Amelioration of human lupus-like phenotypes in MRL/lpr mice by over expression of IL-27Rα (WSX-1)

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Objective.

Investigation of the role of IL-27/IL-27Rα (WSX-1) in the development of autoimmune disorders in MRL/lpr mouse, which is considered as an experimental model of systemic lupus erythematosus in humans.

Methods.

We generated two strains of WSX-1 transgenic mice in the MRL/lpr background with different expression levels of WSX-1, and investigated the effect of WSX-1 overexpression on survival, glomerulonephritis, and immunological properties.

Results.

In comparison with wild-type MRL/lpr (WT) and Tg Low (TgL) mice, Tg High (TgH) mice exhibited a prolonged lifespan and no apparent development of autoimmune nephritis. Production of anti-dsDNA antibody and total IgG and IgG2a were significantly lower in TgH mice than those of TgL and WT mice. The expressed amounts of IFN-γ and IL-4 mRNA by CD4+ T cells from Tg mice decreased in a dose-dependent fashion. CD4+ splenic lymphocytes in TgH mice were more subject to the IL-27-mediated suppression of cytokine production. In vitro stimulation of CD4+ T cells by IL-27 resulted in over phosphorylation of STAT3 in TgH cells than in WT cells.

Conclusion.

WSX-1 over-expression in the MRL/lpr background rendered the autoimmune prone mice protected from the development of autoimmune diseases. Our results suggest that IL-27 signal may be a therapeutic target against autoimmune diseases including human SLE.
Interleukin-27 is a member of the IL-6/IL-12 family and is composed of p28 subunit and Epstein-Barr virus-induced gene 3, polypeptides structurally related to p35 and p40 of IL-12, respectively.[1] IL-27 is produced by activated antigen-presenting cells and induces proliferation of and T-bet expression in naïve CD4+ T cells.[1, 2] WSX-1, which was cloned as a homologue of gp130 of the IL-6 receptor,[3] constitutes a functional signal-transducing receptor for IL-27 with gp130.[4] WSX-1 is highly expressed in CD4+ T cells as well as in natural killer (NK)/natural killer T (NKT) cells and macrophages.[3, 5, 6] Analysis of mice deficient for WSX-1 infected with Leishmania major revealed the critical role of WSX-1 in the initial mounting of proper Th1 responses.[6] In infection with Trichuris muris, a nematode whose clearance depends on Th2 responses, WSX-1-deficient mice showed impaired Th1 responses with augmented Th2 responses resulting in more efficient expulsion of the worms than that in wild-type mice, confirming its role for Th1 development.[7, 8]

Recent lines of evidence, however, have shown a distinct role of WSX-1 and its ligand, IL-27, as an attenuator of inflammatory responses. In Toxoplasma gondii or Trypanosoma cruzi infection, CD4+ T cells as well as NKT cells and macrophages in WSX-1-deficient mice overproduced several inflammatory cytokines, resulting in devastating inflammation in the liver and other organs.[9, 10] The suppressive role of WSX-1 was also observed in various experimental settings such as Con A-induced hepatitis, Mycobacterium tuberculosis infection, allergic asthma model, and experimental autoimmune encephalomyelitis.[11-15] These data clearly demonstrated that IL-27/WSX-1 plays an inhibitory role by regulating cell activation and cytokine production.[16]

Systemic lupus erythematosus (SLE) is a multisystem disease that is caused by tissue damage resulting from autoantibody and complement-fixing immune complex deposition. Lupus nephritis manifests considerable heterogeneity in phenotype and histology. In particular, diffuse proliferative glomerulonephritis (DPGN) and membranous glomerulonephritis (MGN) represent two histologic forms that are polar opposites.[17, 18] The pathogenesis of DPGN is associated with predominance of Th1 cytokines,[19] while that of MGN with predominantly Th2 cytokine response.[20] MRL/lpr mice develop a systemic autoimmune disease, which is reminiscent of SLE in humans. In MRL/lpr mice, Fas-mediated apoptosis of activated lymphocytes was severely impaired, and T cell-dependent production of auto-antibodies results in immune complex-mediated glomerulonephritis and vasculitis.[21, 22] The kidney disease of MRL/lpr mouse is a particularly suitable model of DPGN. Intriguingly,
disruption of the WSX-1 gene changed the pathophysiology of glomerulonephritis developing in MRL/lpr (WT) mice. WSX-1−/− MRL/lpr mice developed a disease resembling human MGN with augmented Th2 responses, confirming that the Th1/Th2 cytokine balance is a key to the pathogenesis of differential types of glomerulonephritis.[23] In this study, we generated lines of WSX-1 transgenic MRL/lpr mice to further investigate roles of IL-27/WSX-1 in the development of autoimmune disorders in MRL/lpr mice.

**Methods**

**Generation of WSX-1 transgenic MRL/lpr mice**

WSX-1 transgenic mice in MRL/lpr background were produced by crossing WSX-1 transgenic BALB/c mice[24] into the MRL/lpr background more than six times (continual backcrossing: 98.44% in MRL/lpr background). Genotyping for lpr alleles was performed by PCR as described previously.[23] We generated two strains of WSX-1 transgenic mice in the MRL/lpr background (TgH and TgL) depending on different expression levels of WSX-1. Female mice from the same litters were used in the present study. 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.

**Western Blotting**

We evaluated the production of WSX-1 protein in the transgenic mice using anti-TCCR (WSX-1) antibody (Abcam, Cambridge, USA), anti-β-actin antibody (SIGMA, Saint Louis, USA), and anti-mouse IgG-HRP antibodies (Amersham Biosciences, Piscataway, USA). They were visualized with the ECL detection system (Amersham Biosciences, Piscataway, USA).

**Laboratory assessments.**

For serum chemistry, total protein, blood urea nitrogen (BUN), and creatinine (Cr)[8] levels were assessed in the sera from 10 mice in each group at 24 weeks. Urinary protein:urinary Cr ratio were also determined. Anti-nuclear antibodies (ANA) were detected by indirect immunofluorescence using HEp-2 substrate slides (Orgentec, Mainz, Germany) with fluorescein isothiocyanate-conjugated AffiniPure donkey
anti-mouse IgG (Jackson ImmunoResearch, West Grove, USA). Serum anti-double-stranded DNA (anti-dsDNA) antibodies (Abs) were analyzed by enzyme-linked immunosorbent assay (ELISA) (Shibayagi, Gunma, Japan). For serum Ig, determination ELISA was performed using the following antibodies rat anti-mouse IgG1 (Zymed Laboratories, San Francisco, USA), rat anti-mouse IgG1-HRP (BioSource International, Camarillo, USA), goat anti-mouse IgG2a (Bethyl Laboratories, Montgomery, USA), rabbit anti-mouse IgG2a-HRP (Cappel Lab, Durham, USA), and goat anti-mouse IgE Ab (Bethyl Laboratories, Montgomery, USA).

**Histopathological and immunohistopathological studies of kidneys**

The severity of glomerulonephritis was evaluated as described previously. For immunohistochemical staining, kidneys were snap frozen in optimal cutting temperature compound (Sakura, Osaka, Japan). To detect IC deposits, cryostat sections (2 µm) were fixed in chilled acetone and stained with FITC-conjugated goat polyclonal anti-mouse IgG Abs (Organon Teknika, Scarborough, USA), a FITC-conjugated goat anti-mouse IgG1 Ab and a FITC-conjugated goat anti-mouse IgG2a Ab (Southern Biotechnology Associates, Birmingham, USA). For negative controls, sections were treated with normal goat IgG (Santa Cruz Biotechnology, Santa Cruz, USA). The fluorescence strength was analyzed using scion image (Scion Cooperation, Frederick, USA).

**Real-time quantitative PCR and TaqMan primers and probes**

Expression levels of *IFN-γ* and *IL-4* in CD4+ T cells were determined using TaqMan-PCR and an ABI prism 7700 sequence detection system (Applied Biosystems Japan, Tokyo, Japan). The relative expression of each mRNA was determined and normalized to the expression of the internal housekeeping gene GAPDH. Primer and probe sequences are described previously.

**Activation of CD4+ T cells**

CD4+ T cells were purified from splenic extracts using magnetic beads (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany). Purified CD4+ T cells were activated with plate-bound anti-CD3 Ab (1 µg/ml) plus soluble anti-CD28 Ab (1 µg/ml) (BD Biosciences, San Jose, USA) for 2 days, transferred to a new plate without antibodies and additionally cultured for 5 days as a total of 7 days either in the presence or absence of IL-27. Culture supernatants containing recombinant murine IL-27 were
prepared as described previously.[16] The cells were then washed and re-stimulated either with anti-CD3 Ab plus anti-CD28 Ab for cytokine production or with IL-27 for signal transducer and activator of transcription (STAT) activation. Anti-STAT1, anti-phosphotyrosine (pY)-STAT1, and anti-pY-STAT3 Abs were purchased from New England BioLabs (Beverly, USA). Anti-STAT3 Ab was purchased from Santa Cruz Biotechnology.

**Measurement of cytokines**

Cytokines in culture supernatants of CD4+ T cells were analyzed using a micro bead-based ELISA system (Multiplex Antibody Bead Kits, Biosource, Camarillo, USA) according to the manufacturer’s directions with Luminex 100 (Luminex, Austin, USA). Cytokines in the sera were measured by ELISA kit (Genzyme, R&D Systems, and eBioscience, Los Angels, USA) for detection of IFN-γ, IL-4, IL-17A and IL-2.

**Statistical analyses**

For survival of mice, Kaplan-Meier analysis was carried out using the STATVIEW software package (SAS Institute Japan Ltd., Tokyo, Japan). Other quantitative data were expressed as the mean ± SD. Mann-Whitney rank sum test was performed to analyze the difference between two groups, while for individual comparisons among the three groups Kruskal-Wallis test, followed by the Scheffe’ test was performed. All tests were two-tailed. A P value of less than 0.05 was considered statistically significant.
Table 1. Clinical Manifestations and serum chemistry in female WT, TgL, and TgH mice

<table>
<thead>
<tr>
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<th>MRL/lpr 24W</th>
<th>WT</th>
<th>TgL</th>
<th>TgH</th>
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<tr>
<td>Body weight (g)</td>
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<td>Spleen weight (g)</td>
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<td>Total LN weight (g)</td>
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<td>Urinary protein:creatinine ratio</td>
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<td>15.98 ± 10.6</td>
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<td>Serum protein (g/dl)</td>
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<tr>
<td>Blood Urea Nitrogen (mg/dl)</td>
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<tr>
<td>Serum creatinine (mg/dl)</td>
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<td>0.36 ± 0.14</td>
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Values are the mean ± SD. LN=lymph node

*P<0.05 versus WT

Results

Prolonged survival in TgH mice, not in TgL mice

The MRL/lpr mice develop a rapid and fluminant autoimmune nephritis with 50% mortality at 6 months of age.[28] In this study MRL/lpr mice (WT mice) died after birth at a rate described above. Although TgL mice died at the similar rate as WT mice, TgH mice showed significantly extended survival (0% dead at 24 weeks after birth; p=0.000037 over WT mice) (Fig. 1A).

Improvement of clinical features and parameters in TgH mice

Given the striking improvement of survival in TgH mice, clinical features and serum chemistry parameters of 24-week-old mice were examined. TgH mice showed significantly lower BUN, compared with WT and TgL mice (Table 1). And urinary protein:creatinine ratio was also lower in TgH mice than in TgL or WT mice. These data indicated amelioration of kidney function in TgH mice. The parameters of 36-week-old TgH mice were comparable with those of 24-week-old TgH mice (data not shown). Of note, both splenomegaly and lymphadenopathy were also significantly reduced in TgH mice.

Amelioration of glomerulonephritis in TgH mice
Since the kidney dysfunction is the primary cause for death in MRL/lpr mice, histopathological examination of kidneys from the three groups of 24-wk-old mice was performed. Typical histological features of DPGN were observed in WT and also in TgL mice, including inflammatory cell infiltration, glomerular sclerosis, mesangial proliferation, and crescent formation (Fig. 2A and 2B). In striking contrast, TgH mice showed drastic attenuation of inflammatory and proliferative changes (Fig. 2C). The score of glomerular proliferative activity of TgH was significantly decreased comparing with that of WT. (Fig. 2M. p=0.0074 TgH vs. WT).

Deposition of immunoglobulin is one of the hallmarks of glomerulonephritis. [15] We then performed immunofluorescent staining of the kidneys to detect Ig in glomeruli in the three groups of mice. An intense IgG deposition was detected in mesangial lesions and along the capillary walls of glomeruli in WT and TgL mice. The isotypes of the deposited Ig were mainly IgG2a and IgG1 in part (Figure 2D, 2G, 2J, 2E, 2H, and 2K). In contrast, IgG deposition was hardly observed in TgH mice, and the deposition of both IgG1 and IgG2a was remarkably decreased (Figure 2F, 2I and 2L), and the fluorescent strength score was significantly decreased. (p=0.0035 TgH vs. WT).

**Decreased production of ANA, anti-dsDNA antibodies and immunoglobulins**

To further examine the immunological changes in TgH mice, the level and the nature of serum Ig and autoantibodies were evaluated. Positive staining for ANA was detected in 16-week-old WT, TgL mouse sera at a 1:200 dilution, as determined by indirect immunofluorescence (n = 10 per group), whereas sera from 16-week-old TgH mice were negative for ANA (n = 8) (Figure 3A). The sera from 24- and 36-week-old TgH mice were also negative (data not shown). Production of anti-dsDNA Abs was significantly lower in TgH mice than those of WT and TgL mice (Figure 3B). While there were no significant differences in the levels of IgG1 and IgE, the levels of total IgG and IgG2a were significantly lower in TgH mice than in other groups (Figure 3C).

**Reduced cytokine production by CD4+ T cells from TgH mice**

Expression of both IFN-γ and IL-4 in splenic CD4+ T cells decreased in a manner dependent on the expression levels of WSX-1 (Fig. 3D). Similarly, expression of IL-12b and IL-10 in spleen cells depleted of B220+CD3+ cell was also decreased in WSX-1 transgenic mice.

**Decrease in CD3+B220+CD4 CD8- T cell, and increase in CD4+ and CD8+ T cell in the WSX-1 TgH MRL/lpr mouse**
Given the significant improvement of splenomegaly and lymphadenopathy in TgH mice (Table 1), cellular composition in the spleen was analyzed. The percentage of CD3+CD20+CD4-CD8- T cells in TgH mouse was greatly diminished over WT mice and in TgL mice (Fig. 4A and data not shown). Concomitantly, percentages of CD4+ and CD8+ T cells increased compared with WT mice.

**Expression of activation markers in CD4+ T cells in WSX-1 TgH MRL/lpr mice**

To clarify the activation status of CD4+ T cells in the mice, expression of several cell surface activation markers was evaluated (Figure 4B). In both WT and TgH mice, most of the CD4+ T cells expressed CD69 on their cell surface, due presumably to their possible autoreactivity. More than 10% of CD4+ T cells were positive for CD25 expression in both WT and TgH mice. While some 40% of CD4+ T cells didn’t express CD62L in WT mice, approximately 80% of CD4+ T cells didn’t express CD62L. Interestingly, however, less CD4+ T cells expressed CD44 in TgH mice than in WT mice. These data demonstrated that while most of CD4+ T cells were in activated status by CD69 expression in both WT and TgH mice, more cells in TgH mice showed activated phenotype by CD62L expression. However, because much fewer cells expressed CD44 in TgH than in WT mice, CD4+ T cells in TgH mice appeared to be in an activation status different from that in WT mice. Although the percentage of CD25+ cells in CD4+ T cell population was higher in TgH mice than in WT mice, there was no significant difference in the expression levels of FoxP3 in CD4+ T cells between these mice (data not shown).

**CD4+ T cells from WSX-1 TgH mice were more subject to the IL-27-mediated suppression of cytokine production**

To examine the effect of IL-27 on lymphocytes activity in the mice, cytokine production by in vitro activated CD4+ T cells either in the presence or absence of IL-27 was examined (Fig. 4A). CD4+ T cells from TgH mice produced more IFN-γ and less IL-4 than those from WT mice. Nonetheless, CD4+ T cells from TgH mice were more sensitive to IL-27 mediated suppression of cytokine production. Although both WT and TgH CD4+ T cells were subject to IL-27-mediated suppression of IFN-γ, and IL-4 production, the suppressive effect was prominent in TgH cells over WT cells. Production of both IFN-γ and IL-4 was strikingly suppressed to barely detectable levels in TgH CD4+ T cells. IL-17 production by TgH CD4+ T cells was lower than WT cells even without IL-27 addition, and was suppressed to a barely detectable level in TgH cells. Interestingly, IL-2 production was not affected by WSX-1 overexpression and both CD4+ cells from WT and TgH mice were similarly subject to IL-27-mediated
suppression.

Downstream of the IL-27R (WSX-1 plus gp130), both STAT1 and STAT3 are activated[16, 24]. When CD4+ T cells were isolated and immediately examined for STAT1/3 phosphorylation, phosphorylation of both STAT1 and 3 was apparent in TgH CD4+ T cells as compared with WT cells (Fig. 5B). When these cells were stimulated with IL-27 for one hour, further phosphorylation of both STAT1 and STAT3 was observed in both WT and Tg cells. While the levels of STAT1 phosphorylation were comparable between WT and Tg cells, STAT3 phosphorylation was higher in Tg cells than in WT cells.

DISCUSSION.

In this study we generated lines of Tg mice that over-expressed WSX-1 in T cells to examine the impact of WSX-1 overexpression on pathophysiology and autoimmune status of MRL/lpr mice. We demonstrated that overexpression of WSX-1 suppressed the development of autoimmune nephritis in WT mice, and ANA, the values of anti-dsDNA Ab, serum Ig, and expression of various cytokines, significantly decreased in of the Tg mice. While CD4+ T cells in TgH mice were in a distinct activation status from those in WT mice, these cells were more subject to the effects of IL-27 in vitro. These results strongly suggested that increased expression of WSX-1 suppressed the autoimmune reaction and the subsequent glomerulonephritis in WT mice.

We originally demonstrated the pivotal role of WSX-1 in the initial mounting of Th1 differentiation via T-bet induction,[2] and in mice deficient in the WSX-1 gene, proper Th1 differentiation was impaired with Th2 skewing during protozoan infection.[6] In line with these findings, we revealed that disruption of the WSX-1 gene drastically changed the histologic features of glomerulonephritis developing in MRL/lpr mice from DPGN to MGN accompanied by impaired IFN-γ production with predominance Th2-dependent IgG1 deposition and increased levels of IgG1 and IgE in the sera.[23] T cells in WSX-1−/− MRL/lpr mice displayed spontaneous skewing of autoimmune responses toward Th2 type. Thus, our previous reports suggested that immune status, or more specifically, the balance between Th1 and Th2 responses, is a key determinant for the pathogenesis of the glomerulonephritis. Counterinstitutively, transgenic overexpression of WSX-1 gene resulted in amelioration of glomerulonephritis in MRL/lpr mice. It would be reasonable to assume this was the result of the suppressive effects of IL-27 since the cytokine expression by CD4+ T cells as well as autoimmune reaction was suppressed in TgH mice.

CD4+ T cells from TgH mice activated in vitro by produced more IFN-γ and
less IL-4 than those from WT mice and showed Th1 phenotype, which was in line with the Th1-promoting function of IL-27. However, IL-27 addition strikingly suppressed production of both IFN-γ and IL-4 to barely detectable levels in Tg CD4+ T cells (Figure 5A). These results confirmed the suppressive effect of IL-27 on cytokine production and also revealed that the suppressive effect was much higher for CD4+ T cells of TgH than for those of WT mice. Such suppression of cellular response was largely consistent with the diminished pathophysiology of glomerulonephritis in TgH mice. We have recently reported that IL-27 exerts its suppressive effects preferentially on activated CD4+ T cells, and that STAT3 activation in response to IL-27 stimulation of activated T cells is, at least partially, responsible for the IL-27-mediated suppression of cytokine production.[16] This is quite consistent with our present finding that TgH mice CD4+ T cells were more sensitive to IL-27 stimulation by STAT3 activation (Fig. 5B). Ohwaki et al reported the involvement of STAT1 in IL-27-mediated suppression of IL-2 production by naïve T cells,[29] although Villarino et al reported that IL-27-mediated suppression in activated T cells is independent of STAT1.[30] The discrepancy may be ascribed to the activation status of the cells.

In summary, we demonstrated that WSX-1 overexpression in the MRL/lpr background rendered the autoimmune prone mice protected from the development of autoimmune disease. Further elucidation of the molecular mechanisms underlying the IL-27-mediated cytokine suppression and detailed dissection of the situations where IL-27 differentially exerts it two roles will no doubt help development of new therapies against various diseases by suppressing excess of cell responses.

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FIGURE LEGENDS

Fig. 1. Prolonged survival of MRL/lpr mice with high expression of WSX-1. (A) The cumulative survivals of WT, TgL, and TgH mice were monitored weekly (n = 20 per group). *, p < 0.05 by the Kaplan-Meier method. There was no difference in the survival rate between WT and TgL mice. (B) Western blot analysis of the transgenic expression of WSX-1 in CD4+ T cells from WT mice and two Tg-positive MRL/lpr mouse lines, TgL and TgH. The lysates of purified splenic CD4+ T cells from 16-wk-old WT, TgL and TgH mice were separated by SDS-PAGE under reducing condition and transferred to membranes. Expression levels for β-actin protein was visualized for each sample as a loading control. The transgene products demonstrate robust, moderate, and faint expression of WSX-1 in TgH, TgL, and WT mice T cells, respectively.

Fig. 2. Amelioration of glomerulonephritis and decreased Ig deposition in TgH mice. The kidneys were fixed in 10% Formalin for 24 h at 4°C. Paraffin sections (4 µm) were stained either with H&E, periodic acid Schiff stain, or periodic acid-methenamine silver (PAM). Microscopic examination of the kidney glomerulus of a 24-wk-old WT mouse (A), TgL mouse (B) and TgH mouse(C) (periodic acid Schiff staining; original magnification, x400). IgG (D, E, and F), IgG1 (G, H, and I), and IgG2a (J, K, and L) deposits in the glomeruli of a 24-wk-old WT, TgL, and TgH mice were visualized using immunofluorescent anti-Ig staining (x400) and quantitative analyzed by image software. WT: IgG (83.8±7.25), IgG1(68.4±6.4), and IgG2a(76.8±4.9), TgL: IgG(81.0±8.48), IgG1(71.4 ± 7.3), and IgG2a(73.0 ± 4.6), TgH: IgG(25.2 ± 9.65), IgG1(22.8 ± 6.45), IgG2a(19.6±3.2). These representative data obtained from 30 glomerular cross sections per kidney (six mice per group) with similar staining patterns. (M) The severity of glomerulonephritis was evaluated by the score of glomerular proliferative activity. The scores of glomerular proliferative activity of 24-wk-old WT, TgL, and TgH mice were 2.38±0.356, 1.92±0.564, and 0.74±0.378, respectively.

Fig. 3. Decreased autoreactive immune responses in TgH mice. (A) Sera collected from 16-wk-old WT, TgL and TgH mice were examined for ANA as described in Materials and methods. The same sera in (A) were measured for anti-dsDNA antibodies (B) or for Ig levels (C). (D) The expression levels of IFN-γ and IL-4 mRNA in CD4+ T cells and those of IL-12 and IL-10 mRNA in B220−CD3+ cell-depleted splenocytes were examined by quantitative PCR. Data shown are mean
value ± SD of 10 mice per group. *, p < 0.05 by unpaired $t$ test.

**Fig.4. Decrease in CD3⁺B220⁺CD4⁻CD8⁻ T cell and up-regulated expression of activation markers on TgH CD4⁺ T cells.** (A) Spleens were removed from 16-wk-old WT and TgH mice, and single cell suspensions were stained for the expression of surface markers, followed by flow cytometry. Numbers are the percentage of CD3⁺B220⁺CD4⁺CD8⁻ T cell, CD8⁺ T cells, and CD4⁺ T cells against total cell populations. (B) Surface expression levels of CD69, CD25, CD62L, and CD44 were analyzed by flow cytometry. Numbers are the percentage of the total CD4⁺ T cells.

**Fig. 5. IL-27 suppression of cytokine production by activated CD4⁺ T cells.**

(A) CD4⁺ T cells from WT and TgH mice were stimulated with plate-bound anti-CD3 antibody plus soluble anti-CD28 antibody (1µg/ml) in the absence or presence of IL-27 for 24 hrs. Culture supernatants were measured for the production of respective cytokines. Data shown are mean value ± SD of 10 mice per group. *, p < 0.05 by unpaired $t$ test. (B) CD4⁺ T cells from WT and TgH mice were stimulated as in Materials and methods. Phosphorylation of STAT1 or STAT3 in whole cell lysates in WT and TgH mice was analyzed with anti-phosphotyrosine (α-pY) -STAT1 or anti-pY-STAT3 antibodies, respectively. The filter was stripped and re-probed with anti-STAT1 or anti-STAT3 antibodies to ensure the same amounts of samples were loaded. Experiments were repeated three times with similar results.
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Figure 2. Sugiyama et al.
Figure 3. Sugiyama et al.
Figure 4. Sugiyama et al.
Figure 5. Sugiyama et al.
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