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IL-21 Has a Pathogenic Role in a Lupus-Prone Mouse Model and Its Blockade with IL-21R.Fc Reduces Disease Progression

Deborah Herber,1* Thomas P. Brown, † Spencer Liang,* Deborah A. Young,* Mary Collins,* and Kyri Dunussi-Joannopoulos*

Systemic lupus erythematosus is a complex autoimmune disease characterized by dysregulated interactions between autoreactive T and B lymphocytes and the development of anti-nuclear Abs. The recently described pleiotropic cytokine IL-21 has been shown to regulate B cell differentiation and function. IL-21 is produced by activated T lymphocytes and its interactions with IL-21R are required for isotype switching and differentiation of B cells into Ab-secreting cells. In this report, we studied the impact of blocking IL-21 on disease in the lupus-prone MRL-<sup>Faslpr</sup> mouse model. Mice treated for 10 wk with IL-21R.Fc fusion protein had reduced proteinuria, fewer IgG glomerular deposits, no glomerular basement membrane thickening, reduced levels of circulating dsDNA autoantibodies and total sera IgG1 and IgG2a, and reduced skin lesions and lymphadenopathy, compared with control mice. Also, treatment with IL-21R.Fc resulted in a reduced number of splenic T lymphocytes and altered splenic B lymphocyte ex vivo function. Our data show for the first time that IL-21 has a pathogenic role in the MRL-<sup>Faslpr</sup> lupus model by impacting B cell function and regulating the production of pathogenic autoantibodies. From a clinical standpoint, these results suggest that blocking IL-21 in systemic lupus erythematosus patients may represent a promising novel therapeutic approach. The Journal of Immunology, 2007, 178: 3822–3830.

**S**ystemic lupus erythematosus (SLE) is a complex autoimmune disease with considerable heterogeneity in clinical manifestations, ranging from nonspecific symptoms to life-threatening renal and CNS disease. The disease is characterized serologically by the presence of anti-dsDNA Abs, which are considered as a hallmark for pathogenicity, particularly in relation to the renal disease (1). Advances in our understanding of disease pathogenesis and drug development have contributed to improvements in prognosis for SLE patients. However, significant morbidity and mortality are still associated with the disease and there are limited therapeutic options for patients with SLE. Thus, despite improved prognosis, patients with SLE still have a 3- to 5-fold increased mortality compared with the general population (2).

The etiology of SLE is still unknown, however, there is a consensus that interactions between autoreactive T and B cells lead to the generation of autoantibodies and secretion of proinflammatory cytokines, including TNF-α, IL-6, and IL-10 (3). Recently, the novel cytokine IL-21 has been found to have a central role in the differentiation and function of B cells (see Leonard and Spolski for review in Ref. 4), raising the possibility that IL-21 may contribute to the pathology of B cell-mediated autoimmune disease. IL-21 is a member of the IL-2 family of cytokines that use the common γ-chain receptor subunit (5). IL-21 is produced by activated CD4<sup>+</sup> T cells and regulates the growth, survival, and function of B, T, and NK cells, which widely express its specific receptor (IL-21R). Early pro-T and B cells lack IL-21R, however, as T and B cells mature, expression levels increase and are further enhanced once the cells become activated, with the highest expression found on activated B cells (6). IL-21 differentially affects B cell functions depending on the nature of the antigenic stimulus and activation through costimulatory pathways. For example, IL-21 enhances anti-CD40-mediated B cell proliferation, whereas it inhibits proliferation mediated by anti-IgM plus IL-4 (5). In resting B cells, IL-21 has been shown to be a proapoptotic factor, yet it is also a key factor in promoting growth and differentiation of B cells into plasma cells (7, 8). IL-21 plays a critical role in regulating Ab production, not only by inducing production of all IgG isotypes, but also by acting as a specific switch factor for IgG1 and IgG3 production (7, 9). Mice deficient in the IL-21R have normal lymphocyte population, but have increased serum IgE and decreased IgG1 and IgG2b levels (9, 10), suggesting IL-21 has a regulatory role in lymphoid function, but not development.

The role of IL-21 in autoimmune diseases is now starting to be explored (11–13). Given the strong regulatory influence IL-21 has on B cells, it is reasonable to examine whether IL-21 has a pathogenic role in B cell-driven autoimmunity models, such as the MRL-<sup>Faslpr</sup> mouse model. MRL-<sup>Faslpr</sup> mice develop a spontaneous autoimmune disease that closely resembles human SLE. Disease in this model is relatively fast and predictable, with hallmark features including autoreactive B and T cells, increased autoantibodies to dsDNA, immune complex-type glomerulonephritis (lupus nephritis), skin lesions, marked lymphadenopathy, splenomegaly, and tissue inflammation (14–16). B cells have a central role in the development of disease in these mice as depicted in the B cell-deficient MRL-<sup>Faslpr</sup> strain. These mice are completely protected from kidney disease and have reduced activated memory T cells, CD4<sup>+</sup> and CD8<sup>+</sup> T cell populations (17). Data from these mice and others suggest that B cells are not only responsible for increased Ab production in MRL-<sup>Faslpr</sup> mice, but also serve as APCs for activation of autoreactive T cells, promote the expansion

*Abbreviations used in this paper: SLE, systemic lupus erythematosus; m, murine; CHO, Chinese hamster ovary; PAS, periodic acid-Schiff; DN, double negative.

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of the normal T cell population, and secrete a variety of cytokines and chemokines, all of which contribute to the pathology (18). Targeting cytokines and factors that regulate B cell functions in lupus has the potential to greatly impact disease (19–23). More recently, it has been reported that lupus-prone BXSB-Yaa mice have elevated circulating levels of IL-21, raising the possibility that IL-21 may be involved in the pathogenesis of the disease (8).

In this study, we investigate the role of IL-21 in the progression of autoimmune disease in MRL-Fas<sup>br</sup> mice by targeting IL-21 with an IL-21R.Fc fusion protein. We demonstrate that IL-21 plays a pathogenic role in disease progression in MRL-Fas<sup>br</sup> mice and is therefore an attractive potential therapeutic target in SLE patients.

Materials and Methods

**Mice**

MRL/MpJ-Fas<sup>br</sup>/Faslpr (MRL-Fas<sup>br</sup>), C3H/HeJ, and MRL/MpJ mice were purchased from The Jackson Laboratory and housed in a pathogen-free animal facility. The Institutional Animal Care and Use Committee approved all animal procedures. All animals were routinely serologically tested and were negative for common pathogens.

**In vitro studies**

The impact of IL-21 on B and T lymphocyte responses was determined in lupus-prone and control mice. Spleens from untreated 8-wk-old MRL-Fas<sup>br</sup>, MRL/MpJ, and C3H/HeJ mice were dissociated into single-cell suspensions. B lymphocytes were isolated from these suspensions using magnetic cell sorting CD19 MicroBeads (Miltenyi Biotec) according to the manufacturer’s instructions. Isolated cells were plated onto anti-mouse CD3 precoated plates (BD Biosciences) at 2 × 10⁵ cells/well, and cultured in RPMI 1640 medium plus 10% FBS for 3 days in the presence or absence of anti-CD28 (BD Pharmingen). Supernatant was collected and IL-21 was measured by ELISA using rat capture mAb (AF594) and biotinylated goat Ab (BAF594) specific for murine IL-21 (R&D Systems).
to minimize Fc binding and complement fixation as described (24). The resulting construct was subcloned into a mammalian dihydrofolate reductase expression vector and transfected into Chinese hamster ovary (CHO) cells using standard methods (25, 26). Concentrated CHO-conditioned medium was loaded onto a protein affinity column, washed with 50 mM Tris·HCl (pH 8.0), 150 mM NaCl, and eluted with 0.1 M HAc (pH 3.5), and 150 mM NaCl. Fractions were neutralized with 1 M Tris (pH 9). Peak fractions were pooled and the buffer was exchanged into PBS. The protein was >95% pure by SDS/PAGE.

Cell-based assays

The ability of IL-21R.Fc to block IL-21/IL-21R interaction was tested in cell-based assays. Briefly, the full-length cDNA for the mIL-21R was isolated from a mouse T lymphocyte cell line (27) and subcloned into a retroviral expression vector. The resulting virus was isolated using published methods (28). BaF/3 cells were infected to generate stable cell lines expressing FLAG-tagged IL-21R. After 3 days of culture, the FLAG-tagged receptor was pulsed with 0.5 μg of anti-FLAG Ab (Sigma-Aldrich), or serum samples serially diluted in PBS, sera was added to the plate at dilutions starting at 1/150. The standard curve was obtained from a mouse anti-dsDNA ELISA kit (Kamiya Biomedical). IgG and C3 deposits in renal glomeruli were measured by exposing a 96-well plate (Immulon 1B; Thermolab Systems) to UV light overnight and coating it with 2 μg/ml calf-thymus DNA (Sigma-Aldrich). Following blocking with 1% BSA/PBS, sera was added to the plate at dilutions starting at 1/150. The standard was obtained from a mouse anti-dsDNA ELISA kit (Kamiya Biomedical). IgG was detected with goat anti-mouse detection Abs conjugated with HRP (Southern Biotechnology Associates). The ELISA was developed with TMB peroxidase substrate (BD Pharmingen) and absorbance was measured at 450 nm.

Anti-dsDNA Ab ELISA

Circulating levels of anti-dsDNA Ab isoforms (IgG1, IgG2a, IgG2b, IgG3) in treated mice were measured by exposing a 96-well plate (Immunob 1B; Thermolab Systems) to UV light overnight and coating it with 2 μg/ml calf-thymus DNA (Sigma-Aldrich). Following blocking with 1% BSA/PBS, sera was added to the plate at dilutions starting at 1/150. The standard was obtained from a mouse anti-dsDNA ELISA kit (Kamiya Biomedical). IgG was detected with goat anti-mouse detection Abs conjugated with HRP (Southern Biotechnology Associates). The ELISA was developed with TMB peroxidase substrate (BD Pharmingen) and absorbance was measured at 450 nm.

IgG and C3 deposits in renal glomeruli

Deposits of IgG in the glomeruli were detected by incubating acetone fixed 5-μm-thick cryostat sections of kidney in 20% normal goat serum for 30 min, followed by a 1-h incubation with FITC-conjugated goat anti-mouse IgG (1/500; Southern Biotechnology Associates). Fluorescence in glomerular capillary walls and in the mesangium was subjectively scored blindly on a scale of 0–3 (0, none; 1, weak; 2, moderate; 3, strong); 10 glomeruli per section were analyzed.

Renal pathology

From each mouse sacrificed, the right and left kidneys were collected, and portions were fixed by immersion in 10% neutral-buffered formalin. Fixed tissues were trimmed, processed, embedded routinely in paraffin, and

![FIGURE 2. IL-21R.Fc reduces phenotypic disease in MRL-Fas<sup>bw</sup> mice.](http://www.jimmunol.org/ Downloaded from http://www.jimmunol.org/)

TREATMENT OF LUPUS-PRONE MICE WITH IL-21R.Fc

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<sup>a</sup> Anti-E. tenella, n = 12; IL-21R.Fc, n = 12; saline, n = 8.

<sup>b</sup> Value of p ≤ 0.05; comparing phenotypic score of IL-21R.Fc vs anti-E. tenella and saline groups at week indicated.

FIGURE 2. IL-21R.Fc reduces phenotypic disease in MRL-Fas<sup>bw</sup> mice. Treating MRL-Fas<sup>bw</sup> mice for 10 wk with IL-21R.Fc reduced proteinuria (score 0–4), skin lesions (score 0–3), and lymphadenopathy (score 0–3) compared with anti-E. tenella and saline-treated mice. *, p ≤ 0.05 anti-E. tenella and saline groups. Values are means ± SEM.
stained with either H&E or periodic acid Schiff’s reagent (PAS). Stained slides were evaluated and scored blindly by a board certified veterinary pathologist. Glomerular, interstitial, and vascular findings in each kidney were scored as 0, no significant findings; 1, minimal; 2, mild; 3, moderate; or 4, severe, as previously described (29).

Flow cytometry

Spleens were harvested from treated and control animals at necropsy and dissociated into single-cell suspensions. RBC were lysed by treatment with Tris/ammonium chloride (RBC lysis buffer; Sigma-Aldrich) per the manufacturer’s instructions. Cell debris and aggregates were removed by passage through a 70-μm cell strainer (BD Biosciences). Cells were incubated with Fc block (anti-CD16/32; BD Pharmingen) for 15 min on ice, stained with B220-FITC, anti-CD3-PE, anti-CD4-allophycocyanin and/or anti-CD8-PerCP (BD Pharmingen) for 30 min on ice and analyzed on a FACSCalibur (BD Biosciences).

In vitro IgG secretion assay

B lymphocytes were isolated from the spleens of anti-E. tenella, IL-21R.Fc-, and saline-treated MRL-Fas+/− mice at the time of sacrifice as described above. Cells were plated into a 96-well plate at 4 × 10⁵ cells/well and incubated with anti-IgM (10 μg/ml), anti-CD40 (2.5 μg/ml), and in the presence or absence of IL-21 (50 ng/ml) for 96 h. The supernatant from each well was collected and its IgG concentration was determined using an IgG ELISA kit (Bethyl) according to the manufacturer’s instructions.

Statistics

Data are presented as mean ± SEM. We determined statistical significance between groups using the Student t test.

FIGURE 3. Circulating autoantibody to dsDNA are reduced in IL-21R.Fc-treated MRL-Fas+/− mice. Ig specific for dsDNA IgG1 and IgG2b are reduced in the sera of MRL-Fas+/− mice receiving IL-21R.Fc for 10 wk compared with anti-E. tenella or saline-treated mice. *, p ≤ 0.05 anti-E. tenella and saline groups. Values are means ± SEM.

FIGURE 4. Circulating Ig levels are reduced in IL-21R.Fc-treated MRL-Fas+/− mice. IL-21R.Fc treatment reduced total IgG1 and IgG2a in the sera of MRL-Fas+/− mice receiving IL-21R.Fc for 10 wk compared with anti-E. tenella or saline-treated mice. *, p ≤ 0.05 anti-E. tenella and saline groups. Values are means ± SEM.
FIGURE 5. IL-21R.Fc does not affect serum IgM or IgE levels. Treating MRL-Faslpr mice with IL-21R.Fc for 10 wk did not affect serum IgM or IgE compared with anti-E. tenella or saline-treated groups. Each point represents an individual mouse with mean of the group represented by a bar.

Results

IL-21 increases in vitro B lymphocyte proliferation and IgG secretion

B lymphocytes isolated from MRL-Faslpr mice had a greater proliferative response of B lymphocytes when cocultured with IL-21, especially in the presence of IL-21R.Fc-stimulated B lymphocytes in all three strains of mice by ~2-fold. The proliferation of MRL-Faslpr B lymphocytes in the presence of IL-21 was significantly greater than that seen in C3H/HeJ or MRL-MPJ mice. The proliferative responses we observed in the C3H/HeJ mice are in agreement with those reported for C57BL/6 B lymphocytes (8). The hyperresponsiveness of MRL-Faslpr B lymphocytes to stimulation and the further enhancement of this response in the presence of IL-21 may contribute to the severity of disease in these mice.

IgG secretion by B lymphocytes is a key factor in progression of disease in lupus-prone mice (17). MRL-Faslpr B lymphocytes secreted a greater amount of IgG than C3H/HeJ or MRL-MPJ B lymphocytes (Fig. 1b), even in the absence of stimuli (medium alone). Further stimulation of such cells with anti-IgM and anti-CD40 did not significantly increase IgG production over medium alone. However, the addition of IL-21 in these cultures greatly increased IgG secretion to 90 pg/ml in C3H/HeJ, MRL-MPJ, and MRL-Faslpr B lymphocytes, respectively. These results show a dramatic in vitro response of B lymphocytes when cocultured with IL-21, especially in MRL-Faslpr B lymphocytes, suggesting that this cytokine has the potential to greatly enhance IgG production in vivo and thus could contribute to disease progression in this model.

IL-21 production by CD4+ T lymphocytes

IL-21 is preferentially expressed by activated CD4+ T lymphocytes differentiated toward Th2 (30). In this study, we investigated whether costimulation in the absence of Th2 skewing conditions was capable of inducing IL-21 secretion in CD4+ T lymphocytes. When CD4+ T lymphocytes from normal and lupus-prone mice were stimulated with anti-CD3 alone, we detected <2 pg/ml IL-21 in all strains of mice (Fig. 1c). However, when costimulated with anti-CD28, IL-21 secretion increased 10-fold in MRL-Faslpr CD4+ T lymphocytes. Costimulation did not have any effects on IL-21 from C3H/HeJ or MRL-MPJ CD4+ T lymphocytes.

IL-21R.Fc reduced phenotypic disease severity

IL-21 was targeted in vivo by using a murine soluble IL-21R.Fc fusion protein that effectively neutralizes IL-21-mediated proliferation of Baf/3 cells engineered to express the mouse IL-21R (data not shown). Escalating urinary protein levels, skin lesions, and lymphadenopathy are characteristics of the SLE-like disease induced in MRL-Faslpr mice (14). Weekly evaluation of these phenotypic parameters is shown in Table I. Over the course of the study, the percentage of mice with significant proteinuria (score of ≥2) and skin lesions increased over time in all three groups, however, in the IL-21R.Fc-treated group onset of these conditions occurred later. For example, by week 12, 17% of the control anti-E. tenella group and 25% of the saline-treated mice had urinary protein score ≥2 (≥100 mg/dl), whereas none of the IL-21R.Fc group had this level of protein in their urine. The percentage of mice with lymphadenopathy score ≥2, reflecting enlarged LN at two or more sites, at the end of the study reached 58 and 38% in the control anti-E. tenella and saline group, respectively, whereas in the IL-21R.Fc-treated group none of the mice developed this degree of lymphadenopathy. Moreover, at the end of the study, the IL-21R.Fc-treated mice had significantly less proteinuria, skin lesions, and lymphadenopathy compared with the anti-E. tenella or saline-treated mice (Fig. 2).

IL-21R.Fc reduced circulating level of autoantibodies and total Ig

MRL-Faslpr mice have elevated levels of autoantibodies in their serum as early as 6 wk of age. These autoantibodies play a primary role in the pathogenesis of their disease (31). IL-21 promotes differentiation of B lymphocytes into plasma cells and is a switch for IgG1 production (7, 8). Treating MRL-Faslpr mice with IL-21R.Fc

FIGURE 6. IL-21R.Fc diminishes IgG glomerular deposits in MRL-Faslpr mice. Frozen kidney sections from IL-21R.Fc, anti-E. tenella, and saline-treated mice were stained for IgG deposits with anti-mouse IgG-FITC Ab. a, Representative photomicrographs of a glomerulus from each treatment group. b, Fluorescent intensity was scored on scale of 0–3. Each point represents an individual mouse. *, p ≤ 0.05 anti-E. tenella and saline groups.
reduced both IgG1 and IgG2b dsDNA-specific Abs (Fig. 3). Furthermore, total circulating IgG1 and IgG2a were reduced in mice given IL-21R.Fc compared with those given anti-E. tenella or saline (Fig. 4) demonstrating that IL-21 increases IgG1 and IgG2 production in MRL-Faslpr mice. Anti-E. tenella and saline-treated mice produced similar levels of autoantibodies and total Ig of all isotypes investigated. Treating MRL-Faslpr mice for 10 wk with IL-21R.Fc did not affect serum IgM or serum IgE levels (Fig. 5).

**Glomerular IgG deposits and kidney pathology are reduced in IL-21R.Fc-treated MRL-Faslpr mice**

The formation of glomerular immune deposits in the kidney is a distinct feature of lupus. Treatment of MRL-Faslpr mice with IL-21R.Fc for 10 wk significantly reduced glomerular IgG deposits compared with mice given anti-E. tenella or saline (Fig. 6). Additionally, IL-21R.Fc-treated mice displayed no thickening in glomerular basement membranes by histological evaluation. Because such thickenings were observed in age-matched mice given anti-E. tenella Ab or saline (Fig. 7), this absence was considered to result from IL-21R.Fc treatment. Glomerular cellularity and perivascular lymphocyte infiltration were not affected by the IL-21R.Fc treatment (Fig. 7).

**IL-21R.Fc reduced splenic T lymphocytes**

IL-21 enhances proliferation and survival of CD4+ and CD8+ T lymphocytes (32). Splenomegaly in MRL-Faslpr mice is due, in part, to an influx of these cells and of a unique B220+CD3+CD4−CD8− (double negative (DN)) T lymphocyte subset into the spleen as disease progresses. Administration of IL-21R.Fc to MRL-Faslpr mice reduced the number of splenic CD4+ and CD8+ T lymphocytes compared with mice receiving anti-E. tenella or saline (Fig. 8, a and b). IL-21R.Fc treatment did not affect the number of DN T lymphocytes (data not shown).

**IL-21R.Fc reduced the ex vivo Ab production of splenic lymphocytes**

B lymphocytes isolated from the spleens of IL-21R.Fc-treated mice produced less IgG after stimulation in the presence of IL-21 compared with B lymphocytes from anti-E. tenella Ab- or saline-treated mice (87 ± 10; 129 ± 16; 150 ± 26 ng/ml, respectively) (Fig. 8c). In the absence of IL-21, B lymphocytes from IL-21R.Fc-treated mice produced similar levels of IgG than anti-E. tenella or saline-treated B lymphocytes (29 ± 5 vs 41 ± 3 and 40 ± 15 ng/ml, respectively).
FIGURE 8. IL-21R.Fc treatment alters splenic T population and B lymphocyte ex vivo function. MRL-Faslpr mice treated with IL-21R.Fc for 10 wk had reduced number of CD4+ (a) and CD8+ (b) T lymphocytes in their spleens compared with mice given anti-E. tenella or saline. c. B lymphocytes from MRL-Faslpr mice that received IL-21R.Fc secrete less IgG ex vivo after IL-21 stimulation than B lymphocytes from mice who received anti-E. tenella or saline. Points represent individual mice with mean of group represented by a bar.

Discussion

Our findings reported here demonstrate that IL-21 contributes to disease progression in MRL-Faslpr lupus-prone mice and that blocking IL-21 with IL-21R.Fc fusion protein reduces disease in these mice. We show that IL-21 greatly enhances B lymphocyte proliferation and IgG secretion from both MRL-Faslpr and control mice. Blocking IL-21 with IL-21R.Fc in MRL-Faslpr mice reduces renal disease (including proteinuria, IgG deposits, and glomerular basement membrane thickening), lymphadenopathy, skin lesions, and circulating autoantibodies and IgG. IL-21R.Fc-treated MRL-Faslpr mice have fewer splenic CD4+ and CD8+ T lymphocytes and their B lymphocytes secreted less IgG ex vivo compared with mice treated with either control Ab or saline. These data suggest that IL-21 has a pathogenic role in the MRL-Faslpr mouse model.

Autoantibodies are thought to have a primary role in the pathogenesis of SLE. Levels of circulating autoantibodies correlate with disease severity in both SLE patients and MRL-Faslpr mice, with IgG1 and IgG2 being the predominant subclasses found (33, 34). These autoantibodies are thought to play a causative role in the occurrence of disease given that a rise in circulating autoantibodies is seen in SLE patients before renal relapses and before any disease is evident in lupus-prone mice (31, 35). Therefore, factors affecting B lymphocyte function may greatly impact disease progression. We noted here that B lymphocytes from MRL-Faslpr mice proliferate and secrete more IgG ex vivo than B lymphocytes from normal mice after stimulation. By wk of age, the B and T lymphocytes in these mice are already in a state of activation in vivo that is most likely carried over to an ex vivo setting (36, 37). We find that IL-21 further enhances the increased proliferation and IgG secretion seen in B lymphocytes from MRL-Faslpr mice, compared with normal mice, suggesting that IL-21 has the potential to greatly impact Ab production in these mice. Targeting IL-21 in vivo by treating MRL-Faslpr mice with IL-21R.Fc reduced circulating levels of dsDNA autoantibodies and total Ig, in particular IgG1 and IgG2 in these mice. This is in agreement with the recently described properties of IL-21 related to B lymphocyte function, in that IL-21 has been shown to have a nonredundant role in Ig class switching and in differentiation of B lymphocytes into Ab-producing plasma cells, resulting in increased IgG production (7, 9). Interestingly, IL-21 has been shown to also modulate serum IgE and IgM levels (8, 38), however, treating MRL-Faslpr mice with IL-21R.Fc did not affect these levels.

The reduction of serum autoantibodies in IL-21R.Fc-treated mice is most likely a major factor contributing to the reduced disease in MRL-Faslpr mice. The impact of autoantibodies on disease was demonstrated by infusing monoclonal autoantibody-secreting hybridoma cells into normal mice which resulted in the development of glomerular immune deposits, renal lesions, proteinuria, and skin vasculitis (39–41). In MRL-Faslpr mice, IgG deposits are prevalent in the kidney, but their presence is also closely associated with skin lesions, as they are detected in the dermoeidermal junction in the skin of older MRL-Faslpr mice and thought to be directed against desmoglein 3 in the skin (42). B cell-deficient MRL-Faslpr mice have abrogated skin lesions, although this is likely due to the B cell-mediated activation of T cells and not Ig production (18). Therefore, parameters impacting B cell function or circulating Ig are likely to reduce lesions in the kidney as well as the skin. In agreement with this, IL-21R.Fc-treated MRL-Faslpr mice with reduced Ig levels in this study also had reduced glomerular immune complex deposits, glomerular basement membrane thickening, proteinuria, as well as reduced skin lesions.

Lymphoproliferation in the MRL-Faslpr mice is due to the accumulation of CD4+, CD8+, and a B220+CD3+CD4+CD8− (DN) T lymphocyte population (33). The expansion of these cells in the periphery occurs in part by the absence of Fas on these cells, which is normally the primary mechanism responsible for limiting clonal proliferation of activated and autoreactive T lymphocytes (44, 45). However, cytokines and B lymphocyte-dependent activation can also expand these T lymphocyte subsets by enhancing their proliferation, activation, and survival (46, 47). IL-21 acts as a proliferation and survival factor for CD8+ T lymphocytes to a lesser extent CD4+ T lymphocytes. The IL-21R, which is expressed on CD4+ and CD8+ T lymphocytes, is up-regulated in the presence of IL-21, (4) thus further enhancing its effects. A reduction in the bioavailability of IL-21 would therefore stand to impact these cell populations. DN T lymphocytes, in contrast, do not express the IL-21R and it is thought that the cytokine does not have an essential role in T cell development (6, 9). The reported differential effect of IL-21 on CD4+ and CD8+ T lymphocytes is...
supported by our data showing that treatment with IL-21R.Fc resulted in reduced splenic CD4⁺ and CD8⁺ T lymphocytes, with no change in DN T lymphocytes. Although lupus is often considered a B cell disorder, T cells also contribute to disease in the MRL-Faslpr mouse. CD4⁺ T lymphocytes are key regulators in the production of the pathogenic anti-dsDNA autoantibodies, arthritis, vasculitis, and Ig-induced nephritis in the MRL-Faslpr mouse (48, 49). Likewise, CD8⁺ lymphocytes have also been shown to contribute to autoantibody production and glomerular nephritis in this mouse (50). By treating MRL-Faslpr mice with IL-21R.Fc we observed a reduction in both of these T lymphocyte population in the spleen which is likely contributing to the reduced disease we observe in these mice (50, 51). These data are interesting in that IL-21R.Fc treatment is impacting both T and B lymphocytes and gives further support of the importance of IL-21 on T lymphocyte biology and SLE.

IL-21 is involved in both cell–mediated and humoral responses. Through its actions on T and NK cells, administration of IL-21 or overexpression of IL-21 increases the severity of experimental autoimmune encephalomyelitis, assists in tumor clearance, is implicated in Crohn’s disease, and expands T lymphocyte populations responsible for autoimmune diabetes (11–13, 52–54). Increased levels of IL-21 have been detected in the serum of XBX-Yaa mice, another model of SLE (8). Although we were not able to detect IL-21 in the sera of MRL-Faslpr mice in this study (data not shown), we did see secretion of IL-21 by MRL-Faslpr CD4⁺ T lymphocytes beyond that seen in normal mice in ex vivo experiments. Furthermore, the efficacy of treatment with IL-21R.Fc in MRL-Faslpr mice suggests that endogenous IL-21 is contributing to the pathogenesis of this disease model. IL-21R.Fc maybe expressed in the context of a tissue microenvironment where T and B lymphocyte interactions occur, resulting in the contribution of this cytokine to disease progression in this lupus model.

In summary, our data provide strong evidence that IL-21 has a pathogenic role in the disease progression the MRL-Faslpr mouse model, primarily by enhancing the function of autoreactive B lymphocytes, but also by having an impact on T lymphocytes and other immune mechanisms underlying autoimmunity. Extension of similar studies to other models of autoimmunity will provide a better understanding of the multifaceted role of IL-21 in the maintenance of self-tolerance.

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Disclosures

The authors have no financial conflict of interest.

References

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