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RESEARCH ARTICLE

Adaptive Immunity

Plasmacytoid Dendritic Cells Facilitate Th Cell Cytokine Responses throughout *Schistosoma mansoni* Infection

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ABSTRACT

Plasmacytoid dendritic cells (pDCs) are potent producers of type I IFN (IFN-I) during viral infection and respond to IFN-I in a positive feedback loop that promotes their function. IFN-I shapes dendritic cell responses during helminth infection, impacting their ability to support Th2 responses. However, the role of pDCs in type 2 inflammation is unclear. Previous studies have shown that pDCs are dispensable for hepatic or splenic Th2 responses during the early stages of murine infection with the trematode *Schistosoma mansoni* at the onset of parasite egg laying. However, during *S. mansoni* infection, an ongoing Th2 response against mature parasite eggs is required to protect the liver and intestine from acute damage and how pDCs participate in immune responses to eggs and adult worms in various tissues beyond acute infection remains unclear. We now show that pDCs are required for optimal Th2 cytokine production in response to *S. mansoni* eggs in the intestinal-draining mesenteric lymph nodes throughout infection and for egg-specific IFN-γ at later time points of infection. Further, pDC depletion at chronic stages of infection led to increased hepatic and splenic pathology as well as abrogated Th2 cell cytokine production and activation in the liver. In vitro, mesenteric lymph node pDCs

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Abbreviations used in this article: cDC, conventional dendritic cell; d32, day 32; DC, dendritic cell; dLN, draining LN; DTR, diphtheria toxin receptor; DTx, diphtheria toxin; huCD2, human CD2; IFN-I, type I IFN; ILC2, group 2 innate lymphoid cell; LN, lymph node; MHC II, MHC class II; MLN, mesenteric LN; pDC, plasmacytoid dendritic cell; PLN, popliteal LN; SEA, soluble egg Ag; Teff, T effector cell; Treg, T regulatory cell; WT, wild-type.

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supported Th2 cell responses from infection-experienced $CD4^+$ T cells, a process dependent on pDC IFN-I responsiveness, yet independent of Ag. Together, these data highlight a previously unappreciated role for pDCs and IFN-I in maintaining and reinforcing type 2 immunity in the lymph nodes and inflamed tissue during helminth infection. *ImmunoHorizons*, 2021, 5: 721–732.

INTRODUCTION

Plasmacytoid dendritic cells (pDCs) are known for potent production of type I IFN (IFN-I), which is especially important for innate activation during viral infection (1, 2). Recent studies demonstrated that pDCs can develop from a lymphoid, as well as myeloid precursor population, and thus, the majority of pDCs belong to a separate lineage from conventional dendritic cell (cDC) subsets (3, 4). Although pDCs can express MHC class II (MHC II) and costimulatory molecules (5, 6), they do not stably express MHC II and exogenous Ag on their surface, they lack essential Ag-processing machinery (7, 8), and they express a very restricted suite of pattern-recognition receptors (9), making their relevance as an APC population uncertain. Thus, more broadly, the role of pDCs and their function beyond provision of IFN-I in settings outside of viral infection, such as during type 2 inflammation, remains unclear.

pDCs can be found in the T cell zones of peripheral lymph nodes (LNs) in the steady state, and their migration into this site is enhanced under inflammatory conditions, such as during bacterial or viral infection (10, 11). Additionally, pDCs home to the intestines in specific settings (10, 12) and exert influence on intestinal immune status, for example, by orchestrating tolerance against orally delivered Ags in the mesenteric LNs (MLNs) (12, 13). The gastrointestinal tract, along with the liver, is one of the primary sites affected by infection with the medically important helminth *Schistosoma mansoni* (14), in which egg transit through tissues causes significant damage and leads to the development of a Th2 response (14, 15). These observations suggest a potential role for pDCs in modulating immune responses in the tissue during helminth infection. As we have recently shown that cDC populations induce egg-specific Th2 responses in the MLNs (16), pDCs are likely not important APCs in this context, and the role of pDCs in supporting Th2 response to eggs and their Ags remains unknown.

pDCs are the primary producers of IFN-I in antiviral responses (7), and pDC activation and function, including IFN-I production, is dependent on an IFN-I autocrine feedback loop via pDC expression of IFNAR, the IFN-I receptor (17–19). pDC-derived IFN-I can also drive optimal cDC function during viral infection (20). IFN-I activation in mice also occurs in response to several helminths and their Ags, including *S. mansoni*, *Heligmosomoides polygyrus*, and *Nippostrongylus brasiliensis* (21–24). Further, cDCs require IFN-I signaling via the IFNAR to initiate type 2 responses against type 2 Ag (21, 22). In response to *S. mansoni* egg Ag, cDCs, not pDCs, are the primary source of IFN-I (21). However, whether IFN-I influences pDC function during type 2 inflammation or pDCs are required for Th2 responses during active *S. mansoni* infection at the egg laying or chronic stages remains unclear.

In murine models of allergic airway inflammation, rather than acting as APCs in priming type 2 immunity, pDCs can negatively regulate Th2 activation (25, 26). TLR7/8 activation can initiate pDC production of IFN-I that inhibits the function of lung group 2 innate lymphoid cells (ILC2s), reducing airway hypersensitivity (27). In contrast, a recent report suggested that pDC accumulation in the lung during viral infection facilitates type 2 allergic airway exacerbations in an OVA-driven model, with Ab-mediated depletion of pDCs reducing type 2 cytokines in the draining LNs (dLNs) (28). In humans, along with cDCs, pDC numbers in the blood are increased during infection with the soil-transmitted nematode *Strongyloides stercoralis*, compared with numbers in endemic healthy and anthelmintic-treated individuals (29). We previously demonstrated that unlike reports from allergic airway models, negative regulation of type 2 inflammation is not a hallmark pDC function in the early stages of *S. mansoni* infection (6). Further, pDCs are not essential for promoting Th2 responses in the liver or spleen during the early stages of infection when egg laying has just recently commenced (6). Despite this, hepatic pDCs isolated ex vivo are able to support OVA-specific TCR transgenic $CD4^+$ T cell production of IL-13 in vitro. These data implicated a role for pDCs in promoting development or maintenance of the adaptive immune response against *S. mansoni*.

In this study, we have addressed the impact of pDC depletion on T cell cytokine production in the intestine-draining MLNs during active *S. mansoni* infection. We found that pDCs supported optimal Th2 cytokine production at this site at the onset of parasite egg laying, as well as during the shift to chronic infection. However, pDCs were not fundamentally required for Th2 priming in the skin-draining popliteal LNs (PLNs) following s.c. injection of *S. mansoni* eggs, an in vivo model of acute egg challenge. Nevertheless, a role for pDCs during active *S. mansoni* infection was also evident in other tissue sites at later stages of infection, with hepatic and splenic pathology exacerbated by increased parasite egg burdens, and the hepatic Th2 response severely abrogated, by pDC depletion later in infection. Together, these data suggest that pDCs have a dynamic role to play at both priming and effector sites affected by egg transit during *S. mansoni* infection. Importantly, MLN pDCs capably supported type 2 cytokine production by infection-derived MLN $CD4^+$ T cells, dependent on pDC expression of IFNAR1, a subunit of the IFNAR. The role of pDCs was not limited to enhancing Th2 responses, as optimal IFN-γ production in response to egg challenge and patent *S. mansoni* infection also depended on the presence of pDCs in the dLNs. Thus, to our knowledge, we have identified a previously unappreciated role for pDCs in supporting established $CD4^+$ T cell effector responses during chronic helminth

infection and demonstrate a novel requirement for pDC IFN-I responsiveness to enable optimal Th2 responses.

MATERIALS AND METHODS

Mice, infections, and immunizations

BDCA-2–diphtheria toxin receptor (DTR), *Ifnar1*$^{-/-}$, and KN2 female mice, on the C57BL/6 background, were bred and maintained at the University of Manchester under specific pathogen–free conditions (30–32). Control C57BL/6 (wild-type [WT]) mice were either bred and maintained in-house or obtained commercially from Harlan. Experimental mice were infected percutaneously with ~40–80 cercariae. *S. mansoni* eggs were isolated from C57BL/6 mouse livers and stored at −80°C before being used to immunize mice s.c. in each rear footpad, with 2500 eggs in 50 μl PBS. For pDC depletion, mice were injected i.p. every 48 h from day 32 (d32) to d42 or d42 to d56 with 4 ng/g diphtheria toxin (Sigma-Aldrich) in PBS. Endotoxin-free soluble egg Ag (SEA) from *S. mansoni* was prepared in-house as previously described (33). All experiments were conducted under a license granted by the Home Office of the United Kingdom, in accordance with local guidelines.

Provision of parasite material

The life cycle of the Naval Medical Research Institute (Puerto Rican) strain of *S. mansoni* was maintained by routine infections (~180 cercariae) of female HsdOla:TO (Tuck Ordinary, Envigo) mice obtained from Harlan and *Biomphalaria glabrata* (NMRI albino and pigmented hybrid) snails (12 miracidia per snail) at Aberystwyth University. All procedures performed on mice adhered to the United Kingdom Home Office Animals (Scientific Procedures) Act of 1986 (project license PPL 40/3700 and P3B8C46FD) as well as the European Union Animals Directive 2010/63/EU and were approved by Aberystwyth University's Animal Welfare and Ethical Review Body.

Flow cytometry

Following cell isolation, cell populations were assessed by flow cytometry. Cells were first stained with LIVE/DEAD Fixable Aqua or UV (Thermo Fisher Scientific) and, following Fc receptor block, were stained with combinations of the following mAb: CD4, CD11b, CD11c, CD40, CD44, CD45R, CD64, CD80, CD86, CD205, CD317, lineage (CD3, CD19, CD49b, Ly-6G, and NK1.1), F4/80, Foxp3, human CD2 (huCD2), Ly-6C, MHC II, TCRβ, and XCR1. All Abs for flow cytometry were purchased from BD Biosciences, Thermo Fisher Scientific, or BioLegend. Samples were acquired on an FACS LSR or LSRFortessa flow cytometer using BD FACS Diva Software and analyzed with FlowJo 9 software (Tree Star) to ascertain proportional expression of markers. When gating on dendritic cell (DC) populations, cells were gated on live, singlet, lineage^{-} cells, and monocytes (Ly-6C^{hi}CD11b^{hi}) and macrophages (F4/80^{+}CD64^{+}) were first excluded from analysis. pDCs were gated as Ly-6C^{mid}CD11b^{-}CD11c^{mid}CD317^{+}CD45R^{+}, cDC1s as CD11c^{hi}MHC II^{hi}CD11b^{lo}CD205^{hi}/XCR1^{hi}, and cDC2s as CD11c^{hi}MHC II^{hi}CD11b^{hi}XCR1^{lo}/CD205^{lo}. T regulatory cells (Tregs) were gated as TCRβ^{+}NK1.1^{-}CD4^{+}Foxp3^{+} and T effector cells (Teff) as TCRβ^{+}NK1.1^{-}CD4^{+}Foxp3^{-}CD44^{hi}CD62L^{lo}.

S. mansoni *egg counts*

Livers and intestines from infected mice were digested in 4% potassium hydroxide (15 ml/g liver tissue; 7.5 ml/g intestine tissue) at 37°C overnight. One-hundred-microliter aliquots of the digests were evaluated on gridded petri dishes, and the eggs were counted at 10× magnification. Each digest was examined in triplicate, and the mean results were used to extrapolate the total number of eggs per gram of tissue.

Restimulation assays

Livers were perfused, diced, and digested at 37°C for 45 min using 0.4 U/ml Liberase (Roche Diagnostics) and 80 U/ml DNase I Type IV (Sigma-Aldrich). 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 centrifugation 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 ammonium–chloride–potassium lysed, and cells were counted and resuspended for use. Single-cell suspensions of PLN, MLN, or liver leukocytes (1×10^6 cells/ml) were cultured in X-VIVO 15 Medium (Lonza) containing 2 mM L-glutamine and 50 μm 2-ME (Thermo Fisher Scientific) in 96-well plates at 37°C 5% CO_2 with, or without, 15 μg/ml SEA. After 72 h, supernatants were harvested and analyzed for IL-4, IL-5, IL-10, IL-13, and IFN-γ using paired mAb and recombinant cytokine standards (BioLegend and PeproTech). Medium alone values were subtracted from Ag-restimulated cytokine levels for each sample.

Coculture assays

pDCs and T cells were FACS sorted from the MLNs of *S. mansoni*–infected animals. This assay was set up as previously described (21, 34). Fifty thousand CD4^{+} T cells were cultured in 96-well plates for 3 d with 2500 pDCs with, or without, 1 μg/ml anti-CD3 (produced in-house). On d3, either supernatants were taken for cytokine analysis by ELISA or cells were analyzed for huCD2 expression (IL-4 production) by flow cytometry.

Statistical analysis

Statistical analysis was carried out using JMP software (SAS), analyzed using linear mixed effects models with a fixed effect of experimental group and a random effect of experiment day, to account for any significant differences between experiments. Model assumptions of normality and homogeneous variance were assessed by a visual analysis of the raw data and the model residuals. Right-skewed data were log or square root transformed. Experimental groups were considered statistically

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significant if the fixed effect F test p value was ≤0.05. Post hoc pairwise comparisons between experimental groups were made using Tukey honest significance difference multiple-comparison test. Statistical outliers were identified using the extreme studentized deviate method and omitted prior to the mixed effect model analysis. Graphs of results were shown as mean ± SEM of untransformed data using Prism version 6 (GraphPad).

RESULTS

S. mansoni infection alters the proportion, number, and activation status of MLN DC subsets

During *S. mansoni* infection, a high proportion of eggs released by adult female worms are forced into the liver by blood flow in the portal vein. However, to perpetuate the helminth lifecycle, some eggs must rupture through the intestinal wall to exit the host (14, 15). Egg transit causes significant pathology in the intestine, as well as the liver, over the course of infection. We have previously shown that egg injection into the intestinal serosa elicits Ag-bearing cDCs to migrate to the MLNs, where they promote Th2 polarization (16). However, little is known about which DC subsets are evident in the MLNs over the course of active *S. mansoni* infection.

To address this question, we harvested MLN cells at different time points throughout *S. mansoni* infection (Fig. 1A). Assessment began at d28 of infection, around the time that adult female worms start to deposit eggs that transit through the tissues (14). The window between d28 and d42 of infection also induces "acute" Th2 priming, predominantly in response to egg Ag. By later, "chronic," time points from around d56 of infection, mice develop extensive Th2-driven immunopathology and display a more regulatory immunophenotype (14). Comparing d28, d42, and d56 postinfection, we found that the proportion of pDCs ($CD317^+Ly\text{-}6C^+CD11c^+$; Fig. 1B) within the MLNs was significantly increased in mice at both d28 and d56 of infection compared with naive mice and that the total numbers of pDCs were increased compared with naive at d42–56 of infection (Fig. 1C). Frequencies of cDC subsets, defined as cDC1s or cDC2s according to their differential expression of surface markers CD11b and CD205/XCR1 (Fig. 1B) (35), did not change throughout infection as a proportion of all live cells. Similarly, the total number of cDC1s was significantly increased at d42–56 of infection compared with naive animals, whereas cDC2s transiently increased in number at d42 of *S. mansoni* infection (Fig. 1C). At every time point, pDCs remained a smaller percentage of total MLN cells and a smaller number of cells than cDC1s or cDC2s, as previously shown in the liver during *S. mansoni* infection (6).

Although cDC expression of MHC II and costimulatory molecules is essential for $CD4^+$ T cell priming (36), minimal upregulation of these surface markers has been reported against helminths, including in splenic cDCs and pDCs during *S. mansoni* infection (37). In agreement with this, we observed no significant increase in MHC II levels (geometric mean fluorescence

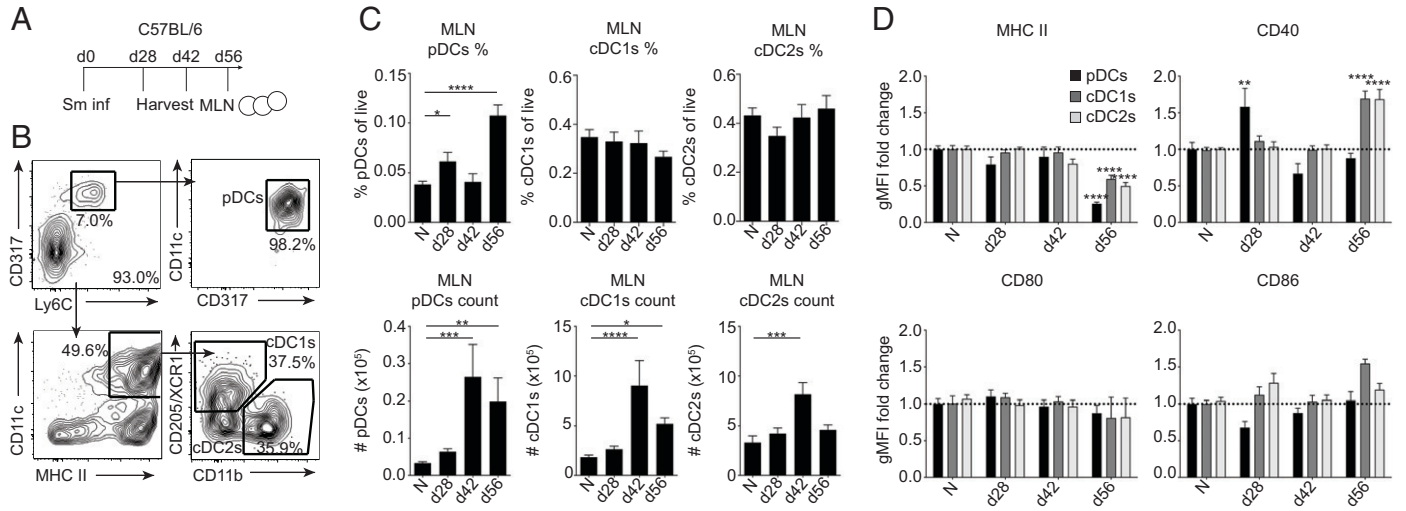

FIGURE 1. The kinetics and activation of DC populations over the course of *S. mansoni* infection.

(**A**) C57BL/6 WT mice were infected with ∼40–80 *S. mansoni* cercariae on d0, and MLNs harvested from naive (N) mice, and at d28, d42, and d56 of infection. (**B**) Representative plots of MLN DC gating. (**C**) Percentage of total live cells and total numbers of pDCs, cDC1s, and cDC2s in N or *S. mansoni*–infected (Sm) MLNs. (**D**) Fold change in geometric mean fluorescence intensity (gMFI) of MHC II, CD40, CD80, and CD86 on the surface of DC subsets over the course of infection, normalized to expression levels on cells from control mice. Results are mean ± SEM. Data from two experiments pooled (n = 4–13 mice per group) and analyzed using a linear fixed effect model with pairwise comparison. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

intensity) on all DC subsets in the MLNs of infected compared with naive mice (Fig. 1D), with MHC II levels in fact decreasing in mice between d42 and d56 of infection. MLN pDCs significantly upregulated CD40 expression on their surface at d28 of infection compared with cells from naive mice, whereas cDC subsets only upregulated CD40 compared with controls around the development of chronic infection at d56. Thus, the MLN pDC population was responsive to *S. mansoni* infection during the early stages of the immune response, before egg production, highlighting the potential for a previously unappreciated role for pDCs at this site following schistosome infection.

pDCs are required for optimal Th cytokine responses in the gut-draining LN during S. mansoni *infection*

pDC depletion early in *S. mansoni* infection (d32–42 postinfection) has no significant effect on Th2 development in the liver or spleen (6), but the impact of pDCs on the Th2 response at other sites affected by egg transit or at other time points after *S. mansoni* infection has remained unclear. To assess the influence of pDCs on Th cell development in the gut-draining MLNs during *S. mansoni* infection, we used BDCA-2–DTR, pDC-depletable mice that express the human DTR gene under control of the promoter for BDCA-2, a marker of human pDCs, for effective and specific pDC depletion (30). We first depleted pDCs between d32 and d42 of *S. mansoni* infection (Fig. 2A), a timeframe that correlates with the onset of egg deposition and the commencement of Th2 priming and our previous assessment of the impact of pDCs and CD11c$^+$ cell depletion on hepatic and splenic Th2 responses during infection (6, 38). The proportion of pDCs from total live cells was variable in the MLNs of WT and diphtheria toxin (DTx) treated animals (Fig. 2B); thus, calculated pDC depletion at this site was roughly 50% in naive mice and 35–40% depletion in *S. mansoni*–infected animals (Fig. 2C). Despite incomplete depletion of pDCs in the MLNs, levels of parasite-specific IL-4 and IL-13 produced by MLN cells, as measured by ELISA of cell-free supernatants, were significantly impaired in infected animals following pDC depletion on d32–42 postinfection (Fig. 2D), suggesting a potential site-specific role for pDCs in promotion of the Th2 cell response. To investigate the role of pDCs later in infection, when the Th2 response is in a more chronic phase (14), we next depleted pDC populations from d42 to d56 of infection (Fig. 2E), 2–4 wk after initial onset of egg deposition. This led to more robust pDC depletion in the MLNs at this time point (Fig. 2F, 2G) and defective *S. mansoni* egg Ag-specific recall responses in the MLNs, including a significant reduction in IL-5, IL-13, and IFN-γ production (Fig. 2H). Although Ag-specific IL-4 from MLN cells was reduced following pDC depletion earlier in infection, at this time point, egg-specific IL-4 was not significantly impacted.

Parasitemia was unaltered after pDC depletion by DTx treatment at the onset of egg laying, with WT and BDCA-

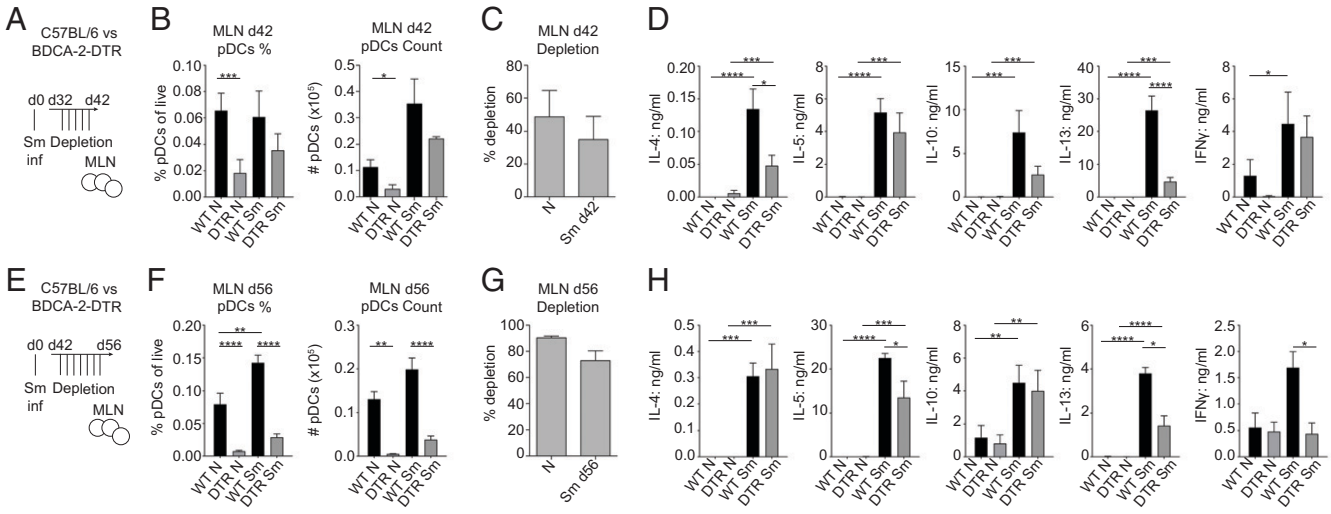

FIGURE 2. pDCs are required for optimal T cell cytokine production in the gut-draining LN throughout *S. mansoni* infection.
WT or BDCA-2–DTR (DTR) mice were infected with ∼40–80 *S. mansoni* (Sm) cercariae on d0 (naive [N]). For assessment of pDCs in acute infection from d32 until d42 (**A**), or for assessment at the onset of chronic infection, from d42 until d56 (**E**), mice were treated i.p. with DTx before MLN harvest. (**B** and **F**) Proportion and total numbers of pDCs from MLN cells on d42 (B) or d56 (F) of infection. (**C** and **G**) pDC depletion in DTR mice calculated compared with pDC percentages in WT mice on d42 (C) or d56 (G) of infection. (**D** and **H**) After SEA restimulation, or culture with medium alone, MLN cell-free supernatants were analyzed by ELISA for a schistosome egg-specific recall cytokine response. Medium alone values were subtracted from Ag-restimulated samples. Results are mean ± SEM. Data from three experiments pooled (n = 4–12 mice per group) analyzed using a linear fixed effect model with pairwise comparison. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

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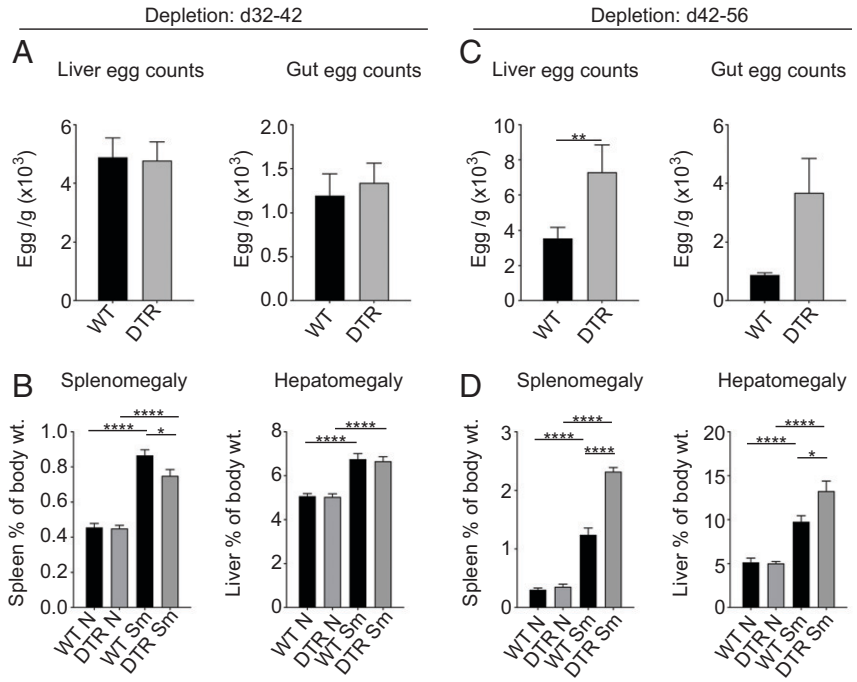

FIGURE 3. pDCs are required to restrain parasite egg laying and host pathology during chronic *S. mansoni* infection.

Naive (N) and *S. mansoni*–infected WT or DTR mice were treated with DTx, as in Fig. 1. On d42 (**A**) or d56 (**C**) of *S. mansoni* infection, livers and small intestines were isolated from infected animals, and parasite egg counts were performed. (**B** and **D**) Splenomegaly and hepatomegaly determined by spleen and liver weights as a percentage of total body weight on d42 (B) or d56 (D) of *S. mansoni* infection. Results are mean ± SEM. Data from two (C and D) or four (A and B) experiments pooled (n = 7–20 mice per group) analyzed using a linear fixed effect model with pairwise comparison. *$p < 0.05$, **$p < 0.01$, ****$p < 0.0001$.

2–DTR mice displaying similar egg burdens in the liver and gut (Fig. 3A) and similar or diminished immunopathology at d42 of *S. mansoni* infection (Fig. 3B). In contrast, there was a significant increase in liver egg counts and a trend for increased intestinal egg deposition following depletion at the later time point (Fig. 3C), accompanied by significantly heightened splenomegaly and hepatomegaly (Fig. 3D). Thus, defective Th2 cytokine levels in the absence of pDCs could impact the ability of the host to tolerate infection as it reaches chronicity.

We addressed the possibility that other DC populations could be altered in response to pDC depletion and found that DTx treatment of WT or BDCA-2–DTR animals had no impact on monocyte, macrophage (6), or cDC subset frequencies in d42 *S. mansoni*–infected mice (Supplemental Fig. 1A), although there was a minor decrease in cDC1s in the MLNs of some naive DTx-treated BDCA-2–DTR mice. At d56 of infection, there was a significant increase in cDC2s in the MLNs and significant decrease in liver cDC1s in both WT and BDCA-2–DTR mice compared with naive controls (Supplemental Fig. 1B). Thus, non-pDC DC subset alteration does not likely influence the deficit in Th2 cytokine responses in the MLN following pDC depletion at d32–42 or d42–56 postinfection. Together, these data demonstrated that pDCs were required for optimal $CD4^+$ T cell Th2 development in the gut-draining MLNs over the course of active *S. mansoni* infection, from early after commencement of egg laying. Further, pDCs were required for IFN-γ production at later stages of infection.

pDCs are not required for Th2 priming against S. mansoni *eggs*

$CD11c^+$ myeloid cells are required for optimal Th2 induction following murine *S. mansoni* infection or egg injection (38), with pDCs specifically promoting $CD4^+$ T cell cytokine responses to eggs Ags in the MLNs during infection (Fig. 2). To directly address whether pDCs are required for Th2 priming against schistosome eggs, we injected PBS- or DTx-treated WT or BDCA-2–DTR mice s.c. with PBS or *S. mansoni* eggs (Supplemental Fig. 2A), a system widely used to investigate acute type 2 response induction to eggs in the dLNs without the additional complexities involved in ongoing infection (34, 38). DTx treatment had no significant impact on Th2 development or egg-specific IFN-γ production following s.c. *S. mansoni* egg injection (Supplemental Fig. 2B), even though PLN pDCs were efficiently depleted in egg-injected mice (Supplemental Fig. 2C–E), with no impact on cDC populations (Supplemental Fig. 2F, 2G). Thus, pDCs were not required for Th cell polarization in a model of acute egg challenge but rather were a key contributing cell type for optimal Th2 and Th1 cytokine responses during patent *S. mansoni* infection (Fig. 2).

pDCs sustain MLN $CD4^+$ T cell responses during chronic S. mansoni *infection*

To better understand the function of pDCs in Th responses during *S. mansoni* infection, we began by analyzing the influence of pDC depletion on $CD4^+$ T cell subpopulations in this context. There was no significant impact of pDC depletion on proportions or total numbers of $CD44^+CD62L^{lo}CD4^+$ Teff or $Foxp3^+$ Treg in the MLNs of infected mice at d42 after pDC depletion (Fig. 4A, 4B). At d56 of infection, there was a comparable increase in the proportion of Teff, but not Tregs, in both WT and BDCA-2–DTR mice compared with naive controls (Fig. 4C). However, when total numbers of $CD4^+$ T cell populations were assessed at this time point, there was a significant

impairment in expansion of both Teff and Tregs in the MLNs of DTx-treated *S. mansoni*–infected BDCA-2–DTR mice compared with WT controls (Fig. 4D). These data demonstrate that pDCs have a key role to play in the development or maintenance of both effector and regulatory $CD4^+$ T cell populations as *S. mansoni* infection progresses from d42 to d56.

pDCs can support Th2 cytokine production by MLN T cells isolated from S. mansoni *infection*

Having demonstrated that pDCs were essential both for optimal Th2 cytokine and IFN-γ output by MLN cells (Fig. 2), we next addressed whether MLN pDCs from active *S. mansoni* infection could directly enhance cytokine production by infection-derived MLN $CD4^+$ T cells. To do so, pDCs sorted from the MLNs of naive mice or mice at d42 of *S. mansoni* infection were cultured with MLN $CD4^+$ T cells also isolated from d42 infected mice (Fig. 4E). MLN pDCs from either naive or infected mice significantly enhanced levels of Th2 cytokine production by $CD4^+$ T cells isolated from infected animals, compared with that observed from T cells cultured alone (Fig. 4F). These data demonstrate that pDCs, although incapable of priming Th2 polarization of naive T cells (6), could competently support Th2 cytokine production from infection-derived, Ag-experienced, $CD4^+$ T cells. Of note, in this context, pDCs failed to significantly enhance $CD4^+$ T cell IFN-γ production.

IFN-I signaling via IFNAR can promote optimal DC activation and function during inflammation (17–20), including during type 2 immune activation driven by helminth Ag, in which cDC induction of Th2 responses is dependent on their ability to respond to IFN-I (21, 22). Further, IFN-I is produced in vivo in response to helminths, including following immunization with *S. mansoni* egg Ag and during active *S. mansoni* infection (21, 24). To investigate whether pDC promotion of type 2 cytokine production from $CD4^+$ T cells was also facilitated by IFN-I responsiveness, we isolated pDCs from the MLNs of WT or *Ifnar1*$^{-/-}$ *S. mansoni*–infected mice and cocultured them with $CD4^+$ T cells isolated from infected WT KN2 cytokine reporter mice, which express huCD2 on their surface when IL-4 is produced (21, 32). Optimal pDC-mediated support of type 2 cytokine production from infection-derived $CD4^+$ T cells was dependent on the ability of pDCs to respond to IFN-I, as the percentage of $huCD2^+$ $CD4^+$ T cells was significantly reduced when pDCs were Ifnar1 deficient, in comparison with WT cells (Fig. 4G). Thus, the ability of pDCs to facilitate type 2 cytokine production by MLN T cells from infection was promoted by pDC responsiveness to IFN-I.

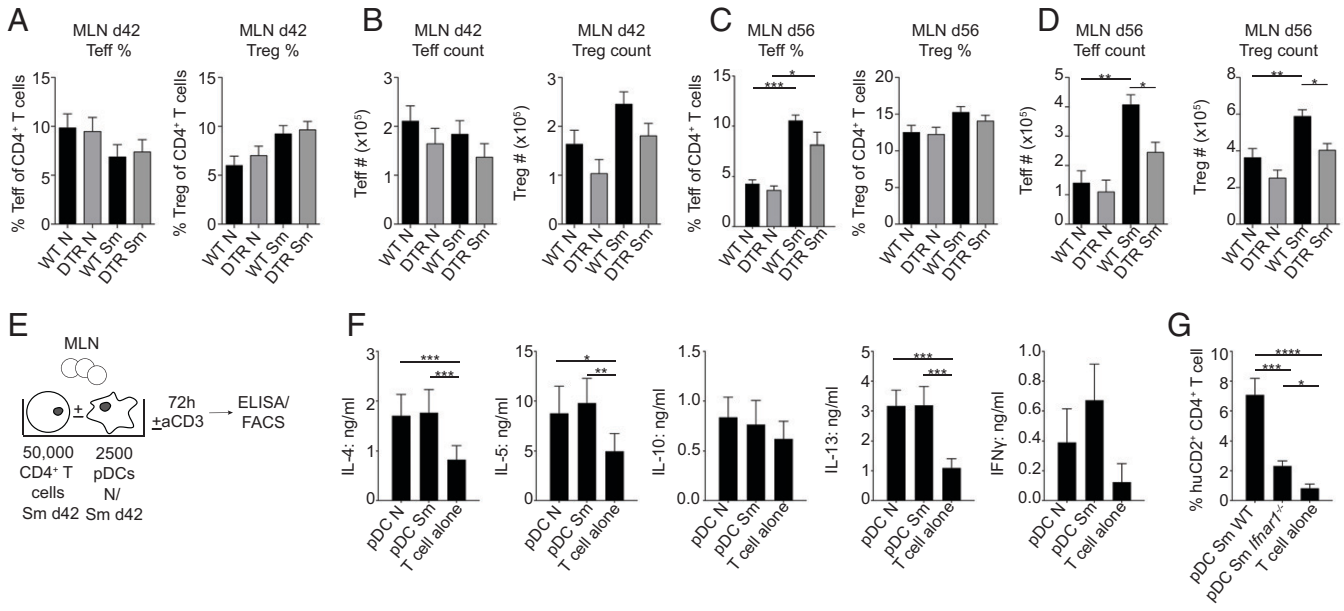

FIGURE 4. pDCs from *S. mansoni*–infected mice support optimal $CD4^+$ T cell responses in vivo and Th2 polarization in vitro.

MLNs were harvested from naive (N) and *S. mansoni*–infected WT and DTR mice on d42 or d56 of infection, after pDC depletion from d32 to d42 or d42 to d56, respectively, and analyzed for $CD4^+$ T cell populations. (**A** and **C**) Percentage of $CD4^+$ T cells on d42 (A) or d56 (C) of *S. mansoni* infection that are $CD44^+$ Teff and $Foxp3^+$ Tregs in the MLN following DTx treatment. (**B** and **D**) Total numbers of T cell populations in the MLN at d42 (B) or d56 (D) of infection following pDC depletion. (**E**) Schematic to show coculture regimen. T cells from d42 *S. mansoni*–infected mice were cultured for 3d with, or without, MLN pDCs from N or d42 *S. mansoni*–infected mice, in the presence, or absence, of anti-CD3 (aCD3). (**F**) On d3 of culture, supernatants were collected from cultures and assessed for cytokine production by ELISA. Media alone values were subtracted from anti-CD3-stimulated samples. (**G**) KN2 MLN T cells from *S. mansoni* mice were cultured in the presence or absence of WT or *Ifnar1*$^{-/-}$ MLN *S. mansoni* pDCs with aCD3. On d3, $CD4^+$ T cells were stained for huCD2 to identify the proportion of $huCD2^+$ ($IL\text{-}4^+$) T cells. Results are mean ± SEM. Data from two to three experiments pooled [n = 6–7 mice per group (A–D), n = 8–14 wells per group (F and G)] analyzed using a linear fixed effect model with pairwise comparison. *$p < 0.05$, **$p < 0.05$, ***$p < 0.001$, ****$p < 0.0001$.

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pDCs support hepatic Th2 cytokine responses as S. mansoni *infection progresses*

We hypothesized that the impaired T cell cytokine response evident in the MLNs of pDC-depleted mice during *S. mansoni* infection might have a subsequent impact on the immune function in the effector sites, such as the liver, at later stages of infection. This hypothesis was supported by increased egg burden and pathology after depletion later in infection (Fig. 3C, 3D). DTx treatment between d42 and d56 of infection (Fig. 5A) led to effective depletion of hepatic pDCs in BDCA-2–DTR mice (Fig. 5B, 5C) but did not significantly reduce hepatic cDC1s or cDC2s during infection (Supplemental Fig. 1B) (39). When we depleted pDCs at this stage of infection (from d42), egg Ag-induced IL-4 levels in the supernatants of cultured liver cells were significantly reduced at d56, with a trend for decreased IL-13 levels compared with WT controls (Fig. 5D). IL-5 and IL-10 were not significantly affected at this site or time point, and pDCs were not required for maintaining Ag-specific IFN-γ in the liver (Fig. 5D), in contrast to the MLN phenotype at this time point (Fig. 2). The decrease in parasite-specific IL-4 and IL-13 from liver cells following pDC depletion was consistent with increased splenomegaly and hepatomegaly (Fig. 3C, 3D), the defects in MLN Th2 cytokines (Fig. 2H) and $CD4^+$ T cell responses (Fig. 4C, 4D) evident at this time point. Despite a minor increase in the percentage of Tregs in pDC-depleted naive mice compared with controls, the proportion of hepatic Teff and Tregs at d56 of *S. mansoni* infection was unaffected by pDC depletion (Fig. 5E). However, the total numbers of Tregs and Teff were significantly diminished in infected pDC-depleted mice compared with WT animals during this phase of infection (Fig. 5F).

These data demonstrate that in addition to playing a key role in supporting Teff responses in the MLN early during *S. mansoni* infection, pDCs enhance type 2 cytokine production and $CD4^+$ T cell responses at the liver effector site at later stages of infection, including effective Th2 function and Treg generation. They highlight for the first time, to our knowledge, a key role for pDCs in supporting optimal effector $CD4^+$ T cell responses to parasite egg Ag during *S. mansoni* infection in both priming and effector sites in the MLNs and liver, respectively. In the absence of pDCs, abrogated $CD4^+$ T cell–mediated adaptive immunity leads to increased immunopathology, with the potential to impact the long-term fitness and survival of the host.

DISCUSSION

We have identified that pDCs are essential for optimal $CD4^+$ T cell responses during chronic *S. mansoni* infection. An LN-specific impairment in Th2 cytokines during early infection was associated with defective accumulation of $CD44^+$-activated Teff and Tregs (Figs. 2, 4)—the $CD4^+$ T cell subsets involved in orchestrating, and regulating, the Th2 response in the tissue as infection progresses (40). This then also led to a defect in Th2 cytokine output in the effector site, the liver, at the onset of chronic infection (Fig. 5). This defect in $CD4^+$ T cell–driven type 2 immunity in the absence of pDCs was associated with, and likely caused, increased parasite egg deposition and exacerbated infection-driven pathology, resulting in worsened splenomegaly and hepatomegaly (Fig. 3). All together, these findings highlight a role for pDCs in supporting optimal $CD4^+$ T cell function during ongoing *S. mansoni* infection.

Our data suggest that there are site-specific differences in the timing of a requirement for pDCs to promote optimal immune activation against *S. mansoni*, comparing the liver and MLNs. This dichotomy may relate to differences in pDC localization, as well as tissue signals and/or architecture in the MLNs and liver during *S. mansoni* infection. During viral infection, pDC recruitment by CCL3/4 chemokines to T cell–cDC1 clusters support T cell priming in the LN paracortex, and formation of these clusters is essential for optimal T cell function (20). Our in vitro coculture assays suggest that when pDCs and $CD4^+$ T cells are in close proximity, pDCs from naive as well as *S. mansoni*–infected mice can enhance type 2 cytokine production (Fig. 4). Thus, as infection progresses, increased immune cell infiltration, changes in liver architecture (Fig. 3), and chemokine production (14) may lead to an increased role for pDCs in promoting $CD4^+$ T cell function at this site. Intriguingly, *S. mansoni* eggs produce a chemokine-binding protein that blocks chemokines, including CCL3, from binding their receptors (41). Additionally, high CCL3 levels are associated with increased disease severity in *S. mansoni*–infected mice and chronic infection in humans (42), supporting a role for these signals in regulating immune function during *S. mansoni* infection that has yet to be fully explored. Further, pDCs infiltrate into the skin following tissue injury, where pDC IFN-I production enhances wound healing, a process promoted by the release of host-derived nucleic acids from damaged tissue (43). Thus, increased tissue damage in the liver and release of host damage signals and immune signals at later stages of infection may promote pDC function at this site as chronic *S. mansoni* infection develops.

Importantly, pDC depletion was not always complete in the MLNs, with naive and d42 infected animals showing depletion levels below 50% in some instances, compared with the average ~80% depletion we observed in other contexts (Fig. 2, Supplemental Fig. 2). This may reflect the fact that pDCs are a very small population in this tissue under these conditions and so are difficult to quantify accurately. Despite this, incomplete pDC depletion still had a significant impact on Th2 cytokine production from MLN cells at early time points of infection (Fig. 2D), as well as later in infection, when pDC depletion was more consistent (Fig. 2H). pDC depletion earlier in infection led to decreased egg-specific IL-4 and IL-13 in the MLN, whereas depletion at d42–56 caused a significant defect in MLN IL-5, IL-13, and IFN-γ, but not IL-4 (Fig. 2). This discrepancy may reflect dynamic changes in the pDC and/or $CD4^+$ T cell populations in response to chronic levels of parasite egg deposition, evidenced by differences in absolute

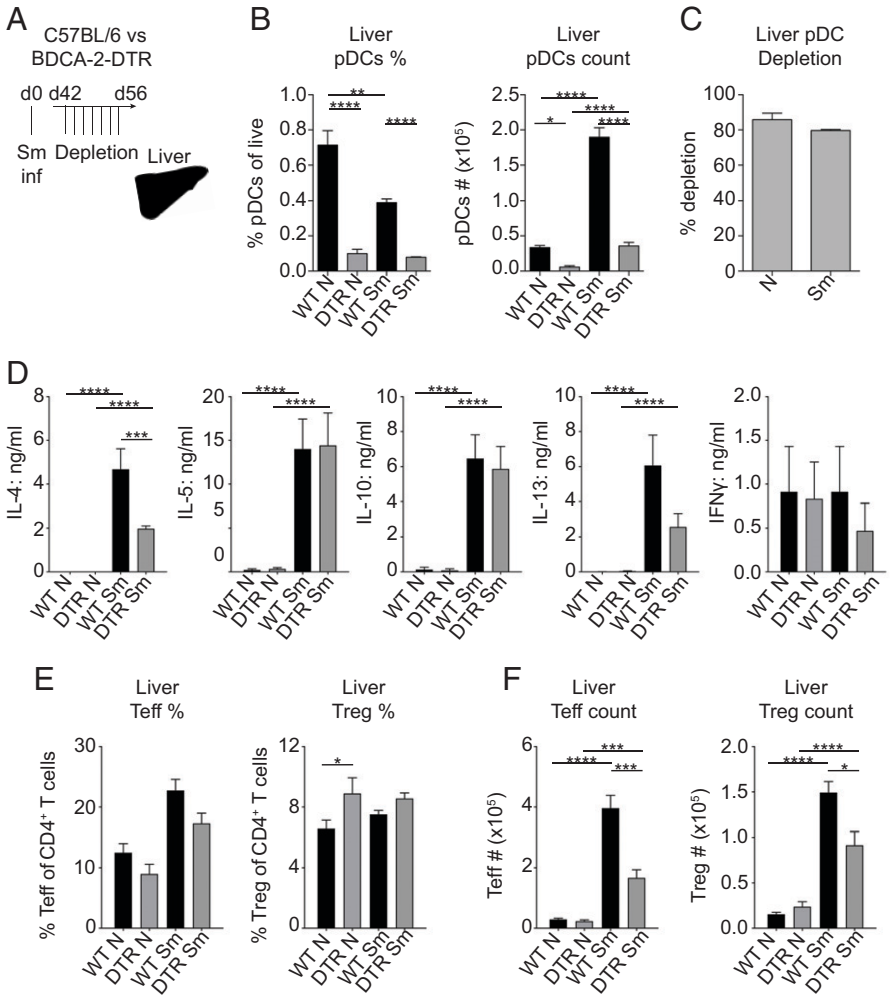

FIGURE 5. pDCs are essential for effective Th2 cytokine production in the liver in chronic *S. mansoni* infection.

(**A**) Livers were harvested from DTx-treated naive (N) and *S. mansoni*–infected WT or DTR mice on d56 of infection, as in Fig. 1C. (**B**) Liver cells were purified and analyzed by flow cytometry for the presence of pDCs, shown as a percentage of all live leukocytes in the liver and as total numbers. (**C**) pDC depletion in N and *S. mansoni* DTR mice calculated by comparing pDC percentages in WT and DTR mice. (**D**) Supernatants from liver cells of N and *S. mansoni* WT/DTR mice were cultured with SEA or medium (M) alone and analyzed by ELISA for schistosome egg-specific recall cytokine responses. M alone values were subtracted from Ag-restimulated samples. (**E** and **F**) Percentage of liver $CD4^+$ T cells (E) or total cell numbers (F) that are $CD44^{hi}$ Teff and $Foxp3^+$ Tregs from N/*S. mansoni* mice on d56 of infection. Results are mean ± SEM. Data from two experiments pooled (n = 6–10 mice per group), analyzed using a linear fixed effect model with pairwise comparison. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

cytokine levels at different time points of infection. In this work, we have primarily focused on the role of pDCs in activating T cell production of type 2 cytokines, but our data also strongly support a role for pDCs in the generation of parasite-specific IFN-γ. pDCs act in a supporting role to cDC1s in IFN-γ induction in other settings (20), and thus, they may co-operate with cDC1s to promote Th1 cytokine production during *S. mansoni* infection (44).

We have not assessed in this study the impact of defective Th2 polarization in the MLNs on the effector response in the intestinal lamina propria because of the technical difficulty of isolating immune cells from *S. mansoni*–infected intestinal tissue. However, we hypothesize a similar impact on Th2 cytokine responses at this site given that pDCs are found abundantly in the small intestine in naive mice (12) and can acquire gut tropism under inflammatory conditions (10, 12, 45). Thus, future studies should address whether pDCs also play a role in intestinal Th2 function during *S. mansoni* infection.

Th2 polarization against acute egg challenge did not fundamentally require pDCs, at least within the first week following exposure to *S. mansoni* eggs (Supplemental Fig. 2). Thus, it may take several weeks of continuous egg deposition during infection for pDCs to become important during Th2 maintenance in the dLNs, with this only becoming evident in effector sites at later time points. In *S. mansoni* egg–driven pulmonary fibrosis, macrophages are important for maintaining T cell responses in the tissue, but not the dLNs (46), whereas ILC2s, basophils, and cDCs have been implicated to play a role in promoting the maintenance of Th2 cells and Tregs at sites of inflammation and at mucosal barriers (47–50). This highlights a potential role for other innate cell populations in promoting optimal Th2 cytokine production in the liver at early time points. Many pDC studies have failed to identify a role for these cells in direct $CD4^+$ T cell priming (7). Our coculture system demonstrated that pDCs are effective at facilitating Th2 cytokine responses in a population of Ag-experienced, or preactivated by

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infection, $CD4^+$ T cells without exogenous Ag (Fig. 4) (6), suggesting that Ag presentation by pDCs is less important. Of note, recent studies highlight that the majority of pDC precursors are lymphoid, rather than myeloid (3, 4), with the suggestion that pDCs may be redesignated as an innate lymphoid cell population (4). If this is indeed the case, pDCs could be compared with ILC2s in their capacity to support Th2 function, rather than myeloid DCs. How pDCs promote optimal $CD4^+$ T cell function during *S. mansoni* infection remains to be determined. Our coculture experiments suggest that any direct pDC–T cell interaction may not depend on Ag (Fig. 4); indeed, pDCs from naive and *S. mansoni*–infected mice fail to support Ag-specific T cell proliferation in vitro (6). Thus, pDCs may act in other ways to potentiate Th cytokine production and $CD4^+$ T cell subset expansion, for example, by driving T cell survival or cytokine production.

Prior work addressing pDC function in type 2 immunity has focused on the lung, particularly in the context of allergic airway models. Early studies using Ab depletion of pDCs in OVA-driven allergic airway inflammation suggested an anti-inflammatory role for these cells (25, 26). In agreement with this, we show that pDCs are required for Treg recruitment and/or expansion in the MLNs and liver during chronic *S. mansoni* infection (Figs. 4, 5). However, a coincident reduction in effector T cell populations (Figs. 4, 5) and Th2 cytokine production (Figs. 2, 5) in the MLNs and liver is inconsistent with regulation being the only role for pDCs in type 2 immunity. In agreement with our results, a more recent study highlighted an influx of pDCs into the lung following OVA-allergen challenge, with Ab depletion of pDCs leading to reduced Th2 cytokines and type 2–mediated inflammation (27). This study highlighted a role for OX40L and IL-25 in regulating pDC function in this context. However, IL-25–deficient mice do not display any significant exacerbation in liver granuloma responses during chronic *S. mansoni* infection (51). Thus, further work is needed to address the importance of different type 2–associated signals for mediating pDC function in *S. mansoni* infection.

We have highlighted a previously unappreciated role for IFN-I signaling in directing pDC function in type 2 inflammation (Fig. 4). Our work emphasizes that DC subsets depend on IFN-I not only for type 2 priming by cDCs (21, 22) but also for optimal Th2 cytokine responses supported by pDCs. We and others have previously shown that several facets of cDC function are altered in the absence of IFN-I responsiveness, including surface activation and migratory capacity (21, 22). In contrast, studies investigating the role of pDCs in ILC2-mediated allergic lung inflammation in response to *Alternaria* fungus with IFN-I–inducing TLR ligands suggested that pDC IFN-I inhibits ILC2-mediated type 2 immunity (28). This study is perhaps more applicable to situations in which tissues are responding to type 1 pathogens that drive high-level IFN-I production alongside a type 2–promoting pathogen or allergen, for example, during virus and helminth coinfection (52). Thus, further work is needed to address the signals that promote pDC potentiation of Th2 cytokine output, including the importance of parasite or host-derived Ag and the broader role of IFN-I in type 2 immunity. In particular, it remains to be determined whether pDCs (17–20) or other cell types are the primary source of IFN-I during helminth infection and whether pDC-derived IFN-I plays a role in promoting $CD4^+$ T cell function. Other potential cellular sources include cDCs—shown to produce IFN-α6 in response to *S. mansoni* egg Ag (21) or stromal and epithelial cells that release innate IFNs in response to damage (53, 54).

In summary, we have revealed that pDCs play an integral role in optimal Th2 cytokine responses and Treg accumulation in both LNs and effector sites during *S. mansoni* infection (Figs. 2 4, 5). Impairment in immune activation following pDC depletion was accompanied by a significant increase in splenic and hepatic pathology during chronic infection, likely because of a dramatic increase in egg deposition in the liver and gut (Fig. 3). Further work will be needed to identify the mechanisms employed by pDCs to influence Th2 and Treg responses, such as whether pDCs are required for the proliferation, recruitment, maintenance, or survival of T cells during *S. mansoni* infection. Our work highlights a novel role for pDCs and IFN-I during *S. mansoni* infection, which may have implications for the future development of targeted immunotherapies and vaccines designed to combat the debilitating disease caused by this important human pathogen.

DISCLOSURES

The Manchester Collaborative Centre for Inflammation Research is a joint venture between the University of Manchester and Glaxo Smith Kline. The authors have no individual financial conflicts of interest.

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