

Basophils and the T helper 2 environment can promote the development of lupus nephritis

Nicolas Charles¹, Donna Hardwick², Eric Daugas³, Gabor G Illei⁴ & Juan Rivera¹

In systemic lupus erythematosus (SLE), self-reactive antibodies can target the kidney (lupus nephritis), leading to functional failure and possible mortality. We report that activation of basophils by autoreactive IgE causes their homing to lymph nodes, promoting T helper type 2 (T_H2) cell differentiation and enhancing the production of self-reactive antibodies that cause lupus-like nephritis in mice lacking the Src family protein tyrosine kinase Lyn (*Lyn*^{-/-} mice). Individuals with SLE also have elevated serum IgE, self-reactive IgEs and activated basophils that express CD62 ligand (CD62L) and the major histocompatibility complex (MHC) class II molecule human leukocyte antigen-DR (HLA-DR), parameters that are associated with increased disease activity and active lupus nephritis. Basophils were also present in the lymph nodes and spleen of subjects with SLE. Thus, in *Lyn*^{-/-} mice, basophils and IgE autoantibodies amplify autoantibody production that leads to lupus nephritis, and in individuals with SLE IgE autoantibodies and activated basophils are factors associated with disease activity and nephritis.

SLE is a complex disease affecting various organs and may result in death when kidney damage (lupus nephritis) is severe^{1,2}. Lupus nephritis is characterized by IgM-, IgG- and IgA-containing immune complexes deposited in the glomeruli. These immune complexes are formed by autoantibodies with specificity to nuclear components (antinuclear antibodies (ANA)) or to nucleic acids (such as double-stranded DNA (dsDNA)). However, little is known about how B cells are activated in SLE and thus, increased understanding of this process may uncover new therapeutic strategies.

Although there is considerable evidence for the role of T_H1, T_H17 and regulatory T cells in SLE^{3–10}, several studies suggest a possible T_H2 contribution^{11–13}. Given that SLE is a disease with a strong humoral response^{14,15}, it seems reasonable that SLE may have a T_H2 component, as increases in IgE concentration and the presence of autoreactive IgE in the sera of some people with SLE, without any associated increased atopy or allergy, have been reported¹⁶. Nonetheless, there is considerable uncertainty as to whether T_H2 cytokines (such as interleukin-4 (IL-4)) and IgE contribute to SLE and what cell type might be responsible for such contribution.

We and others^{17–19} have previously reported that *Lyn*^{-/-} mice develop a strong and constitutive T_H2 skewing in early life and show exacerbated responses to T_H2 challenges. In late life, *Lyn*^{-/-} mice develop an autoimmune disease that mimics some of the features of human SLE^{20–22}. *Lyn*^{-/-} mice have circulating autoantibodies to dsDNA and ANA. Glomerular deposition of circulating immune complexes (CICs) in these mice results in kidney damage and ultimately in death. Notably, a genetic association of *LYN* with SLE, in a European-American population, was recently reported²³.

Additionally, B cells from some individuals with SLE express reduced levels of Lyn kinase²⁴. Thus, *Lyn*^{-/-} mice provide a reasonable model to explore the influence of a T_H2 environment on the development of lupus-like nephritis.

We explored whether the T_H2 skewing of *Lyn*^{-/-} mice has a role in the development of late-life lupus-like nephritis and whether similar characteristics might be seen in people with SLE. We found that the T_H2 phenotype is a contributory factor in the development of lupus-like nephritis in *Lyn*^{-/-} mice and is also associated with lupus nephritis in human SLE. Of note, the findings identify basophils and self-reactive IgE as key components in the development of autoantibody-mediated kidney disease.

RESULTS

IL-4- and IgE-dependent lupus-like nephritis in *Lyn*^{-/-} mice

Consistent with our previous results¹⁸, basophil-dependent T_H2 skewing was still present in aged *Lyn*^{-/-} mice (**Supplementary Fig. 1**) that develop an SLE-like disease. To study the role of the T_H2 environment in the development of the SLE-like phenotype, we used mice deficient in both IgE and Lyn (*Igh*^{-7/-}; *Lyn*^{-/-}), both IL-4 and Lyn (*Il4*^{-/-}; *Lyn*^{-/-}) or both mast cells and Lyn (*Kit*^{W-sh/W-sh}; *Lyn*^{-/-})¹⁸. *Igh*^{-7/-}; *Lyn*^{-/-}, *Il4*^{-/-}; *Lyn*^{-/-} and *Kit*^{W-sh/W-sh}; *Lyn*^{-/-} mice developed a peripheral B cell defect that was comparable to *Lyn*^{-/-} mice and showed high IgM and IgA concentrations in the serum (**Supplementary Figs. 2–6**), indicating that IL-4 and IgE were not involved in these abnormalities. The levels of IgE and IgG in *Igh*^{-7/-}; *Lyn*^{-/-} and *Il4*^{-/-}; *Lyn*^{-/-} mice showed a similar trend to the phenotype reported for *Igh*-7 and *Il4* single-deficient mice^{25,26} and differed from

¹Laboratory of Molecular Immunogenetics, National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS), National Institutes of Health (NIH), Bethesda, Maryland, USA. ²Office of the Clinical Director, NIAMS, NIH, Bethesda, Maryland, USA. ³Institut National de la Santé et de la Recherche Médicale U699, Department of Nephrology, Assistance Publique–Hôpitaux de Paris, Université Paris Diderot, Hôpital Bichat, Paris, France. ⁴Sjogren's Syndrome Clinic, Molecular Physiology and Therapeutics Branch, National Institute of Dental and Craniofacial Research, NIH, Bethesda, Maryland, USA. Correspondence should be addressed to J.R. (juan_rivera@nih.gov).

Received 16 October 2009; accepted 23 April 2010; published online 30 May 2010; doi:10.1038/nm.2159

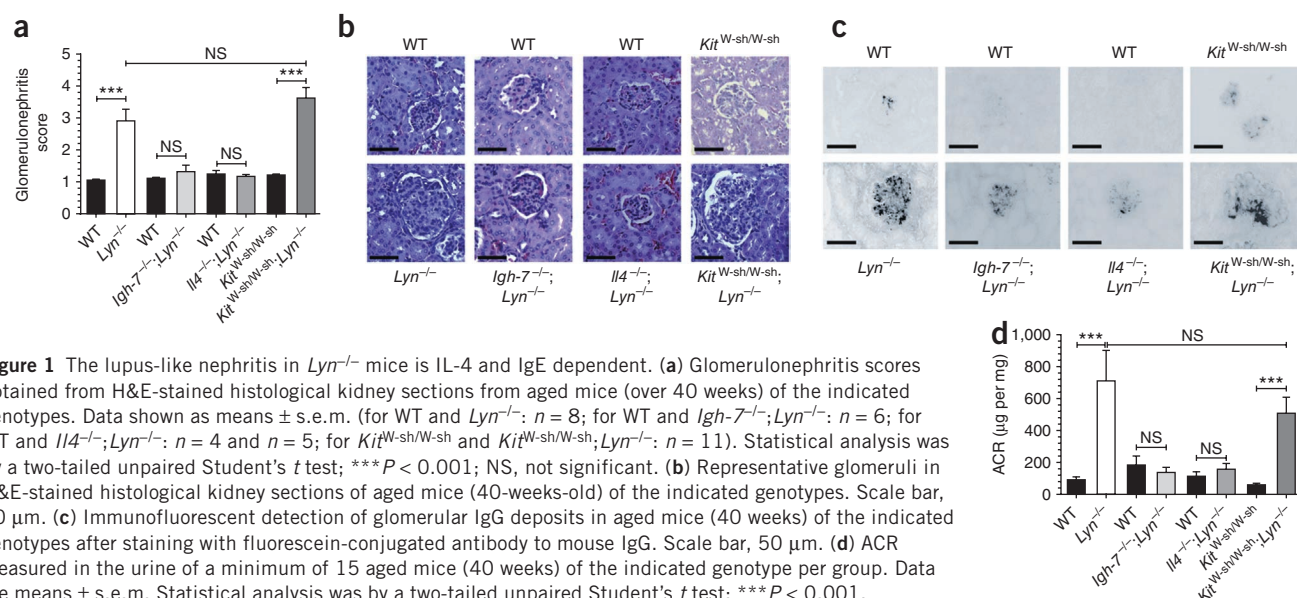


Figure 1 The lupus-like nephritis in *Lyn*^{-/-} mice is IL-4 and IgE dependent. (a) Glomerulonephritis scores obtained from H&E-stained histological kidney sections from aged mice (over 40 weeks) of the indicated genotypes. Data shown as means ± s.e.m. (for WT and *Lyn*^{-/-}: *n* = 8; for WT and *Igh-7*^{-/-}; *Lyn*^{-/-}: *n* = 6; for WT and *Il4*^{-/-}; *Lyn*^{-/-}: *n* = 4 and *n* = 5; for *Kit*^{W-sh/W-sh} and *Kit*^{W-sh/W-sh}; *Lyn*^{-/-}: *n* = 11). Statistical analysis was by a two-tailed unpaired Student's *t* test; ****P* < 0.001; NS, not significant. (b) Representative glomeruli in H&E-stained histological kidney sections of aged mice (40-weeks-old) of the indicated genotypes. Scale bar, 50 μm. (c) Immunofluorescent detection of glomerular IgG deposits in aged mice (40 weeks) of the indicated genotypes after staining with fluorescein-conjugated antibody to mouse IgG. Scale bar, 50 μm. (d) ACR measured in the urine of a minimum of 15 aged mice (40 weeks) of the indicated genotype per group. Data are means ± s.e.m. Statistical analysis was by a two-tailed unpaired Student's *t* test; ****P* < 0.001.

the levels in *Lyn*^{-/-} mice (Supplementary Fig. 5). IgE also contributed to the previously reported¹⁷ increase in mast cell numbers seen in *Lyn*^{-/-} mice (Supplementary Fig. 7), consistent with a role for IgE in mast cell survival^{27,28}. In contrast, the previously described basophilia in *Lyn*^{-/-} mice was independent of both IL-4 and IgE¹⁸ (Supplementary Fig. 7).

Unlike *Lyn*^{-/-} and *Kit*^{W-sh/W-sh}; *Lyn*^{-/-} mice, *Igh-7*^{-/-}; *Lyn*^{-/-} and *Il4*^{-/-}; *Lyn*^{-/-} mice did not develop glomerulonephritis (Fig. 1a,b and Supplementary Fig. 8). Glomerular deposits of CICs containing IgG (Fig. 1c), IgM, IgA and complement factor 3 (C3) (Supplementary Fig. 9a–c) were markedly reduced in the kidneys of *Igh-7*^{-/-}; *Lyn*^{-/-} and *Il4*^{-/-}; *Lyn*^{-/-} mice but were still present in the kidneys of *Kit*^{W-sh/W-sh}; *Lyn*^{-/-} at comparable levels to *Lyn*^{-/-} mice (Fig. 1c and Supplementary Fig. 9a–c). Kidney function (as measured by the albumin-to-creatinine ratio (ACR) in the urine) was rescued in *Igh-7*^{-/-}; *Lyn*^{-/-} and *Il4*^{-/-}; *Lyn*^{-/-} mice, whereas the ACR was elevated to a similar degree in both *Kit*^{W-sh/W-sh}; *Lyn*^{-/-} and *Lyn*^{-/-} mice (Fig. 1d). These findings show that the lupus-like nephritis observed in *Lyn*^{-/-} mice is dependent on IgE and IL-4 but independent of mast cells.

Basophils support autoreactive plasma cells in *Lyn*^{-/-} mice

Aged *Lyn*^{-/-} mice produce large amounts of autoantibodies against dsDNA and nuclear antigens (Fig. 2a,b), which cause the damage seen in the kidney^{29,30}. We explored if the recovery of kidney function in *Igh-7*^{-/-}; *Lyn*^{-/-} and *Il4*^{-/-}; *Lyn*^{-/-} mice was associated with a concomitant decrease in autoantibody production and found a two fold decrease in anti-dsDNA and ANA when compared to *Lyn*^{-/-} and *Kit*^{W-sh/W-sh}; *Lyn*^{-/-} mice (Fig. 2a,b). Depletion of basophils in aged *Lyn*^{-/-} mice (>32 weeks) or in younger *Kit*^{W-sh/W-sh}; *Lyn*^{-/-} mice (~20 weeks) markedly reduced the amount of ANA autoantibodies (Fig. 2c,d). Loss of basophils also decreased the proportion of plasma cells in the spleen (Fig. 2e) and reduced the pro-inflammatory environment in the kidney (Fig. 2f and Supplementary Fig. 10). Collectively, the findings show that basophils support plasma cells in the spleen and amplify the production of autoantibodies in an IL-4 and IgE-dependent manner, leading to kidney disease in *Lyn*^{-/-} mice.

Lyn^{-/-} mice produce basophil-activating self-reactive IgE

Our findings showed that the SLE-like phenotype depends on IgE, thus we investigated whether self-reactive IgE could be found in the circulation of these mice, and whether these activate FcεRI-bearing basophils. Sera from *Lyn*^{-/-} and *Kit*^{W-sh/W-sh}; *Lyn*^{-/-} mice had high levels of dsDNA-specific IgE (Fig. 3a) and ANA-specific IgE (data not shown) as compared to their wild-type (WT) counterparts. The amount of self-reactive IgEs was reduced in *Il4*^{-/-}; *Lyn*^{-/-} mice and, as expected, self-reactive IgEs were not detected in *Igh-7*^{-/-}; *Lyn*^{-/-} mice (Fig. 3a). We purified CICs as previously described³¹ and found IgE-containing CICs (IgE-CICs) in varying amounts in all of the sera from *Lyn*^{-/-} and *Kit*^{W-sh/W-sh}; *Lyn*^{-/-} mice (Fig. 3b and Supplementary Fig. 11a,d), whereas the sera of *Il4*^{-/-}; *Lyn*^{-/-} and *Igh-7*^{-/-}; *Lyn*^{-/-} mice were essentially devoid of IgE-CICs (Fig. 3b and Supplementary Fig. 11d). We observed IgG-containing (Fig. 3b) and IgM (Supplementary Fig. 9) and IgA-containing CICs in all of the mutant strains of mice, but we also observed a marked reduction in these CICs in *Il4*^{-/-}; *Lyn*^{-/-} and *Igh-7*^{-/-}; *Lyn*^{-/-} mice, correlating with the reduced amount of autoantibodies found in these mice (Fig. 3c and Supplementary Fig. 11b,c,e).

We next examined whether IgE or IgG immune complexes could stimulate basophil IL-4 production. Whereas IgE immune complexes were able to induce IL-4 production by basophils, IgG immune complexes failed to stimulate basophil IL-4 production (Fig. 3d and Supplementary Fig. 12a,b). Moreover, basophils from *Lyn*^{-/-} mice showed increased sensitivity to IgE immune complexes as compared to their WT counterparts (Fig. 3d and Supplementary Fig. 12a). Notably, all of the stimuli tested (phorbol 12-myristate 13-acetate plus ionomycin, dinitrophenyl-specific IgE plus dinitrophenyl-HSA (antigen), IgE immune complexes and IgG immune complexes) failed to induce IL-12 p40 or interferon-γ (IFN-γ) production by basophils from WT or *Lyn*^{-/-} mice (Supplementary Fig. 12c,d). These findings demonstrate that the presence of IgE immune complexes (which are present as circulating IgE-CICs in *Lyn*^{-/-} mice) can lead to basophil activation and selective T_H2 cytokine expression.

Lyn^{-/-} basophils express immunoregulatory molecules

We next explored whether basophils can home to the secondary lymphoid tissues of *Lyn*^{-/-} mice, where they might influence

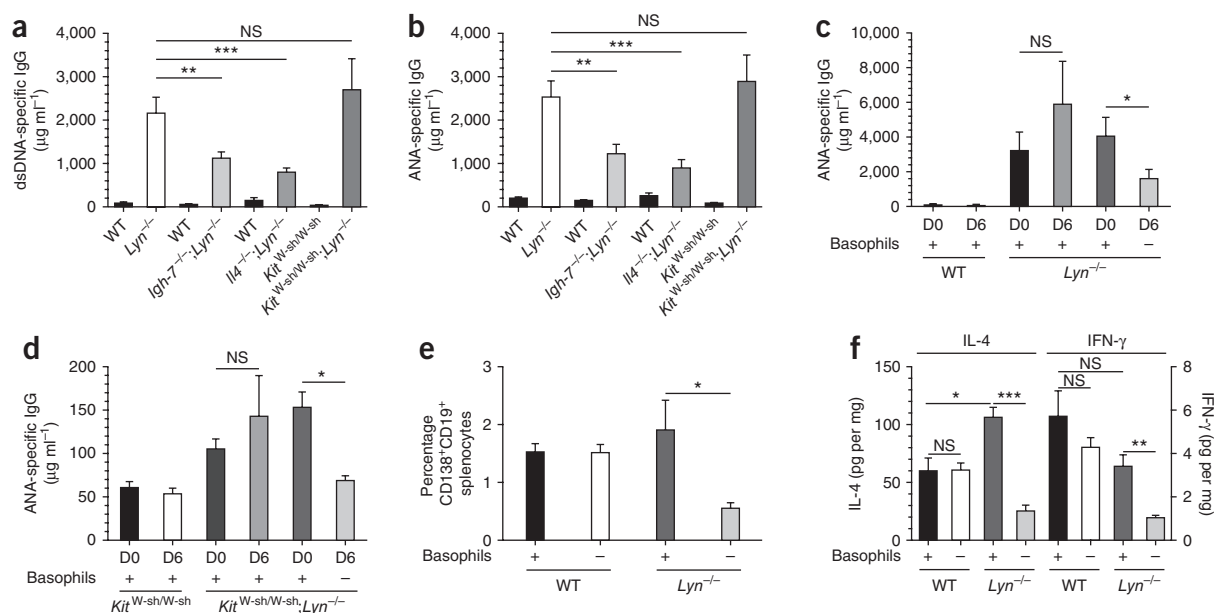


Figure 2 IgE, basophils and IL-4 regulate autoantibody production in *Lyn*^{-/-} mice, and basophils alter the kidney cytokine environment. (a) Quantification of dsDNA-specific IgG in the serum of aged mice (40 weeks) of the indicated genotype. Data are means $\pm$ s.e.m. (at least 15 mice per group). (b) Quantification of ANA-specific IgG in the mice studied in a. (c) Quantification of ANA-specific IgG autoantibodies in the serum of aged mice (32 weeks) of the indicated genotypes before (D0) and six days after (D6) injection of the basophil-depleting antibody MAR-1 (-) or isotype control (+). Data are means $\pm$ s.e.m. (WT: $n = 3$; *Lyn*^{-/-} (+): $n = 4$; *Lyn*^{-/-} (-): $n = 5$). (d) Same quantification as in c for the serum of mice (20-weeks-old) of the indicated genotypes. Data are means $\pm$ s.e.m. (for each group, $n = 3$). (e) Proportion of splenic CD138⁺CD19⁺ plasma cells determined by flow cytometry in mice 6 d after basophil depletion (-) or isotype injection (+). (f) Quantification of IL-4 (left) and IFN- γ (right) in kidney homogenates from 40-week-old WT and *Lyn*^{-/-} mice 6 d after basophil depletion (-) or isotype injection (+). Cytokine amounts were normalized to the total protein content of the respective homogenates. In e and f, data are means $\pm$ s.e.m. (WT and *Lyn*^{-/-}, at least $n = 4$ per group). Statistical analysis was by a two-tailed unpaired (a,b,e,f) or paired (c,d) Student's *t* test; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

B and T cell responses. Circulating basophils from *Lyn*^{-/-} mice showed increased expression of CD62L (L-selectin) (Fig. 4a), which allows for the homing of leukocytes to secondary lymphoid tissues. In the context of *Lyn* deficiency, the absence of IL-4 or IgE (*Il4*^{-/-}; *Lyn*^{-/-} and *Igh*-7^{-/-}; *Lyn*^{-/-} mice), but not of mast cells (*Kit*^{W-sh/W-sh}; *Lyn*^{-/-} mice), inhibited the expression of CD62L on

circulating basophils (Fig. 4b). *Lyn*^{-/-} mice had high numbers of basophils in both the lymph nodes (cervical and inguinal) and spleen (Fig. 4c,d). In the lymph nodes, the proportion of basophils was markedly reduced when IL-4 or IgE were also absent (*Il4*^{-/-}; *Lyn*^{-/-} and *Igh*-7^{-/-}; *Lyn*^{-/-} mice) but not when mast cells were absent (*Kit*^{W-sh/W-sh}; *Lyn*^{-/-} mice) (Fig. 4c). We also observed some

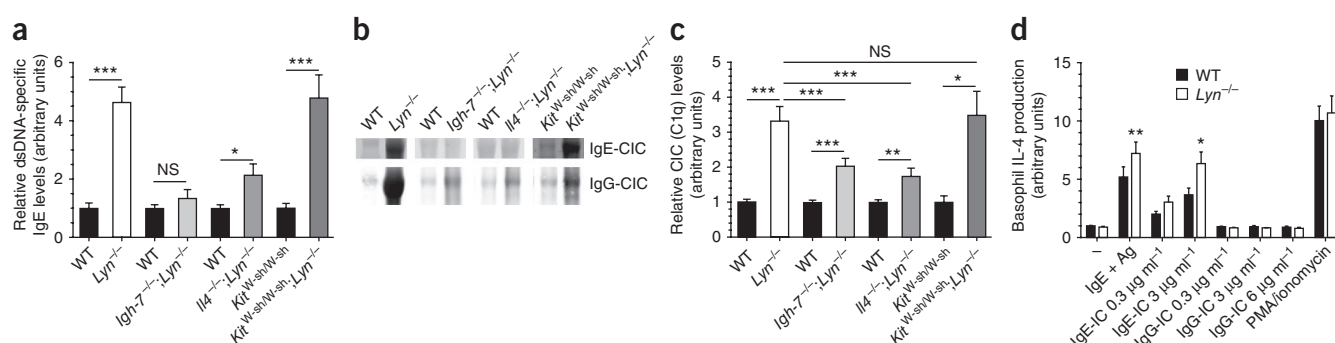


Figure 3 Autoreactive IgEs and IgE-CICs are present in the sera of aged *Lyn*^{-/-} mice. (a) Quantification of dsDNA-specific IgE in the sera of aged mice (40 weeks) of the indicated genotypes, as determined by semiquantitative ELISA. Data are means $\pm$ s.e.m. (more than ten mice per group) normalized to the respective WT control and expressed as arbitrary units. Statistical analysis was by a two-tailed unpaired Student's *t* test; * $P < 0.05$; *** $P < 0.001$. (b) IgE-CICs and IgG-CICs from serum samples of *Igh*-7^{-/-}; *Lyn*^{-/-} and *Il4*^{-/-}; *Lyn*^{-/-} aged mice (>30-weeks-old) are reduced. Western blots were probed with antibody to mouse IgE or antibody to mouse IgG. One representative of at least ten mice per genotype is shown. (c) Serum levels of CICs (IgA, IgM and IgG), as determined by semiquantitative ELISA from at least ten aged mice per genotype on complement factor 1q (C1q)-coated plates. Data are means $\pm$ s.e.m. normalized to levels in WT mice and reported as arbitrary units. Statistical analysis was by a two-tailed unpaired Student's *t* test; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. (d) IL-4 production from (bone marrow-derived) basophils induced by the indicated stimuli. PMA + ionomycin, phorbol 12-myristate 13-acetate (20 nM) plus ionomycin (400 nM). The mean fluorescence intensity (MFI) detected by intracellular staining of IL-4 was normalized to the response of the unstimulated (-) control and expressed as arbitrary units. Data are means $\pm$ s.e.m. ($n = 6$ per condition for three independent experiments). Statistical analysis was by a two-tailed paired Student's *t* test; * $P < 0.05$; ** $P < 0.01$.

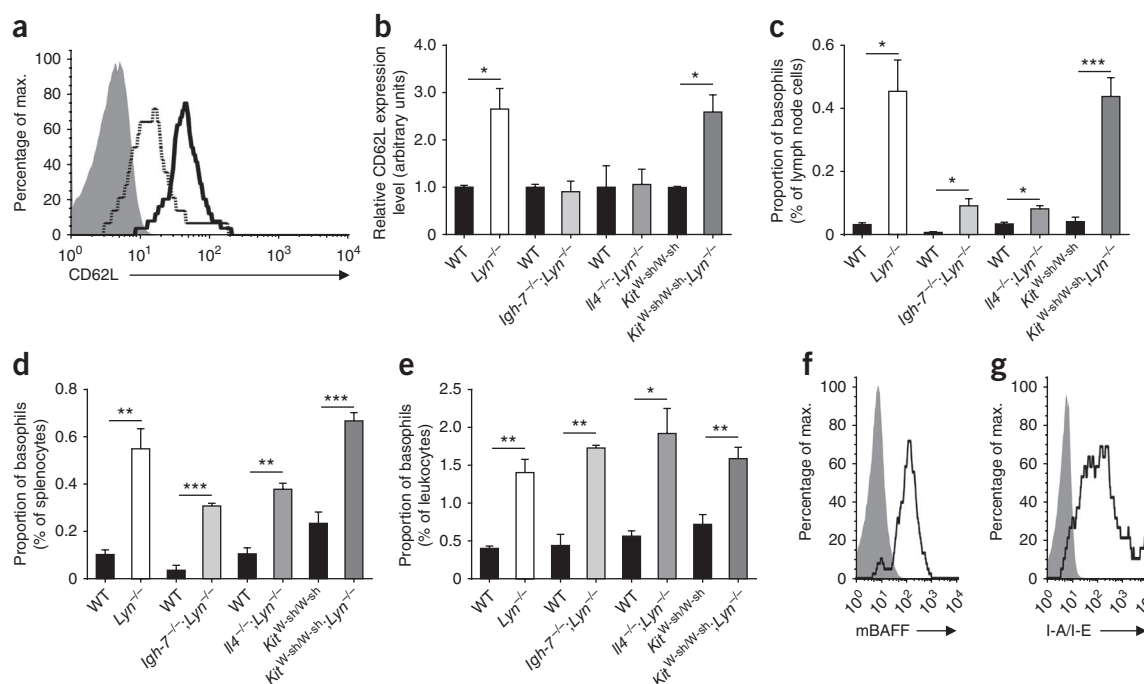


Figure 4 Basophils from aged *Lyn*^{-/-} mice upregulate CD62L expression, home to secondary lymphoid tissues and express membrane BAFF and MHC II. (a) Representative flow cytometric analysis of blood basophil CD62L expression in aged (40 weeks) WT (gray dashed line) and *Lyn*^{-/-} mice (black line) relative to isotype control (gray fill). (b) Pooled data from all experiments performed as in a from aged mice of the indicated genotype. The mean fluorescence intensity (MFI) of CD62L expression on blood basophils was normalized to corresponding WT controls and expressed as means $\pm$ s.e.m. in arbitrary units (WT and *Lyn*^{-/-}: $n = 4$ and $n = 7$; WT and *Igh-7*^{-/-}; *Lyn*^{-/-}: $n = 3$; WT and *I14*^{-/-}; *Lyn*^{-/-}: $n = 3$; *Kit*^{W-sh/W-sh} and *Kit*^{W-sh/W-sh}; *Lyn*^{-/-}: $n = 4$ and $n = 7$). Statistical analysis was by a two-tailed unpaired Student's *t* test; * $P < 0.05$. (c–e) Flow cytometric analysis of basophils (defined as FcεR1⁺CD11b⁺CD49b⁺ cells) in lymph nodes (cervical and inguinal) (c), spleen (d) and blood (e) of the indicated mouse strains relative to the total cell number. (f,g) Representative flow cytometric analysis of basophil membrane BAFF (f) or MHC II (I-A/I-E) (g) expression in the lymph nodes of *Lyn*^{-/-} mice (black line) relative to isotype control (gray fill).

reduction in basophil numbers in the spleen, but it was not as marked as in the lymph nodes (Fig. 4d).

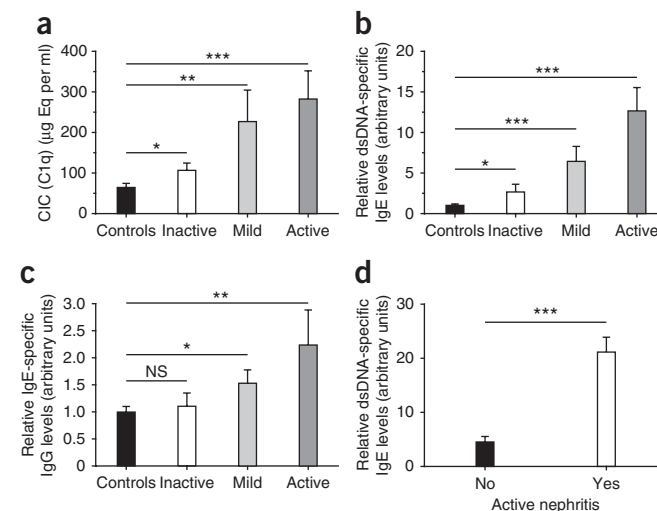
Owing to the basophilia seen in the absence of *Lyn*, there was no significant difference in the proportion of circulating basophils for any of the studied strains (Fig. 4e). We also found that lymph node-resident basophils expressed membrane-associated B cell activating factor belonging to the TNF family (BAFF) (Fig. 4f), which was not accounted for by the low amounts of BAFF receptor expressed on these cells (data not shown), demonstrating the potential of lymph node-resident basophils to influence B cell survival and differentiation. Moreover, both lymph

node- (Fig. 4g) and spleen- (Supplementary Fig. 13) resident basophils from *Lyn*^{-/-} mice showed high MHC II expression. These findings indicate that *Lyn*^{-/-} basophils upregulate CD62L expression and home to the lymph nodes and spleen, where increased expression of MHC II^{32–34}, BAFF or both may allow communication with T and B cells.

Self-reactive IgE is associated with SLE and lupus nephritis

The cohort of subjects with SLE analyzed had large amounts of C1q-reactive CICs that can activate the classical complement pathway (Fig. 5a), a feature previously described for subjects with SLE^{2,30}.

Figure 5 dsDNA-specific IgE and IgE-specific IgG are associated with human SLE disease activity and lupus nephritis. (a) Total CICs in serum from healthy controls ($n = 37$), individuals with inactive SLE (SLEDAI = 0) ($n = 13$), individuals with mild disease (SLEDAI = 2.0 to ≤ 4.0) ($n = 15$) and individuals with active disease (SLEDAI > 4) ($n = 15$), as measured by ELISA. Data are means $\pm$ s.e.m. Statistical analysis was by a two-tailed unpaired Student's *t* test; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. (b) dsDNA-specific IgE levels, as determined by semiquantitative ELISA. dsDNA-coated plates were incubated with sera from healthy controls and subjects with SLE (the same populations as in a). Data are means $\pm$ s.e.m. (same n as in a) normalized to healthy controls. Statistical analysis was by a two-tailed unpaired Student's *t* test; * $P < 0.05$; *** $P < 0.001$. (c) IgE-specific IgG levels, as determined by incubating sera from healthy controls and individuals with SLE on human IgE-coated plates. IgE-specific IgG was detected with antibody to human IgG (Fcγ specific). Data are means $\pm$ s.e.m. (same n as in a) normalized to healthy controls. Statistical analysis was by a two-tailed unpaired Student's *t* test; * $P < 0.05$; ** $P < 0.01$. (d) dsDNA-specific IgE in sera of subjects with SLE classified on the basis of active nephritis (yes, $n = 8$) or not (no, $n = 34$) (see Supplementary Methods). Data are means $\pm$ s.e.m. Statistical analysis was by a two-tailed unpaired Student's *t* test.



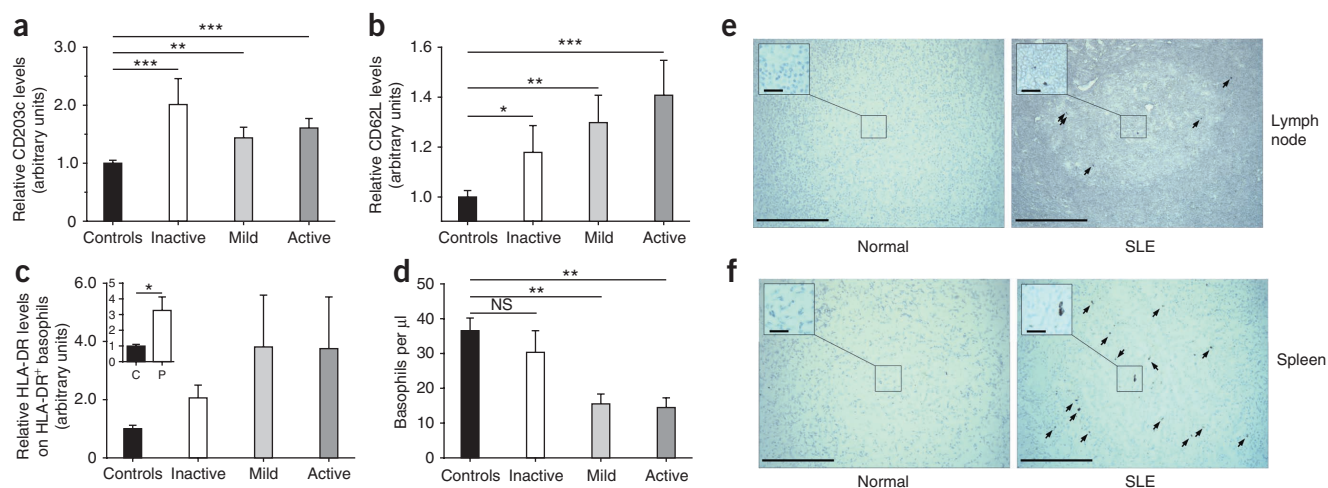


Figure 6 Basophils in individuals with SLE are active, upregulate CD62L and HLA-DR and home to secondary lymphoid organs. **(a)** Flow cytometric analysis of the levels of activated blood basophils (CD203c expression) relative to disease intensity from subjects with inactive, mild or active SLE ($n = 13$, $n = 15$ and $n = 15$, respectively) as defined in the legend for **Figure 5a** compared to controls ($n = 41$). Data are the ratio of CD203c mean fluorescence intensity (MFI) normalized to controls and expressed in arbitrary units. **(b)** Flow cytometric analysis of CD62L expression (MFI) on blood basophils in subject groups as in **a**. Data are normalized as in **a** and are expressed as means $\pm$ s.e.m. in arbitrary units (AU) (healthy controls: $n = 14$; inactive SLE: $n = 4$; moderate SLE: $n = 6$; active SLE: $n = 6$). **(c)** Flow cytometric analysis of relative HLA-DR levels on HLA-DR⁺ blood basophils (MFI) from subjects with SLE (P) compared to healthy controls (C). Data are normalized as in **a** and expressed as means $\pm$ s.e.m. in AU (healthy controls: $n = 13$; SLE patients: inactive/mild/active $n = 4/6/6$). **(d)** Absolute number of blood basophils (healthy controls: $n = 41$; inactive SLE: $n = 13$; moderate SLE: $n = 15$; active SLE: $n = 15$) as determined by flow cytometry. Data are means $\pm$ s.e.m. In **a–d**, statistical analysis was by a two-tailed unpaired Student's *t* test; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. **(e,f)** Immunohistochemistry (with the 2D7 monoclonal antibody) of basophils in the lymph nodes **(e)** or spleen **(f)** of healthy (normal) controls or subjects with SLE ($n = 2$). Basophils were found in the B cell zone of lymph node germinal centers in individuals with SLE only **(e)**. A spleen biopsy from healthy (normal) controls or from an individual with SLE shows the localization of basophils in the germinal centers of subjects with SLE but not normal controls **(f)**. Similar results were obtained with a second basophil-specific antibody (BB1) (data not shown). Original magnification, $\times 20$. Scale bar, 200 μ m. Insets show the boxed area of the larger images. Original magnification, $\times 40$. Scale bars, 25 μ m.

When analyzed relative to disease activity (on the basis of SLE disease activity index (SLEDAI) scores)³⁵, C1q-reactive CICs were strongly elevated in mild (SLEDAI score of 1.0–4.0) and active disease (SLEDAI score of >4.0). Subjects with SLE also had self-reactive IgEs recognizing dsDNA, and the levels of these IgEs were associated with increased disease activity (**Fig. 5b**).

IgGs directed against IgE were also present in the sera of subjects with SLE, with significantly elevated levels in subjects with active disease (**Fig. 5c**). High levels of dsDNA-specific IgEs were associated with active lupus nephritis (**Fig. 5d**). Moreover, subjects with SLE had high total IgE levels, which were associated with disease activity, and showed a modest to strong IgG1, IgG3 and IgE autoantibody response (**Supplementary Fig. 14a–c**). Thus, individuals with SLE have autoantibodies associated with T_H1- and T_H2-responses, and self-reactive IgEs and IgGs specific for IgE that are associated with increased disease activity and active nephritis.

SLE basophils express HLA-DR and home to lymphoid tissues

To investigate the activation state of basophils in individuals with SLE, we determined the expression of the marker CD203c, as it is upregulated in activated basophils³⁶. All subjects with SLE showed high CD203c expression relative to healthy controls, indicating that their basophils are activated (**Fig. 6a**). Expression of CD62L (**Fig. 6b**) and HLA-DR (**Fig. 6c**) was also elevated on SLE basophils, and were associated with increased disease activity.

The absolute numbers of basophils in the circulation was decreased in subjects with SLE (**Fig. 6d**). Although this decrease was associated with immunosuppressive treatment (**Supplementary Fig. 15**), immunosuppressive treatment had no effect on the activation state of basophils (as indicated by the presence of HLA-DR). Of note, we

found basophils in the lymph nodes and spleen of the two subjects tested with SLE, but not in control subjects without SLE (**Fig. 6e,f**). The findings suggest that basophils in individuals with SLE are activated, home to secondary lymphoid organs and express the appropriate molecules for antigen presentation. This is associated with the presence of self-reactive IgE in individuals with SLE.

DISCUSSION

Although SLE has long been considered a B cell disease, self-reactive T cells that promote B cell class switching³⁷, and other cell types such as dendritic cells and macrophages^{38,39}, have also been implicated in the disease, for example, through secretion of factors influencing B cell survival and differentiation, such as BAFF and a proliferation-inducing ligand (APRIL)⁴⁰. Here we demonstrate that basophils are contributors to the production of self-reactive antibodies in SLE. Our findings in *Lyn*^{−/−} mice show that depletion of basophils or the absence of IL-4 or IgE causes a marked reduction in autoantibody production and preserves kidney function. This suggests that without basophils the levels of autoantibodies are insufficient to cause kidney disease. Thus, basophils function to amplify the preexisting loss of B cell tolerance.

Basophils have long been associated with allergy^{41,42}. However, the role of basophils in immunity remains unclear. The recent discoveries that basophils can induce T_H2 cell differentiation *in vivo*^{18,43}, amplify humoral memory responses⁴⁴ and present antigen via MHC II^{32,34,43}, provide evidence of a role for this cell type in regulating T_H2 immunity. In the *Lyn*^{−/−} mouse model, T_H2 skewing is driven by the absence of Lyn kinase in basophils, resulting in upregulation of GATA-3 in these cells and an enhanced production of IL-4 *in vivo*¹⁸. In humans, our preliminary analysis of the amount of Lyn in the basophils of

subjects with SLE did not reveal substantial differences relative to healthy controls (data not shown). However, there is increasing evidence of a role for Lyn kinase in SLE, particularly in populations of European descent^{23,24}. Thus, further studies are required to determine the role of Lyn in human SLE.

Of note is the finding that basophils contribute to the production of autoantibodies that cause lupus-like nephritis in the *Lyn*^{-/-} mice. Activation of these cells caused enhancement of CD62L expression and their accumulation in the lymph nodes of *Lyn*^{-/-} mice and in subjects with SLE. MHC II expression on mouse and human basophils was increased, and in mice we observed expression of membrane-bound BAFF, similar to what has been described in human basophils after engagement of IgD on their cell surface⁴⁵. Depletion of basophils in *Lyn*^{-/-} mice decreased the counts of splenic plasma cells and suppressed autoantibody production, which drives lupus nephritis^{29,30}. Depletion of basophils also reduced the production of IL-1 β , IL-4, IL-6, IL-13 and IFN- γ in the kidney of *Lyn*^{-/-} mice. Thus, a reduction in the proinflammatory milieu in the kidney suggests a possible therapeutic benefit from basophil inactivation or depletion.

Our findings show that IgE immune complexes can activate basophils, and removal of self-reactive IgEs that form functional CICs (by deletion of the *Igh*-7 locus or by eliminating IL-4 production) prevents kidney disease. These IgE-CICs were also associated with lupus nephritis in both *Lyn*^{-/-} mice and human subjects with SLE. Since circulating IgE levels can be reduced by an existing antiallergy drug, omalizumab, an IgE-specific antibody that lowers circulating IgE levels and decreases Fc ϵ RI expression on basophils⁴⁶, this drug may be of therapeutic benefit to patients with SLE with elevated IgE levels. Although in individuals with SLE the association of increased levels of dsDNA-specific IgE with increased disease activity and active lupus nephritis argues for a link between increased T_H2 responses and the development of nephritis, it is clear that T_H1-mediated responses are also found in this population. The presence of increased circulating IgG1 and IgG3 autoantibodies indicates a strong T_H1 component. This suggests that direct modulation of the T_H2 response, through the use of IL-4 and IL-13 receptor antagonists⁴⁷, as a therapeutic strategy could have the unwanted effect of exacerbating disease by shifting toward a T_H1 (or possibly T_H17) phenotype. Nonetheless, as we did not find IgE-CICs in the kidneys of *Lyn*^{-/-} mice (data not shown), it seems that these CICs do not contribute to the kidney pathology *per se* but instead play a part in basophil activation. Thus, the strategy of IgE or basophil depletion may avoid complications associated with altering the T_H1-T_H2 balance.

The view of SLE as a disease with a T_H2 component has been controversial. There is considerable evidence for the involvement of T_H1 and possibly T_H17 cells in SLE^{11,12,48–50}, as well as for the alteration or loss of regulatory T cell activity^{9,51}. Some mouse models where spontaneous genetic mutations or alterations cause lupus-like disease, such as BXS^B and MRL-*Fas*^{lpr} mice, show a T_H1 cytokine IFN- γ -dependent disease. Deletion of the gene encoding IFN- γ in mice with these backgrounds was shown to eliminate disease^{4,49}. Nonetheless, it is less well known that many of the spontaneous mouse models of lupus-like disease have high circulating levels of IgE⁵², suggesting the possibility of a T_H2 component contributing to pathogenesis in these models. Humans with SLE showed both T_H1 and T_H2 responses, and both IgG-CICs and IgE-CICs were associated with increased disease activity. Several studies have suggested that the balance between T_H1 and T_H2 cell responses may determine the phenotype of lupus nephritis^{3,11–13,48}. A strong T_H1 response was shown to be associated with diffuse proliferative lupus nephritis, whereas a dominant T_H2

response was associated with a membranous lupus nephritis^{3,11–13,48}. These observations argue that both T_H1 and T_H2 responses can contribute to lupus nephritis, but the disease may manifest differently depending on the dominance of one or the other response.

Our findings show that basophils and the T_H2 environment influence the production of autoantibodies and that depletion of basophils or deletion of the *Igh*-7 or *Il4* gene, in the context of Lyn deficiency, causes a reduction in the circulating levels of these self-reactive antibodies. In individuals with SLE, self-reactive IgE was associated with active disease and active lupus. Their basophils were active and were found in the secondary lymphoid tissues, of two tested individuals, where they may influence T and B cell function. Thus, our findings suggest the possibility that decreasing the circulating levels of self-reactive IgE or the dampening of basophil activity might have therapeutic benefit in lupus nephritis.

METHODS

Methods and any associated references are available in the online version of the paper at <http://www.nature.com/naturemedicine/>.

Note: Supplementary information is available on the Nature Medicine website.

ACKNOWLEDGMENTS

We thank J. Daruwalla and G. Souto-Adeva (Office of the Clinical Director, NIAMS) for human samples, data input and analysis and H.C. Oettgen (Harvard University) for providing the *Igh*-7^{-/-} mice. We also thank M. Hourseau for human sample preparation and L.B. Schwartz (Virginia Commonwealth University) and A.F. Walls (University of Southampton) for the gift of human basophil-specific monoclonal antibodies. We acknowledge the support of the Laboratory Animal Care and Use Section and the Flow Cytometry Section of the Office of Science and Technology, NIAMS. This research was supported by the intramural programs of NIAMS and National Institute of Dental and Craniofacial Research, NIH.

AUTHOR CONTRIBUTIONS

N.C. and J.R. conceived and directed the project, designed experiments and wrote the manuscript. N.C. conducted experiments. D.H., E.D. and G.G.I. provided SLE patient history, samples and analysis.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

Published online at <http://www.nature.com/naturemedicine/>.

Reprints and permissions information is available online at <http://npg.nature.com/reprintsandpermissions/>.

1. Rahman, A. & Isenberg, D.A. Systemic lupus erythematosus. *N. Engl. J. Med.* **358**, 929–939 (2008).
2. Moser, K.L., Kelly, J.A., Lessard, C.J. & Harley, J.B. Recent insights into the genetic basis of systemic lupus erythematosus. *Genes Immun.* **10**, 373–379 (2009).
3. Masutani, K. *et al.* Predominance of T_H1 immune response in diffuse proliferative lupus nephritis. *Arthritis Rheum.* **44**, 2097–2106 (2001).
4. Balomenos, D., Rumold, R. & Theofilopoulos, A.N. Interferon- γ is required for lupus-like disease and lymphoaccumulation in MRL-lpr mice. *J. Clin. Invest.* **101**, 364–371 (1998).
5. Peng, S.L., Szabo, S.J. & Glimcher, L.H. T-bet regulates IgG class switching and pathogenic autoantibody production. *Proc. Natl. Acad. Sci. USA* **99**, 5545–5550 (2002).
6. Zeng, D., Liu, Y., Sidobre, S., Kronenberg, M. & Strober, S. Activation of natural killer T cells in NZB/W mice induces T_H1-type immune responses exacerbating lupus. *J. Clin. Invest.* **112**, 1211–1222 (2003).
7. Nalbandian, A., Crispin, J.C. & Tsokos, G.C. Interleukin-17 and systemic lupus erythematosus: current concepts. *Clin. Exp. Immunol.* **157**, 209–215 (2009).
8. Pernis, A.B. T_H17 cells in rheumatoid arthritis and systemic lupus erythematosus. *J. Intern. Med.* **265**, 644–652 (2009).
9. Valencia, X., Yarboro, C., Illei, G. & Lipsky, P.E. Deficient CD4⁺CD25^{high} T regulatory cell function in patients with active systemic lupus erythematosus. *J. Immunol.* **178**, 2579–2588 (2007).
10. Zhao, X.F. *et al.* Increased serum interleukin 17 in patients with systemic lupus erythematosus. *Mol. Biol. Rep.* **37**, 81–85 (2010).
11. Akahoshi, M. *et al.* T_H1/T_H2 balance of peripheral T helper cells in systemic lupus erythematosus. *Arthritis Rheum.* **42**, 1644–1648 (1999).

12. Heine, G. *et al.* A shift in the T_H1/T_H2 ratio accompanies the clinical remission of systemic lupus erythematosus in patients with end-stage renal disease. *Nephrol. Dial. Transplant.* **17**, 1790–1794 (2002).
13. Shimizu, S. *et al.* Membranous glomerulonephritis development with T_H2 -type immune deviations in MRL/lpr mice deficient for IL-27 receptor (WSX-1). *J. Immunol.* **175**, 7185–7192 (2005).
14. Tiller, T. *et al.* Autoreactivity in human IgG⁺ memory B cells. *Immunity* **26**, 205–213 (2007).
15. Tsuiji, M. *et al.* A checkpoint for autoreactivity in human IgM⁺ memory B cell development. *J. Exp. Med.* **203**, 393–400 (2006).
16. Atta, A.M., Sousa, C.P., Carvalho, E.M. & Sousa-Atta, M.L. Immunoglobulin E and systemic lupus erythematosus. *Braz. J. Med. Biol. Res.* **37**, 1497–1501 (2004).
17. Odom, S. *et al.* Negative regulation of immunoglobulin E-dependent allergic responses by Lyn kinase. *J. Exp. Med.* **199**, 1491–1502 (2004).
18. Charles, N. *et al.* Lyn kinase controls basophil GATA-3 transcription factor expression and induction of T_H2 cell differentiation. *Immunity* **30**, 533–543 (2009).
19. Beavitt, S.J. *et al.* Lyn-deficient mice develop severe, persistent asthma: Lyn is a critical negative regulator of T_H2 immunity. *J. Immunol.* **175**, 1867–1875 (2005).
20. Hibbs, M.L. *et al.* Multiple defects in the immune system of Lyn-deficient mice, culminating in autoimmune disease. *Cell* **83**, 301–311 (1995).
21. Nishizumi, H. *et al.* Impaired proliferation of peripheral B cells and indication of autoimmune disease in lyn-deficient mice. *Immunity* **3**, 549–560 (1995).
22. Yu, C.C., Yen, T.S., Lowell, C.A. & DeFranco, A.L. Lupus-like kidney disease in mice deficient in the src family tyrosine kinases Lyn and Fyn. *Curr. Biol.* **11**, 34–38 (2001).
23. Lu, R. *et al.* Genetic associations of LYN with systemic lupus erythematosus. *Genes Immun.* **10**, 397–403 (2009).
24. Liossis, S.N. *et al.* B-cell kinase lyn deficiency in patients with systemic lupus erythematosus. *J. Invest. Med.* **49**, 157–165 (2001).
25. Kopf, M. *et al.* Disruption of the murine IL-4 gene blocks T_H2 cytokine responses. *Nature* **362**, 245–248 (1993).
26. Oettgen, H.C. *et al.* Active anaphylaxis in IgE-deficient mice. *Nature* **370**, 367–370 (1994).
27. Asai, K. *et al.* Regulation of mast cell survival by IgE. *Immunity* **14**, 791–800 (2001).
28. Kalesnikoff, J. *et al.* Monomeric IgE stimulates signaling pathways in mast cells that lead to cytokine production and cell survival. *Immunity* **14**, 801–811 (2001).
29. Seshan, S.V. & Jennette, J.C. Renal disease in systemic lupus erythematosus with emphasis on classification of lupus glomerulonephritis: advances and implications. *Arch. Pathol. Lab. Med.* **133**, 233–248 (2009).
30. Sinico, R.A. *et al.* Anti-C1q autoantibodies in lupus nephritis. *Ann. NY Acad. Sci.* **1173**, 47–51 (2009).
31. Toran, E.J. & Lee, C.M. Isolation and analysis of nephritic-producing immune complexes in *Plasmodium berghei*-infected mice. *J. Natl. Med. Assoc.* **87**, 693–699 (1995).
32. Perrigoue, J.G. *et al.* MHC class II-dependent basophil-CD4⁺ T cell interactions promote T_H2 cytokine-dependent immunity. *Nat. Immunol.* **10**, 697–705 (2009).
33. Sokol, C.L. *et al.* Basophils function as antigen-presenting cells for an allergen-induced T helper type 2 response. *Nat. Immunol.* **10**, 713–720 (2009).
34. Yoshimoto, T. *et al.* Basophils contribute to T_H2 -IgE responses *in vivo* via IL-4 production and presentation of peptide-MHC class II complexes to CD4⁺ T cells. *Nat. Immunol.* **10**, 706–712 (2009).
35. American College of Rheumatology Ad Hoc Committee on Systemic Lupus Erythematosus Response Criteria. The American College of Rheumatology response criteria for systemic lupus erythematosus clinical trials: measures of overall disease activity. *Arthritis Rheum.* **50**, 3418–3426 (2004).
36. Hauswirth, A.W. *et al.* Recombinant allergens promote expression of CD203c on basophils in sensitized individuals. *J. Allergy Clin. Immunol.* **110**, 102–109 (2002).
37. Singh, R.R., Ebling, F.M., Sercarz, E.E. & Hahn, B.H. Immune tolerance to autoantibody-derived peptides delays development of autoimmunity in murine lupus. *J. Clin. Invest.* **96**, 2990–2996 (1995).
38. Kyttaris, V.C., Katsiari, C.G., Juang, Y.T. & Tsokos, G.C. New insights into the pathogenesis of systemic lupus erythematosus. *Curr. Rheumatol. Rep.* **7**, 469–475 (2005).
39. Holmdahl, R., Tarkowski, A. & Jonsson, R. Involvement of macrophages and dendritic cells in synovial inflammation of collagen induced arthritis in DBA/1 mice and spontaneous arthritis in MRL/lpr mice. *Autoimmunity* **8**, 271–280 (1991).
40. Levesque, M.C. Translational mini-review series on B cell-directed therapies: recent advances in B cell-directed biological therapies for autoimmune disorders. *Clin. Exp. Immunol.* **157**, 198–208 (2009).
41. Schroeder, J.T. & MacGlashan, D.W. New concepts: the basophil. *J. Allergy Clin. Immunol.* **99**, 429–433 (1997).
42. Mukai, K. *et al.* Basophils play a critical role in the development of IgE-mediated chronic allergic inflammation independently of T cells and mast cells. *Immunity* **23**, 191–202 (2005).
43. Sokol, C.L., Barton, G.M., Farr, A.G. & Medzhitov, R. A mechanism for the initiation of allergen-induced T helper type 2 responses. *Nat. Immunol.* **9**, 310–318 (2008).
44. Denzel, A. *et al.* Basophils enhance immunological memory responses. *Nat. Immunol.* **9**, 733–742 (2008).
45. Chen, K. *et al.* Immunoglobulin D enhances immune surveillance by activating antimicrobial, proinflammatory and B cell-stimulating programs in basophils. *Nat. Immunol.* **10**, 889–898 (2009).
46. Lin, H. *et al.* Omalizumab rapidly decreases nasal allergic response and FcεR1 on basophils. *J. Allergy Clin. Immunol.* **113**, 297–302 (2004).
47. Burmeister Getz, E., Fisher, D.M. & Fuller, R. Human pharmacokinetics/pharmacodynamics of an interleukin-4 and interleukin-13 dual antagonist in asthma. *J. Clin. Pharmacol.* **49**, 1025–1036 (2009).
48. De Carli, M., D'Elia, M.M., Zancuoghi, G., Romagnani, S. & Del Prete, G. Human T_H1 and T_H2 cells: functional properties, regulation of development and role in autoimmunity. *Autoimmunity* **18**, 301–308 (1994).
49. Kono, D.H., Balomenos, D., Park, M.S. & Theofilopoulos, A.N. Development of lupus in BXSB mice is independent of IL-4. *J. Immunol.* **164**, 38–42 (2000).
50. Peng, S.L., Moslehi, J. & Craft, J. Roles of interferon- γ and interleukin-4 in murine lupus. *J. Clin. Invest.* **99**, 1936–1946 (1997).
51. Lee, H.Y. *et al.* Altered frequency and migration capacity of CD4⁺CD25⁺ regulatory T cells in systemic lupus erythematosus. *Rheumatology (Oxford)* **47**, 789–794 (2008).
52. Miyajima, H. *et al.* IgE allotypes in sera of mice with autoimmune diseases and in mice with graft-versus-host disease after transfusion or bone marrow transplantation. *Int. Arch. Allergy Immunol.* **111**, 152–155 (1996).

ONLINE METHODS

Mice. All mice used in the present study were described previously¹⁸. Unless otherwise noted, mice were 32–40-weeks-old and were age-matched for group comparisons. Mice were maintained in specific pathogen-free conditions and used in accordance with NIH guidelines and NIAMS-approved animal study proposal A007-03-01.

Human subjects. Samples were collected from adult subjects enrolled in a long-term natural history study of SLE. The study was approved by the Institutional Review Board of NIAMS. All subjects fulfilled the American College of Rheumatology classification criteria for SLE^{53,54}. Subject characteristics and lupus activity scoring system are shown in **Supplementary Table 1** and in the **Supplementary Methods**. Control samples were obtained from healthy blood donors. All subjects provided written informed consent.

Antibodies and flow cytometry. Dinitrophenyl-specific mouse IgE was produced as previously described⁵⁵. All other antibodies were from commercial sources and are described in **Supplementary Table 2**. Flow cytometry acquisition was done with a FACSCalibur machine (BD Biosciences) as previously described¹⁸. Data analysis was with Flowjo software (Treestar).

In vivo basophil depletion and ex vivo analysis of splenic T cells. *In vivo* basophil depletion and *ex vivo* analysis of splenic T cells (CD4⁺) were previously described¹⁸.

Glomerulonephritis, analysis of glomerular deposition of circulating immune complexes and kidney function. Aged (~40-week-old) mice were killed, and kidneys were removed. One kidney was fixed with 10% buffered formalin (Sigma), embedded in paraffin, sectioned and stained with H&E (American Histolabs). The other kidney was placed in a vinyl mold in optimal cutting temperature medium, and the sample was frozen in liquid nitrogen. Four-micrometer-thick frozen sections were fixed in cold acetone, blocked in PBS containing 1% BSA and stained in the same buffer with the specific fluorescein-conjugated antibodies or isotype controls (see **Supplementary Table 2** for antibodies used).

For assessment of kidney function the albumin/creatinine ratio (ACR) was determined. Urine was collected from at least ten aged mice per genotype and the albumin concentration was measured with a mouse albumin ELISA (Bethyl laboratories). A creatinine assay (R&D systems) was used to determine urine creatinine concentrations. Results are expressed as ACR in μg of albumin per mg of creatinine.

Methods for assessment of glomerulonephritis and cytokine content in the kidney are described in **Supplementary Methods**.

Measurement of autoantibodies, circulating immune complexes and precipitation of circulating immune complexes. Mouse IgG specific for dsDNA, mouse ANA-specific IgG and mouse CICs ((C1q) IgG, IgA and IgM) ELISA kits were from Alpha Diagnostic. The ELISA kit for human circulating immune complexes (C1q-coated plates) was from ALPCO, and the ELISA kit for human IgE was from Mabbiotech. All commercial ELISAs were performed according to the manufacturer's instructions. To measure both human and mouse

dsDNA-specific IgE and dsDNA-specific IgG subclasses, dsDNA-coated plates (Calbiotech) were incubated with serial dilutions of serum in PBS containing 10% FCS (Invitrogen). The corresponding horseradish peroxidase-conjugated secondary antibodies were used (**Supplementary Table 2**). Optical density at 450 nm was measured after tetramethylbenzidine substrate incubation (Invitrogen). Data shown are from 1 in 200 dilution plates (with which the best signal-to-noise ratio was obtained). The same approach was used to measure the amounts of circulating IgE-specific IgGs in subjects and healthy controls, using plates coated with human IgE (Abbiotec) at $2 \mu\text{g ml}^{-1}$ in PBS.

CICs were precipitated from sera of aged mice as described previously³¹. Samples were analyzed by SDS-PAGE followed by western blotting with the indicated antibodies (**Supplementary Table 2**). The LiCor Odyssey System was used to detect signal.

Basophil cultures, basophil detection and measurement of interleukin-4 production. Bone marrow-derived cultured basophils have been previously described¹⁸. At day 9 of culture, cells were washed, resuspended at one million cells per ml in medium containing only IL-3 (Peprotech) and incubated overnight at 37 °C. Cells were then resuspended in the same medium at five million cells per ml and stimulated as indicated in **Figure 3**. For IgE and antigen stimulation, cells were sensitized with $1 \mu\text{g ml}^{-1}$ of dinitrophenyl-specific IgE for 30 min, washed and then stimulated with 20 ng ml^{-1} of dinitrophenyl-HSA (Sigma). For IgE-immune complex and IgG-immune complex stimulations, IgE- or IgG-containing immune complexes were prepared by incubating either IgE and antibody to mouse IgE or IgG1 and antibody to mouse IgG1 at a 1:2 ratio for 30 min at 37 °C (see **Supplementary Table 2**). The indicated concentrations of immune complexes (**Fig. 3**) were then added to the cells for 4 h at 37 °C. Two hours before the end of this incubation, $10 \mu\text{M}$ monensin (Sigma) was added to the cells. Intracellular staining was done as previously described¹⁸.

Immunohistochemistry for basophil detection was performed as previously described^{56,57}.

Statistical analyses. For comparisons between two populations, an unpaired two-tailed Student's *t* test was performed, unless otherwise specified. When three or more populations were compared, a one-way analysis of variance test was first performed, and, if significance was reached ($P < 0.05$), an unpaired two-tailed Student's *t* test was performed between each compared population, unless otherwise indicated. Statistical analysis was performed with GraphPad Prism 5.01 software.

53. Hochberg, M.C. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum.* **40**, 1725 (1997).
54. Tan, E.M. *et al.* The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum.* **25**, 1271–1277 (1982).
55. Liu, F.T. *et al.* Monoclonal dinitrophenyl-specific murine IgE antibody: preparation, isolation and characterization. *J. Immunol.* **124**, 2728–2737 (1980).
56. Kopley, C.L., Craig, S.S. & Schwartz, L.B. Identification and partial characterization of a unique marker for human basophils. *J. Immunol.* **154**, 6548–6555 (1995).
57. McEuen, A.R., Buckley, M.G., Compton, S.J. & Walls, A.F. Development and characterization of a monoclonal antibody specific for human basophils and the identification of a unique secretory product of basophil activation. *Lab. Invest.* **79**, 27–38 (1999).

NATURE MEDICINE

SUPPLEMENTAL DATA

**BASOPHILS AND THE T HELPER 2 ENVIRONMENT CAN PROMOTE THE
DEVELOPMENT OF LUPUS NEPHRITIS**

Nicolas Charles¹, Donna Hardwick², Eric Dugas³, Gabor G. Illei⁴, and Juan Rivera^{1,*}

¹Laboratory of Molecular Immunogenetics and the ²Office of the Clinical Director,
National Institute of Arthritis and Musculoskeletal and Skin Diseases, the ⁴Sjogren's
Syndrome Clinic, Molecular Physiology and Therapeutics Branch, National Institute of
Dental and Craniofacial Research, National Institutes of Health, Bethesda, Maryland
20892, USA, and the ³INSERM U699, Department of Nephrology, Assistance Publique -
Hôpitaux de Paris, Université Paris Diderot, Hôpital Bichat, Paris, France.

Basophils and the T helper 2 environment can promote the development of lupus nephritis

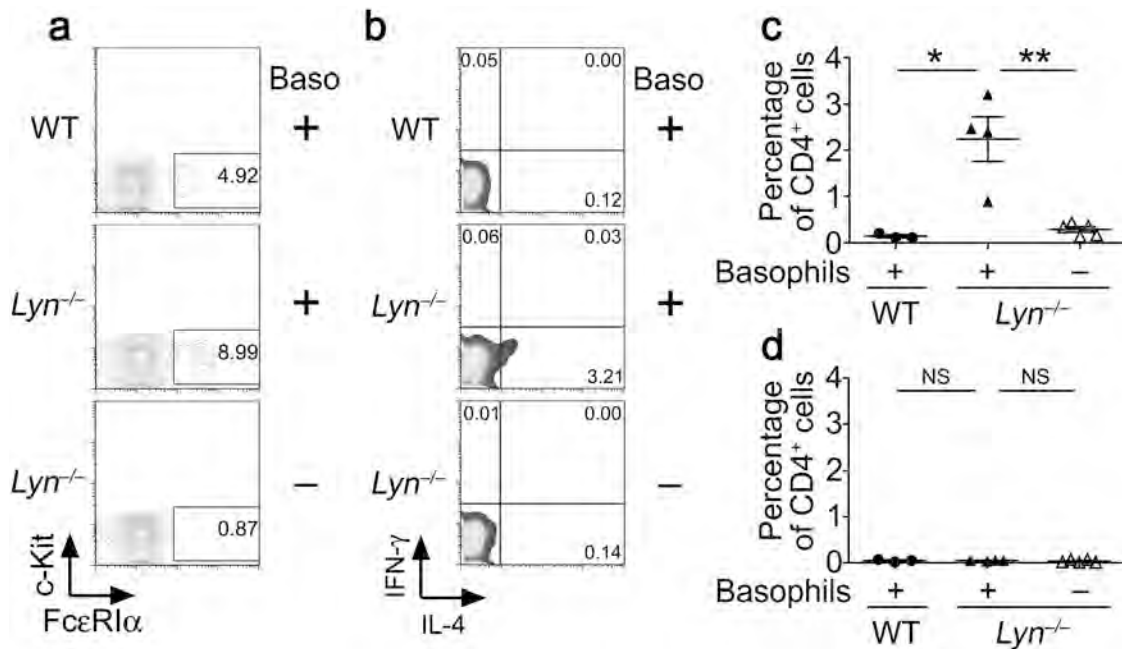


Figure S1. Basophil-dependent T_H2 skewing is a prominent feature of aged *Lyn*^{-/-} mice.

(a) Flow cytometric analysis of non-depleted (Baso +) or basophil-depleted (Baso -) peripheral blood cells from aged (30 weeks) WT and *Lyn*^{-/-} mice. Data shown are representative of at least 3 animals per group. Data was collected on CD11b⁺ leukocytes. (b-d) Splenocytes from WT and *Lyn*^{-/-} mice were harvested, incubated with 10 μM monensin, labeled with a fluorescent anti-CD4 antibody, and stained for intracellular IL-4 and IFN-γ. (b) Representative flow cytometric analysis of CD4⁺ cells from WT or *Lyn*^{-/-} mice, non-depleted (Baso +) or basophil-depleted (Baso -) (as shown in (a)). (c) Compilation of all individual experiments as in (b) for CD4⁺IL-4⁺ T cells. (d) Compilation of all individual experiments as in (b) for CD4⁺IFN-γ⁺ T cells. Statistical analysis was by a two tailed unpaired student *t* test; *: *p*<0.05; **: *p*<0.01; NS: not significant.

Basophils and the T helper 2 environment can promote the development of lupus nephritis

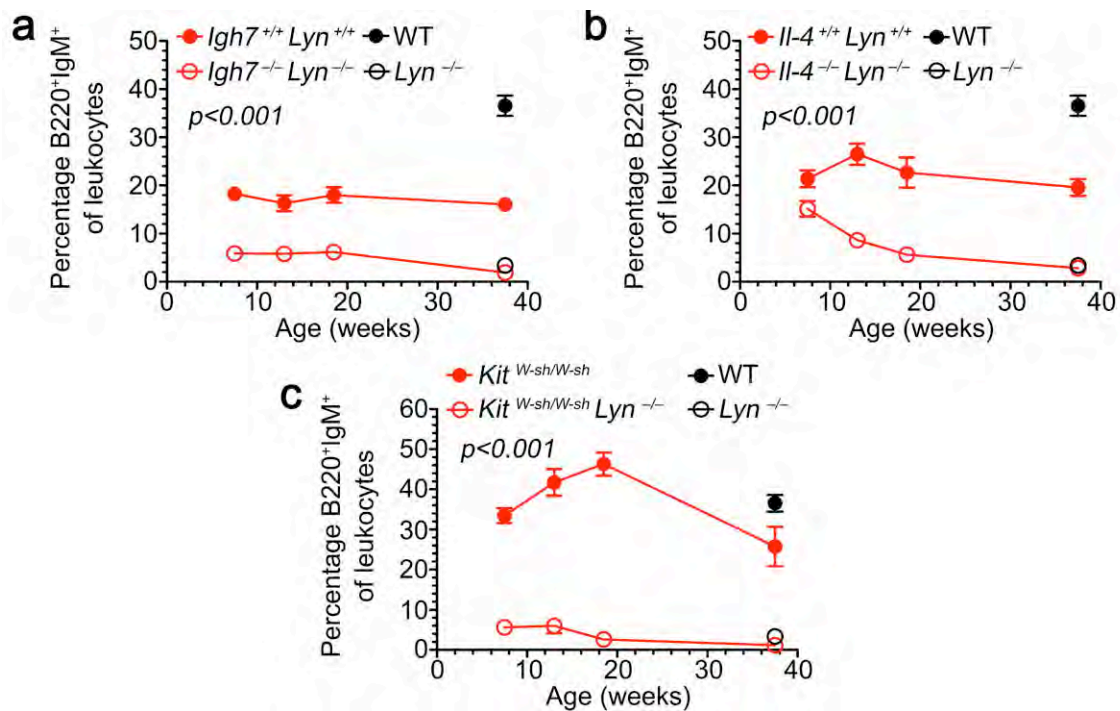


Figure S2. The proportion of blood B cells in $Lyn^{-/-}$ mice is independent of IgE, IL-4 and mast cells.

(a-c) B cell (B220⁺IgM⁺) proportion of the leukocytes was determined by flow cytometry in four distinct age groups in the indicated genotypes. Age group 1: 5 to 10 weeks old (average = 7.5 weeks); age group 2: 12 to 14 weeks old (average = 13 weeks); age group 3: 17 to 20 weeks old (average = 18.5 weeks); age group 4: 35-40 weeks old (average = 37.5 weeks). (a) For $Igh7^{+/+} Lyn^{+/+}$ and $Igh7^{-/-} Lyn^{-/-}$, per group and per genotype, $n=12$; For WT, $n=10$ and $Lyn^{-/-}$ $n=12$. WT and $Lyn^{-/-}$ mice were on a C57BL/6 background. (b) For $Il-4^{+/+} Lyn^{+/+}$ and $Il-4^{-/-} Lyn^{-/-}$, per group and per genotype, $n=12$; For WT, $n=10$ and $Lyn^{-/-}$ $n=12$. (c) For $Kit^{W-sh/W-sh}$ and $Kit^{W-sh/W-sh} Lyn^{-/-}$, group 1: $n=9$ per genotype, group 2: $n=3$ per genotype, group 3: $n=11$ per genotype, group 4: $n=10$ per genotype; For WT $n=10$ and $Lyn^{-/-}$ $n=12$. (a-c) Data are shown as means $\pm$ s.e.m. Statistical analysis was realized by using a two way ANOVA test of variances. The p value shown is the genotype factor p value.

Basophils and the T helper 2 environment can promote the development of lupus nephritis

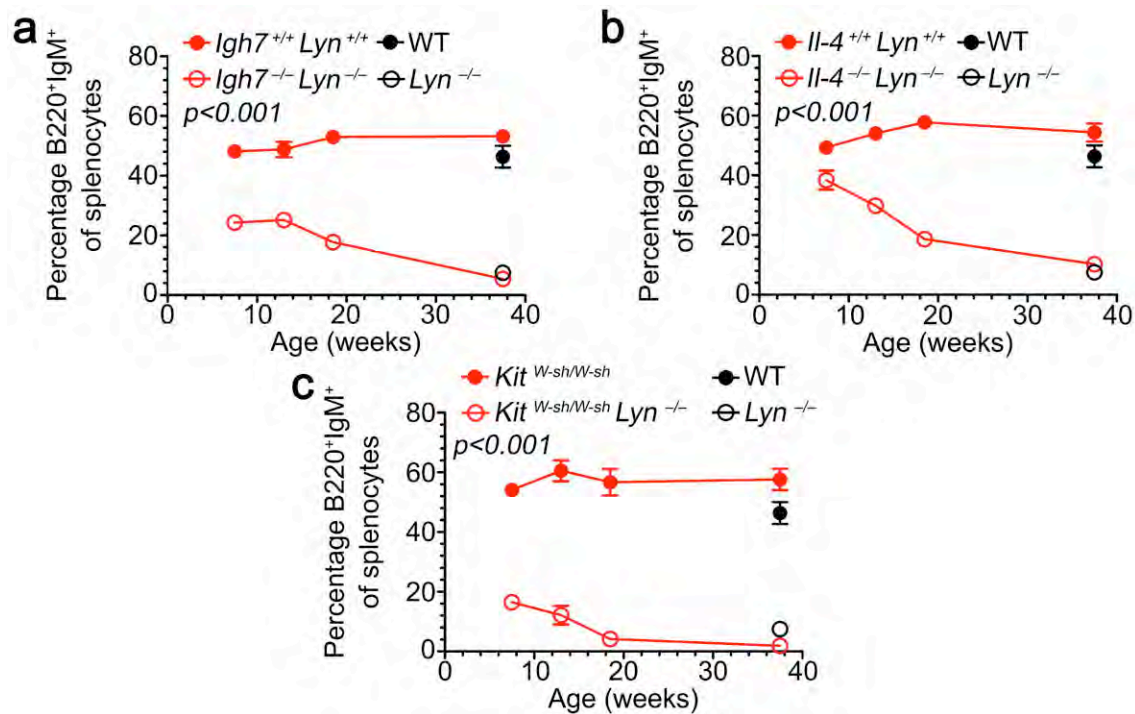


Figure S3. The proportion of spleen B cells in Lyn deficient mice is independent of IgE, IL-4 and mast cells.

(a-c) B cell (B220⁺IgM⁺) proportion of the splenocytes was determined by flow cytometry in four distinct age groups in the indicated genotypes as described in **Supplemental Fig.2**. (a-c) Data are shown as means $\pm$ s.e.m. Statistical analysis was realized by using a two way ANOVA test of variances. The p value shown is the genotype factor p value.

Basophils and the T helper 2 environment can promote the development of lupus nephritis

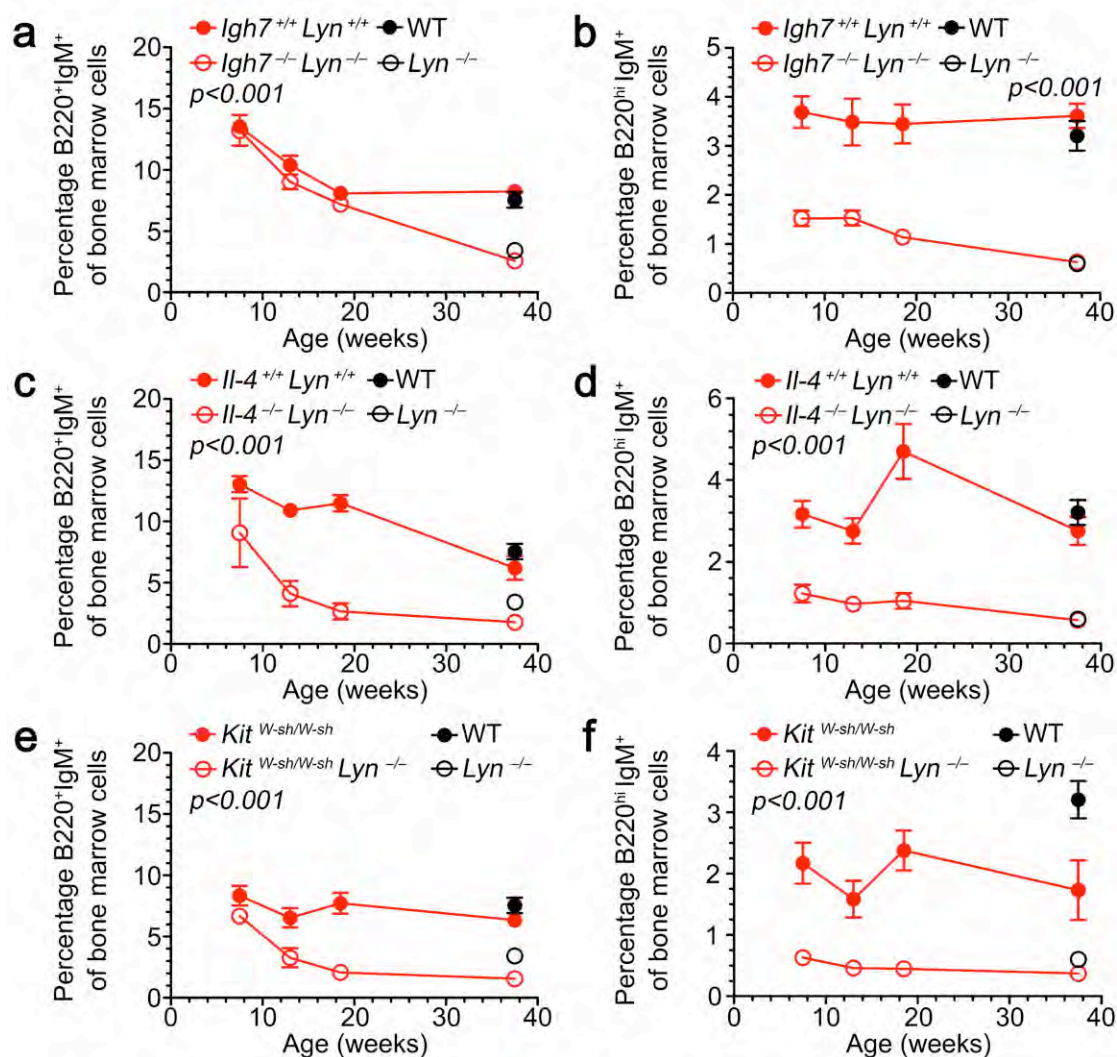


Figure S4. Bone marrow B cell proportion phenotype of *Lyn* deficient mice is independent of IgE, IL-4 and mast cells.

(a, c and e) Total B cell (B220⁺IgM⁺) proportion of the bone marrow cells was determined by flow cytometry in four distinct age groups in the indicated genotypes as described in **Supplemental Fig. 2**. (b, d and f) Recirculating B1 cells (B220^{hi}IgM^{int}) proportion of the BM cells was determined in the same groups. (a-f) Data are shown as means ± s.e.m. Statistical analysis was realized by using a two way ANOVA test of variances. The *p* value shown is the genotype factor *p* value.

Basophils and the T helper 2 environment can promote the development of lupus nephritis

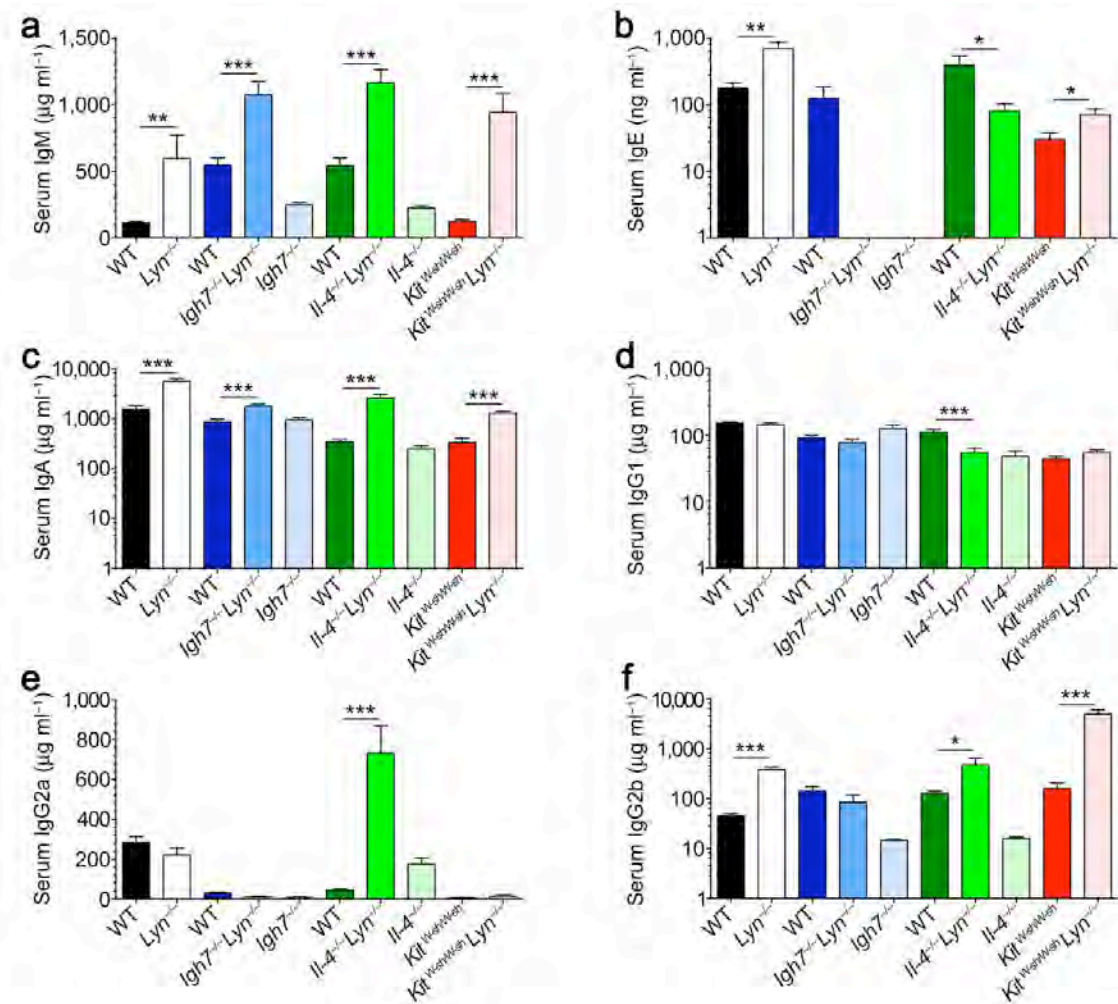


Figure S5. Different immunoglobulin isotypes serum levels in studied mice.

(a-f) Serum quantification by ELISA of circulating IgM (a), IgE (b), IgA (c), IgG1 (d), IgG2a (e) and IgG2b (f) in all the genotypes used in this study. WT, n=35; *Lyn*^{-/-}, n=35; *Igh7*^{+/+}*Lyn*^{+/+}, n=43; *Igh7*^{-/-}*Lyn*^{-/-}, n=41; *Igh7*^{-/-}, n=3; *Il-4*^{+/+}*Lyn*^{+/+}, n=41; *Il-4*^{-/-}*Lyn*^{-/-}, n=42; *Il-4*^{-/-}, n=4; *Kit*^{W-sh/W-sh}, n=18 and *Kit*^{W-sh/W-sh}*Lyn*^{-/-}, n=18. Data shown are means $\pm$ s.e.m. Statistical analysis was by a two tailed unpaired student *t* test; *: *p* < 0.05; **: *p* < 0.01; ***: *p* < 0.001.

Basophils and the T helper 2 environment can promote the development of lupus nephritis

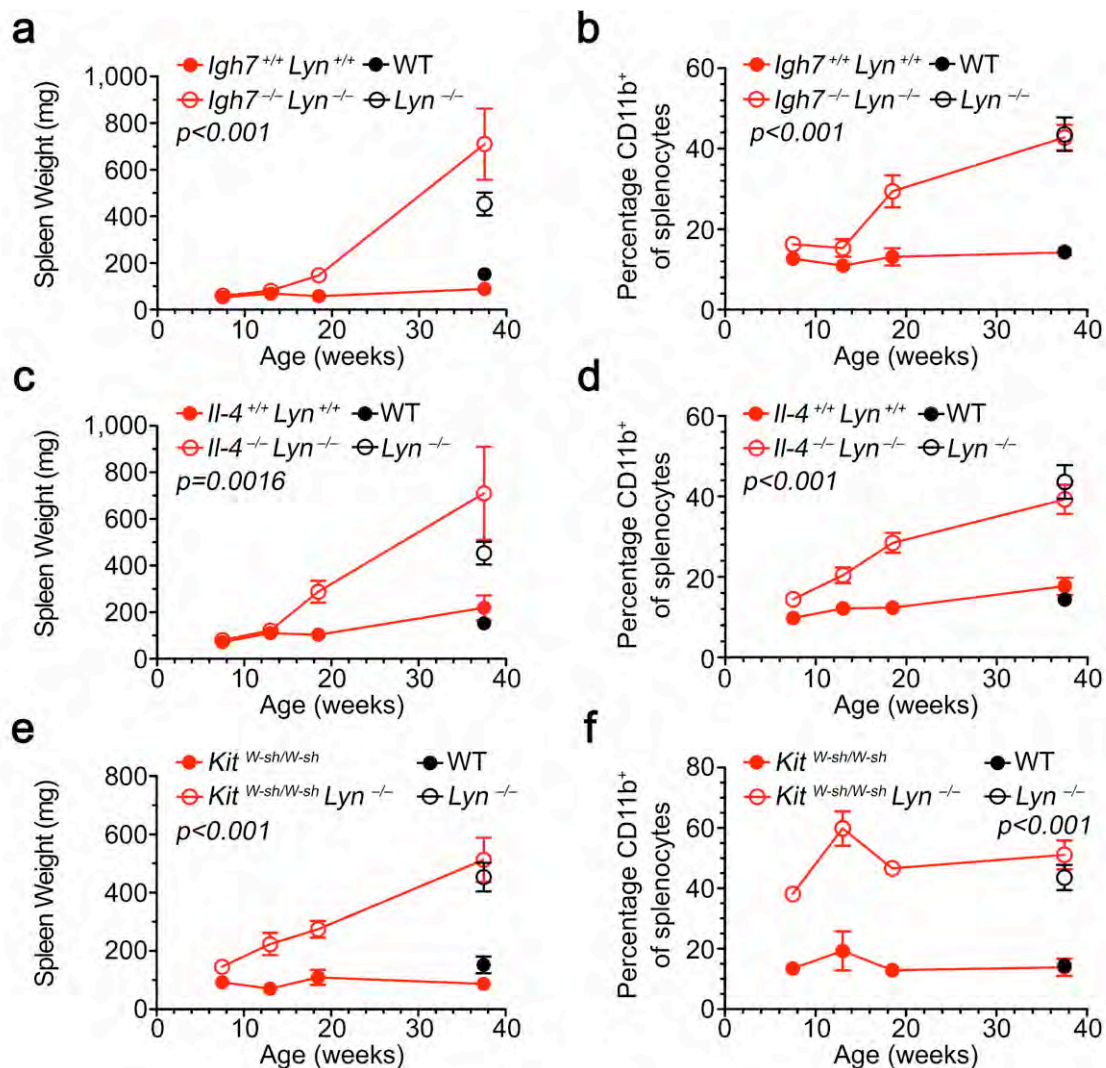


Figure S6. Splenomegaly and spleen proportion of CD11b⁺ cells of Lyn deficient mice are independent of IgE, IL-4 and mast cells.

(a, c and e) Total spleen weight was determined for the spleen from four distinct age groups in the indicated genotypes as described in **Supplemental Fig. 2**. (b, d and f) Proportion of CD11b⁺ cells in the spleen from the same groups was determined by flow cytometry. (a-f) Data are shown as means $\pm$ s.e.m. Statistical analysis was realized by using a two way ANOVA test of variances. The *p* value shown is the genotype factor *p* value.

Basophils and the T helper 2 environment can promote the development of lupus nephritis

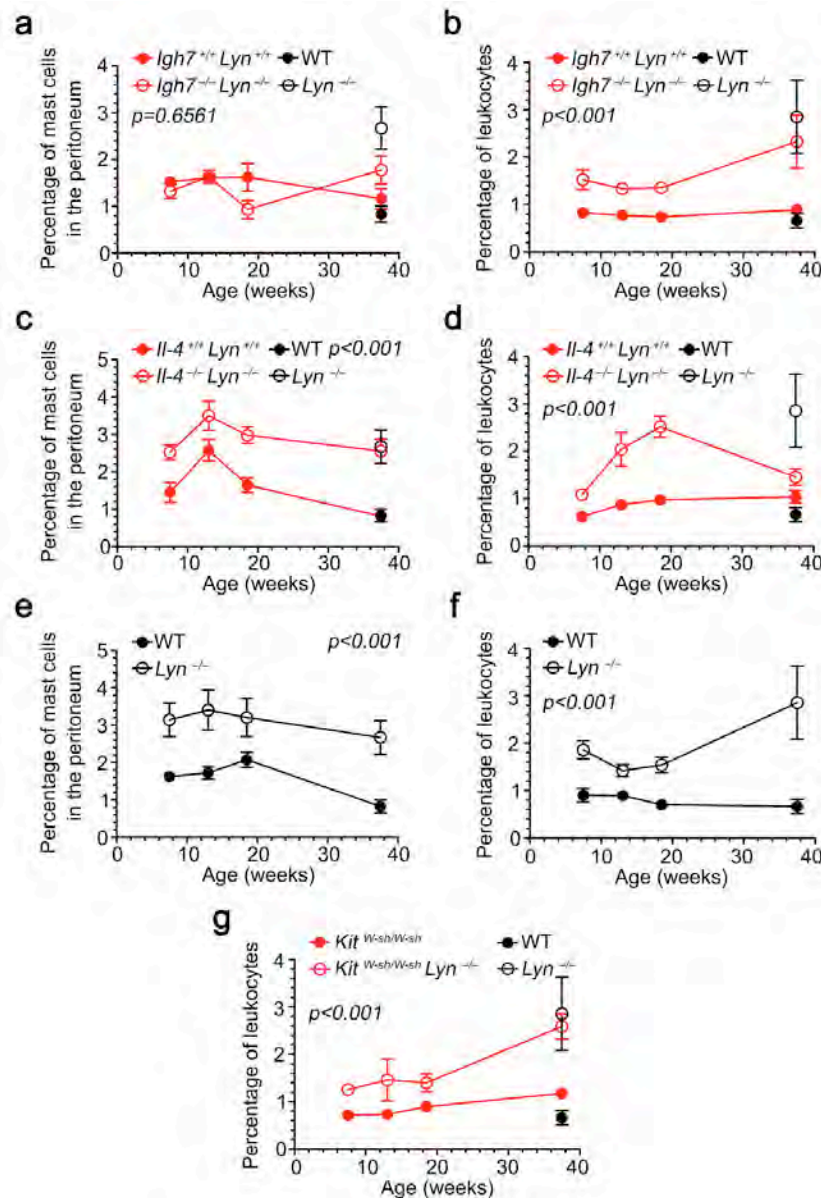


Figure S7. Mast cell but not basophil phenotypes of *Lyn* deficient mice are dependent on IgE, but both are IL-4 independent.

(a, c and e) Proportion of mast cells (FcεRIα⁺CD117⁺) in the peritoneum was determined by flow cytometry after peritoneal lavage in four distinct age groups for the indicated genotypes as described in **Supplemental Fig. 2**. (b, d, f and g) Proportion of basophils (FcεRIα⁺CD49b⁺CD11b⁺CD117⁻) in the total leukocyte population was determined by flow cytometry in the same groups. (a, b) For *Igh7^{+/+}Lyn^{+/+}* and *Igh7^{-/-}Lyn^{-/-}*, per group and per genotype, n=12; For WT, n=10 and *Lyn^{-/-}*, n=12. (c, d) For *Il-4^{+/+}Lyn^{+/+}* and *Il-4^{-/-}Lyn^{-/-}*, per group and per genotype, n=12; For WT, n=10 and *Lyn^{-/-}*, n=12. (e, f) group 1: WT n=8 and *Lyn^{-/-}* n=11, group 2: WT n=14 and *Lyn^{-/-}* n=9, group 3: WT n=14 and *Lyn^{-/-}* n=11, group 4: WT n=10 and *Lyn^{-/-}* n=12. (g) For *Kit^{W-sh/W-sh}* and *Kit^{W-sh/W-sh}Lyn^{-/-}*, group 1: n=9 per genotype, group 2: n=3 per genotype, group 3: n=11 per genotype, group 4: n=10 per genotype; For WT n=10 and *Lyn^{-/-}* n=12. (a-g) Data are shown as means ± s.e.m. Statistical analysis was realized by using a two way ANOVA test of variances. The *p* value shown is the genotype factor *p* value.

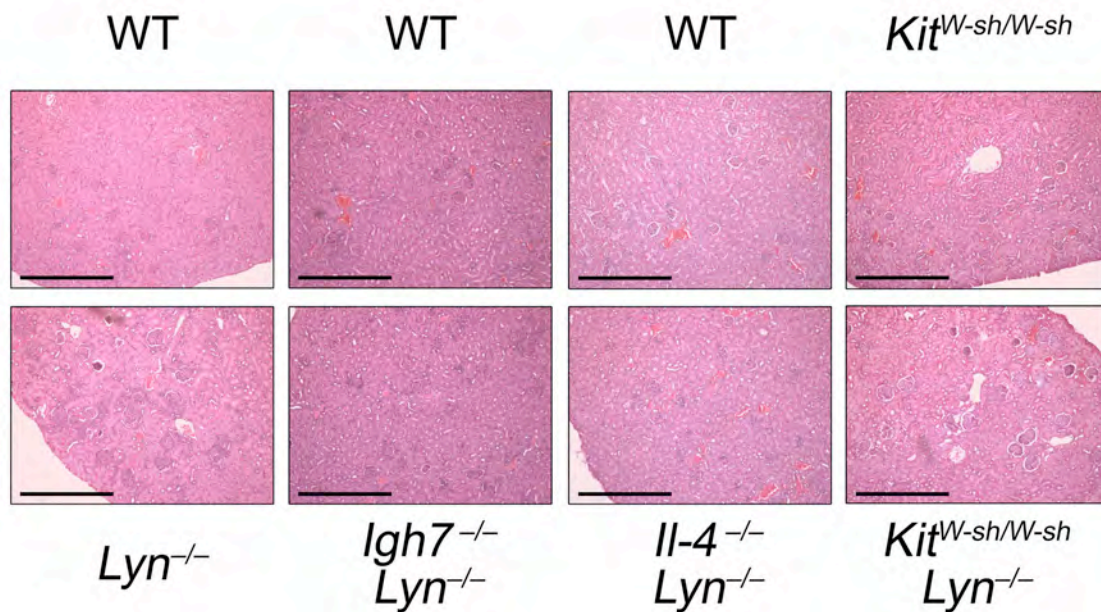


Figure S8: Lupus-like glomerulonephritis in *Lyn*^{-/-} mice is IgE and IL-4 dependent, but mast cell independent.

Representative histological kidney sections from 40 weeks old mice of the indicated genotypes with H&E staining. Original magnification x10. These sections were used to establish the glomerulonephritis score as described in **Fig. 1** where original magnification x40 are shown. Scale bar, 500 μ m.

Basophils and the T helper 2 environment can promote the development of lupus nephritis

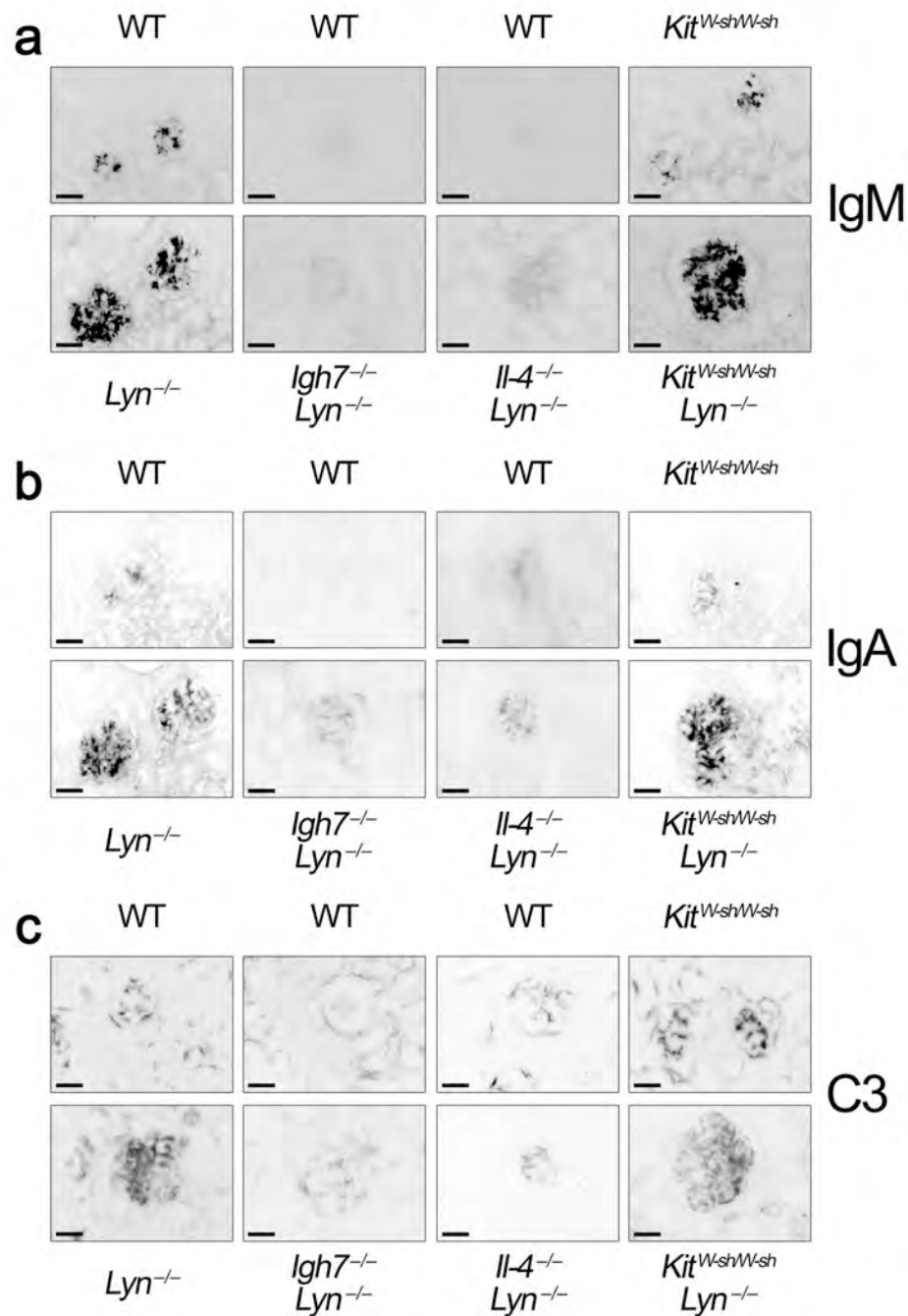


Figure S9. Glomerular immune complexes depositions in Lyn deficient mice are IgE and IL-4 dependent, but mast cell independent.

(a-c) Kidneys from 40 weeks old mice of the indicated genotypes were processed as described in methods. Immunofluorescent staining with fluorescein-conjugated antibodies raised against the indicated antigens was realized (**a**, IgM; **b**, IgA; **c**, complement component 3 (C3)). Pictures shown are representative of over 100 glomeruli per genotype acquired on at least 5 different 40 weeks old mice per genotype. Original magnification x40. Scale bar, 50 μ m.

Basophils and the T helper 2 environment can promote the development of lupus nephritis

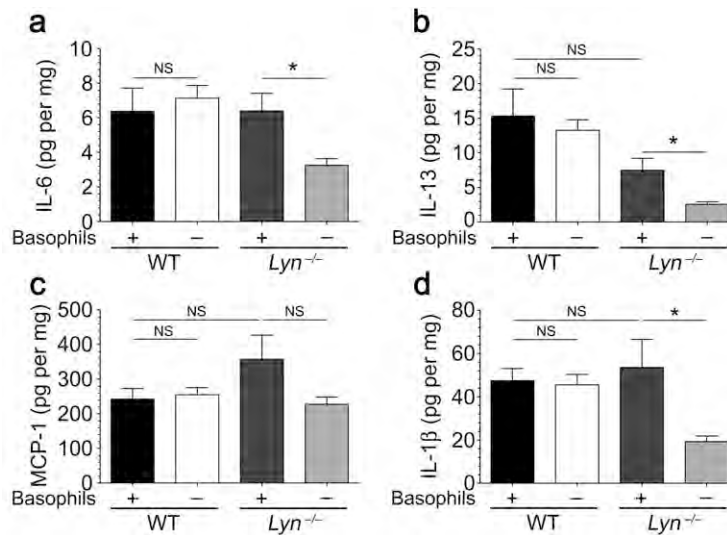


Figure S10. Basophil-depletion reduces the pro-inflammatory cytokine environment in the kidney of *Lyn*^{-/-} mice.

(a-d) ELISA quantitation of the indicated cytokines in kidney homogenates from 40 week old WT and *Lyn*^{-/-} mice 6 days after basophil depletion (MAR-1 injection, basophils -) or not (isotype injection, basophils +) as described in methods. Cytokine amounts were normalized to the total protein content of the respective homogenates. Data are shown as mean ± s.e.m (WT and *Lyn*^{-/-}, at least n=4 per group). Statistical analysis was by a two tailed unpaired student *t* test; NS: not significant, *: *p*<0.05.

Basophils and the T helper 2 environment can promote the development of lupus nephritis

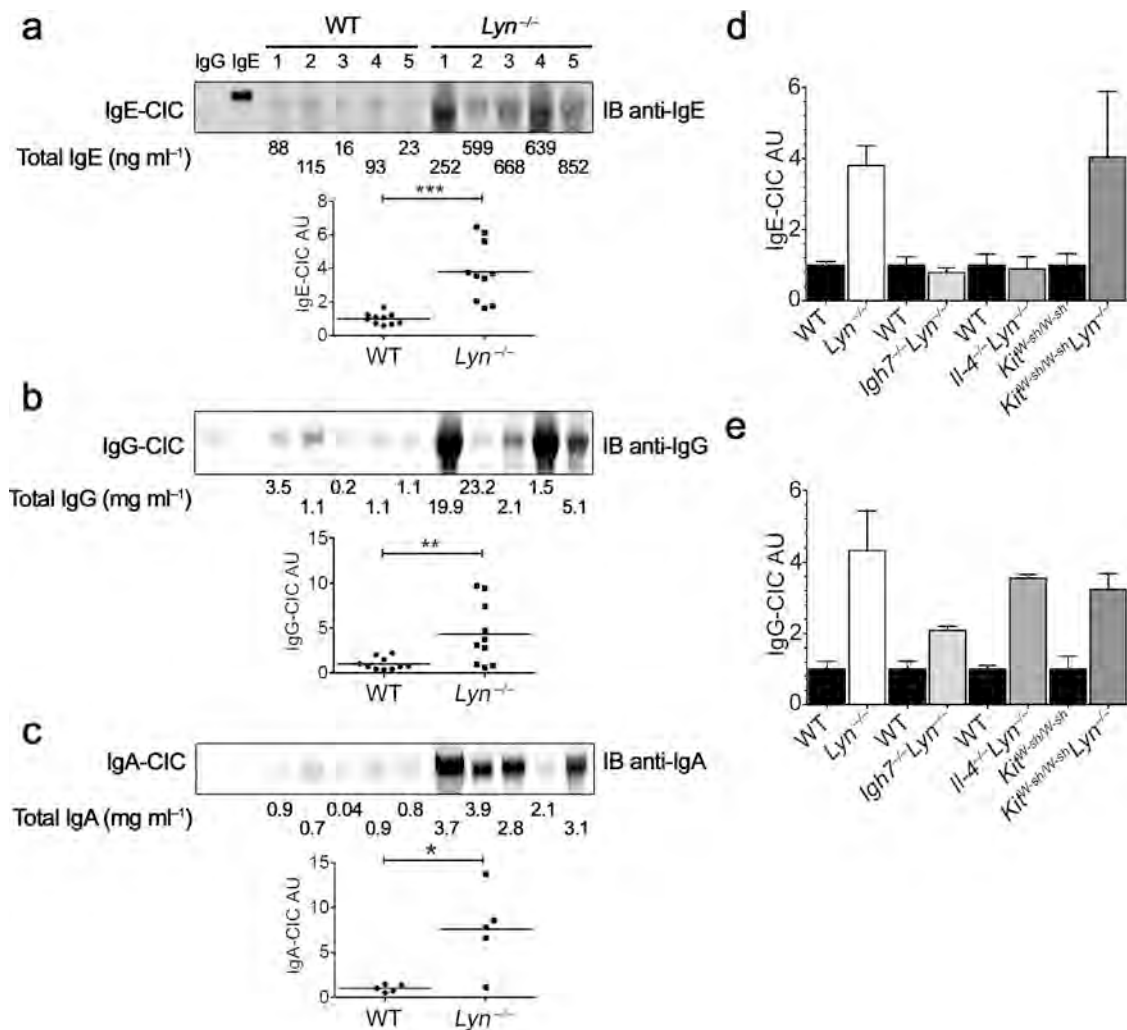


Figure S11. Lupus prone *Lyn*^{-/-} mice contain circulating immune complexes of IgE, IgG, and IgA.

(a-c) Western blot analysis of PEG₆₀₀₀ precipitated circulating immune complexes (CIC) from sera from 5 different 45 weeks old WT and *Lyn*^{-/-} mice (1 to 5 for each genotype).

(a) Upper panel: Rat anti-mouse IgE immunoblot (IgE-CIC). Middle panel: ELISA quantification of total serum IgE in the same mice showing no correlation between the level of total IgE and the amount of IgE-CIC precipitated (upper panel). Lower panel: densitometry analysis of immunoblots similar to the one shown in the upper panel using the NIH Image J software. (b) same as in (a) for IgG-CIC with goat anti-mouse IgG (upper panel), total IgG ELISA (middle panel) and densitometry analysis of IgG-CIC (lower panel). (c) same as in (a) for IgA-CIC with goat anti-mouse IgA (upper panel), total IgA ELISA (middle panel) and densitometry analysis of IgA-CIC (lower panel). (d, e) Immunoblots as the representative ones shown in (a & b) were quantified by densitometry as in (a-c) for IgE-CIC (d) and IgG-CIC (e). All genotypes were analyzed. Data are shown as means $\pm$ s.e.m (WT and *Lyn*^{-/-}, n=10 per group, all other genotypes, n=5 per group). Data presented are representative of at least five independent experiments.

Basophils and the T helper 2 environment can promote the development of lupus nephritis

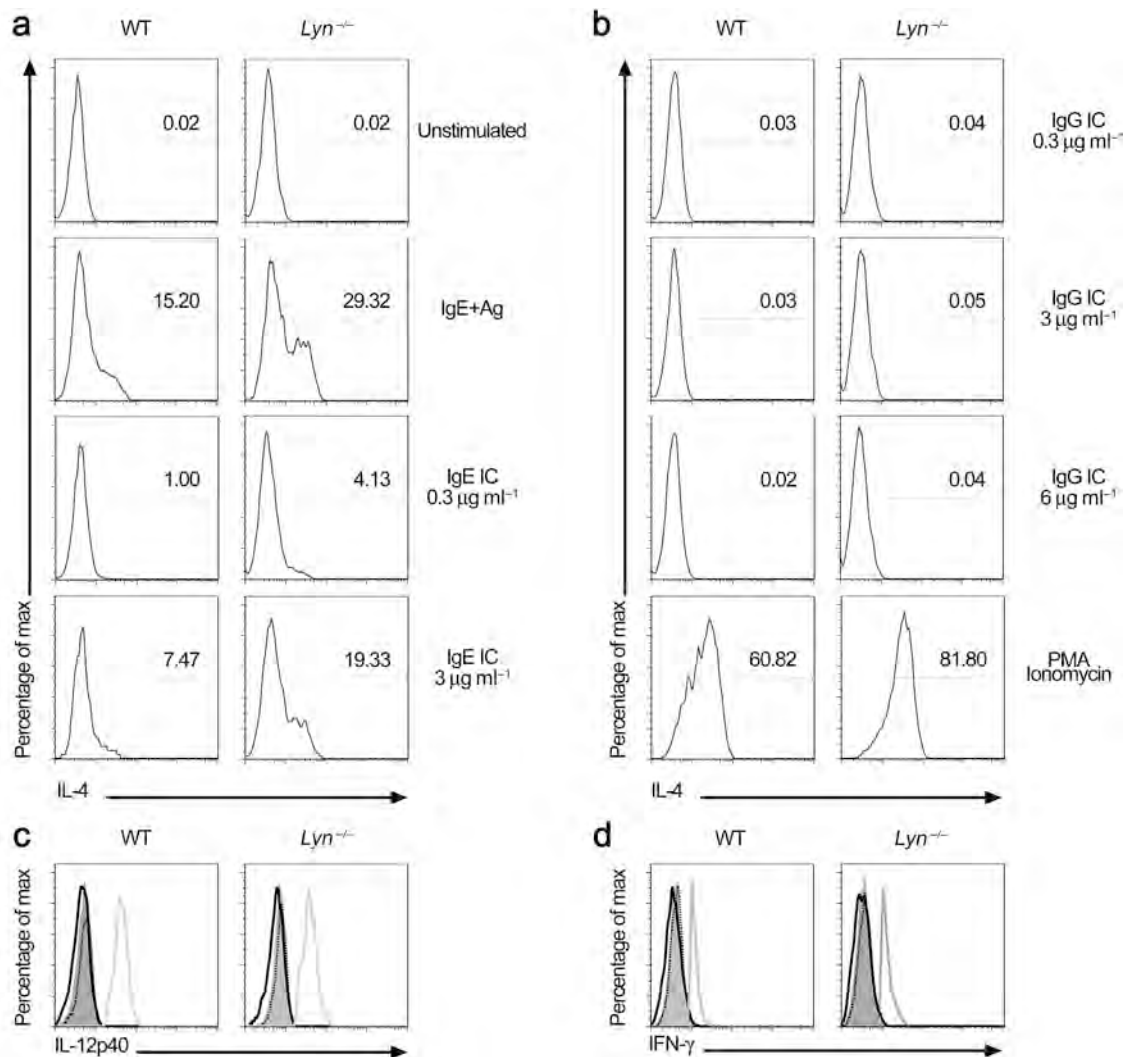


Figure S12. IgE-IC, but not IgG-IC, induces cytokine production by basophils.

(a,b) Representative flow cytometry analysis of IL-4 production by bone-marrow derived basophils (BMBa) in WT and $Lyn^{-/-}$ mice. Cells were stimulated for 4 hours with the indicated stimulus and incubated with 10 μM monensin during the last two hours of stimulation. Cells were then extracellularly stained for mouse basophils markers (CD49b, Fc ϵ RI α and CD11b) and intracellularly stained for IL-4 production. Compilation of all these results is shown in **Fig. 3d**. (c) Same protocol as in (a,b) but cells were intracellularly stained for IL-12p40 production. *Filled*: isotype control, *dashed*: IgE+Ag stimulated BMBa, *solid black*: PMA/ionomycin stimulated BMBa, *solid grey*: BM cell population CD49b $^{-}$ Fc ϵ RI α^{-} (non-basophil, non-mast cell) producing IL-12p40 after PMA/ionomycin stimulation. (d) same as in (c) but cells were intracellularly stained for IFN- γ production. (c,d) None of the stimuli tested (as in a, b) led basophils to produce either IL-12p40 or IFN- γ .

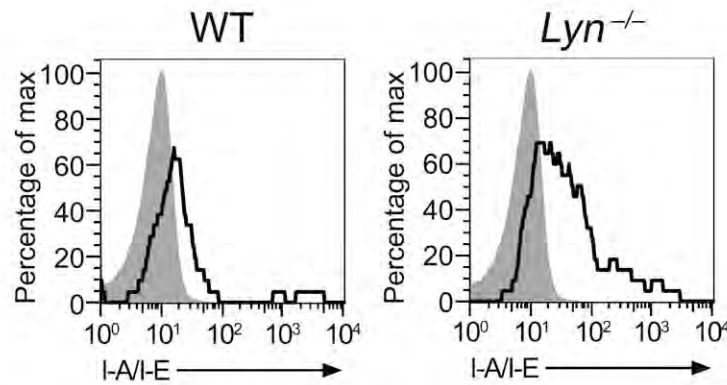


Figure S13. MHC II expression is increased on spleen basophils from *Lyn*^{-/-} mice.

Representative flow cytometric analysis of spleen basophil MHC II expression (I-A/I-E). Basophils were defined as FcεRI⁺ CD117⁻ CD49b⁺ cells, in aged (40 weeks) WT (left panel, black line) and *Lyn*^{-/-} mice (right panel, black line) relative to isotype control (grey fill).

Basophils and the T helper 2 environment can promote the development of lupus nephritis

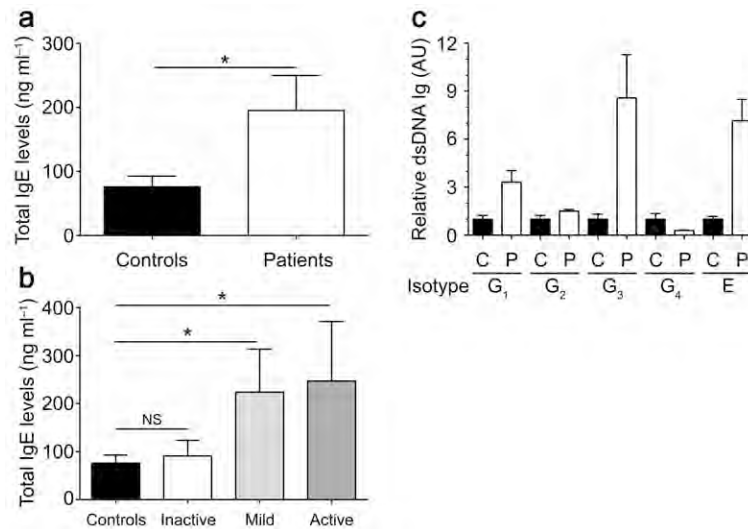


Figure S14. Total IgE levels and dsDNA immunoglobulin subclasses in SLE patients.

(a) Quantitation of total serum IgE levels in healthy controls ($n=27$) and SLE patients ($n=33$) by ELISA. (b) Same measurement as in (a) but showing its relationship to inactive/moderate/active SLE patients ($n=9/13/11$) as described in **Fig. 5a**) versus healthy controls ($n=27$). Data shown are means $\pm$ s.e.m. Statistical analysis was by a two tailed unpaired student t test; NS: not significant, *: $p<0.05$. (c) IgG anti-dsDNA subclasses and IgE anti-dsDNA was determined by semi-quantitative ELISA. dsDNA-coated plates were incubated with sera from healthy controls ($n = 5$) and SLE patients ($n = 43$) and autoreactive IgG1, IgG2, IgG3, IgG4 and IgE were detected with the corresponding specific anti-human Fc portion HRP-conjugated. Data shown are normalized to healthy controls and expressed as means $\pm$ s.e.m.

Basophils and the T helper 2 environment can promote the development of lupus nephritis

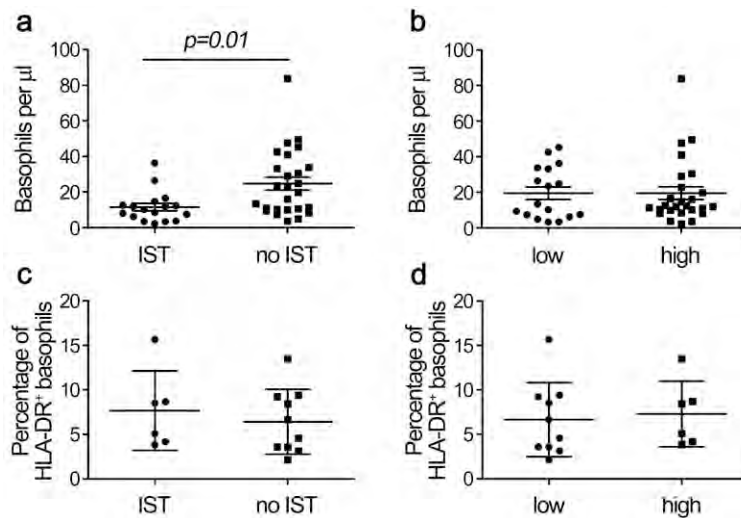


Figure S15. Effect of immunosuppressive treatments on blood basophil numbers and HLA-DR expression.

(a) Peripheral blood basophil counts were significantly lower in patients receiving immunosuppressive treatment (IST) (defined as prednisone more than 15 mg/day and/or cyclophosphamide, azathioprine, mycophenolate mofetil). (b,d) There was no difference on the number of peripheral basophils (b) or HLA-DR expressing basophils (d) among patients receiving low dose (≤ 7.5 mg/day) or medium to high dose (> 7.5 mg/day) prednisone. (c) There was no difference in proportion of HLA-DR⁺ basophils between patients on or off IST. Data shown are means $\pm$ s.e.m. Statistical analysis was by a two tailed unpaired student *t* test.

Supplementary Table 1. Demographic characteristics of SLE patients.

Age (Mean, SD)	38.5±12.9 years
Gender (F/M, n,%)	39(93%) / 3 (7%)
Ethnicity (n, %) Caucasian African American Hispanic Asian	14 (33.3%), 10 (23.8%) 12 (28.6%) 6 (14.3%)
Disease duration (Mean, SD, years)	12.9±10.8
Anti-dsDNA Ab positive (n, %)	33 (55%)
SLEDAI Mean, SD Median, (minimum, maximum)	4.57±5.5 4.0 (0, 24)
Current prednisone dose (mg/day) Mean, SD Median, (minimum, maximum)	12.6±14.9 7.5 (0, 60)
Concurrent immunosuppressive therapy (n, %) hydroxychloroquine mycophenolate mofetil cyclophosphamide azathioprine	35 (83%) 4 (9.5%) 3 (7%) 7 (17%)

Supplementary Table 2: Commercial antibodies used.

Application	Used in Figure #	Description	Conjugate	Clone	Company
Basophil depletion	2, S1	Hamster anti-mouse FcεR1α	-	MAR-1	eBioscience
Basophil depletion (isotype control)	2, S1	Armenian Hamster IgG	-	eBio299Arm	eBioscience
ELISA	5	Human IgE	-	HE1	Abbotec
ELISA	5, S14	Mouse anti-Human IgE	HRP	?	ICL
ELISA	5	Donkey anti-human IgG, Fcy Specific	HRP	Polyclonal	Jackson IRL
ELISA	3	Rat Anti-Mouse IgE	HRP	23G3	Southern Biotech
ELISA	S14	Mouse anti-human IgG1	HRP	HP6070	Invitrogen
ELISA	S14	Mouse anti-human IgG2	HRP	HP6014	Invitrogen
ELISA	S14	Mouse anti-human IgG3	HRP	HP6049	Invitrogen
ELISA	S14	Mouse anti-human IgG4	HRP	HP6023	Invitrogen
Flow cytometry	4, S1, S7	Rat anti-mouse CD117 (c-Kit)	APC	2B8	BD Biosciences
Flow cytometry	S13	Rat anti-mouse CD117 (c-Kit)	PE-Cy5	2B8	BioLegend
Flow cytometry	S1	Rat anti-mouse CD4	PerCP-Cy5.5	RM4-5	BD Biosciences
Flow cytometry	4	Rat anti-mouse CD62L	APC	MEL-14	BD Biosciences
Flow cytometry	S1	Rat anti-mouse Interferon gamma	APC	XMG1.2	BD Biosciences
Flow cytometry	S2, S3, S4	Rat anti-mouse CD45R/B220	APC	RA3-6B2	BioLegend
Flow cytometry	6, S15	Mouse anti-human CD203c	PE	E-NPP3	BioLegend
Flow cytometry	6, S15	Mouse anti-human FcεR1 alpha	FITC	CRA1	BioLegend
Flow cytometry	6, S15	Mouse anti-human CD123	PerCP-Cy5.5	6H6	BioLegend
Flow cytometry	6, S15	Mouse anti-human CD117 (c-kit)	APC	104D2	BioLegend
Flow cytometry	6, S15	Mouse anti-human CD11b	APC	ICRF44	BioLegend
Flow cytometry	6	Mouse anti-human CD62L	APC	DREG-56	BioLegend
Flow cytometry	6, S15	Mouse anti-human HLA-DR	PerCP-Cy5.5	L243	BioLegend
Flow cytometry	S12	Rat anti-mouse IFN-γ	PE	XMG1.2	BioLegend
Flow cytometry	3, S1	Rat anti-mouse interleukin-4 (IL-4)	PE	11B11	BioLegend
Flow cytometry	3, 4, S1, S7, S12, S13	Rat anti-mouse CD49b/Pan-NK Cells	FITC	DX5	BioLegend
Flow cytometry	3, 4, S1, S6, S7, S12	Rat anti-mouse CD11b	PerCP-Cy5.5	M1/70	BioLegend
Flow cytometry	S12	Rat anti-mouse IL-12/IL-23 P40	PE	C15-6	BioLegend
Flow cytometry	4, S1, S7, S13	Hamster anti-mouse FcεR1α	PE	MAR-1	eBioscience
Flow cytometry	S12	Rat anti-mouse interleukin-4 (IL-4)	FITC	BVD6-24G2	eBioscience
Flow cytometry	3, S12	Hamster anti-mouse FcεR1α	Alexa Fluor®-647	MAR-1	eBioscience
Flow cytometry	4	Rat anti-mouse BAFF/BLyS/TNFSF13B	PE	121808	R&D Systems
Flow cytometry	2	Rat anti-mouse CD19	PE-Cy5	6D5	BioLegend
Flow cytometry	2	Rat anti-mouse CD138	PE	281-2	BD Biosciences
Flow cytometry	4, S13	Rat anti-mouse I-A/I-E	Alexa Fluor®-647	M5/114.15.2	BioLegend
Flow cytometry	S1	Rat anti-mouse CD4	PerCP-Cy5.5	RM4-5	BD Biosciences
Flow cytometry (isotype control)	3, S12	Armenian Hamster IgG	Alexa Fluor®-647	eBio299Arm	eBioscience
Flow cytometry (isotype control)	4, S1, S7, S13	Armenian Hamster IgG	PE	eBio299Arm	eBioscience
Flow cytometry (isotype control)	3, 4, S1, S6, S7, S12	Rat IgG2b, k	PerCP-Cy5.5	A95-1	BD Biosciences
Flow cytometry (isotype control)	4, S1, S7	Rat IgG2b, k	APC	A95-1	BD Biosciences
Flow cytometry (isotype control)	4, S13	Rat IgG2b, k	Alexa Fluor®-647	RTK4530	BioLegend
Flow cytometry (isotype control)	S1	Rat IgG2a, k	PerCP-Cy5.5	R35-95	BD Biosciences
Flow cytometry (isotype control)	2	Rat IgG2a, k	PE-Cy5	RTK2758	BioLegend
Flow cytometry (isotype control)	S13	Rat IgG2b, k	PE-Cy5	RTK4530	BioLegend
Flow cytometry (isotype control)	2, 4, S1, S12	Rat IgG2a, k	PE	RTK2758	BioLegend
Flow cytometry (isotype control)	S12	Rat IgG1, k	FITC	R3-34	BD Biosciences
Flow cytometry (isotype control)	3	Rat IgG1, k	PE	R3-34	BD Biosciences
Flow cytometry (isotype control)	4, S1, S2, S3, S4	Rat IgG2a, k	APC	RTK2758	BioLegend
Flow cytometry (isotype control)	3, 4, S1, S7, S12, S13	Rat IgM, k	FITC	RTK2118	BioLegend
Flow cytometry (isotype control)	6, S15	Mouse IgG1, k	PE	MOPC-21	BioLegend
Flow cytometry (isotype control)	6, S15	Mouse IgG2b, k	FITC	MPC-11	BioLegend
Flow cytometry (isotype control)	6, S15	Mouse IgG1, k	APC	MOPC-21	BioLegend
Flow cytometry (isotype control)	6, S15	Mouse IgG2a, k	PerCP-Cy5.5	MOPC-173	BioLegend
Flow cytometry (isotype control)	6, S15	Mouse IgG1, k	PerCP-Cy5.5	MOPC-21	BioLegend
Flow cytometry (isotype control)	6, S15	Mouse IgG2b, k	APC	MG2b-57	BioLegend
Flow cytometry, Immunofluorescence	S9	Rat IgG2a, k	FITC	RTK2758	BioLegend
Flow cytometry, Immunofluorescence	S2, S3, S4, S9	Goat Anti-Mouse IgM (Human Adsorbed)	FITC	Polyclonal	Serotec Inc
Flow cytometry, Immunofluorescence	S2, S3, S4, S9	Goat Control Antibody	FITC	Polyclonal	Serotec Inc
Immune complexes	3, S12	Rat anti-mouse IgE	-	R35-92	BD Biosciences
Immune complexes	3, S12	Mouse IgG1.K	-	MOPC21	Sigma-Aldrich
Immune complexes	3, S12	Goat anti-mouse IgG	-	Polyclonal	Sigma-Aldrich
Immunofluorescence	S9	Rat anti-mouse Complement Component C3	FITC	RmC11H9	Cedarlane Lab.
Immunofluorescence	1, S9	Goat Anti-Mouse IgG, Fc fragment, F(ab') ₂	FITC	Polyclonal	Sigma-Aldrich
Immunofluorescence, western blot	S10	Rat anti-mouse IgE	FITC	R35-72	BD Biosciences
Immunofluorescence, western blot	S9, S10	Goat Anti-Mouse IgA	FITC	Polyclonal	Serotec Inc
Western blot	3, S10	Goat anti-rat IgG	Alexa Fluor®-680	Polyclonal	Invitrogen
Western blot	3, S10	Goat anti-mouse IgG	IR-Dye® 800	Polyclonal	Rockland IC
Western blot	S10	Donkey anti-goat IgG	Alexa Fluor®-680	Polyclonal	Invitrogen

HRP: Horseradish Peroxydase

FITC: Fluorescein

PE: Phycoerythrin

PerCP-Cy5.5: Peridinin-chlorophyll-protein complex - Cyanin 5.5

PE-Cy5: Phycoerythrin - Cyanin 5

APC: Allophycocyanin

Supplemental Methods

Blood, bone marrow and spleen sample preparations for flow cytometric analysis

Mouse Blood

Mice were euthanized by CO₂ according to NIH guidelines. Immediately after death, cardiac puncture was done using a 25G needle, and a minimum of 500 µl of blood was withdrawn in a heparinized tube. Blood samples were then centrifuged at 700 x g at 4 °C for 20 min to obtain the plasma. The latter was kept at –20 °C for further analysis. The harvested blood cells were resuspended in 5 ml of ACK lysing buffer (150 mM NH₄Cl, 12 mM NaHCO₃, 1 mM EDTA, pH 7.4) at room temperature for 3 min, then further incubated for 5 min at 4 °C.

Subsequently, 10 ml of PBS was added and the sample was centrifuged at 500 x g for 5 min. When red blood cells were still present, cells were further incubated in ACK lysing buffer for 5 min at 4 °C and the steps outlined above were repeated until red blood cells were not present. The remaining white blood cells were resuspended in FACS buffer (PBS/ 1%BSA/ 0.05% NaN₃). Basophils were identified as CD49b⁺FcεRIα⁺CD11b⁺CD117[–]. B cells were identified as B220⁺IgM⁺.

Bone Marrow

Both femurs were harvested and the bone marrow was flushed out using a syringe containing 3 ml of FACS buffer equipped with a 30G needle. Recovered cells were centrifuged and red blood cells lysed in 3 ml of ACK lysing buffer for 3

min on ice. Subsequently, 10 ml of PBS was added and the sample was centrifuged (500 x g, 5 min). Cells were then stained for FACS analysis.

Basophils and total B cells were identified as above. Recirculating B cells in the bone marrow were defined as B220^{hi} IgM⁺ (an intermediate mean fluorescence intensity is seen in this IgM⁺ population).

Spleen

The spleen was harvested and weighed as a measure of splenomegaly. The spleen was then homogenized to a single cell suspension by using tweezers. The cell suspension was centrifuged (500 x g, 5 min), and red blood cells were lysed in 5 ml of ACK lysing buffer for 5 min on ice. PBS (20 ml) was added and the sample was again centrifuged (500 x g, 5 min). Cells were then resuspended in FACS buffer (10 ml) and filtered on a 40 µm pore diameter cell strainer (BD Biosciences). 1 ml of this cell suspension was used for FACS staining as indicated. Basophils and B cells were identified as above. For the percent of CD11b⁺ cells in the spleen, the CD11b^{hi} population was gated.

Peritoneum

Proportion of peritoneal mast cells being mast cells were determined as previously described²⁰.

Human blood samples

Blood was harvested in EDTA-coated tubes. 4 ml of blood was used to harvest plasma sample. For this purpose, blood was centrifuged at 600 x g for 20 minutes at 4 °C. Plasma phase was then harvested, and samples kept at –20 °C until further analysis. For basophils analysis, 10 ml of whole blood were added to

20 ml of ACK lysing buffer and incubated 5 min at room temperature and 5 more minutes on ice. 30 ml of PBS was added, and cells were centrifuged (500 x g, 5 min). This step was repeated three times total until no more red blood cells were visible. Cells were then resuspended into 10 ml (original volume) of FACS buffer (PBS/ 1% BSA/ 0.05% NaN₃). Number of leukocytes per ml and viability were assessed with a ViCell cell counter (Beckman and Coulter). Viability was always over 90%. Cells were then processed for extracellular staining with the indicated surface markers. For basophil absolute counts, basophils were identified as FcεR1α⁺CD203c⁺CD123⁺CD11b⁺ cells. For HLA-DR expression analysis, basophils were identified as FcεR1α⁺CD203c⁺CD11b⁺. For CD62L expression analysis, basophils were identified as FcεR1α⁺CD203c⁺CD123⁺.

Histological analysis for glomerular pathological features in mice.

Histological analysis for glomerular pathological features included: inflammation, proliferation, crescent formation, and necrosis. A minimum of thirty glomeruli, of at least ten aged mice per genotype, were scored. For each glomerulus, a score from 1 to 5 (1, normal; 2, moderate; 3, severe; 4, severe with crescent formation and 5, necrosis) was used. Scores from each individual mouse were added and averaged to yield the glomerulonephritis score. All pathological assessments were performed in a blinded fashion.

Assessment of cytokine content in mouse kidney

For, the kidney was homogenized in 800 µl of PBS containing protease inhibitors (Roche) and centrifuged at 10,000 x g for 20 min. Total protein content was determined (Dc protein assay, BioRad) and IL-4 (BD Bioscience), IL-13, IL-6, IL-1β, CCL2, and IFNγ (eBioscience) were measured by ELISA according to the manufacturer's instructions.

Patient's lupus and nephritis activity assessment

Lupus activity was assessed by SELENA-SLEDAI (Safety of Estrogens in Lupus Erythematosus National Assessment Systemic Lupus Erythematosus Disease Activity Index) scores³⁵. Based on the SLEDAI score, lupus activity was classified as inactive (0), mild (1.0-4.0) and active (> 4). Active lupus nephritis was defined by the presence of an active urinary sediment and either a urinary protein creatinine ratio of >1 or immunosuppressive treatment for proliferative lupus nephritis.

Enzyme-linked immunosorbent assays

For the different immunoglobulin isotypes analysis, anti-mouse IgM, IgA, IgG₁, IgG_{2a}, IgG_{2b} and IgE ELISA kits were purchased from Bethyl Laboratories.