Immune complexes stimulate CCR7-dependent dendritic cell migration to lymph nodes

Menna R Clatworthy1,2, Caren E Petrie Aronin2, Rebecca J Mathews1, Nicole Y Morgan3, Kenneth G C Smith4 & Ronald N Germain2

Antibodies are critical for defense against a variety of microbes, but they may also be pathogenic in some autoimmune diseases. Many effector functions of antibodies are mediated by Fcγ receptors (FcγRs), which are found on most immune cells, including dendritic cells (DCs)—important antigen-presenting cells that play a central role in inducing antigen-specific tolerance or immunity1,2. Following antigen acquisition in peripheral tissues, DCs migrate to draining lymph nodes via the lymphatics to present antigen to T cells. Here we demonstrate that FcγR engagement by IgG immune complexes (ICs) stimulates DC migration from peripheral tissues to the paracortex of draining lymph nodes. In vitro, IC-stimulated mouse and human DCs showed greater directional migration in a chemokine (C-C) ligand 19 (CCL19) gradient and increased chemokine (C-C) receptor 7 (CCR7) expression. Using intravital two-photon microscopy, we observed that local administration of IC resulted in dermal DC mobilization. We confirmed that dermal DC migration to lymph nodes depended on CCR7 and increased in the absence of the inhibitory receptor FcγRIIB. These observations have relevance to autoimmunity because autoantibody-containing serum from humans with systemic lupus erythematosus (SLE) and from a mouse model of SLE also increased dermal DC migration in vivo, suggesting that this process may occur in lupus, potentially driving the inappropriate localization of autoantigen-bearing DCs.

Antibodies have direct protective effects via pathogen or toxin neutralization and can activate complement or ligate Fc receptors3,4. FcγRs bind immune-complexed IgG and include activating receptors (in humans: FcγRIIA, FcγRIIIB and FcγRIIIC) and a single inhibitory receptor, FcγRIIB5. DCs express a number of receptors, among them FcγRs, which allow antigen internalization for processing and presentation to T cells1,2,6. Recognition of this displayed antigen may induce T cell tolerance or activation, a cellular decision influenced by the provision of co-stimulatory signals present on mature but not immature DCs7,8. Maturation of DCs may be driven by a variety of stimuli, including Toll-like receptor ligands and proinflammatory cytokines or activating FcγR cross-linking6,10. FcγRIIB provides a basal level of inhibition to DC maturation in the presence of ICs, thereby regulating immunogenic antigen presentation to T cells and providing an important tolerance checkpoint11–15. A number of stimuli preferentially reduce FcγRIIB expression on monocytes, notably interferon-γ (IFN-γ)16, allowing these cells to be activated and matured by subsequent encounters with ICs. Deficiency or dysfunction of FcγRIIB confers susceptibility to the IC-mediated autoimmune disease SLE in both mice and humans17–22.

To permit interactions with T cells, tissue-resident DCs do not only need to be mature but also must relocate from peripheral tissues to lymph nodes23–26. Studies to date on the role of FcγRs in DCs have focused on the effects of antibody and FcγR cross-linking on DC maturation, but none has addressed the question of how antibody engagement of FcγRs might affect the migration and anatomical repositioning of DCs.

To determine if FcγR cross-linking with ICs might alter DC migration from peripheral tissue to lymph nodes, we stimulated bone marrow–derived DCs with ovalbumin (OVA) or IgG-opsonized OVA (ICs) for 24 h and subsequently transferred the DCs subcutaneously to a recipient mouse. We harvested draining lymph nodes 48 h following DC transfer and assessed DC migration by histological quantification. Exposure to OVA alone resulted in a limited number of wild-type DCs within the lymph node paracortex, whereas IC stimulation yielded a substantially greater number (Fig. 1a). We next used FcγRIIB-deficient DCs to model the effect of administering IC to activated DCs (on which FcγRIIB is downregulated11–13) or DCs from individuals with dysfunctional FcγRIIB21. Forty-eight hours after DC transfer, we observed higher numbers of IC-stimulated FcγRIIB−/− DCs than IC-stimulated wild-type DCs in the lymph node paracortex (Fig. 1a–c). To control for variations in DC administration and local tissue anatomy, we also co-transferred wild-type and FcγRIIB-deficient DCs into a single site. These experiments confirmed increased migration of IC-stimulated FcγRIIB−/− DCs to lymph nodes (Fig. 1d,e), as did lymph node disruption and DC enumeration by flow cytometry (Fig. 1f). We verified the viability of migrating DC by intravitral imaging of lymph nodes (Supplementary Video 1) and by flow cytometry (Supplementary Fig. 1).

1Department of Medicine, MRC Laboratory of Molecular Biology, University of Cambridge, Cambridge, UK. 2Laboratory of Systems Biology, Lymphocyte Biology Section, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, USA. 3Biomedical Engineering and Physical Sciences Resource, Microfabrication and Microfluidics Unit, National Institute of Biomedical Imaging and Bioengineering, National Institutes of Health, Bethesda, Maryland, USA. 4Cambridge Institute for Medical Research and Department of Medicine, University of Cambridge School of Clinical Medicine, Cambridge, UK. Correspondence should be addressed to M.R.C. (mrc38@cam.ac.uk) or R.N.G. (germain@nih.gov).

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To dissect the mechanisms underpinning IC-induced DC migration, we considered a variety of factors known to affect this process. In particular, DCs express CCR7, which interacts with CCL19 and CCL21 expressed on lymphatics. IC stimulation resulted in a modest upregulation of CCR7 expression on wild-type DCs but a substantial upregulation on FcγRIIB−/− DCs (Fig. 1g). A second factor we considered was the production of matrix metalloproteinases (MMPs) because migrating DCs secrete MMPs, particularly MMP-2 and MMP-9 (refs. 27,28), to facilitate movement through the extracellular matrix and across basement membranes. Pharmacological inhibition of MMPs (refs. 27,28,31) or genetic deletion of MMPs (refs. 27,28,31) reduces DC migration. In vitro, within 4 h of FcγR cross-linking, wild-type DCs produced proteases capable of gelatin digestion (Fig. 1h and Supplementary Fig. 2). This response was greater in FcγRIIB−/− DCs (Fig. 1h), as were levels of MMP-9 in DC culture supernatants (Fig. 1i). IC-induced MMP-9 production by mouse DCs was abrogated by inhibition of the kinase ERK but not JNK or p38 (Supplementary Fig. 3).

To further explore the possible role of IC-induced upregulation of CCR7 on DC chemotaxis, we used a three-dimensional in vitro model, embedding IC-stimulated DCs within a collagen matrix (Fig. 2a) in a rising soluble CCL19 gradient (C.E.P.A., J.S. Yoon, N.Y.M., T. Prustel, M. Meier-Schellersheim & R.N.G. unpublished results). IC-stimulated wild-type DCs showed greater migration toward CCL19 than OVA-stimulated DCs (Fig. 2b–d, Supplementary Fig. 4).
Supplementary Videos 2 and 3). In the absence of FcγRIIB, IC-stimulated DCs showed even greater directional migration (Fig. 2b–d, Supplementary Fig. 4, Supplementary Videos 4 and 5). The capacity of IC to stimulate mouse DC chemotaxis was dependent on the activating FcγR, FcγRIII (Fig. 2e, Supplementary Fig. 5a), as was IC-mediated upregulation of CCR7 and MMP-9 (Supplementary Fig. 5b,c). CCR7 deficiency abrogated IC-induced DC migration to draining lymph nodes following subcutaneous transfer (Fig. 2f and Supplementary Fig. 6).

To determine the relevance to humans, we generated DCs from peripheral blood monocytes obtained from healthy donors. These DCs demonstrated a significant increase in CCR7 expression post-stimulation with ICs (Fig. 2g), increased directional migration in a CCL19 gradient (Fig. 2h,i, Supplementary Videos 6 and 7) and increased production of MMP-9 (Fig. 2j).

Given our quantitative migration data in the DC transfer model and the in vitro data, we wished to determine whether the administration of ICs was sufficient to stimulate endogenous DC
Figure 3 ICs stimulate CCR7-dependent dermal DC migration to lymph nodes \textit{in vivo}. (a) Footpad dermal DCs (green) were imaged by two-photon microscopy in WT CD11cEYFP (top panels) and Fcgr2b−/− CD11cEYFP mice (bottom panels) 16 h after administration of OVA or IC subcutaneously to the footpad. Blood vessels are shown in red (Qdots intravenous), and cell tracks over an 80-min period are shown in white. Scale bars, 50 µm. (b) Quantification of displacement and persistence index for dermal DCs 16 h after OVA or IC administration. Each symbol represents single DC. Images obtained from the footpads of three mice per experimental group. NS, nonsignificant. (c) Sequential images obtained by intravital two-photon microscopy of the footpad 16 h after IC administration. Movement of a dermal DC (green) tracked (white dashed line) through a dermal lymphatic vessel (LYVE-1, red). Scale bars, 10 µm. Horizontal lines show mean; error bars show s.e.m. (d–g) Representative flow cytometric plots and quantification of live FITC+ MHCIIhigh CD11c+ cells in draining lymph nodes in WT and Fcgr2b−/− mice (d,e) and WT and Ccr7−/− mice (f,g) 48 h after application of FITC to skin followed by intradermal administration of OVA or IC as indicated. Mean and s.e.m. DC number observed in lymph nodes obtained from four mice per experimental condition. P values calculated using a Student’s t-test (two-tailed). EPICAM, epithelial cell adhesion molecule; ND, non-draining lymph node. (h) Quantification of live FITC+ MHCIIhigh CD11c+ cells present in draining lymph nodes of WT mice treated with MMP-9-blocking antibody or isotype control before application of FITC to skin with intradermal administration of OVA or IC. Mean and s.e.m. DC number observed in lymph nodes obtained from four mice per experimental condition. P values throughout calculated using a paired t-test to compare lymph nodes draining skin treated with OVA (left inguinal) and IC (right inguinal) or nondraining lymph nodes (brachial) from the same mice. * P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001, **** P ≤ 0.0001.

migration \textit{in vivo}. Utilizing intravital two-photon microscopy to assess the dynamic behavior of dermal DCs in CD11cEYFP mice, we found that administration of IC into the footpad resulted in mobilization of these cells (consistent with chemotaxis or chemokinesis), whereas injection of OVA alone did not (Fig. 3a,b and Supplementary Videos 8 and 9). These cells did not express granulocyte or monocyte markers (Supplementary Fig. 7), and we observed them clustering around, entering into and traveling within lymphatic vessels following IC stimulation (Fig. 3c, Supplementary Video 10, Supplementary Figs. 8 and 9). IC-induced dermal DC mobilization was even greater in Fcgr2b−/− CD11cEYFP mice (Fig. 3a,b, Supplementary Videos 11 and 12), supporting the idea that human subjects with FcγRIIB dysfunction might demonstrate increased IC-induced DC motility.

Intravital imaging of skin DCs only allows the visualization of the initial phase of DC migration; therefore, we sought to determine whether IC-stimulated dermal DCs complete their journey to draining lymph nodes. We used FITC painting to label dermal DCs, and followed this with local administration of OVA or IC. In wild-type mice, a substantially greater number of FITC+ MHC class IIhigh CD11c+ cells were present in draining lymph nodes after IC stimulation than after OVA stimulation (Fig. 3d,e), and these cells expressed markers typical of skin-resident, migratory DCs (Fig. 3d). We observed greater accumulation of FITC-labeled DCs in Fcgr2b−/− mice following IC stimulation (Fig. 3d,e). CCR7-deficient dermal DCs showed severely impaired migration to draining lymph nodes in response to IC stimulation (Fig. 3f,g and Supplementary Fig. 10). MMP-9 blockade had a less potent effect than CCR7 deficiency on IC-mediated dermal DC migration (Fig. 3h, Supplementary Fig. 11).

Because local administration of IC induced DC migration \textit{in vivo}, we hypothesized that this phenomenon would have relevance to SLE, a disease characterized by IC deposition in tissues, including skin\textsuperscript{12}. Of note, abnormalities in monocytes and DCs have been observed in patients with lupus\textsuperscript{33–36}. To address whether ICs derived from individuals with lupus might stimulate DC mobilization \textit{in vivo}, we injected heat-inactivated serum, taken either from NZB/W F1 mice with antinuclear antibodies and nephritis, a mouse model of human SLE, or from undiseased aged-matched controls, into the footpads of CD11cEYFP mice. Serum from NZB/W F1 mice with lupus stimulated dermal DC mobilization (Fig. 4a,b and Supplementary Video 13), whereas control serum did not (Fig. 4a,b, Supplementary Video 14).
and Supplementary Fig. 12a,b). The stimulatory effect of the serum from NZB/W F1 mice with lupus on dermal DC motility was IgG dependent (Fig. 4c and Supplementary Fig. 12c). We also observed a greater number of FITC-labeled dermal DCs in the draining lymph nodes of mice to which serum from NZB/W F1 mice with lupus had been transferred in a peritoneal cavity than in those of mice given wild-type serum (Fig. 4d,e). In addition, in draining lymph nodes of aged NZB/W F1 mice with lupus nephritis, the number of migratory DCs (CD11c+ MHCIIhigh CD103+) was substantially greater than that of aged-matched NZW control mice, in which such cells were scarcely detectable (Fig. 4f and Supplementary Fig. 13).

To explore the relevance of these observations to human disease, we administered heat-inactivated serum from human patients with lupus into the footpads of CD11cEYFP mice. This resulted in greater dermal DC migration (increased displacement and a higher persistence index) than injection of healthy control serum, particularly when the serum was obtained from patients with high disease activity scores (Fig. 4g,h, Supplementary Fig. 14, Supplementary Videos 15 and 16). In addition, we examined the effect of a polymorphism in human FCGR2B (rs1050501) on DC CCR7 expression. This polymorphism involves an amino acid substitution in the transmembrane domain, a threonine replacing an isoleucine (FcγRIIB-T232), resulting in receptor dysfunction\(^{31,22}\) and contributing to lupus susceptibility\(^{37}\). U937 human monocytic cells stably transfected with FcγRIIB-I232 or FcγRIIB-T232 were differentiated to a DC phenotype and stimulated with IC. Cells transfected with the dysfunctional FcγRIIB-T232 had higher IC-dependent CCR7 expression than cells transfected with FcγRIIB-I232 (Fig. 4i), suggesting that DCs from individuals with the lupus-associated polymorphism would show increased migration when exposed to ICs, analogous to our observations in FcγRIIB-/- mice.

In conclusion, our study demonstrates a previously unknown effect of antibody opsonization, namely increased DC migration to lymph nodes via FcγR-induced upregulation of CCR7 and MMPs. This would allow the optimal positioning of DCs in the lymph node paracortex, acting synergistically with the known effects of FcγR cross-linking on MHC class II and co-stimulatory molecule upregulation on these cells, to promote immunogenic T cell–DC interactions. Furthermore, we
show that dermal DC migration is increased following administration of ICs or autoantibody-containing serum, and this effect is particularly pronounced in the absence of the inhibitory receptor FcγRIIB, a known susceptibility locus in SLE. Together, these data suggest an additional mechanism by which ICs might drive autoimmunity in SLE via the inappropriate localization of autoantigen-bearing DCs and may also have implications for boost strategies in vaccination, since measures to block or downregulate FcγRIIB at the time of boost would enhance the migration of immunogen-bearing DCs to lymph nodes, thereby increasing T cell activation and the magnitude of the vaccine response.

METHODS

Methods and any associated references are available in the online version of the paper.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

M.R.C. conceived of the project, carried out experiments, designed and supervised experiments, analyzed data and wrote the manuscript. C.E.P.A. and M.R.C. conceived of the project, carried out experiments, designed and supervised experiments, analyzed data and wrote the manuscript. K.G.C.S. provided DC chemotaxis assay. K.G.C.S. provided

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Mice. FcRRIIB-deficient mice on a C57BL/6 background19 were kindly provided by J. Ravetch (Rockefeller University) and S. Bolland (US National Institutes of Health, US National Institute of Allergy and Infectious Diseases (NIAID)) and crossed to transgenic mice expressing EGFP under the control of the human ubiquitin C promoter39 (Jackson Laboratories) or transgenic mice expressing Venus EYFP under the control of the CD11c promoter39 (obtained from M. Nussenzweig, Rockefeller University). C57BL/6 mice were obtained from Jackson Laboratories or from Charles River Laboratories (Margate, UK). NZB/W F1 were bred in-house from NZB and NZW mice obtained from Harlan UK. CCR7-deficient mice on a C57BL/6 background (strain B6.129P2(C)-Cec7tm1Rfor/J, stock number 006621, live repository, aged 8 weeks) were purchased from Jackson Laboratories. Age-matched C57BL/6 JAX mice were used as controls. In all experiments, both male and female mice were used. Mice were maintained in specific pathogen–free conditions at an Association for Assessment and Accreditation of Laboratory Animal Care–accredited animal facility at NIAID or at a Home Office–approved facility in the UK. All procedures were approved by the NIAID Animal Care and Use Committee (US National Institutes of Health, Bethesda, MD) or were conducted in accordance with the United Kingdom Animals (Scientific Procedures) Act of 1986.

Antibodies. For immunofluorescence and flow cytometric studies, the following antibodies were used: mouse antibodies (from E Bioscience unless otherwise indicated): anti-B220 antibody (clone RA3-6B2, product code 561877), anti-CD3 antibody (clone 17A2, 561389) (BD Biosciences, San Jose, California), PE-conjugated anti-mouse CCR7 (clone 4B12, 12-1971), PE-Cy7-conjugated anti-CD4 (clone RM4-5, 25-0042), PE-Cy7-conjugated anti-CD19 (clone eBio 1D3, 25-0913), FITC-conjugated anti-CD11b (clone M1/70, 53-0112) Alexa Fluor 780-conjugated CD11c (clone N418, 47-0114), Pacific Blue–conjugated anti-class II (clone AF6-120.1, 48-5320), PE-conjugated anti-CD103 (clone 2E7, 12-1031), APC-conjugated anti-EPcam (clone G8.8, 57-5971), e450-conjugated Gr-1 (clone RB6-8C5, 57-4991) and PE-conjugated Ly6C (Clone HK1.4, 12-5932).

Human antibodies (from BD Pharmingen unless otherwise indicated): PE-Cy7-conjugated anti-CCR7 (clone 3D12, 560922), PE-Cy7– conjugated Rat IgG2a isotype control (clone R35-95, 557855), APC-conjugated CD86 (clone 2331, 560956), FITC-conjugated HLA DR (clone G46-6, 560944).

All antibodies were used at 1/200 dilution (except for the MHC class II antibody, which was used at 1/100). For some flow cytometric studies, a Live/Dead Fixable Aqua Dead Cell Stain Kit (Invitrogen-Molecular Probes) was used.

Immune complexes. Endotoxin-free ovalbumin (EndoGrade Ovalbumin, Hyglos GmbH) was opsonized with a polyclonal rabbit anti-ovalbumin antibody (Sigma–Aldrich) at 37 °C for 1 h.

DC culture and stimulation. We generated primary mouse BMDCs as described previously40. Briefly, bone marrow was flushed from femurs and tibias, plated in regular petri dishes at 2.5 × 10^6 cells per dish and supplemented with 20 ng mL−1 granulocyte–macrophage colony–stimulating factor (GM-CSF, Peprotech) in base media (10% Hyclone FBS, 1% penicillin/streptomycin in RPMI 1640) 3 times over 8 d. On day 9, DCs were transferred to tissue culture plates and incubated overnight with endotoxin-free ovalbumin (EndoGrade Ovalbumin, Hyglos) or immune-complexed ovalbumin for 24 h at 37 °C.

We generated human monocyte–derived DCs from the peripheral blood of healthy volunteers. Samples were obtained with informed consent and the approval of the Cambridge Local Research Ethical committee (reference 08/H0308/176) or from normal volunteers from the US National Institutes of Health Blood Bank. Blood was subjected to Ficoll–Hypaque density separation to isolate peripheral blood mononuclear cells, and monocytes were enriched by negative selection by using a MACS-based monocyte purification kit (Miltenyi Biotec). DCs were generated by culturing the monocytes for 6 d with human GM-CSF (100 ng mL−1, PeproTech) in DMEM supplemented with 10% heat-inactivated calf serum.

For mouse FcγR blocking studies, anti-mouse FcγRIII (CD16, AF1960) or goat anti-mouse isotype control (AB-108-C; both obtained from R&D Systems) were added to cells 1 h before stimulation with IC (final concentration 1.5 µg mL−1). Similarly, the PI3K inhibitor (ZSTK474, Selleckchem, 100 nM) or Akt inhibitor (AKT-XI (3-Formylchromone thiosemicarbazone, Cu(II)Cl2 Complex, FPA-124), Merck Millipore, 2.5 µM) was added to DCs before addition of IC, and cells and supernatants were harvested at 24 h.

Cell lines. The human monocytic cell line U937 (gift from R.A. Floto) stably transfected with vectors containing various isoforms of FcRRIIB (as described previously21) were used. Cells were cultured in RPMI 1640 with 10% FCS (Sigma) and penicillin/streptomycin (Sigma).

In vivo DC migration. 24 h following culture with ovalbumin or immune-complexed ovalbumin, 5 × 10^6 BMDCs were washed in PBS to remove non-internalized ovalbumin or immune complexes and injected subcutaneously in the flank or footpad (depending on the lymph node harvested). 48 h following transfer, lymph nodes were harvested and processed for histological examination or flow cytometric analysis. Cells were labeled with Cell Tracker Orange (Invitrogen Molecular Probes) or Cell tracker Green (Invitrogen Molecular Probes) as indicated in figure legends.

Immunofluorescence microscopy. For quantification of DC migration to lymph nodes, lymph nodes were harvested and incubated in 0.05 M phosphate buffer containing 0.1 M 1,4-lysine, pH 7.4, 2 mg mL−1 NaOAc, and 10 mg mL−1 parafomaldehyde. Twelve hours later, lymph nodes were washed in phosphate buffer and dehydrated in 30% sucrose in phosphate buffer. Tissues were snap frozen in Tissue-Tek (Sakura Finetek). Frozen sections (50 µm) were stained with the indicated antibodies. Confocal images of whole lymph node sections were obtained using the tiling function of a Zeiss 710 or 780 microscope.

Flow cytometry. Cells were stained with the antibodies described above for 1 h at 4 °C, with the exception of CCR7 staining, which was performed at room temperature. Samples were analyzed using an LSR2 flow cytometer (Becton Dickinson). Data were processed using FlowJo software (Treestar).

MMP-9 measurement. Culture supernatants were obtained from BMDCs after stimulation with OVA or IC, as indicated in the figure legends. Total MMP-9 was measured by ELISA (Duoset, R&D Systems) according to manufacturer’s instructions.

Matrix degradation. Glass coverslips were coated with 10 µg mL−1 FITC gelatin (Invitrogen) and cross-linked with 0.5% glutaraldehyde31. 2 × 10^5 DCs were plated onto matrix-coated coverslips and incubated at 37 °C to allow degradation, then fixed with 4% paraformaldehyde (MP-Biomedicals) and loaded into the central channel. EGFP DCs were mixed with Purecol bovine collagen I (final concentration 1.7 mg mL−1). Similarly, the PI3K inhibitor (ZSTK474, Selleckchem, 100 nM) or Akt inhibitor (AKT-XI (3-Formylchromone thiosemicarbazone, Cu(II)Cl2 Complex, FPA-124), Merck Millipore, 2.5 µM) was added to DCs before addition of IC, and cells and supernatants were harvested at 24 h.

In vitro migration assay. The microfluidic-based migration chamber was fabricated using methods established by Haessler et al.42. Briefly, source, sink and cell channels were patterned using commercial film masks generated by high-resolution laser photoplotting (CAD/Art Services). Melted 3% agarose was poured onto the mask to create the desired channel pattern. These agarose stamps were incubated in 10% FBS with media for 24 h. Device assembly included laying agarose stamps, channel side down, onto a glass coverslip. A PMMA manifold containing inlet and outlet ports was placed on top of the agarose stamp, and the entire device was secured together with a metal clamp. EGFPP DCs were mixed with Purecol bovine collagen I (final concentration 1.7 mg mL−1). Advanced Biomatix) and loaded into the central channel. The chamber was incubated for 15 min at 37 °C, inverting once to ensure uniform cell distribution during collagen gelatinization. Following incubation, a rising concentration of CCL19 (maximum concentration 100 ng mL−1) (Peprotech) was applied to the source channel at 5 µl min−1. The sink channel maintained base media under constant flow at 5 µl min−1. Migratory behavior was imaged on an inverted Zeiss 510 microscope under confocal settings with a 20×, 0.9 NA objective.
Intravital imaging of dermal DCs by two-photon microscopy. CD11c-EYFP mice were anaesthetized and Qdots A655 (Invitrogen Molecular Probes) administered intravenously to delineate dermal vasculature. Alternatively, rat anti-mouse LYVE-1 (clone 223322; MAB2125, R&D Systems) was covalently conjugated to HiLyte Fluor 594 using labeling kits (AnaSpec) and was administered into the footpad an hour before imaging. Footpad dermal DCs were imaged at 915 nm using a Zeiss 510 microscope, as described previously43,44. To create a time-lapse sequence, a 70-µm-thick section of the dermis containing lymphatic vessels was scanned at 3 µm steps every 50 s for 1 h 20 min. Imaging alternated between left and right footpads over an 8-h period comparing footpads into which 50 µL−1 of OVA or IC heat-inactivated serum had been injected 16 h previously. Either OVA was placed into the right footpad and IC into the left footpad or control serum was placed in left footpad and lupus serum placed in right footpad, allowing animals to act as their own controls. The investigator performing the imaging was not blinded to this information. The sample size for each experimental group included at least 3 mice; based on previous imaging studies performed in this laboratory this provides data on >50 DCs.

Mouse serum for in vivo studies. Serum was obtained from NZB/W F1 mice with antinuclear antibodies and nephritis (as evidenced by heavy proteinuria) and aged matched WT (C57BL/6) mice. Heat inactivation was performed at 56 °C for 1 h before administration.

Some lupus sera were depleted of IgG using Nab Protein G spin columns (Thermo Scientific) following the manufacturer’s standard protocol.

FITC sensitization dermal DC migration assay. FITC sensitization was used to assess dermal DC migration, as described by Robbiani et al.45. Ovalbumin or immune-complexed ovalbumin was administered subcutaneously to the base of the tail, the flank or the groin on the right and left respectively of C57BL/6 mice. This allows comparison of the two experimental conditions within the same animal. FITC (8 mg mL−1, Sigma) was dissolved in equal volumes of acetone and dibutyl phthalate (Sigma) and applied in 25-µl aliquots to clipped dorsal skin. Forty-eight hours after FITC painting, inguinal lymph nodes were harvested with nondraining brachial lymph nodes for comparison.

Lymph nodes were homogenized, passed through a 70-µm cell strainer, digested in collagenase A (10 mg mL−1)/DNAse I (1 mg mL−1) (Roche) and 2% FBS in PBS for 20 min, and the cells were analyzed by flow cytometry. For the serum transfer model, 200 µL−1 of heat-inactivated WT or lupus serum was administered intraperitoneally to C57BL/6 mice 12 h before FITC painting. For MMP-9 blocking studies, an anti-MMP-9 antibody (Ab-1, Calbiochem/Merck Millipore, UK IM9FL, clone 6.6B) was given at a dose of 4 mg/kg intraperitoneally 12 h before FITC painting and OVA/OVA-IC administration. An isotype control antibody (mouse IgG1, ab37555, Abcam) was used at the same concentration as the anti-MMP-9 antibody.

Human serum for in vivo studies. Serum was obtained from six patients with SLE, three with low BILAG scores (2–9) and three with high BILAG scores (15–21) and three age- and sex-matched healthy controls. Patient characteristics are detailed in Supplementary Table 1. Heat inactivation was performed at 56 °C for 1 h before administration. Samples were obtained with informed consent and approval of the Cambridge Local Research Ethical committee (reference 08/H0308/176).

Image analysis. Both in vitro and in vivo migration movies were processed using Imaris software (version 6.2). The Snapshot tool was used to generate time-lapse movies (with 6–10 fps playback), still images and track histories. To calculate forward protrusion alignment along the dominate CCL19 gradient, independent images from each group were selected, and the angle feature was applied to 20 cells per field to measure the angle between the leading edge and the dominant CCL19 gradient. To further characterize migration behavior, all DC migration data was analyzed using the ‘spots’ feature in Imaris. Specifically, x-y coordinates for each cell tracked were exported to Microsoft Excel and plotted for spatial trajectories. Using the Imaris ‘spots’ feature, values for x displacement and track straightness were calculated for each individual track and plotted in GraphPad PRISM software. Migratory persistence was calculated by dividing displacement values by total path length. Percent distribution of velocities was calculated from the instantaneous velocities of each cell at each time point and binned over selected values. Average speed values were calculated over the entire movie. All chemotactic metrics were calculated in Excel.

Statistical analysis. Statistical analyses were performed using GraphPad PRISM software. A two-tailed Student’s t-test was applied, unless otherwise indicated. Results are expressed as means and s.e.m. All experiments were subject to at least three replicates per experimental parameter. No statistical method was used to predetermine sample size, and the experiments were not randomized.

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