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Migratory CD103$^+$ dendritic cells suppress helminth-driven type 2 immunity through constitutive expression of IL-12

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CD8α$^+$ and CD103$^+$ dendritic cells (DCs) play a central role in the development of type 1 immune responses. However, their role in type 2 immunity remains unclear. We examined this issue using *Batf3*$^{-/-}$ mice, in which both of these DC subsets are missing. We found that Th2 cell responses, and related events such as eosinophilia, alternative macrophage activation, and immunoglobulin class switching to IgG1, were enhanced in *Batf3*$^{-/-}$ mice responding to helminth parasites. This had beneficial or detrimental consequences depending on the context. For example, Batf3 deficiency converted a normally chronic intestinal infection with *Heligmosomoides polygyrus* into an infection that was rapidly controlled. However, liver fibrosis, an IL-13–mediated pathological consequence of wound healing in chronic schistosomiasis, was exacerbated in *Batf3*$^{-/-}$ mice infected with *Schistosoma mansoni*. Mechanistically, steady-state production of IL-12 by migratory CD103$^+$ DCs, independent of signals from commensals or TLR-initiated events, was necessary and sufficient to exert the suppressive effects on Th2 response development. These findings identify a previously unrecognized role for migratory CD103$^+$ DCs in antagonizing type 2 immune responses.

Conventional DCs are highly specialized antigen-presenting cells that play a crucial role in the development of adaptive immune responses. Based on surface marker expression, two main conventional DC subsets can be identified in lymphoid organs and peripheral tissues: CD11b$^+$ and CD8α$^+$ DCs. Although there is a certain degree of functional plasticity among these DC subsets to support the development of different types of T cell responses, it is becoming increasingly clear that specialized DC populations favor the promotion of particular types of T cell responses (Satpathy et al., 2012). For example, CD11b$^+$ DCs are considered to be efficient at priming CD4$^+$ T cells through MHC class II–restricted antigen (Ag) presentation (Dudziak et al., 2007), but poor Ag cross-presenters and therefore inefficient at priming CD8$^+$ T cell responses. Consistent with this, CD11b$^+$ DC subpopulations have been identified that play an essential role in allergy-associated Th2 responses in the lung (Plantinga et al., 2013) and skin (Gao et al., 2013; Kumamoto et al., 2013), or that promote protective Th17 responses in the lung during infection with the fungus *Aspergillus fumigatus* (Schlitzer et al., 2013) and in the gut after pathogenic *Citrobacter rodentium* infection (Satpathy et al., 2013). The extent to which the different Th cell–polarizing properties of CD11b$^+$ DCs are a reflection of functional plasticity or, rather, a result of the presence of separate lineages within the CD11b$^+$ DC compartment is still incompletely understood.

The CD8α$^+$ DC lineage comprises the lymphoid organ–resident CD8α$^+$ DCs and their tissue-resident and migratory counterparts, the CD103$^+$ DCs (Edelson et al., 2010), which are uniquely dependent on basic leucine zipper transcription factor ATF-like 3 (Batf3) for their development. Studies in *Batf3*$^{-/-}$ mice have demonstrated that CD8α$^+$ and CD103$^+$ DCs have superior Ag cross-presenting capabilities and as a result play a critical role in antiviral and antitumor immunity through the generation of cytotoxic T cell responses (Hildner et al., 2008; Fuertes et al., 2011; Zelenay et al., 2012). Moreover, Batf3-dependent DCs represent an obligate source of IL-12 to mount protective type I immunity against the parasitic infections *Toxoplasma gondii* (Mashayekhi et al.,

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Abbreviations used: Ag, antigen; GC, germinal center; GF, germ free; hLN, hepatic LN; mLN, mesenteric LN; pLN, popliteal LN; p-Mac, peritoneal macrophage; SEA, soluble schistosome egg antigen; SPF, specific pathogen free; Tfh, T follicular helper cell; T reg cell, regulatory T cell.

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2011) and *Leishmania major* (Ashok et al., 2014). CD8α$^+$ and CD103$^+$ DCs have also been reported to stimulate de novo induction of regulatory T cells in the spleen (Yamazaki et al., 2008), gut (Coombes et al., 2007; Sun et al., 2007), and lung (Khare et al., 2013), although this property does not appear to be essential for maintenance of self-tolerance (Edelson et al., 2010).

In contrast to the well-established role for Batf3-dependent DCs in Th1 and CD8$^+$ T cell responses, the contribution of these cells to the regulation of Th2 responses is at present unclear. For example, conflicting data exist for allergic asthma, where CD103$^+$ DCs have been reported to either suppress (Khare et al., 2013), be redundant (Plantinga et al., 2013; Zhou et al., 2014), or be essential (Nakano et al., 2012) for induction of Th2 responses. We have addressed this issue by exploring the role of Batf3-dependent DCs in the development of type 2 responses during helminth infection. Helminth parasites are the strongest natural inducers of type 2 responses, which are critical for immunity to these pathogens, but can also cause immunopathology, especially during chronic infections (Ferrick et al., 2008). We found that in the absence of Batf3-dependent DCs, mice mounted broadly stronger type 2 immune responses to helminths. This resulted in heightened resistance to infection with the gastrointestinal parasite *Heligmosomoides polygyrus* and more severe egg-induced liver fibrosis after infection with the intravascular parasite *Schistosoma mansoni*. We identified constitutive production of IL-12 by Batf3-dependent migratory CD103$^+$ DCs as the key mechanism through which these cells suppress type 2 immune responses. Whereas a role for IL-12 in regulating type 2 immunity has been long recognized (Manetti et al., 1993; Oswald et al., 1994), it has been difficult to reconcile a role for this regulatory pathway in helminth infections, as these pathogens inhibit rather than induce the production of IL-12 (Jankovic et al., 2006). Our data reveal that the production of IL-12 by migratory CD103$^+$ DCs in the steady state provides an innate barrier to the development of type 2 immunity after exposure to helminths, and in this way, depending on the parasitic infection, significantly promotes helminth infection chronicity or dampens helminth-induced immunopathology.

RESULTS

Batf3 deficiency augments Th2 responses after subcutaneous immunization with *S. mansoni* eggs

We first used a well-characterized experimental model for studying the induction of Th2 responses by helminth Ag, in which eggs of the trematode parasite *S. mansoni* are injected s.c. into the footpad and responses are measured in draining popliteal LNs 1 wk later (Pearce et al., 1991; Oswald et al., 1994). We found that egg injection in C57BL/6 *Batf3*$^{-/-}$ mice promoted a significantly stronger Th2 response than in WT mice. This was evident as increased expression of IL-4, IL-5, and IL-10 by CD4$^+$ T cells after polyclonal restimulation ex vivo (Fig. 1, A and B), as well as by accumulation of IL-4 and IL-5 in supernatants of LN cell cultures restimulated with soluble schistosome egg Ag (SEA; Fig. 1 C). The enhanced Th2 response in C57BL/6 *Batf3*$^{-/-}$ mice was accompanied by stronger B cell responses, as we observed that a larger proportion of B cells from reactive LNs from immunized *Batf3*$^{-/-}$ mice were expressing germinal center (GC) markers (Fig. 1 D). Furthermore, consistent with increased IL-4 production in these LNs, GC B cells exhibited greater isotype-switching to IgG1 (Fig. 1, E and F). During helminth-induced type 2 immune responses, T follicular helper (Tfh) cells are a significant source of IL-4 produced in reactive LNs. These cells play a central role in regulation GC formation and antibody production (Glatman Zaretsky et al., 2009; King and Mohrs, 2009). However, there was no difference in frequency of Tfh cells between WT and *Batf3*$^{-/-}$ mice (Fig. 1 G), suggesting that the quality (i.e., the amount of cytokine produced), rather than the magnitude of the Tfh response (i.e., cell number), was responsible for the differences in B cell responses between WT and *Batf3*$^{-/-}$ mice. The observed immunological phenotype was not strain-specific, as a similar increase in the magnitude of the Th2 response to injected schistosome eggs was observed in BALB/c *Batf3*$^{-/-}$ mice compared with in WT BALB/c mice, a strain that is known to be inherently more Th2-biased than C57BL/6 mice (Fig. 1 H). Collectively, these results show that Batf3 deficiency results in an augmented Th2 response in mice responding to helminth Ag via skin immunization.

Batf3 deficiency results in stronger Th2 responses accompanied by more severe immunopathology after a natural infection with *S. mansoni*

Based on the results from the egg immunization model, we next examined the effect of Batf3 deficiency on Th2 responses in mice infected with *S. mansoni*. As adults, these parasites live in the portal vasculature, where females produce eggs, which can be carried by the blood flow into the liver, where they become trapped in hepatic sinusoids. Egg antigens induce strong Th2 responses that subsequently orchestrate the development of granulomatous lesions that surround the eggs. Granulomas protect surrounding hepatocytes from toxins made by parasite eggs, but promote hepatic fibrosis, a classic immunopathologic consequence of schistosome infection (Pearce and MacDonald, 2002; Wynn, 2008). The intensity of the Th2 response and associated granulomatous inflammation peaks at 8 wk after infection, ∼2 wk after egg production is initiated, and declines thereafter, despite ongoing infection caused by the development of still incompletely understood immunoregulatory mechanisms (Domingo and Warren, 1968; Boros et al., 1975; Taylor et al., 2009). Consistent with this, we observed a significant increase in cellularity of both the liver and the draining hepatic LN (hLN; Barbier et al., 2012), in WT BALB/c mice at week 8 of infection (Fig. 2, A and B). Whereas a similar increase in overall cellularity was observed in *Batf3*$^{-/-}$ BALB/c, as in WT mice at week 8 after infection in these organs (Fig. 2, A and B), Batf3 deficiency resulted in significantly lower

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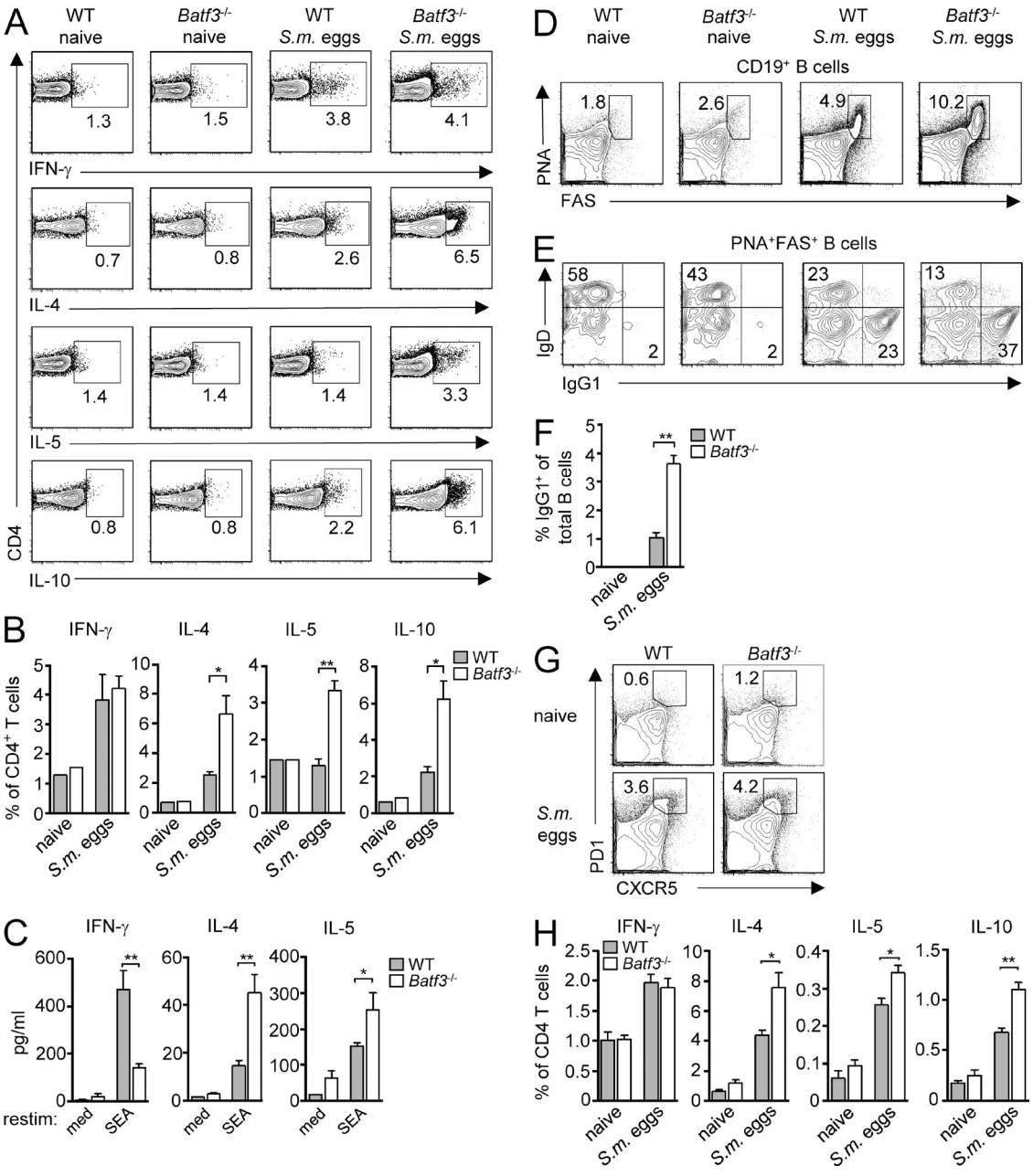

Figure 1. ***Batf3*$^{-/-}$ mice mount stronger Th2 responses after *S. mansoni* egg immunization.** WT or *Batf3*$^{-/-}$ C57BL/6 mice were injected with 5,000 *S. mansoni* eggs in the hind footpad and draining pLNs were analyzed 7 d later. (A and B) pLN cells were restimulated with PMA/Ionomycin in the presence of Brefeldin A and CD4$^+$ T cells were stained for indicated intracellular cytokines. (C) pLN cells were restimulated with water-soluble egg antigens (SEA) for 3 d, and cytokine levels in culture supernatants were determined. (D) GC B cell (CD19$^+$FAS$^+$PNA$^+$) frequency in CD19$^+$ B cell gate from pLNs. (E) Frequency of IgD$^+$ and class-switched IgG1$^+$ GC B cells in pLNs. (F) Frequency of IgG1$^+$ classed-switched GC B cells of total CD19$^+$ B cells in pLNs. (G) Tfh cell (CXCR5$^+$PD1$^+$) frequency in CD4$^+$ T cell gate from pLNs. (H) WT or *Batf3*$^{-/-}$ BALB/c mice were injected with 5,000 *S. mansoni* eggs in the hind footpad and draining pLNs were analyzed as in B 7 d later. Data are concatenated plots (A, D, E, and G) or bar graphs (B, C, F, and H) representing mean ± SEM from three to four mice per group. One of three (A–G) or two (H) experiments is shown. *, $P < 0.05$; **, $P < 0.01$.

frequencies of CD8$^+$ T cells and NK cells in the liver (Fig. 2 C). Alternatively, CD4$^+$ T cell frequencies were unaffected (liver) or increased (hLN) in infected *Batf3*$^{-/-}$ mice relative to infected WT mice (Fig. 2 C). More importantly, CD4$^+$ T cells from infected *Batf3*$^{-/-}$ mice expressed more Th2 cytokines and less IFN-γ compared with infected WT mice (Fig. 2, D and E), suggesting that also during a natural infection with *S. mansoni*, Batf3 deficiency results in

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enhanced Th2 immune responses. Because Batf3-dependent CD103$^+$ DCs have been shown to have the capacity to promote regulatory T (T reg) cell responses (Coombes et al., 2007; Sun et al., 2007), it was possible that the observed increase in Th2 responses may have been secondary to a defect in T reg cell development (Taylor et al., 2006). However, the frequencies of Foxp3-expressing T reg cells in the livers and hLNs of 8-wk–infected *Batf3*$^{-/-}$ mice were comparable to those in WT mice (Fig. 2 F), making this scenario unlikely. Moreover, this difference in Th2 response was not a result of differences in infection intensity, as similar numbers of worms were recovered from infected WT and *Batf3*$^{-/-}$ mice (Fig. 2 G). Despite an elevated Th2 response in infected *Batf3*$^{-/-}$ compared with WT mice, both groups of mice displayed similar degrees of isotype-switching to IgG1 by GC B cells in the hLN in response to the infection (Fig. 2 H) and had comparable SEA-specific IgG1 titers during both acute and chronic stages of infection (Fig. 2 I), suggesting that the enhanced Th2 polarization observed in *Batf3*$^{-/-}$ mice did not grossly influence humoral responses during this infection. Of note, it was recently reported that in *Batf3*$^{-/-}$ mice, DC populations normally dependent on Batf3 are restored after infection with pathogens that induce high levels of IFN-γ (Tussiwand et al., 2012). However, consistent with the fact that helminths generally induce little IFN-γ, Batf3-dependent migratory CD103$^+$ and LN-resident CD8α$^+$ DC populations remained absent during *S. mansoni* infection (Fig. 2 J).

We hypothesized that as a result of the enhanced Th2 profile at 8 wk after infection, infected mice may exhibit more severe granulomatous inflammation. However, we did not observe measurable differences in granuloma size between *Batf3*$^{-/-}$ and WT mice at 8 wk after infection (unpublished data). Interestingly, however, we found that during the chronic phase of the infection (week 16) hLN and hepatic cellularity were significantly higher in infected *Batf3*$^{-/-}$ mice than in infected WT mice (Fig. 2, A and B), indicative of an ongoing exaggerated immune response in the KO mice. In line with this observation, also during this chronic stage of the infection CD4$^+$ T cells from infected *Batf3*$^{-/-}$ mice expressed more Th2 cytokines and less IFN-γ compared with infected WT mice (Fig. 3 A). Consistent with the chronic elevated Th2 response, we found *Batf3*$^{-/-}$ mice to contain large coalescing granulomas in their livers, whereas livers from infected WT mice exhibited the small granulomas typical of chronic infection (Fig. 3 B). A further mark of enhanced type 2 inflammation in the absence of Batf3-dependent DCs was the presence of significantly more eosinophils (Fig. 3 C), and increased fibrosis, measured as increased hydroxyproline (Fig. 3 D), in livers of infected *Batf3*$^{-/-}$ compared with WT mice; in this infection, eosinophil numbers are regulated by IL-5 (Nabors et al., 1995) and fibrosis is promoted by IL-13 (Chiaramonte et al., 1999). Collectively, these data show that during schistosomiasis, the Th2 response and associated changes in eosinophil numbers, fibrosis, and granulomatous pathology are exaggerated in the absence of Batf3-dependent DCs.

Batf3 deficiency results in stronger type 2 immunity and heightened resistance to infection with a gastrointestinal helminth parasite

We next asked whether the enhanced type 2 immunity observed in the absence of Batf3 during *S. mansoni* infection is also apparent when mice are infected with the phylogenetically distinct helminth parasite *H. polygyrus*, which is a well-established murine model for intestinal nematode infections in humans. After ingestion, infectious L3 stage larvae invade the intestinal wall, within which they then develop through several molts before emerging into the intestinal lumen as adults at about day 9 after infection. Egg production begins shortly thereafter. Infection in mice is associated with the development of a type 2 immune response that is generally ineffective at controlling the parasite, leading to chronic infection. However, stronger type 2 responses promoted by the injection of IL-4, or which are apparent during secondary infection, result in reductions in fecundity and in more efficient worm expulsion (Reynolds et al., 2012; Huang et al., 2014). When we infected WT and *Batf3*$^{-/-}$ BALB/c mice with *H. polygyrus*, we found that the cellularity of the reactive mesenteric LN (mLN) was significantly higher in *Batf3*$^{-/-}$ mice than in infected WT mice (Fig. 4 A), without affecting CD4$^+$, CD8$^+$ T cell, B cell, and NK cell frequencies in this LN (Fig. 4 B). Moreover, we found that the Th2 response in mLN of infected *Batf3*$^{-/-}$ mice was significantly stronger than in infected WT mice. This was evident as increased expression of the canonical Th2 cytokines IL-4, IL-5, IL-10, and IL-13 by CD4$^+$ T cells after ex vivo polyclonal restimulation (Fig. 4, C and D), as well as by increased accumulation of all of these cytokines in the medium of mLN cells restimulated with soluble *H. polygyrus* worm Ag (Fig. 4 E). IFN-γ production was barely increased as a result of infection with *H. polygyrus*, and we did not see significant differences in production of this cytokine by cells from infected *Batf3*$^{-/-}$ versus WT mice (Fig. 4, C–E). Furthermore, we observed that in WT mice, *H. polygyrus* infection reduced the frequency of Foxp3-expressing T reg cells in the mLNs (Fig. 4 F). However, because of the strong increase in cellularity of the mLN in infected WT mice over naive mice (Fig. 4 A), a net increase in total numbers of Foxp3-expressing T reg cells was observed in these mice, a finding that is consistent with earlier observations (Finney et al., 2007). The percentages of Foxp3-expressing T reg cells in the mLNs of infected *Batf3*$^{-/-}$ mice were comparable to those of WT mice (Fig. 4 F), suggesting that the enhanced Th2 response is not secondary to a defect in T reg cell responses. Finally, during this infection, the migratory CD103$^+$CD11b$^-$ and resident CD8α$^+$ DC subsets that are dependent on Batf3 also remained absent (Fig. 4 G).

The effector mechanisms of type 2 immunity against *H. polygyrus* are provided by IgG1 antibody and alternatively activated (M2) macrophages (Anthony et al., 2006). We found that the overall percentage of mLN IgG1$^+$ B cells were increased in infected *Batf3*$^{-/-}$ mice (Fig. 5 A), although titers of *H. polygyrus*-worm specific IgG1 in serum were similar

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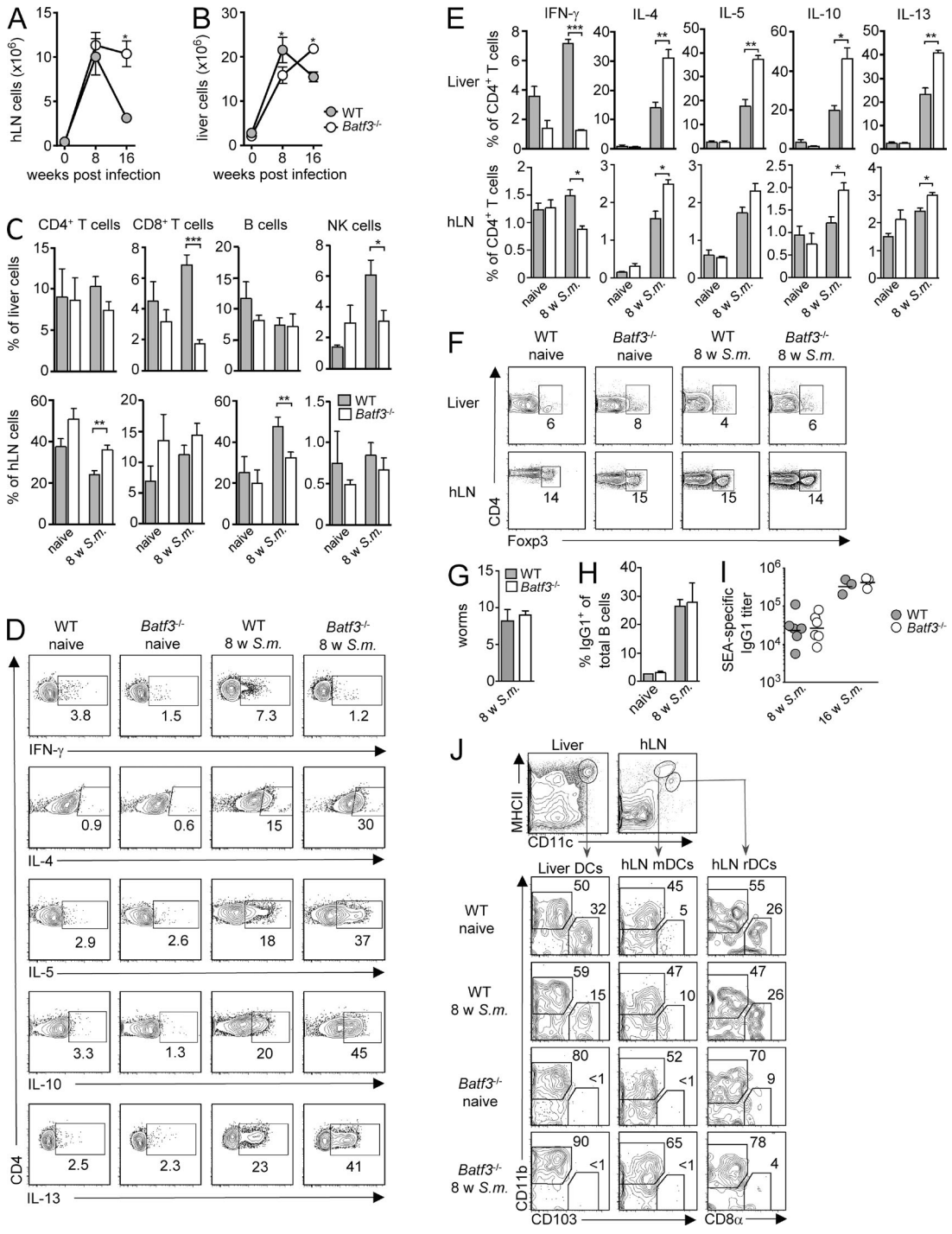

Figure 2. ***Batf3*$^{-/-}$ mice display stronger Th2 responses in response to *S. mansoni* infection.** (A and B) Quantification of total cell numbers in hLNs (A) and livers (B) from naive, 8 wk (acute), and 16 wk (chronic) *S. mansoni*–infected WT and *Batf3*$^{-/-}$ BALB/c mice. Data are representative of 6 (naive and week 8) or 3 (week 16) mice per group. (C) Frequencies of CD3$^+$CD4$^+$ T cells, CD3$^+$CD8α$^+$ T cells, CD19$^+$ B cells, and CD3$^-$DX5$^+$ NK cells in hLNs and livers of 8 wk *S. mansoni*–infected WT and *Batf3*$^{-/-}$ BALB/c mice. Data are representative of six mice per group. Error bars represent mean ± SEM. (D) Liver cells from WT or *Batf3*$^{-/-}$ BALB/c naive mice or mice infected for 8 wk with *S. mansoni* were restimulated with PMA/Ionomycin in the presence of Brefeldin A, after which CD4$^+$ T cells were stained intracellularly for indicated cytokines. Data are concatenated plots from three mice per group. (E) Intracellular cytokine staining of T cells isolated from 8 wk *S. mansoni*–infected mice, as described in D, from liver and hLNs. Error bars represent mean ± SEM from three mice per group. (F) Foxp3 staining in CD4$^+$ T cells isolated from 8 wk *S. mansoni*–infected mice from liver and hLNs. Data are concatenated plots from three mice per group. (G) Worm counts of 8 wk *S. mansoni*–infected WT or *Batf3*$^{-/-}$ BALB/c mice. Data are based on four mice per group. (H) Frequency of IgG1$^+$ classed-switched

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between infected WT and *Batf3*$^{-/-}$ mice (Fig. 5 B). Furthermore, relative to infected WT mice, infected *Batf3*$^{-/-}$ mice exhibited more pronounced increases in peritoneal macrophage (p-Macs) numbers caused by infection (Fig. 5 C). We found that, compared with WT mice, where only a fraction of the p-Macs were M2 activated, nearly all p-Macs in infected *Batf3*$^{-/-}$ mice expressed the M2 marker RELMα (Fig. 5 D), and thus the total number of M2 macrophages in these mice was significantly higher than in infected WT mice (Fig. 5 E). The enhanced M2 activation in infected *Batf3*$^{-/-}$ compared with WT mice was confirmed by staining for an alternative marker for M2 macrophages, CD301 (Fig. 5 F). In contrast, we did not observe enhanced M2 activation of macrophages in the lamina propria of the small intestine of *H. polygyrus*–infected *Batf3*$^{-/-}$ compared with WT mice, as determined by RELMα protein (Fig. 5 G) and levels of mRNA for the M2 markers Arginase-1 (*arg1*) and YM-1 (*chi3l3*; Fig. 5 H), despite elevated IL-13 mRNA expression levels in the knockout mice (Fig. 5 H). Most importantly, *Batf3*$^{-/-}$ mice were more resistant to *H. polygyrus* infection than WT mice, which was made evident by the absence of eggs in the feces throughout infection (Fig. 5 I and not depicted) and the lower number of adult worms that could be recovered from the intestines of these mice (Fig. 5 J).

After they are swallowed, *H. polygyrus* larvae invade the intestinal wall before going through several molts and emerging into the lumen as adult worms. While in the intestinal wall, larvae become surrounded by granulomatous lesions. In susceptible mice, larvae emerge from granulomas, but in resistant mice larvae can become trapped and die within these lesions. Regardless of outcome, the lesions are macroscopically evident on the intestinal wall, and we found similar overall numbers of these between infected WT and *Batf3*$^{-/-}$ mice (Fig. 5 K), indicating that the observed differences in outcome of infection were not caused by differences in the efficacy of the initial infection. In summary, these data indicate that, in the absence of Batf3-dependent DCs, mice mount stronger type 2 immune responses that allow them to more efficiently resist primary infection with *H. polygyrus*.

Migratory CD103$^+$ DCs produce IL-12 at steady state and during helminth infections independently from TLR and commensal signals

Batf3-dependent CD8α$^+$ and CD103$^+$ DCs have been shown to be a critical source of IL-12 to allow for efficient priming of protective type 1 immune responses to intracellular infections or tumors (Hildner et al., 2008; Mashayekhi et al., 2011). In addition, because IL-12 is known to directly inhibit Th2 polarization (Manetti et al., 1993; Finkelman et al., 1994; Oswald et al., 1994), we reasoned that IL-12 production by Batf3-dependent DCs could provide a mechanistic basis for suppression of Th2 cell development. To address this, we first used yet40 C57BL/6 mice that express YFP under control of the IL-12p40 promoter (Reinhardt et al., 2006). In these mice, expression of the YFP reporter is a faithful marker for active IL-12p40 protein expression (Fig. 6 A). In initial experiments, we found that YFP was selectively expressed by CD11c$^+$ cells in hLNs and mLNs from naive mice (Fig. 6 B). Interestingly, a more detailed analysis of the different cell types within the CD11c$^+$ compartment revealed that although there was some YFP expression by resident CD11c^{hi}MHCII$^+$ DCs expressing CD8α$^+$, an observation that is generally consistent with previous studies (Reinhardt et al., 2006; Mashayekhi et al., 2011), it was specifically CD103$^+$CD11b$^-$ DCs with a migratory phenotype (CD11c$^+$MHCII^{hi})—from here on referred to as migratory CD103$^+$ DCs—that were strongly positive for YFP in the steady state (Fig. 6, C and E; and Fig. S1). Consistent with a suppressive effect of helminth infection and their antigens on DC activation (Chiaramonte et al., 1999; de Jong et al., 2002; Ferrick et al., 2008; Everts et al., 2009; Massacand et al., 2009), YFP signal (Fig. 6, C and E) and MHC II expression (Fig. 6, D and F; and Fig. S1) were reduced to various degrees in all DC subsets in the hLN and mLN after *S. mansoni* and *H. polygyrus* infection, respectively. However, significant percentages of migratory CD103$^+$ DCs continued to express YFP after infection (Fig. 6, C and E). As a result, the majority of DCs expressing IL-12p40 in these LNs during both infections were of the migratory CD103$^+$ type (Fig. 6, G and H).

Constitutive expression of IL-12p40 has been reported to occur in CD8α$^-$ CD11b$^-$ DCs in the intestinal lamina propria, and to be dependent on the intestinal microbiota, as it is absent in germfree (GF) mice (Becker et al., 2003). We asked therefore whether the constitutive expression of IL-12 by migratory CD103$^+$ DCs in hLN and mLN is a result of steady state exposure of these cells to products of commensal microbes. However, when we treated naive BALB/c mice with antibiotics to deplete the intestinal microbiota, we did not observe any effect on IL-12p40 expression by migratory CD103$^+$ DCs in mLNs (Fig. 7 A). Likewise, antibiotic treatment of yet40 C57BL/6 mice did not affect YFP signal in migratory CD103$^+$ DCs in mLNs of naive or *H. polygyrus*–infected mice (Fig. 7 B). Consistent with this, migratory CD103$^+$ DCs in mLNs of GF C57BL/6 mice expressed comparable levels of IL-12p40 to mLN CD103$^+$ DCs from mice maintained under specific pathogen–free (SPF) conditions (Fig. 7 C). Moreover, we tested the role of TLR signaling

GC B cells of total CD19$^+$ B cells in hLNs of 8 wk *S. mansoni*–infected mice. Data are based on three mice per group. (I) SEA-specific IgG1 titers in serum of 8 and 16 wk *S. mansoni*–infected WT and *Batf3*$^{-/-}$ BALB/c mice. Dots represent individual mice. (J) Frequencies of DC subsets within the CD11c$^+$MHCII$^+$ DC population in liver, CD11c$^+$MHCII^{hi} migratory DC (mDC) population, and CD11c^{hi}MHCII$^+$ resident DC (rDC) population in hLN from naive and 8 wk infected WT or *Batf3*$^{-/-}$ BALB/c mice. Data are concatenated plots from three mice per group. One of two (C, F, G, and J) or three (A, B, D, E, and H) experiments is shown. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

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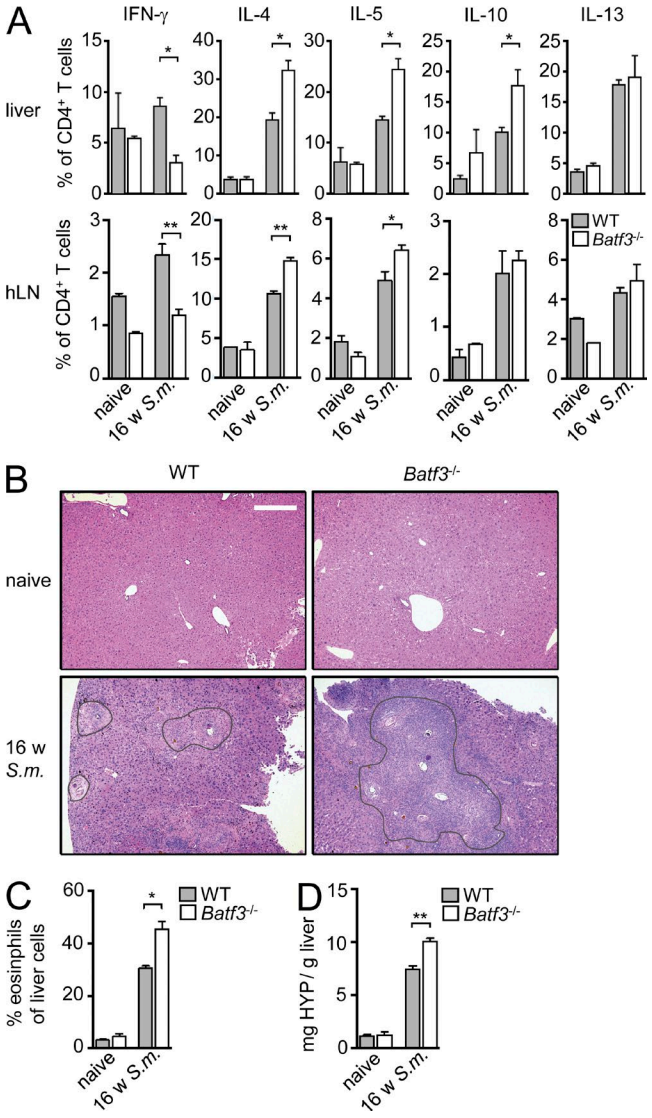

Figure 3. ***Batf3*$^{-/-}$ mice display stronger liver fibrosis in response to chronic *S. mansoni* infection.** (A) Liver and hLN cells from WT or *Batf3*$^{-/-}$ BALB/c naive or 16 wk *S. mansoni*–infected mice were restimulated with PMA/Ionomycin in the presence of Brefeldin A, after which CD4$^+$ T cells were stained intracellularly for indicated cytokines. Error bars represent mean ± SEM from three mice per group. (B) Representative H&E staining of liver sections from naive and 16 wk infected mice. Dashed line marks border of granuloma around the eggs. Bar, 500 µm. Data are representative of three mice per group. (C) Eosinophils (SiglecF$^+$CD11b$^+$F4/80$^-$ SSC^{hi}) frequencies in livers from naive and 16 wk infected mice. Error bars represent mean ± SEM from three mice per group. (D) Hydroxyproline (HYP) levels in livers from naive and 16 wk infected mice. Error bars represent mean ± SEM from three mice per group. (A–D) One of two experiments is shown. *, $P < 0.05$; **, $P < 0.01$.

in the production of IL-12p40 by migratory CD103$^+$ DCs. We found that IL-12p40 expression by migratory CD103$^+$ DCs remained unabated in naive *Trif*$^{-/-}$/*Myd88*$^{-/-}$ double knockout mice (Fig. 7 D). Finally, we generated BM-chimeras in which Batf3-dependent DCs are the only cells deficient for TRIF and MyD88, to specifically address the cell-intrinsic importance of TLR signaling in IL-12p40 expression by migratory CD103$^+$ DCs during helminth infection. In line with the whole body knockouts, IL12p40 expression by migratory CD103$^+$ DCs during *H. polygyrus* infection was not reduced in the absence of TLR signaling (Fig. 7 E). Collectively, these data identify migratory CD103$^+$ DCs as a dominant source of IL-12 that occurs independently of microbial exposure or TLR signaling, both in naive and helminth-infected mice.

IL-12 production by migratory CD103$^+$ DCs impairs type 2 immunity against *H. polygyrus* infection

To start to address the influence of IL-12 derived from Batf3-dependent DCs in Th2 response development, we cultured bulk DCs isolated from mLNs of naive WT or *Batf3*$^{-/-}$ BALB/c mice with naive OVA-specific CD4$^+$ T cells in the presence of OVA-peptide with or without the addition of exogenous IL-12p70 or neutralizing anti–IL-12p70 antibody. Consistent with the in vivo observations, the mLN-derived DC population from *Batf3*$^{-/-}$ mice, from which the IL-12–producing migratory CD103$^+$ DCs are missing, promoted a higher percentage of IL-4–producing T cells, in line with an enhanced Th2-polarized response, compared with DCs from WT mice (Fig. 8 A). Addition of IL-12p70 in the picogram/milliliter range was sufficient to negate the enhanced capacity of mLN-derived DCs from *Batf3*$^{-/-}$ mice to skew the response toward more IL-4–producing T cells. Conversely, blocking IL-12 in the cultures with mLN-derived DCs from WT mice resulted in skewing of the response to a high IL-4/IFN-γ ratio, very similar to that induced by mLN-derived DCs from *Batf3*$^{-/-}$ mice (Fig. 8 A), suggesting that steady-state production of IL-12p70 by Batf3-dependent DCs restrains Th2 differentiation. To assess whether Batf3-dependent DCs dampen Th2 priming solely by acting as a bystander source of IL-12, or by additional mechanisms such as competition for T cells with other (Th2-priming) Ag-presenting cells, we adapted the former co-culture system by using total cells from mLNs of WT or *Batf3*$^{-/-}$ mice instead of purified DCs. In this experiment, Ag presentation is likely to be primarily mediated by B cells that vastly outnumber the Batf3-dependent DCs, thereby creating a situation in which these DCs will not be able to effectively compete for Ag presentation to T cells as a potential mechanism to suppress Th2 polarization. Similar to the co-cultures with purified DCs, we found that, compared with mLN cells from WT mice, total mLN cells from *Batf3*$^{-/-}$ mice promoted a stronger Th2-polarized response, which could be negated by addition of exogenous IL-12p70 (Fig. 8 B). Conversely, blocking IL-12 in the cultures with mLN cells from WT mice resulted in a shift toward Th2 polarization very similar to that induced by mLN cells from *Batf3*$^{-/-}$ mice (Fig. 8 B). We interpret these findings as support for the conclusion that Batf3-dependent DCs dampen Th2 priming by acting as a bystander source of IL-12p70. This conclusion was further corroborated and extended

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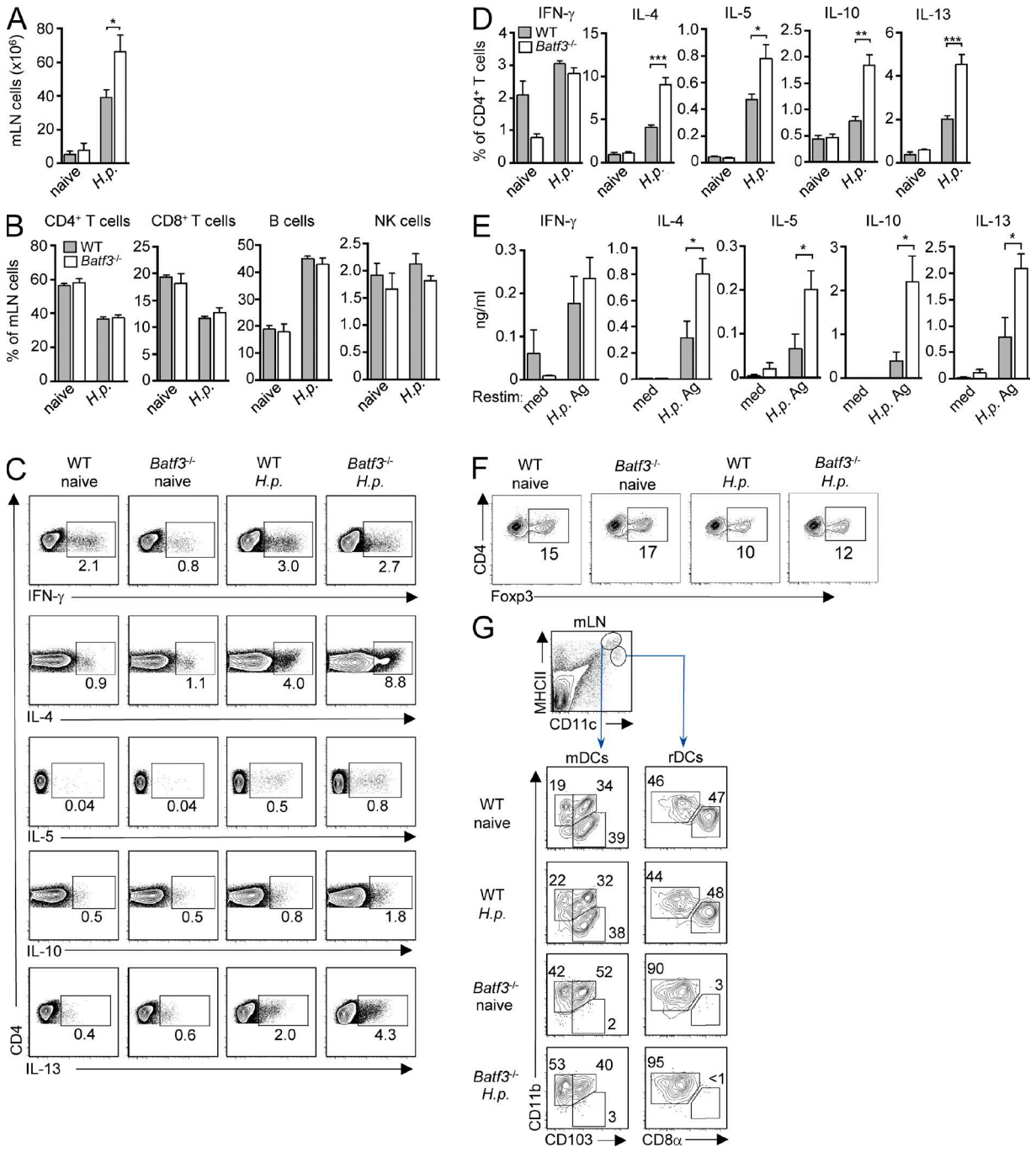

Figure 4. ***Batf3*$^{-/-}$ mice display stronger type 2 immunity to *H. polygyrus* infection.** mLN cell numbers (A) or frequencies (B) of CD3$^+$CD4$^+$ T cells, CD3$^+$CD8α^+ T cells, CD19$^+$ B cells, and CD3$^-$DX5$^+$ NK cells in mLNs from WT or *Batf3*$^{-/-}$ BALB/c naive mice or mice infected for 11 d with *H. polygyrus* (100 L3 stage larvae). (C and D) mLN cells from WT or *Batf3*$^{-/-}$ BALB/c naive mice or mice infected for 11 d as in A were restimulated with PMA/Ionomycin in the presence of Brefeldin A, after which CD4$^+$ T cells were stained intracellularly for indicated cytokines. (E) mLN cells from 15 d *H. polygyrus*–infected mice were restimulated with *H. polygyrus*–derived worm antigen for 3 d and cytokine levels in culture supernatants were determined. (F) Foxp3 staining in T cells isolated from mLNs from mice as described in A. (G) Frequencies of DC subsets within the CD11c$^+$MHCII^{hi} migratory DC (mDC) population and CD11c^{hi}MHCII$^+$ resident DC (rDC) population in mLN from naive and 11 d *H. polygyrus*–infected WT or *Batf3*$^{-/-}$ BALB/c mice. The gating strategy for mDC and rDC subsets is illustrated in the top panel, showing mLN cells from a naive WT mouse and from which CD19$^+$ B cells, which can also be CD11c$^+$MHCII$^+$, have been gated out. Data are (C, F, and G) concatenated plots or (A, B, D, and E) shown as bar graphs representing mean ± SEM from three to six mice per group. One of two (B, F, and G) or three (A and C–E) experiments is shown. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

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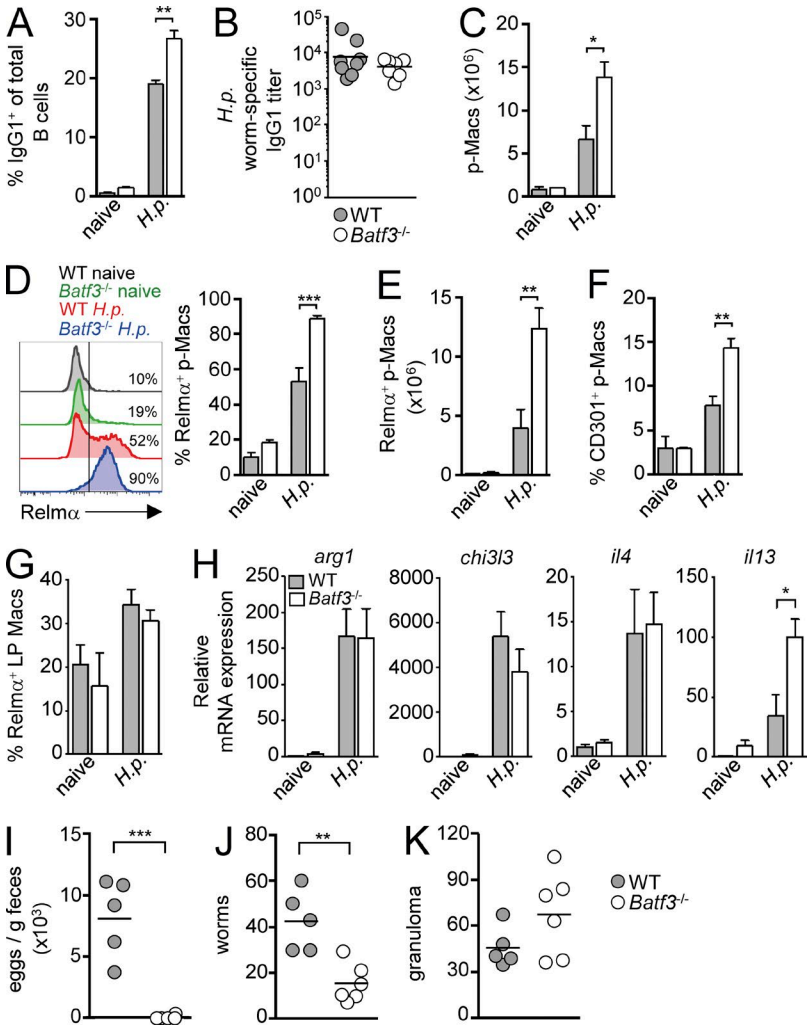

Figure 5. ***Batf3*$^{-/-}$ mice display stronger resistance to *H. polygyrus* infection.** (A) Frequency of IgG1$^+$ class-switched GC B cells of total CD19$^+$ B cells in mLNs from WT or *Batf3*$^{-/-}$ BALB/c naive mice or mice infected for 11 d with *H. polygyrus* (100 L3 stage larvae). (B) *H. polygyrus* worm antigen-specific IgG1 titers in serum of naive and 13 d infected WT or *Batf3*$^{-/-}$ BALB/c mice. (C) Macrophage (F4/80$^+$CD11b$^+$) numbers in peritoneal lavage from mice as described in (A). (D and F) Percentage of p-Macs expressing (D) RELMα or (F) CD301 from mice as described in A, either depicted as histograms or bar graphs. (E) Number of RELMα$^+$ macrophages in peritoneal lavage from mice as described in A. (G) Percentage of CX3CR1$^+$ macrophages expressing RELMα in the lamina propria (LP) of the small intestine from mice as described in A. (H) Relative mRNA expression levels of indicated genes from small intestine of WT or *Batf3*$^{-/-}$ BALB/c naive mice or mice infected for 9 d with *H. polygyrus.* β-Actin was used as housekeeping gene. (I) Fecal eggs from 10 d *H. polygyrus*-infected mice. (J) Number of adult worms isolated from the small intestine of 11 d *H. polygyrus*–infected mice. (K) Number of granuloma present in small intestine of 11 d *H. polygyrus*–infected mice. Data are D concatenated plots or (A, C, and D–H) shown as bar graphs representing mean ± SEM from three to six mice per group. For all data, one of three experiments is shown, except for H, which was done once. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

by experiments in which we cultured purified CD11b$^+$ DCs derived from mLNs of WT C57BL/6 mice with naive OVA-specific CD4$^+$ T cells in the presence of OVA-peptide to which we did, or did not, add CD8α$^+$ DCs or migratory CD103$^+$ DCs isolated from mLNs of WT, *Il12a*$^{-/-}$, or *Mhcii*$^{-/-}$ C57BL/6 mice. *Il12a* encodes for IL-12p35 that heterodimerizes with the IL-12p40 subunit to form bioactive IL-12p70. The addition of migratory CD103$^+$ DCs, whether they were WT or *Mhcii*$^{-/-}$, reduced the percentage of CD4$^+$ T cells, making IL-4 by threefold, and increased the percentage of CD4$^+$ T cells, making IFN-γ by 1.5-fold, whereas the addition of WT CD8α$^+$ DCs or *Il12a*$^{-/-}$ migratory CD103$^+$ DCs had no effect on IL-4 or IFN-γ production (unpublished data). These findings together suggest that spontaneous bystander IL12p70 production specifically by migratory CD103$^+$ DCs restrains Th2 polarization and that this is not dependent on antigen presentation.

We next aimed to test whether this would also hold true in vivo. We found that treatment of WT BALB/c mice with neutralizing anti–IL-12p70 antibody during *H. polygyrus* infection resulted in increased accumulation of RELMα$^+$ p-Macs (Fig. 8 C). Moreover, anti–IL-12p70-treated mice were more resistant to *H. polygyrus* infection than control antibody-treated mice, as evidenced in lower fecal egg counts (Fig. 8 D) and the recovery of lower numbers of adult worms from the intestines of treated infected mice (Fig. 8 E). Thus, neutralization of IL-12p70 in vivo in infected WT mice phenocopied our findings with infected *Batf3*$^{-/-}$ mice. Conversely, daily injections of rIL-12p70 during *H. polygyrus* infection of *Batf3*$^{-/-}$ BALB/c mice were sufficient to reverse the accumulation of RELMα$^+$ p-Macs (Fig. 8 F). As a consequence, *Batf3*$^{-/-}$ BALB/c mice treated with rIL-12p70 partially lost their heightened resistance to *H. polygyrus,* as revealed by restoration of egg production by the worms in these mice (Fig. 8 G). To directly determine whether IL-12p70 expression specifically by Batf3-dependent DCs is important for the effects we observed in these studies, we generated BM chimeric mice by transferring a 1:1 ratio of *Batf3*$^{-/-}$ and *Il12a*$^{-/-}$ C57BL/6 BM into lethally irradiated C57BL/6 recipients. In these mice, Batf3-dependent DCs are the only cells deficient

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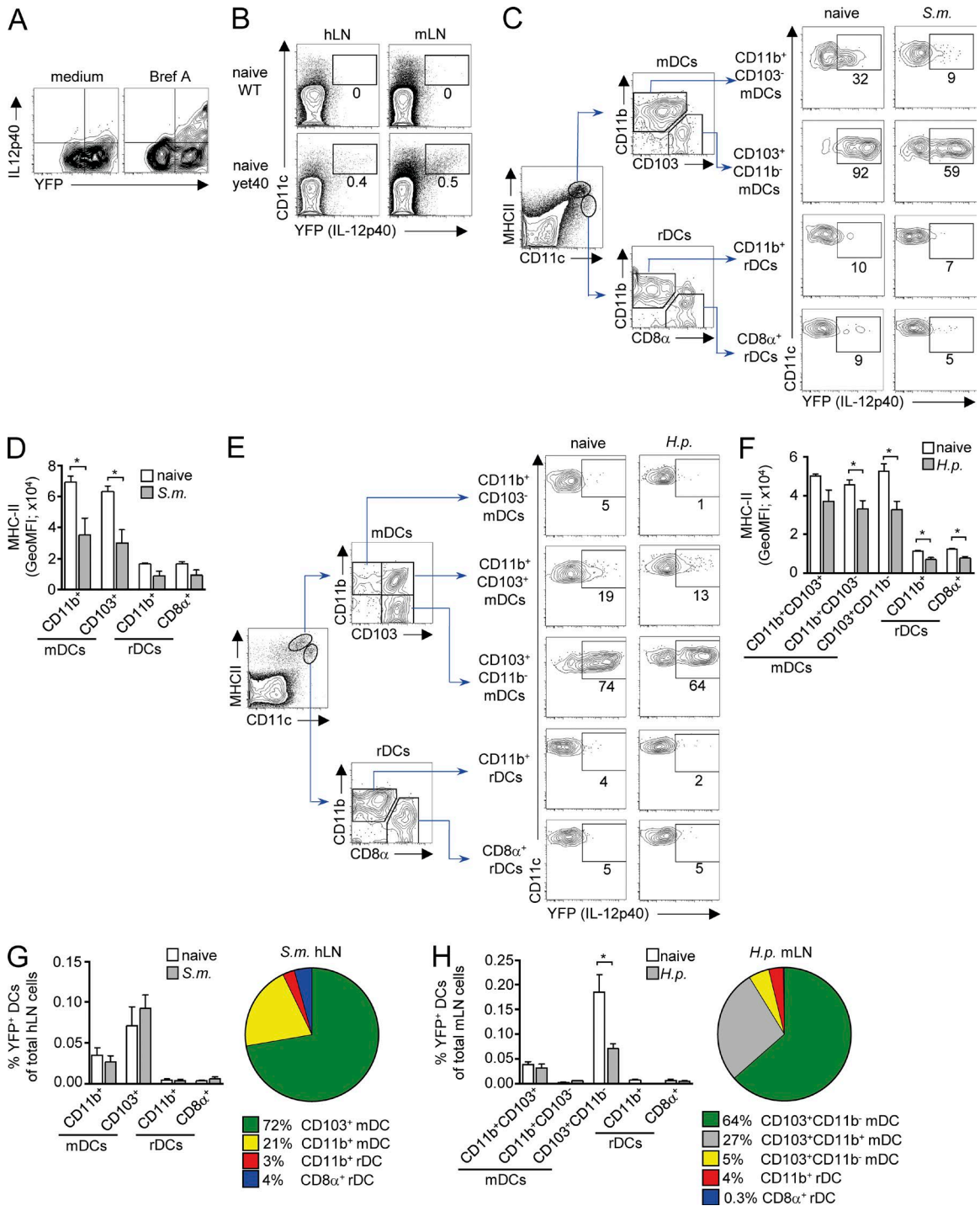

Figure 6. **Batf3-dependent migratory CD103⁺ DCs are major constitutive producers of IL-12 both at steady state and during helminth infections.** (A) mLN cells from naive yet40 mice were cultured for 6 h in the presence or absence of Brefeldin A. YFP signal and IL-12p40 protein expression by CD103⁺ DCs was determined by flow cytometry. (B) hLN and mLN cells from naive C57BL/6 or yet40 IL-12 reporter C57BL/6 mice were analyzed for YFP expression. (C and E) yet40 IL-12 reporter mice were infected with *S. mansoni* for 6 wk (C) or 7 d (E) with *H. polygyrus* and migratory (mDCs; MHCII^{hi}CD11c^{int}) and resident (rDCs; MHCII^{int}CD11c^{hi}) DC subsets in hLNs (C) and mLNs (E) were analyzed for YFP reporter expression. The gating strategy for mDC and rDC subsets is shown in the three left panels. The far left panel shows hLN (C) or mLN (E) cells from a naive WT mouse and from which CD19⁺ B cells, which can also be CD11c⁺MHCII⁺, have been gated out. Data are concatenated plots from three to four mice per group. (D and F) DC subsets as described in C and E were analyzed for MHC-II expression. (G and H) Bar graphs represent frequency of IL-12p40-producing DC subsets of total cells based on YFP expression

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for IL-12p35. Consistent with the results from the IL-12 blocking studies, the chimeric mice mounted a stronger Th2 response in mLNs in response to *H. polygyrus* infection compared with mice harboring IL-12p35–sufficient Batf3-dependent DCs (Fig. 8 H). This was mirrored by increased numbers of IgG^+ GC B cells in mLNs (Fig. 8 I), and increased M2 polarization of p-Macs (Fig. 8 J). Collectively, these data show that IL-12 production by Batf3-dependent $CD103^+$ migratory DCs is the key mechanism through which these cells suppress type 2 immune responses.

DISCUSSION

Batf3-dependent DCs are recognized primarily for their essential role in the priming and regulation of Th1/type 1 immune responses. Here, we provide evidence that these DCs, particularly $CD103^+$ DCs, also play an important function in the regulation of Th2 responses and type 2 immunity during helminth infections. Specifically, we found that Batf3-dependent DCs suppress Th2 responses and type 2 immunity in diverse helminth infection models. The increased Th2 profile is consistent with a study showing that type 2 immune responses were enhanced in a model of allergic asthma in the absence of Batf3-dependent DCs (Khare et al., 2013). In that setting, the suppression in Th2 immune responses by Batf3-dependent DCs was shown to be mediated by $CD103^+$ DC-dependent induction of T reg cells. However, we did not observe major alterations in the T reg cell compartment caused by Batf3 deficiency, a finding that corroborates other studies (Edelson et al., 2010). Instead, we show that Batf3-dependent migratory $CD103^+$ DCs suppress Th2 responses by producing IL-12. Production of this cytokine occurs in naive mice, is independent of TLR signaling, and is largely resistant to suppression by helminth infection. These findings identify Batf3-dependent migratory $CD103^+$ DCs as important negative regulators of type 2 immunity.

It is well recognized that IL-12 from antigen-presenting cells promotes the differentiation of naive Th cells into Th1 cells (Hsieh et al., 1993) and concomitantly inhibits commitment to the Th2 lineage (Manetti et al., 1993; Oswald et al., 1994). More recently, Batf3-dependent DCs were identified as the key cell type to produce IL-12 to support effective type 1 immune responses required for protection against acute *T. gondii* infection (Mashayekhi et al., 2011). In this case, IL-12 secretion by $CD8\alpha^+$ DCs is stimulated by parasite-derived agonists of TLR11 and 12 (Yarovinsky et al., 2005; Amiel et al., 2014). However, we found that IL-12 is also produced in helminth parasite-infected mice, not by $CD8\alpha^+$ DCs, but rather by migratory $CD103^+$ DCs in LNs. This finding contrasts with the well-documented inability of helminth Ag to trigger IL-12 release themselves and instead to suppress IL-12 expression induced by TLR agonists (MacDonald et al., 2001; Ferrick et al., 2008; Everts et al., 2009; Massacand et al., 2009), which has been thought to be a prerequisite for effective induction of Th2 responses (Jankovic et al., 2006). We found that production of IL-12 by $CD103^+$ DCs is independent of TLR signaling or the presence of microbiota and occurs in the steady state in naive mice. This suggests that IL-12 production by migratory $CD103^+$ DCs during helminth infection is a reflection of the steady-state spontaneous IL-12 production by these cells, rather than helminth-induced IL-12 secretion. Moreover, we noted that the proportion of $CD103^+$ DCs expressing IL-12 in helminth-infected mice is lower than in naive mice, indicating that helminth infection has some suppressive effect on IL-12 secretion by these cells in vivo. These findings are generally consistent with previous studies documenting detectable amounts of IL-12 in helminth-infected mice (Wynn et al., 1994; Massacand et al., 2009). However, we have now identified the source of that IL-12, which remained elusive in these earlier studies. Finally, although the expression of IL-12 by migratory $CD103^+$ DCs under steady-state conditions has been documented before (Reinhardt et al., 2006; Dalod et al., 2014), the functional implications of these observations have remained unclear. Now we show that this IL-12 production in the context of helminth infections is of functional significance as it has a marked negative impact on the magnitude of the Th2 response, thereby compromising the ability of the host to expel an intestinal nematode infection or to control Th2-associated immunopathology during a chronic trematode infection.

The basis for the spontaneous expression of IL-12 that appears to underlie the capacity of $CD103^+$ DCs to impair Th2 immunity remains unclear. In contrast to LN-resident DCs, including $CD8\alpha^+$ DCs, $CD103^+$ DCs are migratory cells that have been shown to travel from peripheral tissues into LNs and phenotypically mature even under noninflammatory conditions (Wilson et al., 2003). The spontaneous expression of IL-12 by $CD103^+$ DCs in LNs in naive mice is generally consistent with this. Whereas the stimuli directing steady-state migration and phenotypic maturation of these cells remain to be established, it is reported to be independent from microbial signals (Wilson et al., 2008), which is consistent with our observation that IL-12 production by Batf3-dependent migratory $CD103^+$ DCs is unimpeded in GF mice or in the absence of TLR signaling in both naive and helminth-infected animals. The difference in IL-12 expression between migratory $CD11b^+$ and $CD103^+$ DCs, which have undergone comparable maturation, could reside in the fact that in contrast to $CD11b^+$ DCs, $CD103^+$ DCs express high levels of transcription factor IRF8, an important positive regulator of IL-12p40 and p35 expression (Wang et

within hLNs from naive or *S. mansoni*-infected mice (G) or mLNs from naive or *H. polygyrus*-infected mice (H). Pie charts depict relative contribution of each DC subset to YFP expression within (G) hLNs from *S. mansoni*-infected mice or (H) mLNs from *H. polygyrus*-infected mice. (D and F–H) Data shown as bar graphs represent mean ± SEM from three to four mice per group. One of two (A and B) or three (C–H) experiments is shown. *, $P < 0.05$.

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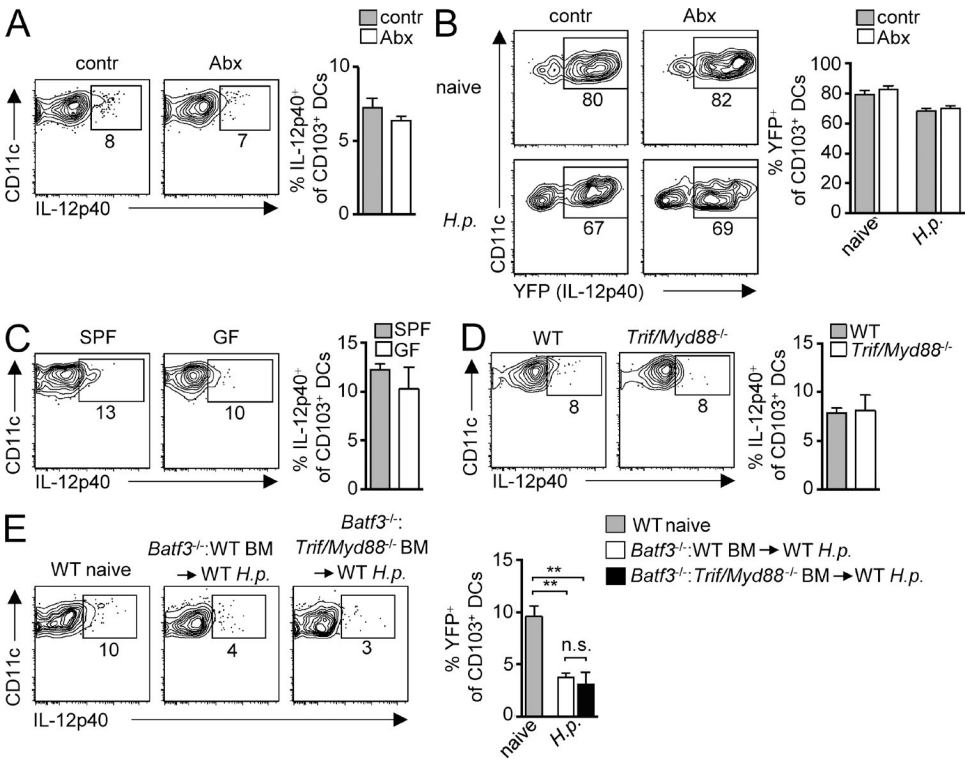

Figure 7. **Migratory CD103⁺ DCs constitutive produce IL-12 independently from TLR or microbial signals.** (A) Naive WT BALB/c mice were treated with a cocktail of antibiotics (Abx) for 3 wk and mLN cells were cultured ex vivo for 6 h in the presence of Brefeldin A. Migratory CD103⁺ DCs were analyzed for expression of IL-12p40 protein by intracellular cytokine staining. (B) Yet40 IL-12p40 reporter mice were infected for 12 d with *H. polygyrus* after pretreatment with antibiotics as in A, and YFP expression by migratory CD103⁺ DCs in mLNs was determined. (C) Migratory CD103⁺ DCs from naive WT C57BL/6 mice maintained under SPF or GF conditions were analyzed as in A. (D) Migratory CD103⁺ DCs from naive WT or *Trif/Myd88*$^{-/-}$ C57BL/6 mice were analyzed as in A. (E) Lethally irradiated C57BL/6 recipient mice received either a 1:1 mixture of WT with *Batf3*$^{-/-}$ BM or *Trif/Myd88*$^{-/-}$ with *Batf3*$^{-/-}$ BM. Chimeric mice were infected for 12 d with *H. polygyrus*, and migratory CD103⁺ DCs in mLNs were analyzed for expression of IL-12p40 protein by intracellular cytokine staining as in A. (A–E) Data are concatenated plots or shown as bar graphs with mean ± SEM from three to four mice per group. One of two experiments is shown (A–D), or the experiment was performed once (E). **, $P < 0.01$.

al., 2000; Liu et al., 2004), which may more readily trigger CD103⁺ DCs to express IL-12.

We found that neutralization of IL-12 both in vitro and in vivo phenocopied the enhanced Th2 polarization due to Batf3 deficiency and, conversely, that IL-12 administration to *Batf3*$^{-/-}$ mice was sufficient to negate enhanced type 2 immunity in the absence of Batf3-dependent DCs. In conjunction with the findings from BM chimera studies in which only Batf3-dependent DCs were IL-12p35 deficient, and in vitro T cell polarization experiments with *Il12a*$^{-/-}$ migratory CD103⁺ DCs, these data provide strong evidence that IL-12 production by migratory CD103⁺ DCs is the key Batf3-dependent event that suppresses type 2 immunity. Moreover, the fact that *Mhcii*$^{-/-}$ migratory CD103⁺ DCs were just as capable of suppressing Th2 differentiation as WT migratory CD103⁺ DCs indicates that their capacity to suppress Th2 responses is independent of Ag presentation. Collectively, the findings suggest that, in LNs, migratory CD103⁺ DCs act as IL-12–producing bystander cells that restrain Th2 induction by other DC subsets. Further studies will be needed to determine whether the production of IL-12 by CD103⁺ DCs in LNs acts directly on CD4⁺ T cells to antagonize Th2 polarization or whether IL-12 licenses NK(T) and CD8⁺ T cells to become activated and to release IFN-γ that subsequently dampens the magnitude of the Th2 response (Oswald et al., 1994; Hildner et al., 2008; Tussiwand et al., 2015). Our observation that Batf3-deficient *S. mansoni*–infected animals show impaired accumulation of CD8⁺ T cells and NK cells in the liver relative to WT animals could provide support for the latter scenario. However, in both helminth infection models, frequencies of CD8⁺ T cells and NK cells in reactive LNs, where most of the CD4⁺ T cell priming is expected to take place, were not affected by Batf3 deficiency, perhaps arguing against this possibility. However, the functionality of these cells was not assessed in this work.

Although the role of particular DC subsets in priming and regulation of Th1 and Th17 responses are well-characterized, there is still an incomplete understanding of DC-mediated polarization and regulation of Th2 responses. Our work now provides evidence that, in addition to specialized DC

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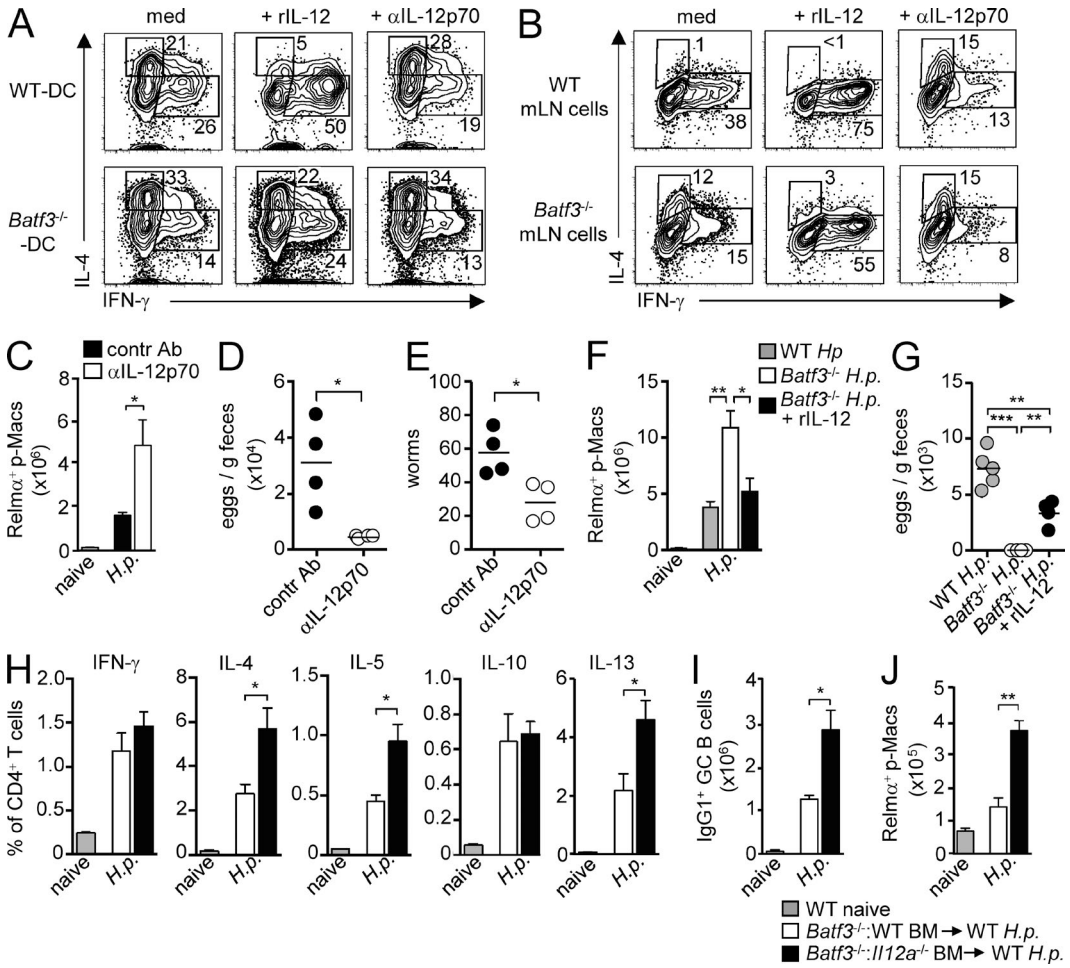

Figure 8. **Batf3-dependent DC-derived IL-12 dampens type 2 immunity during helminth infection.** (A) Total DCs purified from the mLN of naive WT or *Batf3*$^{-/-}$ BALB/c mice were co-cultured for 7 d with DO11.10 OVA-specific CD4$^+$ T cells in the presence of $OVA_{323-339}$ peptide and recombinant IL-12 or neutralizing IL-12 antibody, and subsequently restimulated with PMA/Ionomycin in the presence of Brefeldin A, after which CD4$^+$ T cells were stained intracellularly for indicated cytokines. One of two experiments is shown. (B) As in A, but instead of total DCs purified from the mLN, total mLN cells were used. (C–E) WT BALB/c mice were infected with *H. polygyrus* and at day 0, 3, 6, and 9 after infection injected i.p. with 500 µg control antibody or neutralizing antibody against IL-12p70. 11 d after infection (C), the number of RELMα$^+$ macrophages in peritoneal lavage, (D) fecal egg numbers and (E) number of adult worms isolated from the small intestine were determined. (F and G) WT and *Batf3*$^{-/-}$ BALB/c mice were infected with *H. polygyrus* and injected daily i.p. with 250 ng rIL-12. 13 d after infection the number of RELMα$^+$ macrophages in peritoneal lavage (F) and fecal egg numbers (G) were determined. (H–J) Lethally irradiated C57BL/6 recipient mice received either a 1:1 mixture of WT with *Batf3*$^{-/-}$ BM or *Il12a*$^{-/-}$ with *Batf3*$^{-/-}$ BM. Chimeric mice were infected for 12 d with *H. polygyrus* and mLN cells were restimulated with PMA/Ionomycin in the presence of Brefeldin A (H), after which CD4$^+$ T cells were stained intracellularly for indicated cytokines. (I) Number of IgG1$^+$ classed-switched GC B cell from mLNs isolated from infected chimeric mice. (J) Number of RELMα$^+$ macrophages in peritoneal lavage from infected chimeric mice. Data shown as bar graphs represent mean ± SEM from at least four mice per group. One of two experiments (A–G) is shown, or the experiment was performed once (H–J). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

subsets that promote Th2 responses (Gao et al., 2013; Kumamoto et al., 2013; Plantinga et al., 2013; Tussiwand et al., 2015), there are other subsets that suppress these same responses. This suggests that the magnitude of type 2 immune responses is determined by the balance between DC subsets that promote and antagonize them, and implies that for optimal design of DC-based approaches to manipulate type 2 immune responses for therapeutic purposes, there needs to be a focus on targeting not only Th2-promoting DC subsets but also CD103$^+$ DCs. Another example of potential clinical relevance of our findings comes from a recent study describing immunocompromised individuals with defects in their DC compartment as a result of mutations in the gene encoding IRF8 (Hambleton et al., 2011). DCs of these patients were found to have an impaired capacity to produce IL-12, which was correlated to an increased susceptibility to Bacillus Calmette–Guérin disease. These types of case studies provide evidence for the existence of mutations in genes that dramatically affect the biology of Batf3-dependent DCs in the human population. This is likely to not only have a profound

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effect on the outcome of diseases in which type 1 immune responses are important, but also in which type 2 immunity is involved. The latter would be particularly pertinent to people living in countries where helminth parasites are endemic.

MATERIALS AND METHODS

Mice and parasites. C57BL/6, BALB/c, OT-II, and DO11.10 mice expressing I-A^b and I-A^d–restricted OVA-specific TCRs, respectively, C57BL/6 *Il12a*$^{-/-}$, and C57BL/6 *H2*$^{-/-}$ (*Mhcii*$^{-/-}$) mice were purchased from The Jackson Laboratory. C57BL/6 *Batf3*$^{-/-}$ and BALB/c *Batf3*$^{-/-}$ were generated as previously described (Hildner et al., 2008), and C57BL/6 IL-12p40 reporter mice (yet40; Reinhardt et al., 2006) and C57BL/6 *Trif/Myd88*$^{-/-}$ double knockout mice were gifts from Drs. A. Sher (Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD) and W. Yokoyama (Rheumatology Division, Washington University School of Medicine, St. Louis, MO), respectively. Mice were bred and/or maintained under specific pathogen–free (SPF) or, where indicated, germ-free (GF) conditions under protocols approved by the institutional animal care at Washington University School of Medicine or Leiden University Medical Center, and were used at 6–12 wk of age. To clear mice from gut microbiota, mice were orally gavaged every 48 h with 400 µl of 1 mg/ml ampicillin, 1 mg/ml neomycin, 1 mg/ml metronidazole, and 0.5 mg/ml vancomycin (all from Sigma-Aldrich). Mice were infected with *S. mansoni* (Puerto Rican strain; Naval Medical Research Institute) by percutaneous exposure to 60 cercariae. Adults *S. mansoni* worms residing in the portal vasculature were enumerated after perfusion of the portal vein. For *S. mansoni* egg immunizations, eggs were isolated from the livers of *S. mansoni*–infected mice, and stored at −70°C in PBS until use, as previously described (Taylor et al., 2006). 5,000 eggs/50 µl were injected s.c. in the hind footpad of mice. For infection with *H. polygyrus bakeri,* mice were orally gavaged with 100 infective L3 stage larvae (L3). For quantification of parasite burden, intestines were removed from mice, opened longitudinally, and placed into a metal strainer on top of a 50-ml tube filled with PBS for 4 h at 37°C. Parasites were allowed to migrate through the filter into the tube and were recovered for counting on a dissecting microscope. Parasite eggs were enumerated by floating eggs in feces collected from individual mice on saturated sodium chloride before collection and counting under a microscope. In indicated experiments, mice were injected i.p. with anti–IL-12p70 (500 µg, clone R2-9A5; BioXcell) or isotype control antibody (500 µg, clone LTF-2; BioXcell) on day 0, 3, 6, and 9 after *H. polygyrus* infection, or daily i.p. with recombinant murine IL-12p70 (250 ng; BioLegend). Soluble Egg Antigen (SEA) from *S. mansoni* eggs and soluble antigen from *H. polygyrus* worms were prepared as previously described (MacDonald et al., 2001; Wilson et al., 2005).

Generation of mixed BM chimeras. BM from femurs and tibias from female CD45.2$^+$ donor mice were harvested, red blood cells were lysed in ACK lysis buffer (0.15 M NH_4Cl, 1 mM $KHCO_3$, 0.1 mM EDTA) and filtered through 70-µm strainers. To prevent a graft-versus-host response, CD8$^+$ T cells were depleted from the BM using CD8 microbeads (Miltenyi Biotec). Male CD45.1$^+$ recipient mice were irradiated with 1,000 rads of whole-body irradiation. 24 h after irradiation, the recipients were injected intravenously with 2 × 10^6 BM cells from either a single donor or a 1:1 mixture from two donors. Mice were allowed to reconstitute for 8 wk after donor cell transfer, and subsequently bled to determine chimerism based on the congenic markers CD45.1 and CD45.2 using flow cytometry.

Cell isolation. Livers were removed from HBSS-perfused animals, mashed, and incubated in RPMI containing 0.2% Collagenase D (Roche) and 10 µg/ml DNase I at 37°C for 45 min under constant rotation. The resulting suspension was disrupted through a 100 µm cell strainer and centrifuged twice at 20 *g* for 10 min in PBS/0.5% BSA/2 mM EDTA to remove hepatocytes. Erythrocytes were lysed using ACK lysis buffer. The remaining cell pellet was washed twice in PBS/0.5% BSA/2 mM EDTA and cells were subsequently counted and used for analyses. LNs were harvested and digested at 37°C for 20 min in RPMI with Collagenase D (1 mg/ml; Roche) in the presence of DNase I (10 µg/ml; Roche). LNs were put through 70-µm strainers to generate single-cell suspensions. Cells were subsequently counted and used for analyses. Peritoneal exudate cells (PECs) were harvested from by peritoneal lavage with 10 ml of sterile PBS/5% FBS/2 mM EDTA. For isolation of lamina propria cells, the small intestine was washed in HBSS, Peyer's patches were excised, and the remainder of the tissue was cut into small pieces and incubated 2 × 20 min in predigestion solution (HBSS with 10 mM Hepes, 5 mM EDTA, 5% FBS, and 1 mM DTT) to remove epithelial cells and intraepithelial lymphocytes. Cells for analysis were then isolated using the lamina propria dissociation kit for mice (Miltenyi Biotec) according to the manufacturer's recommendations.

Cell culture. Antigen-specific recall responses were determined by culturing 3 × 10^5 LN cells per well in 96-well roundbottom plates in 200 µl complete medium (RPMI containing 10% fetal calf serum, 100 U/ml penicillin/streptomycin, and 2 mM L-glutamine) in the presence of 20 µg/ml SEA or 20 µg/ml *H. polygyrus* worm antigen. 2.5 µg/ml IL-4R blocking antibody (M1) was added to the cultures to retain IL-4 in culture supernatants. 72 h later, culture supernatants were stored for cytokine determination. For T cell polarization experiments, OVA-specific CD4$^+$ T cells were purified from spleens from BALB/c (DO11.10) or C57BL/6 (OT-II) mice using CD4 microbeads (Miltenyi Biotec), according to the manufacturer's recommendations. 5 × 10^4 DO11.10 CD4$^+$ T cells were then co-cultured with either 5 × 10^5 total

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mLN cells or 5 × 10^3 FACS-purified mLN-derived DCs from WT BALB/c mice in a 96-well roundbottom plate in 200 µl of complete medium in the presence of 1 µg/ml OVA peptide, with or without anti-IL12p70 (20 µg/ml, clone R2-9A5, BioXcell), or recombinant murine IL-12p70 (50 pg/ml; BioLegend). In some of these experiments, 5 × 10^4 OT-II $CD4^+$ T cells were co-cultured with 2.5 × 10^3 FACS-purified migratory $CD11b^+$ DCs together with resident $CD8\alpha^+$ DCs or 2.5 × 10^3 migratory $CD103^+CD11b^-$ DCs sorted from mLNs of naive C57BL/6 mice. After 3 d, 10 U/ml recombinant IL-2 (PeproTech) was added and, 7 d after the start of the culture, T cells were assayed for cytokine production by intracellular staining. Assessment of cytokine production by intracellular staining of T cells from in vitro cultures or isolated from LNs or livers was determined after polyclonal restimulation in 96-well roundbottom plates for 5 h with PMA (phorbol 12-myristate 13-acetate; 50 ng/ml) and ionomycin (1 µg/ml) in the presence of Brefeldin A (10 µg/ml; all from Sigma-Aldrich) for that last 3 h.

Flow cytometry. Antibodies used for flow cytometry analysis were as follows: CD11c (N418), CD8α (53–6.7), TNF (TN3–19), FoxP3 (FJK-16s), and F4/80 (BM8), obtained from eBioscience; MHCII (I-A/E^b; M5/114.15.2), CD103 (2E7), CD49b (DX5) and CX3CR1 (SA011F11), purchased from BioLegend; CD301 (ER-MP23) from AbD Serotec, and CD11b (M1/70), CD44 (IM7), IL-12p40/70 (C15.6), IL-5 (TRFK5), IL-10 (JES5-16E3), IL-13 (eBio13A), IL-4 (11B11), IFN-γ (XMG1.2), FAS/CD95 (Jo2), Siglec-F (E50-2440), CD4 (RM4-5), CD19 (1D3), IgG1 (A85-1), IgD (11–26), IgM (11/41), PD-1 (J43), CXCR5 (2G8), and CD3 (17A2), obtained from BD. Lectin PNA from *Arachis hypogaea* (Molecular Probes) was used to stain GC B cells. Fc-block (anti–mouse CD16/32 clone 93) was used at 5 µg/ml in experiments with macrophages to minimize nonspecific signal. For intracellular staining, cells were fixed for 15 min in 4% ultrapure paraformaldehyde, and stained for 1 h on ice in 0.2% saponin buffer. For intracellular staining of RELMα, rabbit anti-RELMα (PeproTech) was used, followed by incubation with fluorochrome-conjugated anti–rabbit IgG (both from Jackson ImmunoResearch Laboratories). For staining of T cells for FoxP3, a FoxP3 staining kit was used (eBioscience). Cell culture supernatants were analyzed for cytokines using the Cytokine Bead Array (BD) according to the manufacturer's recommendation. Samples were analyzed on a BD Canto II or BD Fortessa Flow Cytometer.

RT-qPCR. RNA was isolated from tissues by homogenization in TRIzol (Invitrogen). Contaminating DNA was removed using Turbo DNase treatment (Ambion). Single-strand cDNA was synthesized using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Real-time PCR was performed by the TaqMan method using an Applied Biosystems 7000 sequence detection system. The expression levels of mRNA were normalized to the expression of β-actin.

Antigen-specific antibody titers. SEA- and *H. polygyrus* worm antigen-specific serum IgG1 endpoint titers were determined by ELISA using the IgG1-specific mAb X56 (BD). Immulon 4HBX plates (Thermo Fisher Scientific) were coated overnight at 4°C with 2 µg/ml of SEA or 2 µg/ml *H. polygyrus* worm antigen, blocked with FBS, and incubated with serial dilutions of sera, followed by a peroxidase coupled anti–mouse IgG1 and ABTS substrate.

Histology. Livers were collected from HBSS-perfused animals and immediately fixed in 10% neutral buffered formalin. Tissues were embedded and sectioned, and sections were stained with hematoxylin and eosin. Hydroxyproline levels were determined in livers using the Hydroxyproline colorimetric assay kit (Biovision), as per the recommendations of the manufacturer.

Statistical analysis. Data were analyzed using GraphPad Prism (v5). Two-group comparisons were assessed using unpaired or, where indicated, paired two-tailed Student's *t* tests. The use of these tests was justified based on assessment of normality and variance of the distribution of the data. Differences were considered significant when p-values were <0.05.

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SUPPLEMENTAL MATERIAL

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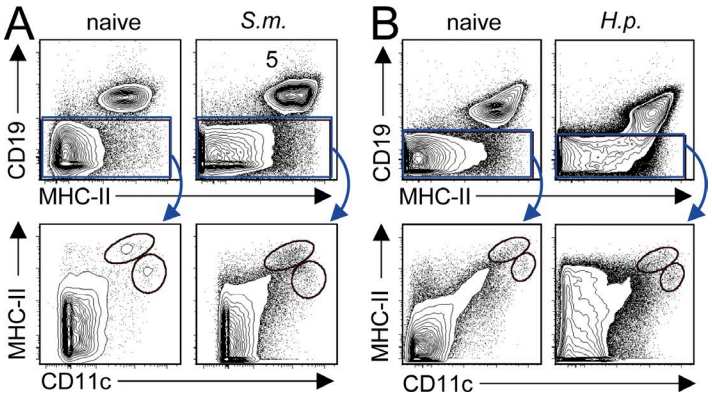

Figure S1. **Gating strategy for migratory and resident DC populations in naive and helminth-infected mice.** (A and B) Representative plots of the migratory and resident DC populations in hLNs from naive BALB/c mice or from mice infected with *S. mansoni* for 8 wk (A) and mLNs from naive BALB/c mice or from mice infected with *H. polygyrus* for 10 d (B). Live single cells were gated for $CD19^{-}$ population to exclude B cells that can also be $CD11c^{+}MHCII^{+}$ under inflammatory conditions (top). The $CD19^{-}$ gate was used to identify migratory (mDCs; $MHCII^{hi}CD11c^{int}$) and resident (rDCs; $MHCII^{int}CD11c^{hi}$) DC subsets, which can be identified on both naive and infected animals, although in the latter case overall MHCII expression by both DC populations was reduced (bottom). Data are concatenated plots from three to four mice per group from one out of three experiments.