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Type 2 Innate Immunity in Helminth Infection Is Induced Redundantly and Acts Autonomously following CD11c⁺ Cell Depletion

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Infection with gastrointestinal helminths generates a dominant type 2 response among both adaptive (Th2) and innate (macrophage, eosinophil, and innate lymphoid) immune cell types. Two additional innate cell types, CD11c<sup>high</sup> dendritic cells (DCs) and basophils, have been implicated in the genesis of type 2 immunity. Investigating the type 2 response to intestinal nematode parasites, including *Heligmosomoides polygyrus* and *Nippostrongylus brasiliensis*, we first confirmed the requirement for DCs in stimulating Th2 adaptive immunity against these helminths through depletion of CD11c<sup>high</sup> cells by administration of diphtheria toxin to CD11c<sup>DOG</sup> mice. In contrast, responsiveness was intact in mice depleted of basophils by antibody treatment. Th2 responses can be induced by adoptive transfer of DCs, but not basophils, exposed to soluble excretory-secretory products from these helminths. However, innate type 2 responses arose equally strongly in the presence or absence of CD11c<sup>high</sup> cells or basophils; thus, in CD11c<sup>DOG</sup> mice, the alternative activation of macrophages, as measured by expression of arginase-1, RELM-α, and Ym-1 (Chi3L3) in the intestine following *H. polygyrus* infection or in the lung following *N. brasiliensis* infection, was unaltered by depletion of CD11c-expressing DCs and alveolar macrophages or by antibody-mediated basophil depletion. Similarly, goblet cell-associated RELM-β in lung and intestinal tissues, lung eosinophilia, and expansion of innate lymphoid ("nuocyte") populations all proceeded irrespective of depletion of CD11c<sup>high</sup> cells or basophils. Thus, while CD11c<sup>high</sup> DCs initiate helminth-specific adaptive immunity, innate type 2 cells are able to mount an autonomous response to the challenge of parasite infection.
In this study, we therefore set out to establish the relative importance of DCs and basophils in the generation of these diverse innate and adaptive type 2 responder populations. Studying both infection and immunization with nematode-secreted Th2-disposing antigens, we showed that DCs are indeed essential for the Th2 response, while basophils are not required for this activity, in either setting. Critically, however, the depletion of DCs or basophils did not compromise expansion of AAMs, eosinophils, or innate type 2 lymphoid cells. These data suggest that multiple populations of innate cells act autonomously by adopting a type 2 program under the conditions of helminth infection, perhaps reflecting the imperative to evolve redundant immune mechanisms to protect against parasite invasion (2).

MATERIALS AND METHODS

Mice, parasites, and antigens. Wild-type BALB/c and C57BL/6 mice and CD11c-DOG mice on the C57BL/6 background, which express human diphtheria toxin receptor and ovalbumin amino acids (aa) 140 to 386 under the control of the CD11c promoter (19, 20), were bred and maintained in a specific-pathogen-free facility at the University of Edinburgh. Mice were injected subcutaneously (s.c.) with 250 N. brasiliensis infective third-stage larvae (L3) or with 200 H. polygyrus bakeri (7, 30) L3 under a gavage tube, or with 50 μg N. brasiliensis into the hind foot, and lymph node (LN) cells were recovered 5 to 7 days later. Excretory-secretory antigens from adult H. polygyrus (HES) and N. brasiliensis (NES) were prepared as previously described (18, 21).

In vivo depletion of dendritic cells and basophils. CD11c-DOG mice were depleted of CD11c<sup>hi</sup> dendritic cells by intraperitoneal (i.p.) injection of 8 ng/g diphtheria toxin daily from day −1 to 6 after infection (19, 20, 45). Efficacy of depletion was assessed by flow cytometry of splenocytes and Liverse-digested mesenteric LN cells (MLNC) (see Fig. 1A and B). BALB/c mice were depleted of basophils by i.p. injection of 10 μg MAR-1 antibody or Armenian hamster IgG isotopy control (eBiosciences) on days 0, 1, and 2 postinfection. Check blood was taken on day 4 postinfection to assess basophil depletion by flow cytometry.

Bone marrow-derived DCs and basophils. DCs and basophils were generated in vitro from femoral bone marrow cells in the presence of 20 ng/ml granulocyte-macrophage colony-stimulating factor (GM-CSF) and 5 ng/ml IL-4, respectively, replenishing the medium at days 3, 6, and 8 for DCs and days 3 and 8 for basophils, before harvesting on day 10. Basophils were purified by flow sorting as DX<sup>+</sup> ckit<sup>−</sup> Gr<sup>−1</sup> cells. Cells were incubated for 18 h in medium containing 20 μg/ml antigen and 5 μg/ml GM-CSF or 5 ng/ml IL-4, DCs were >85% CD11c<sup>+</sup> major histocompatibility complex (MHC) class II<sup>+</sup>, whereas basophils were >95% CD49b<sup>+</sup> ckit<sup>−</sup> and expressed high levels of FceRI but no detectable MHC class II following culture. Cells were then washed in phosphate-buffered saline (PBS) and transferred into the hind foot at 2.5 × 10<sup>6</sup> cells per foot. Popliteal LNs (PLNs) were harvested at day 5 posttransplantation and antigen-specific restimulations performed in Ex-Vivo medium containing penicillin-streptomycin and l-glutamine.

Flow cytometry and LN cytokine assays. MLNs were removed into Hanks balanced salt solution (HBSS) before being digested in 250 μg/ml Liberase TL (Roche) for 30 min at 37°C in a shaking incubator with the addition of 0.02 M EDTA (pH 7.3) for the final 5 min. MLNs were then washed and homogenized in HBSS and centrifuged at 400 × g for 5 min before being resuspended in fluorescence-activated cell sorter (FACS) buffer (0.5% bovine serum albumin [BSA], 0.05% sodium azide, 1× PBS). The left lobe of the lung was prepared similarly by dissection into small pieces in PBS containing 250 μg/ml Liberase and 80 U/ml DNase I (Sigma). Following digestion and homogenization, the cell suspension was treated with red cell lysis buffer, and cells were washed, counted, and stained for flow cytometry. Cells were stained with surface and lineage markers as follows: phycoerythrin (PE)-conjugated Siglec F (clone E50-2440; BD Pharmingen), PE-Cy7-conjugated F4/80 (clone BM8; Biocon), allopheocyanin (APC)-conjugated CD11c (clone N418; eBioscience), fluorescein isothiocyanate (FITC)-conjugated MHC class II (clone M5/114.15.2; BioLegend), and Pacific Blue-conjugated CD11b (clone M1/70; Biolegend) antibodies. Lung samples were then fixed with 1× Foxp3 fixation buffer (eBioscience) before intracellular staining was performed with antibodies to murine Resistin (RELN-α) at 2 μg/ml (Peptech) and 0.72 μg/ml biotinylated antibody to mouse chitinase 3-like 3/EC-F-L (Ym-1; R&D) in Foxp3 permeabilization buffer before a secondary stain with 0.67 μg/ml Zenon rabbit IgG Alexa Fluor 488 (Invitrogen) and 1/200 streptavidin-peridinin chlorophyll protein (PerCP) (Biolegend). Staining was compared to that for isotype controls of pooled naïve and infected samples.

Check blood cells were sensitized with 12 μg/ml recombinant murine IgE (Pharmingen) and then stained using a combination of 1/125 biotin anti-mouse IgE (Pharmingen) and 1/200 streptavidin-APC (Biolegend) with FITC-conjugated CD3 (clone 17A2; Biolegend), PE-Cy7-conjugated CD49b (clone DX5; eBioscience), PerCP-conjugated B220 (clone RA3-6B2; Biolegend), and PE-conjugated CD117 (c-kit) (clone ACK2; Biolegend) antibodies. Red blood cells were lysed using 1× BD FACS red cell lysing solution before acquisition.

For intracellular staining, 6 × 10<sup>6</sup> cells/well were plated in a 24-well plate with 0.5 μg/ml phorbol myristate acetate (PMA) and 1 μg/ml ionomycin for 1 h before addition of 10 μg/ml brefeldin A, which was left for a further 3 h. Cells were then washed and blocked by resuspension in FACS buffer containing FcR block for 15 min. After washing, cells were incubated with 1/200 anti–CD8-APC–FITC and anti–CD4–PerCP for 20 min, washed again, and then fixed for 20 min with 200 μl Fix/Perm buffer (BD Pharmingen). Fixation buffer was removed with two washes with permeabilization buffer (BD Pharmingen), and samples were split and subsequently stained for intracellular cytokines using 1/200 anti-gamma interferon (IFN-γ)–APC, anti-IL-4–PE, anti-IL-10–APC, anti-IL-13–APC, or the relevant isotype control for 20 min in Perm buffer. After another wash in Perm buffer, samples were resuspended in FACS buffer and analyzed by flow cytometry using a Becton Dickinson Canto or LSR-II flow cytometer.

For antigen-specific restimulation, 10<sup>6</sup> mesenteric or popliteal lymph node cells per well were plated in the presence of medium, 5 μg/ml NES, or 1 μg/ml HES for 72 h at 37°C with 5% CO<sub>2</sub> before centrifuging at 400 × g for 5 min and freezing the supernatants at −20°C, which were then analyzed for IFN-γ, IL-4, IL-5, IL-10, IL-13, and IL-17 by commercially available enzyme-linked immunosorbent assay (ELISA) (BD Pharmingen).

RT-PCR. Lung and intestinal tissues were prepared for reverse-transcriptase PCR (RT-PCR) by immersion in TRIzol (Invitrogen), and RNA extraction was performed following the manufacturer’s protocol, transcribing 1 μg RNA using Moloney murine leukemia virus (MMLV) RT (Stratagene). Ym-1, RELM-α, RELM-β, arginase-1, and IL-13 mRNA levels were measured by real-time PCR using a Roche Light Cycler real-time PCR machine (47). The hypoxanthine-guanine phosphoribosyltransferase (HPRT) gene was used as the reference gene. Light Cycler PCR amplifications were carried out in 10-μl mixtures containing 4 μl cDNA, 0.3 μM primers, and 2× Light Cycler-DSY SYBR green 1 mix (Roche). PCR was performed using the following conditions: 5 min of denaturation at 95°C, 10 s of annealing of primers at 60°C, and 20 s of elongation at 72°C, for 40 cycles. The fluorescent DNA binding dye SYBR green
CD11c\(^{+}\) cell depletion ablates adaptive Th2 responsiveness. CD11c.DOG mice were given 8 ng/g DTx (or PBS as a control) daily from day 1 to 6 of mouse-adapted N. brasiliensis (Nb) infection, and MLNC were harvested at day 7 postinfection. (A and B) DC depletion in the mesenteric lymph node (MLN) as staining for CD11c and MHC class II, shown as a representative bivariant plot scaled to 210,000 events (A) and a graphical summary of all data (B). (C) Intracellular IL-4 expression by MLN CD4\(^{+}\) T cells in PBS- and DTx-treated uninfected and infected (Nb) mice. (D to I) Cytokine release by cultured MLNC stimulated with NES (black bars) or medium (open bars), in PBS- and DTx-treated uninfected and infected (Nb) mice, assayed for IL-4 (D), IL-10 (E), IL-5 (F), IL-13 (G), IFN-\(\gamma\) (H), and IL-17 (I). Data presented are means ± standard errors (SE) for LNC from 3 to 5 individual mice per group and are representative of two similar experiments. Statistically significant differences are shown as * (\(P < 0.05\)), ** (\(P < 0.01\)), or *** (\(P < 0.001\)).

As expected from our previous data using S. mansoni and H. polygyrus infections, CD11c\(^{+}\) cell depletion significantly ablated the Th2 response to N. brasiliensis, as measured by polyclonal CD4\(^{+}\) IL-4\(^{+}\) cell percentages (Fig. 1C) as well as the antigen-specific IL-4 and IL-10 cytokine response from MLNC cultured in vitro with N. brasiliensis excretory-secretory antigen (NES) (Fig. 1D and E). IL-5 secretion by MLNC from infected mice in vitro could be observed at two levels: a constitutive response, which did not require the presence of NES antigen, and an antigen-dependent element. The latter was more severely diminished in mice following DC depletion (Fig. 1F). Interestingly, there was a trend, but not a significant one, for reduced production of antigen-specific IL-13 following depletion of CD11c\(^{+}\) cells in N. brasiliensis-infected mice (Fig. 1G), while a sharp increase in the Th1 (IFN-\(\gamma\)) profile was observed following DC depletion (Fig. 1H), perhaps reflecting the loss of antigen-specific IL-10 production in the CD11c\(^{+}\) DC-depleted mice. While no significant change in Foxp3\(^{+}\) Treg numbers is observed in N. brasiliensis infection (data not shown), we also found that the IL-17 recall response of MLNC to NES was significantly depressed in CD11c\(^{+}\) DC-depleted mice (Fig. 1I).

The ability of helminths to stimulate Th2 responses can be reproduced by certain helminth-derived products given in soluble form to naïve mice (11, 21, 59). Th2 responses can also be induced by adoptive transfer into naïve hosts of CD11c\(^{+}\) bone marrow-derived DCs exposed to helminth molecules (5, 29), including NES (21). In view of recent reports that Th2 responses can be induced by basophils, even in the absence of DCs, we reevaluated the ability of NES-pulsed cells to drive Th2 differentiation in vivo. DCs and basophils were generated from murine bone marrow with GM-CSF or IL-3, respectively; basophils were flow sorted for CD49b\(^{+}\) c-kit\(^{+}\) Gr-1\(^{+}\), and then both cell types were cultured overnight with medium or NES and adaptively transferred into

**FIG 1** CD11c\(^{+}\) cell depletion ablates adaptive Th2 responsiveness. CD11c.DOG mice were given 8 ng/g DTx (or PBS as a control) daily from day 1 to 6 of mouse-adapted N. brasiliensis (Nb) infection, and MLNC were harvested at day 7 postinfection. (A and B) DC depletion in the mesenteric lymph node (MLN) as staining for CD11c and MHC class II, shown as a representative bivariant plot scaled to 210,000 events (A) and a graphical summary of all data (B). (C) Intracellular IL-4 expression by MLN CD4\(^{+}\) T cells in PBS- and DTx-treated uninfected and infected (Nb) mice. (D to I) Cytokine release by cultured MLNC stimulated with NES (black bars) or medium (open bars), in PBS- and DTx-treated uninfected and infected (Nb) mice, assayed for IL-4 (D), IL-10 (E), IL-5 (F), IL-13 (G), IFN-\(\gamma\) (H), and IL-17 (I). Data presented are means ± standard errors (SE) for LNC from 3 to 5 individual mice per group and are representative of two similar experiments. Statistically significant differences are shown as * (\(P < 0.05\)), ** (\(P < 0.01\)), or *** (\(P < 0.001\)).
the hind feet of naïve mice. Five days following transfer, popliteal lymph node (PLN) cells were recovered and stimulated with NES antigen or anti-CD3 in vitro. While DC transfer induced strong antigen-specific and polyclonal cytokine responses of popliteal LN at 5 days following transfer of bone marrow-derived DCs or basophils, pulsed with medium alone or with *N. brasiliensis* ES (NES) antigen. Cells were challenged in vitro with medium alone (open bars), 5 μg/ml NES (black bars), or 1 μg/ml anti-CD3 (gray bars) and supernatants collected after 72 h. (A) NES-specific IL-4 responses or medium-alone controls; (B) polyclonal IL-4 responses (NES responses are shown for comparison); (C) NES-specific IL-10 responses; (D) NES-specific IL-17 responses. (E and F) Depletion of peripheral blood CD3⁺CD19⁻IgE⁺CD49b⁺c-kit⁺ basophils in naïve and 4-day *N. brasiliensis*-infected mice treated with isotype control or MAR-1 antibody. (G and H) NES-specific IL-4 and IL-10 production in response to medium (open bars) or NES (black bars) by MLN from naïve, isotype-treated, and MAR-1-treated *N. brasiliensis*-infected (Nb) mice. (I and J) RT-PCR for arginase-1 and Ym-1 expression from lung homogenates from naïve, isotype-treated, and MAR-1-treated *N. brasiliensis*-infected (Nb) mice. (K) Intracellular RELM-α staining of CD11b⁺F4/80⁺SiglecF⁺AAMs from naïve, isotype-treated, and MAR-1-treated *N. brasiliensis*-infected (Nb) mice. (L) Eosinophil populations identified as CD11b⁺Siglec F⁻ cells in the lungs of naïve, isotype-treated, and MAR-1-treated *N. brasiliensis*-infected (Nb) mice. For transfer experiments, data presented are means ± SE for LNC from 4 individual mice and are representative of two similar experiments. For infection experiments, MLNC and lungs were harvested at 7 days following infection. Data presented are means from 4 or 5 individual mice and are representative of two similar experiments. Statistically significant differences are shown as * (P < 0.05), ** (P < 0.01), or *** (P < 0.001).
FcεRI⁺ CD49b⁺ basophils in blood (Fig. 2E and F). Consistent with previous reports, basophil-depleted N. brasiliensis-infected mice showed no loss of antigen-specific Th2 responsiveness, with strong IL-4, IL-10, and IL-13 production when MLNC were cultured in vitro with NES (Fig. 2G and H; see Fig. S1A and B in the supplemental material). In addition, the substantial generation and persistence of AAMs in the lung was unaffected by basophil depletion, with high levels of mRNA transcripts for arginine-1 (Fig. 2I) and Ym-1 (chitinase 3-like protein 3 [Chi3L3]) (Fig. 2I) in pulmonary tissue. In particular, we noted that following infection, some 20 to 30% of CD11b⁺ CD11c⁻ SiglecF⁻ nonalveolar macrophages expressed the AAM product RELM-α by intracellular staining, which was not significantly reduced in the absence of basophils (Fig. 2K). Similarly, increased pulmonary (Fig. 2L) eosinophilia in N. brasiliensis-infected mice was observed irrespective of the basophil status of the host.

We next tested basophil depletion during infection with H. polygyrus, which normally survives for ≥10 weeks in C57BL/6 mice, generating a mixed Th2/Treg response (13, 31, 49). The results closely paralleled those observed in N. brasiliensis infection, with similar in vivo depletion (see Fig. S1C in the supplemental material) and indistinguishable IL-4, IL-10, and IL-13 responses to H. polygyrus ES (HES) challenge in vitro (Fig. 3A and B; see Fig. S1D in the supplemental material). The proportions of Th2 cytokine-synthesizing cells identified by intracellular staining were also unchanged in basophil-depleted mice (see Fig. S1E in the supplemental material). Because H. polygyrus remains in the gastrointestinal tract, AAM responses were measured in the intestine and showed marked elevation of arginine-1 (Fig. 3C) and RELM-α and Ym-1 (see Fig. S1F in the supplemental material) in both isotype- and MAR-1 antibody-treated mice. An important innate effector in immune defense against H. polygyrus is RELM-β, an IL-13- and STAT6-regulated product of mucosal epithelial cells (16, 34). RELM-β expression was found to be highly elevated in intestinal tissue in both basophil-depleted and control mice (Fig. 3D). Increases in eosinophilia in the draining lymph nodes following H. polygyrus infection were also unchanged following administration of MAR-1 antibody (Fig. 3E).

We then analyzed stimulation of innate immune cell populations following CD11c⁺ cell depletion in N. brasiliensis-infected mice, given the high expression of this marker by dendritic cell and alveolar macrophage populations in the lung. Because N. brasiliensis traverses the lung when migrating from the skin site of infection to the small intestine, we first examined pulmonary cell suspensions and tested lung homogenates for quantitative PCR estimations of mRNA levels. Lung eosinophilia, which is known to be Th2 independent in this system (32, 52), was clearly undiminished in DTx-treated CD11c.DOG mice (Fig. 4A and B). In contrast, there was total loss of CD11c⁺ alveolar macrophages, which express the highest levels of CD11c (Fig. 4A and C; see Fig. S2A in the supplemental material), as previously reported for an independently constructed CD11c.DOG transgenic mouse (66). Despite the ablation of alveolar macrophages following DTx treatment, a population of CD11b⁺ CD11c⁻ SiglecF⁻ macrophages expressing intermediate levels of F4/80 remained (Fig. 4D); this subset expresses high levels of RELM-α in N. brasiliensis-infected mice (Fig. 2G), even following depletion of CD11c⁺ cells (Fig. 4E; see Fig. S2B and C in the supplemental material). Overall expression of the AAM genes for Ym-1 and RELM-α (Fig. 4F and G) and arginine-1 (see Fig. S2D in the supplemental material) increased in the lungs of infected mice irrespective of CD11c⁺ cell depletion, showing that alveolar macrophages are not the only source of products of alternative activation during N. brasiliensis infection.

Similar evidence for unabated AAM development in the CD11c⁺ cell-depleted setting was also obtained from the small intestine, in which comparable levels of Ym-1 were observed in both groups of infected mice (Fig. 5A). Moreover, this tissue showed similar expression of the epithelial cell product RELM-β associated with antihelminth immunity (16) in the presence or absence of CD11c⁺ cells (Fig. 5B). In the MLNs, eosinophilia in response to infection was also unaltered by CD11c⁺ cell depletion (Fig. 5C). Finally, we also investigated innate non-B, non-T cell sources of cytokines by gating on CD3⁻ CD19⁻ cells in infected and DTx-treated mice. We noted that while both IL-4⁺ and IL-13⁺ NBNNT cell frequencies increased with N. brasiliensis infection, their numbers were not significantly reduced in CD11c⁺
cell-depleted mice (Fig. 5D and E). Surface phenotyping revealed that the CD3-CD19-IL-13+ cells were CD8+, TCRβ+, c-kitint, CD90+, T1/ST2+, Sca-1+ and CD4+, similar to the case for innate helper cells or nuocytes (Fig. 5G).

DISCUSSION
Type 2 immunity integrates multiple components of both innate and adaptive natures, embracing both antigen-specific Th2 lymphocytes and a range of innate inducer and effector cell types (2, 42, 53, 68). Although a prominent question in recent years has been the nature of the innate cell type that drives Th2 differentiation (28, 42), a broader issue is how innate type 2 populations are themselves elicited. For adaptive T cell populations, Th2 induction can now be unequivocally attributed to the action of CD11cint DCs, both because antigen-pulsed DCs are sufficient for Th2 induction and because DC depletion results in profound ablation of the adaptive Th2 response (15, 45). While an essential role for basophils in Th2 stimulation has been suggested (44, 58), previously, it had been reported that alveolar macrophages express AAM markers within 48 h of N. brasiliensis infection (50) and that AAMs maintain their phenotype in a CD4+ T cell-replete environment (27, 50). Interestingly, we show that the generation of lung AAMs occurs even when resident alveolar macrophages are efficiently deleted and that their numbers are undiminished even after 7 days in a DC- and Th2-depleted environment.

It is well established that AAMs can arise in lymphopenic environments such as RAG−/− and SCID mice, which have no adaptive immunity, and that they are prominent in the innate response to injury (27, 50). In general, adaptive Th2-derived IL-4/IL-13 is necessary to maintain the AAM population (27, 50), although eosinophil-derived IL-4 is sufficient for AAM induction in adipose tissue (69). Our studies indicate that innate IL-4, and particularly IL-13, is sufficiently robust in the absence of DCs to stimulate AAM development during helminth infection. One source of DC-independent IL-13 in infection may be the innate helper cell or nuocyte, which, interestingly, displays a CD4+ phenotype in N. brasiliensis infection, implying that not all CD4+ sources of type 2 cytokines are conventional T cells.

Our data indicate that tissue and lymph node eosinophilia is
also independent of basophils, supporting previous studies (39, 40, 60). As CD4⁺ IL-5 responses were reduced by DC depletion, production of this cytokine by non-T cells may be sufficient to drive eosinophil development, as observed in earlier studies of T cell-deficient nude rodents infected with nematodes such as *Acaris suum* (48) and *Toxocara canis* (62). Eosinophils are not required for Th2 generation (as shown in both *N. brasiliensis* [67] and *S. mansoni* [61]), although, as with basophils, their production of IL-4 can make a significant contribution to the pace and intensity of the Th2 response.

Despite the accumulating data showing that basophils are not necessary to raise the first alarm, and indeed are not well-equipped to do so due to their short life span (40) and absence of antigen presentation machinery (38), there are many examples in acquisitive helminth immunity and, more broadly, in allergy and IgG responses for protective immunity. As CD4⁺ T cells in affected tissue but not in inflamed lymph nodes (23).

Reporter and lineage deletion strains offer elegant tools to analyze the contribution of key cell types to immunity, while the use of depleting antibodies allow experiments in a genetically unmanipulated host. While the MAR-1 antibody can raise neutrophil numbers (22) and partially deplete FcεR1⁺ mast cell populations (35) depending on the route of administration, our data showing intact type 2 responsiveness are unlikely to be confounded by these effects. The long-term effects of MAR-1 administration and the individual contribution of mast cells to *H. polygyrus* fecundity, expulsion, and antibody formation are currently being explored. Similarly, while MAR-1 has also been reported to ablate FcεR1⁺ inflammatory dendritic cells in lymph nodes draining the allergic airways (35), the unaltered Th2 response in MAR-1-treated mice suggests that inflammatory DCs do not contribute to Th2 induction in a number of helminth infection models.

The ability of helminths to stimulate Th2-driving pathways in vivo can be reproduced by pulsing DCs with helminth products in vitro (5, 29), as we have confirmed with NES. Interestingly, molecules from the strongly Th2-inducing *N. brasiliensis* and *S. mansoni* parasites show common effects such as suppression of Toll-like receptor (TLR)-stimulated IL-12p70 release (5, 8, 28). Importantly, in *H. polygyrus* infection, Th2 responsiveness is counterbalanced by a strong regulatory response and additional changes to DC function. Moreover, the DC subset which dominates in draining lymph nodes recovered from chronically infected mice can induce Foxp3⁺ Tregs (56) and inhibit antibacterial immunity (9), while *H. polygyrus* ES (HES) treatment of DCs renders them less immunogenic when transferred into naïve hosts (55). Thus, while diverse helminth species show similar initiation of type 2 immunity, there are sharp contrasts in the subsequent course of infection as well as the functional phenotype of DC populations.

We have analyzed in this report the relative importance of two major innate immune populations in the initiation of type 2 immunity against helminth infection within both innate and adaptive settings. Our results conclusively show that the adaptive Th2 response in these systems is dependent upon CD11c⁺ DCs and that basophils are not required for this outcome. Our data also clearly highlight DC-independent pathways to mobilize innate type 2 immunity which operate autonomously during helminth infection. Such redundancy within the innate type 2 compartment may serve to safeguard the host from the range of helminth pathogens now known to interfere with normal DC function (12, 17, 56) and provide an alternative means to mobilize essential effector functions for protective immunity.
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