

CD11c depletion severely disrupts Th2 induction and development in vivo

Alexander T. Phythian-Adams,¹ Peter C. Cook,¹ Rachel J. Lundie,¹ Lucy H. Jones,¹ Katherine A. Smith,¹ Tom A. Barr,¹ Kristin Hochweller,³ Stephen M. Anderton,² Günter J. Hämmerling,³ Rick M. Maizels,¹ and Andrew S. MacDonald¹

¹Institute of Immunology and Infection Research and ²Centre for Inflammation Research, University of Edinburgh, Edinburgh, EH9 3JT, Scotland, UK

³Department of Molecular Immunology, German Cancer Research Centre, 69120 Heidelberg, Germany

Although dendritic cells (DCs) are adept initiators of CD4⁺ T cell responses, their fundamental importance in this regard in Th2 settings remains to be demonstrated. We have used CD11c-diphtheria toxin (DTx) receptor mice to deplete CD11c⁺ cells during the priming stage of the CD4⁺ Th2 response against the parasitic helminth *Schistosoma mansoni*. DTx treatment significantly depleted CD11c⁺ DCs from all tissues tested, with 70–80% efficacy. Even this incomplete depletion resulted in dramatically impaired CD4⁺ T cell production of Th2 cytokines, altering the balance of the immune response and causing a shift toward IFN- γ production. In contrast, basophil depletion using Mar-1 antibody had no measurable effect on Th2 induction in this system. These data underline the vital role that CD11c⁺ antigen-presenting cells can play in orchestrating Th2 development against helminth infection in vivo, a response that is ordinarily balanced so as to prevent the potentially damaging production of inflammatory cytokines.

CORRESPONDENCE

Andrew S. MacDonald:
andrew.macdonald@ed.ac.uk

Abbreviations used: Ab, antibody; DTx, Diphtheria toxin; pLN, popliteal LN; SEA, soluble egg antigen from *Schistosoma mansoni*.

DCs are the most capable APCs in the immune system, experts at recognizing and responding to pathogens and inflammation, and then efficiently priming and directing the function of T cells (Banchereau and Steinman, 1998; Kapsenberg, 2003). Although the importance of DCs in orchestration of Th1 immune responses against viral and bacterial pathogens is clear, their activation and function in response to Th2-inducing pathogens is less well defined (Pearce and MacDonald, 2002; MacDonald and Maizels, 2008). Notably, it is evident from a large body of work that conventional DCs are sufficient for Th2 priming against helminths (Perona-Wright et al., 2006; MacDonald and Maizels, 2008), pathogens that have evolved with their hosts to generate potent “appropriate” Th2 responses (Maizels and Yazdanbakhsh, 2003). However, it has not yet been shown whether they are actually necessary for this process. This work, along with parallel studies into aberrant Th2 response development against allergens (Lambrecht and Hammad, 2009), has established the ability of DCs to prime Th2 responses, although the actual mechanism of DC Th2 induction is still poorly

understood (Jankovic et al., 2006; MacDonald and Maizels, 2008).

We have addressed the fundamental role of CD11c⁺ DCs in Th2 response initiation in vivo using murine infection with the parasitic helminth *Schistosoma mansoni*. This intravascular trematode is an important cause of chronic human disease and a prime example of a helminth that provokes a strong Th2 response intricately linked to the pathology that develops in infection of both mice and humans (Pearce and MacDonald, 2002; Wynn et al., 2004). In addition, studies using *S. mansoni* have been instrumental in revealing the ability of helminths to confer on DCs Th2 inductive ability in vitro and after adoptive transfer in vivo (Perona-Wright et al., 2006).

Murine DCs are characterized by high-level expression of the integrin CD11c, which has allowed the development of inducible models of depletion in vivo (Bar-On and Jung, 2010).

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We have used mice that allow long-term depletion of CD11c⁺ DCs (Hochweller et al., 2008) to directly address their importance during CD4⁺ T cell priming in the chronic Th2 infection setting induced by *S. mansoni*. While focusing on CD11c⁺ DCs during Th2 induction in vivo, we have also addressed the possible role of basophils in this process, as recent work has suggested that Th2 responses may be initiated by these granulocytes rather than by DCs (Perriou et al., 2009; Sokol et al., 2009; Yoshimoto et al., 2009). Although IL-4 production by basophils can potentiate Th2 development in some settings, a general role for these cells as APCs seems unlikely given their questionable ability to process and present complex antigens, their relatively low level of expression of MHCII and other molecules required for efficient naive T cell priming, and the apparently transient nature of their recruitment to lymphoid tissues (Finkelman, 2009; Lambrecht and Hammad, 2009; Voehringer, 2009; Paul and Zhu, 2010).

Our results demonstrate for the first time that, after depletion of CD11c⁺ cells, Th2 responses are severely impaired either after *S. mansoni* egg injection or during active *S. mansoni* infection. In contrast, depletion of basophils using Mar-1 anti-Fc ϵ R1 α antibody (Ab) has no significant effect on the Th2 response in this system. This suggests that, in this strong Th2 setting, CD11c⁺ DCs are critical for Th2 induction and development and that other CD11c⁻ APC types, such as basophils, cannot fulfill this role.

RESULTS AND DISCUSSION

To assess the importance of CD11c⁺ DCs in Th2 priming in a relevant infection system, we required a model capable of their inducible depletion. We used a recently developed BAC transgenic mouse model with the human diphtheria toxin (DTx) receptor (DTR) under control of the CD11c promoter (CD11c.DOG mice; Hochweller et al., 2008), allowing depletion of CD11c⁺ DCs by administration of DTx. In contrast to previously published CD11c.DTR mice, where repeat injections of DTx are lethal after several days unless BM chimeras are used (Bar-On and Jung, 2010), CD11c.DOG mice allow depletion for up to 11 d without toxicity (Hochweller et al., 2008).

The impact of CD11c or basophil depletion on Th2 induction in response to *S. mansoni* eggs

S. mansoni eggs are the major stimulus for Th2 cytokines during infection (Pearce and MacDonald, 2002), and their injection provides a controlled system for Th2 induction in the draining LN without the additional complexities of active infection. To address the relative importance of CD11c⁺ DCs and basophils for Th2 induction against this challenge, we administered DTx or Mar-1 anti-Fc ϵ R1 α Ab, alone or in combination, to CD11c.DOG x 4get (IL-4-eGFP) reporter mice that were then immunized with *S. mansoni* eggs. CD11c^{Hi}MHCII⁺ DCs were strikingly depleted in the popliteal LNs (pLN) of mice receiving DTx (~80% efficacy; Fig. 1, A and B). Similarly, eGFP⁺B220⁻CD4⁻CCR3⁻CD117⁻ basophils (Perona-Wright et al., 2008) were depleted in Mar-1

Ab-treated animals (~90% efficacy; Fig. 1, C and D). Notably, DTx caused no measurable decrease in basophils and Mar-1 caused no measurable decrease in DCs in this system.

To evaluate the impact of CD11c or basophil depletion on Th2 initiation, we harvested pLN 7 d after schistosome egg injection and cultured the LN cells with *S. mansoni*-soluble egg Ag (SEA). CD11c depletion dramatically impaired production of the Th2 cytokines IL-4 and IL-10, coincident with increased IFN- γ secretion (Fig. 1, E and F). In contrast, basophil depletion did not significantly alter levels of any cytokines measured, and concurrent depletion of basophils and CD11c⁺ cells failed to further reduce the Th2 response remaining after CD11c depletion alone (Fig. 1 E).

To specifically gauge the impact of CD11c or basophil depletion on CD4⁺ T cells, we assessed IL-4-eGFP levels in pLN CD4⁺ T cells. In keeping with impaired IL-4 secretion after LN cell culture (Fig. 1 E), only CD4⁺ T cells from CD11c-depleted mice displayed significantly reduced eGFP expression, with basophil depletion having no obvious effect on this, either alone or in combination with DTx administration (Fig. 1, G and H).

These data suggest that CD11c⁺ DCs, not basophils, play a major role in Th2 induction in response to the potent stimulus provided by *S. mansoni* eggs. Defective Th2 cytokine production was evident despite incomplete depletion (~80%), with residual DCs likely explaining the minor Th2 response remaining after DTx treatment. The data additionally suggest that CD11c depletion alters the balance of the immune response, causing an increased ratio of IFN- γ to IL-4, a pattern associated with damaging immunopathology during active *S. mansoni* infection (Stadecker et al., 2004). This did not reflect a switch from Th2 to Th1, as CD4⁺ T cell IFN- γ production was also impaired after CD11c depletion (Fig. S1 A), and could simply indicate decreased counter-regulation of non-CD4⁺ T cell IFN- γ when Th2 cytokine production (particularly IL-10) is impaired.

The impact of CD11c depletion on Th2 induction during *S. mansoni* infection

Having established that CD11c depletion has a major impact on Th2 induction in vivo using the egg injection model, we next asked whether this was also the case in the more relevant, but more complex, setting of active *S. mansoni* infection. In this well-characterized model, production of the Th2-driving egg stage of the parasite starts ~28 d after infection. Until this point, the immune response against developing worms consists of a low-level mixed Th1/Th2 profile, with the eggs being the main trigger for induction of a Th2-dominated cytokine response (Pearce and MacDonald, 2002). Because our intention was to address the role of DCs in Th2 priming rather than maintenance or regulation, we targeted depletion of CD11c⁺ cells to the earliest possible onset of egg production (4 wk after infection).

Daily administration of DTx from day 28 to 39 significantly depleted splenic CD11c^{Hi}MHCII⁺ DCs in naive or *S. mansoni*-infected mice (Fig. 2, A and B; ~70% efficacy).

DCs from the CD11b⁺CD8⁻, CD11b⁻CD8⁺ and CD11c^{Int}B220^{Int}MHCII⁺CD11b⁻ subsets were all effectively depleted (Fig. S2, A–C). CD11c depletion was also measured in the spleen by quantitative PCR (qPCR), as well as in the liver and gut, the major sites of egg deposition during *S. mansoni* infection (Fig. S2 D).

To explore whether CD11c depletion affected the cytokine balance during infection, we assessed splenocyte responses after DTx administration. Similar to the egg injection model (Fig. 1), production of IL-4, as well as IL-5 and IL-13, was significantly reduced in CD11c-depleted infected mice

(Fig. 2 C). In contrast to what was seen with the eggs alone (Fig. 1 F), this reduced Th2 response did not result in a compensatory increase in IFN- γ secretion, with this cytokine being maintained at a similar level to PBS controls (Fig. 2 D). No IL-17 was detected, in keeping with studies suggesting that C57BL/6 mice make little of this cytokine in response to *S. mansoni* (Rutitzky et al., 2005).

Thus, in stark contrast to reduced production of Th2 cytokines in both egg injection and infection systems, IFN- γ secretion after CD11c depletion was not impaired and was instead either elevated (egg injection; Fig. 1 F) or remained intact

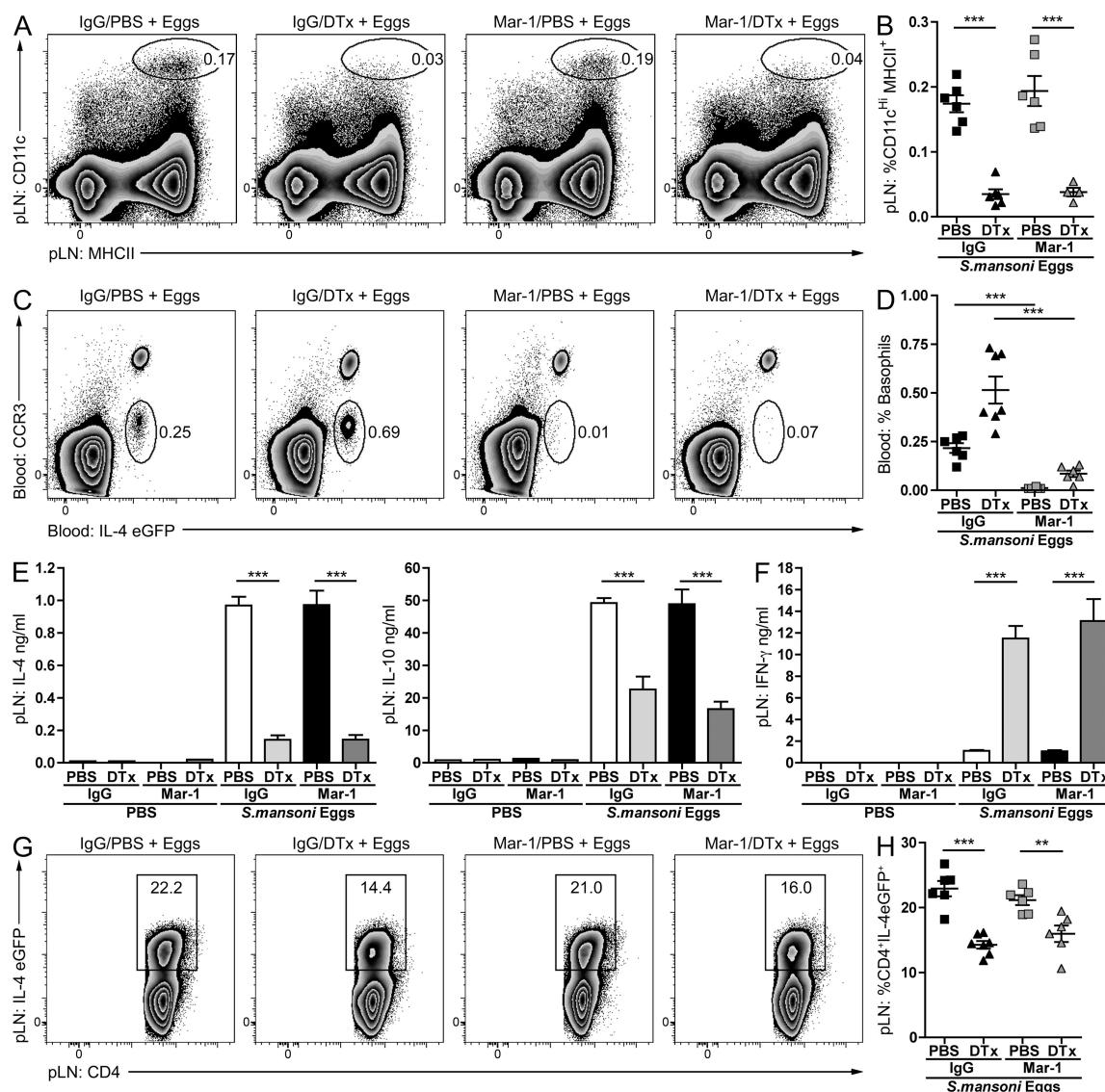


Figure 1. CD11c, not basophil, depletion disrupts Th2 induction in schistosome egg-challenged mice. CD11c.DG x 4get mice were treated daily with PBS (squares) or DTx (triangles) from day –2 to 6. On day –1, 1, and 3 mice were also treated with IgG (black symbols) or Mar-1 (gray symbols). On day 0, mice were challenged with *S. mansoni* eggs or PBS. pLN CD11c⁺MHCII⁺ cell depletion was assessed on day 7 (A and B), and IL-4-eGFP⁺B220⁺CD4⁺CCR3⁺CD117[–] basophil depletion in the blood on day 4 (C and D), after egg injection. On day 7, pLN cells from naive or egg-injected, PBS (white or black bars) or DTx (gray bars), IgG or Mar-1-treated mice were cultured for 72 h with SEA or medium alone. The supernatants were collected, and SEA-specific cytokine production (medium alone values subtracted) was assessed by ELISA (E and F). pLN cells were also assessed on day 7 for IL-4-eGFP expression (G and H). One of three experiments. Error bars are mean \pm SEM of four to seven mice/group.

(infection; Fig. 2 D). The different levels of IFN- γ observed in the two models may be caused by regulatory networks in place by week 4 of active infection not being developed in the acute egg injection system, or it may be that eggs alone provide a more robust stimulus for IFN- γ than infection. Irrespective of this, the cumulative effect in both models was a shift in the cytokine balance from Th2 to IFN- γ , a profile previously shown to result in increased pathology and mortality during murine schistosome infection (Stadecker et al., 2004).

To specifically assess the impact of CD11c depletion on Th2 cells, splenic CD4 $^{+}$ T cells were purified from infected mice and stimulated in medium alone or with SEA in the presence of irradiated splenocytes from naive mice. Similar to the

pattern observed in bulk splenocyte cultures (Fig. 2 C), purified CD4 $^{+}$ T cells displayed severely impaired IL-4, IL-5, IL-10, and IL-13 production (Fig. 2 E). This impairment was not restricted to the Th2 cytokines, but also affected CD4 $^{+}$ cell secretion of the Th1 cytokine IFN- γ (Fig. 2 F and Fig. S1 B), suggesting a non-CD4 $^{+}$ T cell source for a proportion of the IFN- γ detected after stimulation of splenocytes from infected DTx-treated mice (Fig. 2 D). The identity of the non-CD4 $^{+}$ IFN- γ producers evident after CD11c depletion of egg injected or infected mice remains to be determined, but is likely an innate cell type less reliant on DCs for activation than T cells.

To further investigate the ramifications of CD11c depletion on the CD4 $^{+}$ T cell response in infected mice, we

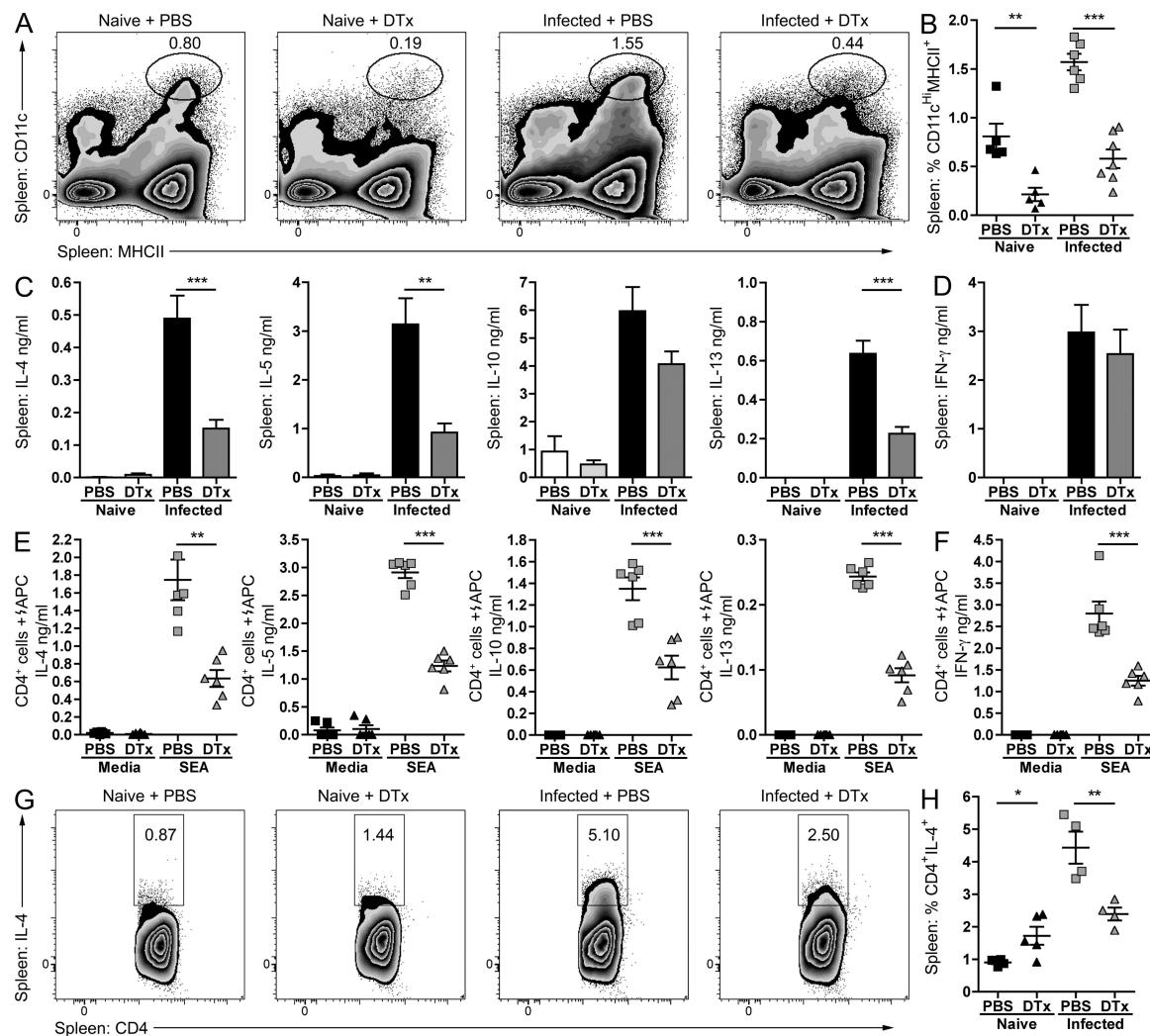


Figure 2. CD11c depletion compromises Th2 induction and development during schistosome infection. CD11c.DOG mice were treated daily with PBS (squares) or DTx (triangles) from day 28 to 39 of *S. mansoni* infection. Naive (black symbols) and infected (gray symbols) mice were assessed for splenic CD11c^{hi}MHCII^{hi} cell depletion on day 40 (A and B) and splenocytes (C and D) from naive or infected PBS (white or black bars)- or DTx (gray bars)-treated mice, or purified CD4 $^{+}$ T cells and irradiated splenocytes (E and F), were cultured for 72 h with SEA or medium alone. The supernatants were collected, and cytokine production was assessed by ELISA. Splenic CD4 $^{+}$ T cell IL-4 production by naive (black symbols) or infected (gray symbols) mice was also assessed by intracellular cytokine staining (G and H). One out of six (A and B), three (C and D), or two (E-H) experiments. Error bars are mean \pm SEM of four to seven mice/group (B and H) or SEA-specific cytokine production (medium alone values subtracted) by 4 to 7 naive or infected mice/group (C and D) or 6 technical replicates for each group of pooled purified CD4 $^{+}$ T cells from infected mice, cultured in medium or SEA (E and F).

assessed ex vivo cytokine production by CD4⁺ T cells, and their activation phenotype, by flow cytometry. CD11c depletion significantly reduced IL-4 production by splenic CD4⁺ T cells from infected mice (Fig. 2 G and H), with a similar result also seen for IL-13 (not depicted). Impaired expression of IL-4 mRNA was also evident in spleen tissue isolated from infected, DTx-treated mice, as determined by qPCR (Fig. S3 A).

Not unlike the pLN after egg injection (Fig. 1), or spleens during infection (Fig. 2), DTx treatment significantly depleted CD11c^{Hi}MHCII⁺ DCs in the liver, one of the main sites of egg exposure during *S. mansoni* infection (Fig. 3, A and B; ~70% efficacy). Consequently, secretion of IL-4, IL-5, IL-10, and IL-13 by cultured liver cells was reduced in CD11c-depleted mice (Fig. 3 C), whereas IFN- γ was not significantly affected (Fig. 3 D). IL-4 production ex vivo was also impaired in effector CD4⁺ T cells from livers of infected, DTx-treated mice, as measured by flow cytometry (Fig. 3, E and F), and in liver tissue, as determined by qPCR (Fig. S3 B). Similar results were seen for IL-13 (unpublished data).

In addition to dramatically reducing T cell cytokine production, CD11c depletion altered proportions of both effector

and regulatory T cells in naive or infected mice. The percentage of splenic CD4⁺ or CD8⁺ T cells was significantly reduced in naive or *S. mansoni*-infected mice after DTx treatment (Fig. S4, A and B). This proportional decrease was also reflected in effector T cell activation, with a reduced proportion of total cells expressing CD4 along with CD25, CD69, or CD44 (unpublished data). Importantly, the lower Th2 response after DTx treatment was likely not caused by increased regulatory T cell activity because these cells were also impaired in CD11c-depleted mice, with a reduced proportion of total (not depicted) and gated CD4⁺ T cells expressing CD25 and FoxP3, and lower levels of FoxP3 per CD4⁺ cell (Fig. S4 C). Together, this suggests that CD11c depletion impairs induction of both effector and regulatory CD4⁺ T cells during the initiation of the response against *S. mansoni* infection.

As well as having a dramatic impact on T cell activation, CD11c depletion reduced the proportional decrease in marginal zone B cells (Fig. S5 A) and increase in follicular B cells (Fig. S5 B) seen in infection, while having little effect on the proportions of total or transitional splenic B cells (Fig. S5, C and D). This supports a role for CD11c⁺ cells

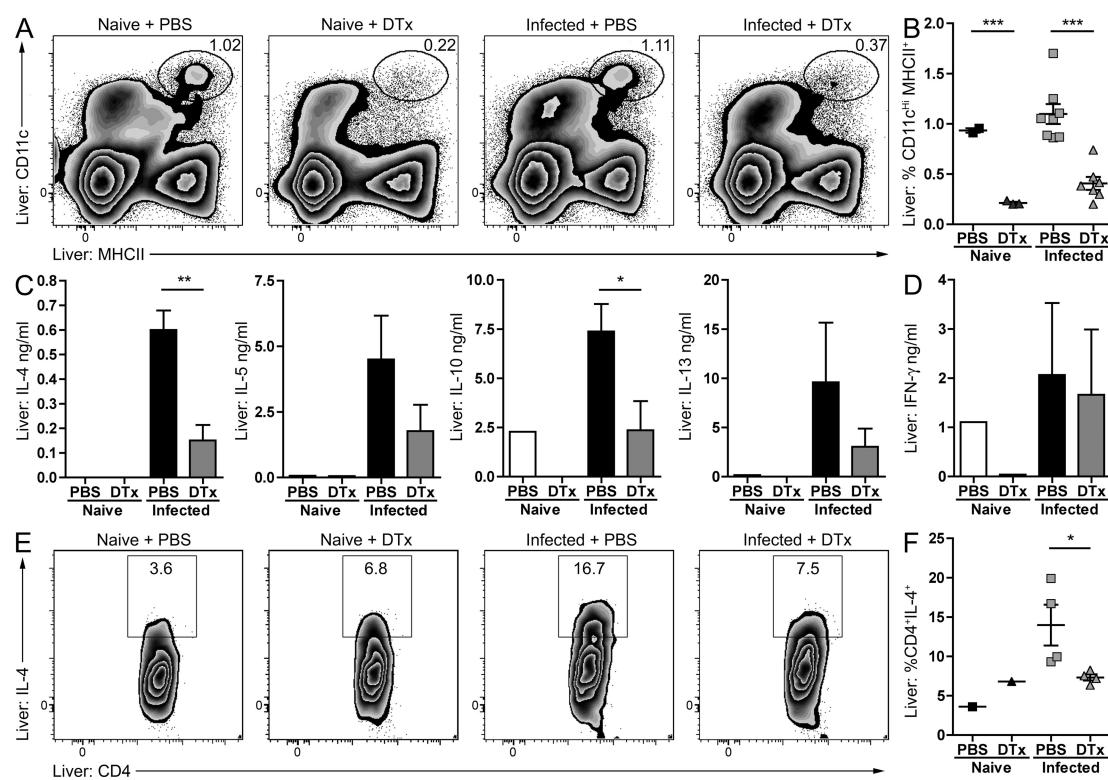


Figure 3. CD11c depletion during schistosome infection impairs the liver Th2 response. CD11c.DOG mice were treated daily with PBS (squares) or DTx (triangles) from day 28 to 39 of *S. mansoni* infection. Naive (black symbols) and infected (gray symbols) mice were assessed for liver CD11c^{Hi}MHCII⁺ cell depletion on day 40 (A and B), and liver cells from naive or infected PBS (white or black bars)- or DTx (gray bars)-treated mice cultured for 72 h with SEA or medium alone. The supernatants were collected, and cytokine production was assessed by ELISA (C and D). Liver CD4⁺ T cell IL-4 production by naive (black symbols) or infected (gray symbols) mice was also assessed by intracellular cytokine staining (E and F). One of three (A and C–F) experiments, or combined data from three experiments (B). Error bars are mean \pm SEM of four to seven mice/group, with liver cells combined in groups where cell numbers were restrictive.

in the coordination of B cell migration from the marginal zone to the follicles, likely via their activation of CD4⁺ Th cells.

In terms of Ab production, and indicative of altered Th2 development, serum levels of IgE were significantly reduced in infected DTx-treated mice (Fig. S5 E). However, IgM, IgG1, and IgG2c were not significantly altered (Fig. S5, F–H). This suggests that CD11c⁺ DCs are not critical for IgM or IgG production, or could reflect a longer half-life of IgM and IgG versus IgE in serum, with 11-d depletion not being sufficient to see a difference in IgM or IgG profiles. Additionally, the Ab response against larvae and adult worms in the 4 wk preceding DTx treatment is likely to cross-react against egg Ag.

The impact of CD11c depletion on Th2 induction could not be attributed to differences in Ag load or pathology, as DTx-treated mice showed no significant alteration in parasitaemia (adult worm numbers, or eggs in liver or gut tissue), weight loss, splenomegaly, or hepatomegaly (unpublished data). At this time point, pathology and granuloma formation was minimal in both control and CD11c-depleted mice, as fibrotic disease takes several more weeks of egg exposure to develop. However, we predict that the eventual outcome of impaired Th2 function, reduced regulation, and less restrained innate cell IFN- γ production in DC-depleted infected animals would be inflammatory pathology later in infection.

We also assessed the impact of CD11c depletion on several other innate cell types that could be involved in the Th2 induction process during schistosome infection. A clear role for macrophages in regulation of Th2 immunity has been described (Martinez et al., 2009), not least during *S. mansoni* infection (Herbert et al., 2004), but evidence to support an important role for macrophages in Th2 priming remains to be provided. Similarly, although eosinophils can express MHCII, process and present Ag (Spencer and Weller, 2010), and are a major innate cell type associated with Th2 settings, eosinophil-ablated mice develop expected pathology and intact Th2 responses during *S. mansoni* infection (Swartz et al., 2006). Finally, although basophils are transiently recruited to the dLN after injection of *S. mansoni* eggs (Perrigoue et al., 2009), and may be involved in Th2 promotion during nematode infection or in response to proteases (Perrigoue et al., 2009; Sokol et al., 2009; Yoshimoto et al., 2009), it is not yet clear whether they are a key player in this process (Finkelman, 2009; Lambrecht and Hammad, 2009; Voehringer, 2009; Kim et al., 2010; Paul and Zhu, 2010). In schistosomiasis this seems unlikely, given our results showing that administration of Mar-1 Ab to deplete Fc ϵ R1 α^+ cells has no significant effect on the Th2 response induced by schistosome eggs (Fig. 1).

The proportion of CD11b⁺F4/80⁺CD11c^{–Dim} macrophages was not reduced, and indeed increased, after DTx treatment of CD11c.DOG mice (Fig. S6). Similarly, in the infection setting, the proportion of eosinophils (Siglec F⁺Gr1^{Int}) or basophils (CD19[–]Siglec F[–]CD117[–]Fc ϵ R1 α^+) observed in CD11c.DOG mice that had received DTx was not adversely affected (Fig. S6), although Th2 cytokines were markedly impaired (Fig. 2 and Fig. 3). So, although we

cannot exclude a contribution by these cell types in the Th2 process, a major role during induction is unlikely.

In relation to this, previous work has demonstrated unimpaired Th2 development in *S. mansoni*-infected basophil (FcR γ chain)-deficient mice (Jankovic et al., 1998) or in *Nippostrongylus brasiliensis*-infected Mar-1-treated or IL-3 deficient mice (Kim et al., 2010), suggesting that the lack of requirement for basophils that we have observed is neither a peculiarity of our laboratory, nor restricted to schistosome infection. It is possible that basophils may only be important for Th2 development in unusual settings, such as protease-driven responses, or in the case of helminths that are relatively poor inducers of Th2 immunity (Finkelman, 2009).

Concluding remarks

Our data strongly indicate that CD11c⁺ DCs are the key initiators of the CD4⁺ T cell response to *S. mansoni* and that their depletion results in a strikingly impaired Th2 cytokine profile and a shift toward IFN- γ production by non-CD4⁺ cells. Remarkably, even in the complex setting of schistosome infection, this was apparent despite incomplete depletion of DCs, suggesting that other APCs are unable to substitute for them in the Th2 priming role. It will be interesting to see if CD11c depletion has a similar impact on CD4⁺ T cell effector function at later stages of infection, where APCs such as B cells might be expected to take on a more dominant role.

Although CD11c depletion had a major effect on Th2 response induction in both egg injection and infection experiments, it was not completely ablated in either setting. It is likely that the low level Th2 response remaining after DTx treatment of egg-injected or infected mice will have been primed by residual DCs, and could be further enhanced during infection by cross-reactive CD4⁺ T cell responses generated against larval and adult parasites before the start of CD11c depletion.

It is probable that there will be variability in the level of requirement for CD11c⁺ APCs for Th2 induction depending on the system in study, to reflect the multiplicity of Th2 environments that can be generated, a consequence of which may be equal diversity in inductive processes. However, our data support previous studies using similar depletion strategies that indicate that CD11c⁺ cells are important for Th2 Ab in allergy (Kool et al., 2008) and for expulsion of *N. brasiliensis* (Ohnmacht et al., 2009). Further, we have established that CD11c depletion using the CD11c.DOG mice also dramatically reduces Th2 induction against both *N. brasiliensis* and *Heligmosomoides polygyrus* nematode infections (unpublished data).

The data presented in this report demonstrate that CD11c⁺ DCs are not redundant during initiation of Th2 immune responses, supporting the wide literature available illustrating the ability of murine or human DCs to prime Th2 responses against helminth Ag or allergens. Thus DCs, in addition to being sufficient for Th2 induction in many systems, can also be a fundamental requirement for it.

MATERIALS AND METHODS

Animals, infections, and immunizations. Experiments were performed using CD11c.DOG (Hochweller et al., 2008) x C57BL/6 or CD11c.DOG x 4get IL-4-eGFP (Mohrs et al., 2001) F1 mice, which were maintained under specific pathogen-free conditions at the University of Edinburgh Animal Facilities and used at 8–12 wk of age. *Biomphalaria glabrata* snails infected with *S. mansoni* were obtained from F. Lewis (Biomedical Research Institute, Rockville, MD). Experimental mice were infected percutaneously with ~80 cercariae. *S. mansoni* eggs were isolated from C57BL/6 mouse livers and stored at –80°C. Mice were immunized s.c. in each rear footpad with 2,500 eggs in 50 µl PBS. For CD11c depletion, mice were injected i.p. daily with 8 ng/g diphtheria toxin (Sigma-Aldrich) in PBS or with PBS alone. For basophil depletion, mice were injected i.p. on days –1, 1, and 3 with 10 µg hamster IgG or anti-FcεR1α (Mar-1; eBioscience) Ab. Endotoxin-free soluble egg Ag (SEA) from *S. mansoni* was prepared in-house as previously described (MacDonald et al., 2001). All experiments were approved under a Project License granted by the Home Office (UK) and conducted in accordance with local guidelines.

Cell isolation and culture. Single-cell suspensions were prepared using the following methods. Spleens and LNs were diced and digested at 37°C for 15 min with 1.75 Wunsch Units/ml Liberase CI (Roche) and 80 Kunitz Units/ml DNase I type VI (Sigma-Aldrich) in HBSS (Sigma-Aldrich) containing 50 U/ml penicillin and 50 µg/ml streptomycin (Invitrogen). 100 µl 0.1 M, pH 7.3, EDTA (Ambion) stop solution per milliliter was then added, and the tube was topped off with DME containing 50 U/ml penicillin and 50 µg/ml streptomycin. The resulting suspension was then passed through a 70-µm cell strainer to obtain a single-cell suspension. For splenocytes, RBCs were lysed and cells counted and resuspended for use. Livers were perfused, diced, and digested at 37°C for 30 min using the aforementioned method. The digested liver was then passed through a 100-µm cell strainer with the aid of a syringe plunger. Leukocytes were separated from other liver cells by resuspension in 33% isotonic Percoll (GE Healthcare) and centrifuged at 700 g. Pelleted cells were resuspended and passed through a 40-µm cell strainer to obtain a single-cell suspension and remove *S. mansoni* eggs. RBCs were lysed and cells were counted and resuspended for use. Single-cell suspensions of splenocytes (2 × 10⁶ cell/ml) or LN or liver cells (10⁶ cell/ml) were cultured in X-vivo 15 medium (BioWhittaker) containing 2 mM L-Glutamine and 50 µM 2-ME (Invitrogen) in 96-well plates at 37°C in a humidified atmosphere of 5% CO₂ with or without 15 µg/ml SEA. Supernatants were harvested from the cultures after 72 h. Purified CD4⁺ T cell were restimulated with irradiated splenocytes (1 CD4⁺ T:10 splenocytes). Purifications were performed using Miltenyi Biotec CD4 beads and LS columns according to the manufacturer's instructions (> 90% purity).

Flow cytometry. After FcR-Block (2.4G2), cell surface markers for several different cell populations were analyzed. Staining for CD11c⁺ subsets was performed using the following mAb conjugations: CD11c-APC, MHCII-PerCP/Cy5.5, B220-APC/eFluor780, CD11b-PE, and CD8-PE/Cy7. In pLN and spleen, DCs were defined as CD11c^{hi}MHCII⁺, and in the liver as CD11c^{hi}MHCII⁺CD11b^{lo}. For egg injection experiments, IL-4-eGFP⁺ basophils were identified by exclusion of cells expressing CD4-APC, CCR3-PE, CD117-PerCP/Cy5.5, or B220-APC/eFluor780. For infection experiments, FcεR1α-FITC⁺ basophils were identified after surface staining to exclude cells expressing Siglec-F-PE, CD19-Alexa Fluor 700 or eFluor450, and CD117-PerCP/Cy5.5. Macrophages were identified using F4/80-FITC, CD11b-PE, and CD11c-APC, and eosinophils with Siglec-F-PE and Gr-1-APC. T cells were stained with CD4-APC/eFluor780, CD8-PE/Cy7, CD25-PerCP/Cy5.5, and FoxP3-APC. FoxP3 staining was performed as per manufacturer instructions (eBioscience). B cells were stained with B220-APC/eFluor780, CD21-FITC, and CD23-PE. IL-4-eGFP was measured on LN cells directly ex vivo, with naïve mice expressing negligible levels of eGFP (0.5–1.5% of CD4⁺ T cells). Intracellular cytokine production was measured either directly ex vivo or after restimulation. Intracellular cytokine staining of splenocytes or liver leukocytes ex vivo was set

up as follows: cells were rested overnight, stimulated with 10 ng/ml PMA and 1 µg/ml Ionomycin (Sigma-Aldrich) for 2 h, and then stimulated with Golgi Stop (BD) for an additional 3 h. After restimulation, intracellular cytokine staining was set-up as follows: after 72 h, 10 ng/ml murine rIL-2 (Peprotech) was added to pLN cell cultures. After an additional 18 h, cells were stimulated with PMA and ionomycin and treated with GolgiStop. After FcR-Block, cells were stained with CD4-FITC or CD4-APC/eFluor780, fixed with 1% isotonic formaldehyde, permeabilized with BD Perm/Wash buffer (BD), and stained with IL-4-APC, IFN-γ-Alexa Fluor 488, or IFN-γ-APC in BD Perm/Wash buffer. Identification of intracellular cytokine-positive cells was determined using appropriate isotype controls. All Abs for flow cytometry were purchased from BD, eBioscience, or BioLegend. Samples were acquired using a FACS LSR II or FACSCanto II flow cytometer using BD FACSDiva software and analyzed with FlowJo v.8 software (Tree Star, Inc.).

Cytokine and serum Ab analysis. Cytokines and serum antibodies were measured by ELISA. Paired capture and detection Abs (produced from hybridomas in-house or purchased from R&D Systems, BD, or eBioscience) were used for analysis of murine IL-4, IL-5, IL-10, IL-13, and IFN-γ. Plates (NUNC Maxisorp) were washed with 0.05% Tween 20 in PBS and blocked with 10% NCS/PBS. Recombinant cytokine standards (Peprotech or BD) were used to determine quantity using a standard curve. Plates were developed by incubation with 50 µl 1:1,000 HR-peroxidase-labeled streptavidin (KPL), and absorbance was read at 450 nm after addition of 100 µl TMB substrate solution (Sigma-Aldrich) and 100 µl 0.18 M H₂SO₄ using a Laboratory Systems Multiskan Ascent plate reader. Serum was collected 1 d before the end of infection experiments. Total IgE was measured using paired capture and detection antibodies (BD) and recombinant murine IgEκ (BD) to assess quantity using a standard curve. Plates were blocked, washed, developed, and read as above. SEA-specific IgG1, IgG2c, and IgM Ab titers were determined using endpoint dilutions measured by ELISA. Plates were coated with 0.25 µg/well of SEA in 0.1 M, pH 9.6, Carbonate/Bicarbonate buffer, washed, and blocked with 1% BSA/PBS. Serum samples were analyzed using serial twofold dilutions. SEA-specific isotypes were detected using alkaline phosphatase-conjugated goat anti-mouse IgG1, IgG2c, or IgM antibodies (SouthernBiotech). Absorbance at 405 nm was determined as above after addition of 50 µl PNPP substrate (SouthernBiotech).

Real-time RT-PCR. Total RNA was extracted from spleen, liver, or gut (ileal) tissue samples that had been snap frozen individually in 0.5 ml of TRIzol reagent (Invitrogen). Samples were defrosted, homogenized using a QIAGEN TissueLyser, and total RNA was extracted as per manufacturer instructions. cDNA was then generated from individual RNA samples using Superscript III Reverse Transcriptase and Oligo (dT) (Invitrogen). Quantitative RT-PCR was performed using a Light Cycler 480 II Real-Time PCR machine and software 1.5.0 SP3 (Roche). The relative concentration of mRNA for the genes of interest was assessed using LightCycler-DNA master SYBR Green I (Roche) and by comparison to a serially diluted standard of pooled cDNA. The mean concentration for each sample was determined from two technical repeats, with mRNA levels for each sample normalized to GAPDH to give a value for the gene of interest in arbitrary units. Primers used for the detection of gene expression were as follows: CD11c, sense 5'-ATGGAGCCTCAAGACAGGAC-3', antisense 5'-GGATCTGGATGCTGAAATC-3'; IL-4, sense 5'-GAGAGATC-ATCGGCATTTGA-3', anti-sense 5'-TCTGTGGTCTTCGT-TGC-3'; GAPDH, sense 5'-AATGTGCCGTGGATCT-3', anti-sense 5'-CCCAGCTCTCCCCATACATA-3'.

Statistical analysis. Statistical analyses were performed using GraphPad Prism 4 software. Experimental groups were compared by one-way ANOVA for the absence of significant variance before collation. Student's *t* tests were used where necessary to determine if there were any significant differences between sample groups. *, P < 0.05; **, P < 0.01; ***, P < 0.001.

Online supplemental material. Fig. S1 shows reduced CD4⁺ T cell IFN-γ production after CD11c depletion of schistosome egg-injected or infected

mice. Fig. S2 details the impact of CD11c depletion on DC subsets during infection. Fig. S3 depicts impaired IL-4 mRNA expression in spleens and livers of infected mice after CD11c depletion. Fig. S4 demonstrates that CD11c depletion impacts both effector and FoxP3⁺ regulatory T cells during infection. Fig. S5 shows that CD11c depletion during infection alters the proportions of splenic B cells, and reduces serum levels of IgE. Fig. S6 illustrates that the proportions of macrophages, eosinophils, and basophils found during infection are not reduced after CD11c depletion. Online supplemental material is available at <http://www.jem.org/cgi/content/full/jem.20100734/DC1>.

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REFERENCES

Banchereau, J., and R.M. Steinman. 1998. Dendritic cells and the control of immunity. *Nature*. 392:245–252. doi:10.1038/32588

Bar-On, L., and S. Jung. 2010. Defining dendritic cells by conditional and constitutive cell ablation. *Immunol. Rev.* 234:76–89. doi:10.1111/j.0105-2896.2009.00875.x

Finkelman, F.D. 2009. Basophils as T(H)2-inducing APCs: the dog can sing but is it a diva? *Immunol. Cell Biol.* 87:568–570. doi:10.1038/icb.2009.67

Herbert, D.R., C. Holscher, M. Mohrs, B. Arendse, A. Schwegmann, M. Radwanska, M. Leeto, R. Kirsch, P. Hall, H. Mossmann, et al. 2004. Alternative macrophage activation is essential for survival during schistosomiasis and downmodulates T helper 1 responses and immunopathology. *Immunity*. 20:623–635. doi:10.1016/S1074-7613(04)00107-4

Hochweller, K., J. Striegler, G.J. Hammerling, and N. Garbi. 2008. A novel CD11c-DTR transgenic mouse for depletion of dendritic cells reveals their requirement for homeostatic proliferation of natural killer cells. *Eur. J. Immunol.* 38:2776–2783. doi:10.1002/eji.200838659

Jankovic, D., A.W. Cheever, M.C. Kullberg, T.A. Wynn, G. Yap, P. Caspar, F.A. Lewis, R. Clynes, J.V. Ravetch, and A. Sher. 1998. CD4+ T cell-mediated granulomatous pathology in schistosomiasis is downregulated by a B cell-dependent mechanism requiring Fc receptor signaling. *J. Exp. Med.* 187:619–629. doi:10.1084/jem.187.4.619

Jankovic, D., S. Steinfelder, M.C. Kullberg, and A. Sher. 2006. Mechanisms underlying helminth-induced Th2 polarization: default, negative or positive pathways? *Chem. Immunol. Allergy*. 90:65–81. doi:10.1159/000088881

Kapsenberg, M.L. 2003. Dendritic-cell control of pathogen-driven T-cell polarization. *Nat. Rev. Immunol.* 3:984–993. doi:10.1038/nri1246

Kim, S., M. Prout, H. Ramshaw, A.F. Lopez, G. LeGros, and B. Min. 2010. Cutting edge: basophils are transiently recruited into the draining lymph nodes during helminth infection via IL-3, but infection-induced Th2 immunity can develop without basophil lymph node recruitment or IL-3. *J. Immunol.* 184:1143–1147. doi:10.4049/jimmunol.0902447

Kool, M., T. Soullie, M. van Nimwegen, M.A. Willart, F. Muskens, S. Jung, H.C. Hoogsteden, H. Hammad, and B.N. Lambrecht. 2008. Alum adjuvant boosts adaptive immunity by inducing uric acid and activating inflammatory dendritic cells. *J. Exp. Med.* 205:869–882. doi:10.1084/jem.20071087

Lambrecht, B.N., and H. Hammad. 2009. Biology of lung dendritic cells at the origin of asthma. *Immunity*. 31:412–424. doi:10.1016/j.jimmuni.2009.08.008

MacDonald, A.S., and R.M. Maizels. 2008. Alarming dendritic cells for Th2 induction. *J. Exp. Med.* 205:13–17. doi:10.1084/jem.20072665

MacDonald, A.S., A.D. Straw, B. Bauman, and E.J. Pearce. 2001. CD8-dendritic cell activation status plays an integral role in influencing Th2 response development. *J. Immunol.* 167:1982–1988.

Maizels, R.M., and M. Yazdanbakhsh. 2003. Immune Regulation by helminth parasites: cellular and molecular mechanisms. *Nat. Rev. Immunol.* 3:733–744. doi:10.1038/nri1183

Martinez, F.O., L. Helming, and S. Gordon. 2009. Alternative activation of macrophages: an immunologic functional perspective. *Annu. Rev. Immunol.* 27:451–483. doi:10.1146/annurev.immunol.021908.132532

Mohrs, M., K. Shinkai, K. Mohrs, and R.M. Locksley. 2001. Analysis of type 2 immunity in vivo with a bicistronic IL-4 reporter. *Immunity*. 15:303–311. doi:10.1016/S1074-7613(01)00186-8

Ohnmann, C., A. Pullner, S.B. King, I. Drexler, S. Meier, T. Brocker, and D. Voehringer. 2009. Constitutive ablation of dendritic cells breaks self-tolerance of CD4 T cells and results in spontaneous fatal autoimmunity. *J. Exp. Med.* 206:549–559. doi:10.1084/jem.20082394

Paul, W.E., and J. Zhu. 2010. How are T(H)2-type immune responses initiated and amplified? *Nat. Rev. Immunol.* 10:225–235. doi:10.1038/nri2735

Pearce, E.J., and A.S. MacDonald. 2002. The immunobiology of schistosomiasis. *Nat. Rev. Immunol.* 2:499–511. doi:10.1038/nri843

Perona-Wright, G., S.J. Jenkins, and A.S. MacDonald. 2006. Dendritic cell activation and function in response to *Schistosoma mansoni*. *Int. J. Parasitol.* 36:711–721. doi:10.1016/j.ijpara.2006.02.003

Perona-Wright, G., K. Mohrs, J. Taylor, C. Zaph, D. Artis, E.J. Pearce, and M. Mohrs. 2008. Cutting edge: Helminth infection induces IgE in the absence of mu- or delta-chain expression. *J. Immunol.* 181:6697–6701.

Perrigoue, J.G., S.A. Saenz, M.C. Siracusa, E.J. Allenspach, B.C. Taylor, P.R. Giacomini, M.G. Nair, Y. Du, C. Zaph, N. van Rooijen, et al. 2009. MHC class II-dependent basophil-CD4+ T cell interactions promote T(H)2 cytokine-dependent immunity. *Nat. Immunol.* 10:697–705. doi:10.1038/ni.1740

Rutitzky, L.I., J.R. Lopes da Rosa, and M.J. Stadecker. 2005. Severe CD4 T cell-mediated immunopathology in murine schistosomiasis is dependent on IL-12p40 and correlates with high levels of IL-17. *J. Immunol.* 175:3920–3926.

Sokol, C.L., N.Q. Chu, S. Yu, S.A. Nish, T.M. Laufer, and R. Medzhitov. 2009. Basophils function as antigen-presenting cells for an allergen-induced T helper type 2 response. *Nat. Immunol.* 10:713–720. doi:10.1038/ni.1738

Spencer, L.A., and P.F. Weller. 2010. Eosinophils and Th2 immunity: contemporary insights. *Immunol. Cell Biol.*

Stadecker, M.J., H. Asahi, E. Finger, H.J. Hernandez, L.I. Rutitzky, and J. Sun. 2004. The immunobiology of Th1 polarization in high-pathology schistosomiasis. *Immunol. Rev.* 201:168–179. doi:10.1111/j.0105-2896.2004.00197.x

Swartz, J.M., K.D. Dyer, A.W. Cheever, T. Ramalingam, L. Pesnicak, J.B. Domachowske, J.J. Lee, N.A. Lee, P.S. Foster, T.A. Wynn, and H.F. Rosenberg. 2006. *Schistosoma mansoni* infection in eosinophil lineage-ablated mice. *Blood*. 108:2420–2427. doi:10.1182/blood-2006-04-015933

Voehringer, D. 2009. The role of basophils in helminth infection. *Trends Parasitol.* 25:551–556. doi:10.1016/j.pt.2009.09.004

Wynn, T.A., R.W. Thompson, A.W. Cheever, and M.M. Mentink-Kane. 2004. Immunopathogenesis of schistosomiasis. *Immunol. Rev.* 201:156–167. doi:10.1111/j.0105-2896.2004.00176.x

Yoshimoto, T., K. Yasuda, H. Tanaka, M. Nakahira, Y. Imai, Y. Fujimori, and K. Nakanishi. 2009. Basophils contribute to T(H)2-IgE responses in vivo via IL-4 production and presentation of peptide-MHC class II complexes to CD4+ T cells. *Nat. Immunol.* 10:706–712. doi:10.1038/ni.1737