Supplemental Experimental Procedures

Mice  DC-SIGN−/− mice were from the Consortium for Functional Glycomics (Scripps Res. Inst., La Jolla, CA). Flt3−/− (I. R. Lemischka, Mount Sinai School of Medicine), GMCSF-R−/− (G. Begley, Amgen), MyD88−/− (S. Akira, Univ. of Osaka) and MyD88−/− x Trif−/− (E. Pamer, Memorial Sloan-Kettering Cancer Center) were provided by M. Nussenzweig (Rockefeller Univ.) and iDTR mice by A. Waisman (Univ. of Mainz), and FcR γ−/− mice by J. Ravetch (Rockefeller Univ.). C57BL/6 (CD45.1 or CD45.2), C3H/HeJ, chemokine receptor (CCR2, CCR5, CCR6, and CCR7), Lysozyme-M Cre (LysMcre), and CD14−/− mice were from Jackson Labs and C3H/HeN and splenectomized mice from Taconic Farms. Mice in specific pathogen-free conditions were studied at 6-10 wks according to institutional guidelines of the Rockefeller University.

Lipopolysaccharide (LPS) and bacteria. LPS from E.coli 055:B5 (Sigma) was given i.v., s.c., or i.p. at a dose of 5 µg to induce Mo-DCs. For optimal LPS activity, stocks had to be dissolved at 10 µg/µl or higher. Other TLR agonists were purchased from Invivogen and injected i.v. at 5 µg/mouse. We also tested bacteria at a dose of 5×10^6 per mouse, both heat-killed and living bacteria (E.coli DH5a, B.subtilis). To evaluate presentation of proteins from bacteria, recombinant E.coli expressing OVA was used.

Bone marrow monocytes and DCs Monocytes were sorted on a FACS-Aria (BD Biosciences) as SSClo, CD11bhi, Ly6Chi or as Ly6G−, CD11bhi, Ly6Chi cells, the latter ensuring higher yields. To generate Mo-DCs, monocytes were cultured with cytokines (M-CSF, GM-CSF, GM-CSF, IL-4; PeproTech) at 20 ng/ml or Flt3L at 200 ng/ml in RPMI with 5% FBS, and Antibiotic-Antimycotic plus β-mercaptoethanol (Invitrogen). At 4-7 days, nonadherent cells were removed to test function, or for M-CSF, adherent cells were recovered with Cellstripper™ non-enzymatic cell dissociation solution (Mediatech).
Alternatively, to generate DCs, total bone marrow was cultured with Flt-3L (400 ng/ml) for 9 days as described (Naik et al., 2005), and the equivalents of CD8⁺ and CD8⁻ spleen DCs were sorted as CD24hi, CD11blo and CD24lo CD11bhi cells respectively.

**Monocyte and bone marrow transfer** 2×10⁶ CD45.2⁺ marrow monocytes were transferred to 4-6 wk CD45.1⁺ mice (>8 wks gave poor results). For mixed marrow chimeras, 50:50 mixtures of KO and WT marrow were injected i.v. into lethally irradiated (5.5 Gy twice, 3 h apart) mice. To deplete monocytes, DT (Sigma) in PBS (1 μg/μl, stored at -80 °C) was injected i.v. to LysMcrexIDTR mice at 25 ng/g weight (~500 ng/mouse).

**Antibodies, flow cytometry and microscopy** Rabbit polyclonal antibody to a 14 amino acid cytoplasmic domain peptide of DC-SIGN, and mAbs to DC-SIGN (BMD10, BMD30, and MMD3) were described (Cheong et al., 2010). MAbs were conjugated with biotin or Alexa 647 (Invitrogen) following manufacturer’s instructions. These bound specifically to CHO cells stably expressing mouse DC-SIGN. 22D1 (α-SIGN-R1/CD209b), SER4 (α-CD169), L31 (α-CD207), NLDC145 (α-DEC-205/CD205), N418 (α-CD11c), KL295 (α-MHC II I-Aβd), GL117 (rat IgG2a control), and MEL-14 (α-CD62L) mAbs were purified from hybridoma supernatants or purchased from eBioscience, and tested to be endotoxin-free (QCL-1000 kit, BioWhittaker). We purchased mAbs conjugated to different fluorochromes to CD19, CD3, NK1.1, DX-5, CD206, CD11b, I-A/I-E (MHC II), CD135, CD172a, CD14, and Ly6G from BD Bioscience; MMR/CD206 from Biolegend; PE-α-mouse CD115, CD8α, Gr-1, CD11b, CD40, CD24, Mac-3, CD62L, and CD14 from eBioscience; F4/80 and Ly6C (PE or Alexa647) from AbD Serotec. For BrdU labeling, 200 μl of 10 mg/ml of BrdU were injected i.p. for 12 h; staining followed manufacturer’s instruction (FITC BrdU flow kit, BD).
**Lymph node cells and sections** Skin draining nodes were treated with collagenase D (400 U/ml) for 30 min at 37 °C. Cells were preincubated 10 min with 2.4G2 mAb at 4 °C to block Fc receptors, stained with fluorescent mAbs, acquired on a BD-LSRII, and analyzed using flowjo (Treestar). To label Mo-DCs in vivo, we injected 10 µg of Alexa 647-MMD3 α-DC-SIGN or control mouse IgG2c mAb along with LPS. Lymphocytes (CD3⁺, CD19⁺, DX5⁺ or NK1-1⁺) and B220⁺ plasmacytoid DCs were excluded, and 3 populations of CD11c⁺ cells were separated as DC-SIGN⁺, DEC-205⁺ (Alexa 488-NLDC145 mAb) and DEC-205⁻ DC-SIGN⁻ DCs. CD19⁺ cells were also sorted. 10 µ OCT embedded lymph node sections were acetone-fixed, stained with BMD10 or BMD30 CD209a mAb for 1 h at room temperature or 4 °C overnight, followed by mouse anti-rat IgG2a-HRP for 30 min and Tyramide-signal amplification (Invitrogen). B220-Alexa 647 stained B cell areas in confocal microscopy (LSM510, Zeiss). We also injected into live mice 30 µg Alexa 488 MMD3 anti-DC-SIGN or isotype control mAb i.v. Tissues were fixed in 4% HCHO/PBS 20 min, then 0.5% Triton-X100 15 min, and stained with rabbit anti-Alexa 488 and anti-rabbit HRP to label using TSA Alexa 488. For live cell DIC imaging, Mo-DCs were seeded on Glass Bottom Culture Dishes (MatTek) and examined in an Olympus LCV110U incubator fluorescence microscope. Confocal and live cell images were analyzed with MetaMorph software (Universal Imaging).

**Splenic monocytes and DCs** These were sorted from collagenase digested spleen as monocytes (CD19⁻ CD3⁻ DX-5⁻ CD11b⁺ CD11c<sup>dim</sup> Ly6G⁻ Ly6C⁺) and two classical DC subsets (CD19⁻ CD3⁻ DX-5⁻ CD11c<sup>hi</sup> and either DEC-205⁺ or DEC-205⁻ cells).

**Antigen presentation** T cells specific for OVA (OT-I, OT-II) or malarial (P.yoelii) circumsporozoite protein (CSP) were cultured with graded doses of DCs or B cells. OVA (LPS-free, Seikagaku Corp.) or CSP (Choi et al., 2009) was added in graded doses but usually at 40 µg/ml in vitro, or the proteins were injected for 2 h in vivo (50 µg/foot...
pad) during LPS mobilization of Mo-DCs. In some experiments, we used irradiated CHO cells stably transduced with OVA as the source of antigen. Splenic transgenic T cells were enriched after Fe block by excluding B220+, F4/80+, NK1.1+, I-Ab+, and CD4+ or CD8+ T cells using anti-rat IgG Dynabeads (Invitrogen), labeled with 5 μM CFSE (Invitrogen) and added to round bottom microtest wells at 50,000/well. After 3 days for OT-I or 4 days for OT-II and CS T cells, proliferation of live (Aqua dye negative, Invitrogen) T cells was evaluated by CFSE dilution and staining with mAb to Vα2 for the OT-I or OT-II TCR, and Vβ8.1/8.2 for CSP. For the MLR, DCs from C57BL/6 mice were added in graded doses to CFSE-labeled BALB/c T cells (NK1.1, I-A, B220, F4/80 negative cells) and assayed at d4.

**Quantitative PCR for TLR and CD14 expression by monocytes and Mo-DCs** Taqman probes (AssayID) were used for TLR4 (Mm00445273_m1), TLR2 (Mm00442346_m1), TLR3 (Mm00628112_m1), TLR7 (Mm00446590_m1), TLR9 (Mm00446193_m1) and CD14 (Mm00438094_g1) from Applied Biosystems. The relative expression was normalized by TATA-box Binding protein (TBP) housekeeping gene expression. All qPCR experiments were performed with LightCycler® 480 Real-Time PCR System (Roche).

**SUPPLEMENTAL INFORMATION** Supplemental Information includes 7 figures and one movie and can be found with this article online at xxxxx.

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