Supporting Information

Cook et al. 10.1073/pnas.1121231109

SI Materials and Methods

Infections. Mice were infected with *L. sigmodontis* for 10 d or with *S. mansoni* for 8 wk, as previously described (1, 2).

BMDC Stimulation and DC: T-Cell Coculture. In selected experiments, DCs were exposed to endotoxin-free soluble egg Ag [SEA, 25 μg/mL, prepared as described elsewhere (3)], heat-killed *Propionibacterium acnes* (Pa, 10 μg/mL), lipopolysaccharide (LPS, 250ng/mL, Sigma) or CpG oligodeoxynucleotide 1826 (ODN 1826, 5 μg/mL, InvivoGen). To assess further activation, CD40 ligation with αCD40 mAb was carried out as previously described (4). In vitro coculture polarization experiments were performed with 50,000 IL10-eGFP$^-$CD4$^+$ or KN2 CD4$^+$. T cells were cultured in 96-well plates for 3–4 d with 2,500 RELMα sufficient or *Retnla*$^{-/-}$ BMDCs, 1 μg/mL anti-CD3, with or without IL-4 (20 ng/mL). For CFSE dilution assays, CD4$^+$ OT-II TCR Transgenic T cells were purified from spleen and LN using CD4$^+$ Dynabeads (Invitrogen) following the manufacturer's protocol. T cells were labeled with 5 μM CFSE (Invitrogen) for 15 min at 37 °C. Excess CFSE was allowed to leach from the cells before culture with 5×10^4 WT, *Retnla*$^{-/-}$, or *Il4ra*$^{-/-}$ BMDCs in the presence of 0.01 μg/mL OVA$_{323–339}$ or 5 μg/mL OVA protein (Sigma), which had been endotoxin depleted in-house. Cultures were incubated at 37 °C for 4 d before assessment of CFSE dilution by flow cytometry.

Flow Cytometry. In some experiments, cells were first stained with LiveDead aqua (Invitrogen). Following FcR-Block (2.4G2), cells were surface stained using the following mAb: CD4-PE, CD11c-APC/eFluor780, MHCII-PerCP/Cy5.5, CD11b-eFluor450, CD80-APC, CD40-PE, CD19-AlexaFluor700, CD86-FITC, Dectin1-APC, IL-4Rα-biotin, SiglecF-PE, F4/80-PE/Cy7, Gr1-PerCP/Cy5.5, and HuCD2-APC. For intracellular staining, cells were fixed in 1% PFA, permeabilized using Cytoperm (BD PharMingen), and stained with anti-RELMα, and anti-Ym1 biotin followed by AlexaFluor 488–conjugated anti-rabbit Ab and streptavidin-APC. To assess Ag uptake, 2×10^5 BMDCs were incubated with 200 μg FITC-conjugated dextran (Sigma) for 30 min at 37 °C or on ice before acquisition.

Splenic DC Purification. Splenic DCs were enriched from five to six naive C57BL/6 mice using NycoDenz density gradient separation and CD11c^{hi}B220$^-$ cDCs were sorted from B220$^+$CD11c^{mid} pDCs using a BD FACs Aria II. After sorting, 5×10^4 DCs were cultured overnight with 20 ng/mL IL-4, before RNA extraction, reverse transcription, and quantification of transcript levels using quantitative PCR.

Arginase Activity Assay. Arginase activity was measured as described previously (5). One unit of arginase enzyme activity is defined as the amount of enzyme that catalyzed the formation of 1 μmol urea per minute at 37 °C.

Quantitative PCR. Relative quantification of the gene of interest was performed by qPCR analysis using Roche Light Cycler 480, with LightCycler SYBR Green I Master mix, compared with a serially diluted standard of pooled cDNA. Expression was normalized to hypoxanthine-guanine phosphoribosyltransferase (*Hprt*).

Primers were as follows:

Hprt-F: 5′-TCCTCCTCAGACCGCTTTT-3′,
Hprt-R: 5′-CCTGGTTCATCATCGCTAATC-3′,
Retnla-F: 5′-TATGAACAGATGGGCCTCCT-3′,
Retnla-R: 5′-GGCAGTTGCAAGTATCTCCAC-3′,
Chi3l3-F: 5′-GAACACTGAGCTAAAAACTCTCCTG-3′,
Chi3l3-R: 5′-GACCATGGCACTGAACGAG-3′,
Clec7a-F: 5′-ATGGTTCTGGGAGGATGGAT-3′,
Clec7a-R: 5′-GCTTTCCTGGGGAGCTGTAT-3′,
Mrc1-F: 5′-TCATTGGAAGATCCACTCTGG-3′,
Mrc1-R: 5′-CAGCGCTTGTGATCTTCATTATAG-3′,
Arg1-F: 5′-GTCTGTGGGGAAAGCCAAT-3′,
Arg1-R: 5′-GCTTCCAACTGCCAGACTGT-3′.
Ccl24-F: 5′-GCAGCATCTGTCCCAAGG-3′,
Ccl24-R: 5′-GCAGCTTGGGGTCAGTACA-3′.

1. Jenkins SJ, et al. (2011) Local macrophage proliferation, rather than recruitment from the blood, is a signature of TH2 inflammation. *Science* 332:1284–1288.
2. Phythian-Adams AT, et al. (2010) CD11c depletion severely disrupts Th2 induction and development in vivo. *J Exp Med* 207:2089–2096.
3. MacDonald AS, Straw AD, Bauman B, Pearce EJ (2001) CD8- dendritic cell activation status plays an integral role in influencing Th2 response development. *J Immunol* 167:1982–1988.
4. Perona-Wright G, et al. (2009) A pivotal role for CD40-mediated IL-6 production by dendritic cells during IL-17 induction in vivo. *J Immunol* 182:2808–2815.
5. Mylonas KJ, Nair MG, Prieto-Lafuente L, Paape D, Allen JE (2009) Alternatively activated macrophages elicited by helminth infection can be reprogrammed to enable microbial killing. *J Immunol* 182:3084–3094.

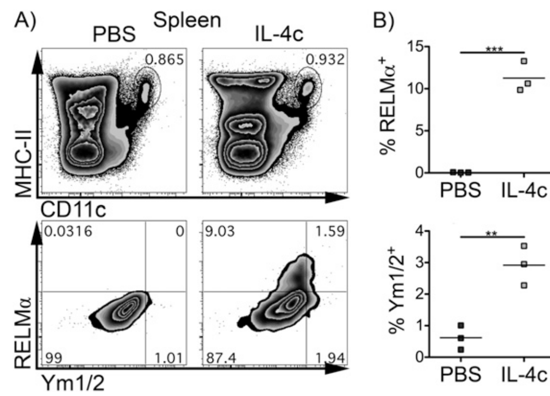

Fig. S1. IL-4c injection induces alternative activation of splenic DCs. Splenic DCs (MHC-II+ CD11c^{hi}) from PBS- or IL-4c–treated mice (2×5 μg, d 0 and d 2) were harvested at d 4 and stained for intracellular expression of RELMα and Ym1/2 (*A* and *B*). Data are representative of at least three experiments. Graphs show percent expression for individual mice, three per group.

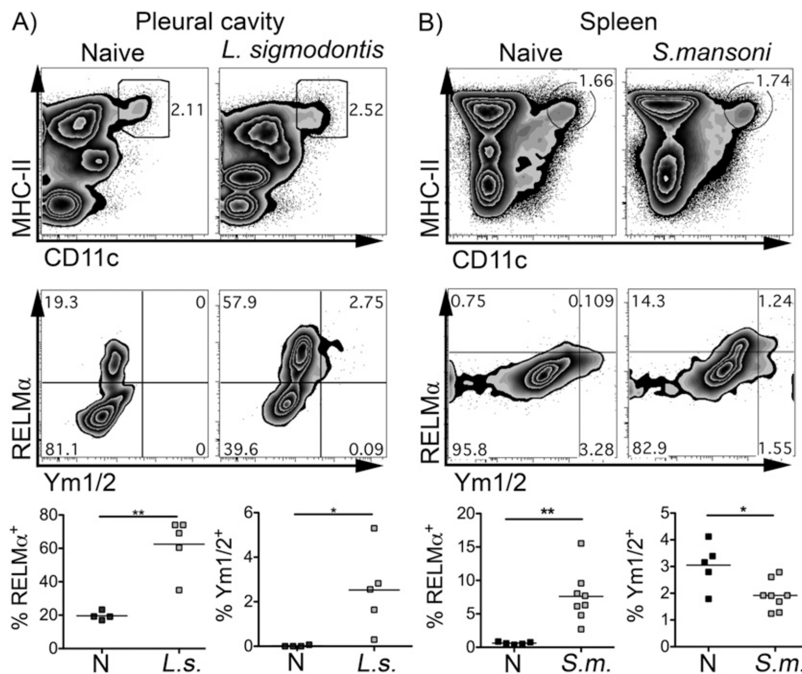

Fig. S2. Parasitic helminth infection induces alternative activation of DCs at multiple tissue sites. Pleural cavity (*A*) or splenic (*B*) DCs (MHC-II+ CD11c^{hi}) from naive mice infected with *L. sigmodontis* (*L.s.*) (*A*) or *S. mansoni* (*S.m.*) (*B*) were stained for intracellular expression of RELMα and Ym1/2. Data are representative of three to six experiments. Graphs show percent expression for individual mice, four to eight per group.

Fig. S3. IL-4–stimulated BMDCs do not dramatically alter their activation phenotype. WT BMDCs were stimulated overnight with 20 ng/mL IL-4, and their expression of costimulatory molecules was assessed by flow cytometry (*A*). Shaded area indicates isotype control; black line indicates media; gray line indicates +IL-4. Geometric mean fluorescence intensity (MFI) of marker expression by CD11c^{+} cells (*B*). Data are representative of more than five experiments. Error bars represent SEM of triplicate wells.

Fig. S4. IL-4 alternatively activates splenic ex vivo DCs in vitro. Following enrichment for DC populations, splenic cDCs (CD11c^{hi}B220^{-}) from naive mice were separated from pDCs (B220^{+}CD11c^{mid}) (*A*) and cultured overnight in the presence of 20 ng/mL IL-4. IL-4 increased mRNA expression of alternative activation markers (*B*). Data are representative of two experiments, with three to four replicate wells per group. a.u., Arbitrary units; N.D., not detected.

Fig. S5. Defined TLR ligands and pathogen products modulate IL-4–driven RELMα and Ym1 expression by BMDCs. BMDCs were cultured overnight in the presence or absence of IL-4 and TLR agonists CpG 1826 (TLR9) or LPS (TLR4) (*A* and *B*) or Pa or SEA (*C–E*). IL-12p70, IL-10, RELMα, Ym1/2, IL-1β, and IL-6 secretion was assessed by ELISA. Error bars represent SEM of triplicate wells. Data are representative of four (*A–D*) or two (*E*) experiments.

Fig. S6. WT, *Il4ra*$^{-/-}$, and *Retnla*$^{-/-}$ DCs are equally capable at antigen uptake. WT, *Il4ra*$^{-/-}$ or *Retnla*$^{-/-}$ BMDCs were incubated with FITC-conjugated dextran at 37 °C, and Ag uptake was assessed by flow cytometry (*A* and *B*). Shaded area indicates 0 °C; black line indicates 37 °C. Data are representative of two experiments, five to six replicate wells per group.

Fig. S7. Ag processing and presentation is unimpaired in *Il4ra*$^{-/-}$ and *Retnla*$^{-/-}$ DCs. WT, *Il4ra*$^{-/-}$, or *Retnla*$^{-/-}$ BMDCs were cultured for 4 d with CFSE-labeled OT-II TCR Tg T-cells in the presence of OVA peptide (*A*) or protein (*B*), and the ability to stimulate proliferation was assessed by flow-cytometric analysis of CFSE dilution. Shaded area indicates no peptide/protein; black line indicates 0.01 μg/mL OVA peptide (*A*) or 5 μg/mL OVA protein (*B*). Data are representative of two experiments, five replicate wells per group.

Fig. S8. IL-4 alters the response of DCs following CD40 ligation. BMDCs cultured overnight with IL-4 and Pa or SEA were washed and recultured with agonistic anti-CD40 (■) or control antibody (□) for another 24 h, and cytokine (*A*), RELMα, and Ym1/2 (*B*) secretion was assessed by ELISA. Data are representative of three experiments. Error bars represent SEM of triplicate culture wells.