SUPPLEMENTAL MATERIAL

Barrett et al., http://www.jem.org/cgi/content/full/jem.20100793/DC1
Figure S1. Dectin-2 expression in GM-CSF–expanded BM cultures. Day-7 BMCs\textsuperscript{GM-CSF} were harvested and stained for the cell surface expression of CD11c, MHCII, and Dectin-2 and analyzed by flow cytometry. (A) Left, a representative histogram is shown of CD11c and MHCII expression, as gated by the isotype controls. Gating on a CD11c\textsuperscript{hi}MHCII\textsuperscript{lo} and a CD11c\textsuperscript{+}MHCII\textsuperscript{hi} subset is also indicated (small box and small rectangle, respectively). MHCII staining was performed with saturating antibody concentrations. Right, Dectin-2 expression on the CD11c\textsuperscript{hi}MHCII\textsuperscript{lo} subset (thick black line) and the CD11c\textsuperscript{+}MHCII\textsuperscript{hi} subset (thin black line) is shown, as well as the isotype control staining for each population (thick dashed and thin dashed lines, respectively). (B) Top, a representative histogram is shown of CD11c and Dectin-2 expression, as gated by the isotype controls. Bottom, CD11b and Ly6C expression on the Dectin-2\textsuperscript{+}CD11c\textsuperscript{−} population. (C) Dectin-2 expression on cytospin preparations from day-7 BMCs\textsuperscript{GM-CSF} detected by immunocytochemistry. Positive staining is indicated in brown. Left, anti-Dectin-2. Right, rat IgG2a control. Results are representative of four cultures from two independent experiments.
Figure S2. Cys-LT and cytokine production by BMCsGM-CSF after lentiviral knockdown. BMCsGM-CSF were infected with viral particles containing Dectin-2 shRNA (kdBMCsGM-CSF), an empty vector control (cBMCsGM-CSF), or a nontargeting vector control (ntcBMCsGM-CSF), selected with puromycin, and harvested at day 7. (A) BMCsGM-CSF were stimulated for 30 h with Df at the indicated concentrations, and cytokines in the supernatant were measured by ELISA. Results are means ± SEM (n = 5–6 mice per group) from three independent experiments. *, P = 0.01; **, P = 0.004; ***, P = 0.002. Significance was determined with an unpaired Student’s t test. (B) Left, TNF production from cBMCsGM-CSF (black) or kdBMCsGM-CSF (white) in response to Df, curdlan, or LPS at 4 h. Results are means ± SEM (n = 5–6) from three independent experiments. *, P = 0.04. Significance was determined with an unpaired Student’s t test. Right, Cys-LT production from cBMCsGM-CSF (black) or kdBMCsGM-CSF (white) in response to 10 and 100 µg/ml Df or 20 and 200 µg/ml curdlan at 60 min. Results are means ± SEM (n = 4–5) from two independent experiments. *, P = 0.02. Significance was determined with an unpaired Student’s t test.
Figure S3. Df-elicited CD4+ Th2 cell recruitment to the lung. WT mice were sensitized with saline-pulsed or Df-pulsed WT or Ltc4s−/− BMCsGM-CSF and challenged with Df as described in the Fig. 4 legend. Pulmonary mononuclear cells were isolated and stimulated with 50 ng/ml PMA and 1 µM ionomycin for 2 h. 2.5 µM monensin was added and cells were stimulated for an additional 8 h, fixed, permeabilized, stained for cell surface expression of CD4 and CD8 and for intracellular expression of IL-4, IL-5, IL-17A, and IFN-γ, and analyzed by flow cytometry. (A) Cell size (top) and CD4 and CD8 expression (bottom) from mice sensitized with Df-pulsed WT (left) or Ltc4s−/− (right) BMCsGM-CSF. (B) Intracellular cytokine staining in CD4+ cells in the lymphocyte gate from mice sensitized with Df-pulsed WT (left) or Ltc4s−/− (right) BMCsGM-CSF with the percentage of cytokine-positive cells noted.
Figure S4. BMCSGM-CSF MHCII expression is required for Df-elicited pulmonary inflammation and CD4+ T cell cytokine generation. WT C57BL/6 mice were sensitized with intranasal administration of 10^4 Df-pulsed WT or MHCII(Δ/Δ) BMCSGM-CSF, challenged with 10 µg Df on day 22, day 24, and day 26, and analyzed on day 27. (A) Cells from the BAL fluid were counted and 400 cells/slide in cytopsins were counted for specific cell types. Results are means ± SEM (n = 8–10 mice per group) from two experiments. *, P = 0.0005; **, P = 0.0002; ***, P = 0.0001. Significance was determined with an unpaired Student’s t test. (B) Parabronchial lymph node cells were isolated, stimulated as in Fig. S3 for a total of 6 h, stained for cell surface expression of CD4 and CD8 and for intracellular expression of IL-4, IL-5, IL-17A, and IFN-γ, and analyzed by flow cytometry. Representative histograms show intracellular cytokine staining on CD4+ cells in the lymphocyte gate from WT recipients of Df-pulsed WT (left) or MHCII(Δ/Δ) (right) BMCSGM-CSF.
Figure S5. WT and Ltc4s−/− BMCsGM-CSF have comparable cell surface expression of costimulatory markers. WT and Ltc4s−/− BMCsGM-CSF were grown to day 7, pulsed with 100 µg/ml Df for 24 h, harvested, and stained for the cell surface expression of CD11c, MHCII, CD80, CD86, CD40, OX40L, CCR7, PD-L1, and ICOSL, and analyzed by flow cytometry. Representative histograms are shown of costimulatory marker expression on CD11c+MHCII+ BMCsGM-CSF. Specific stainings are shown for WT (thick black lines) and Ltc4s−/− (thick gray lines) BMCsGM-CSF, and isotype control stainings are shown for WT (dotted black lines) and Ltc4s−/− (dotted gray lines) BMCsGM-CSF.
Figure S6. WT and \textit{Ltc4s$^{-/-}$} BMCs\textsubscript{GM-CSF} generate comparable concentrations of \textit{Df}-elicited cytokines at 4 and 24 h. WT (black bars) and \textit{Ltc4s$^{-/-}$} (white bars) BMCs\textsubscript{GM-CSF} were grown to day 7 and stimulated with \textit{Df} at the indicated concentrations for either 4 or 24 h, and cytokines in the supernatants were measured by ELISA. Results are means ± SEM (\(n = 4\) mice per group) from two independent experiments.
Figure S7. WT and Ltc4s−/− BMCsGMCs show comparable migration to the lung-draining lymph nodes. Day-7 BMCsGMCs were stimulated with 100 μg/ml of unlabeled Df or Alexa Fluor 647-Df for 18 h, and 10⁶ cells were transferred intranasally into WT recipients. 24 h later, the draining lymph nodes were harvested and digested, and single cell suspensions were stained for CD11c-PE-Cy7 and MHCII-PE and analyzed by flow cytometry. The percentage of CD11c⁺MHCII⁺ cells that were Alexa Fluor 647 positive is shown. Results are means ± SEM (n = 4–5 mice per group) from two independent experiments.