Supplemental Experimental Procedures

Animals. Mice were purchased from either Jackson Laboratory (Bar Harbor, ME; WT: C57BL/6J; CD86–/–: B6.129S4-*Cd86*^{tm1Shr}/J; CD28–/–: B6.129S2-*Cd28*^{tm1Mak}/J; ICOS–/–: B6.129P2-*Icos*^{tm1Mak}/J; CD80–/–: B6.129S4-*Cd80*^{tm1Shr}/J; MHCII–/–: B6.129S2-*H2*$^{dlAb1\text{-}Ea}$/J; ICOSL–/–: B6.129P2-*Icosl*^{tm1Mak}/J; IL-10–/–: B6.129P2-*Il10*^{tm1Cgn}/J; TLR2–/–: B6.129-*Tlr2*^{tm1Kir}/J; BDCA2-DTR: C57BL/6-Tg(CLEC4C-HBEGF)956Cln/J; CD45.1+ SJL/J.B6: B6.SJL-Ptprc^{a}Pepc^{b}/BoyJ) or Taconic Farms (Germantown, NY; WT: C57BL/6NTac). The genotype of C57BL/6-Tg(CLEC4C-HBEGF)956Cln/J mice was confirmed by polymerase chain reaction (PCR) using the following primers: transgene forward, -GCC CCC GGG CAG CAC AGC CAC TGC CGG TCC; transgene reverse, -GGC CAA GCG CTT GGG CAC TGT TCC CTC CCT; internal positive control forward, -CAA ATG TTG CTT GTC TGG TG; internal positive control reverse, -GTC AGT CGA GTG CAC AGT TT). PCR was performed with Taq2X Master Mix (New England Biolabs, Ipswich, MA) and 0.2 μM of each primer for 35 cycles (94^{o}C/30", 55^{o}C/30", 72^{o}C/30"). All genetically deficient mice and their respective controls were age-matched (6- to 8-week-old) males and were co-housed under specific pathogen–free (SPF) conditions. All experiments on animals were approved by the Harvard Medical Area Standing Committee on Animals (animal protocol numbers 8604781 and 04591).

Unconjugated pure antibodies and other reagents. PDC-depleting functional-grade mAb (anti-mPDCA-1 IgG, clone JF05-1C2.4.1) was purchased from Miltenyi Biotec (Auburn, CA), and the corresponding isotype control IgG (rat IgG2b, clone LTF-2) was purchased from Bio X Cell (West Lebanon, NH). Two injections of antibody (250 μg/injection) were given 1 and 2 days, respectively, before intrarectal administration of TNBS in the murine model of colitis. In the EAE model, four injections of antibody (300–400 μg/injection) were given: the first, 1 day before the first PSA/PBS oral gavage; the second and third, 1 day and 2 days before PLP injection, respectively; and the fourth, 1 day before the final PSA/PBS oral gavage. DT (200 ng/dose) from *Corynebacterium diphtheriae* (Sigma, St. Louis, MO) was administered to BDCA2-DTR mice six times, with two doses on successive days prior to the first PSA gavage, two doses prior to presensitization, and two doses prior to intrarectal administration of TNBS.

Inhibitory antibodies used *in vivo* (anti-ICOSL IgG, clone HK 5.3; anti-CD86 IgG, clone GL-1; and isotype control rat IgG2a, clone 2A3) were purchased from Bio X Cell. Three antibody injections (400 μg/injection) were given: the first, 1 day before the first PSA/PBS oral gavage; the second, 1 day before presensitization; and the third, 1 day before intrarectal administration of TNBS in the murine model of colitis. 1-Methyl-D-tryptophan (Sigma) was used *in vitro* at a previously reported concentration (250 μM) to inhibit IDO (Colvin et al., 2009; Ito et al., 2007). TLR1/TLR2 ligand Pam3CSK4.3HCl (Imgenex, San Diego, CA), TLR2/TLR6 ligand synthetic diacylated lipoprotein FSL-1 (InvivoGen, San Diego), and TLR2 ligand lipomannan from *M. smegmatis* (InvivoGen) were used at the highest dose recommended by the manufacturers.

Fluorochrome-conjugated antibodies and flow cytometry. Mouse-specific IgG mAbs were purchased from either BioLegend (San Diego; biotin-conjugated anti-CD11c, anti-MHCII, anti-ICOSL, anti-CD86, anti-CD80, anti-CD40 and anti-TLR2; allophycocyanin-conjugated anti-B220; fluorescein-conjugated anti-TLR2, anti-SH, anti-PDCA-1, anti-CD11b; and phycoerythrin-conjugated anti-PDCA-1 and anti-SH) or eBioscience (San Diego; phycoerythrin-conjugated anti-TLR2). Streptavidin conjugated with peridinin-chlorophyll-protein complex and allophycocyanin-conjugated anti-CD11c were purchased from BD Biosciences (San Jose, CA). Single-cell suspensions from primary tissues were stained with a suitable combination of fluorochrome-conjugated antibodies, and data were acquired with a BD FACSCalibur flow cytometer (Becton, Dickinson). The data were analyzed with BD CellQuest Pro software. For counting of cells by flow cytometry, Flow-Count Fluorospheres (Beckman Coulter, Brea, CA) were used as per instructions provided by the manufacturer.

BMDCs. DCs were derived from bone marrow from untreated mice as previously described (Gehrie et al., 2011). In brief, bone marrow was collected from femurs and tibias and was treated with red-cell lysis buffer to remove red blood cells. The remaining cells were counted and cultured in RPMI-1640 medium [supplemented with 10 mM HEPES, penicillin-streptomycin (50 units/ml and 50 μg/ml, respectively), 50 μM 2-mercaptoethanol, and 10% fetal bovine serum] at a density of 1.5×10^6/ml with recombinant mouse Flt3L (100 ng/ml; R & D systems, Minneapolis, MN) at 37°C in an atmosphere of 5% CO_2 for 10 days. On the fifth day, ~45% of the medium was carefully replaced with fresh medium containing Flt3L.

Tissue isolation and single-cell suspension. For conversion into single-cell suspensions, splenic and MLN tissues were either treated with collagenase type IV (1 mg/ml; Sigma) for 30 minutes at 37°C in an atmosphere of 5% CO_2 or physically teased and passed through 70-µm mesh. Red blood cells were depleted with lysis buffer. To obtain colonic lamina propria cells, the colon was stripped of stool content and mesentery. The tissue was first cut transversely into small pieces and then cut longitudinally to expose the lumen. Pieces were washed in 1 mM dithiothreitol (Sigma) in PBS for 10 minutes and then in PBS. Three washes of 8 minutes each in 30 mM EDTA in PBS were then employed to strip off epithelial cells. After one more wash in PBS, tissue was treated with RPMI medium containing 5% fetal bovine serum and collagenase type IV (1 mg/ml) for 1 hour at 37°C in 5% CO_2. Finally, tissue sections were teased through mesh (100- and 70-µm) to yield single-cell suspensions.

***In vitro* IFNα release assay.** BMDCs or isolated PDCs were cultured in the presence of 2.5 µM CpGA (InvivoGen) and/or PSA (100 µg/ml). After 20 or 44 hours, culture supernatants were assessed for IFNα with a VeriKine Mouse Interferon-Alpha ELISA Kit (PBL Interferon Source, Piscataway, NJ) according to the manufacturer's instructions.

Supplemental References

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