Published in final edited form as:

Clin Immunol. 2015 June ; 158(2): 221–230. doi:10.1016/j.clim.2015.04.004.

Signal Transducer and Activator of Transcription (STAT) 3 Inhibition Delays The Onset Of Lupus Nephritis In MRL/lpr Mice

Lindsay J. Edwards, Ph.D.^{1,2}, Masayuki Mizui, M.D.^{1,2}, and Vasileios Kyttaris, M.D.^{1,2}

¹Division of Rheumatology, Beth Israel Deaconess Medical Center, 330 Brookline Ave, Boston, MA 02215

²Harvard Medical School, Boston, MA 02215

Abstract

The transcription factor STAT3 is overexpressed and hyperactivated in T cells from SLE patients. STAT3 plays a central role in T cell differentiation into Th17 and T follicular helper cells, two subsets that orchestrate autoimmune responses in SLE. Moreover, STAT3 is important in chemokine-mediated T cell migration. To better understand its role in SLE, we inhibited STAT3 in lupus-prone mice using the small molecule Stattic. Stattic-treated mice exhibited delayed onset of proteinuria (3 weeks later than controls), and had lower levels of anti-dsDNA antibodies and inflammatory cytokines. Inhibitor treatment reduced lymphadenopathy, resulted in a 3-fold decrease in total T cell number, and a 4-fold decrease in the numbers of T follicular helper cells. *In vitro* experiments showed that Stattic-treated T cells exhibited decreased proliferation and a decrease in ability to migrate to CXCL12. We propose that STAT3 inhibition represents a therapeutic target in SLE, particularly lupus nephritis.

Keywords

T cells; SLE; STAT3; murine lupus; MRL/lpr

1. Introduction

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease that results from aberrant activation of both the innate and adaptive immune systems (1). Dysfunctional T cell activity plays an important role in SLE pathogenesis in three ways: by providing help to B cells, producing inflammatory cytokines and infiltrating target tissues (1). Despite the approval of belimumab (2), SLE and lupus nephritis in particular, remain areas of significant

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Corresponding Author: Vasileios C. Kyttaris, M.D., Assistant Professor of Medicine, Harvard Medical School, Division of Rheumatology, Beth Israel Deaconess Medical Center, 330 Brookline Ave, CLS-936, Boston, MA 02215, tel: 617-735-4162 fax: 617-735-4170, vkyttari@bidmc.harvard.edu.

The authors declare no conflicts of interest.

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therapeutic need. Small molecules targeting intracellular signaling, such the Jak-STAT pathway, have proven to be effective in rheumatoid arthritis and represent promising novel treatments for SLE (3).

Signal Transducer and Activator of Transcription 3 (STAT3) is a transcription factor that is activated downstream of a host of growth factors, cytokines and chemokines. Binding of a ligand to its receptor results in dimerization of the receptor and activation of Jak kinases, which in turn phosphorylate and activate STAT proteins. Phosphorylated STAT proteins can then form either hetero- or homodimers, translocate to the nucleus and mediate gene transcription (4).

Previous studies have demonstrated in both humans and lupus prone mouse models that total levels of STAT3 as well as STAT3 activation are increased relative to healthy controls (5, 6). Aberrant activation of this pathway in autoimmune disease could impact the immune response in several different ways. In T follicular helper cells (Tfh), which provide help to B cells within the germinal center, STAT3 is activated downstream of cytokines such as IL-6 and IL-21, and is important for the differentiation of these cells (7). IL-21 also plays a role in stimulating autoantibody production through a STAT3 dependent pathway in B cells (8, 9). Activation of STAT3 is also critical for the differentiation of IL-17 secreting T cells, which play a role in lupus pathogenesis, particularly lupus nephritis (10–13). Furthermore, STAT3 expression in T cells is crucial for the development of several other autoimmune diseases (14, 15). In addition to its role in cytokine signaling, STAT3 is also activated downstream of several chemokine receptors (16, 17). Importantly our group has shown that siRNA-mediated knockdown of STAT3 *in vitro* inhibited SLE T cell migration (5).

Because STAT3 is crucial for T cells to provide B cell help, the development of pathogenic Th17 cells and T cell migration, we sought to determine the effects of inhibiting this pathway both *in vitro* and *in vivo*. Our results indicated that inhibition of STAT3 signaling delayed the onset of autoimmunity in lupus prone mice, mitigated kidney damage, decreased lymphocyte accumulation and inhibited T cell migration. We therefore propose that STAT3 signaling represents a therapeutic target in SLE.

2. Materials and Methods

2.1. Subjects

Patients who fulfilled the American College of Rheumatology criteria for the diagnosis of SLE (18) and healthy individuals were enrolled in the study by donating 50 mL of blood in heparin-lithium tubes. The institutional review board of Beth Israel Deaconess Medical Center approved the study protocol and informed consents were obtained from all study subjects. All SLE patients were clinically and immunologically active (SLEDAI 4–8) and positive for antibodies to dsDNA.

For some studies, peripheral blood was obtained from healthy donors from the Blood Donor Center (Boston Children's Hospital). T cells were isolated by negative selection using the RosetteSep T Cell Isolation Kit (StemCell Technologies). PBMCs were isolated using a Ficoll gradient.

2.2 Cell culture and reagents

Culture medium for mouse cells consisted of RPMI supplemented with 10% fetal bovine serum, 5×10^{-5} M 2-ME, 2mM L-glutamine, 100U/ml penicillin and 100µg/ml streptomycin. Culture medium for human cells consisted RPMI supplemented with 10% fetal bovine serum 100U/ml penicillin and 100µg/ml streptomycin. Stattic (Cayman Chemical) was resuspended at 10mg/ml in DMSO and stored in single use aliquots. Treatments for animal studies were prepared fresh immediately prior to administration by dilution of Stattic or an equivalent amount of DMSO into sterile 1x PBS to the appropriate concentration. Recombinant human IL-6 was from R & D Systems. Antibodies to human CD3 (OKT3) and CD28 (CD28.2) were purchased from Bio X Cell and BioLegend respectively.

2.3 Western Blots

Cells were lysed for 30 minutes on ice using RIPA buffer supplemented with a protease inhibitor cocktail. The insoluble fraction was pelleted and lysates were run on a 4–15% gradient gel. Following transfer onto PVDF membranes, blots were blocked with 5% milk in TBST and probed for phospho-Stat3, total Stat3 or actin. Proteins were detected by chemiluminescence using SuperSignal West Pico Substrate (Pierce). Antibodies used included pSTAT3 and STAT3 (Cell Signaling Technologies).

2.4 Flow Cytometry

CellTrace Violet labeling was performed according to the manufacturer's instructions, with the dye added at a concentration of 1µM (life Technologies). For surface staining, cells were incubated with antibody cocktails in PBS for 20 minutes on ice and washed twice with PBS prior to analysis on a BD LSR II cytometer. For intracellular staining, cells were fixed in methanol-free formaldehyde for 10 minutes at room temperature, resuspended in ice cold 100% methanol and stored overnight at -20°C . Cells were then stained for phospho-STAT3. Serum cytokines were quantified using a cytometric bead array (BD Biosciences) performed according to the manufacturer's protocol. Data were analyzed using FlowJo Software (Treestar).

Antibodies used include: CD4 FITC (RM4-5), CD3 APC (145-2C11), CD8 APC-Cy7 (53-6.7), pSTAT3 Pacific Blue (4/P-STAT3), CXCR5 (2G8), PD1 (29F.1A12). Antibodies for flow cytometry were from BD Biosciences or BioLegend. For live/dead cell discrimination, 7AAD was added to samples at a final concentration of 0.25µg/test (BD Biosciences).

2.5 Mice

MRL/lpr mice were purchased from Jackson Laboratories. For treatment studies, mice received 10mg per kilogram body weight Stattic intraperitoneally three times per week. Pooled urine samples were collected weekly following housing in group metabolic cages. Mice were housed in the Beth Israel Deaconess Medical Center Animal Research Facility, and all experiments were performed in accordance with a protocol approved by the Institutional Animal Care and Use Committee of Beth Israel Deaconess Medical Center.

2.6 Histology and Scoring

Kidneys were harvested from exsanguinated mice and immediately fixed in formalin. Tissues were subsequently paraffin embedded, sectioned and stained with H and E. For each animal, 20 glomeruli were scored in a blinded fashion. For immunofluorescence, tissues were snap frozen, sectioned and fixed in acetone. Slides were blocked with 10% BSA and stained with FITC labelled goat anti-mouse IgG or appropriate isotype controls (Jackson ImmunoResearch). Sections were mounted with ProLong Gold with DAPI (life technologies) and imaged using a Nikon Eclipse Ti confocal microscope with EZ-C1 version 3.6 software.

2.7 Urine and serum analyses

Levels of albumin and creatine were assessed in pooled urine using commercially available kits: Albuwell M (Exocell) and Creatinine Parameter Assay Kit (R & D Systems) respectively. IgG specific to dsDNA was quantified by ELISA (Alpha Diagnostics). C3 levels were quantified by ELISA (Quidel).

2.8 Migration assays

Purified T cells or PBMCs were added to the top well of a transwell assay setup (5µm pore size) in RPMI + 0.1% BSA. Recombinant CXCL12 (human or mouse; R & D Systems) was added to the lower chamber at a concentration of 80ng/ml. The assay was incubated for 3 hours at 37°C. Migrated cells were harvested from the lower chamber, stained and analyzed by flow cytometry. Percent inhibition was calculated as [(untreated-Stat1c treated)/(untreated)]*100. Values used in percent inhibition were number of migrated cells with 80ng/ml CXCL12 divided by number migrated in the absence of chemokine. Absolute numbers of cells were quantified using CountBright Absolute Counting Beads (BD Biosciences).

2.9 Statistical analyses

Statistical analysis was performed using GraphPad Prism.

3. Results

3.1 STAT3 inhibition blocks phosphorylation and T cell activation

The small molecule inhibitor Stat1c was previously reported to block the phosphorylation, and thus the subsequent dimerization and nuclear translocation of STAT3 in tumor cells (19). To determine the efficacy of this inhibitor at blocking STAT3 activation in T cells, we pretreated purified T cells from normal human donors with various concentration of the inhibitor and stimulated the cells with IL-6 for 30 minutes to induce STAT3 phosphorylation. Cells not stimulated with IL-6 exhibited no STAT3 phosphorylation, whereas cells treated with DMSO and stimulated with IL-6 induced robust STAT3 phosphorylation. Pre-incubation of the cells with Stat1c inhibited the phosphorylation of STAT3 in a dose dependent manner (Fig. 1a).

Treatment of T cells with Stat1c also inhibited TCR-mediated activation and proliferation. T cells from normal human donors were incubated with various concentrations of Stat1c and

plate-bound antibodies to CD3 and CD28 (5 and 1 $\mu\text{g/ml}$, respectively). Proliferation was measured by dilution of the cytoplasmic dye CellTrace Violet. Stattic doses as low as 0.625 μM were sufficient to completely inhibit T cell proliferation, and lower concentrations exhibited an intermediate effect (Fig. 1b). No significant cell death was observed at any concentration or time point tested (data not shown). These data indicate that Stattic effectively blocks the phosphorylation of STAT3 *in vitro*, and subsequently inhibits T cell proliferation.

3.2 Short term *in vivo* treatment with Stattic delays nephritis

To test the efficacy of this inhibitor in an *in vivo* model of lupus, we treated MRL/*lpr* mice with 10mg/kg of Stattic given intraperitoneally three times per week for two weeks beginning at 8 weeks of age; mice were then followed and monitored for disease activity through 14 weeks of age. An additional group of mice was treated with 25mg/kg Stattic, which resulted in toxicity and death of 60% of animals. Animals that received 10mg/kg Stattic, however, showed no evidence of toxicity. Mice treated with 10mg/kg Stattic developed decreased glomerulonephritis compared with mice treated with vehicle based on kidney histology (Fig. 2a). Onset of proteinuria was delayed by approximately 3 weeks in the treated animals (Fig. 2b), although dsDNA-specific IgG titers were not altered (Fig. 2c). These data suggest that beginning treatment at 8 weeks of age may be insufficient to completely block the onset of autoimmunity but abrogate end organ damage. Interestingly, the absolute number of T cells infiltrating the kidneys was significantly lower in the Stattic treated group, suggesting an impact on the immune response (Fig. 2d). Together, these data indicate that *in vivo* treatment with Stattic prior to the onset of disease was sufficient to delay the onset of nephritis and impact the autoimmune response.

3.3 Long term treatment with Stattic delays onset of autoimmunity

To further characterize the effect of Stattic treatment, MRL/*lpr* mice were treated 3 times per week with 10mg/kg of Stattic or vehicle given intraperitoneally beginning at 6 weeks of age and continuing until 15 weeks of age when tissues were harvested. Following treatment, spleens were harvested, red blood cells were lysed and the remaining cells were fixed, permeabilized and stained for analysis of STAT3 phosphorylation by flow cytometry. Four of five vehicle treated mice exhibited a high frequency of cells positive for phospho-STAT3 immediately *ex vivo*. In contrast, cells from Stattic treated mice had very low frequencies of phospho-STAT3 positive cells, indicating that *in vivo* treatment with Stattic was sufficient to block STAT3 phosphorylation that occurs in the MRL/*lpr* mice (Fig. 3a).

Similar to the results from the shorter treatment, proteinuria was delayed in mice treated long term with Stattic, with the onset of proteinuria occurring approximately 3 weeks later in Stattic treated mice than in vehicle treated controls (Fig. 3b). Treatment of MRL/*lpr* mice with Stattic also delayed the development of antibodies specific for double stranded DNA. Titers of anti-dsDNA IgG in the vehicle treated mice at the mid-point of treatment were dramatically elevated compared to the antibody titers in the Stattic treated mice, which remained at background levels at this time point (Fig. 3c; mean 32174 U/ml vs. 1771 U/ml). Consistent with a delay in disease onset, serum levels of C3 at the endpoint of the experiment were depressed in vehicle treated controls relative to Stattic treated animals (Fig.

3d). Both groups had minor histological changes as the treatment ended at an early stage of the disease (data not shown). Similar to what was observed with the short term treatment, a longer course of treatment with Stattic delayed the onset of proteinuria, decreased levels of autoantibodies and prevented decreases in serum C3 levels, supporting the idea that STAT3 inhibition delays the onset of autoimmunity and specifically nephritis.

3.4 Stattic treatment decreases lymphoaccumulation

Lymphoaccumulation is a key feature of the MRL/*lpr* model of lupus, and we found that this phenotype was attenuated in mice treated long term with the STAT3 inhibitor Stattic. Treated mice had significantly fewer total mononuclear cells in the lymph nodes (Fig. 4a, left). Total splenic cellularity was also decreased in Stattic treated mice relative to controls, but this difference was not statistically significant (Fig. 4a, right). Absolute numbers of T cells in the lymph nodes of vehicle treated mice were 3–4 fold higher than in Stattic treated animals (Fig. 4b). Although the total numbers of T cells were significantly decreased, the relative frequencies of CD4+, CD8+ and CD4–CD8– T cells were unaltered in the treated mice, in both the spleens and lymph nodes (Fig. 4c). We also observed a decrease in the absolute numbers of T follicular helper cells in the spleens and lymph nodes of mice treated with Stattic (Fig. 4d), suggesting that the differentiation of this subset has been impacted by STAT3 inhibition, and may be the underlying cause of the differences in autoantibody titers (Fig. 3c). Taken together, these data support the hypothesis that STAT3 inhibition limits T cell activation and can inhibit the ability of T cells to provide help to B cells.

3.5 Stattic treatment decreases IgG deposition in the kidneys and inflammatory cytokine production

Deposition of autoantibodies in the kidneys is an important pathological mechanism in lupus nephritis. To determine the impact of Stattic treatment on IgG deposition, we performed immunofluorescence on kidneys from mice either treated for short term (a) or long term (b) with Stattic. Staining for IgG revealed substantial antibody deposition in the kidneys of vehicle treated mice (Fig. 5a and b, left panels). Kidneys from Stattic treated mice in both experiments exhibited substantially less IgG deposition (Fig. 5a and b, right panels). Although kidney pathology assessed by H and E stained sections from the kidneys of mice that received long term treatment revealed overall minor damage in both treatment groups (data not shown), the decreased IgG deposition in the Stattic group (Fig. 5b) coupled with the decreased autoantibody titers (Fig. 3c) suggest that the long term treatment with Stattic dramatically impacted both the autoimmune response and the development of nephritis.

Consistent with Stattic treatment limiting the extent of the autoimmune response, levels of inflammatory cytokines in pooled serum at the midpoint of the long term treatment were decreased in Stattic treated mice relative to vehicle treated controls (Fig. 5c). These data provide additional evidence that STAT3 inhibition can delay the development of nephritis and dampen the T cell response, which can subsequently impact the B cell response and the development of autoantibodies.

3.6 STAT3 inhibition blocks T cell migration

Given the effects of Stattic treatment on delaying the development of nephritis and the role of STAT3 in T cell migration, we sought to determine if *in vitro* treatment with Stattic would block the ability of T cells to migrate in response to chemokine stimulation. PBMCs from SLE patients or matched healthy donors were pre-incubated with 0.625 μ M Stattic, and then used in a transwell migration assay. The frequency of T cell migration across the membrane was quantified by flow cytometry, and percent inhibition in the presence of Stattic was calculated as described in the methods section. All samples were normalized to corresponding no chemokine controls. Stattic treatment was sufficient to inhibit the migration of both SLE and normal T cells.

T cells from 16-week old MRL/*lpr* mice, which have severe, active disease, were less susceptible to inhibition by Stattic. At a low concentration of Stattic (0.625 μ M), inhibition of T cell migration was minimal (mean percent inhibition 3.1%), whereas substantial inhibition was achieved with higher doses (20 μ M; mean percent inhibition 81.2%; Fig. 6b). T cells from these mice are highly activated, which may account for this difference, as T cells from younger MRL/*lpr* mice and non-autoimmune prone C57BL/6J mice were susceptible to inhibition at the lower doses (data not shown). STAT3 function appears to be critical for the migration of T cells from both lupus patients and healthy controls in response to CXCL12. However, T cells from MRL/*lpr* mice with active disease were less susceptible to inhibition of migration.

4. Discussion

In this study, we have demonstrated that treatment both *in vitro* and *in vivo* with the small molecule Stattic is sufficient to block the activation of STAT3. Blockade of STAT3 *in vitro* inhibited the proliferation of both mouse and human T cells. Similarly, treatment of lupus prone mice with Stattic decreased lymphoaccumulation and lymphadenopathy. Specifically, this treatment decreased the absolute numbers of T cells in MRL/*lpr* mice, without altering the relative frequencies of CD4⁺, CD8⁺ and CD4[−]CD8[−] T cells. Stattic treatment also decreased the number of Tfh cells (CD4⁺PD-1⁺CXCR5⁺) and delayed the onset of autoantibody production and kidney disease, providing support for the idea that pathways involving STAT3 are crucial for the development of lupus nephritis.

STAT3 is an important regulator of both immune and non-immune processes. Activation of STAT3 plays a substantial role in the differentiation of T follicular helper cells, which promote B cell responses and have been implicated in the pathogenesis of lupus (7, 21, 22). Furthermore, STAT3 is also activated within the B cells themselves, downstream of signals such as IL-21 (8, 9). Thus, inhibition of STAT3 could dampen the B cell response and autoantibody production by both limiting the help provided by Tfh (and perhaps other T helper subsets), as well as inhibiting the responses to these signals in a B cell-intrinsic manner.

In the context of T cell phenotype and pathogenicity, STAT3 plays a pivotal role in the induction and maintenance of IL-17 secreting Th17 cells by virtue of its activation downstream of IL-6 and IL-23 (23, 24). Th17 cells play a pathogenic role in lupus directly

by recruiting monocytes and neutrophils and stimulating the production of various inflammatory mediators (reviewed in (25)). This subset can also contribute to the development of autoimmunity indirectly by supporting formation of germinal centers and provision of help to B cells (26–28). Although we did not directly observe a decrease in the frequency of IL-17-producing T cells, we did see a global reduction in the number of T cells following Stattic treatment. Further studies may more carefully determine the impact of STAT3 inhibition of Th17 differentiation. We also noted that STAT3 inhibition *in vitro* blocks T cell proliferation. The exact mechanism of this observation is unclear. The effect may occur directly downstream of the T cell receptor (20), but more likely is the result of alterations in the ability of the cells to respond to cytokines produced following initial stimulation.

STAT3 is activated downstream of the chemokine receptor CXCR4, which is broadly expressed on T cells (16, 29, 30). The ligand for this receptor, CXCL12/SDF-1, has been found to be overexpressed in nephritic kidneys and may play a role in trafficking of T cells and other inflammatory cells to the site of tissue injury (31, 32).

Both total and phosphorylated STAT3 are increased in T cells from lupus patients (5, 6); increased STAT3 activation is also observed in the B cells from several lupus prone strains of mice (33). These data suggest a global misregulation of signals utilizing STAT3 occurs during lupus, as well as other autoimmune diseases.

Studies of conditional knockouts of STAT3 in other autoimmune diseases, including animal models of multiple sclerosis and uveitis have revealed that T cell intrinsic expression of STAT3 is critical for the development of these diseases (14, 15). The data from these studies demonstrated a decrease in Th17 phenotype cells, as well as a failure of the T cells to traffic to the sites of organ damage. Similarly, we have described a decrease in autoimmunity and alterations in T cell trafficking subsequent to inhibition of STAT3.

In this study, we have demonstrated that inhibition of STAT3 in a mouse model of lupus delays the onset of kidney damage, decreases T cell numbers and delays the production of autoantibodies. Although the majority of these effects are likely to be intrinsic to the T cells, our data do not currently address the impact that this treatment may have on B cells, other immune cells subsets, or non-immune pathways in which STAT3 is activated. These aspects may be more appropriately addressed in future experiments using conditional knockout models in which loss of STAT3 can be limited to specific cell types.

Inhibition of STAT3 appears to be a safe and effective means of treating lupus nephritis in the MRL/*lpr* model. Blocking this pathway provides an approach to dampen T cell activation, T cell help for B cells, and trafficking, thus providing an advantage over approaches that only target one of these aspects. Several inhibitors of the Jak components of the Jak/STAT pathway have been shown to be safe and effective, with one drug approved for use in RA and several others in clinical trials (34). Similarly targeting STAT proteins, as we have done in this study, may also prove both safe and efficacious, and allow for blockade of specific pathways. The impact of STAT3 inhibition on T cell number, particularly the number of T follicular helper cells, may serve to amplify the effect of treatment by

dampening the help provided by T cells to autoreactive B cells. It has to be noted though that Stattic has a narrow therapeutic window and further studies will be needed to address precise dosing of this compound. Moreover, Stattic only delays but not completely abrogates nephritis, raising the possibility of either incomplete STAT3 inhibition or activation of other inflammatory pathways in the absence of STAT3 mediated activation.

In conclusion, our data provide evidence that inhibition of STAT3 activation can delay the onset of lupus nephritis in a mouse model, and provide a proof of concept that targeting this pathway can be both safe and effective.

Acknowledgments

This work was not supported by any commercial interest.

The authors wish to acknowledge Stacy Rivera and Robin Bosse for technical assistance.

This work was supported by: NIH R01AR060849; NIH R01AI42269

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Highlights

- STAT3 is upregulated in SLE and lupus prone mice
- STAT3 plays a role in T:B cell cross-talk, Th17 generation and T cell migration
- Stattic, a small molecule that blocks STAT3 mitigates murine lupus nephritis
- Stattic inhibits T cell migration *in vitro*

Figure 1.

STAT3 inhibition blocks phosphorylation and T cell activation. **A.** T cells were purified from the blood of two healthy donors and pretreated for 1 hour with 5, 10 or 20 μ M Stattic or equivalent amounts of DMSO. Samples were either left unstimulated or were stimulated with 25 ng/ml of recombinant IL-6 for 30 minutes at room temperature. Cells were lysed, samples were run on western blots and probed for phospho-Stat3 or total Stat3. **B.** T cells were purified from the blood of a healthy donor, labeled with CellTrace Violet and stimulated with plate-bound anti-CD3 (5 μ g/ml) and anti-CD28 (1 μ g/ml) in the presence of various concentrations of Stattic (or DMSO) for 5 days. Data are representative of results obtained from 2 different donors.

Figure 2.

Short term (2-week) *in vivo* treatment with Stattic delays nephritis. Mice were treated with 10mg/kg body weight Stattic (or vehicle) from 8 weeks of age to 10 weeks of age. Tissues were harvested for analysis at 14 weeks of age. A. H and E stained sections of kidneys (left) were blinded and scored for pathology (right). Scoring was on a scale of 0–3, with 0 representing no disease. Data are averages $\pm$ SD of 20 glomeruli and were scored for 4 mice in each group. B. Pooled urine from Stattic or vehicle treated mice was collected weekly and analyzed for albumin and creatinine content. C. Serum from mice treated with Stattic or vehicle was analyzed by ELISA to determine the titer of IgG anti-dsDNA antibodies. Samples were analyzed in duplicate; data represent averages of 5 mice per group ($\pm$ SD). D. The absolute numbers of T cells infiltrating the kidney at the termination of the experiment were calculated. Data represent the average of two mice per group from a single experiment ($\pm$ SD).

Figure 3.

Long term treatment with Stattic delays onset of autoimmunity. Mice were treated with 10mg/kg body weight Stattic (or vehicle) from 6 weeks of age to 15 weeks of age. Tissues were harvested for analysis at 15 weeks of age. A. Total splenocytes were fixed, permeabilized, stained for phospho-Stat3 and analyzed by flow cytometry. The x-axis represents individual mice and the y-axis represents intensity of phospho-Stat3 staining. B. Pooled urine from Stattic or vehicle treated mice was collected weekly and analyzed for albumin and creatinine content. C. Serum from mice treated with Stattic or vehicle was analyzed by ELISA to determine the titer of anti-dsDNA IgG. Samples were analyzed in duplicate and were collected prior to beginning treatment, 12 weeks of age and at termination of the experiment; data represent averages of 5 mice per group ($\pm$ SD). D. Serum was collected at 12 weeks of age and analyzed for C3 content by ELISA. Data are averages $\pm$ SD of serum from 5 mice in each group run in triplicate.

Figure 4.

Lymphoaccumulation is decreased in Stattic treated mice. A. Lymph nodes (left) and spleens (right) were harvested from 15 week old mice (Stattic treated from 6–15 weeks of age), red blood cells were lysed and total cellularity was determined by counting samples on a hemocytometer. Data are averages of 5 mice per group $\pm$ SD. B. Total numbers of TCR β + cells in the lymph nodes. Data are averages of 5 mice per group $\pm$ SD. Data were gated on live (7AAD-) singlet events. C. Frequencies of CD4+, CD8+ and CD4-CD8- cells were plotted from the spleens (top) and lymph nodes (bottom) were plotted. Data represent averages of 5 mice per group $\pm$ SD. Data were gated on live (7AAD-) TCR β + singlets. D. Total numbers of Tfh cells in the lymph nodes (left) and spleens (right). Data are averages of 5 mice per group $\pm$ SD. Data were gated on live (7AAD-) TCR β +CD4+ singlets. Data represents CXCR5+PD1+ cells.

Figure 5.

Static treatment decreases IgG deposition in the kidneys and inflammatory cytokine production. A. and B. Immunofluorescence of IgG deposition in the kidneys of mice treated for 2 weeks (8–10 weeks of age; A) or long term (8–15 weeks of age; B). 3–4 images were collected for each mouse at 20x and 60x original magnification; representative images are shown. C. Inflammatory cytokine production was measured in the serum at the midpoint of the time course (12 weeks of age) in the long term treated group. Serum was pooled from 5 mice for each treatment group due to quantity of serum required for the assay.

Figure 6.

STAT3 inhibition blocks T cell migration. Ability of Stattic treatment to inhibit T cell migration was measured in a transwell migration assay using PBMCs from SLE patients and healthy controls treated with 0.625 μM Stattic (A). Data represent averages of 11 patients and matched controls. B. Splenocytes from 16 week old MRL/*lpr* were treated with 0.625 μM and 20 μM Stattic and allowed to migrate (B). Data represent averages of 2 individual mice and are averages $\pm$ SD.