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### Title

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### Permalink

<https://escholarship.org/uc/item/4sp10489>

### Journal

Nature immunology, 10(7)

### ISSN

1529-2908

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### Publication Date

2009-07-01

### DOI

10.1038/ni.1740

Peer reviewed



Published in final edited form as:

*Nat Immunol.* 2009 July ; 10(7): 697–705. doi:10.1038/ni.1740.

## Major histocompatibility complex class II-dependent basophil-CD4<sup>+</sup> T cell interactions promote T<sub>H</sub>2 cytokine-dependent immunity

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### Abstract

Dendritic cells can prime naïve CD4<sup>+</sup> T cells, however we demonstrate that DC-mediated priming is insufficient for the development of T<sub>H</sub>2 cell-dependent immunity. We identify basophils as a dominant cell population that coexpressed MHC class II and *Il4* message following helminth infection. Basophilia was promoted by thymic stromal lymphopoietin (TSLP) and depletion of basophils impaired immunity to helminth infection. *In vitro*, basophils promoted antigen-specific CD4<sup>+</sup> T cell proliferation and IL-4 production and transfer of basophils augmented the expansion of helminth-responsive CD4<sup>+</sup> T cells *in vivo*. Collectively, these studies suggest that MHC class II-dependent interactions between basophils and CD4<sup>+</sup> T cells promote T<sub>H</sub>2 cytokine responses and immunity against helminth infection.

### Keywords

Th2 cells; basophils; MHC class II; helminth infection

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Since the demonstration of specification of CD4<sup>+</sup> T helper (T<sub>H</sub>) cell fates<sup>1</sup>, substantial advances have been made in delineating the regulatory mechanisms that promote distinct modules of CD4<sup>+</sup> T cell-dependent immunity and inflammation<sup>2</sup>. T<sub>H</sub>2 cell differentiation is dependent on interleukin 4 receptor (IL-4R) and the transcription factors STAT6 and GATA3 and their signature cytokine profile is characterized by expression of IL-4 <http://>

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[www.signaling-gateway.org/molecule/query?afcsid=A001262](http://www.signaling-gateway.org/molecule/query?afcsid=A001262), IL-5, IL-9 and IL-133. The hallmarks of T<sub>H</sub>2 cytokine-dependent inflammation at barrier surfaces such as the skin, airway and intestine include the recruitment of CD4<sup>+</sup> T<sub>H</sub>2 cells, eosinophils, mast cells and basophils, coupled with goblet cell hyperplasia, mucus production and increased smooth muscle contractility<sup>4</sup>. Type 2 inflammatory responses are required for immunity and tissue repair following exposure to helminth parasites. However, T<sub>H</sub>2 cytokine responses can also promote pathological changes observed in the context of asthma and allergic diseases<sup>5</sup>.

While the sequelae of type 2 immunity and inflammation in peripheral tissues are well characterized, the innate responses that promote T<sub>H</sub>2 cell development, including the nature of the antigen-presenting cell (APC) involved, the host-microbial receptor-ligand interactions and the APC-derived factors required to initiate and sustain T<sub>H</sub>2 cell differentiation remain less well defined<sup>6</sup>. DCs are the only APC thought to prime naive T cells and the current paradigm suggests that recognition of conserved pathogen-associated molecular patterns via distinct pattern recognition receptors expressed on DCs promote appropriate pathogen-specific CD4<sup>+</sup> T<sub>H</sub> cell responses<sup>7</sup>. Activation of DCs can result in increased surface expression of MHC class II and costimulatory molecules such as CD40, CD80 and CD86 as well as expression of factors that can shape the nature of the developing adaptive immune response<sup>8</sup>. However, the critical DC-derived signals responsible for driving T<sub>H</sub>2 cell responses *in vivo* remain undefined<sup>9</sup>. *In vitro* studies indicate that the requirements for DC-mediated T<sub>H</sub>2 cell differentiation include differential expression of the Notch ligand *jagged* 10 and up-regulation of the costimulatory molecules CD4011 and OX40L12. However whether these pathways are sufficient for DCs to promote CD4<sup>+</sup> T<sub>H</sub>2 cell differentiation *in vivo* is unclear.

The recruitment and activation of mast cells, eosinophils and basophils are hallmarks of T<sub>H</sub>2 cytokine-dependent inflammation in peripheral tissues and earlier studies suggest that these granulocyte populations may function as accessory cells in the initiation of CD4<sup>+</sup> T<sub>H</sub>2 cell responses. For example, mast cells, eosinophils and basophils are competent to produce and secrete IL-4 from intracellular stores, implicating these populations as early sources of IL-4 that could promote CD4<sup>+</sup> T<sub>H</sub>2 cell differentiation<sup>13–15</sup>. In addition, mast cells and eosinophils can express MHC class II, and eosinophils have been implicated as potential APCs in both airway inflammation and helminth infection<sup>16–18</sup>. Basophil frequencies are increased following exposure to allergens and helminth parasites and previous work demonstrated that basophils are a dominant source of IL-4 and IL-13 following helminth infection and contribute to protective immunity<sup>19–21</sup>. Although basophilia is a common feature of T<sub>H</sub>2 cytokine-mediated inflammation, little is known about how these cells are activated and recruited to peripheral tissues. A conserved mechanism for basophil-mediated recognition of parasite products and allergens through protease-dependent activation was recently proposed<sup>22</sup>. In that study, basophils were recruited to the draining lymph node early following allergen exposure and were essential for the generation of T<sub>H</sub>2 cytokine responses elicited following papain immunization. However, the potential accessory cell functions of basophils during CD4<sup>+</sup> T<sub>H</sub>2 cell development remain unknown. Collectively, the inability of DCs to express IL-4 and the lack of defined mechanisms through which DCs

promote T<sub>H</sub>2 cell differentiation have provoked a reassessment of the relative contribution of DCs in promoting T<sub>H</sub>2 cytokine responses *in vivo*.

In this study, we demonstrate that DC-restricted expression of MHC class II was insufficient for the generation of protective CD4<sup>+</sup> T<sub>H</sub>2 cytokine-dependent immunity to the gastrointestinal helminth *Trichuris muris*. Basophils were identified as a dominant accessory cell population that expressed *Il4* message and MHC class II. *In vitro* studies showed that basophils could promote MHC class II-dependent antigen-specific CD4<sup>+</sup> T cell proliferation and T<sub>H</sub>2 cell differentiation. Depletion of basophils *in vivo* resulted in impaired protective immunity to *T. muris*, while adoptive transfer of primary wild-type basophils augmented CD4<sup>+</sup> T cell proliferation in response to *Schistosoma mansoni* egg injection. Taken together, these studies suggest a previously unrecognized role for basophils in MHC class II-dependent cognate interactions with CD4<sup>+</sup> T cells that promote parasite-specific T<sub>H</sub>2 cytokine responses and host protective immunity.

## RESULTS

### CD11c-restricted MHC II is insufficient for type 2 immunity

To test whether antigen presentation by CD11c<sup>+</sup> DCs was sufficient to promote CD4<sup>+</sup> T<sub>H</sub>2 cell-dependent immunity *in vivo*, mice in which MHC class II expression is restricted to CD11c<sup>+</sup> cells (MHC II<sup>CD11c</sup> mice, Supplementary Fig. 1 online) were infected with the intestinal helminth parasite *T. muris*. Expulsion of *T. muris* and protective immunity is dependent on CD4<sup>+</sup> T<sub>H</sub>2 cells while parasite-specific IFN- $\gamma$  production promotes chronic infection<sup>23–25</sup>. *T. muris* infection provides a well-characterized *in vivo* model of T<sub>H</sub>2 cytokine-dependent immunity that offers a functional read-out of the magnitude of the host T<sub>H</sub>2 cytokine response. Since MHC II<sup>CD11c</sup> mice lack MHC class II expression on the thymic epithelium<sup>26</sup> and therefore are unable to positively select CD4<sup>+</sup> T cells in the thymus, mice were given fetal thymic grafts eight weeks prior to infection to provide an endogenous CD4<sup>+</sup> T cell population. Following infection with *T. muris*, littermate control mice developed pathogen-induced T<sub>H</sub>2 cytokine responses characterized by production of IL-4, IL-5 and IL-13 by mesenteric lymph node (mLN) cells (Fig. 1a). In contrast, MHC II<sup>CD11c</sup> mice exhibited minimal infection-induced production of T<sub>H</sub>2 cytokines (Fig. 1a). Histological analysis of intestinal tissues in infected control mice revealed hallmarks of type 2 inflammation including goblet cell hyperplasia and increased mucin production (Fig. 1b), and expression of Gob5, also known as chloride channel calcium activated 3 (mCLCA3) (Fig. 1c), a goblet cell-specific marker regulated by T<sub>H</sub>2 cytokines and associated with type 2 inflammation<sup>27</sup>. Consistent with decreased T<sub>H</sub>2 cytokine production, infected MHC II<sup>CD11c</sup> mice exhibited a marked absence of goblet cells and goblet cell-derived proteins (Fig. 1b,c). T<sub>H</sub>2 cytokine-dependent expression and luminal secretion of goblet cell-derived resistin-like molecule beta (RELM $\beta$ ) in resistant mice provides a non-invasive indicator of the kinetics of T<sub>H</sub>2 cytokine responses in the intestinal microenvironment<sup>28</sup>. As previously reported, luminal RELM $\beta$  protein peaked in resistant control mice between days 12 and 18 post-infection<sup>28</sup>, coincident with worm expulsion (Fig. 1d), while luminal secretion of RELM $\beta$  in infected MHC II<sup>CD11c</sup> mice was severely reduced in magnitude (Fig. 1d). Associated with a polarized T<sub>H</sub>2 cytokine response, littermate control mice also displayed

increased titers of immunoglobulins IgG1 and IgE (data not shown). However, since MHC II<sup>CD11c</sup> mice lack MHC class II expression on B cells<sup>26</sup>, no antigen-specific class-switched antibody was detected in infected MHC II<sup>CD11c</sup> mice (data not shown). Critically, the defect in T<sub>H2</sub> cytokine responses in MHC II<sup>CD11c</sup> mice resulted in susceptibility to infection in mice on a normally genetically resistant background (Fig. 1e). Taken together, these data demonstrate that restriction of MHC class II-dependent antigen presentation to CD11c<sup>+</sup> cells was insufficient to promote CD4<sup>+</sup> T<sub>H2</sub> cell-dependent immunity following intestinal helminth infection.

### Th1 cell differentiation is intact in MHC II<sup>CD11c</sup> mice

The extensive physical and biochemical barriers between antigenic material in the enteric space and lymphocytes in the underlying lymphoid follicles and lamina propria of the intestine create unique challenges in antigen sampling and presentation<sup>29</sup>. Therefore the impaired T<sub>H2</sub> cytokine responses in *T. muris*-infected MHC II<sup>CD11c</sup> mice may indicate that additional APCs are required for either the sampling of *T. muris* antigens or the provision of signals necessary for the priming, proliferation and differentiation of pathogen-specific CD4<sup>+</sup> T cells. However, following *T. muris* infection both littermate control mice and MHC II<sup>CD11c</sup> mice exhibited an infection-induced increase in total CD4<sup>+</sup> T cell numbers in the draining mLNs (Fig. 2a), suggesting that DC-restricted antigen-presentation was sufficient for promoting proliferation of CD4<sup>+</sup> T cells following infection. To determine whether CD4<sup>+</sup> T cells in infected MHC II<sup>CD11c</sup> mice were non-responsive or had received signals for alternative differentiation, mRNA was isolated from mLNs of naive and infected control or MHC II<sup>CD11c</sup> mice and analyzed for expression of IL-10, IL-17, and IFN- $\gamma$  to assess the magnitude of Treg, T<sub>H-17</sub> and T<sub>H1</sub> responses. While there was little to no induction of IL-10 and IL-17 expression in infected control and MHC II<sup>CD11c</sup> mice (Supplementary Fig. 2 online), *Ifng* mRNA was selectively and significantly induced in infected MHC II<sup>CD11c</sup> mice compared to controls (Fig. 2b). Consistent with elevated *Ifng* mRNA expression, the frequency of mLN CD4<sup>+</sup> T cells producing IFN- $\gamma$  (Fig. 2c; bold) as well as the amount of IFN- $\gamma$  made per cell (Fig. 2c; italics) were increased in infected MHC II<sup>CD11c</sup> mice compared to control mice. Secretion of IFN- $\gamma$  was also significantly elevated following *in vitro* stimulation of mLN cells isolated from infected MHC II<sup>CD11c</sup> mice compared to control mice (Fig. 2d). Thus, following intestinal infection, cognate interactions between antigen-specific CD4<sup>+</sup> T cells and CD11c<sup>+</sup> DCs alone were sufficient to promote the priming and expansion of CD4<sup>+</sup> T cells as well as to provide signals necessary for T<sub>H1</sub> cell differentiation but were insufficient for the development of T<sub>H2</sub> cytokine-dependent immunity. These data suggested that CD11c<sup>+</sup> cells may not be required for T<sub>H2</sub> cytokine-dependent immunity. To determine the relative contribution of CD11c<sup>+</sup> cells in immunity to *T. muris* we utilized CD11c-diphtheria toxin receptor (DTR) mice in which delivery of diphtheria toxin to littermate controls has no effect while similar administration to CD11c-DTR mice results in the selective apoptosis of CD11c<sup>+</sup> cells. To avoid the toxicity associated with long-term diphtheria toxin treatment of intact CD11c-DTR mice, we employed bone marrow chimeras of either wild-type or CD11c-DTR donor bone marrow into wild-type recipients. While transient depletion of CD11c<sup>+</sup> cells (Supplementary Fig. 3a online) throughout the course of *T. muris* infection resulted in a significant reduction in mLN CD4<sup>+</sup> T cell numbers (Supplementary Fig. 3b), there was no effect on production of

T<sub>H</sub>2 cytokines or worm burdens (Supplementary Fig. 3c,d). Together these data suggest that CD11c<sup>+</sup> cells may not be essential for protective immunity to *T. muris* and that another APC may be required for the development of T<sub>H</sub>2 cytokine-dependent immunity *in vivo*.

To determine whether alterations in the cytokine milieu could restore immunity in MHC II<sup>CD11c</sup> mice, *T. muris*-infected MHC II<sup>CD11c</sup> mice were treated with a monoclonal anti-IFN- $\gamma$  blocking antibody during the course of infection. Consistent with previous findings (Fig. 1a, Fig. 2d) stimulated T cells isolated from the mLN of infected MHC II<sup>CD11c</sup> mice exhibited a robust IFN- $\gamma$  response with low concentrations of IL-4, IL-5 and IL-13 (Fig. 2e). Associated with the lack of T<sub>H</sub>2 cytokines, control-treated MHC II<sup>CD11c</sup> mice exhibited decreased goblet cell responses and susceptibility to *T. muris* infection (Fig. 2f,g). Anti-IFN- $\gamma$  treatment of MHC II<sup>CD11c</sup> mice resulted in a significant reduction in IFN- $\gamma$  production and the emergence of a T<sub>H</sub>2 cytokine response characterized by significantly increased IL-4, IL-5 and IL-13 production by mLN cells, goblet cell hyperplasia and recovery of immunity to infection (Fig. 2e–g). Taken together, these data suggest that following blockade of a non-protective T<sub>H</sub>1 cytokine response, CD11c<sup>+</sup> cells alone can provide the antigen-specific interactions to drive CD4<sup>+</sup> T<sub>H</sub>2 cell differentiation and protective immunity. However, in the presence of endogenous IFN- $\gamma$  signals, non-DC populations are required for the development of protective T<sub>H</sub>2 cytokine responses *in vivo*.

### Basophils express MHC class II and *Il4* mRNA

In addition to DC, macrophages and B cells are professional APCs involved in the development of adaptive CD4<sup>+</sup> T cell-dependent immunity. However, clodronate-loaded liposome depletion of macrophages had no effect on cytokine-dependent inflammation or worm expulsion (Supplementary Fig. 4a–c online). Previous work demonstrated that adoptive transfer of CD4<sup>+</sup> T cells alone into mice lacking both B and T cells was sufficient to restore immunity to *T. muris*<sup>30</sup>. Mice deficient in B cells ( $\mu$ MT) also exhibited intact T<sub>H</sub>2 cytokine-dependent goblet cell responses and protective immunity (Supplementary Fig. 4d–f). Collectively, these data suggest that while macrophages and B cells may contribute to immunity to *T. muris* in a physiologic setting, they do not have essential non-redundant roles in host protective immunity. We therefore focused on the identification of innate immune cells that could both express MHC class II and provide an innate source of IL-4 following *T. muris* infection. We previously employed IL-4–eGFP (4-get) reporter mice to track emerging CD4<sup>+</sup> T<sub>H</sub>2 responses following *T. muris* infection<sup>31</sup>. 4-get mice contain an internal ribosomal entry site (IRES)-enhanced green fluorescent protein (eGFP) element within the *Il4* locus allowing direct *ex vivo* analysis of cells competent to express IL-4<sup>32</sup>. We utilized the same *in vivo* approach to identify non-B non-T cells that co-expressed *Il4* mRNA and MHC class II molecules. Gating on non-B non-T cells, we identified an IL-4–eGFP<sup>+</sup> cell population (Fig. 3a) that expressed MHC class II (Fig. 3b). Previous studies have shown that mast cells and eosinophils can express MHC class II<sup>16–18</sup> and are competent to produce IL-4<sup>13,14</sup>. However, classical mast cells (c-Kit<sup>+</sup> SSC<sup>hi</sup>) were not found following infection with *T. muris* and frequencies of siglec-F<sup>+</sup> SSC<sup>hi</sup> eosinophils were decreased after infection (Fig. 3c). In contrast, CD49b<sup>+</sup> Fc $\epsilon$ RI<sup>+</sup> basophils emerged as a dominant cell type expressing both *Il4* mRNA and MHC class II following *T. muris* infection (Fig. 3c), consistently comprising 40% of IL-4–eGFP<sup>+</sup> MHC class II<sup>+</sup> cells. Although MHC class II

was not expressed as abundantly as in professional APCs such as B cells, basophils expressed intermediate amounts of MHC class II compared to MHC class II-deficient basophils (Fig. 3d). While there have been previous reports of MHC class II expression on eosinophils<sup>17,18</sup>, this is the first report we are aware of demonstrating MHC class II expression on basophils and suggests a potential accessory cell function for this cell population during helminth infection.

### Basophils depletion impairs immunity to *T. muris*

To determine whether basophils play a role in the development of T<sub>H</sub>2 cytokine-dependent protective immunity, wild-type C57BL/6 mice were infected with *T. muris* and treated with either control Ig or a monoclonal antibody against the FcεRI (MAR-1). Previous studies have demonstrated efficient depletion of basophils for up to 10 days following i.p. injection of MAR-133 and we observed greater than 90% depletion of basophils at day 21 post-infection (Fig. 4a) following MAR-1 treatment. Depletion of basophils in infected mice resulted in decreased *Il4* mRNA expression (Fig. 4b), a marked reduction in T<sub>H</sub>2 cytokine-dependent goblet cell hyperplasia (Fig. 4c) and a decrease in luminal secretion of RELMβ in the intestine (Fig. 4d). Loss of basophils and impaired T<sub>H</sub>2 cytokine responses were associated with impaired expulsion of *T. muris* (Fig. 4e). Taken together, these data support a role for basophils in the development of protective type 2 immunity to intestinal helminth infection.

### TSLP selectively elicits basophils

We recently identified essential functions for intestinal epithelial cell (IEC)-derived cytokines IL-25<sup>34</sup> and TSLP<sup>27</sup> <http://www.signaling-gateway.org/molecule/query?afcsid=A002363> in the development of T<sub>H</sub>2 cytokine-dependent immunity to *T. muris*. In addition, IEC-derived IL-33 was shown to promote T<sub>H</sub>2 cytokine responses and worm expulsion<sup>35</sup> and several studies have demonstrated that IL-33 treatment can directly stimulate cytokine and chemokine production from basophils and mast cells *in vitro* <sup>36–38</sup>. To test whether IL-25, IL-33 or TSLP contributed to basophil responses *in vivo*, 4-week mice were injected with recombinant IL-25, IL-33 or TSLP and the peripheral basophil responses examined by flow cytometry. As previously reported, IL-25 treatment elicited a robust population of IL-4-eGFP<sup>+</sup> SSC<sup>hi</sup> cells<sup>39</sup> (Fig. 5a). Treatment with IL-33 also resulted in a marked elevation in the frequency of IL-4-eGFP<sup>+</sup> SSC<sup>hi</sup> cells (Fig. 5a). However, phenotypic analysis of these IL-4-eGFP<sup>+</sup> cells revealed two distinct cell populations selectively elicited by each cytokine. IL-25 treatment resulted in increased frequencies of a non-B non-T c-kit<sup>+</sup> mast cell-like population while IL-33 treatment led to increases in the frequency of CCR3<sup>+</sup> eosinophils (Supplementary Fig. 5 online). While administration of TSLP also resulted in a 3-fold increase in IL-4-eGFP<sup>+</sup> cells over PBS-treated controls (Fig. 5a), unlike IL-25 and IL-33, TSLP treatment selectively elicited CD49b<sup>+</sup> FcεRI<sup>+</sup> basophils (Fig. 5b). These data suggest that while IEC-derived IL-25, IL-33 and TSLP promote the expansion of diverse innate cell populations competent to produce IL-4, only TSLP promotes basophil population expansion.



## Basophils promote CD4<sup>+</sup> Th2 cell differentiation

Demonstration that depletion of basophils resulted in impaired immunity to *T. muris* (Fig. 4) coupled with the co-expression of MHC class II and *Il4* mRNA (Fig. 3) suggested that they may also participate in MHC class II-dependent cognate interactions with CD4<sup>+</sup> T cells to promote T<sub>H</sub>2 cell differentiation. To test whether basophils could present antigen, an *in vitro* co-culture system was adopted in which antigen-pulsed purified basophils were activated with recombinant IL-3, to provide survival signals and promote IL-4 production, and co-cultured with purified CFSE-labeled ovalbumin (OVA)-specific DO11.10 CD4<sup>+</sup> T cells. Sorted basophils exhibited characteristic multi-lobed nuclei and expressed both MHC class II and IL-4-eGFP (Fig. 6a). While minimal proliferation was detected in the absence of OVA peptide, approximately 75% of CD4<sup>+</sup> T cells co-cultured in the presence of antigen-pulsed basophils had diluted CFSE, consistent with proliferation (Fig. 6b). Basophil-induced CD4<sup>+</sup> T cell proliferation was dependent on MHC class II expression as addition of a blocking antibody against MHC class II abrogated these responses (Fig. 6b). To determine whether basophils could influence CD4<sup>+</sup> T<sub>H</sub>2 cell differentiation following antigen-specific stimulation of T cells, intracellular cytokine staining for IL-4 was performed (Fig. 6b) and IL-4 secretion measured in supernatants from co-cultured cells (Fig. 6c). Supernatants from basophil-T cell co-cultures in the absence of antigen contained basal amounts of IL-4 (Fig. 6c) and upon addition of OVA peptide, there was a 2- to 3-fold increase in secreted IL-4 that was abrogated in the presence of anti-MHC class II (Fig. 6c). Therefore, MHC class II-dependent cognate interactions between basophils and CD4<sup>+</sup> T cells can promote antigen-specific T<sub>H</sub>2 cell differentiation *in vitro*.

## *S. mansoni* eggs recruit MHC class II<sup>+</sup> basophils to LN

We sought to determine whether the recruitment of IL-4-eGFP<sup>+</sup> MHC class II<sup>+</sup> basophils was unique to *T. muris* infection or whether they were common events following exposure to other helminth parasites. To test this, we employed footpad injection of *Schistosoma mansoni* eggs whereby delivery of *S. mansoni* eggs results in an acute and synchronous T<sub>H</sub>2 cytokine responses in the draining popliteal lymph node (pLN). In previous studies we have demonstrated robust proliferation of CD4<sup>+</sup> T cells following egg injection with greater than 40% of pLN CD4<sup>+</sup> T cells becoming BrdU<sup>+</sup> and 20% expressing IL-4-eGFP40 providing a powerful *in vivo* model to examine helminth-induced innate and adaptive responses. *S. mansoni* eggs were delivered into the footpad of 4-week mice and pLN harvested at various time points post-injection. A transient recruitment of basophils to the draining pLN occurred by day 2 post-injection with a greater than 20-fold increase in frequency (Fig. 7a) and number (Fig. 7b) that was absent by day 5 (data not shown). Sorted IL-4-eGFP<sup>+</sup> basophils from *S. mansoni* egg-injected mice exhibited characteristic multi-lobular nuclei by cytoplasm (Fig. 7c), were FcεRI<sup>+</sup>, and expressed MHC class II by both flow cytometry (Fig. 7d) and immunofluorescence (Fig. 7e). We next investigated whether helminth-elicited basophils could influence CD4<sup>+</sup> T cell proliferation *in vivo*. To address this, CFSE-labeled CD4<sup>+</sup> T cells were adoptively transferred into MHC II<sup>CD11c</sup> mice. We then utilized the *S. mansoni* egg injection model to assess whether adoptive transfer of basophils influenced helminth-induced proliferation of CD4<sup>+</sup> T cells in the draining pLN. Sorted wild-type basophils from *S. mansoni* egg-injected mice were adoptively transferred into naive MHC II<sup>CD11c</sup>



recipients. We have previously observed that in the absence of additional antigen stimulation in recipient mice, transfer of basophils alone does not induce the recruitment of antigen-specific T cells to the pLN (data not shown). We therefore challenged MHC II<sup>CD11c</sup> mice that had received both T cells and basophils with *S. mansoni* eggs in the footpad. Following egg injection, MHC II<sup>CD11c</sup> mice that received eggs alone exhibited a four- to five-fold increase in total CD4<sup>+</sup> T cell numbers in the draining pLN compared to the non-draining pLN (Fig. 7f) and 50% of pLN CD4<sup>+</sup> T cells were CFSE<sup>lo</sup> (Fig. 7g). In contrast, proliferation of CD4<sup>+</sup> T cells was substantially augmented in MHC II<sup>CD11c</sup> mice that had received activated basophils. At day 4 following delivery of *S. mansoni* eggs, there was a greater than 14-fold increase in total pLN CD4<sup>+</sup> T cells (Fig. 7f) and nearly 70% of CD4<sup>+</sup> T cells were CFSE<sup>lo</sup> (Fig. 7g). This proliferation was consistent with the magnitude of CD4<sup>+</sup> T cell responses we previously observed in egg-injected wild-type mice<sup>40</sup>. In addition, in earlier studies we found that unlike adoptively transferred T cells in *Rag1*<sup>-/-</sup> or *Rag2*<sup>-/-</sup> recipient mice, donor CD4<sup>+</sup> T cells delivered into MHC II<sup>CD11c</sup> mice do not undergo homeostatic proliferation, likely due to the fact that MHC II<sup>CD11c</sup> mice have a normal CD8<sup>+</sup> T cell compartment<sup>26,41</sup>. Collectively, these data demonstrate that MHC class II<sup>+</sup> basophils are rapidly recruited to the lymph node following exposure to helminth antigens and suggest potential cooperation between basophils and DCs in the efficient expansion of helminth-responsive CD4<sup>+</sup> T cells *in vivo*.

## DISCUSSION

Basophils are rare circulating cells that make up less than 0.5% of total blood leukocytes yet are evolutionarily conserved across all vertebrate species and can accumulate in peripheral tissues in multiple settings associated with type 2 inflammation. Although basophils were first described 130 years ago<sup>42</sup>, their scarcity, coupled with a paucity of reagents, has made it difficult to study their function *in vivo*. Availability of new reagents has revealed distinct non-redundant roles for basophils in augmenting CD4<sup>+</sup> T<sub>H</sub>2 cytokine responses<sup>22,43</sup>, in providing B cell help for IgE class-switch recombination and enhanced humoral immune responses<sup>33,44</sup> and in the initiation and maintenance of chronic allergic inflammation<sup>45</sup>. To these functions, the results of the present study add a previously unrecognized role for basophils as accessory cells that can promote CD4<sup>+</sup> T<sub>H</sub>2 cell differentiation in part through MHC class II-dependent cognate interactions as indicated by basophil-CD4<sup>+</sup> T cell co-culture experiments. A critical question that emerges from these findings is where functional basophil-T cell cognate interactions occur *in vivo*. Basophils are readily found in the blood and spleen but have been reported to be excluded from lymph nodes, where CD4<sup>+</sup> T cell priming is likely to take place<sup>46</sup>. However, basophils have recently been shown to be transiently recruited to draining lymph nodes following allergen exposure<sup>22</sup>. In this study we demonstrate that basophils are rapidly recruited to the lymph node following exposure to *S. mansoni* eggs. Basophils that accumulated in lymph nodes co-expressed MHC class II and *il4* mRNA, suggesting that they have the capacity to directly interact with naive T cells in peripheral lymphoid tissues. Consistent with a role in the development of T<sub>H</sub>2 cell responses, *in vivo* depletion of basophils resulted in impaired expression of T<sub>H</sub>2 cytokines and host protective immunity while adoptive transfer of basophils augmented helminth-induced CD4<sup>+</sup> T cell proliferation.

In addition to a potential role in the initial priming of naive CD4<sup>+</sup> T cells in the lymph node, basophils may act as accessory cells at the site of inflammation, where activated T cells may require additional cognate interactions to promote or maintain T<sub>H</sub>2 cell differentiation and effector function. Supporting this notion, a previous report utilizing cytokine reporter mice demonstrated that cytokine mRNA and protein expression are uncoupled following priming and expansion of naive T cells, suggesting that additional activation at the site of infection may be required to license effector function<sup>47</sup>. Depletion of basophils also suggests that these cells may provide chemotactic factors, either directly or indirectly, that are required for the recruitment of eosinophils to peripheral tissues<sup>48</sup>. Microarray analyses of basophils sorted from the lung during *Nippostrongylus brasiliensis* infection also revealed high expression of the chemokines CCL3 (MIP1 $\alpha$ ), CCL4, (MIP1 $\beta$ ), CCL6 (C10), and CCL17 (TARC), supporting a role for basophils in the recruitment of activated CD4<sup>+</sup> T cells to the site of infection<sup>21</sup>. Therefore, identifying the factors that regulate basophil proliferation and recruitment could be an important target for modulating early events in the generation of CD4<sup>+</sup> T<sub>H</sub>2 cell-dependent immunity and inflammation.

Previous reports identified T cell-derived IL-3 as a critical cytokine for basophilia during intestinal helminth infection<sup>49</sup>. However, whether other innate cell-derived cytokines contribute to early basophil responses is unclear. We recently identified a critical role for intestinal epithelial cell (IEC) activation in the generation of protective T<sub>H</sub>2 cytokine-dependent immunity to *T. muris* and demonstrated that TSLP is an important part of the IEC response required for immunity to infection<sup>27,50</sup>. Here we have shown that delivery of recombinant TSLP resulted in the selective accumulation of basophils in the periphery, identifying a previously unappreciated role for TSLP in promoting basophilia. TSLP has previously been implicated in the promotion of type 2 inflammation in the skin and lung through effects on both innate and adaptive immune cells<sup>51,52</sup>. Although no analysis of basophil responses were conducted in the TSLP-transgenic mice used in those earlier studies, it is tempting to speculate that a component of the enhanced type 2 inflammation observed could be a consequence of elevated basophil responses.

In addition to TSLP, the IEC-derived cytokines IL-25 and IL-33 have also been implicated in the promotion of type 2 inflammation and IL-33 can directly activate basophils<sup>34,36–38,53–56</sup>. However, we demonstrate that these cytokines, although capable of promoting the accumulation of IL-4-eGFP<sup>+</sup> innate cells, do not promote basophilia *in vivo*. Rather, IL-25 promoted the proliferation and/or accumulation of c-Kit<sup>+</sup> cells while IL-33 promoted peripheral eosinophilia. While TSLP appears to have a selective effect on basophil responses, the influence of TSLP, IL-25, and IL-33 in combination with other stimuli such as IL-3, IL-18, TLR ligation, and Fc $\epsilon$ RI crosslinking on basophil cytokine production, lymph node recruitment and APC function remains to be determined.

In addition to IEC-derived cytokines, there is evidence to suggest that basophils can be directly activated by either helminth-derived products or allergens that may act as ‘superallergens’ to stimulate Fc $\epsilon$ RI cross-linking in a non-antigen-specific manner<sup>57</sup>. For example, IPSE $\alpha$ 1, a glycoprotein derived from *S. mansoni* eggs, has been shown to directly stimulate the production of IL-4 from basophils by an IgE-dependent but non-antigen-specific mechanism<sup>58</sup>. Thus, a combination of non-hematopoietic and innate immune cell-

derived cytokines, coupled with direct stimulation by helminth products or allergens, may act together to elicit basophil proliferation and activation *in vivo*.

In addition to the identification of a role for basophils in MHC class II-dependent promotion of T<sub>H</sub>2 cell differentiation and immunity to *T. muris* infection, Medzhitov and colleagues found a critical role for basophils in the development of allergen-specific T<sub>H</sub>2 cytokine responses. In those studies, allergen-stimulated basophils expressed MHC class II, CIITA, the invariant chain and co-stimulatory molecules and promoted allergen-specific CD4<sup>+</sup> T<sub>H</sub>2 cell differentiation (personal communication, R. Medzhitov). Taken together, these findings indicate that basophil-mediated recognition of allergens and helminth-derived products, coupled with their MHC class II-dependent promotion of T<sub>H</sub>2 cell responses, may be an evolutionarily conserved pathway that plays a cardinal role in the development of type 2 inflammation at mucosal sites.

## METHODS

### Mice and parasites

6–8 week-old C57BL/6 mice and timed pregnant female B6.SJL mice were obtained from The Jackson Laboratories. MHC II<sup>CD11c</sup> (also known as CD11c-A $\beta$ <sup>b</sup>; generated as previously described<sup>26</sup>), MHC class II-deficient (*H2Ab1*<sup>-/-</sup>), 4-get-IL-4eGFP (C.129-*IL4<sup>tm1Lky</sup>/J*) (from M. Mohrs, Trudeau Institute), B cell-deficient ( $\mu$ MT), CD11c-DTR, and DO11.10 mice were bred and housed in specific pathogen-free conditions at the University of Pennsylvania. Littermate control mice were sham-grafted and MHC II<sup>CD11c</sup> mice were given subcutaneous thymic grafts from neonatal (0–2 days) B6.SJL mice at 4–6 weeks of age and allowed 8 weeks to reconstitute CD4<sup>+</sup> T cells before experimental use. Bone marrow chimeras were generated by i.v. injection of  $5 \times 10^6$  bone marrow cells from wild-type or CD11c-DTR mice into irradiated (2 times 500 rads) wild-type recipients. Recipient mice were on antibiotics for two weeks and allowed 8 weeks to reconstitute. All experiments were performed following the guidelines of the University of Pennsylvania Institutional Animal Care and Use Committee. *T. muris* was maintained in genetically susceptible mouse strains and eggs harvested as previously described<sup>31</sup>. Mice were infected by oral gavage with 200–300 embryonated *T. muris* eggs. *S. mansoni* eggs were prepared as previously described<sup>40</sup>. Mice were injected in the footpad with 2500 eggs in 50  $\mu$ l PBS.

### Polyclonal T cell stimulation

mLNs were harvested and single-cell suspensions prepared in complete media (DMEM supplemented with 10% heat-inactivated FBS, 2 mM glutamine, 100 U/ml penicillin, 100  $\mu$ g/ml streptomycin, 25 mM HEPES, and 50  $\mu$ M beta-mercaptoethanol). mLN cells were seeded in 48-well plates at  $2.5 \times 10^6$  per well and incubated with either media or 1  $\mu$ g/ml soluble anti-CD3 and anti-CD28 (eBioscience) for 48 h. Cell-free supernatants were harvested and cytokine production determined by sandwich ELISA (all antibody pairs purchased from eBioscience: clones AN-18, R4-6A2 (IFN- $\gamma$ ), 11B11, BVD6-24G2, (IL-4), TRFK5, TRFK4 (IL-5) and eBio13A, eBio 1316H (IL-13)).

### Immunoblot

Fecal protein isolation was performed as previously described<sup>28</sup>. 30 µg of protein was loaded per sample for analysis by SDS-PAGE and immunoblotted for RELMβ using a polyclonal rabbit α-murine RELMβ (Peprotech).

### Real-time PCR

RNA was isolated from intestinal tissues of mice using a TRizol extraction (Invitrogen) and from mLN cells using RNEasy Spin Columns (Qiagen). Tissues were disrupted in a tissue homogenizer (TissueLyzer, Qiagen) and cDNA synthesized using Superscript Reverse Transcriptase (Invitrogen). Quantitative real-time PCR was carried out on cDNA samples using commercial primer sets (Qiagen *il4* QT00160678, *ifng* QT01038821, *il10* QT00106169, *il17a* QT00103278) and SYBR Green chemistry. All reactions were run on an ABI 7500 Fast Real-Time PCR System (Applied Biosystems). Samples are normalized to naive controls unless otherwise stated.

### Histology and immunofluorescence

Cecal tips were fixed in 4% paraformaldehyde (PFA) and embedded in paraffin. 5 µm sections were cut and stained for hematoxylin and eosin (H&E) or Alcian blue-periodic acid-Schiff. Unstained sections prepared on immunoslides were stained for Gob5 by immunofluorescence as previously described<sup>27</sup>. Briefly, samples were de-paraffinized by consecutive methanol and ethanol rinses, boiled in citric acid buffer and stained with an antibody against Gob5 at 4 °C overnight followed by staining with a Cy2-conjugated anti-goat antibody. Sorted basophils were subjected to cytopspin and fixed in 2% PFA at 4 °C overnight. Slides were washed in PBS, permeabilized in Triton-X, blocked with streptavidin and biotin, and stained with anti-MHC class II-biotin and anti-GFP at 4 °C overnight. Slides were then washed with PBS and stained with streptavidin-Cy3 and donkey anti-rabbit-Cy2 for 2 h at 25 °C, washed with PBS, and nuclei stained with DAPI.

### Neutralizing and depleting antibodies and recombinant cytokines

Neutralizing monoclonal antibody against IFN-γ (XMG-6) was purified from ascites (grown by Harlan Bioscience) by ammonium sulfate precipitation and dialyzed against PBS. Mice were given 1 mg antibody i.p. every 3–5 days during the course of infection starting at day 0. Basophils were depleted by i.p. injection of 10 µg anti-FcεRI (MAR-1, eBioscience) on days 0, 1, 2 and 10, 11, 12 post-infection. Recombinant murine IL-25 (4 µg/ml), IL-33 (20 µg/ml), and TSLP (0.1 mg/ml) were all purchased from R&D Systems and 100 µl in PBS was injected i.p. once daily for four days.

### Basophil isolation and CD4<sup>+</sup> T cell co-culture

CD4<sup>+</sup> T cells were isolated from spleens by negative selection via incubation with hybridoma supernatants (αB220, αF<sub>C</sub>R, αCD8, αMHCII) followed by magnetic bead purification (Qiagen). To obtain purified basophils, blood, spleen, and mLN cells were isolated from 4-get IL-4–eGFP reporter mice injected with 10 µg rTSLP (R&D System) i.p. once daily for 4 days to enrich for basophils, positively selected for CD49b expression by MACs column purification (Milltenyi) and stained with fluorochrome-conjugated mAbs

against B220 (RA3-6B2), CD3 $\epsilon$  (145-2C11), c-Kit (2B8), CD49b (HM $\alpha$ 2), and Fc $\epsilon$ RI (MAR-1) (BD Bioscience and eBioscience). Basophils were sorted based on negative staining for B220, CD3 and c-kit, positive staining for CD49b, Fc $\epsilon$ RI and expression of IL-4-eGFP using a FACS Aria (BD Bioscience). Following purification, sorted basophils were resuspended at  $1 \times 10^5$  cells/ml in complete medium. 100  $\mu$ l of basophils were used for cytospin and stained by Diffquick to confirm cellular morphology. Between  $5 \times 10^3$  –  $1 \times 10^4$  basophils were co-cultured with  $2 \times 10^5$  purified, CFSE-labeled DO11.10 CD4 $^+$  T cells with 10 ng/ml rIL-3 (RnD Systems) in the presence or absence of 1  $\mu$ g per ml OVA peptide and 5  $\mu$ g/ml blocking antibody for MHC class II (M5/114). After four days of culture, cells were stimulated with PMA, ionomycin, and brefeldin A for 4 h. Cells were pelleted at 485 g for 5 min. Supernatants were collected for ELISA and cells washed in FACS buffer, incubated with Fc Block (2.4G2 and rat IgG) for 10 min at 4  $^{\circ}$ C, stained with fluorochrome-conjugated monoclonal antibodies against CD4 (RM4-5) and fixed with 2% paraformaldehyde. Cells were permeabilized with 0.4% saponin in FACS buffer and stained for intracellular cytokines using fluorochrome-conjugated monoclonal antibodies against IL-4 (11B11) and IL-13 (eBio13A) (eBioscience).

### Adoptive transfer of basophils

C57BL/6 mice were injected with  $2.5 \times 10^3$  *S. mansoni* eggs in each footpad, popliteal lymph nodes, spleen, and blood pooled two days later, and basophils purified by sequential CD49b enrichment and cell sorting as described above. Recipient MHC II $^{CD11c}$  mice were given  $1 \times 10^7$  purified CFSE-labeled CD4 $^+$  T cells from naive C57BL/6 mice one day prior to egg injection. Sorted basophils were resuspended in a PBS plus *S. mansoni* egg suspension and each recipient MHC II $^{CD11c}$  mouse given either  $5 \times 10^4$  basophils and  $2.5 \times 10^3$  *S. mansoni* eggs or  $2.5 \times 10^3$  *S. mansoni* eggs alone in the right footpad in a volume of 50  $\mu$ l. Draining and non-draining pLN cells were isolated 4 days post-egg injection, stained with fluorochrome-conjugated monoclonal antibodies and analyzed by flow cytometry on a FACSCanto II (BD Biosciences).

### Macrophage and dendritic cell depletion

PBS- or Clodronate-loaded liposomes were prepared as previously described<sup>59</sup>. 150  $\mu$ l of liposomes were injected i.v. every 2 days during the course of infection. Clodronate was a gift from Roche Diagnostics GmbH (Mannheim, Germany). DC were depleted in the CD11c-DTR mice by injection of 100ng diphtheria toxin i.p. per mouse every 3 days during the course of infection.

### Statistics

Results represent the mean  $\pm$  SEM unless otherwise stated. Statistical significance was determined by the Student's *t* test.

### Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

The authors would like to thank A. Troy for critical reading of this manuscript, E. Tait for performing the bone marrow chimeras, and the University of Pennsylvania Flow Cytometry Core and Center for Molecular Studies in Digestive and Liver Diseases Morphology Core for assistance with sorting and immunofluorescence staining. Research in the Artis lab is supported by the National Institutes of Health (NIH), AI61570, AI074878 (D.A.), NIH T32 Training Grant AI007532-08 (J.G.P.), NIH F31 Training Grant GM082187 (S.A.S.), the Burroughs Wellcome Fund (Investigator in Pathogenesis of Infectious Disease Award to D.A.), the Pilot Feasibility Program of the National Institute of Diabetes and Digestive Kidney Diseases (NIDDK) DK50306, the Crohns and Colitis Foundation of America's William and Shelby Modell Family Foundation Research Award (D.A., M.G.N.), and pilot grants from the University of Pennsylvania (University Research Foundation Award, PGI Pilot Grant, and UPenn Center for Infectious Diseases Pilot Grant to D.A., Department of Medicine to D.A. and T.M.L.). Work in the Pearce lab is supported by NIH grants AI32573 and AI53825 Work in the Laufer lab is supported by the NIH. Michael Comeau is employed by Amgen.

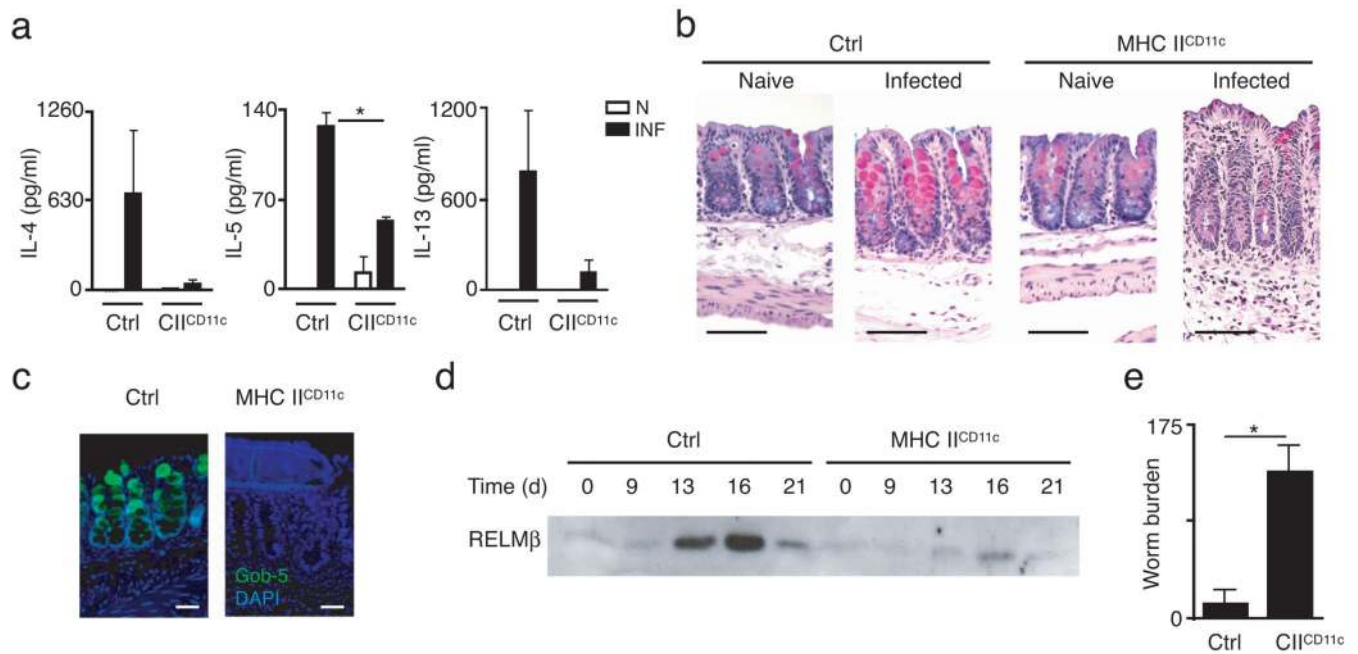
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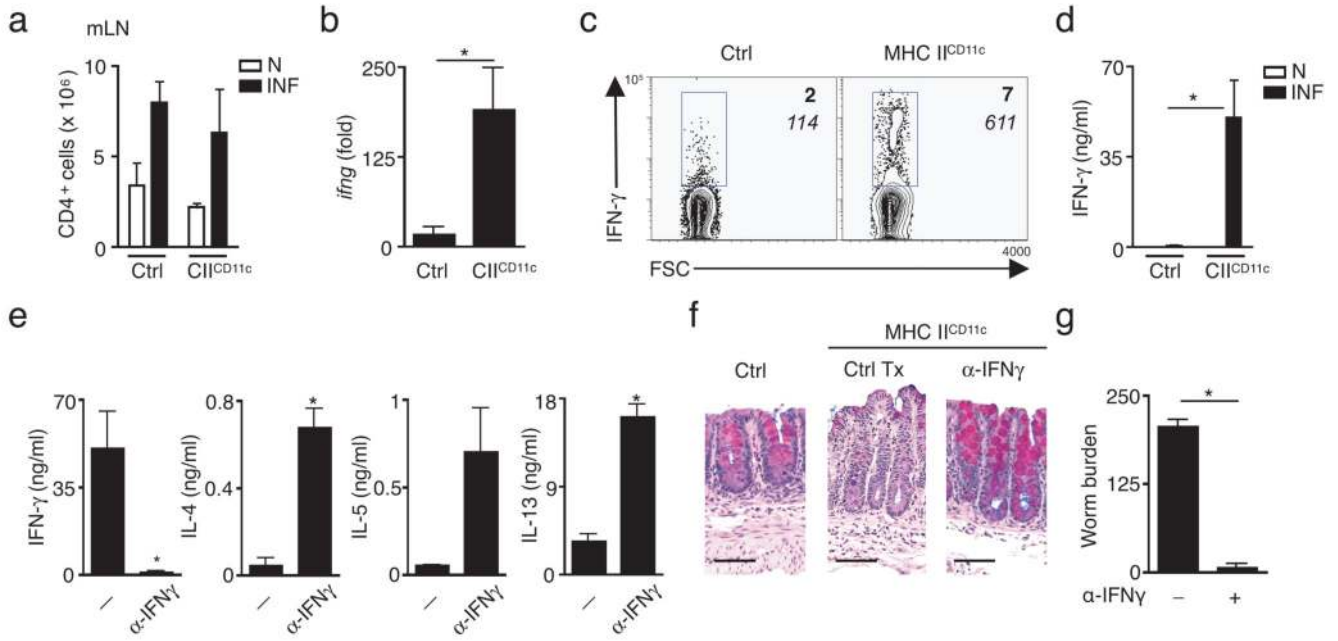
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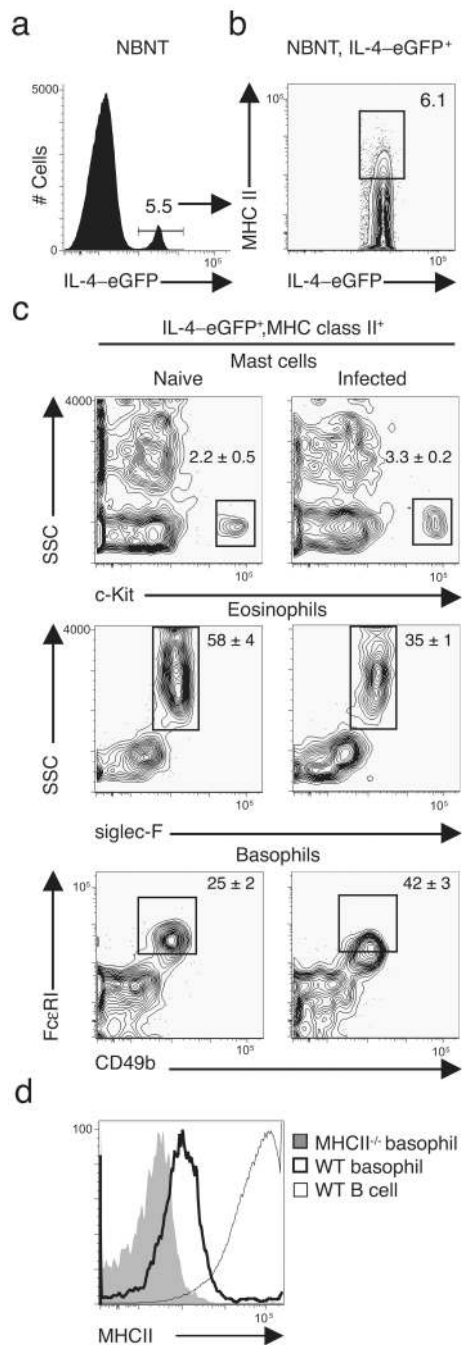
**Figure 1. MHC class II expression restricted to CD11c<sup>+</sup> DC is insufficient to promote type 2 immunity to intestinal helminth infection**

(a–e) Littermate control and MHC II<sup>CD11c</sup> mice were infected with *T. muris* eggs and sacrificed on day 21 post-infection. (a) mLN cells from naïve (N, open bars) and infected (INF, filled bars) mice were cultured *in vitro* for 48 h and supernatants were assayed by ELISA for IL-4, IL-5, and IL-13 secretion. \* $P = 0.003$  (b) Cecal sections from naïve and infected control or MHC II<sup>CD11c</sup> mice were stained with Alcian blue/periodic-acid Schiff reagent to detect mucins. Bar = 20  $\mu\text{m}$  (c) Cecal sections from day 21 infected control and MHC II<sup>CD11c</sup> mice were stained by immunofluorescence for Gob-5 (green) and DAPI (blue). (d) Protein extracted from pooled fecal pellets of control and MHC II<sup>CD11c</sup> mice collected on the indicated days post-infection was analyzed by immunoblot to assess luminal secretion of RELM $\beta$ . (e) Cecal worm burdens from infected control and MHC II<sup>CD11c</sup> mice were determined microscopically at day 21 post-infection. \* $P = 0.0006$  Results are representative of three independent experiments with  $n = 3\text{--}5$  mice per group (a–e). Graphs represent mean  $\pm$  SEM of  $n = 3\text{--}5$  mice per group (a,e).



**Figure 2. Blockade of IFN $\gamma$  in MHC II<sup>CD11c</sup> mice recovers Th2 cytokine-dependent immunity to *T. muris* infection**

(a) mLN cells from naïve (N, open bars) or *T. muris*-infected (INF, filled bars) littermate control and MHC II<sup>CD11c</sup> mice were counted and total numbers of CD4<sup>+</sup> T cells determined by flow cytometry. Values are mean  $\pm$  SEM of three mice per group representative of three independent experiments of  $n = 3-5$  mice per group. (b) *Ifng* mRNA expression in mLN was determined by quantitative real-time PCR. Values represent mean  $\pm$  SEM of the fold-increase over naïve controls. \* $P = 0.03$  (c) Flow cytometry of intracellular IFN- $\gamma$  staining of mLN cells from infected control or MHC II<sup>CD11c</sup> mice. Plots are gated on CD4<sup>+</sup> T cells. Numbers indicate cells within the gated area. IFN- $\gamma$ <sup>+</sup>, italics = mean fluorescence intensity. Plots representative of three independent experiments of  $n = 3-5$  mice per group. (d) IFN- $\gamma$  secretion by mLN cells isolated from naïve (N, open bars) and infected (INF, filled bars) mice was examined by ELISA. \* $P = 0.03$ . (e) Cytokine production by stimulated mLN cells isolated from control-treated (Tx) or anti-IFN $\gamma$ -treated MHC II<sup>CD11c</sup> mice was examined by ELISA. IFN- $\gamma$  \* $P = 0.03$ , IL-4 \* $P = 0.002$ , IL-13 \* $P = 0.001$ . (f) Cecal sections from littermate control, control-treated or anti-IFN $\gamma$ -treated MHC II<sup>CD11c</sup> mice stained for mucins with Alcian blue-periodic-acid Schiff. Images representative of  $n = 3-4$  mice per group. (g) Worm burdens from infected control-treated or anti-IFN $\gamma$ -treated MHC II<sup>CD11c</sup> mice at day 21 post-infection. \* $P < 0.001$ . Results are presented as mean  $\pm$  SEM  $n = 3-4$  mice per group (e.g).



### Figure 3. FcεRI<sup>+</sup> CD49b<sup>+</sup> basophils co-express MHC class II and IL-4/eGFP

Flow cytometry of splenocytes isolated from naïve and *T. muris*-infected 4-get mice at day 14 post-infection. (a) IL-4-eGFP<sup>+</sup> cells were identified from a non-B non-T (NBNT) cell gate (CD3<sup>-</sup>B220<sup>-</sup>CD19<sup>-</sup>) and (b) analyzed for co-expression of MHC class II and IL-4-eGFP. (c) IL-4-eGFP<sup>+</sup>, MHC class II<sup>+</sup> NBNT cells were characterized for expression of c-Kit, siglec-F, FcεRI, and CD49b. Plots representative of *n* = 3 mice per group (a–c); numbers are mean ± SEM (c). (d) MHC class II expression on WT basophils (heavy line),

MHC class II<sup>-/-</sup> basophils (grey filled), or WT B cells (light line) isolated from naïve mice.  
Plots representative of  $n = 3$  mice per group.

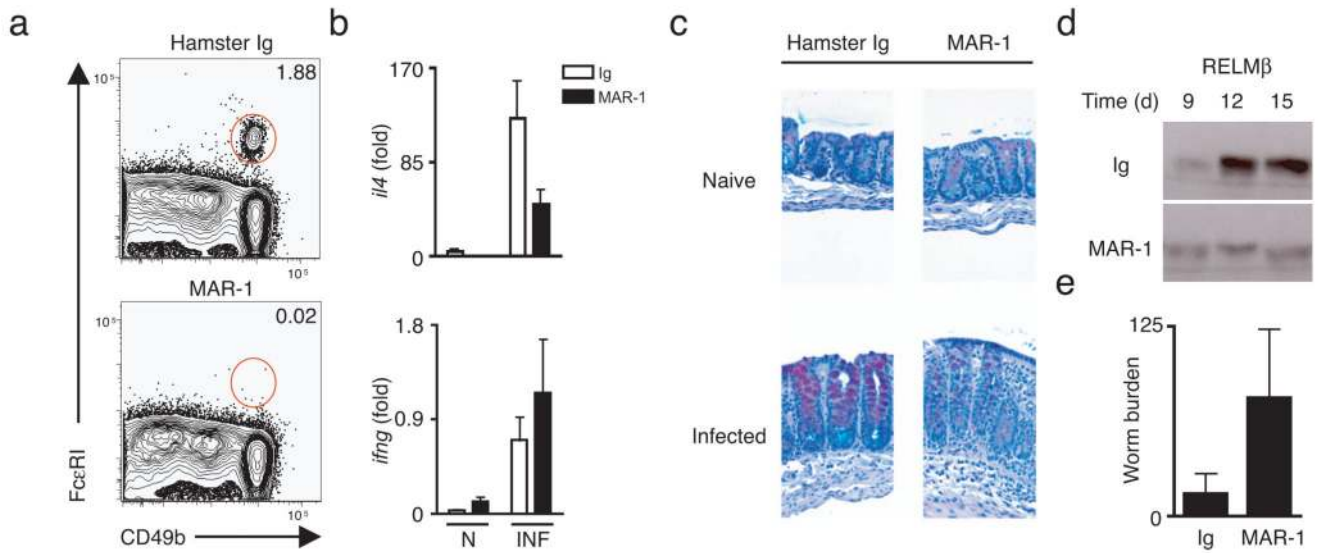
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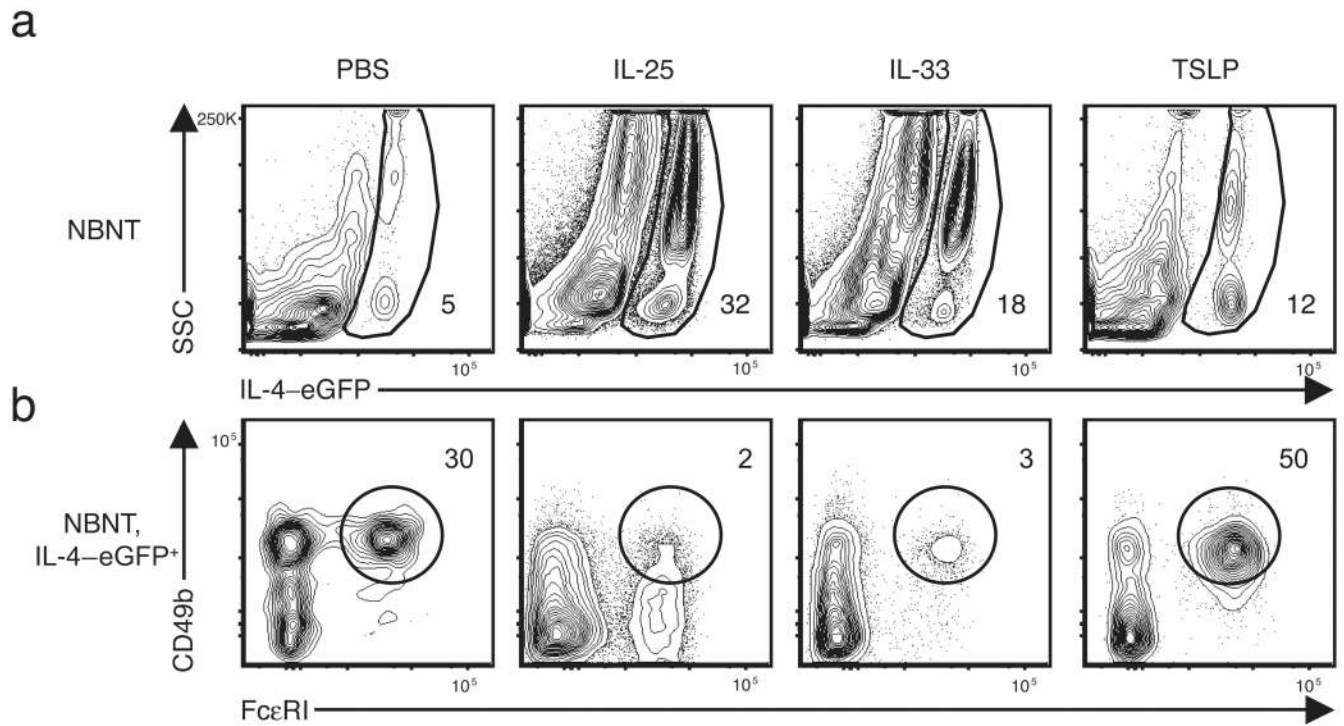
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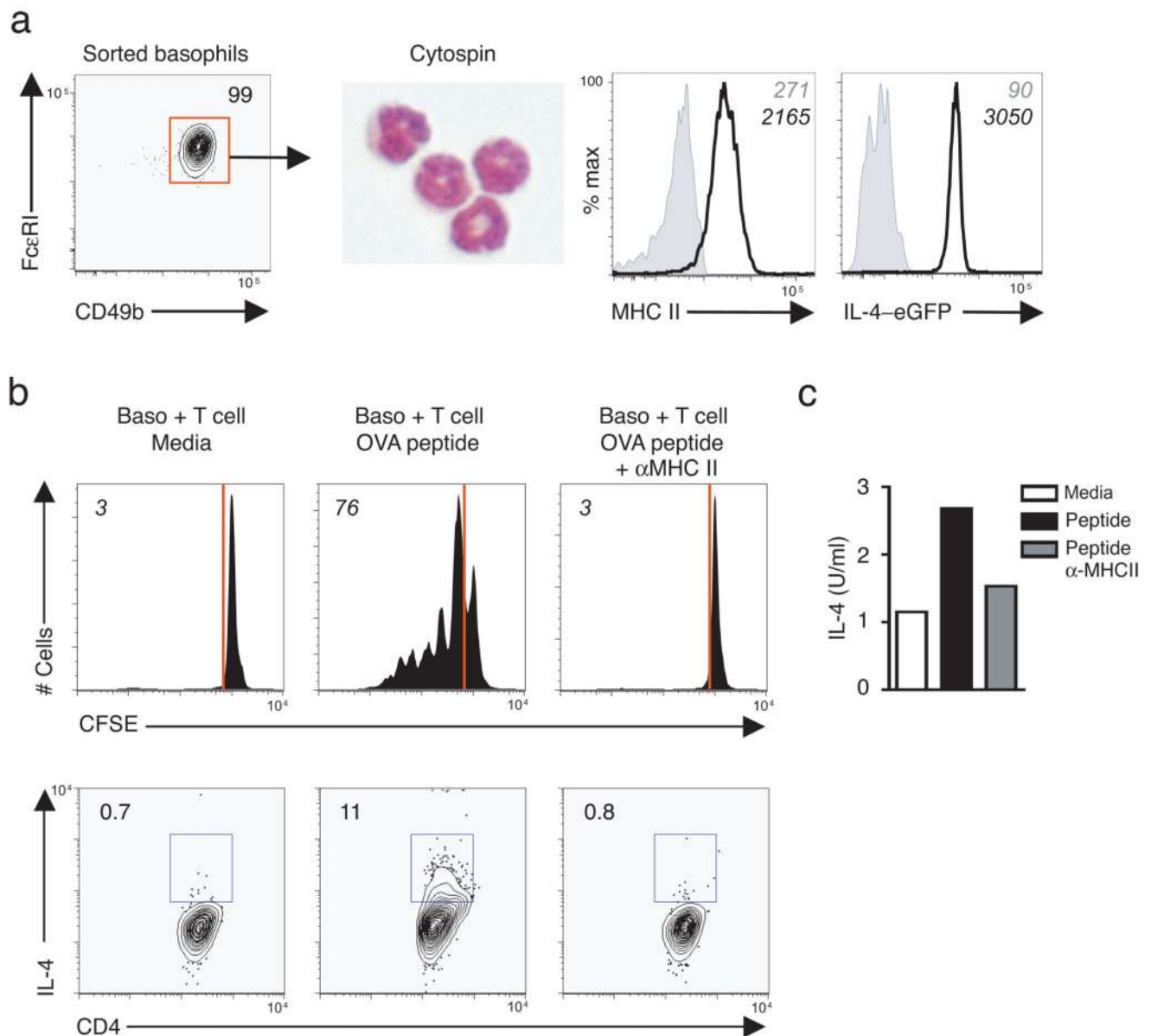


**Figure 4. Depletion of FcεRI<sup>+</sup> cells *in vivo* results in impaired immunity to *Trichuris* infection**  
 (a) Flow cytometric analysis of splenic basophils from control Ig-treated or MAR-1-treated mice at day 21-post-infection. Plots gated on CD3<sup>-</sup>B220<sup>-</sup>CD19<sup>-</sup> non-B non-T cells. (b) Real-time quantitative PCR of colon tissue from naïve and infected Ig-treated or MAR-1-treated mice at day 21-post-infection; results represented as fold increase over naïve Ig-treated controls. (c) Cecal sections from naïve and infected Ig-treated or MAR-1-treated mice at day 21-post-infection stained for mucins with Alcian blue/periodic-acid Schiff. (d) Immunoblot of protein extracted from pooled fecal pellets of Ig-treated or MAR-1-treated mice at indicated days post-infection and immunoblotted for RELMβ. (e) Cecal worm burdens at day 21 post-infection. Results are representative of two independent experiments of  $n = 3-4$  mice per group (a-e).



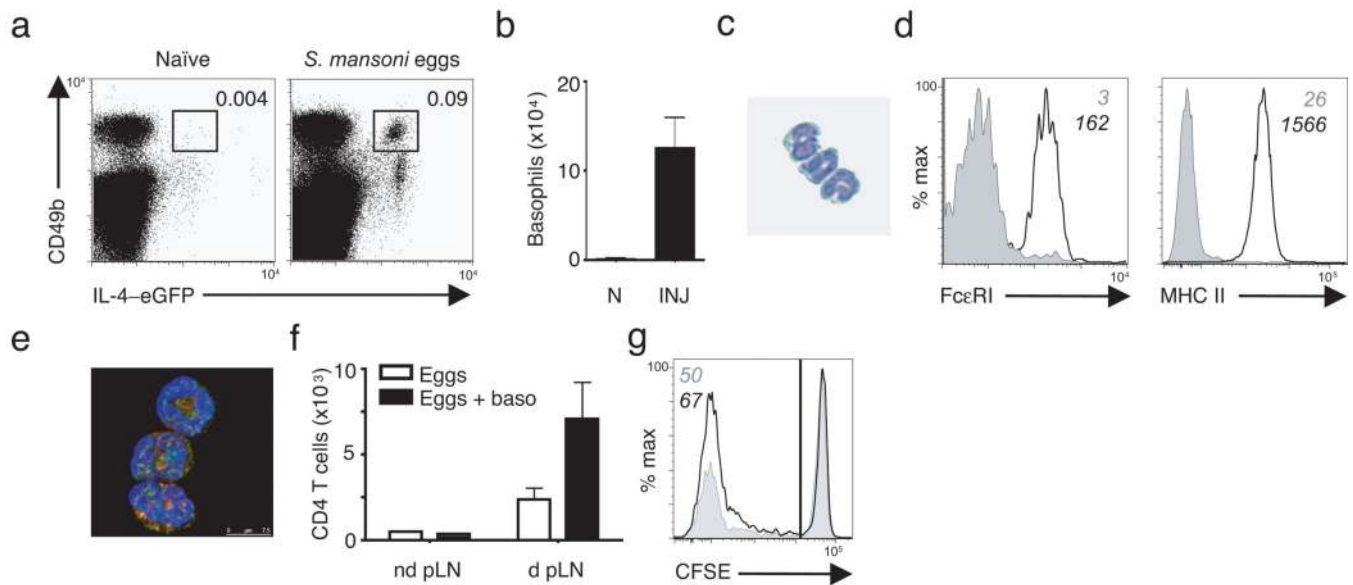
**Figure 5. TSLP treatment selectively increases basophil frequencies *in vivo***

Flow cytometric analysis of basophil frequencies in the blood of mice treated daily for four days with rIL-25, rIL-33 or rTSLP. (a) Non-B non-T cells from the peripheral blood were analyzed for expression of IL-4-eGFP. Numbers indicate frequency of gated population. (b) Basophil frequencies in IL-4-eGFP<sup>+</sup> non-B non-T cell populations. Numbers indicate frequency of gated population. Results representative of at least two independent experiments with  $n = 3$  mice per group.



**Figure 6. Basophils promote MHC class II-dependent antigen-specific CD4<sup>+</sup> T cell proliferation and Th2 cytokine production *in vitro***

(a) Sorted TSLP-elicited basophils were subjected to cytopsin and stained with DiffQuick or examined by flow cytometry for expression of MHC class II and IL-4-eGFP (solid line) over fluorescence minus one (FMO) controls (shaded histograms). Numbers in italics are mean fluorescence intensity. Plots are representative of four independent experiments with  $n = 5-10$  mice pooled per experiment. (b) CFSE-dilution of DO11.10 CD4<sup>+</sup> T cells following 4 day co-culture with basophils in media, OVA peptide, or OVA peptide plus MHC class II blocking antibody (M5114), top. Lower panel indicates frequencies of IL-4<sup>+</sup> CD4<sup>+</sup> T cells by intracellular cytokine staining. (c) Supernatants from basophil-CD4<sup>+</sup> T cell co-cultures in were analyzed for IL-4 secretion by ELISA. Results are representative of two independent experiments (b,c).



**Figure 7. IL-4-eGFP<sup>+</sup> MHC class II<sup>+</sup> basophils are recruited to the draining LN following exposure to *Schistosoma mansoni* eggs and augment CD4<sup>+</sup> T cell proliferation *in vivo***

(a) Flow cytometry of basophil frequencies in popliteal LN from naïve or *S. mansoni* egg-injected 4-get mice day 2 post-injection. (b) Total numbers of basophils in the lymph node of naïve (N) or *S. mansoni* egg injected (INJ) mice. (c) Cytospin of sorted *S. mansoni*-elicited basophils stained by Diffquick. (d) Flow cytometry of sorted *S. mansoni*-elicited basophils stained for FcεRI or MHC class II. Grey histograms are expression on CD3<sup>+</sup> cells, black lines are sorted basophils. Numbers indicate mean fluorescence intensity. (e) Confocal microscopy of sorted *S. mansoni*-elicited basophils. GFP (green); MHC class II (red); DAPI (blue). Results are representative of five independent experiments with  $n = 3-5$  mice per group (a-e). (f) Total numbers of CD4<sup>+</sup> cells in the non-draining (nd pLN) versus draining (d pLN) popliteal lymph nodes of MHC II<sup>CD11c</sup> that received either *S. mansoni* eggs alone or in combination with basophils. (g) Flow cytometry showing CFSE-dilution of donor CD4<sup>+</sup> T cells from MHC II<sup>CD11c</sup> that received either *S. mansoni* eggs alone (grey histogram) or with basophils (black line histogram). Numbers in italics refers to percent of CFSE<sup>10</sup> cells. Results represent one experiment with  $n = 2$  mice per group (f,g).