SUPPLEMENTAL METHODS

Purification of band cells and neutrophil-DC hybrids. FACSAria (BD Biosciences) was used to sort Gr-1^{high}/CD48^{-} band cells from BM cells with the purity of >99.5%. The following populations were also sorted with the purity of >95%: CD45.2^{+}/CD45.1^{-} cells, Ly6G^{+}/CD11c^{high}/MHC II^{+} neutrophil-DC hybrids, Ly6G^{-}/CD11c^{high}/MHC II^{+} traditional DCs, and Ly6G^{+}/CD11c^{-}/MHC II^{-} neutrophils. The sorting procedure was repeated if necessary to achieve the above purity.

Antibodies and flow cytometry. Samples were suspended in HBSS with 2% bovine serum, treated with anti-CD16/32 monoclonal antibody (mAb) for Fc blocking, and then stained with mAbs against the following antigens purchased from BD Biosciences, eBioscience, R&D Systems, BioLegend, and AbD Serotec: CD3ε (clone 145-2C11), CD11a (2D7), CD11b (M1/70), CD11c (HL3), CD24 (M1/69), CD62L (MEL-14), CD80 (16-10A1), CD86 (GL1), 7/4 (7/4), Ly6C (AL-21), Ly6G (1A8), F4/80 (BM8, CI:A3-1), MHC II (AF6-120.1, 2G9, M5/114.15.2), and TCR Vα2 (B20.1). The samples were then analyzed by FACSAria (≥5-color analyses) or by FACSCalibur (up to 4-color analyses), in which only the propidium iodide-negative populations were included in the analyses. The data were processed by CellQuest Pro (BD Biosciences), FACSDiva (BD Biosciences), or Flow Jo (Tree Star).

Bacterial culture. The following *E. coli* K-12 strains were used in the present study: TOP10 (Invitrogen) expressing enhanced GFP cDNA or OVA cDNA under the control of the lac promoter, and DH5α (Invitrogen) expressing OVA cDNA under the control of the *nir*15 promoter. These bacteria were grown in LB medium for 12 hours at 37ºC under aerobic

conditions, except the strain containing pnir15.OVA, which for OVA production was cultured under anaerobic conditions.

Experimental inflammation models. Acute peritonitis was induced in C57BL/6 mice by i.p. injection of 3% thioglycollate (BD Biosciences) (1 ml/animal) or live *E. coli* K-12 (5×10^6 CFU/animal). The PECs were collected by washing the peritoneal cavity with 7 ml ice-cold PBS. To analyze the emergence of hybrids in inflammatory skin lesions, ear skin samples harvested from KC-Tie2 TG mice (CD1 background) or age-matched wild-type (WT) CD1 mice were treated with 0.5% dispase II (Roche) for 45 minutes at 37°C. After removal of epidermis, the dermis was further treated with 1,000 U/ml collagenase IV (Worthington) and 1,000 U/ml hyaluronidase (Sigma) in the presence of 0.1% DNase I (Roche) for 60 minutes to prepare single cell suspensions. Acute lung inflammation was induced in BALB/c mice by intratracheal administration of GC frass extracts (40 μg/animal). Single cell suspensions were prepared from the lung samples harvested 48 hours after GC frass instillation by 40 minute incubation with 0.5% collagenase IV and 0.05% DNase I. C57BL/6 mice received s.c. injection of *E. coli* (10^7 CFU/animal) into the ear skin, and cervical LNs harvested 24 hours later were examined for the numbers of neutrophil-DC hybrids.

LN-directed homing assay. $Ly6G^+/CD11c^{high}$ hybrids and $Ly6G^-/CD11c^{high}$ traditional DCs were FACS-purified from GM-CSF-supplemented BM culture. CpG oligonucleotide (100 nM) and LPS (100 ng/ml) were added to the culture during the last 24 hours. After labeling with 2.5 μM CFSE, the cells were s.c. injected (2×10^5 cells/animal) into the ear skin of C57BL/6 mice, and cervical LNs were harvested 48 hours later to count the number of $CFSE^+$ cells by

FACSCalibur. Cryostat sections were examined under fluorescence microscopy after staining with APC-conjugated anti-CD3 mAb.

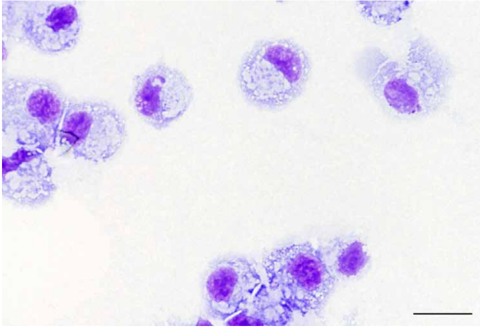
Measurement of GM-CSF in peritoneal fluid. C57BL/6 mice received i.p. injection of thioglycollate together with neutralizing mAb against GM-CSF receptor (clone 698423, R&D Systems) (50 μg/animal). Peritoneal fluid samples harvested 2 hours later were examined for GM-CSF using an ELISA kit (R&D Systems).

SUPPLEMENTAL FIGURE LEGEND

Supplemental Figure S1. Morphology and surface phenotype of macrophages isolated from thioglycollate-induced peritonitis lesions. After i.p. injection of thioglycollate into C57BL/6 mice, PECs harvested on Day 3 were incubated for 2 hours on tissue culture plates. After removal of floating cells, tightly adherent cells were harvested by scraping as macrophages. Data shown are the microscopic image of the cytospin preparation after HEMA-3 staining (A, bar = 20 μm) and the staining profiles with mAbs against the indicated markers (red) or isotype-matched control IgG (B). The data are representative of two independent experiments.

Figure S1

A



B