NATURE MEDICINE

SUPPLEMENTAL DATA

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

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Figure S1. Basophil-dependent T\(_h2\) skewing is a prominent feature of aged \(\text{Lyn}^{-/-}\) mice.
(a) Flow cytometric analysis of non-depleted (Baso +) or basophil-depleted (Baso −) peripheral blood cells from aged (30 weeks) WT and \(\text{Lyn}^{-/-}\) mice. Data shown are representative of at least 3 animals per group. Data was collected on CD11b\(^+\) leukocytes. (b-d) Splenocytes from WT and \(\text{Lyn}^{-/-}\) mice were harvested, incubated with 10 \(\mu\)M 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 \(\text{Lyn}^{-/-}\) 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-\(\gamma\)\(^+\) T cells. Statistical analysis was by a two tailed unpaired student \(t\) test; *: \(p<0.05\); **: \(p<0.01\); NS: not significant.
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Figure S2. The proportion of blood B cells in Lyn<sup>−/−</sup> mice is independent of IgE, IL-4 and mast cells. (a-c) B cell (B220<sup>+</sup>IgM<sup>+</sup>) 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<sup>+/+</sup>Lyn<sup>+/+</sup> and Igh7<sup>−/−</sup>Lyn<sup>−/−</sup>, per group and per genotype, n=12; For WT, n=10 and Lyn<sup>−/−</sup> n=12. WT and Lyn<sup>−/−</sup> mice were on a C57BL/6 background. (b) For Il-4<sup>+/+</sup>Lyn<sup>+/+</sup> and Il-4<sup>−/−</sup>Lyn<sup>−/−</sup>, per group and per genotype, n=12; For WT, n=10 and Lyn<sup>−/−</sup> n=12. (c) For Kit<sup>W<sub>sh</sub>/W<sub>sh</sub></sup> and Kit<sup>W<sub>sh</sub>/W<sub>sh</sub>Lyn<sup>−/−</sup></sup>, 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<sup>−/−</sup> n=12. (a-c) 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 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 ± 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 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^hiIgM^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.
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; Kitw−/−W-sh, n=18 and Kitw−/−W-sh/Lyn−/−, n=18. Data shown are means ± s.e.m. Statistical analysis was by a two tailed unpaired student t test; *: p<0.05; **: p<0.01; ***: p<0.001.
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 ± 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.
Supplemental Figures

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**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) For *Kit−Wsh/WshLyn−/−*, 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<sup>−/−</sup> 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 µm.
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 µm.
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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.
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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 ± 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.
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, FcεRIα 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-12 p40 production. Filled: isotype control, dashed: IgE+Ag stimulated BMBa, solid black: PMA/ionomycin stimulated BMBa, solid grey: BM cell population CD49b−FcεRIα− (non-basophil, non-mast cell) producing IL-12 p40 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-12 p40 or IFN-γ.
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**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).
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 ± 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 ± s.e.m.
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 ± 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              | 14 (33.3%)       |
| African American       | 10 (23.8%)       |
| Hispanic               | 12 (28.6%)       |
| Asian                  | 6 (14.3%)        |
| **Disease duration (Mean, SD, years)** | 12.9±10.8 |
| **Anti-dsDNA Ab positive (n, %)** | 33 (55%) |
| **SLEDAI**             |                  |
| Mean, SD               | 4.57±5.5         |
| Median, (minimum, maximum) | 4.0 (0, 24) |
| **Current prednisone dose (mg/day)** | 12.6±14.9 |
| Mean, SD               | 7.5 (0, 60)      |
| Median, (minimum, maximum) |            |
| **Concurrent immunosuppressive therapy (n, %)** | 35 (83%) |
| hydroxychloroquine     | 4 (9.5%)         |
| mycophenolate mofetil  | 3 (7%)           |
| cyclophosphamide       | 7 (17%)          |
| azathioprine           |                  |
Supplementary Table 2: Commercial antibodies used.

| Application | Used In Figure # | Description | Conjugate | Clone | Company |
|-------------|------------------|-------------|-----------|-------|---------|
| Basophil depletion | S1 | Armenian Hamster IgG | - | eBo299Arm | eBioscience |
| Basophil depletion (isotype control) | S1 | Rat anti-mouse FcR/ IgG | - | eBo299Arm | eBioscience |
| ELISA | 5 | Human IgE | - | HE1 | Abbiotec |
| ELISA | 7 | Mouse anti-human IgG | HRP | ICL | Invitrogen |
| ELISA | 5 | Donkey anti-human IgG, Fc-specific | HRP | Jackson-R | Invitrogen |
| ELISA | 3 | Rat Anti-Mouse IgE | HRP | 23G3 | Southern Biotech |
| ELISA | S14 | Mouse anti-human IgG1 | HRP | H6070 | Invitrogen |
| ELISA | S14 | Mouse anti-human IgG2 | HRP | H6014 | Invitrogen |
| ELISA | S14 | Mouse anti-human IgG3 | HRP | H6049 | Invitrogen |
| ELISA | S14 | Mouse anti-human IgG4 | HRP | H6023 | Invitrogen |
| Flow cytometry | 4, 5 | Rat anti-mouse CD117 (c-Kit) | APC | 2B8 | BD Biosciences |
| Flow cytometry | 13 | Rat anti-mouse CD117 (c-Kit) | PE-Cy5 | 2B8 | BD Biosciences |
| 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 | XM31.2 | BD Biosciences |
| Flow cytometry | S2, S3, S4 | Rat anti-mouse CD45RB/220 | APC | 74-4-B220 | BD Biosciences |
| Flow cytometry | S6, S15 | Mouse anti-human CD20/3c | PE | E-NPPC | BioLegend |
| Flow cytometry | S15 | Mouse anti-human FcεR alpha | FITC | CRA1 | BioLegend |
| Flow cytometry | S2, S5, S17 | Mouse anti-human CD123 | PerCP-Cy5.5 | 6B6 | BioLegend |
| Flow cytometry | S15 | Mouse anti-human CD117 (c-Kit) | APC | 104D2 | BioLegend |
| Flow cytometry | S15 | Mouse anti-human CD11b | APC | ICRF44 | BioLegend |
| Flow cytometry | 6 | Mouse anti-human CD62L | FITC | DREG-56 | BD Biosciences |
| Flow cytometry | S15 | Mouse anti-human HLA-DR | PerCP-Cy5.5 | L243 | BD Biosciences |
| Flow cytometry | S12 | Rat anti-mouse IP-10 | PE | XMG1.2 | BioLegend |
| Flow cytometry | 3 | Rat anti-mouse interleukin-4 (IL-4) | PE | 11B11 | BioLegend |
| Flow cytometry | 3, 4, 5 | Rat anti-mouse CD48b/Pan-NK Cells | FITC | DX5 | BD Biosciences |
| Flow cytometry | 3, 4, 5 | Rat anti-mouse CD11b | PerCP-Cy5.5 | M170 | BioLegend |
| Flow cytometry | S10 | Rat anti-mouse IL-12/IL-23, P40 | PE | C56-5 | BioLegend |
| Flow cytometry | 4, 5, S7, S13 | Hamster anti-mouse FcR/ | PE | XMG1.2 | BD Biosciences |
| Flow cytometry | S12 | Rat anti-mouse interleukin-4 (IL-4) | FITC | BV6/246Z | BD Biosciences |
| Flow cytometry | S12 | Rat anti-mouse interleukin-4 (IL-4) | FITC | BVD6-246Z | BD Biosciences |
| Flow cytometry | 3 | Rat anti-mouse FcR/ | Alexa Fluor®647 | MAR1 | BD Biosciences |
| Flow cytometry | 4 | Rat anti-mouse BAFF/Blys/TNFSF13B | PE | 121080 | R&D Systems |
| Flow cytometry | 2 | Rat anti-mouse CD18 | PE-Cy5 | 6D5 | BioLegend |
| Flow cytometry | 2 | Rat anti-mouse CD13B | PE | 2B1-2 | BD Biosciences |
| Flow cytometry | 4, S13 | Rat anti-mouse I-A/E | Alexa Fluor®647 | M5114 16.2 | BioLegend |
| Flow cytometry (isotype control) | S1 | Rat anti-mouse CD4 | PerCP-Cy5.5 | RM4-5 | BD Biosciences |
| Flow cytometry (isotype control) | S12 | Armenian Hamster IgG | Alexa Fluor®647 | eBo299Arm | eBioscience |
| Flow cytometry (isotype control) | 4, 5 | Armenian Hamster IgG | PerCP-Cy5.5 | A95-1 | BD Biosciences |
| Flow cytometry (isotype control) | 3, 4, S1, S6, S7, S12 | Rat IgG2b, k | PE-Cy5 | A95-1 | BD Biosciences |
| Flow cytometry (isotype control) | 4, S1, S7 | Rat IgG2b, k | Alexa Fluor®647 | RTK4350 | BioLegend |
| Flow cytometry (isotype control) | S1 | Rat IgG2b, k | PerCP-Cy5.5 | R35-5 | BD Biosciences |
| Flow cytometry (isotype control) | 2 | Rat IgG2a, k | PE-Cy5 | RTK2758 | BioLegend |
| Flow cytometry (isotype control) | S1 | Rat IgG2a, k | Alexa Fluor®647 | RTK4350 | BioLegend |
| Flow cytometry (isotype control) | 2, 4, S1, S12 | Rat IgG2a, k | PE | RTK2758 | BioLegend |
| Flow cytometry (isotype control) | S12 | Rat IgG2a, k | FITC | R3-34 | BD Biosciences |
| Flow cytometry (isotype control) | 3 | Rat IgG1, k | FITC | 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, S11, S13 | Rat IgG1, k | FITC | RTK2118 | BioLegend |
| Flow cytometry (isotype control) | S6, S15 | Mouse IgG1, k | PE | MOPC-21 | BD Biosciences |
| Flow cytometry (isotype control) | S6, S15 | Mouse IgG2b, x | FITC | MPC-11 | BD Biosciences |
| Flow cytometry (isotype control) | S6, S15 | Mouse IgG1, x | APC | MOPC-21 | BD Biosciences |
| Flow cytometry (isotype control) | S6, S15 | Mouse IgG2a, x | PerCP-Cy5.5 | MOPC-173 | BD Biosciences |
| Flow cytometry (isotype control) | S6, S15 | Mouse IgG1, x | PerCP-Cy5.5 | MOPC-21 | BD Biosciences |
| Flow cytometry (isotype control) | 6, S15 | Mouse IgG2b, x | PE | MOPC-21 | BD Biosciences |
| Flow cytometry, immunofluorescence | S9 | Rat anti-mouse IgG | FITC | RTK2758 | BioLegend |
| Flow cytometry, immunofluorescence | S2, S3, S4, S9 | Goat Anti-Mouse IgG (human Adsorbed) | FITC | Polyicnic | Serotec Inc |
| Flow cytometry, immunofluorescence | S2, S3, S4, S9 | Goat Anti-Mouse IgM (human Adsorbed) | FITC | Polyicnic | Serotec Inc |
| Flow cytometry, immunofluorescence | S2, S3, S4, S9 | Goat Anti-Mouse IgE | FITC | Polyicnic | Serotec Inc |
| Flow cytometry, immunofluorescence | S2, S3, S4, S9 | Goat Anti-Mouse IgG | FITC | Polyicnic | Serotec Inc |
| Flow cytometry, immunofluorescence | S2, S3, S4, S9 | Goat Anti-Mouse IgM (human Adsorbed) | FITC | Polyicnic | Serotec Inc |
| Flow cytometry, immunofluorescence | S2, S3, S4, S9 | Goat Anti-Mouse IgE | FITC | Polyicnic | Serotec Inc |
| Flow cytometry, immunofluorescence | S2, S3, S4, S9 | Goat Anti-Mouse IgG | FITC | Polyicnic | Serotec Inc |
| Immune complexes | S3, S12 | Rat anti-mouse IgG | - | R3-35 | BD Biosciences |
| Immune complexes | S3, S12 | Mouse IgG1, k | MOPC-21 | Sigma-Aldrich |
| Immune complexes | S3, S12 | Mouse IgG1, k | MOPC-21 | Sigma-Aldrich |
| Immune complexes | S3, S12 | Rat anti-mouse IgG | Polyclonal | Sigma-Aldrich |
| Immune complexes | S3, S12 | Rat anti-mouse IgG | Polyclonal | Sigma-Aldrich |
| Immune complexes | S3, S12 | Rat anti-mouse IgG | Polyclonal | Sigma-Aldrich |
| Immunofluorescence | S9 | Rat anti-mouse Complement Component C3 | FITC | RhC119 | Cederlane Lab |
| Immunofluorescence | S9 | Goat Anti-Mouse IgG, Fc fragment (Fab')2 | FITC | Polyicnic | Sigma-Aldrich |
| Immunofluorescence, western blot | S10 | Goat anti-mouse IgG | FITC | R3-35 | BD Biosciences |
| Immunofluorescence, western blot | S10 | Goat anti-mouse IgG | FITC | R3-35 | BD Biosciences |
| Immunofluorescence, western blot | S10 | Goat anti-mouse IgG | FITC | R3-35 | BD Biosciences |
| Western blot | S10 | Goat anti-mouse IgG | Alexa Fluor®680 | Polyicnic | Invitrogen |
| Western blot | S10 | Goat anti-mouse IgG | Alexa Fluor®680 | Polyicnic | Invitrogen |

HRP: Horseradish Peroxidase
FITC: Fluorescein
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 CO\textsubscript{2} 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 NH\textsubscript{4}Cl, 12 mM NaHCO\textsubscript{3}, 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% NaN\textsubscript{3}). Basophils were identified as CD49b\textsuperscript{+}Fc\varepsilon RI\alpha\textsuperscript{+}CD11b\textsuperscript{+}CD117\textsuperscript{−}. B cells were identified as B220\textsuperscript{+}IgM\textsuperscript{+}.

**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
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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\textsuperscript{hi} IgM\textsuperscript{+} (an intermediate mean fluorescence intensity is seen in this IgM\textsuperscript{+} 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\textsuperscript{+} cells in the spleen, the CD11b\textsuperscript{hi} population was gated.

**Peritoneum**

Proportion of peritoneal mast cells being mast cells were determined as previously described\textsuperscript{20}.

**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 centrifugated 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) scores\(^{35}\). 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, IgG\(_1\), IgG\(_{2a}\), IgG\(_{2b}\) and IgE ELISA kits were purchased from Bethyl Laboratories.