Supplementary Materials for

The tyrosine kinase Lyn limits the cytokine responsiveness of plasma cells to restrict their accumulation in mice

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Fig. S1. Lyn−/− mice accumulate plasma cells with an increased abundance of Blimp-1. Splenocytes were isolated from WT and Lyn−/− mice at 5 and 20 weeks of age and then were analyzed by flow cytometry. Cells were gated first for viability and then for their expression of CD138 and GFP (a surrogate marker for Blimp-1). Plots show the percentages of GFP⁺CD138⁺ cells of the indicated mice and are representative of at least five mice of each genotype.
Fig. S2. B cells from Lyn−/− and wild-type mice differentiate similarly in vitro. (A and B) B cells were purified from the spleens of WT mice (open bars) and Lyn−/− mice (gray bars) with magnetic beads and then were stimulated in triplicate cultures with CD40L, IL-4, and IL-5 in the absence or presence of IL-6 for 3 days. (A) The percentages of plasma cells were determined by flow cytometric analysis of CD138 staining. (B) The numbers of plasma cells were measured by adding a known number of beads to each well and then analyzing the samples by flow cytometry to calculate the total number of plasma cells in each well. Data are means ± SEM of triplicate cultures from a single experiment, which is representative of four independent experiments.
Fig. S3. Analysis of spleen size and B cell numbers in chimeric mice generated by bone marrow reconstitution. (A) and (B) Chimeric mice were generated by bone marrow reconstitution as described in the Materials and Methods. (A) The numbers of splenocytes in the chimeric mice was determined 15 weeks after reconstitution. The genotypes of bone marrow donors in the mixed chimeras is indicated below the graph, with B-lineage cells derived obligatorily from the 20% donor (WT or Lyn^−/−), whereas all other lineages derived from the 80% μMT donor (WT or Lyn^−/−). Data are means ± SEM from four mice for each condition in a single experiment, which is representative of two independent experiments. (B) The numbers of B cells (defined as CD19^+ cells) in the spleens of the indicated chimeric mice were determined by flow cytometric analysis. Data are means ± SEM from three mice for each condition from a single experiment, which is representative of two independent experiments.
Fig. S4. The cell surface abundance of CXCR4 is similar on plasma cells from wild-type and Lyn−/−Il6−/− mice. Plasma cells from the spleens of WT, Lyn−/−, and Lyn−/−Il6−/− mice, as indicated, were analyzed by flow cytometry such that plasma cells from WT and Lyn−/− mice were gated as CD138+GFP^{hi} (Blimp-1^{hi}), whereas plasma cells (PC) from Lyn−/−Il6−/− mice were gated as B220^{int}/CD138^{+}. The MFIs for CXCR4 on these cells are shown, with each mouse represented by an individual symbol. Means are shown as horizontal bars, and at least three mice of each genotype were analyzed.
Fig. S5. Plasma cells from Lyn−/− and wild-type mice exhibit similar survival kinetics in the presence of IL-21, BAFF, and Flt3L. Plasma cells sorted from the spleens of WT and Lyn−/− reporter mice as CD138+Blimp-1hi (GFPhi) cells were cultured for 3 days in medium alone or in medium containing IL-6 (50 ng/ml), BAFF (500 ng/ml), IL-21 (20 ng/ml), or Flt3L (125 ng/ml). The numbers of plasma cells at day 3 were measured by adding a known number of beads to each well and then analyzing the samples by flow cytometry to calculate the total number of viable plasma cells in each well, which was then expressed as the percentage of starting cells that survived. Data are means ± SD from triplicate wells from a single experiment and are representative of two independent experiments.
Fig. S6. Analysis of the cell surface abundance of CD123 on plasma cells. (A) Splenocytes isolated from WT and Lyn−/− mice were analyzed by flow cytometry and gated as viable CD138+Blimp-1hi (mature plasma cells, GFPhi) and CD138+Blimp-1int (less mature plasma cells, GFPint). Each population was then analyzed for the cell-surface abundance of CD23 with a specific antibody (gray line), which was compared to staining with an isotype control antibody (dotted line). (B) Splenic basophils gated as CD49b+CD45flowFcεR1+ served as a positive control for CD123 staining, whereas B cells served as a negative control. For (A) and (B), a representative histogram is shown (from one of two mice per condition), and data are representative of three independent experiments.
Fig. S7. JAK1/2 inhibition in Lyn<sup>−/−</sup> mice with AZD1480. (A to D) Lyn<sup>−/−</sup> mice were treated with the JAK1/2 inhibitor AZD1480 (60 mg/kg) or with vehicle for 4 days. The inhibitor- and vehicle-treated mice were then analyzed to determine (A) the numbers of plasma cells in the spleen, (B) the total numbers of splenocytes, (C) the total numbers of thymocytes, and (D) the numbers of B cells in the spleen. Data are means ± SEM of the numbers of the indicated cells from at least six mice for each condition from two independent experiments. Each mouse is represented by an individual symbol. Data were analyzed by the student’s t-test. (E) Plasma cells (CD138<sup>+</sup>Blimp-1<sup>+</sup>) isolated from WT and Lyn<sup>−/−</sup> mice were left untreated or were pretreated for 15 min with 1 µM AZD1480 or DMSO before being stimulated with IL-6. Cells were then analyzed by flow cytometry to determine the MFI of pSTAT3. Histograms are from a single experiment and are representative of two independent experiments.
Fig. S8. Effects of treatment of plasma cells with PP2 in vitro. (A and B) Plasma cells (CD138\(^{+}\)Blimp-1\(^{+}\)) sorted from the spleens of WT and Lyn\(^{+}\) mice were left untreated or were pre-treated with 50 nM PP2 or DMSO for 15 min before being stimulated with IL-6 (50 ng/ml). Cells were then analyzed by flow cytometry to detect pSTAT3. (A) Flow cytometry plots are representative of two independent experiments. (B) The MFIs of pSTAT3 for the indicated samples were determined. Data are representative of two independent experiments. (C) Splenocytes isolated from three mice were left untreated or were pre-treated with 50 nM PP2 before being stimulated for 5 min with anti-IgM F(ab')2 (40 µg/ml). Cells were then analyzed by flow cytometry to determine the MFI of pERK. Data are means ± SEM from three mice for each condition and are representative of three independent experiments.