IL-21 Receptor Is Required for the Systemic Accumulation of Activated B and T Lymphocytes in MRL/MpJ-Fas<sup>lpr/lpr</sup>/J Mice

Andrew L. Rankin, Heath Guay, Deborah Herber, Sarah A. Bertino, Tatyana A. Duzanski, Yijun Carrier, Sean Keegan, Mayra Senices, Nancy Stedman, Mark Ryan, Laird Bloom, Quintus Medley, Mary Collins, Cheryl Nickerson-Nutter, Joe Craft, Deborah Young and Kyri Dunussi-Joannopoulos

J Immunol 2012; 188:1656-1667; Prepublished online 9 January 2012; doi: 10.4049/jimmunol.1003871
http://www.jimmunol.org/content/188/4/1656

Supplementary Material
http://www.jimmunol.org/content/suppl/2012/01/09/jimmunol.1003871.DC1

References
This article cites 81 articles, 47 of which you can access for free at:
http://www.jimmunol.org/content/188/4/1656.full#ref-list-1

Subscription
Information about subscribing to The Journal of Immunology is online at:
http://jimmunol.org/subscription

Permissions
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
IL-21 Receptor Is Required for the Systemic Accumulation of Activated B and T Lymphocytes in MRL/MpJ-Fas<sup>lpr/lpr</sup>/J Mice

Andrew L. Rankin,*,1 Heath Guay,*,1,2 Deborah Herber,* Sarah A. Bertino,‡
Tatyana A. Duzanski,* Yijun Carrier,* Sean Keegan,* Mayra Senices,* Nancy Stedman,‡
Mark Ryan,* Laird Bloom,§ Quintus Medley,* Mary Collins,* Cheryl Nickerson-Nutter,*
Joe Craft,‡ Deborah Young,* and Kyri Dunussi-Joannopoulos* 

MRL/MpJ-Fas<sup>lpr/lpr</sup>/J (MRL<sup>lpr</sup>) mice develop lupus-like disease manifestations in an IL-21–dependent manner. IL-21 is a pleiotropic cytokine that can influence the activation, differentiation, and expansion of B and T cell effector subsets. Notably, autoreactive CD4<sup>+</sup> T and B cells spontaneously accumulate in MRL<sup>lpr</sup> mice and mediate disease pathogenesis. We sought to identify the particular lymphocyte effector subsets regulated by IL-21 in the context of systemic autoimmunity and, thus, generated MRL<sup>lpr</sup> mice deficient in IL-21R (MRL<sup>lpr</sup>/IL-21R<sup>−/−</sup>). Lymphadenopathy and splenomegaly, which are characteristic traits of the MRL<sup>lpr</sup> model were significantly reduced in the absence of IL-21R, suggesting that immune activation was likewise decreased. Indeed, spontaneous germinal center formation and plasma cell accumulation were absent in IL-21R–deficient MRL<sup>lpr</sup> mice. Correspondingly, we observed a significant reduction in autoantibody titers. Activated CD4<sup>+</sup>CD44<sup>+</sup>CD62Llo T cells also failed to accumulate, and CD4<sup>+</sup> T cell differentiation was impaired, as evidenced by a significant reduction in CD4<sup>+</sup> T cells that produce the particular lymphocyte effector subsets regulated by IL-21 in the context of systemic autoimmunity and, thus, generated MRL<sup>lpr</sup> mice. Importantly, we demonstrated that T extrafollicular helper cells are dependent on IL-21R for their generation. Together, our data highlighted the novel observation that IL-21 is a critical regulator of multiple pathogenic B and T cell effector subsets in MRL<sup>lpr</sup> mice. The Journal of Immunology, 2012, 188: 1656–1667.

MRL/MpJ-Fas<sup>lpr/lpr</sup>/J (MRL<sup>lpr</sup>) mice spontaneously develop systemic autoimmunity that is characterized by generalized activation of B and T cells and pathologic features resembling those observed in patients with systemic lupus erythematosus (SLE). Hallmark features of disease include the development of glomerulonephritis and cutaneous lesions and logic features resembling those observed in patients with systemic lupus erythematosus (SLE). Multiple lymphocyte effector populations contribute to disease pathogenesis in MRL<sup>lpr</sup> mice. Both B cells and CD4<sup>+</sup> T cells are critical for the development of renal pathology and autoantibody production (3–7). B cells promote the development of disease features via both Ab-dependent and -independent mechanisms and are required for the accumulation of activated memory CD4<sup>+</sup> and CD8<sup>+</sup> T cells (7, 8). The predominant source of somatically mutated autoantibodies in MRL<sup>lpr</sup> mice derives from extrafollicular foci that require CD4<sup>+</sup> T extrafollicular helper (T<sub>hf</sub>) cells for their formation (9, 10). CD4<sup>+</sup> T<sub>hf</sub> cells develop via an ICOS–dependent mechanism in MRL<sup>lpr</sup> mice, and MRL<sup>lpr</sup>/ICOS<sup>−/−</sup> mice develop reduced autoantibody titers (10–12). Nevertheless, development of renal pathology is only modestly impacted in MRL<sup>lpr</sup>/ICOS<sup>−/−</sup> mice, consistent with an Ab-independent role for the development of nephritis, and accumulation of activated lymphocyte populations still occurs (7, 11, 12). In addition, activated CD4<sup>+</sup> T cells from MRL<sup>lpr</sup> mice produce high levels of IFN-γ, which is a cytokine that is necessary for the development of renal pathology, suggesting that CD4<sup>+</sup> T cell-derived IFN-γ may promote the development of renal pathology in MRL<sup>lpr</sup> mice (13–16). Thus, multiple lymphocyte populations exhibit spontaneous activation and accumulation in MRL<sup>lpr</sup> mice; however, the molecular mechanisms underlying these processes, which ultimately contribute to disease pathogenesis, remain poorly understood.

IL-21 is the most recently discovered member of the type I cytokine family. IL-21 binds the IL-21R, which is a heterodimer composed of IL-21Rα and the common γ-chain that is shared with receptors specific for IL-2, IL-4, IL-7, IL-9, and IL-15 (17–19). IL-21R is expressed by lymphoid cells, including B cells and activated CD4<sup>+</sup> T cells (17, 20). Its expression is also found on myeloid cells, such as monocytes and dendritic cells (21, 22). IL-21 is produced by activated CD4<sup>+</sup> T cells and NK T cells and exhibits complex context-dependent biologic activity. For instance, in vitro IL-21

*Inflammation and Immunology, Pfizer Research, Cambridge, MA 02140;
†Department of Immunobiology, Yale University School of Medicine, New Haven, CT 06520; ‡Drug Safety and Metabolism, Pfizer Research, Andover, MA 01810; and §Global Biotherapeutics Technologies, Pfizer Research, Cambridge, MA 02140

A.L.R. and H.G. contributed equally to this work.

Received for publication November 24, 2010. Accepted for publication December 4, 2011.

Address correspondence and reprint requests to Andrew L. Rankin, Pfizer Research, 200 Cambridgepark Drive, Cambridge, MA 02140. E-mail address: Andrew.Rankin@pfizer.com

The online version of this article contains supplemental material.

Abbreviations used in this article: ANA, anti-nuclear autoantibody; DN, double negative; GBML, glomerular basement membrane; GC, germinal center; KO, knockout; MFI, mean fluorescence intensity; MRL<sup>lpr</sup>/J, MRL/MpJ-Fas<sup>lpr/lpr</sup>/J; PAS, periodic acid–Schiff; PNA, peanut agglutinin; SLE, systemic lupus erythematosus; T<sub>hf</sub>, T extrafollicular helper; T<sub>hf</sub>/T<sub>hf</sub>, T extrafollicular helper; WT, wild-type.

Copyright © 2012 by The American Association of Immunologists, Inc. 0022-1767/12 $16.00

www.jimmunol.org/cgi/doi/10.4049/jimmunol.1003871
induces apoptosis of resting B cells but drives enhanced proliferation of B and T cells stimulated via their AgRs (20, 23). In vivo IL-21 is required for the normal accumulation of early Ab-secreting cells in response to immunization with T-dependent Ags (24, 25). Consistent with this observation, costimulation of B cells by their BCR and IL-21 promotes plasma cell differentiation in vitro (26, 27). IL-21 is also required for normal germinal center (GC) formation via a B cell-intrinsic pathway (25, 28–30). IL-21 was also recently shown to be produced by T follicular helper (Tfh) cells, which are a specialized subset of activated CD4+ T cells that promote GC-dependent Ab responses (31, 32). In some experimental systems, IL-21 is required for the differentiation of Tfh cells (33–35). Tfh cells produce IL-21 and promote Ab production in an IL-21–dependent manner; however, it still remains unclear whether IL-21–derived signals are required for their formation (10). In addition, Th17 cells, which have recently been associated with SLE in human patients and mouse models, can also use IL-21 for their differentiation (32, 36–38).

Recent candidate gene studies identified polymorphisms in both the IL-21 and IL-21R genes that associate with development of SLE (39–41). Moreover, IL-21 promotes disease pathogenesis in preclinical lupus models, such as the BXSB-Yaa and MRL+/− mice (42, 43). We sought to identify the particular lymphocyte effector subsets regulated by IL-21 in the context of systemic autoimmunity and, thus, generated MRL+/− mice deficient in IL-21R (MRL+/−IL-21R−/−). Lymphadenopathy and splenomegaly were significantly reduced and corresponded to dramatic reductions in the absolute numbers of CD4+ T, double-negative (DN) T, and B cells. Notably, the spontaneous accumulation of GC B cells and plasma cells is completely abrogated in MRL+/−IL-21R−/− mice. Similarly, activated CD4+CD62L−CD4+ T cells accumulate in an IL-21R–dependent manner. Multiple T effector subsets are correspondingly impacted by loss of IL-21R signaling. Notably, the frequency of IFN-γ–producing CD4+ T cells was significantly reduced in IL-21R−/− deficient MRL+/− mice. In addition, we demonstrated that Tfh cells require IL-21R for their development in MRL+/− mice. Overall, these studies highlighted that IL-21R signaling is necessary for the spontaneous accumulation of multiple B and T cell effector populations in MRL+/− mice.

Materials and Methods

Mice

IL-21R knockout (KO) mice were generated, as previously described (44). MRL+/− mice deficient in IL-21R were generated by backcrossing C57BL/6. IL-21R−/− mice onto the MRL+/− background (The Jackson Laboratory). Congenic MRL+/− mice were generated using a speed-congenic MaxBax backcrossing strategy (Charles River Laboratories). Mice were considered fully backcrossed after obtaining 99% MRL+/− genomic sequence, as assessed by 81 microsatellite markers covering the entire murine genome. Age- and sex-matched MRL/MpJ mice were obtained from The Jackson Laboratory. All animals were housed in a pathogen-free animal facility at Pfizer. Mice were used according to protocols approved by the Pfizer Animal Care and Use Committee.

Evaluation of disease progression

To evaluate the role of IL-21R in the development of lupus-like disease in MRL+/−IL-21R−/− and wild-type (WT) MRL+/− IL-21R−/− mice, littermate animals were scored blindly for proteinuria, skin lesions, lymphadenopathy, and splenomegaly. Proteinuria was measured using Albustix (Bayer) and scored on a scale of 0–5; 0, none; 1, trace; 2, 30 mg/dl; 3, 100 mg/dl; 4, 300 mg/dl; and 5, ≥2000 mg/dl. Lymph nodes were palpated, and lymphadenopathy was scored on a scale of 0–3; 0, none; 1, small; 2, moderate, at two different sites; and 3, large, at three or more different sites. Skin lesions were assessed by gross pathology and scored on a scale of 0–3; 0, none; 1, small (face, ears); 2, moderate (<2 cm face, ears, and back); and 3, severe (>2 cm face, ears, and back). Splenomegaly was measured, and splenocytes were counted to assess splenomegaly.

Flow cytometry

Single-cell suspensions of splenocytes were prepared, treated with RBC lysis buffer (Sigma), and then washed with FACS buffer (PBS plus 1% FCS). Cells were then incubated with purified anti-CD16/32 (Fc block; clone 2.4G2; BD Pharmingen), washed, and stained with primary Abs diluted in FACS buffer for 30 min at 4˚C. Intracellular staining of cytokines was performed using the BD intracellular FACS kit, according to the manufacturer’s protocol (BD Biosciences). Abs used in these studies included anti-T and B cell-activating Ag “GL7” (clone GL7), CD4 (clone GK1.5), CD3 (clone 145-2C11), CD8a (clone 53-6.7), CD138 (clone 28-1B2), B220 (clone RA3-6B2), CD19 (clone 1D3), CD44 (clone IM7), CD62L (clone MEL-14), IL-21R (A49), TCRG (clone H57-587), FSG1 (clone 4R4A10), PD1 (clone J43), CXCR5 (clone 2G8), CXCR4 (clone 2B11), CD38 (clone 90), CD278/ICOS (clone C93.84a), IL-17 (clone TC11-18H10), IL-4 (clone 1B11), and IFN-γ (clone XMG1.2). Unlabeled anti-CXCR5 mAb was visualized using allophycocyanin-goat anti-rat-Ig (BD Biosciences). Cells were also stained with live/dead Fixable Aqua dead cell stain (Invitrogen), as per the manufacturer’s directions. At least 100,000 events were counted using either a FACSCalibur or LSRII (Becton Dickinson) and analyzed using FlowJo software (Tree Star).

Flow cytometric analysis of kidney infiltrates was performed as follows. Mice were perfused with 30 ml HBSS containing 2 mM EDTA. Kidneys were isolated, capsules were removed, and the tissues were processed using a gentleMACS tissue dissociator (Miltenyi Biotec). Tissue homogenates were filtered through a 70-μm nylon mesh filter, and leukocytic infiltrates were isolated by centrifugation over a Percoll gradient. Cells were then washed with FACS buffer, counted, and stained, as above.

In vitro B cell stimulation

Spleens from untreated 8-wk-old mice were dissociated into single-cell suspensions and lysed with RBC lysis buffer (Sigma). B lymphocytes were isolated using magnetic cell-sorting CD19 MicroBeads (Miltenyi Biotec), according to the manufacturer’s instructions. Purified B cells were cultured in RPMI 1640 medium plus 10% FBS in a 96-well plate and incubated with 2.5 μg/ml purified anti-mouse CD40 (BD Pharmingen) and 10 μg/ml PibAb (goat anti-mouse IgM (Jackson ImmunoResearch Laboratories) in the presence or absence of 50 ng/ml IL-21 (R&D Systems). Supernatants were collected after 96 h of incubation and analyzed for IgG content using an IgG ELISA kit (Bethyl).

Serum Ig ELISAs

Serum Ig was measured by ELISA. Briefly, Costar plates (Thermolab Systems) were coated overnight with 1 μg/ml goat anti-mouse Ig(H+L) (Southern Biotech). Plates were blocked the next day with PBS plus 1% BSA, serially diluted serum samples were added (1:100 starting dilution), and bound Ab was detected with HRP-conjugated goat Abs directed against mouse IgM, IgG1, IgG2a, IgG2b, or IgG3 (Southern Biotech). Purified unlabelled mouse IgM, IgG1, IgG2a, IgG2b, or IgG3 Ab (Southern Biotech) was used as a standard to quantify serum Ig concentration. Plates were developed using 3,3′,5′,5′-tetramethyl-benzidine (KPL), and reactions were stopped with 2 N sulfuric acid. Absorbances were read at 450 nm using a SpectraMax Plus 384 microplate reader (Molecular Devices) and SoftMax Pro software.

Serum anti-dsDNA ELISA

Serum anti-dsDNA IgG Abs were measured by ELISA. Briefly, Immulon 1B plates (Thermolab Systems) were UV irradiated overnight and then coated with 2 μg/ml calf thymus DNA (Sigma) for 1 h at room temperature. Plates were blocked with PBS plus 1% BSA, serially diluted serum samples were added (1:100 dilution), and bound Ab was detected with HRP-conjugated goat Abs directed against mouse IgM, IgG1, IgG2a, IgG2b, or IgG3 (Southern Biotech). Purified unlabelled mouse IgM, IgG1, IgG2a, IgG2b, or IgG3 Ab (Southern Biotech) was used as a standard to quantify serum Ig concentration. Plates were developed using 3,3′,5′,5′-tetramethyl-benzidine (KPL), and reactions were stopped with 2 N sulfuric acid. Absorbances were read at 450 nm using a SpectraMax Plus 384 microplate reader (Molecular Devices) and SoftMax Pro software.

Anti-nuclear Ab assay

Serum anti-nuclear Abs (ANAs) were measured using a Hep-2 ANA kit (Antibodies), according to the manufacturer’s protocol. Briefly, serum samples were diluted to 1:100 in sample-dilution buffer, bound Ab was detected using a FITC–anti-mouse IgG Ab (Southern Biotech), and images were captured using a Nikon Eclipse E800 fluorescent microscope. To quantitate serum ANA, mean fluorescence intensity (MFI) was measured for ≥10 cells/sample using ImagePro Plus software, and average MFI was determined.
Histopathological evaluation of H&E- and periodic acid-Schiff–stained tissue sections

Routine H&E and periodic acid-Schiff (PAS) staining was performed on formalin-fixed, paraffin-embedded sections of kidney. To assess inflammation, the total numbers of inflammatory foci were counted in the kidneys and salivary glands in H&E-stained tissue sections. If foci had coalesced, an estimate was made of the numbers of individual foci present. To assess glomerular morphology, PAS was performed on PAS-stained tissue sections of kidney using a commercial image-analysis software package (Image-Pro Plus v.5.1; Media Cybernetics, Silver Spring, MD). Ten glomeruli from each kidney were photographed at 40× magnification in 24-bit color at 1388 × 1040 resolution using a Zeiss Axio Imager.A1 microscope and a Zeiss Axiocam HRc digital microscope camera (Carl Zeiss Microimaging, Thornwood, NY). Using Image-Pro Plus, glomeruli captured in digital photomicrographs were evaluated morphometrically by manually tracing the circumference of the glomerular tuft and measuring the total area of the traced glomerular tuft. The total areas of PAS-positive mesangium (as a measure of immune complex deposition and basement membrane thickening) and nuclei (as an estimate of cellularity) in the traced glomerular tuft were also measured in Image-Pro Plus using color segmentation.

Immunohistochemistry and immunofluorescence Ab staining

Immunohistochemical staining for IL-21R (polyclonal Ab; cat. #14-6469, Biocare Medical), IgD (clone 11-26c.2a; BD Biosciences), biotinylated peanut agglutinin (PNA; Vector Labs), IgG and IgM (polyclonal Abs; Jackson ImmunoResearch, West Grove, PA), and complement C3 (ICN/Cappel) was performed using commercially available cryosections of kidney specimens and a Nemesis 7200 autostainer (Biocare Medical, Concord, CA). TBST was applied to HRP-labeled sections, and Permanent Red Chromagen (DakoCytomation) was used. Streptavidin:HRP was quenched with 3% H2O2 in 70% methanol for 30 min. Anti–IL-21R Ab was used at 1:200 in blocking solution; F4/80 Alexa Fluor 488 (clone: BM8; eBiosciences), B220 Biotin (clone: RA3-6B2; BD Pharmingen), CD4 Pacific Blue (BD Pharmingen), and IgM FITC (BD Pharmingen) were applied for 2 h at 1:200 in blocking solution; PNA/647 (clone: BM8; eBiosciences), B220 Biotin (clone: RA3-6B2; BD Pharmingen), CD4 Pacific Blue (BD Pharmingen), and IgM FITC (BD Pharmingen) were applied for 1 h in a dark, humid chamber. Sections were rinsed in TBST, mounted with VECTASHIELD DAPI mounting media (Vector Laboratories, Burlingame, CA), and examined using a Nikon E800 microscope and a 40× plan Apo objective (Nikon, Melville, NY).

Immunofluorescent staining for F4/80, B220, CD4, and IgM was performed on acetone-fixed cryosections of spleen sections. Sections were protein blocked (3% BSA; Sigma) for 20 min, and primary rat anti-mouse Abs were applied for 2 h at 1:200 in blocking solution; F4/80 Alexa Fluor 647 (clone: BM8; eBiosciences), B220 Biotin (clone: RA3-6B2; BD Pharmingen), CD4 Pacific Blue (BD Pharmingen), and IgM FITC (BD Pharmingen). Sections were rinsed in washing solution (1% BSA), and secondary reagents, which included rabbit IgG anti-FITC Alexa Fluor 488 (1:200, Invitrogen) or streptavidin Alexa Fluor 555 (1:500; Invitrogen), were applied for 1 h. Sections were rinsed with washing solution, followed by 1× PBS, and examined using a Leica TCS SP5 Spectral Confocal Microscope, 405UV and a 40× Apo objective. The area (mm2) of extrafollicular regions was calculated, using ImageJ software. Plasmablasts localized within the extrafollicular region were blindly manually counted, and numbers of plasmablasts/mm2 of extrafollicular region were also measured in Image-Pro Plus using color segmentation.

Ex vivo intracellular cytokine analysis

For the detection of Th1, Th2, and Th17 cells, ex vivo, freshly isolated splenocytes were stimulated with PMA (50 ng/ml) and ionomycin (1 μg/ml) in the presence of 1 μM GolgiStop (BD Biosciences) for 4 h and then examined for production of IFN-γ, IL-17, and IL-4 by intracellular flow cytometry. Cells were analyzed on a FACSCalibur flow cytometer (BD Biosciences).

Results

Nephritis and skin lesions develop in an IL-21R–dependent manner in MRLpr mice

We sought to better understand the impact of IL-21R deficiency on immune activation in the MRLpr mouse model of lupus. To these ends, we generated MRLpr/IL-21R−/− mice and first assessed the development of disease features in both MRLpr/IL-21R−/− and MRLpr/IL-21R+/− animals. By 3 mo of age, MRLpr/IL-21R+/− mice developed proteinuria, lymphadenopathy, and splenomegaly, which increased dramatically in severity with age (Fig. 1A, 1B, data not shown). Also, 86% of 5-mo-old MRLpr/IL-21R−/− mice exhibited severe skin lesions around the face, back, and neck (Fig. 1C, data not shown). Notably, the severity of these disease features was significantly reduced in aged MRLpr/IL-21R−/− mice compared with IL-21R+/− littermates (Fig. 1C). Three-month-old MRLpr/IL-21R−/− mice exhibited reduced levels of lymphadenopathy, splenomegaly, and proteinuria; interestingly, the severity of these features did not increase from 3 to 5 mo of age in MRLpr/IL-21R−/− mice (Fig. 1A, 1B, data not shown). In addition, only 69% of MRLpr/IL-21R−/− mice developed skin disease by 5 mo of age, which was limited to small lesions and hair loss on the nose and face (Fig. 1C, data not shown).

We also assessed the degree of renal pathology present in kidneys obtained from MRLpr/IL-21R−/− and IL-21R−/− mice. Deposition of immune complexes in the glomeruli is a prominent disease feature of lupus nephritis and is a critical mediator of kidney damage in MRLpr mice (45, 46). IgM, IgG, and C3 deposits were readily observed in the glomeruli of 5-mo-old IL-21R−/− WT MRLpr mice (Fig. 1D, 1E). Kidneys obtained from MRLpr/IL-21R−/− mice also contained numerous monoclonal inflammatory cell infiltrates and had thickening of the glomerular basement membrane (GBM), which is consistent with the development of lupus nephritis (Fig. 1F, 1G). By contrast, kidneys obtained from MRLpr/IL-21R−/− mice had significantly reduced levels of IgG and C3 deposition in their glomeruli (Fig. 1D, 1E). Moreover, inflammatory cell infiltrates were dramatically reduced in the kidneys of MRLpr/IL-21R−/− mice, as was GBM thickening (Fig. 1F, 1G). The decreased renal pathology observed in IL-21R−/− mice correlated well with reduced proteinuria in 5-mo-old MRLpr/IL-21R−/− mice relative to IL-21R+/− littermates (Fig. 1A). Deposition of IgM was not affected in the IL-21R−/− background; however, this isotype does not appear to play a pathogenic role in the MRLpr model (Fig. 1E) (47). We also examined salivary glands, because inflammatory infiltrates also accumulate in this organ as MRLpr mice age; we found that the number of inflammatory foci were significantly reduced in MRLpr/IL-21R−/− mice (data not shown).

Because loss of IL-21R signaling in MRLpr mice dramatically limited the development of renal pathology, we subsequently examined the expression levels of IL-21R in kidneys obtained from aged MRLpr mice to determine whether IL-21R signaling locally might contribute to renal pathology. By quantitative RT-PCR analysis, IL-21R expression levels were significantly increased in MRLpr mice compared with MRLpr mice (Fig. 2A). As expected, expression of IL-21R was undetectable in kidneys obtained from MRLpr/IL-21R−/− mice (Fig. 2A). To determine
the localization of IL-21R–expressing cells, we next performed immunohistochemical analysis on kidney sections. IL-21R expression was observed on a fraction of cells present in the perivascular infiltrates of MRL\textsuperscript{\textit{Il-21r}}/\textit{Il-21r}\textsuperscript{ KO} kidneys. Consistent with the increased numbers of inflammatory foci observed in kidneys obtained from MRL\textsuperscript{\textit{Il-21r}}/\textit{Il-21r}\textsuperscript{ KO} mice, intrarenal leukocyte counts were significantly elevated in MRL\textsuperscript{\textit{Il-21r}}/\textit{Il-21r}\textsuperscript{ KO} mice compared with MRL\textsuperscript{\textit{Il-21r}}/\textit{Il-21r}\textsuperscript{ KO} mice. The majority of the cells isolated from MRL\textsuperscript{\textit{Il-21r}}/\textit{Il-21r}\textsuperscript{ KO} kidneys were CD4\textsuperscript{+} T cells (67\% ± 0.1\%; \textit{n} = 10), with CD19\textsuperscript{+} B cells (0.9 ± 0.1\%; \textit{n} = 10), CD8\textsuperscript{+} T cells (9.0 ± 0.9\%; \textit{n} = 10), and DN T cells (6.5 ± 1.1\%; \textit{n} = 10) making up the remaining minority population. Kidneys from MRL\textsuperscript{\textit{Il-21r}}/\textit{Il-21r}\textsuperscript{ KO} mice contained nearly 20-fold fewer CD4\textsuperscript{+} T cells than were present in kidneys prepared from MRL\textsuperscript{\textit{Il-21r}}/\textit{Il-21r}\textsuperscript{ KO} mice (Fig. 2C). By contrast, the number of CD8\textsuperscript{+} and DN T cells was reduced by <2.5-fold in kidneys obtained from MRL\textsuperscript{\textit{Il-21r}}/\textit{Il-21r}\textsuperscript{ KO} mice, and B cell numbers were unchanged between these strains. Thus, intrarenal CD4\textsuperscript{+} T cell accumulation occurs via an IL-21R–dependent pathway in MRL\textsuperscript{\textit{Il-21r}}/\textit{Il-21r}\textsuperscript{ KO} mice.

IL-21R expression levels were assessed on each of these lymphocyte populations. The anti-IL-21R Ab used for these studies appeared specific for IL-21R, based on its lack of staining of lymphocytes obtained from both the spleen and kidney of MRL\textsuperscript{\textit{Il-21r}}/\textit{Il-21r}\textsuperscript{ KO} mice compared with WT controls (Fig. 2D, data not shown). Notably, CD4\textsuperscript{+}, CD8\textsuperscript{+}, DN T cells, and B cells each had detectable expression of IL-21R. However, B cells (1580 ± 300 MFI; \textit{n} = 9) expressed IL-21R at nearly 5-fold higher levels than did CD4\textsuperscript{+} T cells (328 ± 8.1 MFI; \textit{n} = 9) in the kidney, and CD8\textsuperscript{+} (640 ± 29.5 MFI; \textit{n} = 9) and DN T cells (540 ± 26.2 MFI; \textit{n} = 9) expressed intermediate levels (Fig. 2D). Therefore, the limited distribution of IL-21R staining seen by immunohistochemical staining may reflect detection of the highest surface levels of IL-21R, which was observed on B cells (Fig. 2D). Collectively, these results indicated that IL-21R is required for the pathologic accumulation of intrarenal CD4\textsuperscript{+} T cells and that IL-21R–expressing cells contribute to the inflammatory infiltrates present in the diseased kidneys of MRL\textsuperscript{\textit{Il-21r}}/\textit{Il-21r} mice.

**IL-21R is required for B and T cell accumulation in MRL\textsuperscript{\textit{Il-21r}}/\textit{Il-21r} mice**

Splenomegaly develops in MRL\textsuperscript{\textit{Il-21r}}/\textit{Il-21r} mice as a consequence of accumulation of DN (CD4\textsuperscript{+} CD8\textsuperscript{−} CD3\textsuperscript{−}) T cells and activated CD4\textsuperscript{+} and CD8\textsuperscript{+} T and B cells (1). Notably, IL-21 was shown to enhance both the proliferation and survival of B and T cells (48). To understand the potential contribution of IL-21 to the lymphoproliferative phenotype observed in MRL\textsuperscript{\textit{Il-21r}}/\textit{Il-21r} mice, we examined the cellular composition of spleens obtained from adult MRL\textsuperscript{\textit{Il-21r}}/\textit{Il-21r}\textsuperscript{ KO} and MRL\textsuperscript{\textit{Il-21r}}/\textit{Il-21r}\textsuperscript{ KO} mice. MRL\textsuperscript{\textit{Il-21r}}/\textit{Il-21r}\textsuperscript{ KO} spleens contained half as many total splenocytes as did IL-21R WT MRL\textsuperscript{\textit{Il-21r}}/\textit{Il-21r} spleens, indicating that IL-21R contributes to the development of splenomegaly in MRL\textsuperscript{\textit{Il-21r}}/\textit{Il-21r} mice (Fig. 3). Interestingly, the relative frequency of B cells, CD4\textsuperscript{+} T cells, and DN T cells was similar in spleens obtained from MRL\textsuperscript{\textit{Il-21r}}/\textit{Il-21r}\textsuperscript{ KO} and MRL\textsuperscript{\textit{Il-21r}}/\textit{Il-21r}\textsuperscript{ KO} mice (data not shown). Consequently, the absolute number of B cells, CD4\textsuperscript{+} T cells, and DN T cells was reduced by roughly half in MRL\textsuperscript{\textit{Il-21r}}/\textit{Il-21r}\textsuperscript{ KO} spleens (Fig. 3). CD8\textsuperscript{+} T cells numbers were unaffected by IL-21R deficiency in the MRL\textsuperscript{\textit{Il-21r}} background (Fig. 3). However, it is noteworthy that the DN T cells that accumulate in MRL\textsuperscript{\textit{Il-21r}} mice derive from
activated CD8+ T cells, which suggests that IL-21R signaling in CD8+ T cells is involved in the accumulation of DN T cells (6, 49, 50). Because IL-21 supports accumulation of chronically activated CD8+ T cells in response to viral infection, our observations suggested that IL-21R may play a similar role in autoimmune disease settings (51–53). Collectively, these results indicated that IL-21R signaling is critical for the systemic accumulation of B, CD4+ T, and DN T cells subsets in MRL-lpr mice.

IL-21R regulates spontaneous B cell activation and autoantibody responses in MRL-lpr mice

IL-21 promotes the differentiation of B cells to plasma cells and isotype switching (26, 27). Because autoantibody production by B cells contributes to renal pathology in MRL-lpr mice, this suggested that signaling via IL-21R on B cells could promote B cell-activation pathways in MRL-lpr mice (7). GC-independent and dependent B cell responses were reported to be activated in MRL-lpr mice (9, 54). We assessed the extent of B cell activation by determining the absolute numbers of splenic GC B cells and plasma cells. We included MRL/MpJ mice in these analyses to better assess the background level of B cell activation in the absence of the lpr mutation. GC B cells were identified using a combination of cell surface markers that included GL7, PNA, and CD38. Indeed, the percentage and absolute number of splenic GC GL7+ PNA+ B cells was significantly reduced in MRL-lpr,IL-21R+/− mice compared with MRL-lpr,IL-21R+/+ mice (Fig. 4A, 4B). These GL7+ PNA+ B cells also expressed low levels of CD38, which is consistent with B cells bearing a GC phenotype (55) (data not shown). Immunohistochemical staining for PNA revealed GCs localized to the B cell follicles in MRL-lpr,IL-21R+/+ mice (Fig. 4E). GCs were not observed in spleen sections prepared from MRL-lpr,IL-21R−/− mice (Fig. 4E). The percentage and absolute number of splenic plasma cells were also significantly reduced in MRL-lpr,IL-21R−/− mice compared with MRL-lpr,IL-21R+/+ mice (Fig. 4C, 4D). Strikingly, the absolute numbers of GC B cells and plasma cells were similar to those present in MRL/MpJ mice, which lack the lpr mutation. In MRL-lpr mice, plasmablasts accumulate in the extrafollicular space and play a critical role in production of autoantibody (9). Immunofluorescence staining identified large plasmablastic foci in the extrafollicular space in MRL-lpr,IL-21R+/+ mice (Fig. 4F). By contrast, extrafollicular plasmablasts were significantly reduced in MRL-lpr,IL-21R−/− mice, consistent with the flow cytometric results (Fig. 4F, 4G). Collectively, these results highlighted that signaling via IL-21R is critical for the spontaneous accumulation of activated B cell subsets in MRL-lpr mice.

The significant reduction in total plasma cell numbers in MRL-lpr,IL-21R−/− mice suggested that Ab production may be similarly affected. Indeed, IgG1, IgG2a, IgG3, and IgM serum titers were all significantly reduced in MRL-lpr,IL-21R−/− mice (Fig. 5A, Supplemental Fig. 1). Levels of anti-nuclear and anti-dsDNA autoantibodies were likewise reduced in MRL-lpr,IL-21R−/− mice.
In MRL<sup>lpr</sup> mice, the spleens of these animals (Fig. 5). Stimulation of IL-21R WT mice expressed a similar phenotype (Fig. 6, Supplemental Fig. 2). To further corroborate these observations, we next examined the ability of IL-21R WT and MRL<sup>lpr</sup> IL-21R<sup>-/-</sup> B cells to secrete Ig following stimulation in vitro. Notably, even in the absence of exogenous stimulation, IL-21R WT MRL<sup>lpr</sup> B cells secreted detectable levels of IgG Ab, consistent with the increased numbers of plasma cells observed in the spleens of these animals (Fig. 5E). Stimulation of IL-21R WT MRL<sup>lpr</sup> B cells with anti-IgM and anti-CD40 resulted in an increase in detectable levels of Ab, which could be enhanced by addition of exogenous IL-21 (Fig. 5E). By contrast, IgG was barely detectable in culture supernatants when MRL<sup>lpr</sup> IL-21R<sup>-/-</sup> B cells were stimulated under any of these conditions (Fig. 5E). Collectively, these data demonstrated that IL-21R plays a critical role in regulating the spontaneous B cell activation and autoantibody production in MRL<sup>lpr</sup> mice.

**IL-21R is required for the development of T<sub>fh</sub> cells responses in MRL<sup>lpr</sup> mice**

IL-21 can also induce proliferation and differentiation of CD4<sup>+</sup> T cells. Notably, CD4<sup>+</sup> T cells are required for the development of lupus nephritis and autoantibody production in MRL<sup>lpr</sup> mice (5, 6). Moreover, accumulation of activated CD4<sup>+</sup> T cells, including IFN-γ-producing CD4<sup>+</sup> T cells and T<sub>fh</sub> cells that produce IL-21, is a prominent feature in MRL<sup>lpr</sup> mice (10, 13). We next assessed CD4<sup>+</sup> T cell activation in MRL<sup>lpr</sup> mice lacking IL-21R by first examining cell surface levels of CD44 and CD62L. Approximately 75% of CD4<sup>+</sup> T cells in MRL<sup>lpr</sup> IL-21R<sup>-/-</sup> mice appeared activated, bearing a CD44<sup>hi</sup> CD62L<sup>lo</sup> cell surface phenotype (Fig. 6A). By contrast, 39% of CD4<sup>+</sup> T cells present in spleens obtained from MRL<sup>lpr</sup> IL-21R<sup>-/-</sup> mice expressed a similar phenotype (Fig. 6A). Remarkably, the percentage of activated CD4<sup>+</sup> T cells in control MRL/MpJ mice, which lack the lpr mutation, was indistinguishable from that of MRL<sup>lpr</sup> IL-21R<sup>-/-</sup> mice (Fig. 6A). Absolute numbers of CD4<sup>+</sup> CD62L<sup>lo</sup> CD4<sup>+</sup> T cells were also significantly reduced in MRL<sup>lpr</sup> IL-21R<sup>-/-</sup> mice compared with MRL<sup>lpr</sup> IL-21R<sup>-/-</sup> mice and reached values similar to those observed in MRL/MpJ mice, indicating that activated CD4<sup>+</sup> T cells also accumulate in MRL<sup>lpr</sup> mice via an IL-21R-dependent process (Fig. 6A, 6B).

Cytokine production by Th1, Th2, and Th17 CD4<sup>+</sup> Th subsets has also been implicated in the development of pathologic features in MRL<sup>lpr</sup> mice (56, 57). To determine whether loss of IL-21R signaling in MRL<sup>lpr</sup> mice may impact cytokine production by CD4<sup>+</sup> T cells, we examined the ex vivo cytokine profiles of splenic CD4<sup>+</sup> T cells. Indeed, IFN-γ–producing CD4<sup>+</sup> T cells constituted nearly 50% of total CD4<sup>+</sup> cells in MRL<sup>lpr</sup> mice, and this population was reduced by 50% in spleens obtained from MRL<sup>lpr</sup> IL-21R<sup>-/-</sup> mice (Fig. 6C, 6D). IL-17 and IL-4 were produced by <5% of total CD4<sup>+</sup> T cells in MRL<sup>lpr</sup> IL-21R<sup>-/-</sup> and MRL<sup>lpr</sup> IL-21R<sup>-/-</sup> mice (Fig. 6C). Interestingly, the percentage of IL-17–producing CD4<sup>+</sup> T cells was increased by 2-fold in MRL<sup>lpr</sup> IL-21R<sup>-/-</sup> mice compared with MRL<sup>lpr</sup> IL-21R<sup>-/-</sup> mice; however, similar absolute numbers of these cells were present in the two strains as a consequence of CD4<sup>+</sup> T cell accumulation in the MRL<sup>lpr</sup> IL-21R<sup>-/-</sup> mice (Fig. 6C, data not shown). This suggested that Th17 cells in MRL<sup>lpr</sup> mice do not require IL-21R for their formation and that they are not required for disease progression. Consistent with this observation, IL-17 transcripts could not be detected in mRNA isolated from either kidneys or ears from diseased MRL<sup>lpr</sup> IL-21R<sup>-/-</sup> or MRL<sup>lpr</sup> IL-21R<sup>-/-</sup> mice (data not shown). Moreover, CD4<sup>+</sup> T cells from MRL<sup>lpr</sup> IL-21R<sup>-/-</sup> spleens also produced IL-17 under Th17-skewing conditions (Fig. 6E). The percentage of IL-4–producing CD4<sup>+</sup> T cells was reduced by 50% in MRL<sup>lpr</sup> IL-21R<sup>-/-</sup> mice compared with MRL<sup>lpr</sup> IL-21R<sup>-/-</sup> mice (Fig. 5C). Collectively, these data indicated that IL-21R supports the accumulation of IFN-γ and IL-4–producing Th subsets in MRL<sup>lpr</sup> mice.

We next quantified the numbers of T<sub>fh</sub> and T<sub>het</sub> cells present in MRL<sup>lpr</sup> IL-21R<sup>-/-</sup> and MRL<sup>lpr</sup> IL-21R<sup>+</sup> mice. T<sub>fh</sub> and T<sub>het</sub> cells are CD4<sup>+</sup> T effector subsets that are specialized for promoting GC-dependent Ab responses and extrafollicular Ab responses, respectively. The relative contribution of T<sub>fh</sub> and T<sub>het</sub> cells to the autoantibody production observed in MRL<sup>lpr</sup> mice remains controversial, with T<sub>fh</sub> cells reported to be the predominant inducer of Ab production in MRL<sup>lpr</sup> mice (10). However, the presence of GC B cells in MRL<sup>lpr</sup> mice in our study suggested that T<sub>fh</sub> CD4<sup>+</sup> T cells may also contribute (Fig. 4B, 4E). T<sub>fh</sub> and T<sub>het</sub> CD4<sup>+</sup> T cells can be identified by their cell surface phenotypes. Both populations belong to a subset of activated CD4<sup>+</sup> T cells that bear a CD4<sup>+</sup> TCR<sup>B</sup> B220<sup>+</sup> CD44<sup>hi</sup> CD62L<sup>lo</sup> PD1<sup>+</sup> and PSGL1<sup>lo</sup> phenotype (10, 30). We observed that MRL<sup>lpr</sup> mice had an increased percentage of CD4<sup>+</sup> T cells bearing a CD62L<sup>lo</sup> CD44<sup>hi</sup> PSGL1<sup>lo</sup> phenotype compared with MRL/MpJ and MRL<sup>lpr</sup> IL-21R<sup>-/-</sup> mice, which suggested that IL-21R may contribute to an increased representation of T<sub>fh</sub> cells in MRL<sup>lpr</sup> mice (Fig. 7A). We then assessed the numbers of T<sub>fh</sub> and T<sub>het</sub> cells based on their unique patterns of chemokine receptor expression, because T<sub>fh</sub> cells bear a CXCR5<sup>+</sup> CXCR4<sup>-</sup> cell surface phenotype, and T<sub>het</sub> cells express CXCR5<sup>-</sup> CXCR4<sup>+</sup> (10). Indeed, MRL<sup>lpr</sup> mice contained a population of T<sub>fh</sub> cells that constituted roughly 0.75% of CD4<sup>+</sup> T cells (Fig. 7B, 7C). Notably, this percentage did not differ between strains (Fig. 7B, 7C). Nevertheless, the absolute number of T<sub>fh</sub> cells was significantly reduced in MRL<sup>lpr</sup> mice, consistent with the profound reduction in GC B cells seen by flow cytometry- and immunohistochemical-based analyses (Figs. 4A, 4E, 7D). This result suggested that the accumulation of T<sub>fh</sub> cells in MRL<sup>lpr</sup> mice is also IL-21R dependent (Fig. 7D). In agreement with previous reports, T<sub>het</sub> cells constituted a much larger percentage of total CD4<sup>+</sup> T cells in MRL<sup>lpr</sup> mice: ~2.5% versus 0.75% of CD4<sup>+</sup> T cells were T<sub>het</sub> and T<sub>fh</sub> cells, respectively (Fig. 7E).

Strikingly, MRL<sup>lpr</sup> IL-21R<sup>-/-</sup> mice exhibited a 5-fold reduction in the percentage of CD4<sup>+</sup> T cells bearing a T<sub>het</sub> phenotype (Fig. 7E). Moreover, the absolute number of T<sub>het</sub> cells was reduced by >17-fold in MRL<sup>lpr</sup> IL-21R<sup>-/-</sup> mice compared with MRL<sup>lpr</sup> IL-21R<sup>-/-</sup> mice and by 2-fold compared with MRL/MpJ.
mice (Fig. 7F). Thus, T_{het} cell accumulation in MRL^{lpr} mice is IL-21R dependent.

Discussion
Our studies highlighted the novel finding that IL-21R is critical for the characteristic lymphoaccumulation and activation observed in MRL^{lpr} mice. Specifically, we demonstrated that spontaneous GC formation and plasma cell accumulation are completely abrogated in MRL^{lpr} mice. Consequently, total Ig levels and serum autoantibody titers are significantly reduced. We also showed that accumulation of activated CD4^{+} T cells is dependent on IL-21R. T_{het} cells were reported to be the primary inducers of Ab production in
MRL<sup>lpr</sup> mice. Our studies highlighted that this unique Th population requires IL-21R for its development. Consistent with the pleiotropic abilities described for IL-21, our studies highlighted that IL-21R–derived signals are vital for activation of multiple disease-associated effector pathways in MRL<sup>lpr</sup> mice.

We previously showed that neutralizing the activity of IL-21 by therapeutic administration of IL-21R:Fc abrogated renal and serologic disease manifestations in MRL<sup>lpr</sup> mice (43). In this study, we extended these observations by demonstrating that congenital deficiency of IL-21R similarly limited the development of renal pathology and autoantibody production, firmly establishing the IL-21/IL-21R pair as key inducer of the pathologic features observed in MRL<sup>lpr</sup> mice. Notably, the primary source of IL-21R is reported to be T<sub>hf</sub> cells; however, the factors regulating the development and maintenance of this population remain poorly understood (10). Our results demonstrated that T<sub>hf</sub> cells require IL-21R for their accumulation in MRL<sup>lpr</sup> mice. Cognate interactions with Ag-specific B cells are required for the accumulation of T<sub>hf</sub> cells, and an analogous process may support T<sub>hf</sub> cells in this model (58). Indeed, B cells promote accumulation of activated CD4<sup>+</sup> T cells in MRL<sup>lpr</sup> mice; thus, the accumulation of T<sub>hf</sub> cells seen in MRL<sup>lpr</sup> mice may occur indirectly via the effects of IL-21 on B cells (7). Alternatively, a positive feedback loop may promote the development of these cells, as was reported for T<sub>hf</sub> cells (34, 35).

T<sub>hf</sub> cells require ICOS for their development, because ICOS-deficient MRL<sup>lpr</sup> mice fail to accumulate T<sub>hf</sub> cells, similar to MRL<sup>lpr</sup>/IL-21R<sup>−/−</sup> mice (10). Disease development in MRL<sup>lpr</sup>/ICOS<sup>−/−</sup> mice was reduced, but it exhibited notable differences from that seen in MRL<sup>lpr</sup>/IL-21R<sup>−/−</sup> mice (11, 12). Proteinuria and cutaneous lesions were unaffected in the absence of ICOS; additionally, accumulation of B cells, DN T cells, and activated CD4<sup>+</sup> T cells still occurred (11, 12). This suggested that T<sub>hf</sub> cells are not the sole source of IL-21 in this model. We did identify a small population of T<sub>hf</sub> cells in MRL<sup>lpr</sup> mice, which was not observed by investigators examining MRL<sup>lpr</sup>/ICOS<sup>−/−</sup> mice; this may have resulted from environmental variation between laboratories and/or differences in the sensitivities of the staining protocols used (10). Notably, disease features were maintained in the absence of T<sub>hf</sub> and T<sub>hf</sub> cells in MRL<sup>lpr</sup>/ICOS<sup>−/−</sup> mice, suggesting that IL-21 derived from alternative cellular sources, including the Th1, Th2, and Th17 populations present in MRL<sup>lpr</sup> mice, may support lymphocyte accumulation, development of proteinuria, and skin lesions. Further studies to identify potential cellular sources of IL-21 will be dependent on the generation of IL-21 reporter animals or validated intracellular staining reagents.

Expression of IL-21R is increased in the kidneys of MRL<sup>lpr</sup> mice, and immunohistochemical staining revealed IL-21R expression on a subset of cells in the perivascular infiltrates. By flow cytometric analysis, we determined that CD19<sup>+</sup> B cells made up a small fraction of intrarenal leukocytes that expressed the highest levels of IL-21R, possibly explaining the limited distribution of IL-21R staining observed by immunohistochemistry. Nevertheless, all intrarenal lymphocyte populations examined, which also included CD4<sup>+</sup>, CD8<sup>+</sup>, and DN T cells, expressed IL-21R at varying levels. These observations suggested that IL-21 may promote pathology locally via effects on renal infiltrates. Notably, activated CD4<sup>+</sup> T cells accumulate in the kidneys of MRL<sup>lpr</sup> mice, and a subset of these cells bear a Th5-like phenotype and, thus, may function as a local source of IL-21 (11, 59). We observed that CD4<sup>+</sup> T cells made up the predominant infiltrating cell type in kidneys of MRL<sup>lpr</sup> mice, and, notably, IL-21R was critical for their pathologic accumulation. Using quantitative RT-PCR analysis, we were unable to detect IL-21 message in kidneys obtained from MRL<sup>lpr</sup> mice. However, this is not necessarily indicative of local IL-21 protein levels, because secretion of IL-21 by T<sub>hf</sub> cells that accumulate in secondary lymphoid organs might also lead to elevated circulating levels of cytokine (10).

Autoantibodies to nuclear Ags, particularly those reactive with dsDNA, are thought to play a primary role in the pathogenesis of SLE (60, 61). In MRL<sup>lpr</sup> mice, these autoantibodies are often somatically mutated and affinity matured (62–64). Recent studies identified autoreactive B cells at extralymphocytic sites that are somatically mutated and clonally related, suggesting that autoantibody responses can also develop via an extralymphocytic pathway in MRL<sup>lpr</sup> mice (9, 65). We showed that spontaneous GC formation and plasma cell accumulation requires IL-21R in MRL<sup>lpr</sup> mice. Consistent with this observation, early extralymphocytic Ab-secreting cell responses and GC formation are impaired in IL-21R/IL-21–deficient animals following immunization with T-dependent Ags (24, 25, 28–30). By contrast, the spontaneous formation of GCs in the lupus-prone sanroque model occurs in an IL-21R–independent manner, thus indicating that both IL-21-dependent and -independent mechanisms can regulate GC activity (66). GC B cells were shown to express IL-21R, thus suggesting that the profound loss of GC B cells in MRL<sup>lpr</sup>/IL-21R<sup>−/−</sup> mice is mediated via direct effects of IL-21 on these cells (31). Interestingly, immunization of IL-21R–deficient animals with the T-dependent Ag NP-CGG resulted in impaired GC formation, as well as accelerated memory B cell accumulation (25). Moreover, it is noteworthy that the affinity of the Abs produced by post-GC B cells was significantly reduced in IL-21R<sup>−/−</sup> mice immunized with a T-dependent Ag (25). Therefore, it seems possible that loss of IL-21R signaling in
MRL<sup>lpr</sup> mice may not only impact the spontaneous accumulation of GC B cells, it may also restrict the quality of GC-derived class-switched autoantibodies, which would have important implications for therapeutic blockade of IL-21/IL-21R in B cell-mediated autoimmune diseases. Whether IL-21R could function similarly in the extrafollicular autoantibody response observed in MRL<sup>lpr</sup> mice remains to be determined.

Activated CD44<sup>+</sup> CD62L<sup>lo</sup> CD4<sup>+</sup> T cells accumulated in an IL-21R–dependent manner in MRL<sup>lpr</sup> mice. Previous studies showed that activated CD4<sup>+</sup> T cells express elevated levels of IL-21R, and in vitro IL-21 provides a comitogenic stimulus for anti-CD3–treated T cells (23). In addition, treatment of NOD mice with IL-21 resulted in enhanced division and accumulation of CD4<sup>+</sup> CD44<sup>+</sup> T cells (67). These observations suggested that IL-21 may

**FIGURE 6.** IFN-γ–producing CD4<sup>+</sup> T cells are reduced in MRL<sup>lpr</sup> IL-21R<sup>−/−</sup> mice. A, Cell surface expression of CD44 and CD62L on CD4<sup>+</sup> B220<sup>−</sup> splenocytes from MRL/MpJ (left panel), MRL<sup>lpr</sup> IL-21R<sup>+/+</sup> (middle panel), and MRL<sup>lpr</sup> IL-21R<sup>−/−</sup> (right panel) mice. B, Absolute numbers of CD4<sup>+</sup> B220<sup>−</sup> splenocytes that are CD44<sup>hi</sup> CD62<sup>lo</sup> from MRL/MpJ (MpJ), MRL<sup>lpr</sup> IL-21R<sup>+/+</sup> (WT), and MRL<sup>lpr</sup> IL-21R<sup>−/−</sup> (KO) mice. Symbols represent individual mice. Lines show the mean ± SEM. **p = 0.0003, Mann–Whitney U test. C, Expression levels of IFN-γ, IL-17A, and IL-4 by CD4<sup>+</sup> T cells stimulated directly ex vivo. D, Percentage of CD4<sup>+</sup> splenocytes that express IFN-γ following ex vivo stimulation of splenocytes obtained from MRL<sup>lpr</sup> IL-21R<sup>+/+</sup> and MRL<sup>lpr</sup> IL-21R<sup>−/−</sup> mice. Bars show the mean value obtained from four mice/group, and error bars indicate the SEM. *p = 0.02, Mann–Whitney U test. E, Expression levels of IL-17 by CD4<sup>+</sup> T cells from MRL<sup>lpr</sup> IL-21R<sup>+/+</sup> (left panels) or MRL<sup>lpr</sup> IL-21R<sup>−/−</sup> (right panels) mice stimulated under Th0-skewing (bottom row) or Th17-skewing (top row) conditions. Percentage of cells falling in the indicated gate is shown in C and E.
mediate accumulation of activated CD4+ T cells in MRL\textsuperscript{lpr} mice by promoting proliferation. IL-21 was reported to impact the differentiation of Th1 and Th17 cells (32, 38, 68, 69). Consistent with these observations, we found that IL-21R–deficient MRL\textsuperscript{lpr} mice had fewer IFN-γ–producing T cells. Elevated levels of plasma IFN-γ correlate with disease activity in both SLE patients and murine models of lupus (70–73). In addition, MRL\textsuperscript{lpr} mice deficient in IFN-γ have delayed mortality, reduced anti-dsDNA Ab titers, and decreased severity of lymphadenopathy and glomerulonephritis (15, 16). This suggests that the decreased renal pathology observed in MRL\textsuperscript{lpr}.IL-21R$^{-/-}$ mice may also result from impaired generation of the Th1 response. IL-21 was also demonstrated as an autocrine growth factor for Th17 cells; however, the role of IL-17 in the pathogenesis of SLE remains unclear. Serum levels of IL-17 are elevated in SLE patients, and an increased frequency of IL-17–producing Th cells in SLE patients has been reported (74–77). In addition, MRL\textsuperscript{lpr} mice deficient in IFN-γ have delayed mortality, reduced anti-dsDNA Ab titers, and decreased severity of lymphadenopathy and glomerulonephritis (15, 16). This suggests that the decreased renal pathology observed in MRL\textsuperscript{lpr}.IL-21R$^{-/-}$ mice may also result from impaired generation of the Th1 response.

IL-21 was also demonstrated as an autocrine growth factor for Th17 cells; however, the role of IL-17 in the pathogenesis of SLE remains unclear. Serum levels of IL-17 are elevated in SLE patients, and an increased frequency of IL-17–producing Th cells in SLE patients has been reported (74–77). Similarly, in the recombinant inbred autoimmune-prone BXD2 strain of mice, it was noted that Th17 cells are present in elevated numbers and that IL-17R deficiency resulted in a significant reduction in autoantibody titers (37). Interestingly, CD4+ T cells from MRL\textsuperscript{lpr} mice also produce IL-17, and the development of renal pathology is IL-23R dependent, suggesting that IL-17 may be required for the development of lupus nephritis in C57BL/6\textsuperscript{acr} mice (78, 79). Surprisingly, we observed a small population of IL-17–producing CD4+ T cells in WT MRL\textsuperscript{lpr} mice that appeared increased in the IL-21R–deficient background. This result indicated that Th17 cell formation is not dependent on IL-21R in MRL\textsuperscript{lpr} mice, which was further confirmed by the observation that MRL\textsuperscript{lpr}.IL-21R$^{-/-}$ splenic T cells could differentiate into IL-17–producing cells in vitro when stimulated under Th17-skewing culture conditions. IL-17 transcripts were undetectable in diseased kidneys and skin from MRL\textsuperscript{lpr} mice (data not shown). Similarly, Bubier et al. (42) recently reported that neither IL-17–producing T cells nor IL-17 transcripts could be detected in kidneys of diseased lupus-prone BSXB-Yaa mice, suggesting that IL-17 is not required for the development of SLE-like disease features in these particular models.

In summary, we showed that the IL-21 pathway drives systemic autoimmunity in the MRL\textsuperscript{acr} model of SLE by promoting both pathogenic B and T cell responses in these mice. Only a single new therapy has been approved by the U.S. Food and Drug Administration in the past 47 y, which is due, in part, to the clinical heterogeneity of the disease. B cells are thought to play...
a central role in the pathogenesis of SLE, and many recently developed experimental therapeutics have focused on either eliminating or limiting the activity of these cells (80). However, T cells are also major contributors to the disease processes via B cell-dependent and -independent pathways (81). The profound dampening of lymphocyte effector activation by inhibition of IL-21 signaling suggests that blockade of the IL-21 pathways may be a promising strategy for the treatment of B and T cell-mediated autoimmune diseases, such as SLE.

Acknowledgments
We thank Dr. Terrie Cunlin-Beamer and Kim Muzzi for assistance with backcrossing and breeding IL-21R−/− and IL-21 WT MRLpr mice and Jameel Syed for expert technical assistance.

Disclosures

References
53. Elsaesser, H., K. Sauer, and D. G. Brooks. 2009. IL-21 is required to control
52. Frohlich, A., J. Kisielow, I. Schmitz, S. Freigang, A. T. Shamshiev, J. Weber,
51. Vlahakos, D. V., M. H. Foster, A. A. Ucci, K. J. Barrett, S. K. Datta, and
46. Vlahakos, D. V., M. H. Foster, S. Adams, M. Katz, A. A. Ucci, K. J. Barrett,
49. Merino, R., L. Fossati, M. Iwamoto, S. Takahashi, R. Lemoine, N. Ibnou-Zekri,
43. Herber, D., T. P. Brown, S. Liang, D. A. Young, M. Collins, and K. Dunussi-
lymphokine that modulates B, T, and natural killer cell responses. J. Allergy Clin.
112: 1033–1045.
4704.
43. Herber, D., T. P. Brown, S. Liang, D. A. Young, M. Collins, and K. Dunussi-
Supplementary Figure 1. Decreased IgG titers in MRL<sup>ipr</sup>,IL21R<sup>−/−</sup> mice. The graphs show the circulating levels of IgG2a (left graph), IgG2b (middle graph) and IgG3 (right graph) antibody present in serum obtained from WT (black bars) and KO (white bars) mice at 6, 12 and 20 weeks of age. Bars indicate the mean for 10 mice per group and error bars indicate the SEM. p-values were calculated using the Student t test.
**Supplementary Figure 2. Decreased anti-dsDNA IgG titers in MRL*pr,IL21R* KO mice.** The graphs show the circulating levels of IgG2a (left graph), IgG2b (middle graph) and IgG3 (right graph) anti-dsDNA antibodies in serum obtained from WT (black circles) and KO (white squares) mice at 6, 12, and 20 weeks of age. Bars indicate the mean of 10 mice per group. p-values were calculated using the Student t test.