} MRL/lpr CD4⁺ T cells than in *WSX-1*^{+/-} MRL/lpr CD4⁺ T cells, although the difference was not statistically significant. There were also no significant differences in the expression of IL-5 and IL-13 mRNA between *WSX-1*^{-/-} MRL/lpr and *WSX-1*^{+/-} MRL/lpr CD4⁺ T cells (data not shown). Finally, the production of both IL-4 and IL-5 in the sera was significantly higher in *WSX-1*^{-/-} MRL/lpr mice than in *WSX-1*^{+/-} MRL/lpr mice (Fig. 5*D*). Taken together with data shown in Fig. 3, these results indicate that Th responses in the absence of *WSX-1* are inclined toward Th2 and are characterized by decreased IFN- γ production and increased IL-4 expression. Such a skewed response is consistent with the patho-

physiology of glomerulonephritis in *WSX-1*^{-/-} MRL/lpr mice, and by extension, with MGN in human SLE.

Discussion

In this study, we examined the effect of an alteration in the Th1/Th2 balance on the manifestation of autoimmune disease in MRL/lpr mice. MRL/lpr mice deficient for *WSX-1* showed significantly better survival than control MRL/lpr mice (Fig. 1). Light microscopic examination of kidney tissues showed generalized diffuse thickening of peripheral capillary walls, revealed by PAM staining to exhibit a spike and dome pattern (Fig. 2*C*). Electron microscopy demonstrated the presence of typical membranous glomerulonephritis with electron-dense deposits in the subepithelial layer (Fig. 2*D*). These findings in *WSX-1*^{-/-} MRL/lpr mice are essentially identical with those observed in human MGN. Other parameters, such as severe proteinuria in proportion to better kidney function and lower anti-dsDNA Abs in *WSX-1*^{-/-} MRL/lpr mice, are also along the lines of clinical manifestations of MGN (19). To our knowledge, this is the first report of the establishment of a spontaneous animal model for human MGN.

Analyses of the expression and production of cytokines and Ig subclasses in the kidneys and serum of MRL/lpr mice strongly indicated a marked skewing of Th responses from Th1 to Th2 in the absence of *WSX-1* (Figs. 2*E*, 3, and 5). In addition, isolated conventional CD4⁺ T cells, but not the abnormal B220⁺CD3⁺ population, shifted from a Th1 pattern of cytokine production to a Th2 pattern in the absence of *WSX-1* (Fig. 5 and data not shown). Thus, the natures of CD4⁺ T cells, on which *WSX-1* is most abundantly expressed, appear to be the primary determinant controlling the phenotype of the glomerular disease that develops in this model. However, despite the striking pathophysiology of *WSX-1*^{-/-} MRL/lpr mice, neither the absolute nor relative numbers of lymphocytes, including the abnormal B220⁺CD3⁺ subset, were altered in either the spleen or the lymph nodes (Table I, Fig. 4, and data not shown).

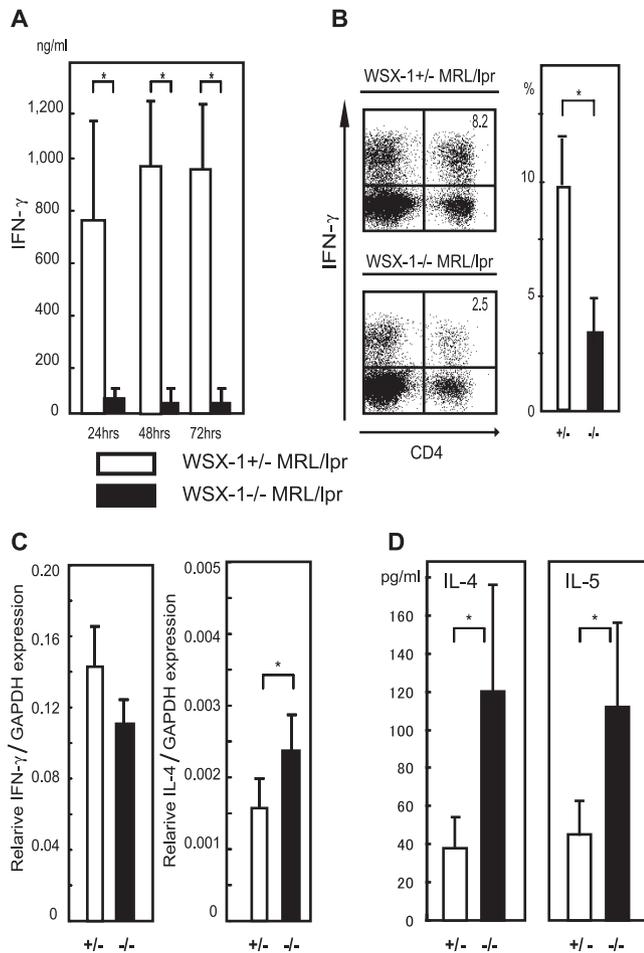


FIGURE 5. Altered IFN- γ and IL-4 production in CD4⁺ T cells of WSX-1^{-/-} MRL/lpr mice. *A*, Decreased IFN- γ production. Purified CD4⁺ T cells from pararenal lymph nodes of WSX-1^{+/-} MRL/lpr and WSX-1^{-/-} MRL/lpr mice (at 16 wk of age) were cultured with Con A for 24, 48, or 72 h, and culture supernatants were analyzed by ELISA. Data shown are mean \pm SD for five mice per group and are representative of three trials. *B*, Decreased numbers of IFN- γ -producing cells. Cells stimulated with PMA plus calcium ionophore for 6 h were stained for intracellular IFN- γ , as described in *Materials and Methods*. *Left panel*, Flow cytometric analysis of IFN- γ -producing CD4⁺ cells as a percentage of total CD4⁺ T cells. *Right panel*, Bar graph representation of data (mean percentage \pm SD) in *left panel* compiled for five mice per group. *, $p < 0.05$. *C*, Increased IL-4 mRNA expression. Data shown are the relative expression levels of IFN- γ and IL-4 mRNAs (normalized to GAPDH mRNA expression) in CD4⁺ T cells from WSX-1^{+/-} MRL/lpr and WSX-1^{-/-} MRL/lpr mice (five mice per group; *, $p < 0.05$). *D*, Augmented IL-4 and IL-5 production in WSX-1^{-/-} MRL/lpr mice. Data shown are mean \pm SD of serum IL-4 and IL-5 levels in WSX-1^{+/-} MRL/lpr and WSX-1^{-/-} MRL/lpr mice (24 wk of age; seven mice per group; *, $p < 0.05$). Experiments were repeated three times with similar results.

We find it astonishing that deficiency for a single gene, WSX-1, completely changed the phenotype of glomerulonephritis in MRL/lpr mice. Our data strongly indicate that this phenotypic shift from DPGN-like to MGN-like disease occurs because the loss of WSX-1 impairs the Th1 response and concomitantly enhances the Th2 response. WSX-1 was originally reported as a critical factor for Th1 differentiation of CD4⁺ T cells, and lack of WSX-1 in mice impaired Th1 differentiation and skewed immune responses to Th2 (14, 15). The following reports on the identification of IL-27, a ligand for WSX-1 (20), and on the clarification of the signal transduction

pathway downstream of WSX-1 (21) also confirmed the important roles of WSX-1 in the Th1 differentiation. WSX-1 deficiency in MRL/lpr mice, therefore, skewed the ongoing immune responses against autoantigens within the mice from Th1 dominant to Th2 dominant, resulting in the dramatic changes in the pathological features of the glomerulonephritis. Although the impaired IFN- γ production by PMA-stimulated T cells is evident (Fig. 5A) and consistent with our previous findings (15, 21), Th2 deviation of immune responses against autoantigens occurring *in vivo* may be less drastic, as demonstrated in Fig. 5, C and D. WSX-1-deficient CD4⁺ T cells stimulated constantly by weak autoantigens *in vivo* may recover IFN- γ productivity while retaining augmented IL-4 productivity, just as observed in WSX-1-deficient CD4⁺ T cells during *L. major* infection (15). From the data shown in Figs. 3 and 5, it is reasonable to assume that autoimmune reactions *in vivo* are persistently skewed toward Th2, albeit small in degree. Recently, IL-27/WSX-1 has been reported to have an inhibitory effect on the production of proinflammatory cytokine production, and, in some situations, WSX-1-deficient cells produced more IFN- γ over wild-type cells (22, 23). Although the reduction in IFN- γ production by WSX-1-deficient cells was temporary in some experimental conditions (15, 24), the impaired IFN- γ production in WSX-1^{-/-} MRL/lpr mice was long lasting and observed throughout the experimental time course (Fig. 5 and data not shown). Presumably, the intensities and/or the natures of the immune responses are distinct between infection-induced ones and autoimmune ones.

Lupus nephritis (WHO class V) is a type of secondary MGN histologically indistinguishable from idiopathic MGN. Mesangial and subepithelial IC deposition, mesangial cell proliferation, and the presence of tubuloreticular structures in glomerular endothelial cells are characteristic of lupus nephritis (25, 26). Notably, the Ig subclass predominantly deposited in lupus and idiopathic MGN is IgG4 (murine IgG1), an isotype typically up-regulated in Th2 responses (27, 28). The abnormal skewing of Th responses toward Th2 has therefore been implicated in MGN pathogenesis (28–32).

The precise mechanistic relationship between IgG4 and MGN is not clear. It has been hypothesized that MGN arises when IgG4 ICs formed between self Ags and low avidity/affinity Abs in the glomerular capillaries accumulate and are deposited in the outer aspect of the glomerular basement membrane (26, 33–35). Indeed, it has been shown that ICs formed from IgG4 and autoantibodies are relatively small and tend to localize in the subepithelial space (36). We observed such subepithelial deposits of ICs in WSX-1^{-/-} MRL/lpr mice. The deposited ICs in these animals contained predominantly IgG1 (human IgG4), an isotype with a lower avidity/affinity than IgG2a (37–39). Thus, although the nature of the Ag has been reported as a major driver in the pathogenesis of MGN (40–43), we now submit that the host Th1/Th2 balance is just as important in determining the kind of glomerulonephritis that develops. Of note, however, Th2 predominance may not be the absolute prerequisite for MGN, because IL-4 overexpression in distinct lupus-prone strains of mice exacerbated glomerulosclerosis (44). Depending on the lupus susceptibility loci involved and genetic background of mice, etiology of autoimmune glomerulonephritis may substantially differ (45).

In addition to the correlation drawn between the predominance of Th2 responses and MGN pathogenesis in humans (32, 46, 47), there is considerable other evidence to suggest that the Th1/Th2 balance can affect disease outcome. Patients with chronic graft-vs-host disease, a disorder in which the Th2 response is dominant,

occasionally show MGN, but rarely DPGN (48–50). Another example can be found in the phenotype of leprosy. In lepromatous leprosy, patients have high specific Ab titers, but weak cell-mediated responses to *Mycobacterium leprae* Ags. In tuberculoid leprosy, patients have strong cell-mediated immunity, but low Ab levels. These two disease phenotypes have been linked to the clear differences in cytokine production and Th1/Th2 balance observed in lepromatous and tuberculoid leprosy patients (51). Consistent with these findings, C57BL/6 mice lacking WSX-1 show impaired granuloma formation with poor cell accumulation in response to *Mycobacterium bovis* BCG infection (15).

Idiopathic MGN is the most frequent cause of nephritic syndrome in adults. It is also a very common cause of end-stage renal disease due to primary glomerulonephritis. In SLE patients, MGN (WHO class V) tends to progress to nephritic syndrome. Our study is the first to provide genetic evidence that the Th1/Th2 balance of immune responses determines the phenotype of glomerulonephritis. We have demonstrated that a single gene disruption resulting in the skewing of immune responses toward Th2 alters the histological features of glomerulonephritis in MRL/*lpr* mice from DPGN to MGN. Further study of WSX-1-deficient MRL/*lpr* mice should facilitate not only the elucidation of the pathogenesis of glomerulonephritis, but also the evidence-based development of new therapies for lupus nephritis and idiopathic MGN.

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Disclosures

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