Functional specialization of intestinal dendritic cell subsets during Th2 helminth infection in mice

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Abbreviations:

Batf3, Basic Leucine Zipper ATF-like Transcription Factor 3; DC, dendritic cell; GFP, green fluorescent protein; IL-4Rα, interleukin 4 receptor alpha; IRF, Interferon Regulatory Factor; MLN, mesenteric lymph node; PDL2, programmed death-ligand 2; SI-LP, small intestine lamina propria; SSC, side scatter; Tfh, T follicular helper; Th2, T helper cell type 2

Abstract

Dendritic cells (DCs) are essential in dictating the nature and effectiveness of immune responses. In the intestine DCs can be separated into discrete subsets, defined by expression of CD11b and CD103, each with different developmental requirements and distinct functional potential. Recent evidence has shown that different intestinal DC subsets are involved in the induction of T helper (Th)17 and regulatory T cell responses, but the cells that initiate Th2 immune responses are still incompletely understood. We show that in the Th2 response to an intestinal helminth in mice, only $CD11b^+$ and not $CD11b^-$ DCs accumulate in the local lymph node, upregulate PDL2 and express markers of alternative activation. An enteric Th1 response instead activated both $CD11b^+$ and $CD11b^-$ DCs without eliciting alternative activation in either population. Functionally, only $CD11b^+$ DCs activated during helminth infection supported Th2 differentiation in naive $CD4^+$ T cells. Together our data demonstrate that the ability to prime Th2 cells during intestinal helminth infection, is a selective and inducible characteristic of $CD11b^+$ DCs.

Introduction

Dendritic cells (DCs) are professional antigen presenting cells, essential for the initiation of adaptive immune responses. Heterogeneous DC populations exist, and discrete subsets have been associated with the development of different T-helper (Th) cell responses. In the spleen, $CD8\alpha^+$ DCs were reported to secrete high levels of IL-12p70 and preferentially induce Th1 responses, while $CD8\alpha^-$ DCs instead expressed IL-10 and promote Th2 differentiation[1]. In the intestine, multiple DC subsets have been defined[2]. Mice have at least three intestinal subsets: a major $CD11b^-$ $CD103^+$ population whose development depends on the transcription factors Basic Leucine Zipper ATF-like Transcription Factor 3 (Batf3) and Interferon Regulatory Factor (IRF)8; a large $CD11b^+$ $CD103^+$ population that instead requires IRF4[2]; and a smaller, less well characterized $CD11b^+$ $CD103^-$ population that is also IRF4-dependent[3]. In the steady state all three subsets migrate from the intestinal lamina propria to the mesenteric lymph node (MLN)[4]. Recent work has begun to elucidate their function under homeostatic conditions[3-7], but their contribution during infection is less well understood.

Helminth parasites are common pathogens of the human intestinal tract, infecting some 2 billion people worldwide[8]. Helminths typically induce strong Th2 cell responses, characterized by the cytokines IL-4, IL-5, and IL-13[9], and DCs are essential to this process[10-12]. Several recent studies have examined whether such Th2 induction is specific to a particular subset of DCs. Mice with selective ablation of $CD11b^+$ $CD103^+$ DCs show reduced Th2 responses in the MLN during infection with the helminths *Nippostrongylus brasiliensis* or *Schistosoma mansoni* [13, 14]. The same mice also show impaired Th2 responses in the context of allergic inflammation[13, 15, 16]. These data suggested that $CD11b^+$ $CD103^+$ DCs may play a unique role in the induction of Th2 cells. However, the same DCs also direct the differentiation of Th17 cells in the lungs during fungal infection[7] and prime Th17 cells in the MLN following CD40 and LPS stimulation[6]. We

therefore hypothesized that CD11b$^+$ CD103$^+$ DCs are not inherently programmed for Th2 induction but instead require external signals to adopt this function.

In a Th2 response, neither the nature of DC activation signals nor the cellular source of these instructions is well understood[17]. Unlike bacteria and viruses, helminths do not typically activate DCs through Toll-like receptors (TLRs)[18]. Instead, C-type lectin receptors[19] or class A scavenger receptors[20] may function as DC pattern recognition receptors (PRR). Host-derived molecules also influence DC function. Tissue damage caused by invading helminth larvae can trigger the release of alarmins such as thymic stromal lymphopoietin (TSLP) and IL-33 that are able to bias DCs toward Th2 induction[21, 22]. In the lung, alarmins also initiate IL-13 production by innate lymphoid cells (ILCs) and this IL-13 has been proposed to stimulate the activation and migration of Th2-driving DCs[23]. IL-4 is also a product of activated ILCs[24] and can support Th2 induction[25]. Early cytokine signals therefore have significant potential to influence DC function.

In the intestine, Batf3-dependent CD11b$^-$ CD103$^+$ DCs are reported to release the pro-inflammatory cytokine IL-12 constitutively, and hence provide a constant deterrent to a Th2 response[26]. Th2 immunity to helminth infection is significantly exaggerated in Batf3-deficient mice[26]. However, it is still not clear which DC subset(s) drive Th2 immunity during intestinal infection, nor whether or how these DCs are altered by intestinal infection. To address this, we saught to elucidate how intestinal DC populations respond to gastrointestinal infection and to identify the activation signals involved. Our data reveal that CD11b$^+$ and CD11b$^-$ DC subsets are differently activated by intestinal helminth infection. CD11b$^+$ but not CD11b$^-$ DCs accumulate in the MLN, increase expression of the costimulatory molecule PDL2, and assume a phenotype reminiscent of alternatively activated macrophages. Only helminth-activated CD11b$^+$ DCs had an enhanced capacity to drive naïve CD4$^+$ T cells toward Th2 differentiation. Interestingly, early IL-4Rα signaling was essential for the alternatively activated phenotype in CD11b$^+$ DCs but was

dispensable for their Th2 induction. Thus a specific population of $CD11b^+$ intestinal DC, activated during intestinal infection with the helminth *H. polygyrus*, specifically directs the differentiation of Th2 cells. Together our data suggest that the ability to prime Th2 cells during intestinal helminth infection is a unique characteristic of $CD11b^+$ DCs and is activated by external instruction.

Results

CD11b$^+$ DCs accumulate in the MLN following *H. polygyrus* infection

To assess the activation of intestinal DCs during helminth infection, we first infected mice with the enteric nematode *Heligmosomoides polygyrus* and quantified the proportion and number of each DC subset in the small intestinal lamina propria (SI-LP) and MLN at day 7 post-infection (pi). We identified DCs as CD11c$^+$ MHCII$^+$ cells that are CD64 negative, excluding macrophages[3], and separated the DCs into distinct subsets based on expression of CD11b and CD103[2] (Fig S1). Consistent with previous reports[3, 4], in naïve mice we distinguished three DC populations in the SI-LP and MLN: a major CD103$^+$ CD11b$^-$, a large CD11b$^+$ CD103$^+$, and a smaller CD11b$^+$ CD103$^-$ subset (Fig 1A,B). Following *H. polygyrus* infection, both the proportion and number of CD11b$^+$ CD103$^+$ DCs decreased in the SI-LP (Fig 1C). This reduction was accompanied by an increase in the proportion and number of these DCs in the MLN (Fig 1D). These data support a hypothesis that CD11b$^+$ CD103$^+$ DCs leave the infection site upon helminth infection, and accumulate in the local lymph node. The proportion and number of CD11b$^+$ CD103$^-$ DCs in the MLN also increased following *H. polygyrus* infection (Fig 1F), although the number of these DCs did not decrease significantly in the SI-LP (Fig 1E). In contrast, there was no change in the number of CD11b$^-$ CD103$^+$ DCs in either the SI-LP or MLN during *H. polygyrus* infection (Fig 1G,H), and the total number of unfractionated DCs in the MLN also did not alter significantly upon infection (Fig S2). Together, these data suggest that only CD11b$^+$ DCs accumulate in the MLN in response to intestinal helminth infection.

Helminth infection induces functional specialization in CD11b$^+$ and CD11b$^-$ DCs

We next asked whether the accumulation of CD11b$^+$ DCs in the MLN during *H. polygyrus* infection was accompanied by DC activation. Certain co-stimulatory molecules including CD40 and OX40L have been associated with Th2 polarization[27, 28], although other reports argue that

both molecules are unbiased co-stimulatory signals[29, 30]. PDL2 expression has also been linked to Th2 polarization[13]. In our experiments all three DC subsets appeared activated in the MLN, expressing higher levels of MHCII than in the SI-LP, but there were no differences in expression of MHCII, CD40 or OX40L between the three populations, neither in naïve nor infected animals (Fig S3 and data not shown). Interestingly, $CD11b^+$ $CD103^-$ and $CD11b^+$ $CD103^+$ DCs in the MLN showed higher expression of PDL2 following *H. polygyrus* infection compared to DCs in naïve mice (Fig 2A,B). PDL2 expression on $CD103^+$ $CD11b^-$ DCs was unaffected by infection, although this subset showed high basal expression (Fig 2A,B). Thus, in the MLN, only $CD11b^+$ DCs upregulate the co-stimulatory molecule PDL2 in response to *H. polygyrus* infection.

To further examine the nature of DC subset activation in response to helminth infection, we assessed the cytokine expression of each population. IL-6 and IL-10 have previously been associated with Th2 development in response to helminth antigens[31, 32]. We analyzed gene expression for the cytokines IL-6, IL-10 and the canonical Th1 polarizing signal, IL-12, in DC subsets from the MLN of naive and day 7 *H. polygyrus*-infected mice. For this analysis we grouped $CD11b^+$ $CD103^-$ and $CD11b^+$ $CD103^+$ DCs together as a single population of $CD11b^+$ DCs, since both populations showed a similar phenotypic response to *H. polygyrus* infection (Fig 1D,F and Fig 2A,B), both subsets are IRF4-dependent, and both share developmental origins[3, 6]. Our sort gates are illustrated in Fig S4. During *H. polygyrus* infection, $CD11b^+$ DCs expressed more *il6* than $CD11b^-$ DCs (Fig 2C). $CD11b^+$ DCs also expressed 2 fold more *il10* than did $CD11b^-$ DCs (Fig 2C). In contrast, *il12p40* and *il12p35* gene expression was 3 fold higher in $CD11b^-$ than in $CD11b^+$ DCs (Fig 2C) and, correspondingly, more $CD11b^-$ DCs than $CD11b^+$ produced IL-12/23 p40 protein (Fig S5). These data indicate that both $CD11b^+$ and $CD11b^-$ DCs express cytokines during helminth infection, but the selection of cytokines is markedly different between the two DC populations.

Helminth infection has been reported to induce markers of alternative activation in unfractionated $CD11c^+$ $MHCII^+$ DCs[25]. In macrophages, alternative activation is associated with distinct functional specialization[33]. We tested each of the intestinal DC subsets for evidence of alternative activation during *H. polygyrus* infection. Our data revealed a clear, 12-fold induction of *Arg1* (Arginase) in response to *H. polygyrus* infection in $CD11b^+$ intestinal DCs (Fig 2D). The $CD11b^+$ DC subsets also expressed *Retnla* (Resistin-like molecule α), *Chi3l3* (Ym-1) and *Mrc1* (Mannose receptor) following *H. polygyrus* infection (Fig 2D). In contrast, expression of all four genes remained low in $CD11b^-$ DCs, even in the presence of *H. polygyrus* infection (Fig 2D). These data suggest that pronounced alternative activation is a distinct characteristic of $CD11b^+$ DCs. Together our findings suggest functional specialization of intestinal DCs in response to helminth infection, whereby only $CD11b^+$ DCs upregulate PDL2, upregulate IL-6 and IL-10, and adopt an alternatively activated phenotype.

Toxoplasma gondii infection activates $CD11b^+$ DCs, but does not elicit alternative activation

Our data suggested that both $CD11b^+$ and $CD11b^-$ DCs in the MLN were activated during *H. polygyrus* infection, but that $CD11b^+$ DCs were more distinctly alternatively activated. To determine whether the alternative activation was an inherent feature of activated $CD11b^+$ DCs or a specific response to helminth infection, we compared the activation status of intestinal DCs in the mixed Th1/Th17 setting of infection with the protozoan *Toxoplasma gondii*. Consistent with data from Denkers and colleagues[34], neither the percentage nor number of any DC subset in the MLN was changed significantly by *T. gondii* infection (Fig S6). Both $CD11b^+$ and $CD11b^-$ DCs increased expression of PDL2 following *T. gondii* infection (Fig 3A,B) suggesting that both populations are activated in response to infection. $CD11b^-$ $CD103^+$ MLN DCs from *T. gondii*-infected mice expressed more IL-12p40 than $CD11b^+$ DCs (Fig 3C) [34], while only $CD11b^+$ DCs increased expression of *il10* in response to infection (Fig 3C). Both $CD11b^+$ and $CD11b^-$ $CD103^+$ DCs increased expression of *il6* (Fig 3C). In contrast to *H. polygyrus*, expression of the alternative

activation markers *Arg1*, *Retnla*, *Chi3l3 and Mrc1* remained low in both $CD11b^+$ and $CD11b^-$ DCs during *T. gondii* infection (Fig 3D). These data suggest that $CD11b^+$ DCs are activated and respond to *T. gondii* infection, but they do not become alternatively activated in this context.

IL-4Rα signaling promotes alternative activation of $CD11b^+$ DCs

The alternative activation of $CD11b^+$ DCs in the MLN during *H. polygyrus* but not *T. gondii* infection suggests that a signal unique to helminth infection may be responsible. IL-4Rα signaling has been reported to induce alternative activation of DCs[25], and IL-4 and IL-13 are produced in the SI-LP by innate lymphoid cells (ILCs) in the early stage of *H. polygyrus* infection[24]. We hypothesized that IL-4 and/or IL-13 promote the alternative activation of $CD11b^+$ DCs during helminth infection. IL-4Rα and IL-13Rα1 were expressed on both $CD11b^+$ and $CD11b^-$ DCs (Fig 4A), suggesting that both populations were capable of responding to IL-4/IL-13 signals. Numbers of each DC subset in the MLN after *H. polygyrus* infection were similar in WT, IL-$4^{-/-}$ and IL-$4R\alpha^{-/-}$ mice, suggesting that DC accumulation in the draining lymph node was not affected by IL-4 or IL-13 signaling (Fig 4B). However, $CD11b^+$ DCs from IL-$4^{-/-}$ and IL-$4R\alpha^{-/-}$ mice failed to induce expression of the alternative activation markers *Arg1*, *Retlna*, *Chi3l3* and *Mrc1* upon *H. polygyrus* infection, compared to $CD11b^+$ DCs from WT mice (Fig 4C). The smaller induction of these alternative activation genes in $CD11b^-$ DCs was also reduced in IL-$4^{-/-}$ and IL-$4R\alpha^{-/-}$ animals (Fig 4C), and their expression in $CD11b^+$ and $CD11b^-$ DCs taken from uninfected mice was low and equivalent in all three genotypes (data not shown). These data demonstrate that IL-4 is required for the alternative activation of $CD11b^+$ DCs during *H. polygyrus* infection.

$CD11b^+$ DCs drive Th2 immune responses

Our data suggest that $CD11b^+$ and $CD11b^-$ DCs are differently activated during helminth infection. To test whether the activated subsets also show distinct functions, we sort-purified $CD11b^+$ and $CD11b^-$ DCs from either naive or *H. polygyrus*-infected mice and cultured them with antigen

specific T cells in the presence of peptide antigen. IL-4 was included as a DC survival factor[35]. All DCs induced T cell activation, measured by CD25 and CD44 upregulation (Fig S7). $CD11b^+$ DCs from *H. polygyrus*-infected mice elicited more of the Th2 cytokines IL-5 and IL-13 than $CD11b^+$ DCs from uninfected mice (Fig 5A and Fig S8). In contrast $CD11b^-$ DCs from *H. polygyrus*-infected mice did not stimulate more IL-5 or IL-13 than naïve CD11b- DCs (Fig 5A), but instead elicited an IFN-γ response (Fig 5B). Interestingly, $CD11b^+$ but not $CD11b^-$ DCs from *H. polygyrus*-infected mice also stimulated IL-17A production, suggesting dual Th2/Th17 polarization (Fig 5C). Control wells containing T cells and DCs without peptide antigen produced very little cytokine, irrespective of DC subset (Fig S9). Thus $CD11b^+$ DCs alone have the ability to prime Th2 immune responses and, in contrast, $CD11b^-$ DCs retain the capacity to promote Th1 responses even in the context of a strongly Th2-polarizing helminth infection.

IL-4Rα signaling promotes Th1 induction, but is not required for Th2 priming by $CD11b^+$ DCs

Our data indicated that helminth-activated $CD11b^+$ DCs uniquely primed Th2 cells, and displayed a pronounced, alternatively activated phenotype induced by IL-4. We hypothesized therefore that IL-4 signaling was responsible for instructing $CD11b^+$ DCs to become Th2-inducing. To test this, we repeated the DC-T cell co-culture experiment using $CD11b^+$ and $CD11b^-$ DCs from WT and IL-$4R\alpha^{-/-}$, *H. polygyrus*-infected mice. Unexpectedly, IL-$4R\alpha^{-/-}$ $CD11b^+$ DCs stimulated similar IL-5 and IL-13 production as did WT $CD11b^+$ DCs (Fig 6A). IL-$4R\alpha^{-/-}$ $CD11b^-$ DCs, however, induced significantly less IFN-γ than WT $CD11b^-$ DCs (Fig 6B), and simultaneously induced IL-5 and IL-13 at levels equivalent to those achieved by WT $CD11b^+$ DCs (Fig 6A). These data suggest that IL-$4R\alpha^{-/-}$ $CD11b^-$ DCs fail to prime Th1 cells and instead induce a Th2 response. Together our data demonstrate that, despite imposing an alternatively activated phenotype, IL-4 is not required for the generation of Th2-polarizing $CD11b^+$ DCs during helminth infection; instead, IL-4 enhances the ability of $CD11b^-$ DCs to polarize a Th1 response.

Discussion

Advances in genetic tools and the use of novel biomarkers such as CD64 have recently enabled the unambiguous identification of *bona fide* DCs in the intestinal tract[3, 36], and three major subsets have been described[2]. The different subsets have been associated with particular T cell responses: $CD11b^+$ $CD103^+$ intestinal DCs are reported to instruct Th17 differentiation, for example[6], while IRF8-dependent, $CD11b^-$ $CD103^+$ DCs promote Th1 immunity[37]. The contribution of DC subsets to intestinal Th2 biology is less well understood. In other tissues, Th2 immunity depends on the same $CD11b^+$ $CD103^+$ DCs as are important in Th17 differentiation[13-16, 38]. In the intestine, $CD11b^-$ $CD103^+$ DCs have been shown to inhibit Th2 reactivity through constitutive release of IL-12[26]. Our aim was to identify the intestinal DC subset(s) responsible for driving a Th2 response, and to determine whether these cells were constitutively Th2-polarizing or whether their Th2 induction was a response to external instruction. Our data demonstrate that, in helminth infection, $CD11b^+$ DCs increased in number in the draining lymph node, acquired key markers of alternative activation, and gained the capacity to polarize naive $CD4^+$ T cells toward Th2 differentiation in an IL-4 setting. Each of these changes represented a definite response of $CD11b^+$ DCs to helminth infection, as neither DCs from uninfected mice nor DCs from *Toxoplasma gondii* infection had these attributes. Together our data indicate that the ability to prime Th2 cells during intestinal helminth infection is both a selective and inducible characteristic of $CD11b^+$ DCs.

The specific molecules used by DCs to polarize naïve T cells towards Th2 differentiation have been notoriously difficult to identify. We observed strong induction of IL-6 and IL-10 in $CD11b^+$ DCs following *H. polygyrus* infection. DC-derived IL-6 can polarize naive CD4 T cells toward Th2 differentiation *in vitro*[39], and helminth-exposed, Th2-driving DCs have been shown to release IL-6[31], but IL-6 can also limit Th2 immunity to helminths *in vivo*[40]. Similarly, DC-derived IL-10 has been argued both to promote and to regulate Th2 responses[41-43]. Our data also indicated that

only CD11b$^+$ DCs increased expression of PDL2 in response to *H. polygyrus* infection, suggesting specific activation of the CD11b$^+$ subsets. DCs expressing high levels of PDL2 have been associated with the induction of Th2 immune responses in a number of settings. In the skin, a subset of Th2-driving DCs were defined by high expression of PDL2 and were shown to stimulate IL-4 production from effector or memory Th2 cells *in vitro*[13]. Our data indicate that PDL2 expression by DCs is not exclusive to a Th2 immune response, as DC activation during the Th1/Th17 immune response to *T. gondii* also featured high PDL2 expresssion. Interestingly, CD4$^+$ T follicular helper cells (Tfh) express high levels of PD-1 and, during helminth infection, Tfh cells are the only cells activated to produce IL-4 protein in the lymph node[44, 45]. It is possible that PDL2 mediates an interaction between CD11b$^+$ migratory DCs and PD-1$^+$ Tfh cells that is required to initiate IL-4 secretion in the lymph node. We show that CD11b$^+$ DCs specifically accumulate in the MLN following *H. polygyrus* infection. Lund and colleagues have argued that migration of DCs during *H. polygyrus* infection depends upon on CXCR5 mediated chemotaxis toward CXCL13 which directs DCs to the interfollicular regions of the lymph node, proximal to B cells and Tfh cells, where DCs promote Th2 differentiation[46]. It will be interesting to determine whether discrete DC subsets occupy different locations within the reactive lymph node, with CD11b$^+$ migratory DC unique in the interfollicular zone.

In our study, we show that CD11b$^+$ DCs from *H. polygyrus*-infected mice are distinctively able to promote Th2 differentiation. These data illustrate functional specialization in intestinal DC subsets. Studies using specific genetic depletion of CD11b$^+$ DCs have also implicated these subsets in the activation of T cell responses during helminth infection[13], and in the development of allergic immune responses in the skin[13, 15] and lungs[38]. However, CD11b$^+$ intestinal DCs can also prime Th17 cells[6], and CD11b$^+$ DCs in the lung are essential in the development of Th17 responses to fungal pathogens[7]. These data suggest that the function of separate DC populations remains flexible, and indeed our study indicates that the ability of CD11b$^+$ DCs to prime Th2 cells

is not inherent but is a specific response to a helminth infection. In support of this proposal, a recent transcriptomic analysis of DCs in skin-draining LNs reported contrasting patterns of gene expression in the same DC subsets activated either by helminth infection or contact sensitization[47]. Our model is that Th2 induction in the intestines is a unique but inducible characteristic of $CD11b^+$ DCs, directed by environmental signals.

Both IL-4 and IL-13 have previously been proposed to influence DC-T cell interactions[23, 25]. A study of papain-induced lung inflammation suggested that ILC-derived IL-13 was required to direct activated dendritic cells to the draining LN to initiate Th2 activation[23]. IL-4 initiates expression of the alternative activation markers Relm-α and Ym-1 in bulk DCs *in vitro* and *in vivo*[25], and DCs expressing these markers have been shown to promote the development of Th2 responses[25, 48]. Our data indicate that, *in vivo*, alternative activation of DCs during *H. polygyrus* infection is a marked characteristic of $CD11b^+$ subsets. Interestingly, our observation of induction of *Arg1* expression in $CD11b^+$ DCs in the draining lymph node of *H. polygyrus*-infected mice contrasts with data from Cook *et al*, who reported that Arg-1 was not among the alternative activation genes expressed in IL-4 treated DCs[25]. In our experiments, the expression of all analysed alternative activation markers was dependent on signaling through IL-4Rα. Surprisingly, however, $CD11b^+$ DCs from infected mice polarized IL-4-conditioned Th2 cells independently of IL-4Rα. These data suggest that, in the context of intestinal helminth infection, neither IL-4 nor IL-13, nor the downstream molecules Relm-α and Ym-1, are essential for activating the Th2-inducing function of DCs.

The production of IL-4 in the lymph node during *H. polygyrus* infection is likely to expose both $CD11b^+$ and $CD11b^-$ DCs to IL-4 signals[49, 50], and we show IL-4Rα expression on the surface of both subsets of DCs. That $CD11b^-$ DCs did not become alternatively activated during helminth infection is further evidence of functional distinction between DC subsets. $CD11b^-$ intestinal DCs

produce IL-12 even in the steady state[26], and our own observations and that of others[34] show that during *T. gondii* infection, $CD11b^-$ $CD103^+$ DCs are the dominant intestinal DC subset producing IL-12. It has recently been argued that the IL-12 constitutively produced by $CD11b^-$ DCs limits Th2 immunity to intestinal helminth infection[26]. We observed that $CD11b^-$ $CD103^+$ DCs promoted an antigen-specific Th1 response even in the context of helminth infection, and indeed the Th1 induction by $CD11b^-$ $CD103^+$ DCs was enhanced by helminth infection in a manner dependent on IL-4Rα signals. Previous experiments *in vitro*[51] and *in vivo*[25] have demonstrated that exogenous IL-4 can enhance IL-12p70 production from bone marrow-derived DCs, forming a negative feedback loop to curtail the Th2 response. Our data are consistent with such a negative feedback loop and, indeed, we suggest that IL-4 may target $CD11b^-$ $CD103^+$ DCs to promote the counter-regulatory Th1 response.

Together our data demonstrate that DC function is determined both by intrinsic subset specialization and by external, environmental instruction. The ability to prime Th2 cells during intestinal helminth infection is a selective and inducible characteristic of migratory, $CD11b^+$ DCs. We show that only $CD11b^+$ DCs accumulate in the draining lymph node, upregulate markers of alternative activation and become licensed for the induction of Th2 immunity. IL-4 signaling is essential for alternative activation of $CD11b^+$ DCs but not for their Th2 induction, and, instead, IL-4Rα signals act on $CD11b^-$ DCs to enhance Th1 activation. Thus, during intestinal helminth infection, the phenotypes and functions of $CD11b^+$ and $CD11b^-$ mucosal DCs are distinct and are directed by external signals.

Materials and Methods

Mice and infections

4get, IL-4$^{-/-}$ (KN2), 4get IL-4Ra$^{-/-}$, 4get DO11.10 and C57BL/6 mice were bred in house and maintained under specific pathogen free conditions at the University of British Columbia. Mice were used at 6-8 weeks of age and were age- and sex-matched within each experiment. All animal work was conducted in accordance with guidelines set by the University of British Columbia Animal Care Committee and the Canadian Council of Animal Care. Mice were infected with 200 *H. polygyrus* L3 larvae or with 10. *T. gondii* cysts by oral gavage. 4get mice were used to facilitate the exclusion of SSC^{hi}, GFP^{+} eosinophils from the intestinal MHCII^{+} CD11c^{+} population.

Cell isolation and restimulation

MLNs were incubated in HBSS containing 400 U/ml Collagenase IV and 100 U/ml DNase for 25 mins at 37^{0}C, plus 5 minutes with 5mM EDTA. Digested MLNs were washed and crushed through a 70-μm cell strainer to obtain single cell suspensions. T and B cells were depleted using biotinylated αCD5 (BD Pharmingen, 53-7.3) and αCD19 (BD Pharmingen, 1D3) with anti-biotin microbeads and MACS LS columns (Miltenyi), and DCs purified by cell sorting. For intracellular cytokine staining, purified DCs were stimulated for 5 hours with 1μg/ml LPS, and 10 μg/ml Brefeldin A (SigmaAldrich) added for the final 2 hours. For isolation of SI-LP mononuclear cells, external adipose tissue and peyers patches were removed. The small intestine was opened longitudinally, washed, incubated for 10 minutes in PBS supplemented with 1 mM DTT, 1 mM EDTA and 5% FCS at 37^{0}C, washed, incubated at 37^{0}C for a further 20 minutes in the same buffer, and washed again. Tissues were then digested in HBSS containing 0.5 mg/ml Collagenase IV and 100 U/ml DNase for 30 mins at 37^{0}C and passed through 70-μm and 40-μm filters to obtain a single cell suspension. For isolation of naive DO11.10 CD4^{+} T cells, spleens and peripheral lymph nodes

were dissociated and $CD4^+$ T cells enriched using an EasySep Mouse $CD4^+$ T cell isolation kit (Stemcell Technologies), before purification by cell sorting.

Flow cytometry and cell sorting

Antibodies included Brilliant Violet 421-conjugated anti-CD11b (M1/70, BD Biosciences), Brilliant Violet 421-conjugated anti-CD4 (RM4-5, BD Biosciences), phycoethyrin-conjugated anti-CD273 (TY25, BD Biosciences), phycoethyrin-conjugated anti-IL-4Rα (mIL-4R-M1, BD Biosciences), phycoethyrin-conjugated anti-IL-13Rα1 (13MOKA, eBioscience), phycoethyrin-conjugated anti-SiglecF (E50-2440, BD Biosciences), phycoethyrin-conjugated anti-IL-12p40/70 (C15.6, BD Biosciences), biotin-conjugated anti-CD103 (M290, BD Biosciences), PE-Cy7-conjugated anti-CD11c (HL3, BD Biosciences), allophycocyanin-conjugated CD64 (X54-5/7.1, BD Biosciences), Alexa Flour 700-conjugated anti-I-A/I-E (M5/114.15.2, eBioscience), APC-eFlour780-conjugated anti-I-A/I-E (M5/114.15.2, eBioscience). Dead cell exclusion used Fixable Viability Dye eFlour 780 (eBioscience). Non-specific binding was blocked with anti-CD16/32 (2.4G2, eBioscience). For intracellular cytokine staining, cells were permeabilized using BD Cytofix/Cytoperm. Flow cytometry was performed using an LSRII running FACS-Diva software (BD Biosciences). Analysis was performed using FlowJo (Treestar). Cell sorting was performed on a BD Influx and a BD FACS Aria II.

***In vitro* cultures and cytometric bead array**

2000 FACS-sorted $CD11b^+$ or $CD11b^-$, $SiglecF^-$ $CD64^-$ $CD11c^+$ cells were cultured for 72 hours at 37^0C with 200,000 FACS-sorted naive DO11.10 $CD4^+$ $MHCII^-$ T cells in RPMI 1640 (Invitrogen) supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, 2mM L-glutamine, NEAA, 1mM Sodium Pyruvate, 0.5 mM β-mercaptoethanol and 5% fetal calf serum (FCS) in the presence of 5 μg/ml $OVA_{323-339}$ peptide and 20 ng/ml recombinant IL-4. Supernatants were analyzed using a Flex Set cytometric bead array (BD Biosciences).

Quantitative RT-PCR

RNA was extracted from sorted cell populations using the RNAqueous Micro Kit (Ambion). RNA concentrations were quantified using a Nanodrop 1000. 0.1-1µg RNA was reverse-transcribed using the iScript cDNA synthesis kit (BioRad). quantitative RT-PCR used SsoFast EvaGreen supermix (BioRad) and a BioRad CFX96 instrument. Gene expression was normalized to GAPDH. Primer sequences: *gapdh*-F 5' GTGTTCCTACCCCCAATGTGT 3'; *gapdh*-R 5' ATTGTCATACCAGGAAATGAGCTT 3'; *il10*-F 5' ATCGATTTCTCCCCTGTGAA 3'; *il10*-R 5' TTCGGAGAGAGGTACAAACGA 3'; *Retnla*-F 5' TATGAACAGATGGGCCTCCT 3'; *Retnla*-R 5' GGCAGTTGCAAGTATCTCCAC 3'; *Chi3l3*-F 5' GAACACTGAGCTAAAAACTCTCCTG 3'; *Chi3l3*-R 5' GACCATGGCACTGAACGAG 3'; *Mrc1*-F 5' TCATTGGAAGATCCACTCTGG 3'; *Mrc1*-R 5' CAGCGCTTGTGATCTTCATTATAG 3'; *Arg1*-F 5' GTCTGTGGGGAAAGCCAAT 3'; *Arg1*-R 5' GCTTCCAACTGCCAGACTGT 3'; *il12p40*-F 5' CATCATCAAACCAGACCCCCCAA 3'; *il12p40*-R 5' AACTTGAGGGAGAAGTACGAATGG 3'; *il12p35*-F 5' GATGACATGGTGAAGACGGCC 3'; *il12p35*-R 5' GGAGGTTTCTGGCGCAGAGT 3'; *il6*-F 5' ACCAGAGGAAATTTTCAATAGGC 3'; *il6*-R 5' TGATGCACTTGCAGAAAACA 3'.

Statistics

Data sets were compared by Students t-test or by ANOVA followed by Tukey's multiple comparison test, using GraphPad Prism 6. Values of $p < 0.05$ were considered significant.

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Conflict of Interest

The authors declare no commercial or financial conflict of interest.

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Figure legends

Figure 1. CD11b$^+$ DCs accumulate in the MLN following *H. polygyrus* infection. 4get BALB/C mice were infected with *H. polygyrus* and the proportion and numbers of CD11b$^+$ CD103$^-$, CD11b$^+$ CD103$^+$ and CD11b$^-$ CD103$^+$ DCs in the SI-LP and MLN were quantified by flow cytometry at day 7 post-infection. Representative plots showing discrete DC subsets in the SI-LP (A), and MLN (B). Graphs illustrate the percentage and number of CD11b$^+$ CD103$^+$ DCs in the SI-LP (C) and MLN (D); of CD11b$^+$ CD103$^-$ DCs in the SI-LP (E) and MLN (F); and of CD11b$^-$ CD103$^+$ DCs in the SI-LP (G) and MLN (H). Data shown in (C-H) are combined from two independent experiments with 4-5 mice per group in each one. Graphs display mean + SD (*$p < 0.05$ Students t-test). Hp, *H. polygyrus*

Figure 2. Helminth infection induces functional specialization in CD11b$^+$ and CD11b$^-$ DCs. 4get BALB/C mice were infected with *H. polygyrus* and MLNs harvested at day 7 post-infection. (A) Histograms showing PDL2 expression by CD11b$^+$ CD103$^-$, CD11b$^+$ CD103$^+$ and CD11b$^-$ CD103$^+$ DCs. (B) Bar graphs showing geometric mean fluorescence intensity (MFI) of PDL2 expression of samples from (A). Graphs display mean + SD. Statistical comparisons were performed using the Students t-test (****$p < 0.0001$). Data in A & B are from a single experiment, representative of two independent experiments with 4-5 mice per group in each one. (C) Gene expression, assayed by qPCR, of *il6*, *il10*, *il12p40*, *il12p35*, and (D) *arg1*, *retnla*, *chi3l3* and *mrc1* within CD11b$^+$ and CD11b$^-$, CD64$^-$ CD11c$^+$ DCs sorted and pooled from 5-8 mice per group. Data are normalized to that of the naïve CD11b+ control, and are from a single experiment representative of two independent experiments. AU, Arbitrary units; Hp, *H. polygyrus*.

Figure 3. *Toxoplasma gondii* infection does not elicit alternative activation in CD11b$^+$ DCs. C57BL/6 mice were infected with 10 *T. gondii* cysts and harvested at day 7 post-infection. (A) Representative histograms showing PDL2 expression by CD11b$^+$ CD103$^-$, CD11b$^+$ CD103$^+$ and CD11b$^-$ CD103$^+$ DC in the MLN. (B) Bar graphs showing geometric mean fluorescence intensity (MFI) of PDL2 expression of samples from (A). Graphs display mean + S.D. Statistical comparisons were performed using the Students t-test (*$p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). Data in A & B are from a single experiment, representative of two independent experiments with 4-5 mice per group in each one. (C) Gene expression, assayed by qPCR, of *il6*, *il10*, *il12p40*, *il12p35*, and (D) *arg1*, *retnla*, *chi3l3* and *mrc1* within CD11b$^+$ and CD11b$^-$, CD64$^-$ CD11c$^+$ DCs sorted and pooled from 5-8 mice per group. Data are normalized to that of the naïve CD11b+ control, and are from a single experiment representative of two independent experiments. AU, Arbitrary units; Hp, *H. polygyrus*.

Figure 4. IL-4Rα signaling promotes alternative activation of CD11b$^+$ DCs. (A) Representative histograms illustrating IL-4Rα and IL-13Rα1 expression on CD11b$^+$ and CD11b$^-$ DCs in the MLN at day 7 of *H. polygyrus* infection. Data are from a single experiment, representative of two independent experiments with 4-5 mice per group in each one. (B) Numbers of each DC subset in the MLNs of day 7 *H. polygyrus*-infected wild type, IL-4$^{-/-}$ and IL-4Rα$^{-/-}$ mice, quantified by flow cytometry. Data are combined from two independent experiments with 4-5 mice per group. Graphs display mean + S.D. Wildtype data from the same experiments are also shown in Figure 1C-H. (C) Gene expression of *arg1*, *retnla*, *chi3l3*, and *mrc1* was assayed by qPCR within CD11b$^+$ and CD11b$^-$, CD64$^-$ CD11c$^+$ DCs sorted from MLNs pooled from *H. polygyrus*-infected WT, IL-4$^{-/-}$ and IL-4Rα$^{-/-}$ mice (5-8 mice per group). Data are normalized to that of the WT, naive CD11b$^+$ control. Data are from a single experiment, representative of two independent experiments. Wildtype data from the same experiment are also shown in Figure 2D. AU, Arbitary units; Hp, *H. polygyrus*.

Figure 5. CD11b$^+$ DCs induce Th2 cytokines in responding T cells in a co-culture system. CD11b$^+$ and CD11b$^-$, CD64$^-$ CD11c$^+$ DCs were sorted from MLNs of naive or *H. polygyrus*-infected mice and cultured with DO11.10 CD4$^+$ T cells in the presence of cognate antigen and IL-4 for 72 hours. Concentrations of the cytokines (A) IL-5 and IL-13, (B) IFNγ and (C) IL-17 were assessed in the culture supernatant by cytometric bead array. Data are from a single experiment, representative of two independent experiments with 4-5 mice per group in each one. Replicates are independent DC-T cell co-cultures using DCs purified from individual mice. Graphs display mean + S.D. Statistical comparisons were performed using an ANOVA followed by Tukeys post hoc test (*p < 0.05, **p < 0.01). Hp, *H. polygyrus*.

Figure 6. IL-4Rα signaling to DCs is required for Th1 induction by CD11b$^-$ DCs, but is dispensible for Th2 priming by CD11b$^+$ DCs. CD11b$^+$ and CD11b$^-$ CD64$^-$ CD11c$^+$ DCs were sorted from MLNs of wild type and IL-4Rα$^{-/-}$ *H. polygyrus*-infected mice and cultured with DO11.10 CD4$^+$ T cells in the presence of cognate antigen and IL-4 for 72 hours. Concentrations of the cytokines (A) IL-5 and IL-13, and (B) IFNγ were assessed in the culture supernatant by cytometric bead array. Data are from a single experiment, representative of two independent experiments with 4-5 mice per group in each one. Replicates are independent DC-T cell co-cultures using DCs purified from individual mice. Graphs display mean ± S.D. Statistical comparisons were performed using an ANOVA followed by Tukeys post hoc test (**p < 0.01, ****p < 0.0001).

Figure 1

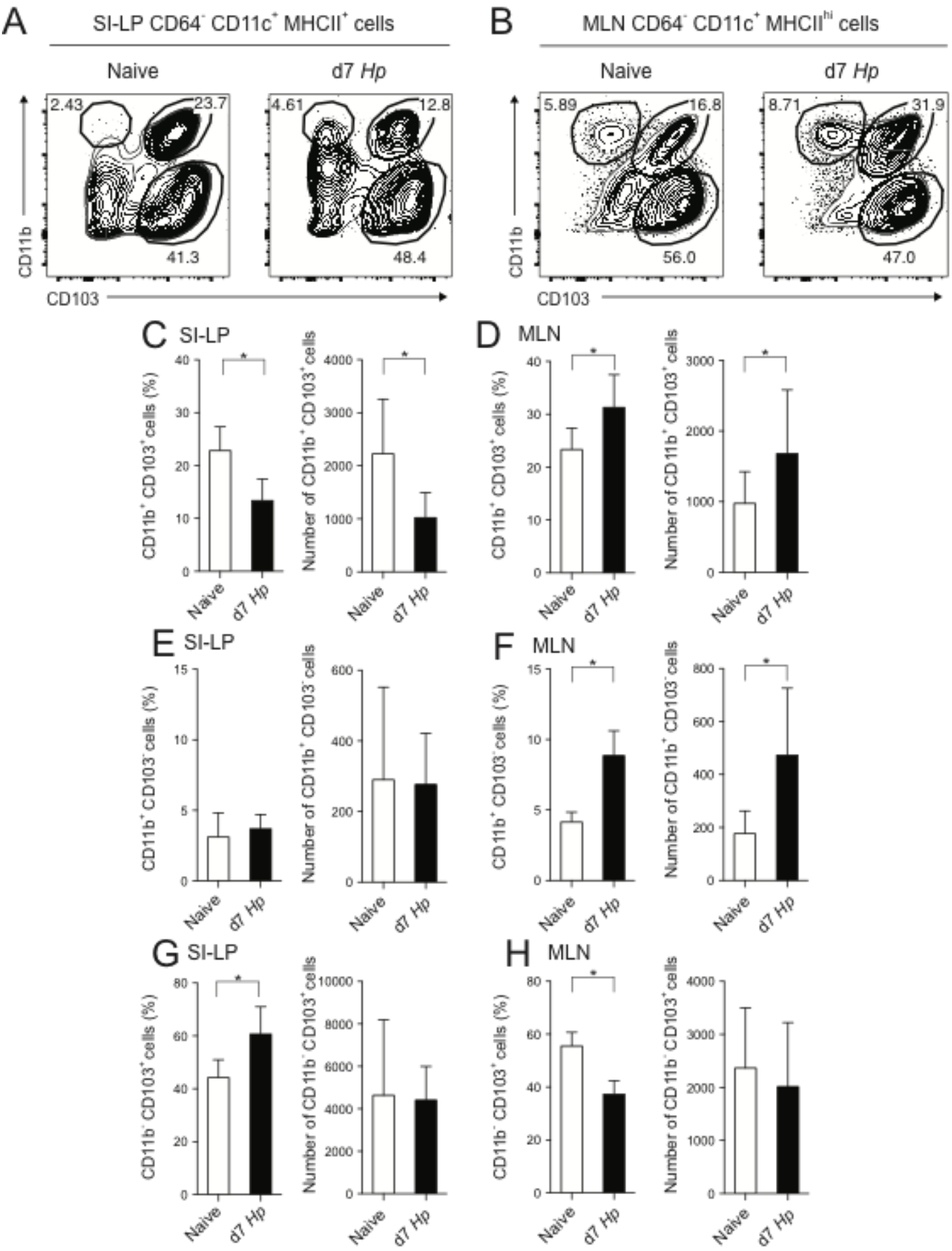


Figure 1. CD11b⁺ DCs accumulate in the MLN following *H. polygyrus* infection. 4get BALB/C mice were infected with *H. polygyrus* and the proportion and numbers of CD11b⁺ CD103⁻, CD11b⁺ CD103⁺ and CD11b⁻ CD103⁺ cells DCs in the SI-LP and MLN were quantified by flow cytometry at day 7 post-infection. (A) Representative plots showing discrete DC subsets in the SI-LP (A), and MLN (B). Graphs illustrate the proportion and number of CD11b⁺ CD103⁺ DCs in the SI-LP (C) and MLN (D); of CD11b⁺ CD103⁻ DCs in the SI-LP (E) and MLN (F); and of CD11b⁻ CD103⁺ DCs in the SI-LP (G) and MLN (H). Data shown are combined from two independent experiments with 4-5 mice per group in each one. Graphs display mean ± SD. (*$p < 0.05$ Students t-test).

Figure 2

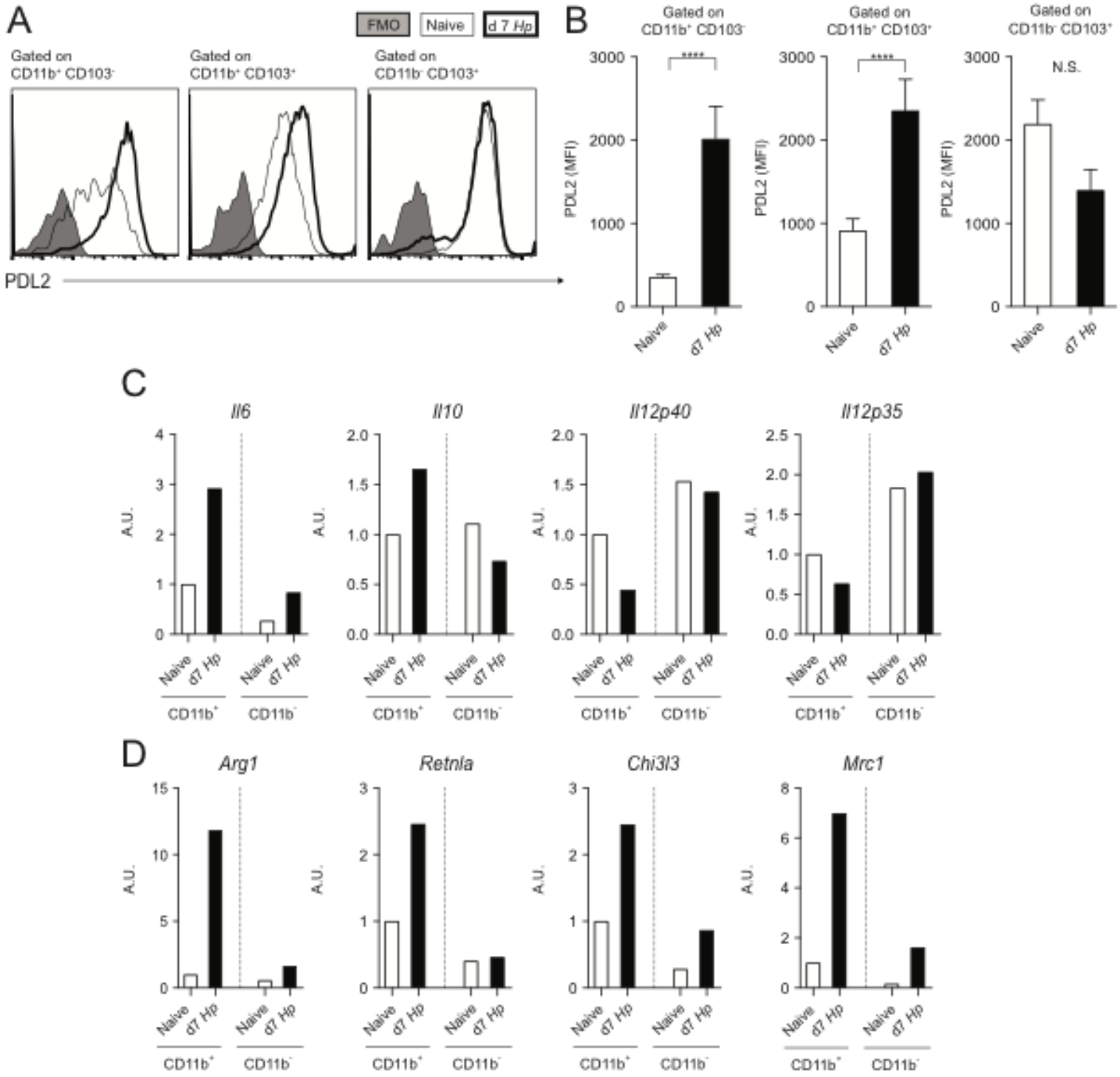


Figure 2. Helminth infection induces functional specialization in CD11b$^+$ and CD11b$^-$ DCs. 4get BALB/C mice were infected with *H. polygyrus* and harvested at day 7 post-infection. (A) Histograms showing PDL2 expression by CD11b$^+$ CD103$^-$, CD11b$^+$ CD103$^+$ and CD11b$^-$ CD103$^+$ DC in the MLN. (B) Bar graphs showing geometric mean flourescence intensity (MFI) of PDL2 expression. Graphs display mean ± SD Statistical comparisons were performed using the Students t-test (****$p < 0.0001$). Data in A & B are from a single experiment, representative of two independent experiments with 4-5 mice per group in each one. (C) Gene expression of *il6*, *il10*, *il12p40* and *il12p35*, and (D) *arg1*, *retnla*, *chi3l3* and *mrc1* within CD11b$^+$ and CD11b$^-$, CD64$^-$ CD11c$^+$ DC sorted from MLNs pooled from 5-8 mice per group. Data are normalized to that of the naïve CD11b$^+$ control, and are from a single experiment representative of two independent experiments. A.U. - Arbitrary units.

Figure 3

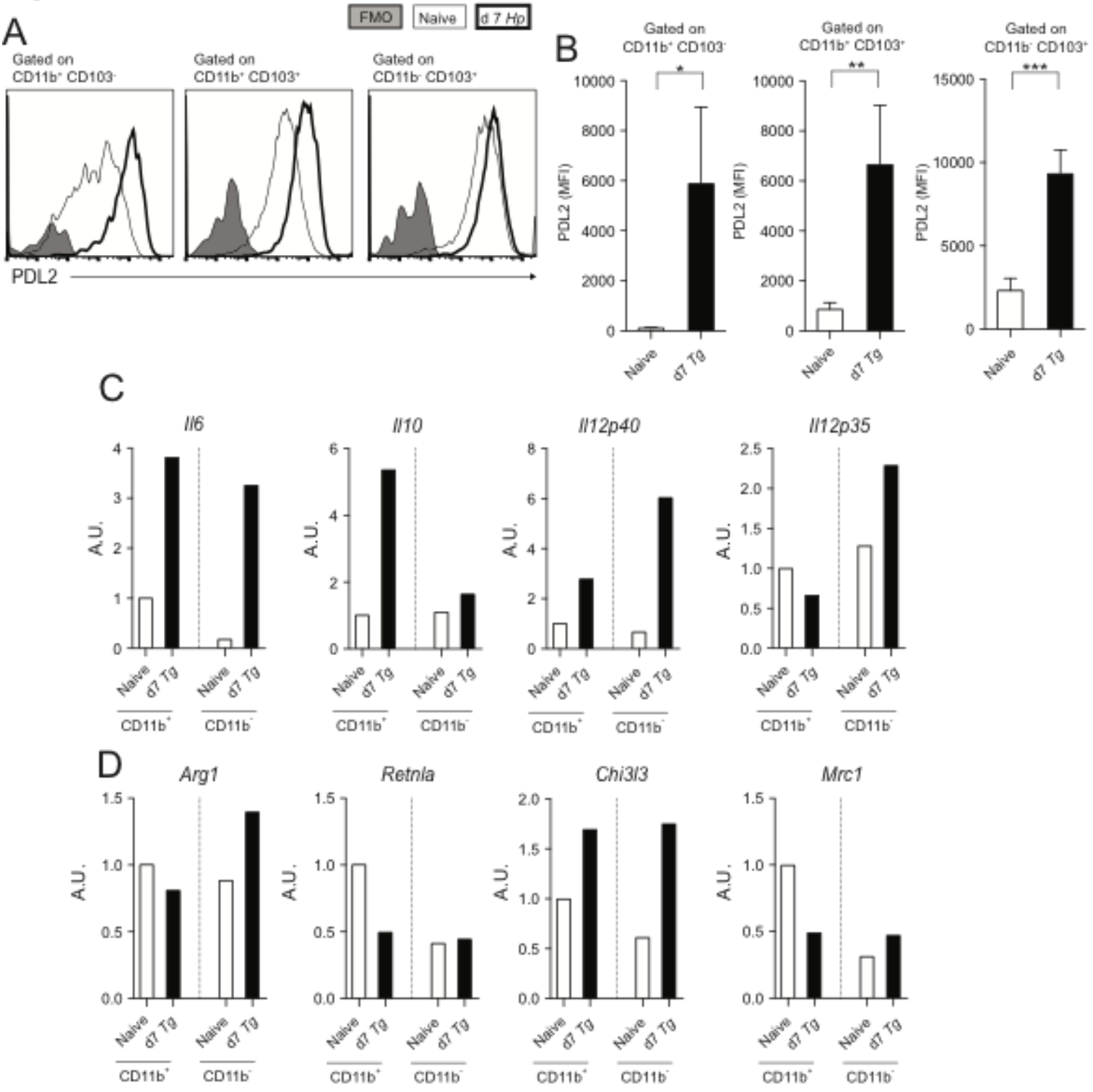


Figure 3. T*oxoplasma gondii* infection does not elicit alternative activation in CD11b⁺ DCs. C57BL/6 mice were infected with 10 *T. gondii* cysts and harvested at day 7 post-infection. (A, B) Histograms and graphs showing PDL2 expression by CD11b⁺ CD103⁻, CD11b⁺ CD103⁺ and CD11b⁻ CD103⁺ DCs in the MLN. Graphs show MFI of PDL2 expression. Data are from a single experiment, representative of two independent experiments with 4-5 mice per group in each one. Graphs display mean ± S.D. Statistical comparisons were performed using the Students t-test (*$p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). (C) Gene expression of *Il6, Il10, Il12p40* and *Il12p35* and (D) *Arg1, Retnla, Chi3l3 and Mrc1* within CD11b⁺ and CD11b⁻, CD64⁻ CD11c⁺ DC sorted from MLNs pooled from 5-8 mice per group. Data are normalized to that of the naive CD11b⁺ control, and are from a single experient representative of two independent experiments. A.U. -Arbitrary units.

Figure 4

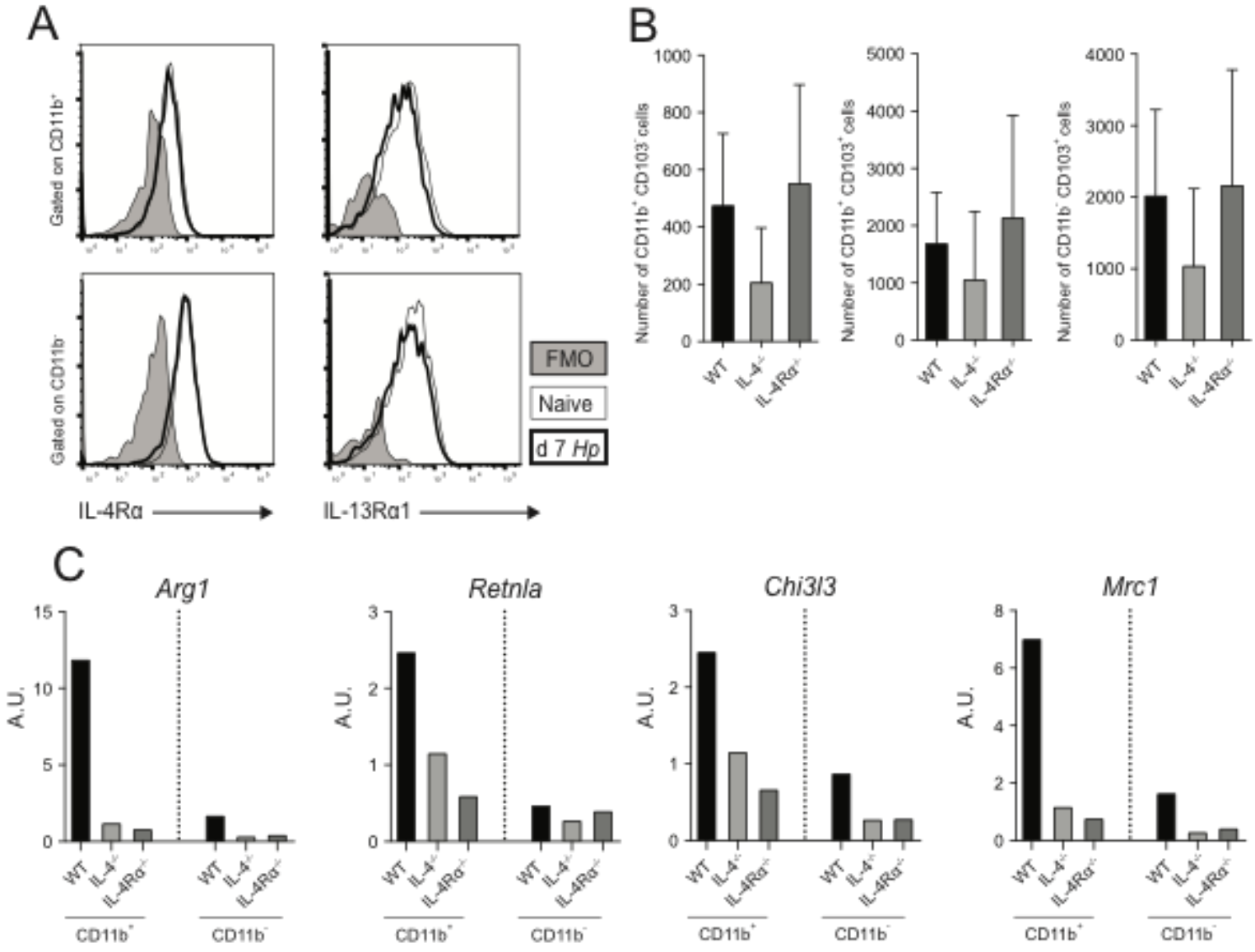


Figure 4. IL-4Rα signalling promotes alternative activation of CD11b⁺ DCs. (A) Histograms illustrating IL-4Rα and IL-13Rα 1 expression on CD11b⁺ and CD11b⁻ DC in the MLN at day 7 of *H. polygyrus* infection. Data are from a single experiment, representative of two independent experiments with 4-5 mice per group in each one. (B) Numbers of each DC subsets in the MLNs of day 7 *H. polygyrus*-infected wild type, IL-4$^{-/-}$ and IL-4Rα$^{-/-}$ mice. Data are combined from two independent experiments with 4-5 mice per group in each one. Graphs display mean ± S.D. Wildtype data from the same experiments are also shown in Figure 1 C-H. (C) Gene expression of *arg1*, *retnla*, *chi3l3*, and *mrc1* within CD11b⁺ and CD11b⁻ CD64⁻ CD11c⁺ DC sorted from MLNs pooled from *H. polygyrus*-infected WT, IL-4$^{-/-}$ and IL-4Rα$^{-/-}$ mice (5-8 mice per group). Data are normalized to the WT, naive CD11b⁺ control. Data are from a single experiment, representative of two independent experiments. A.U. - Arbitary units. Wildtype data from the same experiments are also shown in Figure 2 D.

Figure 5

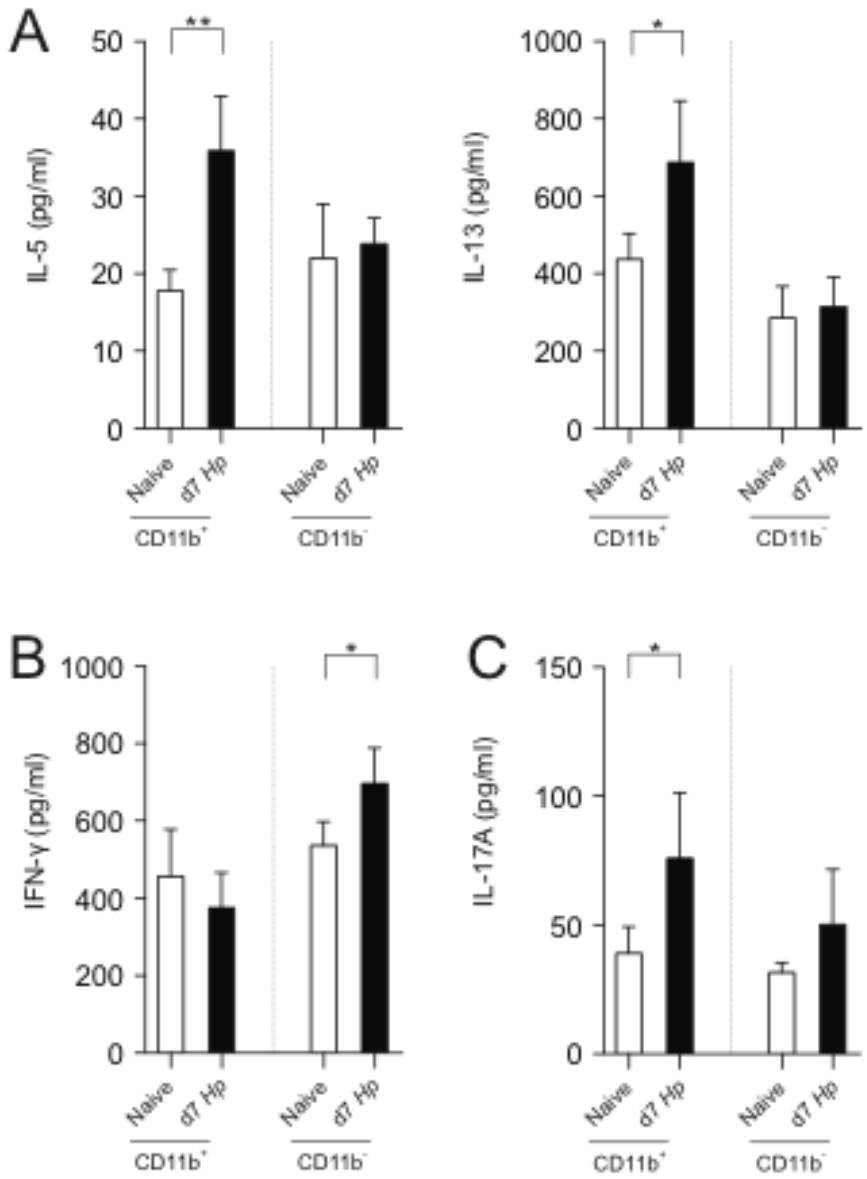


Figure 5. CD11b⁺ DC induce Th2 cytokines in responding T cells in a co-culture system. CD11b⁺ and CD11b⁻ CD64⁻ CD11c⁺ DC were sorted from MLNs of naive or *H. polygyrus*-infected mice and cultured with DO11.10 CD4⁺ T cells in the presence of cognate antigen and IL-4 for 72 hours. Concentrations of the cytokines (A) IL-5 and IL-13, (B) IFNγ and (C) IL-17A were assesed in the culture supernatant. Data shown are from one experiment, representative of two independent experiments with 4-5 mice per group in each one. Replicates are independent DC-T cell co-cultures using DCs purified from indiviudal mice. Graphs display mean ± S.D. Statistical comparisons were performed using an ANOVA followed by Tukeys post hoc test (*$p < 0.05$, **$p < 0.01$).

Figure 6

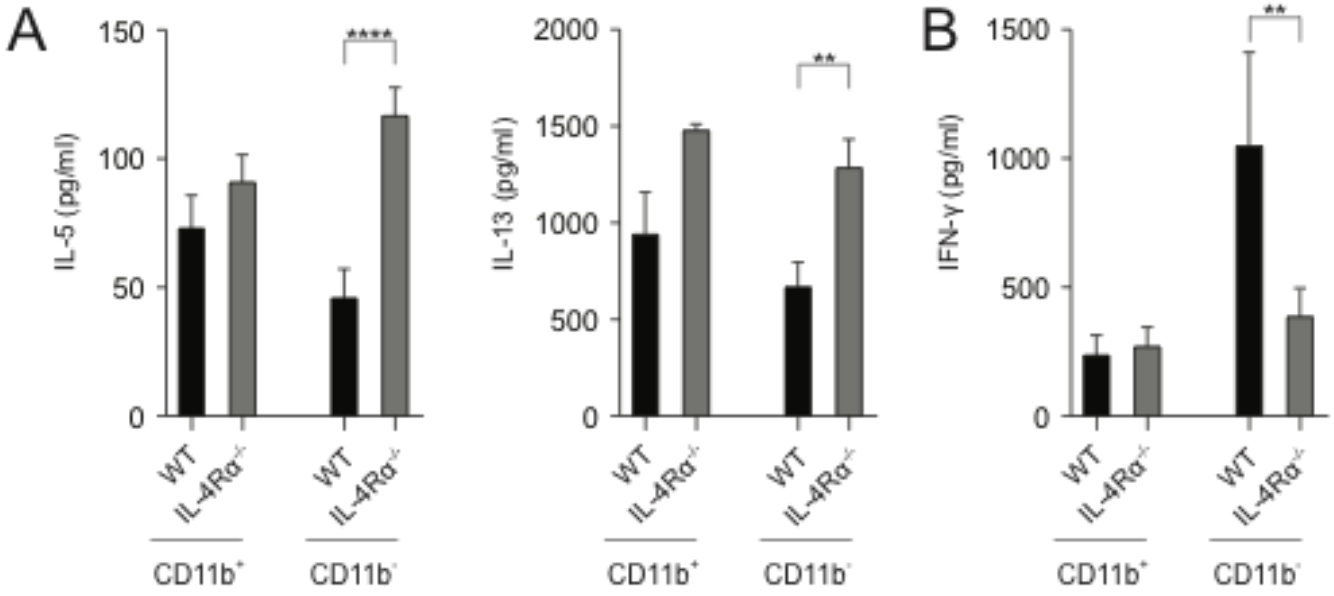


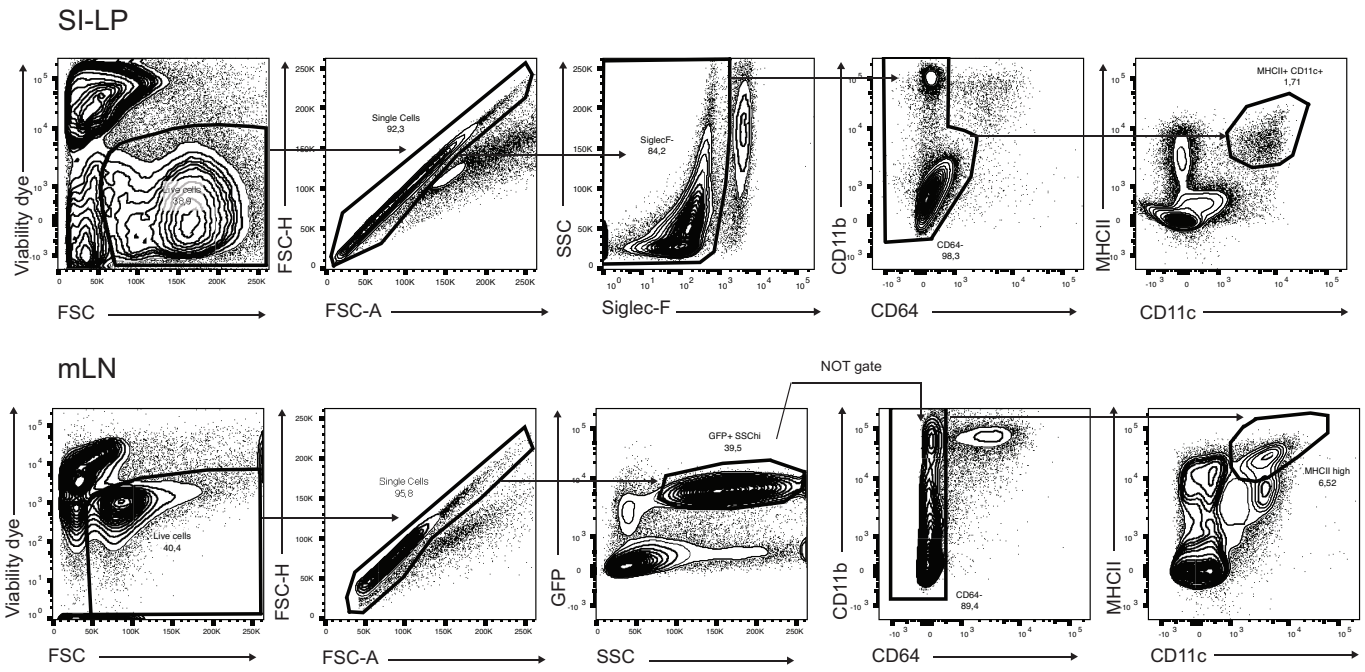
Figure 6. IL-4Rα signaling to DCs is required for Th1 induction by CD11b$^-$ DCs, but is dispensable for Th2 priming from CD11b$^+$ DCs. CD11b$^+$ and CD11b$^-$ CD64$^-$ CD11c$^+$ DC were FACS sorted from MLNs of wild type and IL-4Rα$^{-/-}$ *H. polygyrus* infected mice and cultured with DO11.10 CD4$^+$ T cells in the presence of cognate antigen and IL-4 for 72 hours. Concentrations of the cytokines (A) IL-5 and IL-13, and (B) IFNγ were assesed in the culture supernatant. Data are from a single experiment, representative of two independent experiments with 4-5 mice per group in each one. Replicates are independent DC-T cell co-cultures using DCs purified from individual mice. Graphs display mean ± S.D. Statistical comparisons were performed using an ANOVA followed by Tukeys post hoc test (**$p < 0.01$, ****$p < 0.0001$).

Supplementary Data

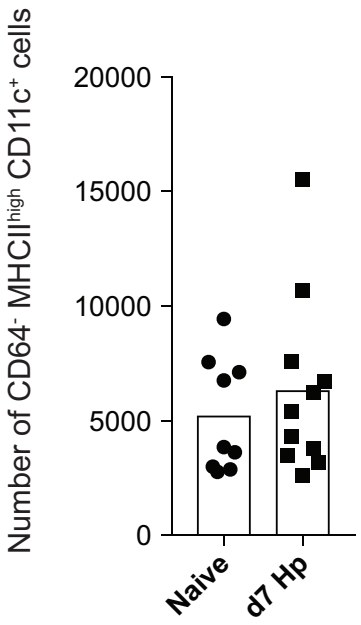
Functional specialization of intestinal dendritic cell subsets during helminth infection

Stephen A. Redpath, Graham A. Heieis, Lisa A. Reynolds, Nicolette M. Fonseca, Sandra S.-Y. Kim, and Georgia Perona-Wright

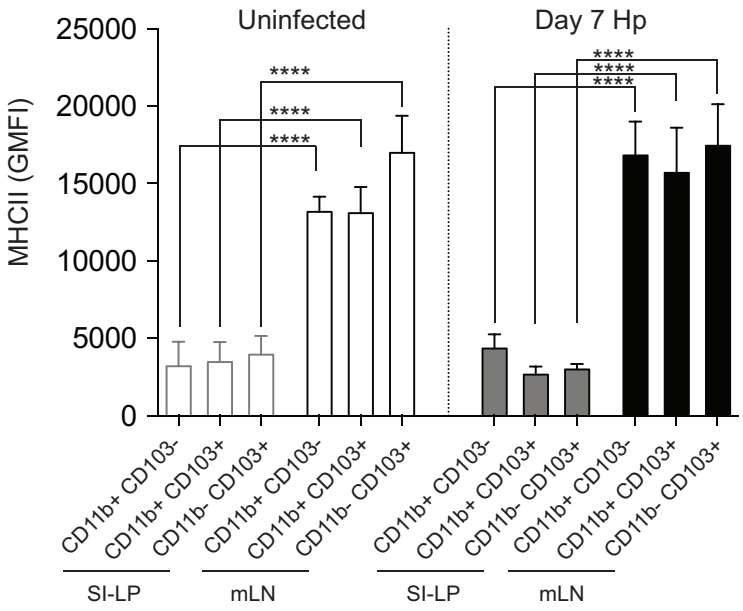
Figures S1 – S9



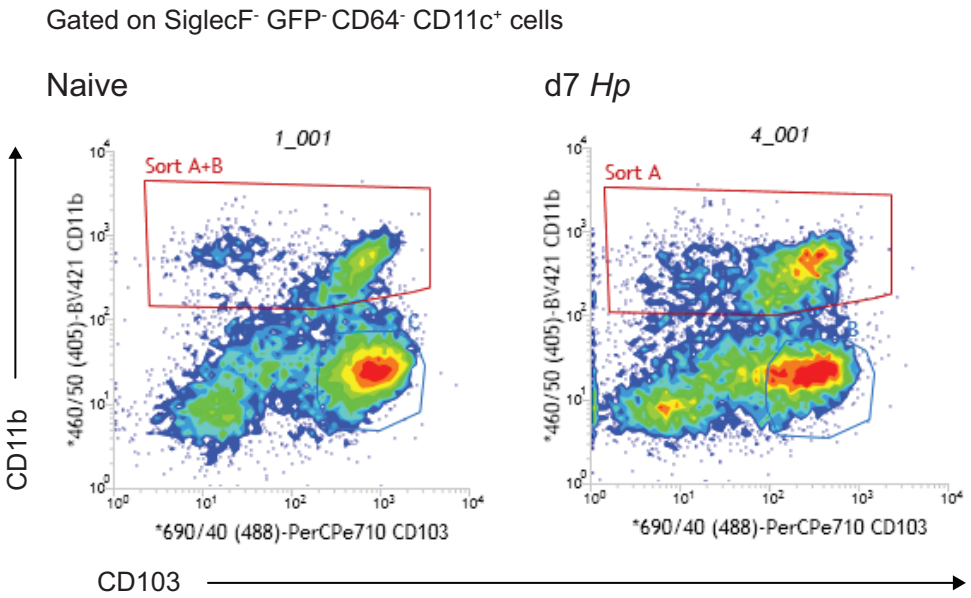
Supplemental Figure 1. Gating strategy used to identify the different DC subsets within MHCII+ CD11c+ or MHCIIhigh CD11c+ cells in the SI-LP and mLN respectively. In the mLN, GFP+ SSChi events were gated out (a NOT gate), to exclude eosinophils. Plots shown are from H. polygyrus infected mice.



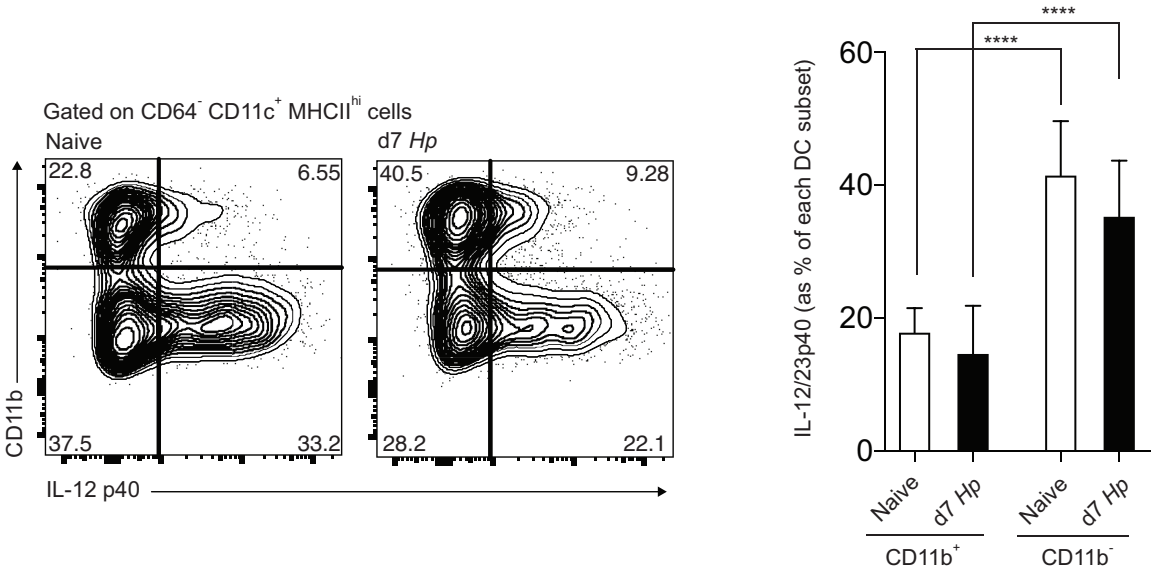
Supplemental Figure 2. The total number of unfractionated DCs in the MLN does not change significantly during *H. polygyrus* infection. Graph showing number of MHCII^{high} CD11c^{+} CD64^{-} DCs in the mesenteric lymph node (mLN) of mice infected with *H. polygyrus* at day 7 post-infection. Numbers were counted after MACS depletion of T and B cells. Data are combined from two independent experiments with 4-6 mice per group in each one. Bars show mean values.



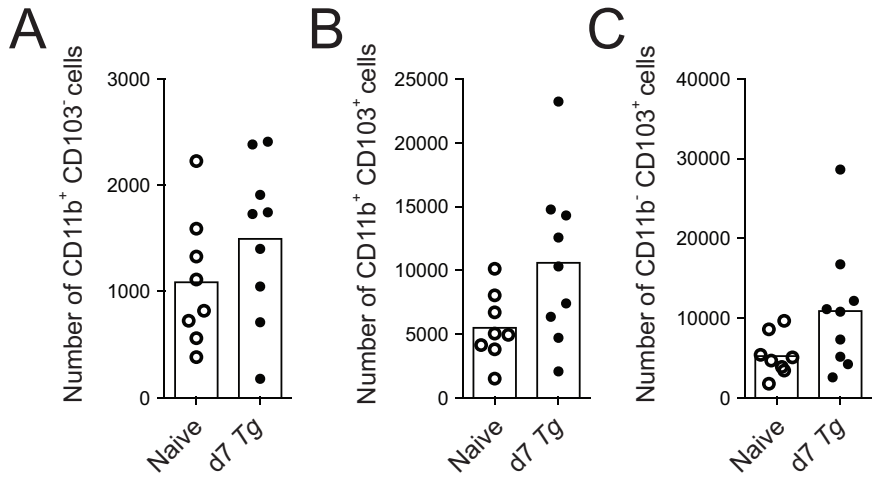
Supplemental Figure 3. MHCII expression on DC subsets in the intestinal tissue versus draining lymph node. Graph showing intensity of MHCII expression on different DC subsets in the SI-LP and mLN. Data are combined from two independent experiments with 4-5 mice per group in each one. Graphs display mean ± SD. Statistical comparisons were performed using an ANOVA followed by Tukeys post hoc test (**** $p < 0.0001$).



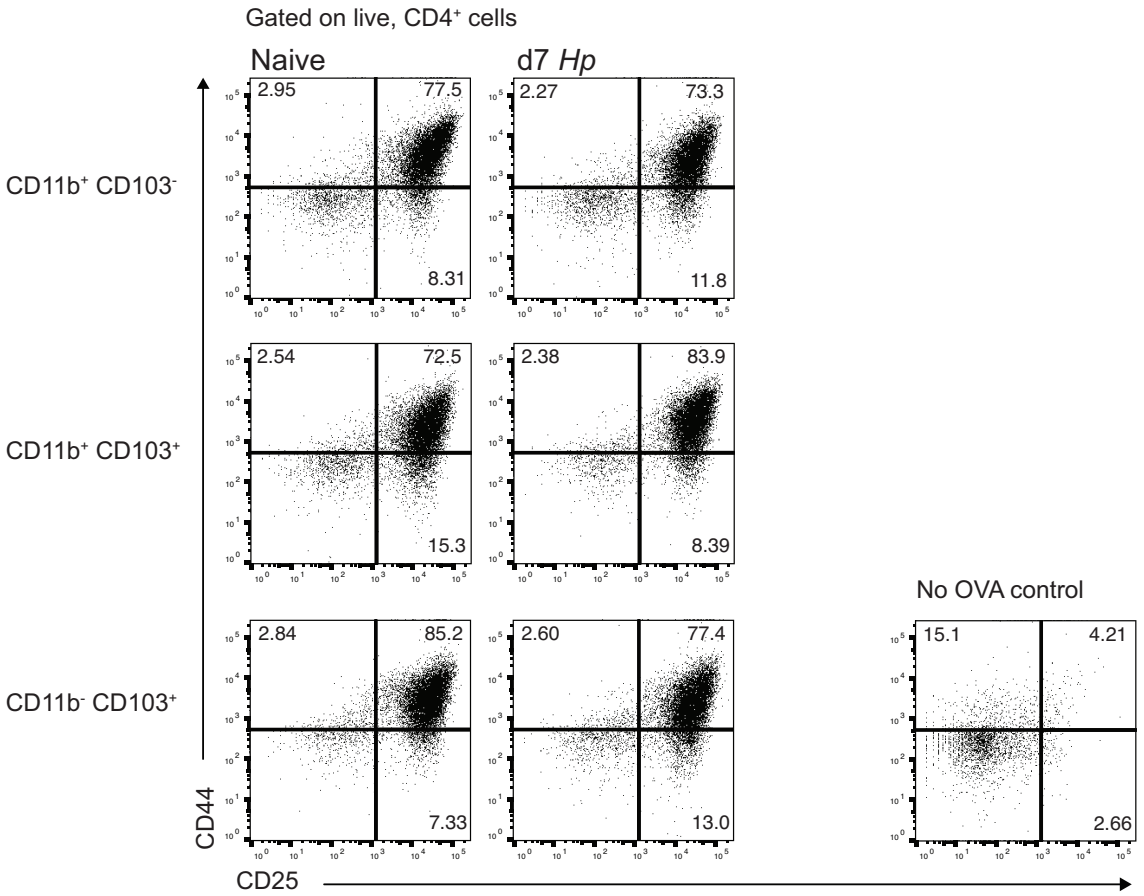
Supplemental Figure 4. FACS plots showing gating strategy used to sort CD11b+ DCs (which were either CD103- or CD103+) and CD11b- DC (which were all CD103+).



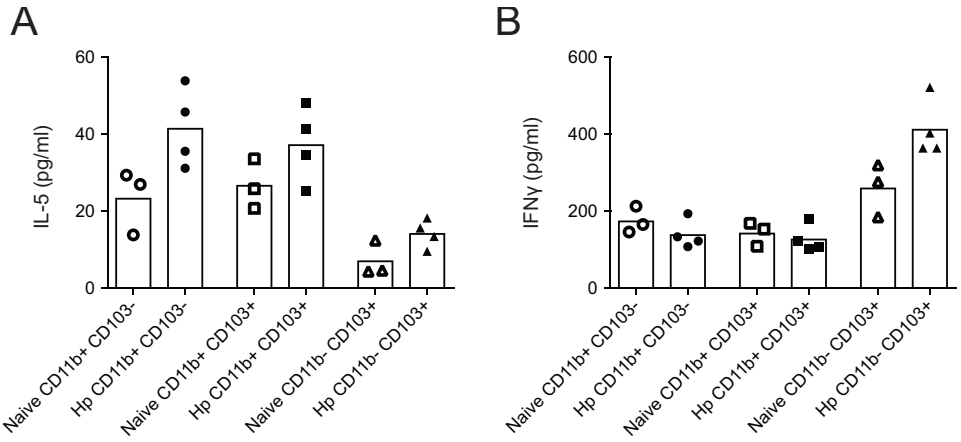
Supplemental Figure 5. CD11b$^-$ DC in the MLN express more IL-12/23p40 protein than CD11b$^+$ DC. Representative FACS plots showing IL-12/23 p40 protein expression within CD11b$^+$ and CD11b$^-$, CD64$^-$ CD11c$^+$ MHCII^{hi} MLN DCs, and graphs showing percentage of IL-12/23 p40 positive cells within the CD11b$^+$ and CD11b$^-$ DC populations. Statistical comparisons were performed using an ANOVA followed by Tukeys post hoc test (****$p < 0.0001$). Data shown are from a single experiment, representative of two independent experiments. Graphs show mean ± S.D.



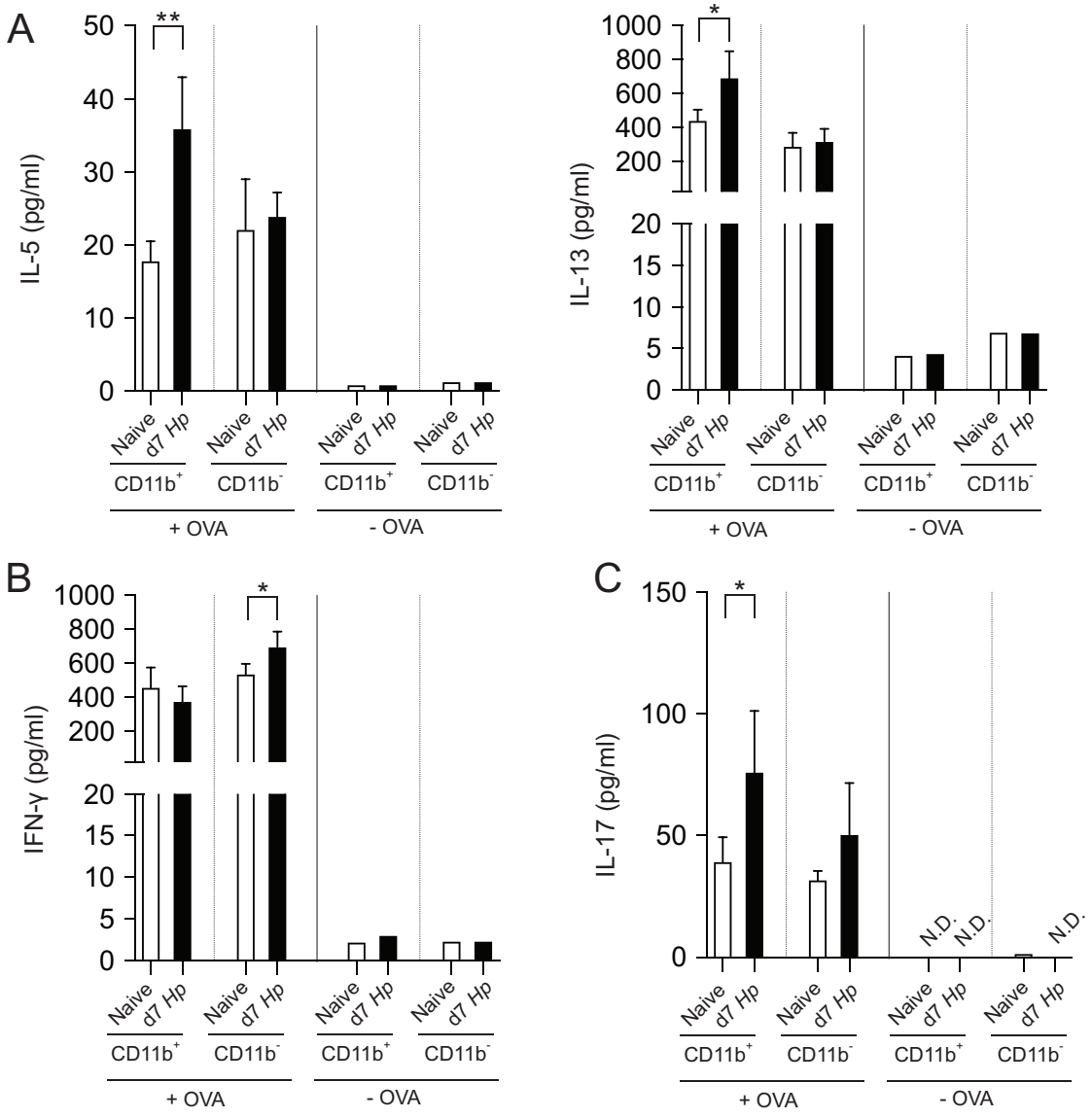
Supplemental Figure 6. The numbers of each DC subset in the MLN are unaffected by *T. gondii* infection. C57BL/6 mice were infected with T. gondii and the numbers of (A) CD11b^{+} CD103^{-}, (B) CD11b^{+} CD103^{+} and (C) CD11b^{-} CD103^{+} cells DCs in the MLN were quantified by flow cytometry at day 7 post-infection. Data are combined from two independent experiments with 4-5 mice per group. Bars show mean values.



Supplemental Figure 7. Both CD11b$^+$ and CD11b$^-$ MLN DCs activate T cells *in vitro*. CD11b$^+$ and CD11b$^-$ CD64$^-$ CD11c$^+$ DCs were sorted from MLNs of naive or *H. polygyrus*-infected mice and cultured with DO11.10 CD4$^+$ T cells in the presence of cognate antigen and IL-4 for 72 hours. Responder CD4$^+$ T cells were surface stained for CD25 and CD44. Representative FACS plots showing CD25 and CD44 expression on CD4$^+$ T cells cultured with either CD11b$^+$ CD103$^-$, CD11b$^+$ CD103$^+$, or CD11b$^-$ CD103$^+$ DCs from naive or *H. polygyrus* infected mice. The no OVA control is also shown. Data are from a single experiment, representative of two independent experiments with 4-5 mice per group in each one. Replicates are independent DC-T cell co-cultures using DCs purified from individual mice.



Supplemental Figure 8. CD103$^-$ CD11b$^+$ and CD103$^+$ CD11b$^+$ DCs behave similarly in DC-T cell co-cultures. CD11b$^+$ CD103$^-$, CD11b$^+$ CD103$^+$, and CD11b$^-$ CD103$^+$ CD64$^-$ CD11c$^+$ DCs were sorted from mLNs of naive or *H. polygyrus* infected mice, and cultured with DO11.10 CD4$^+$ T cells in the presence of cognate antigen and IL-4 for 72 hours. Concentrations of the cytokines (A) IL-5 and (B) IFNγ were assesed in the culture supernatant. Data are from a single experiment, representative of two independent experiments with 3-4 mice per group in each one. Replicates are independent DC-T cell co-cultures using DCs purified from individual mice. Bars represent mean values



Supplemental Figure 9. *In vitro* DC-T cell co-cultures shown with no antigen controls. CD11b$^+$ and CD11b$^-$ CD64$^-$ CD11c$^+$ DCs were sorted from MLNs of naive or *H. polygyrus*-infected mice and cultured with DO11.10 CD4$^+$ T cells in the presence or absence of cognate antigen and IL-4 for 72 hours. Concentrations of the cytokines (A) IL-5 and IL-13, (B) IFN γ and (C) IL-17A were assesed in the culture supernatant. Data from the groups + OVA are the same data as shown in Figure 5. Data are from a single experiment, representative of two independent experiments with 3-4 mice per group in each one. Replicates are independent DC-T cell co-cultures using DCs purified from individual mice. Graphs display mean ± S.D. Statistical comparisons were performed using an ANOVA followed by Tukeys post hoc test (*$p < 0.05$, **$p < 0.01$). N.D. Not determined (below limit of detection).