The role of dendritic cells during infection with the gastrointestinal parasite Trichuris muris

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The role of dendritic cells during infection with the gastrointestinal parasite *Trichuris muris*

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Abstract

Adaptive immune responses are critical for mediating sterilizing immunity and induce immunological memory during infection. Conventional dendritic cells (cDC) play a critical role in the induction of adaptive T cell responses, by acquiring and transporting antigens from tissues to local draining lymph nodes, where they are presented in order to activate and polarize T cells. The nature of the effector T helper (Th) cell response is dictated by signals from the cDC, and recent work has established that distinct cDC subsets can promote different Th cell responses. Adaptive Th2 responses promote immunity to helminth infections, while Th1 responses are associated with chronic infections; however, the role of cDC subsets in the generation of these responses is not fully understood. The aim of this thesis has been to determine the contribution of cDC subsets in the generation of adaptive immune responses to acute and chronic infection with the gastrointestinal nematode *Trichuris muris*.

In the first paper, we examined the role of IRF8-dependent cDC in intestinal homeostasis, by studying mice that are deficient in IRF8 specifically in CD11c-expressing cells, resulting in the absence of IRF8-dependent cDCs. Our results demonstrated a role of IRF8-dependent cDCs in the generation of Th1 responses in the intestinal mucosa. Consistent with this, mice lacking IRF8-dependent cDCs failed to become chronically infected following a Th1-associated low dose-infection with *T. muris*.

In the second paper, we assessed the role of IRF4-dependent cDCs in the generation of colonic and small intestinal Th2 responses, in two complementary models: live infection with *T. muris*, and subserosal injection of Th2-promoting *Schistosoma* egg antigens (SEA). In both models, we could demonstrate that IRF4-dependent cDCs are critical for induction of Th2 responses. In addition, we showed a regionally compartmentalized role of cDCs in this process, such that while $CD11b^{+}$ $CD103^{+}$ cDCs were the Th2-inducing subset after antigen delivery in the small intestine, $CD11b^{+}$ $CD103^{-}$ cDCs performed this task in the colon.

In the third paper, we further investigated the role of IRF4 and IRF8-dependent cDC during induction of adaptive Th1 and Th2 responses. Here, we extended out previous analysis by demonstrating that absence of IRF8-dependent cDCs and failure to support chronic infection indeed was associated with a failure to induce Th1 cells. Interestingly, this was associated with a strong increase in the production of Th2 cytokines, indicating that IRF8-dependent cDCs may act to antagonize Th2 responses. Furthermore, by generating bone marrow chimeric IRF4- and IRF8-double deficient mice, we could demonstrate that Th2 responses can be induced in the absence of IRF4-dependent cDCs, in the concurrent absence of IRF8-dependent cDCs.

Taken together, our work demonstrates that adaptive immune responses to Th2-associated, acute and Th1-associated, chronic infection with *T. muris* is mediated by IRF4- and IRF8-dependent cDC, respectively. In addition, our work highlights a potential antagonistic capacity of these cDC subsets in regulation of the induction of adaptive immune responses. Finally, we demonstrate that although IRF4-dependent cDCs are critical for induction of Th2 responses under normal conditions, this can be accomplished by additional antigen-presenting cells in the combined absence of IRF4- and IRF8-dependent cDCs. This work contributes to the understanding of how adaptive immune responses in the intestinal mucosa are controlled; however, further work will be required to unravel the underlying molecular mechanism controlling this capacity.

Key words: *T. muris*, Dendritic cells, T cells, Th1 and Th2, Intestinal helminth infection

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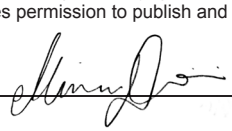
Signature  Date 2017-10-23

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Paper I

IRF8 Transcription-Factor-Dependent Classical Dendritic Cells Are Essential for Intestinal T Cell Homeostasis.

Katarzyna M. Luda, Thorsten Joeris, Emma K. Persson, Aymeric Rivollier, Mimoza Demiri, Katarzyna M. Sitnik, Lieneke Pool, Jacob B. Holm, Felipe Melo-Gonzalez, Lisa Richter, Bart N. Lambrecht, Karsten Kristiansen, Mark A. Travis, Marcus Svensson-Frej, Knut Kotarsky, William W. Agace

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Paper II

Different populations of CD11b+ dendritic cells drive Th2 responses in the small intestine and colon.

Johannes U. Mayer, Mimoza Demiri, William W. Agace, Andrew S. MacDonald, Marcus Svensson-Frej, and Simon W. Milling

*Nature Communication 2017 Jun 9;8:15820**

Paper III

Distinct DC subsets regulate adaptive Th1 and 2 responses during *Trichuris muris* infection.

Mimoza Demiri, Katarzyna Müller-Luda, William W.Agace, Marcus Svensson-Frej

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Abbreviations

APC	Antigen presenting cell
Batf3	Basic Leucine Zipper ATF-Like Transcription Factor 3
BM	Bone marrow
CCR	C-C chemokine receptor
CD	Cluster of differentiation
cDC	Conventional dendritic cell
CDP	Common dendritic cell progenitor
CLR	C-type lectin receptor
CX3CR1	Chemokine (C-X3-C motif) receptor 1
DAMP	Damage-associated molecular patterns
DC	Dendritic cell
DNGR-1	DC NK lectin group receptor-1
E/S	Excretory/Secretory
GALT	Gut associated lymphoid tissue
GM-CSF	Granulocyte-macrophage colony-stimulating factor
Id2	Inhibitor of dna binding 2
IEC	Intestinal epithelial cell
IEL	Intraepithelial lymphocyte
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
ILC	Innate lymphoid cells
ILF	Isolated lymphoid follicle
IRF	Interferon regulatory factor
KO	Knock-out
LI	Large intestine
LN	Lymph node

LP	Lamina propria
MDP	Monocyte-macrophage dendritic cell progenitor
MHC	Major histocompatibility complex
MLN	Mesenteric lymph node
NF-κβ	Nuclear factor kappa-light-chain-enhancer of activated B cells
NLR	Nod like receptor
OVA	Ovalbumin
PAMP	Pathogen associated molecular pattern
PRR	Pathogen recognition receptor
RA	Retinoic acid
RALDH2	Retinaldehyde dehydrogenase 2
RLR	RIG-I-like receptor
SI	Small intestine
SIRPα	Signal regulatory protein α
STAT	Signal transducer and activator of transcription
TCR	T cell receptor
TED	Transepithelial dendrites
TGF	Tumor growth factor
Th	T helper
TLR	Toll-like recetptor
TNF	Tumor necrosis factor
TSLP	Thymic stromal lymphopoietin
WT	Wild type
XCR1	X-C motif chemokine receptor 1
Zbtb46	Zinc finger and BTB domain containing 46

1. The intestinal mucosa and immunity

1.1 The intestinal mucosa

The intestine is a tube-like organ spanning from the oral cavity to the rectum, and represent the body's largest surface towards the environment. The intestine is divided into the small intestine, in which the majority of nutrient uptake occurs, and the large intestine, responsible for the absorption of water and excretion of waste products. In addition, the intestine is also housing a huge amount of commensal bacteria, in particular in the colon, which play important roles in immune development and function. Given the large surface area of the intestinal mucosa, together with the vast amount of food antigens and commensal and pathogenic microbes present in the intestinal lumen, it is not surprising that the intestinal mucosal contains the highest number of immune cells in the body.[1] Because of this enormous exposure of the outside world, there is a high demand on a highly organized and tightly regulated intestinal mucosal immune defences, which can act by either dampening immune responses towards harmless substances, or respond effectively upon pathogen encounter.

The first line of defence in the intestinal immune system consists of non-cellular and cellular barriers, consisting of the mucous layer and a monolayer of epithelial cells that protects the underlying tissue, the lamina propria. The lamina propria is populated by a large variety of immune cells, such as dendritic cells (DC), macrophages, lymphocytes and innate lymphoid cells (ILC), serving to recognize and respond to potential pathogenic insult. To their help, many of these immune cells are equipped with pattern recognition receptors (PRR),[2] germline-encoded receptors that recognize essential and conserved microbial molecules, so called pathogen-associated molecular pattern (PAMPs), e.g. bacterial cell-wall components or viral nucleic acids. PRR can be expressed in the membranes of the cell-surface or vesicles, e.g. toll-like receptors (TLR), or in the cytosol (e.g. NOD-like receptors and RIG-I-like receptors). In addition to microbial products, some PRR can also recognize endogenous molecules associated with tissue damage, damage-associated molecular patterns (DAMP), and so also respond to injury[3]. Binding of ligands to PRR mediates induction of pro-inflammatory responses, e.g.

recruitment and activation of immune cells by production of chemokines and cytokines, which serve to eradicate the infectious agent. In addition, PRR signalling can also trigger migration of DCs from the tissue to local lymphoid inductive sites to induce an adaptive immune response.[4]

1.2 The intestinal barrier

Pathogen entry into the intestinal mucosa is prevented by physiological as well as immunological mechanisms. The first physical barrier that needs to be breached by invading pathogens is the mucous layer, which protects the underlying epithelium from external damage.[5–8] This mucous layer is made up of heavy O-glycosylated glycoproteins, which can either be attached to the epithelial surface via a transmembrane domain, or form a loose gel-like mucus.[5,6] The intestinal epithelial layer itself serves as the second line of defence against the environment and protects the underlying lamina propria cells from direct contact with luminal contents. Epithelial cells are under constant replacement, and are exchanged every 4-5 days.[9] The cell types making up the epithelial layer consists of several distinct types, including enterocytes, enteroendocrine cells, Paneth cells, tuft cells and goblet cells,[10–13] all which are derived from $Lgr5^+$ stem cells located in the basal crypts.[14,15] In order to maintain a protective barrier, the integrity of the epithelial monolayer is further strengthened by various types of epithelial junction complexes which differ in their morphology and function,[16] and prevent diffusion of proteins and lipids across the epithelial layer.[17] Tight junctions, consisting of e.g. claudins and occludins, are critical for maintaining the barrier integrity, while permitting passage of small molecules.[18] Epithelial integrity is essential for homeostasis, and disruption may lead to pathological conditions varying from minor outcomes such as epithelial cell death, to more severe effects that could advance into inflammatory bowel disease (IBD).[19–29] In addition to being a physical barrier, and although not strictly belonging to the innate immune system, intestinal epithelial cells also have functions associated with immune cells. For example, epithelial cells have the ability to act as antigen-presenting cells (APC),[30] and to directly produce and secrete antimicrobial mediators into the intestinal lumen.[31] Furthermore, upon PRR activation, epithelial cells can release a variety of cytokines and chemokines;[32] in this way, the epithelial cells can interact with and direct the function of immune cells in the underlying lamina propria. In addition, antigen-presenting cells in the intestinal lamina propria, including macrophages and DCs, can also take up and present antigens derived from dying epithelial cells.[33,34]

1.3 Antigen recognition and uptake in the intestine

The intestinal lamina propria and epithelium are associated with several immune inductive sites, including the mesenteric lymph nodes (MLN), and gut-associated lymphoid tissues (GALT), consisting of Peyer's patches (PP) and isolated lymphoid follicles (ILFs). Antigen uptake into the intestinal mucosa can occur by a variety of mechanisms and routes. One of the best characterised ways of antigen uptake occurs via so called microfold (M) cells, which are located in the follicle-associated epithelium (FAE), covering Payer's patches and isolated lymphoid follicles. M cells differ in their microvillus structure as well as functionally compared to other epithelial cells,[35–38] and have the ability to transfer antigens or pathogens to immune cells in the underlying lymphoid structure, and even present endocytosed antigens to neighbouring cells.[38–40] Immature DCs, located in the basolateral pocket of M cells or in the subepithelial dome directly beneath the FAE of the PP, can phagocytose and present these delivered antigens to induce effector T cell responses. M cell-mediated antigen delivery and induction of mucosal immune responses towards certain bacteria and other luminal antigens seem to rely heavily on this passage.[41–43] Recently, goblet cells were also implicated as transporters of luminal antigens to DCs in the LP.[44]

In addition, it has been suggested that immune cells in the lamina propria are capable of directly sampling luminal antigens, by extending dendrites between epithelial cells to reach antigens in the lumen.[45,46] This capacity was shown to depend on the chemokine receptor CX3CR1, and was initially ascribed to dendritic cells.[45] However, recent evidence demonstrate that the majority of $CX3CR1^+$ cells in the intestinal lamina propria are tissue-resident macrophages;[47–49] it is therefore more likely that the dendrite-extending cells are macrophages rather than DCs. In line with this, intraepithelial $CD103^+$ DCs were shown to be poor at sampling luminal antigens as compared to tissue resident macrophages.[50] Furthermore, it has been suggested that macrophages that have acquired antigens via transepithelial dendrites can transfer these antigens to lamina propria DCs, via gap junctions.[51] Thus, lamina propria DCs can acquire luminal antigens by a variety of mechanisms, although the entire mechanism of how antigen uptake occurs is not fully understood.

2. Dendritic cells

2.1 Introduction

The discovery of DCs was made by Steinman and Cohn in the early 70's.[52,53] Their work laid the foundation for DC biology for decades to come, and awarded Ralph Steinman Alfred Nobel's prize in Medicine or Physiology in 2011. DCs were defined by their stellate morphology and their functional distinction from macrophages, and later studies have revealed these cells as critical players for initiation of adaptive T cell responses.[54] In addition, Janeway's ideas about self and non-self-antigen recognition also contributed to DC biology research.[55]

DCs can be grouped into two separate lineages, conventional DC (cDC) and plasmacytoid DCs (pDCs), based on ontogeny, transcription factor (TF) dependency and functionality. The identification of cDCs has been far from simple, and many of the markers originally used to separate cDCs from other immune cells, in particular macrophages, are in fact redundantly expressed by several cell types. Thus, both cDCs as well as macrophages express MHC-II and the integrin CD11c.[56] However, the use of a broader panel of markers has allowed distinction between these cell types. Thus, macrophages express F4/80,[57,58] CX3CR1[59–61] and CD64.[47] In contrast, cDCs are MHC-II^{+} CD11c^{+} but lack expression of F4/80[49] and CD64,[47] and do not express high levels of CX3CR1.[48] In addition, some cDCs can be identified by their expression of CD103.[62–64] Finally, in contrast to macrophages, cDCs can express the chemokine receptor CCR7 which allows them to migrate to the MLN where they can induce T cell activation and promote effector T cell migration to the intestinal mucosa by induction of gut-homing receptor expression.[48,59,65–67]

cDCs can be further divided into phenotypically and functionally distinct subsets. However, because surface marker expression may vary depending on activation status and localization, grouping cDCs based on their lineage commitment or requirement for certain transcription factors has been suggested to be a far more suitable method. Using this strategy, cDCs can be separated into two distinct lineages, cDC1 and cDC2, which depend on the transcription factors IRF8[68] and IRF4,[69,70] respectively. Moreover, cDC1 and cDC2 cells can be further distinguished based on their respective expression of XCR1[71,72] and SIRPα.[73]

2.2 Development of dendritic cells

DC progenitors are found in the bone marrow (BM) where they mature from hematopoietic stem cells into precursor cDCs (pre-cDC) in a series of intermediate precursors, including common monocyte progenitors (CMP), monocyte-macrophage DC progenitor (MDP) and common dendritic cell progenitor (CDP). The monocyte/DC axis separates when CDPs diverge from the MDPs, losing monocyte potential but allowing pDC and cDC development.[74–77] CDPs can develop towards either pre-pDCs or pre-cDCs, while later committed cDC precursors lack monocyte and pDC potential.[74,76] Originally, cDC1 and cDC2s were thought to develop into mature subsets after travelling via the blood to peripheral tissue where they would undergo Flt3L-dependent expansion and differentiate into fully mature subsets;[59,76,78–80] however, recent evidence suggests that the commitment to each branch might occur in the BM,[74,80–82] already at the CDP stage,[83] and with cDC1- and cDC2-specific progenitors (Fig 1). Consistently, recent studies identified IRF8 as a critical terminal selector for the maintenance of terminally differentiated cDC1s (Fig 1).[84]

ZBTB46 is currently the only transcription factor that separates committed cDCs from other mononuclear phagocytes.[85,86] The distinct expression of *Zbtb46* in cDCs was defined in *Zbtb46*-DTR mice, which display inhibited development of all cDC subsets.[86] *Zbtb46* gene expression is initiated in pre-cDCs, and maintained in subsequent differentiated cDC subsets. The mechanism by which *Zbtb46* allows for commitment to the cDC lineage is mediated, at least in part, by restricting the expression of myeloid growth factor receptors and thus only allowing cDC commitment.[85] cDCs also require certain growth factors for their development and maturation process. The development of cDCs is dependent on the growth factors GM-CSF and Flt3L,[79,87–90] and mice which are either Flt3L-deficient or receive Flt3L inhibitors have severely reduced numbers of cDCs and pDCs.[47,91,92] Development of cDCs from BM cells *in vitro* can be accomplished by addition of Flt3L[93] or GM-CSF.[88,94,95] While GM-CSF is required for the development of cDCs in peripheral tissue,[59,60,96,97] it does not seem to be important for lymph node (LN)-resident cDCs.[98,99]

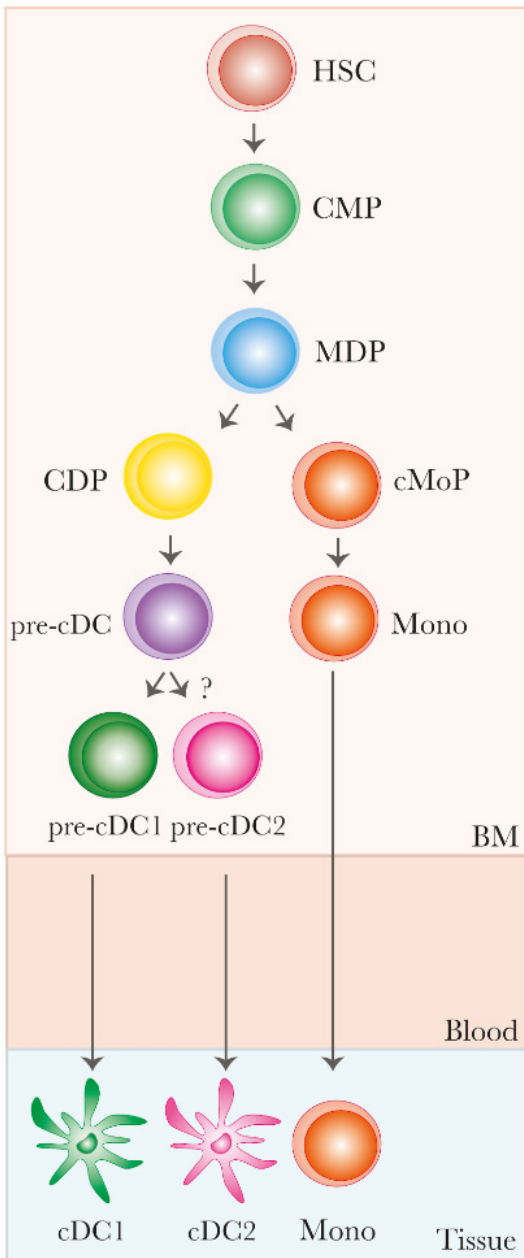

Figure 1. Development of the cDC1 and cDC2 lineage
cDCs develop in a series of events, including the development of common DC progenitors (CDP) from macrophage DC progenitors (MDP), which further develop into pre-cDCs. Lineage commitment to cDC1 and cDC2s is suggested to occur in the BM and not in the periphery, as previously thought.

2.3 Dendritic cell activation, maturation and migration

Pre-cDCs leaving the BM can localize to lymphoid organs to become lymph node-resident cDCs, or enter peripheral organs such as the intestine where microenvironmental cues induce expression of distinct surface markers.[100] LN-resident and migratory cDCs can be separated based on the level of expression of MHC-II, with LN-resident cDCs having intermediate expression of MHC-II (MHC-II^{int}), while cDCs that have migrated from the tissues to local LNs are defined by their high expression of MHC-II (MHC-II^{hi}).[100,101] Immature DCs are efficient at sampling antigens, while being less efficient at antigen-presentation. cDCs mature in a series of events, initiated upon sampling of the environment through PRRs such as TLRs, in addition to the process of antigen uptake and processing.[102–105] The maturation process involves upregulation of the antigen-presenting molecules MHC-I and –II[106,107] and costimulatory molecules, including CD80[108], CD86[109] and CD40.[95,110–112] In addition, maturation also induces increased expression of the chemokine receptor CCR7[113–116] on the surface of cDCs, which allows their recruitment from the periphery, via afferent lymphatics, to the T cell area of the draining lymph nodes by expression of the CCR7 ligands CCL19 and CCL21, released by lymphatic endothelial cells and LN stroma cells.[117–122]

2.4 T cell activation

Activated DCs in the periphery migrate to associated lymphoid tissue and LNs where they present antigen to naïve T cells. Activation of naïve T cells and differentiation of effector cell subsets require three signals. Signal 1 and 2 are provided by the antigen-presenting DC, in the form of antigenic peptide/MHC, and costimulatory molecules, respectively, expression of which are upregulated following signalling via pattern recognition receptors. Cytokines, derived either from the antigen-presenting DC or adjacent cells, and typically referred to as "signal 3", determines the type of effector T cell response induced. CD4$^+$ T helper cells can in this way differentiate into Th1, Th2, Th17, Tfh and Treg cells. In addition, DCs also induce the capacity of effector T cells to home to the relevant tissue, by induction of tissue-specific homing receptor expression during T cell activation.[48,59,65–67]

T helper cell effector responses can be tailored towards the type of infection giving rise to T cell activation. Thus, Th1 cells have the important task of eradicating intracellular pathogens, e.g. by producing IFNγ[123] that has the ability to activate microbicidal activity in macrophages and by inducing isotype class switching to IgG2a/IgG2c.[124] Differentiation of Th1 cells requires IL-12-production by the

antigen-presenting cell,[125] which drives generation of the transcription factor T-bet and in turn mediates IFNγ production.[126] Th2 cells, on the other hand, are associated with multicellular parasite infection, and may also play a pathogenic role during asthma and allergic reactions.[127,128] Th2 cells produce the effector cytokines IL-4, IL-5 and IL-13, which can regulate the function of various immune and non-immune cells, and promote class switching to IgG1 and IgE.[123,124,129–131] Th2 cell development from naïve CD4 T cells is promoted by signalling via the IL-4R, which leads to activation of STAT6 that promotes expression of the Th2-associated transcription factor GATA3.[132–137] Production of IL-4 by T cells is further regulated by the transcription factor c-Maf,[138,139] which promotes Th2 differentiation through an IL-4-dependent mechanism.[140] Finally, Th17 responses are typically associated with infection with fungi and certain extracellular bacteria.[141] In addition, Th17 cells have also been shown to be involved in the pathogenesis of IBD, multiple sclerosis and rheumatoid arthritis.[142] Th17 cells produce the signature cytokine IL-17, which e.g. mediate neutrophil recruitment, and IL-22 that can protect the intestinal epithelium upon injury or infection by bacteria.[143,144] Development of Th17 cells is driven by the transcription factor RORγt[145] and require TGFβ,[146] and occasionally IL-6.[147,148] In addition, IL-23[149] and IL-1β can reinforce an ongoing Th17 response.[148] Finally, the signature cytokines produced by some of these subsets, such as those derived by Th1 and Th2 cells, can antagonize the differentiation and function of the other subsets,[126,150,151] demonstrating the delicate balance between the ratio of these polarizing cytokines.

In addition to Th1, Th2 and Th17 cells that localize to peripheral tissues to exert their effector functions, T helper cells can also differentiate into FoxP3-expressing regulatory T cells (Treg) and T follicular helper cells. Tregs promote regulation and maintenance of peripheral tolerance to prevent autoimmune diseases and inflammatory conditions e.g. by production of IL-10 and TGFβ[152], while Tfh cells provide help during B cell activation.

2.5 Intestinal dendritic cells

In addition to being divided into cDC1 and cDC2 cells, cDCs in the intestinal lamina propria can also be defined and subdivided by their expression of the integrins CD103 and CD11b, into four distinct subsets: $CD103^{+}CD11b^{-}$ (CD103 SP), $CD103^{+}CD11b^{+}$ (DP), $CD103^{-}CD11b^{+}$ (CD11b SP) and $CD103^{-}CD11b^{-}$ (Fig 2).[153] These subsets are unevenly dispersed along the intestinal mucosa,[59,64,101] with DP DCs found at highest frequency in the small intestine, while CD103 SPs are more numerous in the colon.[64,73] DP and CD103 SP DCs are the major populations of cDCs in the small intestinal lamina propria, accounting for 85-90% of migratory

cDCs. In contrast, DP cDCs only account for a minor population of migratory cDCs in the colon, with CD103 and CD11b SPs being the major cDC populations.[101] All four subsets are able to migrate from the intestinal lamina propria to the ascending draining MLN where they prime naïve T cells.[101]

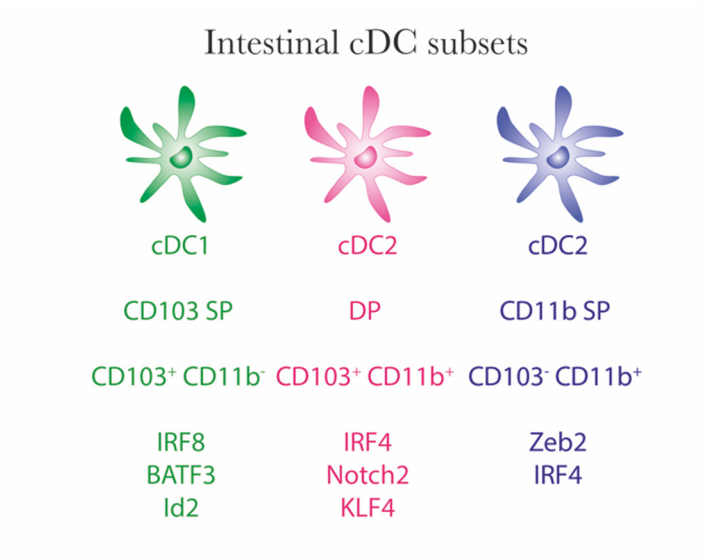

Figure 2. Intestinal cDC subsets and their transcription factor dependency.
Schematic overview of the major cDC subsets found in the small and large intestinal lamina propria and MLN, and their dependency on distinct transcription factors for their development.

2.6 Irf8 and Irf4-dependent cDC1s and cDC2s

IRF8-dependent cDC1s

CD103 SP cDC in the intestine mucosa express XCR1, and are classed as cDC1 cells. Development of cDC1s depend on the transcription factors Batf3, IRF8 and Id2;[154] hence, cDC1s express Batf3 and IRF8, and are completely absent in BAFT3$^{-/-}$ and IRF8$^{-/-}$ mice.[155,156] Several surface markers have been associated with cDC1 cells in addition to CD103,[157,158] including CD8α,[159] XCR1[71,72] and DNGR1.[160–162] The chemokine receptor XCR1 is a highly specific marker and particularly useful marker for cDC1s as it is expression by human as well as mouse cDC1s.[71,163,164] Although DNGR1 has also been proposed as a useful marker for their definition,[160–162] it is however also expressed by all committed cDC precursors.[165] cDC1s found in lymphoid organs such as LNs and the spleen express CD8α.[158]

cDC1s are acknowledged for their efficient presentation of antigens to $CD8^+$ T cells[63,71,155,162,166–171] and as a source of IL-12 during both steady-state conditions as well as during infection or immunological challenge, which is required for efficient Th1 responses.[63,68,172–175] Additionally, cDC1s are critical for tolerogenic responses, accomplished by their superior ability to generate TGFβ and induce Tregs.[176] Thus, cDC1s express high levels of the TGFβ-activating integrin αvβ8,[68,177] which is required for the maturation of inactive TGFβ to its active form.[178,179] Mice with a DC-specific deficiency in αvβ8 develop colitis because of, at least in part, the inability of these DCs to induce or maintain Tregs.[180] $CD103^+$ are also efficient producers of retinoic acid (RA),[68,181] which is required for the generation of Tregs,[182] and critical needed for the imprinting of gut homing molecules on T cells.[183–185]

IRF4-dependent cDC2s

While cDC1 cells in the intestinal mucosa and associated lymphoid tissues appear to consist of one uniform population, cDC2s can be divided into DPs ($CD103^+$ $CD11b^+$) and CD11b SPs ($CD103^-$ $CD11b^+$), based on their surface marker expression. cDC2s can also be defined by expression of SIRPα. cDC2s depend fully or partially on Irf4, Notch2, Zeb2 and Klf4 for their development.[186–190]

IRF4 has a critical role for the homoeostasis of DPs in the intestinal mucosa by regulating the development and survival of this subset.[69,70] In contrast to IRF8, which is critical for the development of cDC1,[68] mice with a CD11c-specific deletion of IRF4 retained a population of DP cDCs in the intestinal lamina propria, although at lower numbers, while the number of DP cDCs in the MLN was severely reduced.[69] This was shown to be due to impaired survival of DP cDCs in the absence of IRF4 expression.[69] Similarly, mice with Notch2- or KLF4-deficiency in $CD11c^+$ cells display a reduced frequency of intestinal DPs,[188,191] while DPs remain unaffected by deficiency in IRF8 or Batf3.[80,158] IRF4 is also required for the functionality of cDCs, including during the migration of cDCs from the skin to the draining lymph nodes via CCR7[192] and for antigen presentation via the MHC-II complex.[193] Although DP cDCs are capable of presenting antigens to $CD8^+$ T cells,[101] they preferentially express antigens to $CD4^+$ T cells.[170] Consistently, IRF4-competent, but not IRF8-competent cDCs, are able to efficiently prime OT-II cells upon OVA administration *in vivo*.[193] Furthermore, cDC2 appear to be preferentially involved in the generation of adaptive Th17 and Th2 responses. Hence, mice with deficiency of IRF4, SIRPα, or Notch2 in $CD11c^+$ cells have impaired intestinal Th17 responses during steady state,[69,70,73,187] while mice with a KLF4-deficiency display aberrant Th2 responses upon infection or immunization with *S. mansoni* soluble egg antigens (SEA).[188]

While CD11b SPs appear to be partly dependent on IRF4, as demonstrated by the reduction of this population in mice with IRF4-deficient $CD11c^+$ cells,[69,194] they are

most likely Notch2-independent.[187,191] Compared to DPs, CD11b SP DC express higher levels of SIRPα in the MLN and intermediate levels of CX_3CR1.[154] Recently, Zeb2-deficiency in $CD11c^+$ cells was shown to be important for the development of CD11b SPs in the intestine, by repressing expression of Id2 that is associated with development of cDC1 cells.[195] Furthermore, a proportion of CD11b SPs express the monocyte-associated chemokine receptor CCR2.[194] CCR2 is well-known for its role during egress of $Ly6C^{hi}$ monocytes from the BM;[196] however, $CCR2^+$ CD11b SPs in the MLN represents true cDCs and do not derive from $Ly6C^{hi}$ monocytes.[194] $CCR2^+$ CD11b SPs are found in both human and mouse intestine and are involved in driving Th17 responses,[194] which is consistent with the observation that KLF4-deficient mice have normal Th17 responses but reduced numbers of DPs, indicating that Th17-induction is indeed IRF4-dependent but may not depend entirely on the DP cDC subset.

3. *Trichuris muris*

3.1 Introduction

Intestinal helminth infections cause severe health issues in over 1 billion people worldwide,[197] with children being the most targeted group.[197,198] Over the past fifty years, infection of rodents with the gastrointestinal nematodes *Nippostrongylus brasiliensis*, *Heligmosomoides polygyrus*, *Trichinella spiralis* and *Trichuris muris* have served as valuable models to study the induction of adaptive immunity and parasite expulsion mechanisms.[199–201] *T. muris* is a large intestinal nematode parasite, closely related to the major human pathogen *T. trichiura* that infect approx. 500 million people worldwide.[202] Similar to many other nematode infections, successful immune responses to *T. muris* infection relies on induction of a Th2 response, in particular by the production of the cytokine IL-13,[203] which ultimately leads to expulsion of the worm prior to adulthood.[204] The Th2 response promotes a variety of effector responses by cells in the local tissue, for example increased epithelial turnover,[205,206] goblet cell hyperplasia[207] and enhanced mucus production,[208–210] which together create an unfavourable niche for the parasite. Induction of adaptive type 2 responses to *T. muris* infection occurs primarily in the local intestine-draining mesenteric lymph nodes,[211,212] and is thought to be mediated by migratory cDCs that localize to these organs after acquiring parasite-derived antigens in the infected tissue,[213] although the precise mechanism by which adaptive Th2 responses are induced, and the role of cDCs and cDC subsets in this process, is not yet fully understood.

T. muris infection is initiated upon ingestion of infectious eggs and proceeds with larvae hatching in the caecum – a process that in mice depends on the presence of bacteria, and is mediated at least partly by bacterial type 1 fimbriae.[214] Next, the anterior end of the larvae invades the epithelial layer of the caecum and proximal colon.[215] This is likely facilitated by the secretion of proteases from the stichosome, an organ in the anterior part of the worm, which can degrade mucins[216] and extracellular matrix proteins.[217] The worms form so called syncytial tunnels by burrowing through adjacent epithelial cells, where they remain embedded throughout their entire life cycle.[215,218,219] Early studies of *T. muris* excretory/secretory (E/S) antigens revealed that soluble antigens from the anterior end of adult worms were the most immunogenic as compared to the posterior end

and whole larvae.[220] In addition to the mucus-degrading proteases, one of the best categorized proteins in *Trichuris* E/S is a pore-forming protein.[221] E/S antigens from swine-specific *T. suis*, are capable of degrading the tight junction proteins between intestinal epithelial cells and in this way weakening the epithelial barrier integrity,[222] however a potential similar function of *T. muris* E/S antigens remains to be demonstrated. Finally, while parasite-derived products from other nematodes have been suggested to have a direct Th2-inducing capacity, such as for example products in *S. mansoni* SEA[223,224], no direct such capacity has been shown for *T. muris*-derived E/S antigens.

3.2 Resistance & Th2 responses

The adaptive immune response towards *T. muris* infection can be of either a Th1 or Th2 type and is determined by several factors including genetic background and infection dose. The genetic background of the infected host is an essential determinant for the outcome of resistance or susceptibility and is driven by MHC-II-linked genes. Alleles promoting either resistance, H-2q, or susceptibility, H-2k, during infection are found in the I-A region.[225–228] The infection dose is also a determinant in the regulation of adaptive immune responses to *T. muris* infection. Thus, the nature of the ensuing adaptive response can be intentionally modulated by infection with a high (typically 100-400 eggs) or low dose (20-40) of eggs, with high dose resulting in generation of a Th2 response and resistance, while low dose infection of normally resistant C57BL/6 and BALB/c mice results in induction of a Th1 response and chronic infection.[229,230] Interestingly, trickle infections, i.e. repeated low dose infections, which are thought to better mimic the parasite exposure in infected humans, leads to the build-up of a large enough egg load to drive induction of a Th2 response and expulsion,[231] with a similar phenomenon also seen in wild-living mice.[232]

Resistance to infection is associated with type 2 immunity and consequently characterized by production of the cytokines IL-4, IL-5, IL-9 and IL-13,[204,233–237] which promotes protective immunity through several mechanisms. IL-4 and IL-13 share the usage of the IL-4Rα receptor,[233,238,239] which has been shown to be a critical for initiation of protective Th2 responses during infection with *T. muris*.[237,240] Thus, antibody-mediated blockade of the IL-4 receptor prevented expulsion, associated with increased IFNγ levels, while reduced levels of Th2-associated cytokines.[237,241] The role of IL-4 was further supported by experiments conducted in genetically susceptible AKR mice, which became resistant when administered with an IL-4 complex, containing a mixture of rIL-4 and anti-IL-4 mAb.[237] Interestingly however, while IL-4 deficiency in C57BL/6,[233] and male

BALB/c mice,[234] rendered the mice susceptible to infection, IL-4-deficient BALB/c females remained capable of mediating expulsion, due to production of high levels of IL-13.[234] The same study found that IL-13-blockade in resistant IL-4-deficient BALB/c females prevented expulsion, while IL-13 administration to IL-4 knock-out males induced resistance.[234] However, while both IL-4 and IL-13 are involved in the Th2 response, IL-13 appears to be more directly involved in mediating worm expulsion. The production of IL-13 drives several responses by the colonic epithelium that promotes expulsion, including goblet cell hyperplasia and mucus production.[233,241,242] Additionally, IL-13 also positively regulates epithelial cell turnover,[205] in balance with IFNγ, which promotes enhanced epithelial differentiation within the crypts of Lieberkühn in the large intestine.[243] Similarly, IL-9 has also been suggested to be involved in the effector response to *T. muris*, by induction of smooth muscle contractility that contributes to a more efficient expulsion of *T. muris* worms.[235]

3.3 Chronicity & Th1 responses

Chronic infection with many human[244] and rodent parasites[245] is often associated with adaptive Th1 responses, with a beneficial outcome for the parasite in which it is able to stay within its host and reproduce. Consistently, adaptive responses to chronic *T. muris* infection in susceptible mice, such as AKR mice, are characterized by production of the typical Th1-associated cytokines IFNγ, IL-12 and IL-18.[229,237,246–251] The role of these cytokines in the establishment or maintenance of chronicity has been elegantly demonstrated in studies using administration of either blocking antibodies or recombinant cytokines. Thus, genetically susceptible AKR mice became resistant and expelled parasite worms upon IFNγ neutralization.[237] IL-12 is similarly important for Th1 responses during infection, as IL-12 administration led to the establishment of chronicity and increased worm burden in normally resistant mice.[248] Interestingly, depletion of IFNγ in IL-12-treated mice inhibited chronicity, demonstrating that IL-12-mediated chronicity was dependent on IFNγ.[248] IL-18 has also been proposed to regulate Th1 responses in *T. muris*-infected mice as IL-18-deficient mice are resistant to infection[247]. However, the role of IL-18 appear to be more associated with downregulation of IL-4 and IL-13, and does not rely on the capacity to induce IFNγ, as demonstrated by unaltered IFNγ levels upon IL-18 administration, while reduced levels of IL-13 and induction of resistance.[247]

3.4 Induction of adaptive immunity to *T. muris* infection

3.4.1 The role of the intestinal epithelium

The immune response to *T. muris* is first initiated at the intestinal epithelium, the first host cell type targeted during infection. However, already the mucus barrier covering the epithelial layer in the intestine plays an important role during infection with *T. muris*.[242] The type 2 response to infection is associated with IL-13-induced goblet cell hyperplasia,[208–210] and increased production of Muc2, the dominant mucin type under normal conditions, in resistant but not susceptible mice.[241] Moreover, the protective response in resistant mice is also associated with production of Muc5ac, a mucin normally not found in the intestinal mucosa, also under the control of IL-13.[241] Importantly, Muc5ac-deficient mice are unable to expel worms despite an ongoing robust Th2 response,[241] demonstrating that the Th2 response is insufficient for expulsion to occur when the mucous barrier is deprived of Muc5ac. Recent studies also suggest that mucin sulphation, regulated by IL-13, promotes expulsion by modification of the mucin so that it becomes less vulnerable for degradation by parasite-derived E/S antigens,[252] thus further high-lighting the importance of a protective mucus layer.

Because of the close association of *T. muris* worms and their intestinal epithelial niche, it is not surprising that various studies have highlighted a critical role for the epithelium in the direction of type 2 responses.[253–258] Although larvae never breach the basement membrane and lives in close contact with the intestinal epithelium in so called syncytial tunnels, they must re-invade new epithelial cells at the apical side to maintain their niche.[215] PRR are found throughout the epithelium and act as important sentinels responsible for recognizing foreign PAMPs, yet, no *T. muris*-associated PAMP or corresponding critical PRR has yet been identified. However, a role for TLR4 and Myd88 signalling has been suggested during chronic *T. muris* infections.[259] Although the underlying mechanism is yet to be determined, it was suggested that TLR4-induced MyD88 signalling was triggered in response to luminal bacteria due to the reduced epithelial integrity.[259] Finally, protective immunity to *T. muris* has been demonstrated to involve NF-κβ activation in epithelial cells, as mice with a loss of NF-κβ signalling in intestinal epithelial cells via a deletion of IKK-β, the catalytic subunit needed for NF-κβ signalling, were susceptible to *T. muris* infection,[256] although the trigger(s) and pathway(s) leading to NF-κβ activation remains to be determined.

The intestinal epithelium is an important regulator of the immune response upon activation by production of chemokines and cytokines that subsequently act by recruiting and activating immune cells.[260] Several chemokines have been implicated during infection with *T. muris*. The expression of the chemokines CCL2, CCL3,

CCL5 and CCL20 are increased in resistant, but not in susceptible mouse strains, and have been shown to attract DCs to migrate towards the epithelial layer in the colon of resistant mice.[213] Furthermore, expression of CCL2 is reduced in Myd88$^{-/-}$ mice,[261] and CCL2$^{-/-}$ mice fail to expel parasite worms and display reduced levels of IL-4, while increased levels of IL-12.[212] Epithelial-derived cytokines may also contribute to the induction of appropriate immune responses during nematode infection.[201,262] The epithelial-derived cytokines IL-25, IL-33 and TSLP have been demonstrated to play an important role for induction of Th2 responses during infection with *T. muris*,. Thus, TSLP promote Th2 responses, as demonstrated by studies showing delayed expulsion and reduced Th2 cytokines following administration of anti-TSLP antibodies or using TSLP-R deficient mice.[258] Similarly, IL-25 knock-out (KO) mice were susceptible to infection and displayed reduced Th2 cytokine responses,[263] while IL-25 administration to normally susceptible AKR mice was sufficient to confer resistance,[253] suggesting that IL-25 is also of importance for protective responses against *T. muris* infection. Furthermore, IL-25 promotes the production of IL-4, IL-5 and IL-13, as demonstrated by the increased secretion of these cytokines, by Th2 cells and ILCs, in lung and intestinal tissue of mice upon administration of IL-25.[257,258,264–268] Recently, epithelial tuft cells were suggested to be the dominant source of IL-25 in the intestine, which could be further enhanced during infection with the nematodes *H. polygyrus*, *N. brasiliensis* and *T. spiralis*.[266–268] However, the role of tuft cells in production of IL-25 during *T. muris* infection has not yet been investigated. In addition to TSLP and IL-25, type 2 responses can also be activated upon release of the alarmin IL-33.[269,270]. Thus, administration of IL-33 in mice induces IL-4, IL-9 and IL-13 production in mucosal tissue.[269–271] Moreover, IL-33 is upregulated early during *T. muris* infection in resistant mice,[255] and has been shown to induce expulsion during infection with some[272–274] but not all intestinal parasite infections.[255,269,273,275] Thus, epithelial-derived mediators are critical players in bridging the innate immune response with adaptive responses, by secreting cytokines and chemokines, which can attract DCs and, directly or indirectly, promote initiation of protective Th2 responses.

3.4.2 Innate type 2 responses and other type 2-accessory cell types

While it is clear that *T. muris* infection causes the production of epithelial cytokines, including IL-25, IL-33 and TSLP, the target cells of these cytokines are not clear. The release of epithelial-derived cytokines is known to influence the function of various cells in the lamina propria, including DCs and ILC2s. ILC2s lack antigen-specific receptors but can instead be activated in response to epithelial cytokines, and in turn promote immunity by production of type 2 cytokines, in particular IL-5 and IL-13.[276–278] Because of their exceptional ability to initiate and amplify type 2

responses, ILC2s have been implicated during infection with several helminth parasites.[263,279–284] While their contribution during infection with *T. muris* is not yet clear, a population of so called MMP^{type2} cells, defined as Lin^{-} $Sca\text{-}1^{+}$ and $c\text{-}kit^{int}$, were shown to be important for protective responses during *T. muris* infection,[263] although it remains to be determined if these cells are true ILC2s.

Mast cells, basophils and eosinophils have the capacity produce IL-4, and may be involved during induction of Th2 responses.[285–291] In fact, basophils were suggested to act as antigen-presenting cells during *T. muris* infection,[292] although later studies has demonstrated that DCs are critical for induction of Th2 responses following *S. manosni* infection,[293] suggesting that basophils are more likely to play an accessory role during induction of Th2 cells. Mast cells play an important role for the protection against a range of intestinal parasites, including infections with *N. brasiliensis,*[294] *H. polygyrus bakeri*[295] and *T. spiralis.*[296–298] While initially shown to be dispensable for *T. muris* expulsion,[299] more recent studies have suggested that they may play a role in the initiation of optimal Th2 responses.[300] In addition, mast cells accumulate in the colonic epithelium during high dose *T. muris* infection and remain there after worm expulsion, indicating a potential role of these cells during the resolution phase.[301] Finally, while IL-4-producing eosinophils are found at increased frequencies in both the MLN and colon during *T. muris* infection they are dispensable for generation of Th2 responses and worm expulsion.[291,302]

3.4.3 The role of DCs

While DCs have been implicated in several helminth infection models,[303] including infection with *S. mansoni* and *H. polygyrus,*[293,304,305] the role of cDCs during infection with *T. muris* has so far been inadequately examined. Previous experiments have demonstrated accumulation of a population of $CD11c^{+}$ $B220^{-}$ cells, suggested to be DCs, in the MLN during *T. muris* infection.[211] Furthermore, $CD103^{+}$ DC are recruited to the colonic epithelium in response to epithelial-derived chemokines selectively in resistant mice.[213,306] More recently, SHIP-1, which regulate the PI3K pathway, was shown to be crucial in DCs for protective Th2 responses during *T. muris* infection, as demonstrated by increased IL-12 production by DCs,[307] however, the role for SHIP-1 in DCs has not yet been determined in depth.

Thus, given the gap in our knowledge on the role of DCs during *T. muris* infection, the aim of this thesis has been to investigate the contribution of cDC and cDC subsets in the induction of adaptive immune responses to chronic or acute *T. muris* infection.

4 Aims of this thesis

The overall aim of this thesis has been to study the role for conventional dendritic cell subsets in the generation of adaptive Th1 and Th2 responses during acute and chronic infection with the gastrointestinal nematode *Trichuris muris*. More specifically, my aims were:

- To determine the role of IRF4-dependent cDCs in the generation of adaptive Th2 responses during acute infection with *T. muris*, through high-dose infections in *CD11c-cre.Irf4*$^{flox/flox}$ mice, which have a substantial reduction of intestinal CD103^{+}CD11b^{+} cDCs.
- To determine the role of IRF8-dependent cDCs in the generation of adaptive Th1 responses during chronic infection with *T. muris*, through low-dose infections in *CD11c-cre.Irf8*$^{flox/flox}$ mice, which lack intestinal CD103^{+}CD11b^{-} cDCs.
- To assess the immune response to *T. muris* infection in the combined absence of the transcription factors IRF4 and IRF8 in CD11c^{+} cells, by low-dose infections of bone marrow-chimeric *CD11c-cre.IRF4*$^{flox/flox}$*.IRF8*$^{flox/flox}$ mice.

5 Summaries of papers I-III

Paper I:

IRF8 Transcription-Factor-Dependent Classical Dendritic Cells Are Essential for Intestinal T Cell Homeostasis.

Background/Aim:

IRF8-dependent $CD103^+$ $CD11b^-$ DC represent one of the major cDC populations in the small and large intestinal mucosa. Despite this, the role of IRF8-dependent cDC in intestinal homeostasis and during induction of adaptive immune responses in intestinal-draining MLN remain largely unknown. Here, we utilized mice with a CD11c-specific deletion of IRF8 to examine the consequence on immune cell homeostasis, and on induction of adaptive T cell responses following antigen immunization and during live infection with the gastrointestinal nematode *T. muris*.

Results:

- CD11c-cre.*IRF8*$^{fl/fl}$ mice lack $CD103^+CD11b^-$ cDC in the small intestinal lamina propria and MLN, and $CD8\alpha^+$ lymph node-resident cDC
- CD11c-cre.*IRF8*$^{fl/fl}$ mice displayed dramatically reduced numbers of $CD45^+$ IEL, including $CD8\alpha\beta^+$ $TCR\alpha\beta^+$ and $CD4^+$ $TCR\alpha\beta^+$ IELs, and fail to generate $CD4^+CD8\alpha\alpha^+$ IELs due to the absence of β8 integrin-expressing IRF8-dependent cDC
- CD11c-cre.*IRF8*$^{fl/fl}$ mice display impaired cross-presentation and induction of gut-homing receptors on $CD4^+$ and $CD8^+$ T cells
- CD11c-cre.*IRF8*$^{fl/fl}$ mice display reduced numbers of $IFN\gamma^+$ cells in the small intestinal lamina propria, and impaired generation of Th1 cells in the MLN
- Finally, CD11c-cre.*IRF8*$^{fl/fl}$ mice fail to support chronic infection and produce parasite-specific IgG2c antibodies following low dose-infection with *T. muris*

Discussion

TCRαβ IELs in the intestinal mucosa are tissue-resident memory T cells which may be equipped with cytolytic functions that can quickly react for the befit to fight pathogens or curtail overt inflammatory responses, thus contributing to a maintained homeostasis.[308] Here, we demonstrate a critical role for $CD103^+CD11b^-$ cDCs in the maintenance of $CD8\alpha\beta^+$ and $CD4^+CD8\alpha\alpha^+$ IELs, as the numbers of these IEL subsets were severely reduced in mice deficient in IRF8-dependent cDC. The reduction of $CD8\alpha\beta^+$ IEL was associated with an impaired capacity of IRF8-dependent cDC to cross-present antigens and induce gut-homing receptor expression on responding $CD8^+$ T cells. In contrast, the reduced number of $CD4^+$ $CD8\alpha\alpha^+$ IEL was caused by the absence of β8 integrin, which is expressed selectively on IRF8-dependent cDC.[68] β8-integrin can mediate activation of the biologically active form of TGFβ, which has previously been shown to be required for induction of $CD4^+$ CD8αα IEL.[309,310] Thus, IRF8-dependent cDCs are required for IEL homeostasis.

We also assessed the capacity to generate Th1 responses in the absence of IRF8-dependent cDCs. Strikingly, $IFN\gamma^+$ T cells were almost absent in the intestinal lamina propria of IRF8-deficient mice, and Th1 induction was impaired in MLN. We therefore utilised low dose infection with *T. muris* to further investigate the induction of Th1 responses, as it is well known that chronic infection with *T. muris* is dependent on generation of a Th1 response and IFNγ production[237,250] and promoted by IL-12,[248] likely produced by the antigen-presenting cell. Mice deficient in IRF8-dependent cDCs failed to become chronically infected following low dose infection and, furthermore, failed to mediate isotype switching to IgG2c, a process known to be dependent on IFNγ, consistent with the impaired capacity to induce Th1 responses.

Taken together, these results demonstrate an essential role for IRF8-dependent cDCs in maintaining intestinal homeostasis, including an essential role for intestinal IEL differentiation and generation of Th1 cells during homeostasis as well as in an infection-context.

Paper II:

Different populations of CD11b$^+$ dendritic cells drive Th2 responses in the small intestine and colon

Background/Aim:

Previous publications have demonstrated the importance of IRF4-dependent cDC in generation of intestinal Th17 responses.[69] In addition, several studies have suggested a role for IRF4-dependent DC in the induction of Th2 responses in the skin and respiratory tract.[311–313] In the current study, our aim was to examine the role of IRF4-dependent DC during induction of Th2 responses in the intestinal mucosa. Therefore, we investigated mice which harboured a targeted deletion of IRF4 selectively in CD11c$^+$ cells in two complementary models to induce intestinal Th2 responses, subserosal injection of Th2-promoting SEA antigens[314,315] and live infection with *T. muris*.

Results:

- IRF4-dependent cDCs were critical for induction of Th2 responses following subserosal injection of SEA and during high dose-infection with *T. muris*
- cDCs transported antigens and triggered CD4$^+$ T cells responses in the MLN after subserosal antigen injection
- IRF4-deficient cDCs were capable of driving Th2 responses after transfer directly into the MLN
- The incapacity of CD11c-cre.*IRF4*$^{fl/fl}$ mice to induce Th2 responses was associated with reduced numbers of antigen-carrying cDC in the MLN, while
- Distinct IRF4-dependent cDCs promoted Th2 response after antigen injection into the small intestine and colon

Discussion

While the mechanism of Th2 induction *in vitro* is relatively well characterized, much less is known about the cell types and mechanism(s) that promote Th2 responses *in vivo*. Our results show that IRF4-dependent cDCs are critical for Th2 responses in the intestine, which is consistent with the findings depicting these cells as critical for induction of Th2 responses in the skin and lung.[311–313] Following *T. muris* infection with a high dose, which normally induces a Th2 response in wild-type mice,[316] we found that IRF4-deficient mice were unable to clear the infection and displayed a marked reduction of Th2-associated cytokines in the MLN. Interestingly, previous reports have suggested that IRF4 expression in cDCs is

important for antigen presentation via MHC-II and in migration to draining lymph nodes, however, we show that IRF4-deficient cDCs can take up antigen and mediate T cell proliferation and Th2 induction. Instead, we find that the number of antigen-carrying cDCs in the MLN are fewer in mice with IRF4-deficient cDCs, which could stem from previously described observations that IRF4 regulates survival[69,70] and migration[192] of cDCs, and suggesting that the reduced Th2 response is due to limited amount of presented antigen. In addition, we found a surprising compartmentalization in the functionality of the two major IRF4-dependent cDC subsets, thus, while DP cDCs were critical for induction of Th2 responses in the small intestine, CD11b SP cDCs mediated this function in the colon,[314] suggesting that functional differences between cDC subsets may not only be dictated by their intrinsic properties, but also through local microenvironmental cues.

Collectively, these results reveal that IRF4-dependent DCs are critical for the induction of intestinal Th2 responses in the small and large intestine of SEA-immunized mice and *T. muris*-infected mice. Additionally, while DPs account for driving Th2 responses in the small intestine, CD11b SPs drive Th2 responses in the large intestine.

Paper III

Distinct DC subsets regulate adaptive Th1 and 2 responses during Trichuris muris infection.

Background/Aim:

In this paper, we aimed to extend our observations from paper I and II, to further characterize the role of cDC subsets in adaptive Th1 and Th2 responses during chronic and acute *T. muris* infection, and to examine the potential synergistic or antagonistic role of these cells during infection. Finally, we investigated the adaptive response in mice with a combined CD11c-specific IRF4- and IRF8-deficiency.

Results:

- cDC accumulate in the MLN following high, but not low dose-infection with *T. muris*
- Consistent with our previous findings, CD11c-cre.*IRF4*$^{fl/fl}$ mice displayed reduced Th2 responses at day 21 p.i. following high dose-infection with *T. muris*
- Mice lacking IRF8-dependent cDC failed to induce Th1 cells and support chronic infection following low dose infection, which was associated with a strong increase in Th2 cytokine production, suggesting a potential Th2 antagonistic function of IRF8-dependent DCs
- Bone marrow chimeric mice with a combined deletion of IRF4 and IRF8 in CD11c^{+} cells induced Th2 responses and mediated worm expulsion following low-dose infection with *T. muris*, indicating that other antigen-presenting cells can compensate for the absence of IRF4-dependent DCs, in the concurrent absence of IRF8-dependent DCs

Discussion

Recent studies by us[68,314] and others[311–313,317] have implied that cDC1 and cDC2s are critical for generation of Th1 and Th2 responses, respectively, and that a potential balance between the two subsets might exists.[317] Our previous findings in paper I indicated a critical role for IRF8-dependent CD103 SP cDCs for the induction of chronic infection with *T. muris*, a phenotype which we confirmed in this paper and by demonstrating that IRF8-deficient mice failed to induce IFNγ^{+} Th1 cells. In addition, interestingly, we found that a strong Th2 response developed in the absence of IRF8-dependent cDCs, suggesting that this subset may be involved in antagonizing Th2 responses. How the absence of one cDC subset may lead to polarization of the adaptive immune response in a certain direction remains to be

assessed, although it seems plausible that the absence of one cDC subset and its secreted mediators, may allow the remaining cDCs to drive T cell polarization. This is consistent with other studies, demonstrating that Batf3-dependent cDC1 are able to antagonize Th2 responses, by production of IL-12.[317] The absence of CD103 SPs and, thus, their ability to generate active TGFβ is also a plausible explanation for the increased Th2 responses seen in these mice, as mice with TGFβ-deficient signalling in cDCs have enhanced Th2 responses and expel *T. muris,*[318] similar to mice with IRF8-deficient DCs.[68] Finally, and much to our surprise, we found that Th2 responses can develop in the absence of IRF4-dependent cDC, in the concurrent absence of IRF8-dependent cDCs. While the underlying mechanism of how Th2 responses develop in these mice remains elusive, these data indicate that other cells ma promote Th2 responses in the absence of IRF4- and IRF8-dependent cDCs.

6 Concluding remarks

Adaptive T helper cell responses are critical for mediating sterilizing immunity and generation of immunological memory. The immune response against gastrointestinal parasite infections is mediated by numerous cell types, which form a complex network in order to mediate efficient immunity and protection, normally accomplished through the induction of Th2 responses. In contrast, secretion of classical Th1-associated cytokines is associated with detrimental outcomes for the host tissue, often with chronic infection as the ultimate outcome. cDCs are at the center of these responses, by first sensing the pathogen or its products, and secondly, by inducing an appropriate adaptive effector T cell response to ensure eradication of the pathogen. However, we still lack detailed understanding of the cellular and molecular requirements for induction of Th2 responses. Furthermore, given the recent identification of cDC subsets, each with a distinct ontogeny and function, additional studies are yet required to fully unravel the role of these cDC subsets in inducing different types of T helper cell responses. The current thesis contributes to this field, and extends prior knowledge about the role of cDC subsets in the generation of adaptive Th1 and Th2 responses, by demonstrating the requirement of IRF8- and IRF4 dependent cDCs in these processes, respectively.

cDC subsets, in addition to their role in inducing different types of T helper cell responses, have also been suggested to antagonize each other, by production of cytokines. Our data from paper III of this thesis is consistent with this hypothesis, as we detected increased secretion of Th2 cytokines in the absence of IRF8-dependent cDC1s. cDC1 cDCs are the major source of IL-12 in the MLN, which has the ability to directly inhibit the differentiation of Th2 cells,[319–321] and studies in *Schistosoma mansoni* and *Heligmosomoides polygyrus* infection have identified a functional role of cDC1-derived IL-12 in dampening type 2 responses.[317] In addition, cDC1s have the highest expression of the TGFβ-activating integrin αvβ8,[68,322,323] required for the generation of active TGFβ. TGFβ signalling in cDCs might be accountable for inhibiting Th2 responses during *T. muris* infection, as infected mice which lack the TGFβ-activating integrin αvβ8 in $CD11c^+$ cells have enhanced Th2 response and display expulsion upon infection.[318] In line with this, αvβ8 is expressed by CD103 SPs,[68] and the lack of these cells, and thus the generation of active TGFβ, leads to the absence of Th1 responses during low-dose infection with *T. muris* and instead induces Th2 responses – in this way TGFβ

signalling might be involved in antagonizing Th2 responses. Conversely, IRF4-dependent cDC2s may also have an antagonistic capacity. CD11b SP cDCs or macrophages has been suggested to be a major source of IL-23 during infection with *C. rodentium*, and IL-23 production was associated with dampened IL-12 production by CD103 SPs,[324] consistent with a potential role of IL-23 in antagonizing Th1 responses. Although the role of IL-23 during *T. muris* infection remains unknown, it is plausible that IL-23 may also promote Th2 responses during infection with *T. muris*. Previous studies have indeed shown that IL-23 signalling can enhance Th2-polarization *in vitro,*[325] as well as *in vivo*, demonstrated by either neutralization or transgenic over-expression of IL-23 or IL-23R.[325,326] However, this concept has not been studied in the intestinal mucosa and thus remains largely unexplored in the context of intestinal Th2 responses.

With these studies implicating an essential role for specific cDC subsets for the induction of adaptive immune responses, another key question remains unanswered, and that is which environmental signals cDCs receive in order to initiate Th2 responses. Thus, clear mechanistic data on how DCs orchestrate Th2 polarization, and whether certain receptors on DCs are required for this process, remains to be determined. While the molecular requirements for induction of adaptive Th1 and Th17 responses are relatively well characterized, comparably little is known with regards to how Th2 responses are generated. Indeed, for Th2 responses, it wasn't even clear that this induction was mediated by cDCs until quite recently.[293] Thus, some studies have depicted basophils[327] or ILCs,[328] instead of DCs, as key antigen-presenting cells that could mediate Th2 differentiation. However, this idea was later dismissed in favour of studies that proved cDCs as critical also for adaptive Th2 responses.[293]

Several hypotheses have been put forward as "the mechanism", including those portraying Th2 responses as default responses triggered only when typical polarizing mediators mediating other types of adaptive responses, such as IL-12, were absent.[329,330] This was however later dismissed when it became apparent that cDCs don't necessarily drive Th2 responses by the mere absence of pro-inflammatory signals.[331] One of the prevailing mechanisms is that the antigen itself may promote induction of a Th2 response. Currently, the most well-described Th2 inducing parasite-derived antigen is the *S. mansoni*-derived ribonuclease Omega-1, which binds to mannose receptors on DCs.[332] *S. mansoni* soluble egg antigen-treated DCs promote induction of Th2 cells *in vitro*[333] through the endonuclease Omega-1,[224] which orchestrate degradation of mRNA and rRNA to inhibit protein synthesis and orchestrate DCs to induce Th2 polarization in a mannose receptor-dependent manner.[332] However, to date, a corresponding Th2-inducing capacity has not been demonstrated for *T. muris*-derived E/S. Alternatively, while the mechanism of induction of Th1 responses through various PRRs on cDCs is comparably well-established, the potential existence of Th2-inducing PRRs remains more unclear.

Several PRRs, including TLR2 and -4, NOD1 and-2 and Fcγ receptors, have been depicted to control Th2 responses. The TLR2 ligand, Pam-3-Cys, induces reduced IL-12 secretion by DCs *in vitro* and favours Th2 differentiation via the ERK-cFos-pathway.[334] In addition, low doses of inhaled LPS[335] or HDM allergens[336] signal through TLR4 on epithelial cells, leading to induction of Th2 responses. Additional studies have implicated the role for some NLRs, CLRs and Fcγ receptors (discussed in greater detail elsewhere),[128,303] yet, these appear largely context-dependent and often leave out the molecular mechanism of Th2 induction via the DC, and do not include the mechanism of intestinal Th2 responses. The role of cDCs in Th2 induction may also be more indirect and occur, for example, via epithelial-derived cytokines and alarmins. The role for the epithelial-derived cytokines IL-25, IL-33 and TSLP have been demonstrated in various models and displayed as strong inducers of type 2 responses by directly or indirectly inducing and amplifying production of IL-4, IL-5, IL-9 and IL-13. Increased TSLP production have been found at barrier surfaces such as in the skin, respiratory tract and intestine upon pathogen invasion.[337,338] TSLP-binding acts primarily on monocytes and DCs to induce STAT5 phosphorylation,[339] leading to increased CD80 and CD40 in addition to the release of chemokines which attracts T cells,[340] or through induction of NF-κβ, leading to upregulation of OX40L[341] and secretion of CCL17.[342,343] Additionally, TSLP may also be involved in antagonizing Th1 responses, by suppression of the p40 subunit of IL-12 which is associated with promoted Th1 responses.[344,345] However, the full details of how intestinal cDCs may be regulated by epithelial-derived cytokines is yet to be explored.

While our studies have shed some light on the role of distinct cDC subsets in the regulation of adaptive immunity against intestinal nematode infection, further studies need to assess the potential interplay and antagonism between these subsets, in addition to determining the molecular pathways underlying induction of adaptive Th2 responses.

7 Populärvetenskaplig sammanfattning

Tarmens slemhinna är kroppens största yta och befinner sig i direkt kontakt med vår omgivning och med allt vi får i oss via födan. Tarmslemhinnan innehåller en stor mängd nyttiga bakterier och är också en angreppspunkt för många virus, bakterier och parasiter vilka har förmågan att orsaka infektion och vävnadsskada. *T. muris* är ett exempel på en parasit som infekterar det enkelskiktade lagret av epitelceller som skyddar den underliggande vävnaden i tjocktarmen hos möss. *T. muris* är snarlik den typ av infektion som sker med den närbesläktade *T. trichiura* som infekterar ofattbara 500 miljoner människor i världen. För att kunna bekämpa masken är det helt avgörande att rätt typ av immunceller aktiveras vid infektion – annars riskerar man att bli kroniskt infekterad med långvarig inflammation som följd.

De vita blodkropparna som utgör immunförsvaret måste inte bara lära sig att svara gentemot inkräktare utan också lära sig att behålla lugnet mot ofarliga ämnen. När de vita blodkropparna inte klarar av att skilja farligt från ofarligt kan sjukdom uppstå. Sjukdomar såsom astma, allergi och inflammatoriska tarmsjukdomar är på stadig uppgång i vår del av världen och är ett resultat av att immunförsvaret aktiverats felaktigt.

Fokus för denna avhandling har varit att studera dendritiska celler (DC), en typ av medfödd immunförsvarscell som finns i nästan alla kroppens vävnader. DCs i tarmslemhinnan har som uppgift att fånga upp främmande ämnen, s.k. antigen, och förflytta sig från vävnaden i tarmen till lymfknutor där de kan presentera antigen för T-celler, vilka utgör en del av det adaptiva immunförsvaret. De T-celler som känner igen antigenet som presenteras aktiveras och utvecklas till olika ”effektorceller”, såsom Th1 och Th2, vilka är anpassade till att bekämpa olika typer av infektioner. Under infektion med *T. muris* är det fördelaktigt att Th2-celler aktiveras, vilka har förmågan att bekämpa parasiter, medan Th1-celler orsakar långvarig infektion och kan orsaka vävnadsskada.

Det finns olika typer av DCs i tarmen som är specialiserade på att aktivera T celler till att utvecklas till exempelvis Th1 eller Th2 celler. De två huvudgrupperna av DCs som återfinns i tarmens slemhinna är IRF4- och IRF8-beroende DCs, vilka har

skilda funktioner. För att dessa DCs ska utvecklas normalt från outvecklade blodceller och reagera med optimal funktion är de beroende av genuttrycket av IRF4 respektive IRF8. Våra studier syftar till att bestämma vilken typ av DC, IRF4- eller IRF8-beroende, som behövs under utvecklingen av Th1- och Th2-svar under infektion med *T. muris*.

Under experimenten har vi använt oss av möss som saknar IRF4- och/eller IRF8-beroende DCs. Genom att undersöka vilka immunsvar mössen utvecklade efter infektion med *T. muris* i avsaknad av en viss typ av DC har vi visat att IRF8-beroende DCs krävs för aktivering av Th1 celler, medans IRF4-beroende DCs är helt nödvändiga för aktivering av Th2 celler. När vi undersökte infekterade möss som saknade både IRF4- och IRF8-beroende DCs fann vi att dessa blev resistenta och uppvisade ett Th2-svar, precis som de möss som endast saknade IRF8-beroende DCs. Dessa resultat tyder, överraskande nog, på att de möss som saknar IRF4-DCs i själva verket har förmåga att utveckla ett Th2 svar i de fall då de samtidigt saknar IRF8-DCs.

Sammanfattningsvis har vi i dessa studier studerat aktiveringen av adaptiva immunsvar under parasitinfektion i tarmens slemhinna och identifierat de DCs som är viktiga för aktivering av Th1- och Th2-svar. En ökad förståelse kring hur dessa processer regleras är viktig för att hitta nya metoder för behandling av såväl tarmsjukdomar som allergier och andra autoimmuna sjukdomar. Förståelsen kring hur immunförsvaret regleras vid parasitinfektion kan vidare understödja nya strategier för vaccinering mot denna typ av vitt utbredda infektioner.

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References

1. May GR, Sutherland LR, Meddings JB. Is small intestinal permeability really increased in relatives of patients with Crohn's disease? *Gastroenterology*. 1993;104(6):1627-1632.
2. Mogensen TH. Pathogen Recognition and Inflammatory Signaling in Innate Immune Defenses. *Clin Microbiol Rev*. 2009;22(2):240-273.
3. Tang D, Kang R, Coyne CB, Zeh HJ, Lotze MT. PAMPs and DAMPs: Signal 0s that Spur Autophagy and Immunity. *Immunol Rev*. 2012;249(1):158-175.
4. Suresh R, Mosser DM. Pattern recognition receptors in innate immunity, host defense, and immunopathology. *Adv Physiol Educ*. 2013;37(4):284-291.
5. Corfield AP. Mucins: A biologically relevant glycan barrier in mucosal protection. *Biochim Biophys Acta - Gen Subj*. 2015;1850(1):236-252.
6. Hattrup C, Gendler S. Structure and function of the cell surface (tethered) mucins. *Annu Rev Physiol*. 2008;70:431-457.
7. Johansson ME V, Phillipson M, Petersson J, et al. The inner of the two Muc2 mucin-dependent mucus layers in colon is devoid of bacteria. *Pnas*. 2008;105(39):15064-15069.
8. Johansson ME V, Larsson JMH, Hansson GC. The two mucus layers of colon are organized by the MUC2 mucin, whereas the outer layer is a legislator of host-microbial interactions. *Proc Natl Acad Sci U S A*. 2011;108 Suppl:4659-4665.
9. Mowat AM, Agace WW. Regional specialization within the intestinal immune system. *Nat Rev Immunol*. 2014;14(10):667-685.
10. Cheng H, Merzel J, Leblond CP. Renewal of Paneth cells in the small intestine of the mouse. *Am J Anat*. 1969;126(4):507-525.
11. Merzel J, Leblond CP. Origin and renewal of goblet cells in the epithelium of the mouse small intestine. *Am J Anat*. 1969;124(3):281-305.
12. Bjerknes M, Cheng H. Clonal analysis of mouse intestinal epithelial progenitors. *Gastroenterology*. 1999;116(1):7-14.
13. Hermiston ML, Gordon JI. Organization of the crypt-villus axis and evolution of its stem cell hierarchy during intestinal development. *Am J Physiol*. 1995;268(5 Pt 1):G813-22.
14. Potten CS, Loeffler M. Stem cells: attributes, cycles, spirals, pitfalls and uncertainties. Lessons for and from the crypt. *Development*. 1990;110(4):1001-1020.
15. Barker N, van Es JH, Kuipers J, et al. Identification of stem cells in small intestine and colon by marker gene Lgr5. *Nature*. 2007;449(7165):1003-1007.

16. Farquhar M, Palade G. Junctional complexes in various epithelia. *J Cell Biol*. 1963;17:375-412.
17. Schneeberger EE, Lynch RD. Structure, function, and regulation of cellular tight junctions. *Am J Physiol*. 1992;262(6 Pt 1):L647-61.
18. Tsukita S, Furuse M, Itoh M. Multifunctional strands in tight junctions. *Nat Rev Mol Cell Biol*. 2001;2(4):285-293.
19. Hermiston ML, Gordon JI. Inflammatory bowel disease and adenomas in mice expressing a dominant negative N-cadherin. *Science*. 1995;270(5239):1203-1207.
20. Hermiston ML, Gordon JI. In vivo analysis of cadherin function in the mouse intestinal epithelium: essential roles in adhesion, maintenance of differentiation, and regulation of programmed cell death. *J Cell Biol*. 1995;129(2):489-506.
21. Barrett JC, Lee JC, Lees CW, et al. Genome-wide association study of ulcerative colitis identifies three new susceptibility loci, including the HNF4A region. *Nat Genet*. 2009;41(12):1330-1334.
22. Vetrano S, Rescigno M, Rosaria Cera M, et al. Unique Role of Junctional Adhesion Molecule-A in Maintaining Mucosal Homeostasis in Inflammatory Bowel Disease. *Gastroenterology*. 2008;135(1):173-184.
23. Laukoetter MG, Nava P, Lee WY, et al. JAM-A regulates permeability and inflammation in the intestine in vivo. *J Exp Med*. 2007;204(13):3067-3076.
24. Suzuki T. Regulation of intestinal epithelial permeability by tight junctions. *Cell Mol Life Sci*. 2013;70(4):631-659.
25. Schulzke JD, Ploeger S, Amasheh M, et al. Epithelial tight junctions in intestinal inflammation. *Ann N Y Acad Sci*. 2009;1165:294-300.
26. Heller F, Florian P, Bojarski C, et al. Interleukin-13 is the key effector Th2 cytokine in ulcerative colitis that affects epithelial tight junctions, apoptosis, and cell restitution. *Gastroenterology*. 2005;129(2):550-564.
27. Hollander D, Vadheim CM, Brettholz E, Petersen GM, Delahunty T, Rotter JI. Increased intestinal permeability in patients with Crohn's disease and their relatives. A possible etiologic factor. *Ann Intern Med*. 1986;105(6):883-885.
28. Buhner S, Buning C, Genschel J, et al. Genetic basis for increased intestinal permeability in families with Crohn's disease: role of CARD15 3020insC mutation? *Gut*. 2006;55(3):342-347.
29. Boirivant M, Amendola A, Butera A, et al. A Transient Breach in the Epithelial Barrier Leads to Regulatory T-Cell Generation and Resistance to Experimental Colitis. *Gastroenterology*. 2008;135(5):1612-1623.
30. Hershberg RM, Mayer LF. Antigen processing and presentation by intestinal epithelial cells: polarity and complexity. *Immunol Today*. 2000;21(3):123-128.
31. Muniz LR, Knosp C, Yeretssian G. Intestinal antimicrobial peptides during homeostasis, infection, and disease. *Front Immunol*. 2012;3:310.
32. Xavier RJ, Podolsky DK. Unravelling the pathogenesis of inflammatory bowel disease. *Nature*. 2007;448(7152):427-434.

33. Nagashima R, Maeda K, Imai Y, Takahashi T. Lamina propria macrophages in the human gastrointestinal mucosa: their distribution, immunohistological phenotype, and function. *J Histochem Cytochem*. 1996;44(7):721-731.

34. Muller AJ, Kaiser P, Dittmar KEJ, et al. Salmonella gut invasion involves TTSS-2-dependent epithelial traversal, basolateral exit, and uptake by epithelium-sampling lamina propria phagocytes. *Cell Host Microbe*. 2012;11(1):19-32.

35. Shimizu Y, Andrew W. Studies on the rabbit appendix. I. Lymphocyte-epithelial relations and the transport of bacteria from lumen to lymphoid nodule. *J Morphol*. 1967;123(3):231-249.

36. Wolf JL, Bye WA. The membranous epithelial (M) cell and the mucosal immune system. *Annu Rev Med*. 1984;35:95-112.

37. Allan CH, Mendrick DL, Trier JS. Rat intestinal M cells contain acidic endosomal-lysosomal compartments and express class II major histocompatibility complex determinants. *Gastroenterology*. 1993;104(3):698-708.

38. Neutra MR, Mantis NJ, Kraehenbuhl JP. Collaboration of epithelial cells with organized mucosal lymphoid tissues. *Nat Immunol*. 2001;2(11):1004-1009.

39. Owen RL. Uptake and transport of intestinal macromolecules and microorganisms by M cells in Peyer's patches--a personal and historical perspective. *Semin Immunol*. 1999;11(3):157-163.

40. Rimoldi M, Rescigno M. Uptake and presentation of orally administered antigens. *Vaccine*. 2005;23(15):1793-1796.

41. Lelouard H, Fallet M, de Bovis B, Meresse S, Gorvel J-P. Peyer's patch dendritic cells sample antigens by extending dendrites through M cell-specific transcellular pores. *Gastroenterologyfile:///Users/mimozademiri/Downloads/nihms380687.pdf*. 2012;142(3):592-601.e3.

42. Wang J, Gusti V, Saraswati A, Lo DD. Convergent and divergent development among M cell lineages in mouse mucosal epithelium. *J Immunol*. 2011;187(10):5277-5285.

43. Kanaya T, Hase K, Takahashi D, et al. The Ets transcription factor Spi-B is essential for the differentiation of intestinal microfold cells. *Nat Immunol*. 2012;13(8):729-736.

44. McDole JR, Wheeler LW, McDonald KG, et al. Goblet cells deliver luminal antigen to CD103+ dendritic cells in the small intestine. *Nature*. 2012;483(7389):345-349.

45. Rescigno M, Urbano M, Valzasina B, et al. Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. *Nat Immunol*. 2001;2(4):361-367.

46. Niess JH, Brand S, Gu X, et al. CX3CR1-mediated dendritic cell access to the intestinal lumen and bacterial clearance. *Science*. 2005;307(5707):254-258.

47. Tamoutounour S, Henri S, Lelouard H, et al. CD64 distinguishes macrophages from dendritic cells in the gut and reveals the Th1-inducing role of mesenteric lymph node macrophages during colitis. *Eur J Immunol*. 2012;42(12):3150-3166.

48. Schulz O, Jaensson E, Persson EK, et al. Intestinal CD103+, but not CX3CR1+, antigen sampling cells migrate in lymph and serve classical dendritic cell functions. *J Exp Med*. 2009;206(13):3101-3114.

49. Bain CC, Scott CL, Uronen-Hansson H, et al. Resident and pro-inflammatory macrophages in the colon represent alternative context-dependent fates of the same Ly6C(hi) monocyte precursors. *Mucosal Immunol*. 2013;6(3):498-510.

50. Farache J, Koren I, Milo I, et al. Luminal bacteria recruit CD103+ dendritic cells into the intestinal epithelium to sample bacterial antigens for presentation. *Immunity*. 2013;38(3):581-595.

51. Mazzini E, Massimiliano L, Penna G, Rescigno M. Oral tolerance can be established via gap junction transfer of fed antigens from CX3CR1(+) macrophages to CD103(+) dendritic cells. *Immunity*. 2014;40(2):248-261.

52. Steinman RM. Identification of a Novel Cell Type in Peripheral Lymphoid Organs of Mice: I. Morphology, Quantitation, Tissue Distribution. *J Exp Med*. 1973;137(5):1142-1162.

53. Steinman BYRM, Cohn ZA. Identification of a novel cell type in peripheral lymphoid organs. 1974;139.

54. Steinman RM, Witmer MD. Lymphoid dendritic cells are potent stimulators of the primary mixed leukocyte reaction in mice Immunology : *October*. 1978;75(10):5132-5136.

55. Rudensky A, Preston-Hurlburt P, Hong SC, Barlow A, Janeway CAJ. Sequence analysis of peptides bound to MHC class II molecules. *Nature*. 1991;353(6345):622-627.

56. Mildner A, Jung S. Development and function of dendritic cell subsets. *Immunity*. 2014;40(5):642-656.

57. Nussenzweig MC, Steinman RM, Unkeless JC, Witmer MD, Gutchinov B, Cohn Z a. Studies of the cell surface of mouse dendritic cells and other leukocytes. *J Exp Med*. 1981;154(1):168-187.

58. Nussenzweig MC, Steinman RM, Witmer MD, Gutchinov B. A monoclonal antibody specific for mouse dendritic cells. *ProcNatlAcadSciUSA*. 1982;79(January):161-165.

59. Bogunovic M, Ginhoux F, Helft J, et al. Origin of the Lamina Propria Dendritic Cell Network. *Immunity*. 2009;31(3):513-525.

60. Varol C, Vallon-Eberhard A, Elinav E, et al. Intestinal Lamina Propria Dendritic Cell Subsets Have Different Origin and Functions. *Immunity*. 2009;31(3):502-512.

61. Weber B, Saurer L, Schenk M, Dickgreber N, Mueller C. CX3CR1 defines functionally distinct intestinal mononuclear phagocyte subsets which maintain their respective functions during homeostatic and inflammatory conditions. *Eur J Immunol*. 2011;41(3):773-779.

62. Uematsu S, Fujimoto K, Jang MH, et al. Regulation of humoral and cellular gut immunity by lamina propria dendritic cells expressing Toll-like receptor 5. *Nat Immunol*. 2008;9(7):769-776.

63. Fujimoto K, Karuppuchamy T, Takemura N, et al. A new subset of CD103+CD8alpha+ dendritic cells in the small intestine expresses TLR3, TLR7, and TLR9 and induces Th1 response and CTL activity. *J Immunol*. 2011;186(11):6287-6295.

64. Denning TL, Norris BA, Medina-Contreras O, et al. Functional specializations of intestinal dendritic cell and macrophage subsets that control Th17 and regulatory T cell responses are dependent on the T cell/APC ratio, source of mouse strain, and regional localization. *J Immunol*. 2011;187(2):733-747.

65. Jaensson E, Uronen-Hansson H, Pabst O, et al. Small intestinal CD103(+) dendritic cells display unique functional properties that are conserved between mice and humans. *J Exp Med*. 2008;205(9):2139-2149.

66. Annacker O, Coombes JL, Malmstrom V, et al. Essential role for CD103 in the T cell-mediated regulation of experimental colitis. *J Exp Med*. 2005;202(8):1051-1061.

67. Johansson-Lindbom B, Svensson M, Pabst O, et al. Functional specialization of gut CD103(+) dendritic cells in the regulation of tissue-selective T cell homing. *J Exp Med*. 2005;202(8):1063-1073.

68. Luda KM, Joeris T, Persson EK, et al. IRF8 Transcription-Factor-Dependent Classical Dendritic Cells Are Essential for Intestinal T Cell Homeostasis. *Immunity*. 2016;44(4):860-874.

69. Persson EK, Uronen-Hansson H, Semmrich M, et al. IRF4 Transcription-Factor-Dependent CD103+CD11b+ Dendritic Cells Drive Mucosal T Helper 17 Cell Differentiation. *Immunity*. 2013;38(5):958-969.

70. Schlitzer A, McGovern N, Teo P, et al. IRF4 Transcription Factor-Dependent CD11b+ Dendritic Cells in Human and Mouse Control Mucosal IL-17 Cytokine Responses. *Immunity*. 2013;38(5):970-983.

71. Bachem A, Güttler S, Hartung E, et al. Superior antigen cross-presentation and XCR1 expression define human CD11c+CD141+ cells as homologues of mouse CD8+ dendritic cells. *J Exp Med*. 2010;207(6):1273-1281.

72. Crozat K, Tamoutounour S, Vu Manh TP, et al. Cutting edge: expression of XCR1 defines mouse lymphoid-tissue resident and migratory dendritic cells of the CD8alpha+ type. *J Immunol*. 2011;187:4411-4415.

73. Scott CL, Zangerle Murray TFP, Beckham KSH, Douce G, Mowat AMI. Signal regulatory protein alpha (SIRPα) regulates the homeostasis of CD103+CD11b+ DCs in the intestinal lamina propria. *Eur J Immunol*. 2014;44(12):3658-3668.

74. Naik SH, Sathe P, Park H-Y, et al. Development of plasmacytoid and conventional dendritic cell subtypes from single precursor cells derived in vitro and in vivo. *Nat Immunol*. 2007;8(11):1217-1226.

75. Onai N, Obata-Onai A, Schmid M a, Ohteki T, Jarrossay D, Manz MG. Identification of clonogenic common Flt3+M-CSFR+ plasmacytoid and conventional dendritic cell progenitors in mouse bone marrow. *Nat Immunol*. 2007;8(11):1207-1216.

76. Liu K, Victora GD, Schwickert TA, et al. In vivo analysis of dendritic cell development and homeostasis. *Science*. 2009;324(5925):392-397.

77. Fogg D, Sibon C, Miled C, Jung S,Aucouturier, Dan R. Littman, Ana Cumano FG. A Clonogenic Bone Marrow Progenitor Specific for Macrophages and Dendritic Cells. *Science (80-)*. 2006;311(March):83-88.

78. Dudziak D, Kamphorst AO, Heidkamp GF, et al. Differential antigen processing by dendritic cell subsets in vivo. *Science (80-)*. 2007;315(5808):107-111.

79. Waskow C, Liu K, Darrasse-Jeze G, et al. The receptor tyrosine kinase Flt3 is required for dendritic cell development in peripheral lymphoid tissues. *Nat Immunol*. 2008;9(6):676-683.

80. Ginhoux F, Liu K, Helft J, et al. The origin and development of nonlymphoid tissue CD103+ DCs. *J Exp Med*. 2009;206(13):3115-3130.

81. Naik SH, Metcalf D, van Nieuwenhuijze A, et al. Intrasplenic steady-state dendritic cell precursors that are distinct from monocytes. *Nat Immunol*. 2006;7(6):663-671.

82. Helft J, Ginhoux F, Bogunovic M, Merad M. Origin and functional heterogeneity of non-lymphoid tissue dendritic cells in mice. *Immunol Rev*. 2010;234(1):55-75.

83. Schlitzer A, Sivakamasundari V, Chen J, et al. Identification of cDC1- and cDC2-committed DC progenitors reveals early lineage priming at the common DC progenitor stage in the bone marrow. *Nat Immunol*. 2015;16(7):718-728.

84. Cells PD, Sichien D, Scott CL, et al. IRF8 Transcription Factor Controls Survival and Function of Terminally Differentiated Conventional Article IRF8 Transcription Factor Controls Survival and Function of Terminally Differentiated Conventional and Plasmacytoid Dendritic Cells , Respectively. 2016;45:1-15.

85. Satpathy AT, Kc W, Albring JC, et al. Zbtb46 expression distinguishes classical dendritic cells and their committed progenitors from other immune lineages. *J Exp Med*. 2012;209(6):1135-1152.

86. Meredith MM, Liu K, Darrasse-Jeze G, et al. Expression of the zinc finger transcription factor zDC (Zbtb46, Btbd4) defines the classical dendritic cell lineage. *J Exp Med*. 2012;209(6):1153-1165.

87. Merad M, Manz MG. Review article Dendritic cell homeostasis. *Cell*. 2009;113(15):3418-3427.

88. Inaba K, Inaba M, Romani N, et al. Generation of Large Numbers of Dendritic Cells from Mouse Bone Marrow Cultures Supplemented with Granulocyte/Macrophage Colony-stimulating Factor. *J Exp Med*. 1992;176(December):1693-1702.

89. Inaba K, Steinman RM, Pack MW, et al. Identification of proliferating dendritic cell precursors in mouse blood. *J Exp Med*. 1992;175(5):1157-1167.

90. Karsunky H, Merad M, Cozzio a., Weissman IL, Manz MG. Flt3 Ligand Regulates Dendritic Cell Development from Flt3+ Lymphoid and Myeloid-committed Progenitors to Flt3+ Dendritic Cells In Vivo. *J Exp Med*. 2003;198(2):305-313.

91. McKenna HJ, Stocking KL, Miller RE, et al. Mice lacking flt3 ligand have deficient hematopoiesis affecting hematopoietic progenitor cells, dendritic cells, and natural killer cells. *Blood*. 2000;95(11):3489-3497.

92. Tussiwand R, Onai N, Mazzucchelli L, Manz MG. Inhibition of natural type I IFN-producing and dendritic cell development by a small molecule receptor tyrosine kinase inhibitor with Flt3 affinity. *J Immunol*. 2005;175(6):3674-3680.

93. Naik SH, Proietto AI, Wilson NS, et al. Cutting edge: generation of splenic CD8+ and CD8- dendritic cell equivalents in Fms-like tyrosine kinase 3 ligand bone marrow cultures. *J Immunol*. 2005;174(11):6592-6597.

94. Caux C, Dezutter-Dambuyant C, Banchereau DS& J. GM-CSF and TNF-alpha cooperate in the generation of dendritic Langerhans cells. 1992:258-261.

95. Sallusto BF, Lanzavecchia A. Efficient Presentation of Soluble Antigen by Cultured Human Dendritic Cells Is Maintained by Granulocyte/Macrophage Colony-stimulating Factor Plus Iuterleukin 4 and Downregulated by Tumor Necrosis Factor alpha. *J Exp Med*. 1994;179(April).
96. Greter M, Helft J, Chow A, et al. GM-CSF Controls Nonlymphoid Tissue Dendritic Cell Homeostasis but Is Dispensable for the Differentiation of Inflammatory Dendritic Cells. 2012;36(6):1031-1046.
97. King IL, Kroenke M a, Segal BM. GM-CSF-dependent, CD103+ dermal dendritic cells play a critical role in Th effector cell differentiation after subcutaneous immunization. *J Exp Med*. 2010;207(5):953-961.
98. Vremec D, J. Lieschke G, R. Dunn A, Robb L, Metcalf D, Shortman K. The influence of granulocyte/macrophage colony-stimulating factor on dendritic cell levels in mouse lymphoid organs. *Eur J Immunol*. 1997;27:40-44.
99. Maraskovsky E, Brasel K, Teepe M, et al. Dramatic increase in the numbers of functionally mature dendritic cells in Flt3 ligand-treated mice: multiple dendritic cell subpopulations identified. *J Exp Med*. 1996;184(5):1953-1962.
100. Merad M, Sathe P, Helft J, Miller J, Mortha A. The Dendritic Cell Lineage: Ontogeny and Function of Dendritic Cells and Their Subsets in the Steady State and the Inflamed Setting. *Annu Rev Immunol*. 2013;31.
101. Cerovic V, Houston SA, Scott CL, et al. Intestinal CD103(-) dendritic cells migrate in lymph and prime effector T cells. *Mucosal Immunol*. 2013;6(1):104-113.
102. Mellman I, Steinman RM. Dendritic cells: Specialized and regulated antigen processing machines. *Cell*. 2001;106(3):255-258.
103. Sporri R, Reis e S. Inflammatory mediators are insufficient for full dendritic cell activation and promote expansion of CD4+ T cell populations lacking helper function. *NatImmunol*. 2005;6(2):163-170.
104. Gatto D, Wood K, Caminschi I, et al. The chemotactic receptor EBI2 regulates the homeostasis, localization and immunological function of splenic dendritic cells. *Nat Immunol*. 2013;14(5):446-453.
105. Steinman RM, Nussenzweig MC. Avoiding horror autotoxicus: the importance of dendritic cells in peripheral T cell tolerance. *Proc Natl Acad Sci U S A*. 2002;99(1):351-358.
106. Cella M, Engering A, Pinet V, Pieters J, Lanzavecchia A. Inflammatory stimuli induce accumulation of MHC class II complexes on dendritic cells. *Nature*. 1997;388(6644):782-787.
107. Sallusto BF, Lanzavecchia A. Dendritic Cells Use Macropinocytosis and the Mannose Receptor to Concentrate Macromolecules in the Major Histocompatibility Complex Class 1I Compartment: Downregulation by Cytokines and Bacterial Products. *J Exp Med*. 1995;179(April).
108. Larsen BCP, Kitchie SC, Pearson TC, Linsley PS, Lowry RP. Functional Expression of the Costimulatory Molecule, B7/BB1, on Murine Dendritic Cell Populations. 1992;176(October).
109. Lenschow DJ, Su GH, Zuckerman LA, et al. Expression and functional significance of an additional ligand for CTLA-4. 1993;90(December):11054-11058.

110. Cella M, Scheidegger D, Palmer-Lehmann K, Lane P, Lanzavecchia A, Alber G. Ligation of CD40 on dendritic cells triggers production of high levels of interleukin-12 and enhances T cell stimulatory capacity: T-T help via APC activation. *J Exp Med*. 1996;184(2):747-752.

111. Caux C, Massacrier C, Vanbervliet B, et al. Activation of human dendritic cells through CD40 cross-linking. *J Exp Med*. 1994;180(4):1263 LP-1272.

112. Kiener PA, Moran-Davis P, Rankin BM, Wahl AF, Aruffo A, Hollenbaugh D. Stimulation of CD40 with purified soluble gp39 induces proinflammatory responses in human monocytes. *J Immunol*. 1995;155(10):4917-4925.

113. Dieu BM, Vanbervliet B, Vicari A, et al. Selective Recruitment of Immature and Mature Dendritic Cells by Distinct Chemokines Expressed in Different. 1998;188(2).

114. Sallusto F, Schaerli P, Loetscher P, et al. Rapid and coordinated switch in chemokine receptor expression during dendritic cell maturation. *Eur J Immunol*. 1998;28(9):2760-2769.

115. Sozzani S, Allavena P, D'Amico G, et al. Differential regulation of chemokine receptors during dendritic cell maturation: a model for their trafficking properties. *J Immunol*. 1998;161(3):1083-1086.

116. Yanagihara S, Komura E, Nagafune J, Watarai H, Yamaguchi Y. EBI1/CCR7 is a new member of dendritic cell chemokine receptor that is up-regulated upon maturation. *J Immunol*. 1998;161(6):3096-3102.

117. Förster R, Schubel A, Breitfeld D, et al. CCR7 Coordinates the Primary Immune Response by Establishing Functional Microenvironments in Secondary Lymphoid Organs. 1999;99:23-33.

118. Martín-fontecha A, Sebastiani S, Höpken UE, et al. Regulation of Dendritic Cell Migration to the Draining Lymph Node : Impact on T Lymphocyte Traffic and Priming. 2003;198(4):615-622.

119. Ohl L, Mohaupt M, Czeloth N, et al. CCR7 Governs Skin Dendritic Cell Migration under Inflammatory and Steady-State Conditions Max-Delbruck-Center for Molecular Medicine. 2004;21:279-288.

120. Sallusto F, Palermo B, Lenig D, et al. Distinct patterns and kinetics of chemokine production regulate dendritic cell function. *Eur J Immunol*. 1999;29(5):1617-1625.

121. Saeki H, Moore AM, Brown MJ, Hwang ST. Cutting edge: secondary lymphoid-tissue chemokine (SLC) and CC chemokine receptor 7 (CCR7) participate in the emigration pathway of mature dendritic cells from the skin to regional lymph nodes. *J Immunol*. 1999;162(5):2472-2475.

122. Vassileva G, Soto H, Zlotnik A, et al. The reduced expression of 6Ckine in the plt mouse results from the deletion of one of two 6Ckine genes. *J Exp Med*. 1999;190(8):1183-1188.

123. Cherwinski HM, Schumacher JH, Brown KD, Mosmann TR. Two types of mouse helper T cell clone. III. Further differences in lymphokine synthesis between Th1 and Th2 clones revealed by RNA hybridization, functionally monospecific bioassays, and monoclonal antibodies. *J Exp Med*. 1987;166(5):1229-1244.

124. Stevens TL, Bossie A, Sanders VM, et al. Regulation of antibody isotype secretion by subsets of antigen-specific helper T cells. *Nature*. 1988;334(6179):255-258.

125. Hsieh CS, Macatonia SE, Tripp CS, Wolf SF, O'Garra A, Murphy KM. Development of TH1 CD4+ T cells through IL-12 produced by Listeria-induced macrophages. *Science*. 1993;260(5107):547-549.

126. Szabo SJ, Kim ST, Costa GL, Zhang X, Fathman CG, Glimcher LH. A novel transcription factor, T-bet, directs Th1 lineage commitment. *Cell*. 2000;100(6):655-669.

127. Licona-Limón P, Kim LK, Palm NW, Flavell R a. TH2, allergy and group 2 innate lymphoid cells. *Nat Immunol*. 2013;14(6):536-542.

128. Hussaarts L, Yazdanbakhsh M, Guigas B. Priming dendritic cells for Th2 polarization: Lessons learned from helminths and implications for metabolic disorders. *Front Immunol*. 2014;5(OCT):1-9.

129. Finkelman FD, Katona IM, Urban JFJ, et al. IL-4 is required to generate and sustain in vivo IgE responses. *J Immunol*. 1988;141(7):2335-2341.

130. Katona IM, Urban JFJ, Kang SS, Paul WE, Finkelman FD. IL-4 requirements for the generation of secondary in vivo IgE responses. *J Immunol*. 1991;146(12):4215-4221.

131. Snapper CM, Finkelman FD, Paul WE. Regulation of IgG1 and IgE production by interleukin 4. *Immunol Rev*. 1988;102:51-75.

132. Zhang DH, Cohn L, Ray P, Bottomly K, Ray A. Transcription factor GATA-3 is differentially expressed in murine Th1 and Th2 cells and controls Th2-specific expression of the interleukin-5 gene. *J Biol Chem*. 1997;272(34):21597-21603.

133. Zheng W, Flavell RA. The Transcription Factor GATA-3 Is Necessary and Sufficient for Th2 Cytokine Gene Expression in CD4 T Cells. *Cell*. 1997;89:587-596.

134. Agarwal S, Rao A. Modulation of chromatin structure regulates cytokine gene expression during T cell differentiation. *Immunity*. 1998;9(6):765-775.

135. Bonecchi R, Bianchi G, Bordignon PP, et al. Differential expression of chemokine receptors and chemotactic responsiveness of type 1 T helper cells (Th1s) and Th2s. *J Exp Med*. 1998;187(1):129-134.

136. Voehringer D, Reese TA, Huang X, Shinkai K, Locksley RM. Type 2 immunity is controlled by IL-4/IL-13 expression in hematopoietic non-eosinophil cells of the innate immune system. *J Exp Med*. 2006;203(6):1435-1446.

137. Voehringer D, Shinkai K, Locksley RM. Type 2 immunity reflects orchestrated recruitment of cells committed to IL-4 production. *Immunity*. 2004;20(3):267-277.

138. Ho IC, Hodge MR, Rooney JW, Glimcher LH. The proto-oncogene c-maf is responsible for tissue-specific expression of interleukin-4. *Cell*. 1996;85(7):973-983.

139. Kim JI, Ho IC, Grusby MJ, Glimcher LH. The transcription factor c-Maf controls the production of interleukin-4 but not other Th2 cytokines. *Immunity*. 1999;10(6):745-751.

140. Ho IC, Lo D, Glimcher LH. c-maf promotes T helper cell type 2 (Th2) and attenuates Th1 differentiation by both interleukin 4-dependent and -independent mechanisms. *J Exp Med*. 1998;188(10):1859-1866.

141. Ouyang W, Kolls JK, Zheng Y. The Biological Functions of T Helper 17 Cell Effector Cytokines in Inflammation. *Immunity*. 2008;28(4):454-467.

142. Lee Y, Awasthi A, Yosef N, et al. Induction and molecular signature of pathogenic TH17 cells. *Nat Immunol*. 2012;13(10):991-999.

143. Zenewicz LA, Yancopoulos GD, Valenzuela DM, Murphy AJ, Stevens S, Flavell RA. Innate and adaptive interleukin-22 protects mice from inflammatory bowel disease. *Immunity*. 2008;29(6):947-957.

144. Sonnenberg GF, Monticelli LA, Elloso MM, Fouser LA, Artis D. CD4(+) lymphoid tissue-inducer cells promote innate immunity in the gut. *Immunity*. 2011;34(1):122-134.

145. Ivanov II, McKenzie BS, Zhou L, et al. The orphan nuclear receptor RORgammat directs the differentiation program of proinflammatory IL-17+ T helper cells. *Cell*. 2006;126(6):1121-1133.

146. Ivanov I, Frutos R de L, Manel N, Yoshinaga K. Specific microbiota direct the differentiation of IL-17-producing T-helper cells in the mucosa of the small intestine. *Cell host*. 2008;4(4):337-349.

147. Hu W, Troutman TD, Edukulla R, Pasare C. Priming microenvironments dictate cytokine requirements for T helper 17 cell lineage commitment. *Immunity*. 2011;35(6):1010-1022.

148. Shaw MH, Kamada N, Kim Y-G, Núñez G. Microbiota-induced IL-1β, but not IL-6, is critical for the development of steady-state T_H17 cells in the intestine. *J Exp Med*. 2012;209(2):251 LP-258.

149. Mangan PR, Harrington LE, O'Quinn DB, et al. Transforming growth factor-beta induces development of the T(H)17 lineage. *Nature*. 2006;441(7090):231-234.

150. Boom WH, Liano D, Abbas AK. Heterogeneity of helper/inducer T lymphocytes. II. Effects of interleukin 4- and interleukin 2-producing T cell clones on resting B lymphocytes. *J Exp Med*. 1988;167(4):1350 LP-1363.

151. Gajewski TF, Fitch FW. Anti-proliferative effect of IFN-gamma in immune regulation. I. IFN-gamma inhibits the proliferation of Th2 but not Th1 murine helper T lymphocyte clones. *J Immunol*. 1988;140(12):4245 LP-4252.

152. Josefowicz SZ, Rudensky A. Control of regulatory T cell lineage commitment and maintenance. *Immunity*. 2009;30(5):616-625.

153. Murphy TL, Grajales-Reyes GE, Wu X, et al. Transcriptional Control of Dendritic Cell Development. *Annu Rev Immunol*. 2016;34(1):93-119.

154. Joeris T, Müller-Luda K, Agace WW, Mowat AM. Diversity and functions of intestinal mononuclear phagocytes. *Mucosal Immunol*. 2017;10(April):845-864.

155. Hildner K, Edelson BT, Purtha WE, et al. Batf3 deficiency reveals a critical role for CD8alpha+ dendritic cells in cytotoxic T cell immunity. *Science*. 2008;322(5904):1097-1100.

156. Suzuki S, Honma K, Matsuyama T, et al. Critical roles of interferon regulatory factor 4 in CD11bhighCD8alpha- dendritic cell development. *Proc Natl Acad Sci U S A*. 2004;101(24):8981-8986.

157. del Rio M-L, Rodriguez-Barbosa J-I, Kremmer E, Forster R. CD103- and CD103+ bronchial lymph node dendritic cells are specialized in presenting and cross-presenting innocuous antigen to CD4+ and CD8+ T cells. *J Immunol*. 2007;178(11):6861-6866.

158. Edelson BT, KC W, Juang R, et al. Peripheral CD103+ dendritic cells form a unified subset developmentally related to CD8alpha+ conventional dendritic cells. *J Exp Med*. 2010;207(4):823-836.

159. Vremec D, Zorbas M, Scollay R, et al. The surface phenotype of dendritic cells purified from mouse thymus and spleen: investigation of the CD8 expression by a subpopulation of dendritic cells. *J Exp Med*. 1992;176(1):47-58.

160. Huysamen C, Willment JA, Dennehy KM, Brown GD. CLEC9A is a novel activation C-type lectin-like receptor expressed on BDCA3+ dendritic cells and a subset of monocytes. *J Biol Chem*. 2008;283(24):16693-16701.

161. Poulin LF, Reyal Y, Uronen-Hansson H, et al. DNGR-1 is a specific and universal marker of mouse and human Batf3-dependent dendritic cells in lymphoid and nonlymphoid tissues. *Blood*. 2012;119(25):6052-6062.

162. Zelenay S, Keller AM, Whitney PG, et al. The dendritic cell receptor DNGR-1 controls endocytic handling of necrotic cell antigens to favor cross-priming of CTLs in virus-infected mice. *J Clin Invest*. 2012;122(5):1615-1627.

163. Caminschi I, Maraskovsky E, Heath WR. Targeting dendritic cells in vivo for cancer therapy. *Front Immunol*. 2012;3(FEB):1-13.

164. Bachem A, Hartung E, Güttler S, et al. Expression of XCR1 characterizes the Batf3-dependent lineage of dendritic cells capable of antigen cross-presentation. *Front Immunol*. 2012;3(JUL):1-12.

165. Schraml BU, Van Blijswijk J, Zelenay S, et al. Genetic tracing via DNGR-1 expression history defines dendritic cells as a hematopoietic lineage. *Cell*. 2013;154(4):843-858.

166. Poulin LF, Salio M, Griessinger E, et al. Characterization of human DNGR-1+ BDCA3+ leukocytes as putative equivalents of mouse CD8alpha+ dendritic cells. *J Exp Med*. 2010;207(6):1261-1271.

167. Bedoui S, Whitney PG, Waithman J, et al. Cross-presentation of viral and self antigens by skin-derived CD103+ dendritic cells. *Nat Immunol*. 2009;10(5):488-495.

168. den Haan JM, Lehar SM, Bevan MJ. CD8(+) but not CD8(-) dendritic cells cross-prime cytotoxic T cells in vivo. *J Exp Med*. 2000;192(12):1685-1696.

169. Haniffa M, Shin A, Bigley V, et al. Human Tissues Contain CD141 hi Cross-Presenting Dendritic Cells with Functional Homology to Mouse CD103 + Nonlymphoid Dendritic Cells. *Immunity*. 2012;37(1):60-73.

170. Pooley JL, Heath WR, Shortman K. Cutting Edge: Intravenous Soluble Antigen Is Presented to CD4 T Cells by CD8- Dendritic Cells, but Cross-Presented to CD8 T Cells by CD8+ Dendritic Cells. *J Immunol*. 2001;166(9):5327-5330.

171. Cerovic V, Houston S a, Westlund J, et al. Lymph-borne CD8α(+) dendritic cells are uniquely able to cross-prime CD8(+) T cells with antigen acquired from intestinal epithelial cells. *Mucosal Immunol*. 2014;8(October 2013):1-11.

172. Maldonado-López R, De Smedt T, Michel P, et al. CD8alpha+ and CD8alpha- subclasses of dendritic cells direct the development of distinct T helper cells in vivo. *J Exp Med*. 1999;189(3):587-592.

173. Pulendran B, Smith JL, Caspary G, et al. Distinct dendritic cell subsets differentially regulate the class of immune response in vivo. *Proc Natl Acad Sci U S A*. 1999;96(3):1036-1041.

174. Sun T, Rojas OL, Li C, Ward LA, Philpott DJ, Gommerman JL. Intestinal Batf3-dependent dendritic cells are required for optimal antiviral T-cell responses in adult and neonatal mice. *Mucosal Immunol*. 2017;10(3):775-788.

175. Conejero L, Khouili SC, Martínez-cano S, et al. Lung CD103 + dendritic cells restrain allergic airway inflammation through IL-12 production. 2017;2(10).

176. Yamazaki S, Dudziak D, Heidkamp GF, et al. CD8+ CD205+ splenic dendritic cells are specialized to induce Foxp3+ regulatory T cells. *J Immunol*. 2008;181(10):6923-6933.

177. Travis MA, Reizis B, Melton AC, et al. Loss of integrin αvβ8 on dendritic cells causes autoimmunity and colitis in mice. *Nature*. 2007;449(7160):361-365.

178. Annes JP, Munger JS, Rifkin DB. Making sense of latent TGFbeta activation. *J Cell Sci*. 2003;116(Pt 2):217-224.

179. Mu D, Cambier S, Fjellbirkeland L, et al. The integrin alpha(v)beta8 mediates epithelial homeostasis through MT1-MMP-dependent activation of TGF-beta1. *J Cell Biol*. 2002;157(3):493-507.

180. Travis MA, Reizis B, Melton AC, et al. Loss of integrin alpha(v)beta8 on dendritic cells causes autoimmunity and colitis in mice. *Nature*. 2007;449(7160):361-365.

181. Yokota A, Takeuchi H, Maeda N, et al. GM-CSF and IL-4 synergistically trigger dendritic cells to acquire retinoic acid-producing capacity. *Int Immunol*. 2009;21(4):361-377.

182. Esterhazy D, Loschko J, London M, Jove V, Oliveira TY, Mucida D. Classical dendritic cells are required for dietary antigen-mediated induction of peripheral T(reg) cells and tolerance. *Nat Immunol*. 2016;17(5):545-555.

183. Iwata M, Hirakiyama A, Eshima Y, Kagechika H, Kato C, Song S-Y. Retinoic acid imprints gut-homing specificity on T cells. *Immunity*. 2004;21(4):527-538.

184. Jaensson-Gyllenback E, Kotarsky K, Zapata F, et al. Bile retinoids imprint intestinal CD103+ dendritic cells with the ability to generate gut-tropic T cells. *Mucosal Immunol*. 2011;4(4):438-447.

185. Svensson M, Johansson-Lindbom B, Zapata F, et al. Retinoic acid receptor signaling levels and antigen dose regulate gut homing receptor expression on CD8+ T cells. *Mucosal Immunol*. 2008;1(1):38-48.

186. Kobayashi T, Walsh PT, Walsh MC, et al. TRAF6 is a critical factor for dendritic cell maturation and development. *Immunity*. 2003;19(3):353-363.

187. Lewis KL, Caton ML, Bogunovic M, et al. Notch2 receptor signaling controls functional differentiation of dendritic cells in the spleen and intestine. *Immunity*. 2011;35(5):780-791.

188. Tussiwand R, Everts B, Grajales-Reyes GE, et al. Klf4 Expression in Conventional Dendritic Cells Is Required for T Helper 2 Cell Responses. *Immunity*. 2015;42(5):916-928.

189. Wu L, D'Amico A, Winkel KD, Suter M, Lo D, Shortman K. RelB Is Essential for the Development of Myeloid-Related CD8alpha Dendritic Cells but Not of Lymphoid-Related CD8alpha Dendritic Cells. *Immunity*. 1998;9(6):839-847.

190. Ichikawa E, Hida S, Omatsu Y, et al. Defective development of splenic and epidermal CD4+ dendritic cells in mice deficient for IFN regulatory factor-2. *Proc Natl Acad Sci U S A*. 2004;101(11):3909-3914.

191. Satpathy AT, Briseño CG, Lee JS, et al. Notch2-dependent classical dendritic cells orchestrate intestinal immunity against attaching and effacing bacterial pathogens. *Nat Immunol*. 2013;14(9):937-948.

192. Bajaña S, Roach K, Turner S, Paul J, Kovats S. IRF4 promotes cutaneous dendritic cell migration to lymph nodes during homeostasis and inflammation. *J Immunol*. 2012;189:3368-3377.

193. Vander Lugt B, Khan A a, Hackney J a, et al. Transcriptional programming of dendritic cells for enhanced MHC class II antigen presentation-Supplemental. *Nat Immunol*. 2014;15(2):161-167.

194. Scott CL, Bain CC, Wright PB, et al. CCR2(+)CD103(-) intestinal dendritic cells develop from DC-committed precursors and induce interleukin-17 production by T cells. *Mucosal Immunol*. 2014;8(2):327-339.

195. Scott CL, Soen B, Martens L, et al. The transcription factor Zeb2 regulates development of conventional and plasmacytoid DCs by repressing Id2. *J Exp Med*. 2016:jem.20151715.

196. Serbina N V, Pamer EG. Monocyte emigration from bone marrow during bacterial infection requires signals mediated by chemokine receptor CCR2. *Nat Immunol*. 2006;7(3):311-317.

197. Hotez PJ, Alvarado M, Basáñez MG, et al. The Global Burden of Disease Study 2010: Interpretation and Implications for the Neglected Tropical Diseases. *PLoS Negl Trop Dis*. 2014;8(7).

198. Weatherhead JE, Hotez PJ. Worm Infections in Children. *Pediatr Rev*. 2015;36(8):341-344.

199. Balic A, Bowles VM, Meeusen ENT. The immunobiology of gastrointestinal nematode infections in ruminants. *Adv Parasitol*. 2000;45:181-241.

200. Grencis RK. Th2-mediated host protective immunity to intestinal nematode infections. *Philos Trans R Soc Lond B Biol Sci*. 1997;352(1359):1377-1384.

201. Maizels RM, Hewitson JP, Smith KA. Susceptibility and immunity to helminth parasites. *Curr Opin Immunol*. 2012;24(4):459-466.

202. Pullan RL, Smith JL, Jasrasaria R, Brooker SJ. Global numbers of infection and disease burden of soil transmitted helminth infections in 2010. *Parasit Vectors*. 2014;7(1):37.

203. Betts J, deSchoolmeester ML, Else KJ. Trichuris muris: CD4+ T cell-mediated protection in reconstituted SCID mice. *Parasitology*. 2000;121 Pt 6:631-637.

204. Blackwell NM, Else KJ. A comparison of local and peripheral parasite-specific antibody production in different strains of mice infected with Trichuris muris. *Parasite Immunol*. 2002;24(4):203-211.

205. Cliffe LJ, Humphreys NE, Lane TE, Potten CS, Booth C, Grencis RK. Accelerated intestinal epithelial cell turnover: a new mechanism of parasite expulsion. *Science (80-)*. 2005;308(2005):1463-1465.

206. Artis D, Potten CS, Else KJ, Finkelman FD, Grencis RK. Trichuris muris: host intestinal epithelial cell hyperproliferation during chronic infection is regulated by interferon-gamma. *Exp Parasitol*. 1999;92(2):144-153.

207. Bell L V, Else KJ. Regulation of colonic epithelial cell turnover by IDO contributes to the innate susceptibility of SCID mice to Trichuris muris infection. *Parasite Immunol*. 2011;33(4):244-249.

208. Bancroft AJ, Grencis RK. Th1 and Th2 cells and immunity to intestinal helminths. *Chem Immunol*. 1998;71:192-208.

209. Else KJ, Finkelman FD. Intestinal nematode parasites, cytokines and effector mechanisms. *Int J Parasitol*. 1998;28(8):1145-1158.

210. Yamauchi J, Kawai Y, Yamada M, Uchikawa R, Tegoshi T, Arizono N. Altered expression of goblet cell- and mucin glycosylation-related genes in the intestinal epithelium during infection with the nematode Nippostrongylus brasiliensis in rat. *APMIS*. 2006;114(4):270-278.

211. Koyama K. Dendritic cell expansion occurs in mesenteric lymph nodes of B10.BR mice infected with the murine nematode parasite Trichuris muris. *Parasitol Res*. 2005;97(3):186-190.

212. deSchoolmeester ML, Little MC, Rollins BJ, Else KJ. Absence of CC chemokine ligand 2 results in an altered Th1/Th2 cytokine balance and failure to expel Trichuris muris infection. *J Immunol*. 2003;170(9):4693-4700.

213. Cruickshank SM, Deschoolmeester ML, Svensson M, et al. Rapid dendritic cell mobilization to the large intestinal epithelium is associated with resistance to Trichuris muris infection. *J Immunol*. 2009;182(5):3055-3062.

214. Hayes KS, Bancroft AJ, Goldrick M, Portsmouth C, Roberts IS, Grencis RK. Exploitation of the intestinal microflora by the parasitic nematode [i]Trichuris muris[/i]. *Science*. 2010;328(5984):1391-1394.

215. Tilney LG, Connelly PS, Guild GM, Vranich KA, Artis D. Adaptation of a nematode parasite to living within the mammalian epithelium. *J Exp Zool A Comp Exp Biol*. 2005;303(11):927-945.

216. Hasnain SZ, McGuckin MA, Grencis RK, Thornton DJ. Serine Protease(s) Secreted by the Nematode Trichuris muris Degrade the Mucus Barrier. *PLoS Negl Trop Dis*. 2012;6(10).

217. Drake LJ, Bianco AE, Bundy DA, Ashall F. Characterization of peptidases of adult Trichuris muris. *Parasitology*. 1994;109 (Pt 5:623-630.

218. Panesar TS, Croll NA. The hatching process in Trichuris muris (Nematoda: Trichuroidea). *Can J Zool*. 1981;59(4):621-628.

219. Wakelin D. Acquired immunity to Trichuris muris in the albino laboratory mouse. *Parasitology*. 1967;57(3):515-524.

220. Wakelin D, Selby GR. Functional antigens of Trichuris muris. The stimulation of immunity by vaccination of mice with somatic antigen preparations. *Int J Parasitol*. 1973;3(6):711-715.

221. Drake L, Korchev Y, Bashford L, et al. The major secreted product of the whipworm, Trichuris, is a pore-forming protein. *Proceedings Biol Sci*. 1994;257(1350):255-261.

222. Hiemstra IH, Klaver EJ, Vrijland K, et al. Excreted/secreted Trichuris suis products reduce barrier function and suppress inflammatory cytokine production of intestinal epithelial cells. *Mol Immunol*. 2014;60(1):1-7.

223. Klaver EJ, Kuijk LM, Lindhorst TK, Cummings RD, van Die I. Schistosoma mansoni Soluble Egg Antigens Induce Expression of the Negative Regulators SOCS1 and SHP1 in Human Dendritic Cells via Interaction with the Mannose Receptor. Gobert GN, ed. *PLoS One*. 2015;10(4).

224. Everts B, Perona-Wright G, Smits HH, et al. Omega-1, a glycoprotein secreted by Schistosoma mansoni eggs, drives Th2 responses. *J Exp Med*. 2009;206(8):1673-1680.

225. Else KJ, Wakelin D, Wassom DL, Hauda KM. The influence of genes mapping within the major histocompatibility complex on resistance to Trichuris muris infections in mice. *Parasitology*. 1990;101 Pt 1:61-67.

226. Else KJ, Wakelin D. Genetically-determined influences on the ability of poor responder mice to respond to immunization against Trichuris muris. *Parasitology*. 1990;100 Pt 3:479-489.

227. Else K, Wakelin D. Genetic variation in the humoral immune responses of mice to the nematode Trichuris muris. *Parasite Immunol*. 1989;11(1):77-90.

228. Else K, Wakelin D. The effects of H-2 and non-H-2 genes on the expulsion of the nematode Trichuris muris from inbred and congenic mice. *Parasitology*. 1988;96 (Pt 3):543-550.

229. Bancroft AJ, Else KJ, Grencis RK. Low-level infection with Trichuris muris significantly affects the polarization of the CD4 response. *Eur J Immunol*. 1994;24(12):3113-3118.

230. Bancroft AJ, Else KJ, Humphreys NE, Grencis RK. The effect of challenge and trickle Trichuris muris infections on the polarisation of the immune response. *Int J Parasitol*. 2001;31(14):1627-1637.

231. Wakelin D. The stimulation of immunity to Trichuris muris in mice exposed to low-level infections. *Parasitology*. 1973;66(1):181–189.

232. Behnke JM, Wakelin D. The survival of Trichuris muris in wild populations of its natural hosts. *Parasitology*. 1973;67(2):157-164.

233. Allison Bancroft GJ, J McKenzie AN, Bancroft AJ, Grencis RK. A Critical Role for IL-13 in Resistance to Intestinal Nematode Infection. *J Immunol Ref*. 1998;160:3453-3461.

234. Bancroft AJ, Artis D, Donaldson DD, Sypek JP, Grencis RK. Gastrointestinal nematode expulsion in IL-4 knockout mice is IL-13 dependent. *Eur J Immunol*. 2000;30(7):2083-2091.

235. Khan WI, Richard M, Akiho H, et al. Modulation of intestinal muscle contraction by interleukin-9 (IL-9) or IL-9 neutralization: Correlation with worm expulsion in murine nematode infections. *Infect Immun*. 2003;71(5):2430-2438.

236. Antignano F, Mullaly SC, Burrows K, Zaph C. Trichuris muris infection: a model of type 2 immunity and inflammation in the gut. *J Vis Exp*. 2011;(51):4-8.

237. Else KJ. Cytokine-mediated regulation of chronic intestinal helminth infection. *J Exp Med*. 1994;179(1):347-351.

238. Lin J-X, Migone T-S, Tseng M, et al. The role of shared receptor motifs and common stat proteins in the generation of cytokine pleiotropy and redundancy by IL-2, IL-4, IL-7, IL-13, and IL-15. *Immunity*. 1995;2(4):331-339.

239. Zurawski SM, Chomarat P, Djossou O, et al. The primary binding subunit of the human interleukin-4 receptor is also a component of the interleukin-13 receptor. *J Biol Chem*. 1995;270(23):13869-13878.

240. Bancroft AJ, McKenzie AN, Grencis RK. A critical role for IL-13 in resistance to intestinal nematode infection. *J Immunol*. 1998;160(7):3453-3461.

241. Hasnain SZ, Evans CM, Roy M, et al. Muc5ac: a critical component mediating the rejection of enteric nematodes. *J Exp Med*. 2011;208(5):893-900.

242. Hasnain SZ, Wang H, Ghia JE, et al. Mucin Gene Deficiency in Mice Impairs Host Resistance to an Enteric Parasitic Infection. *Gastroenterology*. 2010;138(5):1763-1771.e5.

243. Potten CS, Booth C, Pritchard DM. The intestinal epithelial stem cell: the mucosal governor. *Int J Exp Pathol*. 1997;78(4):219-243.

244. Maizels RM, Yazdanbakhsh M. Immune Regulation by helminth parasites: cellular and molecular mechanisms. *Nat Rev Immunol*. 2003;3(9):733-744.

245. Cortés A, Muñoz-Antoli C, Esteban JG, Toledo R. Th2 and Th1 Responses: Clear and Hidden Sides of Immunity Against Intestinal Helminths. *Trends Parasitol*. 2017;xx:1-16.

246. Hurst RJM, Else KJ. Trichuris muris research revisited: a journey through time. *Parasitology*. 2013;140(11):1325-1339.

247. Helmby H, Takeda K, Akira S, Grencis RK. Interleukin (IL)-18 Promotes the Development of Chronic Gastrointestinal Helminth Infection by Downregulating IL-13. *J Exp Med*. 2001;194(3):355-364.

248. Bancroft AJ, Else KJ, Sypek JP, Grencis RK. Interleukin-12 promotes a chronic intestinal nematode infection. *Eur J Immunol*. 1997;27(4):866-870.

249. Else KJ, Grencis RK. Helper T-cell subsets in mouse trichuriasis. *Parasitol Today*. 1991;7(11):313-316.

250. Else KJ, Hultner L, Grencis RK. Modulation of cytokine production and response phenotypes in murine trichuriasis. *Parasite Immunol*. 1992;14(4):441-449.

251. Else KJ, Entwistle GM, Grencis RK. Correlations between worm burden and markers of Th1 and Th2 cell subset induction in an inbred strain of mouse infected with Trichuris muris. *Parasite Immunol*. 1993;15(10):595-600.

252. Hasnain SZ, Dawson PA, Lourie R, et al. Immune-driven alterations in mucin sulphation is an important mediator of Trichuris muris helminth expulsion. *PLoS Pathog*. 2017;13(2):1-20.
253. Owyang AM, Zaph C, Wilson EH, et al. Interleukin 25 regulates type 2 cytokine-dependent immunity and limits chronic inflammation in the gastrointestinal tract. *J Exp Med*. 2006;203(4):843-849.
254. Zhao A, Urban JFJ, Sun R, et al. Critical role of IL-25 in nematode infection-induced alterations in intestinal function. *J Immunol*. 2010;185(11):6921-6929.
255. Humphreys NE, Xu D, Hepworth MR, Liew FY, Grencis RK. IL-33, a Potent Inducer of Adaptive Immunity to Intestinal Nematodes. *J Immunol*. 2008;180(4):2443-2449.
256. Zaph C, Troy AE, Taylor BC, et al. Epithelial-cell-intrinsic IKK-beta expression regulates intestinal immune homeostasis. *Nature*. 2007;446(7135):552-556.
257. Massacand JC, Stettler RC, Meier R, et al. Helminth products bypass the need for TSLP in Th2 immune responses by directly modulating dendritic cell function. *Proc Natl Acad Sci U S A*. 2009;106(33):13968-13973.
258. Taylor BC, Zaph C, Troy AE, et al. TSLP regulates intestinal immunity and inflammation in mouse models of helminth infection and colitis. *J Exp Med*. 2009;206(3):655-667.
259. Helmby H, Grencis RK. Essential role for TLR4 and MyD88 in the development of chronic intestinal nematode infection. *Eur J Immunol*. 2003;33(11):2974-2979.
260. Peterson LW, Artis D. Intestinal epithelial cells: regulators of barrier function and immune homeostasis. *Nat Rev Immunol*. 2014;14(3):141-153.
261. deSchoolmeester ML, Manku H, Else KJ. The innate immune responses of colonic epithelial cells to Trichuris muris are similar in mouse strains that develop a type 1 or type 2 adaptive immune response. *Infect Immun*. 2006;74(11):6280-6286.
262. Hammad H, Lambrecht BN. Barrier Epithelial Cells and the Control of Type 2 Immunity. *Immunity*. 2015;43(1):29-40.
263. Saenz SA, Siracusa MC, Perrigoue JG, et al. IL25 elicits a multipotent progenitor cell population that promotes T(H)2 cytokine responses. *Nature*. 2010;464(7293):1362-1366.
264. Fort MM, Cheung J, Yen D, et al. IL-25 induces IL-4, IL-5, and IL-13 and Th2-associated pathologies in vivo. *Immunity*. 2001;15(6):985-995.
265. Hongjia L, Caiqing Z, Degan L, et al. IL-25 promotes Th2 immunity responses in airway inflammation of asthmatic mice via activation of dendritic cells. *Inflammation*. 2014;37(4):1070-1077.
266. von Moltke J, Ji M, Liang H-E, Locksley RM. Tuft-cell-derived IL-25 regulates an intestinal ILC2–epithelial response circuit. *Nature*. 2015;529(7585):221-225.
267. Howitt MR, Lavoie S, Michaud M, et al. Tuft cells, taste-chemosensory cells, orchestrate parasite type 2 immunity in the gut. *Science*. 2016;351(6279):1329-1333.
268. Gerbe FF, Sidot E, Smyth DJ, et al. Intestinal epithelial tuft cells initiate type 2 mucosal immunity to helminth parasites. *Nature*. 2016;529(7585):226-230.

269. Hung L-Y, Lewkowich IP, Dawson LA, et al. IL-33 drives biphasic IL-13 production for noncanonical Type 2 immunity against hookworms. *Proc Natl Acad Sci U S A*. 2013;110(1):282-287.

270. Wills-Karp M, Rani R, Dienger K, et al. Trefoil factor 2 rapidly induces interleukin 33 to promote type 2 immunity during allergic asthma and hookworm infection. *J Exp Med*. 2012;209(3):607-622.

271. Schmitz J, Owyang A, Oldham E, et al. IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. *Immunity*. 2005;23(5):479-490.

272. Grencis RK, Humphreys NE, Bancroft AJ. Immunity to gastrointestinal nematodes: Mechanisms and myths. *Immunol Rev*. 2014;260(1):183-205.

273. Yasuda K, Muto T, Kawagoe T, et al. Contribution of IL-33-activated type II innate lymphoid cells to pulmonary eosinophilia in intestinal nematode-infected mice. *Proc Natl Acad Sci U S A*. 2012;109(9):3451-3456.

274. Townsend MJ, Fallon PG, Matthews DJ, Jolin HE, McKenzie AN. T1/ST2-deficient mice demonstrate the importance of T1/ST2 in developing primary T helper cell type 2 responses. *J Exp Med*. 2000;191(6):1069-1076.

275. Haraldsen G, Balogh J, Pollheimer J, Sponheim J, Kuchler AM. Interleukin-33 - cytokine of dual function or novel alarmin? *Trends Immunol*. 2009;30(5):227-233.

276. Artis D, Spits H. The biology of innate lymphoid cells. *Nature*. 2015;517(7534):293-301.

277. Nussbaum JC, Van Dyken SJ, von Moltke J, et al. Type 2 innate lymphoid cells control eosinophil homeostasis. *Nature*. 2013;502(7470):245-248.

278. Duerr CU, Fritz JH. Regulation of group 2 innate lymphoid cells. *Cytokine*. 2016;87:1-8.

279. Neill DR, Wong SH, Bellosi A, et al. Nuocytes represent a new innate effector leukocyte that mediates type-2 immunity. *Nature*. 2010;464(7293):1367-1370.

280. Price AE, Liang H-E, Sullivan BM, et al. Systemically dispersed innate IL-13-expressing cells in type 2 immunity. *Proc Natl Acad Sci U S A*. 2010;107(25):11489-11494.

281. Moro K, Yamada T, Tanabe M, et al. Innate production of T(H)2 cytokines by adipose tissue-associated c-Kit(+)Sca-1(+) lymphoid cells. *Nature*. 2010;463(7280):540-544.

282. Turner J-E, Morrison PJ, Wilhelm C, et al. IL-9-mediated survival of type 2 innate lymphoid cells promotes damage control in helminth-induced lung inflammation. *J Exp Med*. 2013;210(13):2951-2965.

283. Hwang YY, McKenzie ANJ. Innate lymphoid cells in immunity and disease. *Adv Exp Med Biol*. 2013;785:9-26.

284. Saenz SA, Noti M, Artis D. Innate immune cell populations function as initiators and effectors in Th2 cytokine responses. *Trends Immunol*. 2010;31(11):407-413.

285. Rivera A, Siracusa MC, Yap GS, Gause WC. Innate cell communication kick-starts pathogen-specific immunity. *Nat Immunol*. 2016;17(4):356-363.

286. Voehringer D. Protective and pathological roles of mast cells and basophils. *Nat Rev Immunol*. 2013;13(5):362-375.

287. Obata-Ninomiya K, Ishiwata K, Tsutsui H, et al. The skin is an important bulwark of acquired immunity against intestinal helminths. *J Exp Med*. 2013;210(12):2583-2595.

288. McLeod JJA, Baker BN, Ryan JJ. Mast Cell Production and Response to IL-4 and IL-13. *Cytokine*. 2015;75(1):57-61.

289. Min B, Prout M, Hu-Li J, et al. Basophils Produce IL-4 and Accumulate in Tissues after Infection with a Th2-inducing Parasite. *J Exp Med*. 2004;200(4):507-517.

290. Piehler D, Stenzel W, Grahnert A, et al. Eosinophils Contribute to IL-4 Production and Shape the T-Helper Cytokine Profile and Inflammatory Response in Pulmonary Cryptococcosis. *Am J Pathol*. 2011;179(2):733-744.

291. Svensson M, Bell L, Little MC, Deschoolmeester M, Locksley RM, Else KJ. Accumulation of eosinophils in intestine-draining mesenteric lymph nodes occurs after Trichuris muris infection. *Parasite Immunol*. 2011;33(1):1-11.

292. Perrigoue JG, Saenz SA, Siracusa MC, et al. MHC class II–dependent basophil–CD4+ T cell interactions promote TH2 cytokine–dependent immunity. *Nat Immunol*. 2009;10(7):697-705.

293. Phythian-Adams AT, Cook PC, Lundie RJ, et al. CD11c depletion severely disrupts Th2 induction and development in vivo. *J Exp Med*. 2010;207(10):2089-2096.

294. Crowle PK, Reed ND. Rejection of the intestinal parasite Nippostrongylus brasiliensis by mast cell-deficient W/Wv anemic mice. *Infect Immun*. 1981;33(1):54-58.

295. Hayes KS, Bancroft AJ, Grencis RK. Immune-mediated regulation of chronic intestinal nematode infection. *Immunol Rev*. 2004;201:75-88.

296. Pennock JL, Grencis RK. In vivo exit of c-kit+/CD49dhi/β7+ mucosal mast cell precursors from the bone marrow following infection with the intestinal nematode Trichinella spiralis. *Blood*. 2004;103(7):2655-2660.

297. Knight PA, Wright SH, Lawrence CE, Paterson YY, Miller HR. Delayed expulsion of the nematode Trichinella spiralis in mice lacking the mucosal mast cell-specific granule chymase, mouse mast cell protease-1. *J Exp Med*. 2000;192(12):1849-1856.

298. Donaldson LE, Schmitt E, Huntley JF, Newlands GF, Grencis RK. A critical role for stem cell factor and c-kit in host protective immunity to an intestinal helminth. *Int Immunol*. 1996;8(4):559-567.

299. Betts CJ, Else KJ. Mast cells, eosinophils and antibody-mediated cellular cytotoxicity are not critical in resistance to Trichuris muris. *Parasite Immunol*. 1999;21(1):45-52.

300. Hepworth MR, Danilowicz-Luebert E, Rausch S, et al. Mast cells orchestrate type 2 immunity to helminths through regulation of tissue-derived cytokines. *Proc Natl Acad Sci U S A*. 2012;109(17):6644-6649.

301. Sorobetea D, Holm JB, Henningsson H. Acute infection with the intestinal parasite Trichuris muris has long-term consequences on mucosal mast cell homeostasis and epithelial integrity. 2017:257-268.

302. Dixon H, Blanchard C, Deschoolmeester ML, et al. The role of Th2 cytokines, chemokines and parasite products in eosinophil recruitment to the gastrointestinal mucosa during helminth infection. *Eur J Immunol*. 2006;36(7):1753-1763.

303. Pulendran B, Tang H, Manicassamy S. Programming dendritic cells to induce T(H)2 and tolerogenic responses. *Nat Immunol*. 2010;11(8):647-655.

304. Smith KA, Harcus Y, Garbi N, Hämmerling GJ, MacDonald AS, Maizels RM. Type 2 innate immunity in helminth infection is induced redundantly and acts autonomously following cd11c+ cell depletion. *Infect Immun*. 2012;80(10):3481-3489.

305. Smith KA, Hochweller K, Hammerling GJ, Boon L, MacDonald AS, Maizels RM. Chronic Helminth Infection Promotes Immune Regulation In Vivo through Dominance of CD11cloCD103- Dendritic Cells. *J Immunol*. 2011;186(12):7098-7109.

306. Bowcutt R, Bramhall M, Logunova L, et al. A role for the pattern recognition receptor Nod2 in promoting recruitment of CD103(+) dendritic cells to the colon in response to Trichuris muris infection. *Mucosal Immunol*. 2014;7(November 2012):1-12.

307. Gold MJ, Antignano F, Hughes MR, Zaph C, Mcnagny KM. Dendritic-cell expression of Ship1 regulates Th2 immunity to helminth infection in mice. *Eur J Immunol*. 2016;46(1):122-130.

308. Cheroutre H, Lambolez F, Mucida D. The light and dark sides of intestinal intraepithelial lymphocytes. *Nat Rev Immunol*. 2011;11(7):445-456.

309. El-Asady R, Yuan R, Liu K, et al. TGF-{beta}-dependent CD103 expression by CD8(+) T cells promotes selective destruction of the host intestinal epithelium during graft-versus-host disease. *J Exp Med*. 2005;201(10):1647-1657.

310. Reis BS, Rogoz A, Costa-Pinto FA, Taniuchi I, Mucida D. Mutual expression of Runx3 and ThPOK regulates intestinal CD4(+) T cell immunity. *Nat Immunol*. 2013;14(3):271-280.

311. Gao Y, Nish SA, Jiang R, et al. Control of T helper 2 responses by transcription factor IRF4-dependent dendritic cells. *Immunity*. 2013;39(4):722-732.

312. Williams JW, Tjota MY, Clay BS, et al. Transcription factor IRF4 drives dendritic cells to promote Th2 differentiation. *Nat Commun*. 2013;4(May):2990.

313. Kumamoto Y, Linehan M, Weinstein JS, Laidlaw BJ, Craft JE, Iwasaki A. CD301b+ dermal dendritic cells drive T helper 2 cell-mediated immunity. *Immunity*. 2013;39(4):733-743.

314. Mayer JU, Demiri M, Agace WW, MacDonald AS, Svensson-Frej M, Milling SW. Different populations of CD11b+ dendritic cells drive Th2 responses in the small intestine and colon. *Nat Commun*. 2017;8(May):15820.

315. Demiri M, Muller-Luda K, Agace WW, Svensson-Frej M. Distinct DC subsets regulate adaptive Th1 and 2 responses during Trichuris muris infection. *Parasite Immunol*. 2017;39(10).

316. Cliffe LJ, Grencis RK. The Trichuris muris system: A paradigm of resistance and susceptibility to intestinal nematode infection. *Adv Parasitol*. 2004;57(4):255-307.

317. Everts B, Tussiwand R, Dreesen L, et al. Migratory CD103+ dendritic cells suppress helminth-driven type 2 immunity through constitutive expression of IL-12. *J Exp Med*. 2015:1-17.

318. Worthington JJ, Klementowicz JE, Rahman S, et al. Loss of the TGFβ-activating integrin αvβ8 on dendritic cells protects mice from chronic intestinal parasitic infection via control of type 2 immunity. *PLoS Pathog*. 2013;9(10).

319. Manetti R, Parronchi P, Giudizi MG, et al. Natural killer cell stimulatory factor (interleukin 12 [IL-12]) induces T helper type 1 (Th1)-specific immune responses and

inhibits the development of IL-4-producing Th cells. *J Exp Med*. 1993;177(4):1199-1204.

320. Oswald IP, Caspar P, Jankovic D, Wynn TA, Pearce EJ, Sher A. IL-12 inhibits Th2 cytokine responses induced by eggs of Schistosoma mansoni. *J Immunol*. 1994;153(4):1707-1713.
321. Finkelman FD, Madden KB, Cheever AW, et al. Effects of interleukin 12 on immune responses and host protection in mice infected with intestinal nematode parasites. *J Exp Med*. 1994;179(5):1563-1572.
322. Esterházy D, Loschko J, London M, Jove V, Oliveira TY, Mucida D. Classical dendritic cells are required for dietary antigen-mediated induction of peripheral Treg cells and tolerance. *Nat Immunol*. 2016;17(5):545-555.
323. Boucard-Jourdin M, Kugler D, Endale Ahanda M-L, et al. beta8 Integrin Expression and Activation of TGF-beta by Intestinal Dendritic Cells Are Determined by Both Tissue Microenvironment and Cell Lineage. *J Immunol*. 2016;197(5):1968-1978.
324. Aychek T, Mildner A, Yona S, et al. IL-23-mediated mononuclear phagocyte crosstalk protects mice from Citrobacter rodentium-induced colon immunopathology. *Nat Commun*. 2015;6(6525):6525.
325. Peng J, Yang XO, Chang SH, Yang J, Dong C. IL-23 signaling enhances Th2 polarization and regulates allergic airway inflammation. *Cell Res*. 2010;20(1):62-71.
326. Wakashin H, Hirose K, Maezawa Y, et al. IL-23 and Th17 cells enhance Th2-cell-mediated eosinophilic airway inflammation in mice. *Am J Respir Crit Care Med*. 2008;178(10):1023-1032.
327. Sokol CL, Chu N-Q, Yu S, Nish SA, Laufer TM, Medzhitov R. Basophils function as antigen-presenting cells for an allergen-induced T helper type 2 response. *Nat Immunol*. 2009;10(7):713-720.
328. Oliphant CJJ, Hwang YYY, Walker JAA, et al. MHCII-mediated dialog between group 2 innate lymphoid cells and CD4+ T cells potentiates type 2 immunity and promotes parasitic helminth expulsion. *Immunity*. 2014;41(2):283-295.
329. Eisenbarth SC, Piggott DA, Bottomly K. The master regulators of allergic inflammation: dendritic cells in Th2 sensitization. *Curr Opin Immunol*. 2003;15(6):620-626.
330. Moser M, Murphy KM. Dendritic cell regulation of TH1-TH2 development. *Nat Immunol*. 2000;1(3):199-205.
331. MacDonald AS, Pearce EJ. Cutting edge: polarized Th cell response induction by transferred antigen-pulsed dendritic cells is dependent on IL-4 or IL-12 production by recipient cells. *J Immunol*. 2002;168(7):3127-3130.
332. Everts B, Hussaarts L, Driessen NN, et al. Schistosome-derived omega-1 drives Th2 polarization by suppressing protein synthesis following internalization by the mannose receptor. *J Exp Med*. 2012;209(10):1753-1767.
333. Thomas PG, Carter MR, Atochina O, et al. Maturation of Dendritic Cell 2 Phenotype by a Helminth Glycan Uses a Toll-Like Receptor 4-Dependent Mechanism. *J Immunol*. 2003;171(11):5837-5841.

334. Dillon S, Agrawal A, Van Dyke T, et al. A Toll-Like Receptor 2 Ligand Stimulates Th2 Responses In Vivo, via Induction of Extracellular Signal-Regulated Kinase Mitogen-Activated Protein Kinase and c-Fos in Dendritic Cells. *J Immunol*. 2004;172(8):4733-4743.
335. Eisenbarth SC, Piggott DA, Huleatt JW, Visintin I, Herrick CA, Bottomly K. Lipopolysaccharide-enhanced, Toll-like Receptor 4–dependent T Helper Cell Type 2 Responses to Inhaled Antigen. *J Exp Med*. 2002;196(12):1645-1651.
336. Hammad H, Chieppa M, Perros F, Willart MA, Germain RN, Lambrecht BN. House dust mite allergen induces asthma via Toll-like receptor 4 triggering of airway structural cells. *Nat Med*. 2009;15(4):410-416.
337. Soumelis V, Liu Y-J. Human thymic stromal lymphopoietin: a novel epithelial cell-derived cytokine and a potential key player in the induction of allergic inflammation. *Springer Semin Immunopathol*. 2004;25(3-4):325-333.
338. Bogiatzi SI, Fernandez I, Bichet J-C, et al. Cutting Edge: Proinflammatory and Th2 cytokines synergize to induce thymic stromal lymphopoietin production by human skin keratinocytes. *J Immunol*. 2007;178(6):3373-3377.
339. Bell BD, Kitajima M, Larson RP, et al. The transcription factor STAT5 is critical in dendritic cells for the development of TH2 but not TH1 responses. *Nat Immunol*. 2013;14(4):364-371.
340. Reche PA, Soumelis V, Gorman DM, et al. Human thymic stromal lymphopoietin preferentially stimulates myeloid cells. *J Immunol*. 2001;167(1):336-343.
341. Ito T, Wang Y-HH, Duramad O, et al. TSLP-activated dendritic cells induce an inflammatory T helper type 2 cell response through OX40 ligand. *J Exp Med*. 2005;202(9):1213-1223.
342. Arima K, Watanabe N, Hanabuchi S, Chang M, Sun S-C, Liu Y-J. Distinct signal codes generate dendritic cell functional plasticity. *Sci Signal*. 2010;3(105):ra4.
343. Soumelis V, Reche PA, Kanzler H, et al. Human epithelial cells trigger dendritic cell mediated allergic inflammation by producing TSLP. *Nat Immunol*. 2002;3(7):673-680.
344. Ito T, Wang YH, Duramad O, et al. TSLP-activated dendritic cells induce an inflammatory T helper type 2 cell response through OX40 ligand. *J Exp Med*. 2005;202(9):1213-1223.
345. Watanabe N, Hanabuchi S, Marloie-Provost M-AA, Antonenko S, Liu Y-JJ, Soumelis V. Human TSLP promotes CD40 ligand-induced IL-12 production by myeloid dendritic cells but maintains their Th2 priming potential. *Blood*. 2005;105(12):4749-4751.

Paper I

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IRF8 Transcription-Factor-Dependent Classical Dendritic Cells Are Essential for Intestinal T Cell Homeostasis

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SUMMARY

The role of dendritic cells (DCs) in intestinal immune homeostasis remains incompletely defined. Here we show that mice lacking IRF8 transcription-factor-dependent DCs had reduced numbers of T cells in the small intestine (SI), but not large intestine (LI), including an almost complete absence of SI CD8$\alpha\beta^+$ and CD4$^+$CD8$\alpha\alpha^+$ T cells; the latter requiring β8 integrin expression by migratory IRF8 dependent CD103$^+$CD11b$^-$ DCs. SI homing receptor induction was impaired during T cell priming in mesenteric lymph nodes (MLN), which correlated with a reduction in aldehyde dehydrogenase activity by SI-derived MLN DCs, and inefficient T cell localization to the SI. These mice also lacked intestinal T helper 1 (Th1) cells, and failed to support Th1 cell differentiation in MLN and mount Th1 cell responses to *Trichuris muris* infection. Collectively these results highlight multiple non-redundant roles for IRF8 dependent DCs in the maintenance of intestinal T cell homeostasis.

INTRODUCTION

The intestinal mucosa contains a diverse array of T cells that play an essential role in maintaining tissue homeostasis, protection from mucosal pathogens and, when inadequately controlled, in driving immune pathology. Deciphering the key cellular and molecular pathways regulating the differentiation and maintenance of intestinal T cell subsets is thus essential to our understanding of mucosal immune homeostasis and disease and for the generation of more effective vaccines.

Intestinal T cells are diffusely distributed throughout the intestinal lamina propria (LP) and epithelium and collectively make up the largest populations of T cells in the body (Cheroutre and Madakamutil, 2004; Mowat and Agace, 2014). LP T cells consist primarily of CD4$^+$ T cells, which enter the intestine following activation in secondary lymphoid organs and might reside within the LP for long periods of time as tissue-resident memory (Trm) cells (Turner and Farber, 2014). They are functionally heterogeneous and include distinct subsets of interleukin-17 (IL-17)-producing T helper 17 (Th17) cells, interferon-γ (IFN-γ)-producing T helper 1 (Th1) cells, and diverse populations of regulatory T cells (Tregs) (Mowat and Agace, 2014). In contrast, small intestinal (SI) intraepithelial lymphocytes (IEL) can be broadly divided into two groups; "unconventional" CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ and CD8$\alpha\alpha^+$TCR$\gamma\delta^+$ IEL and conventional CD8$\alpha\beta^+$TCR$\alpha\beta^+$ and CD4$^+$TCR$\alpha\beta^+$ IEL (Cheroutre et al., 2011). Like LP T cells, conventional IEL derive from lymphoid tissue primed T cells (Cheroutre et al., 2011) and might remain in the epithelium as Trm cells (Cauley and Lefrançois, 2013).

Intestinal dendritic cells (DCs) are located throughout the intestinal LP, in gut-associated lymphoid tissues (GALT), including Peyer's patches (PP) and solitary isolated lymphoid tissues, and in intestinal draining mesenteric lymph nodes (MLN). In addition to LN resident interferon regulatory factor (IRF) 8 and basic leucine zipper transcription factor ARF-like 3 (Batf3) dependent CD8α^+ and IRF4 expressing CD11b$^+$ DCs (Aliberti et al., 2003; Edelson et al., 2010; Persson et al., 2013) MLNs contain a large population of LP-derived CD11c$^+$ MHCII^{hi} migratory DCs that play a key role in the transport and presentation of intestinal derived self and foreign antigen (Huang et al., 2000; Jaensson et al., 2008; Schulz et al., 2009). The majority of DCs in the SI-LP (Johansson-Lindbom et al., 2005), and intestinal draining steady-state lymph (Cerovic et al., 2013; Schulz et al., 2009) express the integrin α_E(CD103)β_7 and can be divided into two major subsets; a Batf3- and IRF8-dependent subset of CD103$^+$CD11b$^-$ DCs

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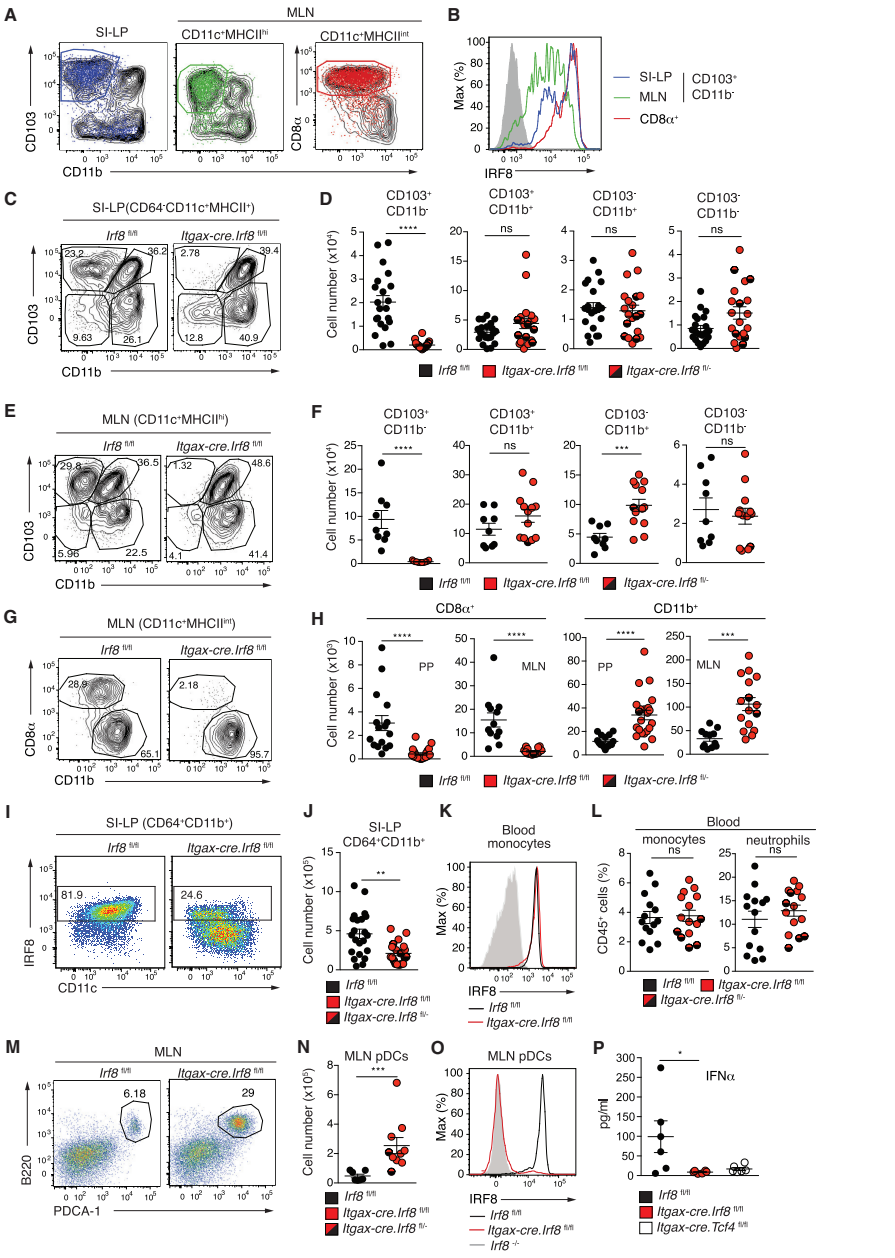

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(Edelson et al., 2010), and an IRF4- and Notch2-dependent subset of CD103$^+$CD11b$^+$ DCs (Lewis et al., 2011; Persson et al., 2013). We, and others, have recently demonstrated that IRF4-dependent DCs have a non-redundant role in driving mucosal Th17 cell and Th2 cell responses (Gao et al., 2013; Persson et al., 2013; Schlitzer et al., 2013). In contrast, although IRF8-dependent DCs are recognized for their capacity to cross-present antigen (Shortman and Heath, 2010), and more recently to serve as a platform for CD4$^+$ T cell dependent CD8$^+$ T cell responses (Eickhoff et al., 2015; Hor et al., 2015), their role in the regulation of intestinal T cell homeostasis remains unknown.

RESULTS

IRF8 Deletion in CD11c$^+$ Cells Leads to a Loss in LN Resident CD8α^+ and Intestinal CD103$^+$CD11b$^-$ DCs

To explore the role of IRF8-dependent DCs in intestinal homeostasis, we initially assessed IRF8 expression in intestinal DC subsets by intracellular flow cytometry analysis. In the SI-LP, IRF8 was expressed by CD103$^+$CD11b$^-$ DCs and a minor subset of CD103$^-$ DCs (Figure 1A). In the MLN, IRF8 was expressed by lymph node resident CD8α^+MHCII^{int} DCs (Figures 1A and 1B) and SI-LP derived CD103$^+$CD11b$^-$ MHCII^{hi} DCs (Figures 1A and 1B). MLN resident CD8α^+MHCII^{int} DCs and SI-LP CD103$^+$ CD11b$^-$ DCs expressed higher amounts of IRF8 than SI-LP derived CD103$^+$CD11b$^-$ DCs in the MLN (Figure 1B), suggesting that IRF8 expression is downregulated upon emigration from the intestine. To assess the role of IRF8 in intestinal DC homeostasis, we crossed *Itgax-cre* mice (Caton et al., 2007) with *Irf8*$^{fl/fl}$ mice. Germline deletion of *Irf8* occurred in a small proportion of offspring and we thus tracked the presence of the deleted and floxed *Irf8* allele in all pups. Quantitative genotyping and further analysis of the *Irf8* locus in these mice can be found in Supplemental Information and Figures S1A and S1B. IRF8 protein was not detected in CD11c$^+$MHCII$^+$ splenic or MLN cells from *Itgax-cre.Irf8*$^{fl/fl}$ and *Itgax-cre.Irf8*$^{fl/-}$ mice (Figure S1C and data not shown) consistent with efficient removal of the flox *Irf8* allele in these cells (Figure S1B). Because *Itgax-cre.Irf8*$^{fl/fl}$ and *Itgax-cre.Irf8*$^{fl/-}$ mice displayed similar phenotypes, data from these groups were pooled throughout. *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ mice lacked CD103$^+$CD11b$^-$ DCs in the SI-LP, LI-LP, and MLN, while the numbers of SI-LP and LI-LP CD103$^-$ and SI-LP CD103$^+$ CD11b$^+$ DCs were similar to those observed in *Irf8*$^{fl/fl}$ mice (Figures 1C–1F, Figures S2A and S2B). Consistent with previous findings in IRF8-deficient mice (Aliberti et al., 2003), *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ mice lacked resident CD8α^+ DCs in Peyer's patch (PP), MLN, and the spleen (Figures 1G and 1H, and Figures S2C and S2D); these tissues also had enhanced numbers of resident CD11b$^+$ DCs (Figure 1H, and Figure S2D). As recently observed in *Irf8*$^{WT/-}$ mice (Grajales-Reyes et al., 2015), *Irf8*$^{fl/-}$ and *Itgax-cre.Irf8*$^{fl/WT}$ mice showed a major reduction in LN resident CD8α^+ DCs (Figures S2E and S2F), with unaltered numbers of migratory CD103$^+$CD11b$^-$ DC (Figures S2E and S2F), indicating differential gene dosage requirements between these populations.

IRF8-deficient mice have reduced numbers of circulating Ly6C^{hi} monocytes (Kurotaki et al., 2013), which can act as precursors of intestinal macrophages (Bain et al., 2014), and increased numbers of circulating neutrophils (Holtschke et al., 1996). We thus assessed whether IRF8 expression and numbers of these cells was altered in *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ mice. IRF8 was expressed in SI-LP CD64$^+$CD11b$^+$ cells and circulating Ly6C^{hi} monocytes, but not in neutrophils (Figures 1I and 1K, Figure S1C), and in *Itgax-cre.Irf8*$^{fl/fl}$ mice IRF8 was deleted in SI-LP CD64$^+$CD11b$^+$ cells expressing the highest amounts of CD11c but not in Ly6C^{hi} monocytes (Figures 1I and 1K). The number of SI-LP CD64$^+$CD11b$^+$ cells was reduced in *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ mice compared with *Irf8*$^{fl/fl}$ mice; however, the proportion of circulating Ly6C^{hi} monocytes and neutrophils was unchanged (Figures 1J and 1L). While the total number of splenic CD64$^+$ and CD64$^+$F4/80$^+$ myeloid cells was unaltered in *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ mice (Figures S2G and S2H), there was a slight yet significant increase in splenic CD64$^+$CD11b$^+$ cells compared with *Irf8*$^{fl/fl}$ mice (Figure S2H).

While IRF8 is expressed by and required for the development of plasmacytoid DCs (pDCs) (Schiavoni et al., 2002), the total number of pDCs was increased in the MLN and spleen of *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ mice compared with *Irf8*$^{fl/fl}$ mice (Figures 1M and 1N, and Figure S2I) despite an absence of IRF8 expression (Figure 1O). CD11c enriched splenic cells from *Itgax-cre.Irf8*$^{fl/fl}$ mice failed to produce IFNα after stimulation with D-type CpG-oligodeoxynucleotide 1585 (Figure 1P), a response that is pDC dependent (Kumagai et al., 2007) and absent in *Itgax-cre.Tcf4*$^{fl/fl}$ mice that display reduced numbers of pDCs (Cisse et al., 2008)(Figure 1P). Thus IRF8 is required for the regulation of pDC homeostasis and function.

Itgax-cre.Irf8$^{fl/fl\ or\ fl/-}$ Mice Have Reduced Numbers of SI-IEL

Next we assessed the impact of IRF8 deficiency in CD11c$^+$ cells on the composition and number of SI-IEL (Figure 2). The total number of CD45$^+$ cells was dramatically reduced in the SI epithelium of

Figure 1. Impact of IRF8 Deletion on Intestinal Mononuclear Phagocyte Subset Composition

(A) Representative flow cytometry plots of SI-LP and MLN DC subsets in C57BL/6 mice. Colored dots in each panel represent IRF8-expressing cells. Cells are pre-gated on live, CD45$^+$ Lin(CD19, B220, NK1.1, TER119)$^-$CD11c$^+$MHCII$^+$Ly6C$^-$CD64$^-$ cells.

(B) Intracellular IRF8 staining within the indicated gated DC populations in (A).

(C–H) Intestinal DC subset composition of *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ and *Irf8*$^{fl/fl}$ mice. (C, E, G) Representative flow cytometry plots and (D, F, H) total cell number of (C) and (D) SI-LP, (E) and (F) MHCII^{hi} MLN DCs, and (G) and (H) MHCII^{int} MLN and PP DCs.

(I–O) (I, K, and O) Intracellular IRF8 staining, (J) and (N) total number or (L) frequency among CD45$^+$ cells of (I) and (J) SI-LP macrophages, (K) and (L) blood monocytes (Ly6G$^-$SiglecF$^-$CD64$^+$CD11b$^+$Ly6C^{hi}, left panel) and neutrophils (Ly6G$^+$CD11b$^+$SSC^{int}, right panel), and (N) and (O) MLN pDCs in *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ and *Irf8*$^{fl/fl}$ littermates. (M) Representative flow cytometry plot of MLN pDCs. (K) FMO staining control (filled histogram).

(P) IFN-α production by MACS enriched splenic CD11c$^+$ cells from indicated mice following stimulation with CpG ODN 1585. Data are from (A, B, E, F, K, P) 2–4 or (C, D, G, H, I, J) 5–8 independent experiments. Each dot represents one mouse. Error bars represent mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, ns, not significant. See also Figure S1 and 2.

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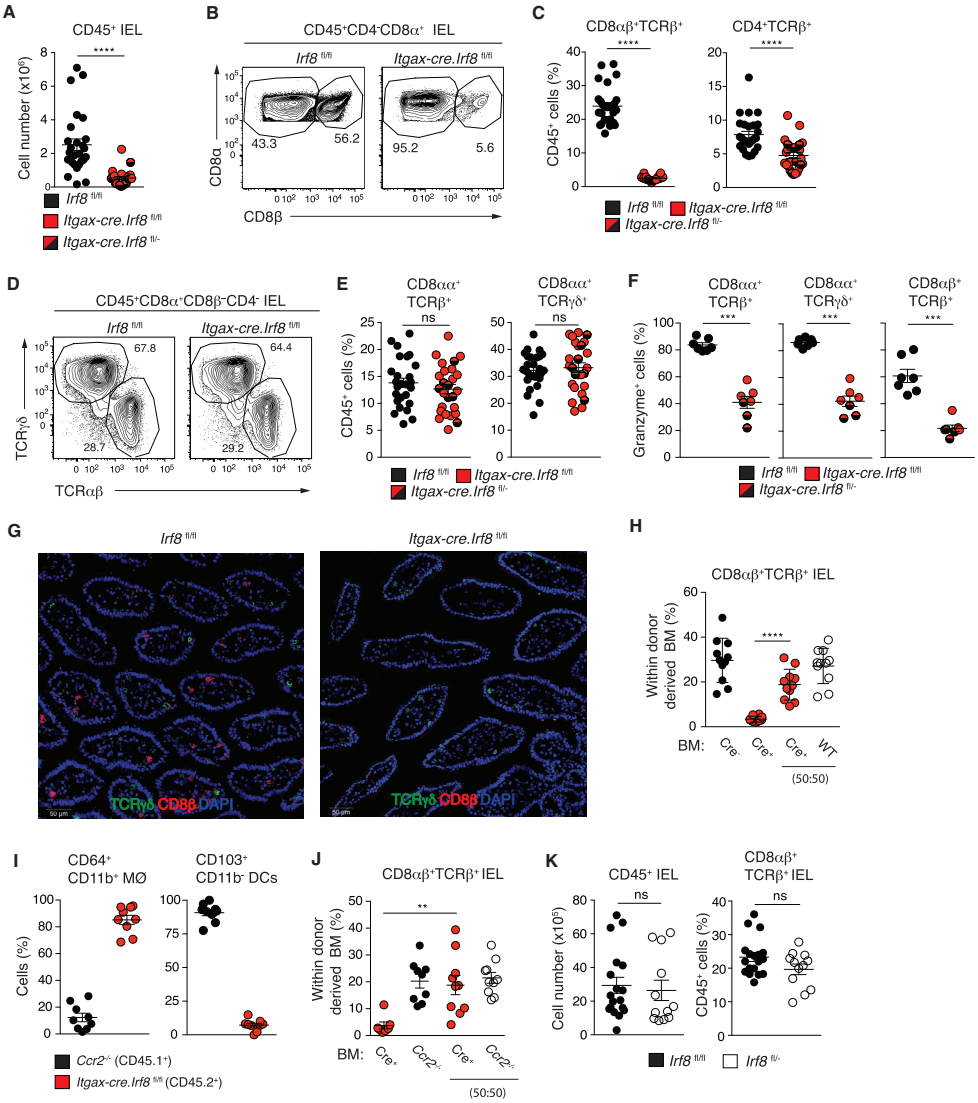

Figure 2. *Itgax-cre.Irf8* $^{fl/fl\ or\ fl/-}$ Mice Have Reduced Numbers of Conventional SI-IEL

(A–E) (A) Total number of CD45$^+$ cells IEL, (B) and (D) representative flow cytometry plots and (C) proportion of CD8$\alpha\beta^+$TCRβ^+, CD4$^+$TCRβ^+, and (E) CD8$\alpha\alpha^+$TCRβ^+ and CD8$\alpha\alpha^+$TCR$\gamma\delta^+$ among total CD45$^+$ SI-IELs in indicated mouse strains.

(F) Proportion of granzyme A$^+$ cells in indicated SI-IEL populations.

(G) Representative immunohistochemical staining of jejunum sections from indicated mice. DAPI, blue; TCR$\gamma\delta$, green; CD8β, red. Scale bar represents 50 μm.

(H) Percentage of CD8$\alpha\beta^+$ IEL within the indicated BM derived population in single and mixed BM chimeras.

(I) Percentage of SI-LP CD64$^+$CD11b$^+$ cells and CD103$^+$CD11b$^-$ MLN MHCII^{hi} DCs deriving from indicated BM from *Ccr2*$^{-/-}$.CD45.1$^+$:*Itgax-cre.Irf8*$^{fl/fl}$ mixed BM chimeras.

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Itgax-cre.Irf8$^{fl/fl\ or\ fl/-}$ compared with *Irf8*$^{fl/fl}$ mice (Figure 2A) including an almost complete absence of CD8αβ$^+$TCRαβ$^+$ IEL together with a reduction in total CD4$^+$TCRαβ$^+$ IEL (Figures 2B and 2C, Figure S3A). *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ mice also had significantly reduced numbers of CD8αα$^+$TCRαβ$^+$ and CD8αα$^+$TCRγδ$^+$ IEL (Figure S3A), although the proportion of CD8αα$^+$TCRαβ$^+$ and CD8αα$^+$TCRγδ$^+$IEL within CD45$^+$ IEL was similar to that of *Irf8*$^{fl/fl}$ mice (Figures 2D and 2E). Granzyme A expression was reduced in remaining CD8αβ$^+$TCRαβ$^+$, CD8αα$^+$TCRαβ$^+$, and CD8αα$^+$ TCRγδ$^+$ IEL in *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ mice (Figure 2F). Analysis of intestinal sections confirmed an almost complete absence of SI CD8αβ$^+$ but not TCRγδ$^+$ IEL in *Itgax-cre.Irf8*$^{fl/fl}$ mice (Figure 2G). Such alterations in SI-IEL composition were not observed in *Itgax-cre.Irf4*$^{fl/fl\ or\ fl/-}$ mice (Figures S3B and S3C) that lack SI-LP derived CD103$^+$CD11b$^+$ MLN DCs (Persson et al., 2013).

Because SI-IEL can express CD11c (Huleatt and Lefrançois, 1995), and we detected both the flox and deleted *Irf8* allele in sorted splenic T cells from *Itgax-cre.Irf8*$^{fl/fl}$ mice (Figure S1C), we determined whether the reduction in conventional CD8αβ$^+$ IEL in *Itgax-cre.Irf8*$^{fl/fl}$ mice was a result of cell extrinsic or intrinsic effects by transferring a 1:1 ratio of BM from WT (CD45.1$^+$) and *Itgax-cre.Irf8*$^{fl/fl}$ (CD45.2$^+$) mice into WT (CD45.1$^+$CD45.2$^+$) recipients. Eight weeks after reconstitution, CD8αβ$^+$ IEL deriving from *Itgax-cre.Irf8*$^{fl/fl}$ BM were readily detected in the SI of the mixed BM chimeras (Figure 2H). Similar results were obtained in mixed *Rag1*$^{-/-}$ and *Itgax-cre.Irf8*$^{fl/fl}$ BM chimeras (data not shown). Thus the paucity of CD8αβ$^+$ IEL in *Itgax-cre.Irf8*$^{fl/fl}$ mice was primarily due to T and B cell extrinsic effects.

Because *Irf8* was deleted in a proportion of SI-LP CD64$^+$ CD11b$^+$ myeloid cells in *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ mice (Figure 1I), we next assessed whether the reduction in CD8αβ$^+$ IEL might be related to IRF8 deficiency in CD64$^+$ cells. To this end, mixed BM chimeras were established by transferring a 1:1 ratio of BM from *Itgax-cre.Irf8*$^{fl/fl}$ (CD45.2$^+$) and *Ccr2*$^{-/-}$(CD45.1$^+$) mice, on the basis that *Ccr2*$^{-/-}$ BM would fail to re-establish IRF8 sufficient intestinal CD64$^+$ cells (Bain et al., 2014). Indeed, analysis of mixed BM chimeras 8 weeks after reconstitution demonstrated that almost all CD64$^+$ cells in the SI-LP derived from *Itgax-cre.Irf8*$^{fl/fl}$ BM whereas migratory CD103$^+$CD11b$^-$ DCs and CD8α$^+$ resident DCs in the MLN derived from *Ccr2*$^{-/-}$ BM (Figure 2I and data not shown). *Itgax-cre.Irf8*$^{fl/fl}$ BM was capable of generating CD8αβ$^+$ IEL in these mixed BM chimeras and granzyme A expression was restored in all subsets of SI-IEL derived from *Itgax-cre.Irf8*$^{fl/fl}$ BM (Figure 2J and data not shown). These results suggest that alterations in SI-IEL composition in *Itgax-cre.Irf8*$^{fl/fl}$ mice is not due to a loss of IRF8 expression in SI-LP CD64$^+$ cells. Further *Irf8*$^{fl/-}$ and *Itgax-cre.Tcf4*$^{fl/fl}$ mice had normal numbers and composition of SI-IEL (Figure 2K, Figures S3D and S3E), suggesting that LN resident CD8α$^+$ DCs and functional pDCs are not critical for maintenance of SI-IEL homeostasis. Collectively these results suggest an important role for intestinal CD103$^+$CD11b$^-$ DCs in intestinal IEL homeostasis.

Itgax-cre.Irf8$^{fl/fl\ or\ fl/-}$ Mice Display Deficiencies in Cross-Presenting Intraperitoneally Injected Soluble and Cellular Antigen

Given the marked reduction of CD8αβ$^+$ IEL in *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ mice, we assessed the ability of *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ and *Irf8*$^{fl/fl}$ mice to mount CD8$^+$ T cell responses to cell-associated and soluble antigen in vivo (Figure S4). Briefly, CellTrace Violet (VCT)-labeled OVA-specific CD8$^+$ (OT-I) T cells were injected intravenously (i.v.) into *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ and *Irf8*$^{fl/fl}$ mice, recipient mice were immunized intraperitoneally (i.p.) with OVA or heat treated H-2^{bm1} MEFs expressing truncated non-secreted OVA (OVA-MEF, (Sancho et al., 2009)), and OT-I cell proliferation was assessed in the MLN and spleen 3 days later by flow cytometry. The sphingosine 1-phosphate receptor agonist FTY720 was administered i.p. to prevent effector lymphocyte egress from LN. OT-I responses to soluble OVA or OVA-MEF was markedly reduced in *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ mice compared to *Irf8*$^{fl/fl}$ mice (Figures S4A and S4B). Consistent with these findings, while endogenous CD8$^+$ T cell numbers were unaffected in the MLN, or marginally lower in the spleen of *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ mice, effector (CD62L$^-$CD44$^+$) CD8$^+$ T cell numbers were markedly reduced in both locations (Figure S4C). In contrast the total number of endogenous CD4$^+$ T cells in the MLN and spleen as well as the number of effector CD4$^+$ T cells in the MLN did not differ between *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ and *Irf8*$^{fl/fl}$ mice (Figure S4C). Thus IRF8-dependent DCs play a non-redundant role in cross-presenting i.p. injected cell-associated and soluble antigen to CD8$^+$ T cells in vivo.

T Cells Primed in Intestinal Inductive Sites of *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ Mice Have a Reduced Capacity to Localize to the Small Intestine

While a reduced cross-presenting capacity of *Itgax-cre.Irf8*$^{fl/fl}$ mice could in part underlie the dramatic reduction in CD8αβ$^+$ T cells in the SI epithelium and LP (Figure 3A), CD8αβ$^+$ T cell numbers were similar in the LI-LP and lung of *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ and *Irf8*$^{fl/fl}$ mice (Figure 3A). Moreover, CD4$^+$ T cell numbers were reduced in the SI (Figure 2C and Figure 3A) but not LI-LP or lung of *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ mice (Figure 3A and Figure S4C), indicating potential tissue specific defects regulating T cell accumulation in the SI. We therefore assessed the capacity of *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ mice to support T cell priming after oral antigen administration and the subsequent migration of these cells to the SI. Briefly, VCT labeled OT-I or OVA specific CD4$^+$ (OT-II) T cells were injected i.v. into recipient *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ or *Irf8*$^{fl/fl}$ mice and recipients were orally immunized with OVA with or without the TLR7 agonist R848. FTY720 was administered i.p. and the number and division history of responding donor T cells in MLN was assessed 3 (OT-I) and 4 (OT-II) days later (Figures 3B–3E). Following oral gavage of OVA OT-I cell numbers in the MLN of *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ were lower than in *Irf8*$^{fl/fl}$ mice and these cells had undergone fewer divisions (Figures 3B and 3C), similar to responses after i.p. OVA administration (Figures S4A and SB). In contrast OT-I cells expanded equally efficiently in the MLN of *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$

(J) Percentage of CD8αβ$^+$ IEL within indicated BM derived CD45$^+$ population in single and mixed BM chimeras.
(K) Total number of CD45$^+$ IEL (left panel) and percentage of CD8αβ$^+$ IEL within CD45$^+$ IEL gate (right panel) in *Irf8*$^{fl/fl}$ and *Irf8*$^{fl/-}$ mice. Data are from (A, C, E) 9, (K) 6, or (B, D, F-J) 2–3 independent experiments performed. Each dot represents one mouse. Error bars represent mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, ns, not significant. See also Figure S3.

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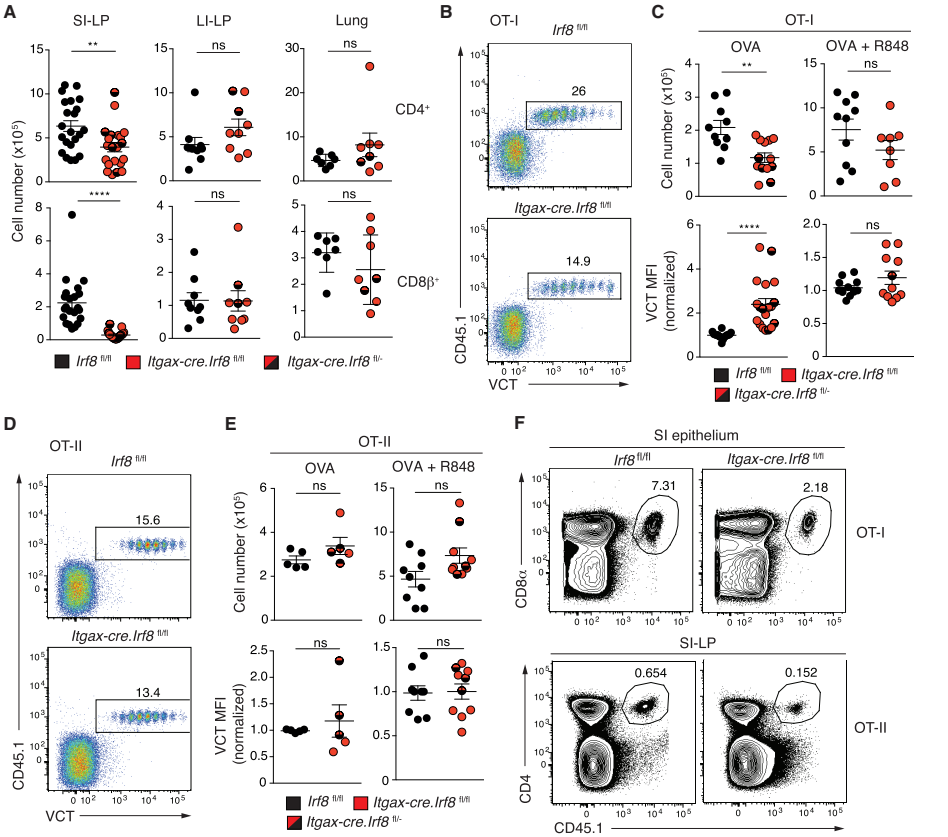

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mice following oral administration of OVA and R848 (Figure 3C). OT-II cells proliferated equally well in *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ and *Irf8*$^{fl/fl}$ mice in response to oral administration of OVA or OVA and R848 (Figures 3D and 3E). To assess whether effector T cells primed in *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ mice were capable of localizing to the SI, we immunized OT-I or OT-II cell recipients orally with OVA and R848 (conditions of equal T cell proliferation) in the absence of FTY720 and their localization to the SI epithelium and LP assessed 4 days later by flow cytometry. OT-I cells primed in *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ mice displayed a reduced ability to mobilize to the SI but not lung or spleen (Figures 3F and 3G) although this was only significant for the SI-epithelium (Figure 3G). OT-II numbers in general were low in the epithelium, consistent with the fact that most SI-IEL are CD8$^+$ T cells, however their accumulation in the SI-LP but not lung or spleen was significantly reduced in *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ compared with *Irf8*$^{fl/fl}$ mice (Figure 3G).

T Cells Primed in the MLN of *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ Mice Have Reduced CCR9 Expression

We hypothesized that *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ mice might differ in their expression of the T cell homing receptor ligands CCL25 and MadCAM-1 in the SI, or that T cells primed in the MLN of these mice might be deficient in their expression of the SI homing receptors, CCR9 and $\alpha4\beta7$ (Agace, 2008). The proportion of CD31$^+$ PDPN$^-$ vascular endothelial cells expressing MadCAM-1 and the amount of *Ccl25* mRNA in the SI of *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ mice were similar to that of *Irf8*$^{fl/fl}$ mice (Figures S5A and S5B) arguing against alterations in homing receptor ligand expression as a cause for reduced T cell localization to this site. To assess SI homing receptor induction, we determined CCR9 and $\alpha4\beta7$ expression on OT-I and OT-II cells in the MLN 3 (OT-I) and 4 (OT-II) days after oral administration of OVA or OVA and R848 and i.p. injection of FTY720 (Figure 4). In both situations, the proportion of OT-I and OT-II cells expressing CCR9, and the amount of CCR9 on CCR9-expressing cells was reduced in the MLN of *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ compared with *Irf8*$^{fl/fl}$ mice (Figures 4A and 4B). The amount of $\alpha4\beta7$ on $\alpha4\beta7$ expressing cells was also reduced, although to a lesser extent (Figure 4C).

Induction of CCR9 and $\alpha4\beta7$ on T cells in vitro requires the vitamin A metabolite retinoic acid (RA) (Iwata et al., 2004; Svensson et al., 2008) and vitamin-A-deficient mice fail to support the generation of CCR9$^+\alpha4\beta7^+$ CD8$^+$ T cells in MLN in vivo (Jaensson-Gyllenbäck et al., 2011). Consistent with this reflecting a T cell intrinsic requirement for RA, CCR9 and $\alpha4\beta7$ were not induced after oral administration of OVA and R848 on adoptively transferred OT-I or OT-II cells that expressed a dominant negative form of the retinoic acid receptor α (RARα) (OT-I.*Cd4-cre.dnRara*$^{lsl/WT}$ or OT-II.*Cd4-Cre.dnRara*$^{lsl/WT}$) (Pino-Lagos et al., 2011)(Figure 4D). We thus hypothesized that T cells primed in the MLN of *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ received less RA. Consistent with this possibility OT-I cells sorted from the MLN of recipient mice 3 days after oral OVA and R848 administration expressed lower amounts of the RA target gene *P2x7r* (Heiss et al., 2008) as well as *Ccr9* (Figure 4E).

The generation of CCR9$^+\alpha4\beta7^+$ T cells by intestinal DCs in vitro requires the activity of retinaldehyde dehydrogenases that convert retinal to retinoic acid (Iwata et al., 2004). We found that SI-LP derived CD103$^+$CD11b$^-$ DCs were significantly enriched in the fraction of MLN DCs displaying the highest aldehyde dehydrogenase (ALDH) activity (Yokota et al., 2009) (Figure 4F) and that MHCII^{hi} MLN DCs in *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ mice displayed significantly reduced ALDH activity compared with *Irf8*$^{fl/fl}$ mice (Figure 4G). Collectively, these results suggest that RA production by IRF8-dependent CD103$^+$CD11b$^-$ migratory DCs is important for the optimal generation of SI homing T cells.

Itgax-cre.Irf8$^{fl/fl\ or\ fl/-}$ Mice Lack CD4$^+$CD8$\alpha\alpha^+$ IEL

Within the SI a subset of CD4$^+$TCR$\alpha\beta^+$ T cells differentiate into CD4$^+$CD8$\alpha\alpha^+$ IEL (Morrissey et al., 1995; Reimann and Rudolphi, 1995), a transition that is associated with induction of the cytotoxic T lymphocyte (CTL) associated runt-related transcription factor 3 (Runx3), and acquisition of MHC II restricted CTL like activity (Mucida et al., 2013; Reis et al., 2013). In addition to a reduction in total SI CD4$^+$TCR$\alpha\beta^+$ IEL (Figure 2C and Figure S3A), the CD4$^+$TCR$\alpha\beta^+$ IEL compartment of *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ mice lacked CD4$^+$CD8$\alpha\alpha^+$ IEL (Figure 5A), expressing granzyme A (Figure 5B), and the NK and memory CD8$^+$ T cell marker CD244 (2B4) (Figure 5C) (Mucida et al., 2013). Among total SI CD4$^+$TCR$\alpha\beta^+$ IEL both CD4$^+$CD8$\alpha\alpha^+$ and, to a lesser extent, CD4$^+$CD8$\alpha\alpha^-$ IEL express CD103 (Figure 5D) (Reis et al., 2013); however, all CD4$^+$TCR$\alpha\beta^+$ IEL lacked CD103 expression in *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ mice (Figure 5E). The percentage of CD8$\alpha\beta^+$TCRβ^+, CD8$\alpha\alpha^+$TCR$\gamma\delta^+$, and CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ IEL expressing CD103, as well as the amount of CD103 expression by CD8$^+$CD103$^+$ IEL, were also significantly reduced in *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ mice (Figure 5F and data not shown). Consistent with a lack of CD4$^+$CD8$\alpha\alpha^+$ IEL, remaining CD4$^+$TCR$\alpha\beta^+$ IEL in *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ mice expressed lower amounts of *Cd8a*, *Cd244*, *Gzma*, *Gzmb*, and *Itgae* mRNA, as well as *Runx3* and *Tbx21* (encoding Tbet) (Figure 5G), a transcription factor associated with the transition of CD4$^+$ T cells into CD4$^+$CD8$\alpha\alpha^+$ IEL (Mucida et al., 2013; Reis et al., 2013). Lack of CD4$^+$CD8$\alpha\alpha^+$ IEL and reduced CD103 expression by CD4$^+$TCR$\alpha\beta^+$ IEL was T cell extrinsic as CD4$^+$CD8$\alpha\alpha^+$ IEL and CD103 expressing CD4$^+$TCR$\alpha\beta^+$ IEL were generated efficiently from *Itgax-cre.Irf8*$^{fl/fl}$ BM in WT (CD45.1$^+$):*Itgax-cre.Irf8*$^{fl/fl}$ (CD45.2$^+$) as well as *Rag1*$^{-/-}$:*Itgax-cre.Irf8*$^{fl/fl}$ mixed BM chimeras (Figure 5H, data not shown). Further, CD4$^+$CD8$\alpha\alpha^+$ IEL and CD103 expressing CD4$^+$TCR$\alpha\beta^+$ IEL deriving from *Itgax-cre.Irf8*$^{fl/fl}$ BM were readily

Figure 3. T Cells Primed in Intestinal Inductive Sites of *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ Display a Reduced Capacity to Localize to the Small Intestine

(A) Total number of CD4$^+$ and CD8$\alpha\beta^+$ T cells in indicated organs of *Irf8*$^{fl/fl}$ and *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ mice.

(B–E) (B) and (D) show representative flow cytometry plots and (C) and (E) show total number and division history of (B and C) OT-I and (D and E) OT-II cells in the MLN of indicated recipient mice (B and C) 3 and (D and E) 4 days after oral gavage with OVA or OVA and R848. MFI, median fluorescence intensity normalized to the median MFI value of the *Irf8*$^{fl/fl}$ group.

(F) Representative flow cytometry plots and (G) total number of OT-I and OT-II cells in the indicated organs 4 days after oral gavage with OVA and R848. Data are from 2–7 independent experiments. Each dot represents one mouse. Error bars represent mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, ns, not significant. See also Figure S4.

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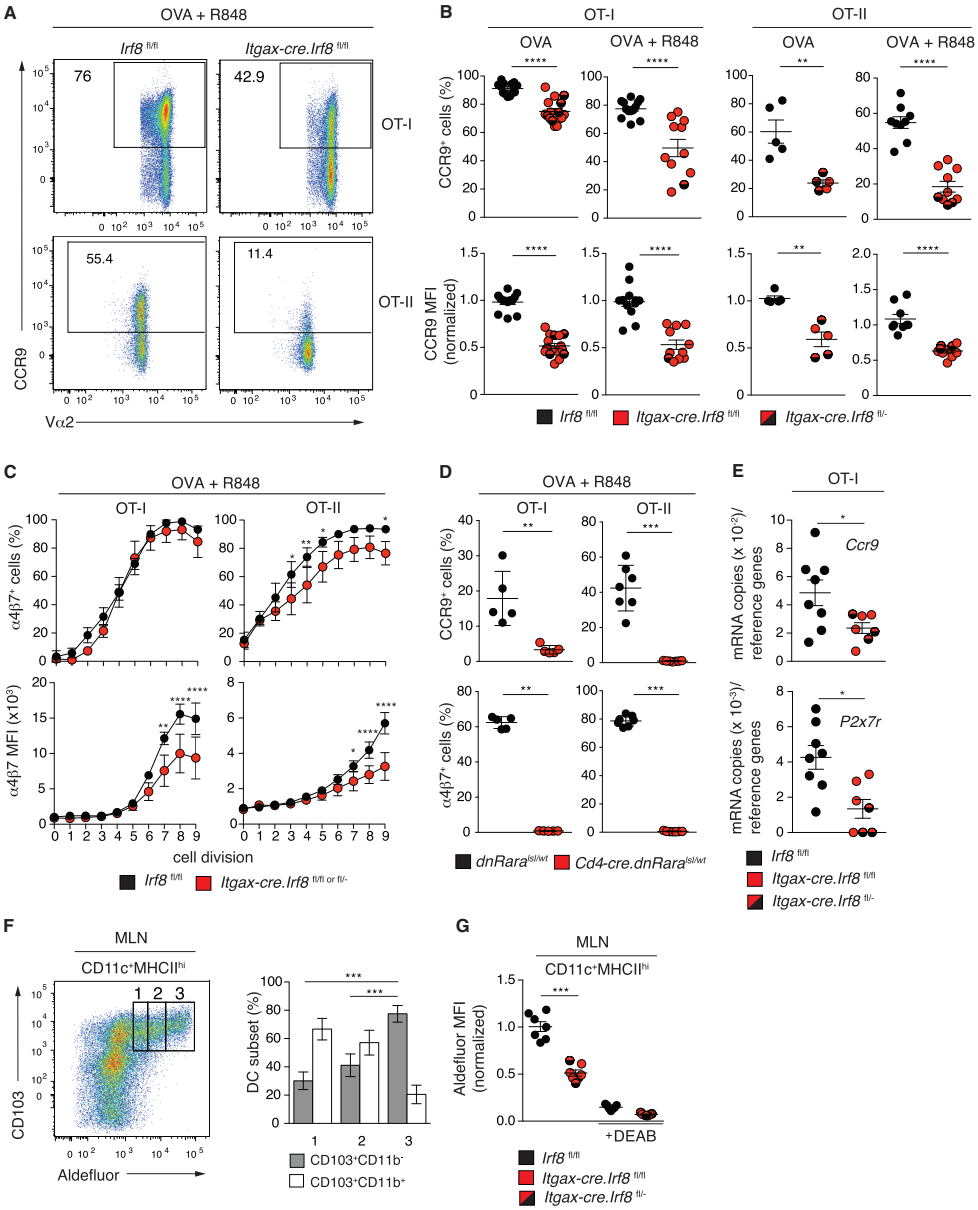

Figure 4. T Cells Primed in the MLN of *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ Mice Have Reduced Expression of Small Intestinal Homing Receptors

(A) Representative flow cytometry plots and (B) pooled data of (A) and (B) CCR9 and (C) α4β7 expression on OT-I or OT-II cells in the MLN of indicated recipient mice 3 (OT-I) or 4 (OT-II) days after oral gavage with OVA or OVA and R848.

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detected in *Ccr2*$^{-/-}$ (CD45.1$^+$):*Itgax-cre.Irf8*$^{fl/fl}$ (CD45.2$^+$) mixed BM chimeras (Figure 5I) and were present in normal numbers in *Itgax-cre.Tcf4*$^{fl/fl}$ mice (Figure S4F).

β8 Integrin Subunit Expression by IRF8 Dependent CD103$^+$CD11b$^-$ DCs Is Required for the Generation of CD4$^+$CD8αα$^+$ IEL

Because the differentiation of CD4$^+$TCRαβ$^+$ T cells to CD4$^+$ CD8αα$^+$ IEL and the induction of CD103 on intestinal IEL is TGF-β dependent (El-Asady et al., 2005; Reis et al., 2013), we next assessed the expression of genes involved in TGF-β production and activation in intestinal DC subsets. qPCR analysis demonstrated that *Itgb8*, encoding the β8 integrin chain involved in the activation of latent TGF-β (Travis and Sheppard, 2014), was expressed selectively by CD103$^+$CD11b$^-$ DCs (Figure 5J). To investigate the functional significance of β8 integrin expression by CD103$^+$CD11b$^-$ DCs in CD4$^+$CD8αα$^+$ IEL development, we generated *Itgax-cre.Itgb8*$^{fl/fl}$ mice. While displaying normal numbers of CD45$^+$ SI-IEL and proportions of CD4$^+$TCRαβ$^+$ SI-IEL (Figure 5K, data not shown), these animals had significantly reduced proportions of CD4$^+$CD8αα$^+$ IEL compared with *Itgb8*$^{fl/fl}$ littermates (Figure 5K, data not shown). Further BM from *Itgb8*$^{fl/fl}$, but not from *Itgax-cre.Itgb8*$^{fl/fl}$ mice rescued the generation of CD4$^+$CD8αα$^+$ SI-IEL and CD103 expression on CD4$^+$TCRαβ$^+$ SI-IEL in mixed BM chimeras with *Itgax-cre.Irf8*$^{fl/fl}$ BM (Figure 5L). Thus β8 integrin expression by IRF8-dependent DCs is required for the generation of CD4$^+$CD8αα$^+$ IEL.

Itgax-cre.Irf8$^{fl/fl\ or\ fl/-}$ Mice Lack Intestinal Th1 Cells

We next compared intestinal LP CD4$^+$ T cell subset composition in *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ and *Irf8*$^{fl/fl}$ mice (Figures 6A and 6B). Strikingly, IFN-γ$^+$IL-17$^-$ CD4$^+$ T cells were almost absent and IFN-γ$^+$IL-17$^+$ CD4$^+$ T cells were reduced in the SI-LP and LI-LP of *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ mice (Figures 6A and 6B). In contrast, *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ mice had elevated proportions of intestinal IL-17$^+$IFN-γ$^-$ cells, while the proportions of Foxp3$^+$CD4$^+$ T cells were slightly reduced in the LI but not SI (Figures 6A and 6B). Consistent with these findings, SI-LP CD4$^+$ T cells from *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ mice expressed lower amounts of *Tbx21*, but not *Rorc* or *Foxp3*, compared with *Irf8*$^{fl/fl}$ mice (Figures 6C). Naive splenic CD4$^+$ T cells from *Itgax-cre.Irf8*$^{fl/fl}$ and *Irf8*$^{fl/fl}$ mice differentiated equally well into IFN-γ producing Th1 cells in vitro (Figure 6D), and IFN-γ producing CD4$^+$ T cells deriving from *Itgax-cre.Irf8*$^{fl/fl}$ BM were present in the SI and MLN of WT (CD45.1$^+$):*Itgax-cre.Irf8*$^{fl/fl}$ (CD45.2$^+$) mixed BM chimeras (Figure 6E). Further, IFN-γ producing SI-LP CD4$^+$ T cells were readily detected in *Itgax-cre.Tcf4*$^{fl/fl}$ mice (Figure 6F). Despite these defects in mucosal T cell homeostasis, the composition of the cecal and colonic microbiota, including amounts of segmented filamentous bacteria, did not differ between *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ and *Irf8*$^{fl/fl}$ mice (Figures S6A–S6D), and *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ mice displayed no major defects in intestinal barrier function as assessed by amounts of serum endotoxin (Figure S6E).

Th1 Cell Differentiation Is Impaired in the MLN of *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ Mice

To assess whether Th1 cell priming in MLN was altered in *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ mice, OT-II cells were injected i.v. into *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ and *Irf8*$^{fl/fl}$ mice and recipients were immunized with OVA, LPS, and αCD40 i.p., a protocol previously demonstrated to generate both IFN-γ and IL-17 producing OT-II populations (Persson et al., 2013). While OT-II cells primed in the MLN of both *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ and *Irf8*$^{fl/fl}$ mice expressed IL-17 (Figures 7A and 7B), the MLN of *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ mice failed to support Th1 cell differentiation (Figures 7A and 7B). *Ccr2*$^{-/-}$ mice, that displayed dramatically reduced numbers of CD64$^+$ CD11b$^+$ cells in the MLN 4 days after immunization (Figure 7C), supported efficient Th1 cell differentiation (Figure 7D), as did *Itgax-cre.Tcf4*$^{fl/fl}$ mice (Figure 7E). Finally, when *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ and *Irf8*$^{fl/fl}$ mice were infected orally with 20 *Trichuris muris* (*T. muris*) eggs, a dose that drives a Th1 cell response resulting in chronic infection in C57BL/6 mice (Bancroft et al., 1994), chronic infection developed in *Irf8*$^{fl/fl}$ mice but not *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ mice (Figure 7F). Further while both sets of mice generated an equivalent *T. muris* specific immunoglobulin G1 (IgG1) response, *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ mice failed to mount a *T. muris*-specific IgG2c response (Figure 7G). Collectively, these findings suggest a role for IRF8-dependent DCs in the generation of intestinal Th1 cell responses and offer one likely mechanism underlying the dramatic reduction in Th1 cells in the SI-LP and LI-LP of *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ mice.

DISCUSSION

Itgax-cre.Irf8$^{fl/fl\ or\ fl/-}$ mice displayed a similar reduction in classical DC subsets to that previously reported in *Irf8*$^{-/-}$ and *Irf8*^{R294C} mice (Aliberti et al., 2003; Edelson et al., 2010). However, in contrast to *Irf8*-deficient mice that lack pDCs (Schiavoni et al., 2002), *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ mice had increased numbers of pDCs, although these appeared dysfunctional. *Irf8*^{R294C} mice have also been reported to have higher numbers of pDCs (Tailor et al., 2008), collectively indicating that distinct IRF8 interacting partners might be required for pDC development versus pDC homeostasis and functionality. Although it remains possible that dysfunctional IRF8-deficient pDCs influence SI T cell homeostasis, our findings provide evidence that it is primarily an absence of classical IRF8-dependent DCs that underlie the defects in intestinal T cell homeostasis observed in *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ mice.

(D) CCR9 and α4β7 expression on OT-I and OT-II.*cd4-cre*. *dnRara*$^{lsl/WT}$ or *dnRara*$^{lsl/WT}$ cells in the MLN of recipient mice 3 (OT-I) or 4 (OT-II) days after oral gavage with OVA and R848.

(E) Relative expression of *Ccr9* and *P2x7r* on divided OT-I cells in the MLN of recipient mice 3 days after oral gavage with OVA and R848.

(F) and (G) ALDH activity of (F) MHCII^{hi} MLN DC subsets in *Irf8*$^{fl/fl}$ mice and (G) MHCII^{hi} MLN DC in *Irf8*$^{fl/fl}$ and *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ mice. (F, left) Representative flow cytometry plot and (F, right) proportion of CD103$^+$CD11b$^-$ and CD103$^+$CD11b$^+$ DCs in the indicated gates in the left hand plot. (G) MFI, mean fluorescence intensity. DEAB (N,N-diethylaminobenzaldehyde, ALDH inhibitor). Data are from (A, B, E–G) 2–4 independent experiments or (C) a representative experiment of three performed or (D) one experiment. (B, D, E, G) Each dot represents one mouse, (C) pooled data from three mice per group. Error bars represent mean ± SEM, (F) mean ± SD. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, ns, not significant. See also Figure S5.

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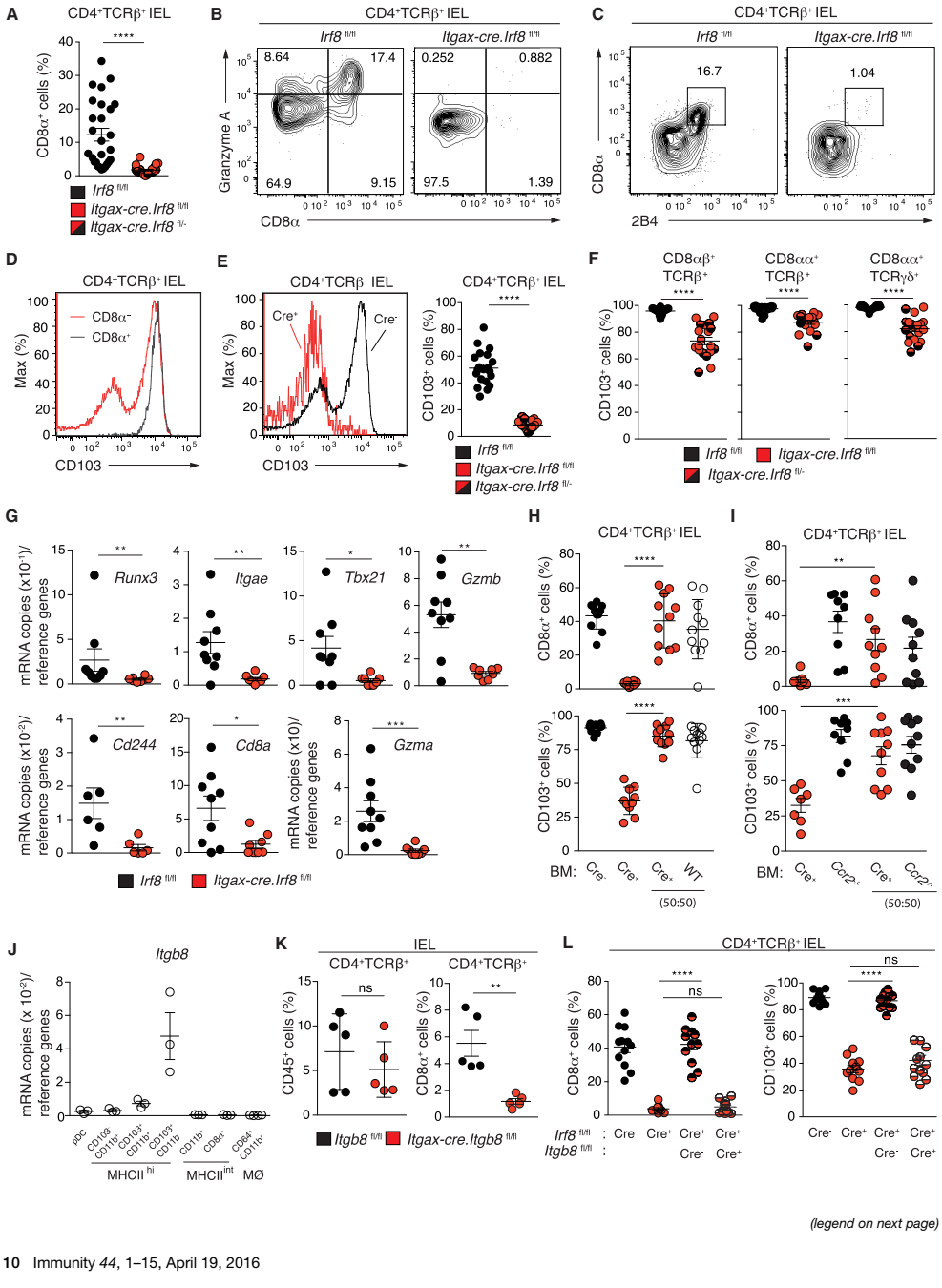

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Our finding that *Irf8*$^{fl/-}$ mice had normal numbers of SI CD8αβ$^+$ T cells, despite a major reduction in LN resident CD8α$^+$ DCs, suggest that a lack of migratory CD103$^+$CD11b$^-$ DCs is responsible for the paucity of SI CD8αβ$^+$ T cells in *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ mice. One likely contributing factor underlying this deficiency is the reduced capacity of *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ mice to cross-present antigen. Nevertheless these mice had similar numbers of CD8αβ$^+$ T cells as controls in the LI and lung, indicating additional tissue specific mechanisms. In this regard, both CD4$^+$ and CD8$^+$ T cells primed in the MLN of *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ mice had reduced expression of the SI homing receptor CCR9 and localized less efficiently to the SI but not lung. Although both SI-derived CD103$^+$CD11b$^+$ and CD103$^+$CD11b$^-$ DCs display retinaldehyde dehydrogenase activity (Cerovic et al., 2013; Denning et al., 2011; Persson et al., 2013), we here demonstrate that SI-derived CD103$^+$CD11b$^-$ DCs display the highest amounts of aldehyde dehydrogenase activity in MLN, and their absence in *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ mice resulted in a decrease in DC-derived aldehyde dehydrogenase activity. We thus hypothesize that optimal generation of "SI tropic" T cells requires RA signals from CD103$^+$CD11b$^-$ DCs, potentially providing an additional explanation for the reduced numbers of SI CD8αβ$^+$ and SI CD4$^+$ T cells in *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ mice. These results do not exclude the possibility that SI-derived CD103$^+$CD11b$^-$ DCs also support "SI tropic" T cell generation in the MLN through additional mechanisms. Notably, SI lymphoid tissues were also recently suggested to be sites of unconventional IEL precursor activation, associated with an enhanced expression of CCR9 and α4β7 (Guy-Grand et al., 2013). Whether a similar mechanism contributes to the reduction in unconventional IEL numbers or whether the development and maintenance of these cells is altered in *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ mice await further study.

In addition to an overall reduction in SI CD4$^+$ T cell numbers CD4$^+$ IEL of *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ mice failed to express CD103 and to differentiate into CD4$^+$CD8αα$^+$ IEL. These results are consistent with a recent study in *Batf3*$^{-/-}$ mice indicating an important role for cell adhesion molecule 1 (Cadm1) expression by Batf3 dependent DCs in driving CD4$^+$CD8αα$^+$ IEL generation (Cortez et al., 2014). Here we show that αvβ8 integrin expression by CD103$^+$CD11b$^-$ DCs also plays a key role in the generation of CD4$^+$CD8αα$^+$ IEL as well as in the induction of CD103 on CD4$^+$ IEL. Whether αvβ8 and Cadm1 function together or independently of one another to promote CD4$^+$CD8αα$^+$ IEL development remains to be determined.

Itgax-cre.Irf8$^{fl/fl\ or\ fl/-}$ mice had normal proportions of intestinal FoxP3$^+$ Treg cells, consistent with previous findings in *Batf3*$^{-/-}$ mice (Edelson et al., 2010) and increased proportions of intestinal IL-17$^+$IFN-γ$^-$ Th17 cells. This phenotype is markedly distinct from that of *Itgax-cre.Itgb8*$^{fl/fl}$ mice that have reduced numbers of colonic FoxP3$^+$ T cells and Th17 cells (Melton et al., 2010; Travis et al., 2007) suggesting that αvβ8 expression by a CD11c$^+$ cell distinct from IRF8-dependent DCs is required for intestinal Th17 cell homeostasis. Alternatively a complete absence of IRF8-dependent DCs might lead to the generation of additional αvβ8 independent signals that promote the generation of these cells. Given our previous work demonstrating reduced numbers of intestinal IL-17$^+$IFN-γ$^-$ Th17 cells in *Itgax-cre.Irf4*$^{fl/fl\ or\ fl/-}$ mice (Persson et al., 2013), we speculate that the increase in Th17 cells observed in *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ mice is due to the enhanced proportions of IRF4 dependent intestinal DCs, as well to the defect in IFN-γ production by IL-17$^+$ cells.

In contrast to the moderate changes in intestinal Th17 cell composition, intestinal Th1 cells were absent and IFN-γ$^+$IL-17$^+$ producing CD4$^+$ T cells severely reduced in both the SI and colon of *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ mice. Consistent with these findings *Batf3*$^{-/-}$ mice appear to display reduced numbers of MLN Th1 cells in steady state (Everts et al., 2016), although intestinal IFN-γ-producing Th1 cells have been observed in these mice (Welty et al., 2013). Although the reason for this discrepancy remains to be determined, *Batf3*$^{-/-}$ mice can develop IRF8-dependent DCs in settings of elevated IL-12 (Tussiwand et al., 2012) and intestinal Th1 cells might have been generated under such conditions. Alternatively, absence of IRF8 in additional CD11c$^+$ cells might contribute to the paucity of intestinal Th1 cells in *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ mice. In contrast to intestinal derived migratory CD103$^+$CD11b$^+$ DCs and MLN resident DC subsets, most LP derived CD103$^+$CD11b$^-$ DCs in the MLN constitutively produce IL-12 (Everts et al., 2016), and we here provide evidence for a role of IRF8-dependent DCs in driving Th1 cell differentiation in MLN, providing one mechanistic explanation underlying this phenotype.

In summary, our findings suggest a key role for IRF8-dependent classical DCs in multiple aspects of intestinal T cell homeostasis that are distinct from those of intestinal Notch2 and IRF4-dependent DCs, whose absence results in a selective reduction in intestinal Th17 cells (Lewis et al., 2011; Persson et al., 2013; Welty et al., 2013) and, as we demonstrate here, does not impact intestinal IEL composition. Such results have important implications not only for the design of DC targeted mucosal vaccines but also for the exploration of

Figure 5. β8 Integrin Subunit Expression by IRF8 Dependent CD103$^+$CD11b$^-$ DCs Is Required for the Generation of CD4$^+$CD8α$^+$ IEL

(A) Percentage of SI CD4$^+$TCRβ$^+$ IEL expressing CD8α and (B) flow cytometry plots of granzyme A and (C) 2B4 expression on SI CD4$^+$TCRβ$^+$ IEL in indicated mice.

(D) Representative CD103 staining on SI CD4$^+$TCRβ$^+$ CD8α$^-$ and CD4$^+$TCRβ$^+$ CD8α$^+$ IEL of *Irf8*$^{fl/fl}$ mice and (E, left) SI CD4$^+$TCRβ$^+$ IEL from *Irf8*$^{fl/fl}$ and *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ mice. Percentage of CD103 expressing SI (E, right) CD4$^+$TCRβ$^+$ IEL, or (F) CD8αβ$^+$TCRβ$^+$, CD8αα$^+$TCRβ$^+$, and CD8αα$^+$TCRγδ$^+$ IEL in indicated mice.

(G) Relative expression of indicated genes in SI CD4$^+$TCRβ$^+$ IEL from *Irf8*$^{fl/fl}$ and *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ mice.

(H and I) Percentage of CD8α (upper panel) or CD103 (lower panel) expressing cells within the indicated BM derived SI CD4$^+$TCRβ$^+$ IEL population in single and mixed BM chimeras.

(J) *Itgb8* expression by MLN DC subsets and SI-LP CD64$^+$ myeloid cells.

(K) Percentage of SI CD4$^+$TCRβ$^+$ IEL among CD45$^+$ IEL (left panel) and CD4$^+$TCRβ$^+$ IEL expressing CD8α (right panel) in 4-week-old *Itgb8*$^{fl/fl}$ and *Itgax-cre.Itgb8*$^{fl/fl}$ mice.

(L) Percentage of SI CD4$^+$TCRβ$^+$ IEL expressing CD8α (left) and CD103 (right) in indicated single and mixed BM chimeras. Results are from 2–9 independent experiments. Each dot represents one mouse. Error bars represent mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, ns, not significant.

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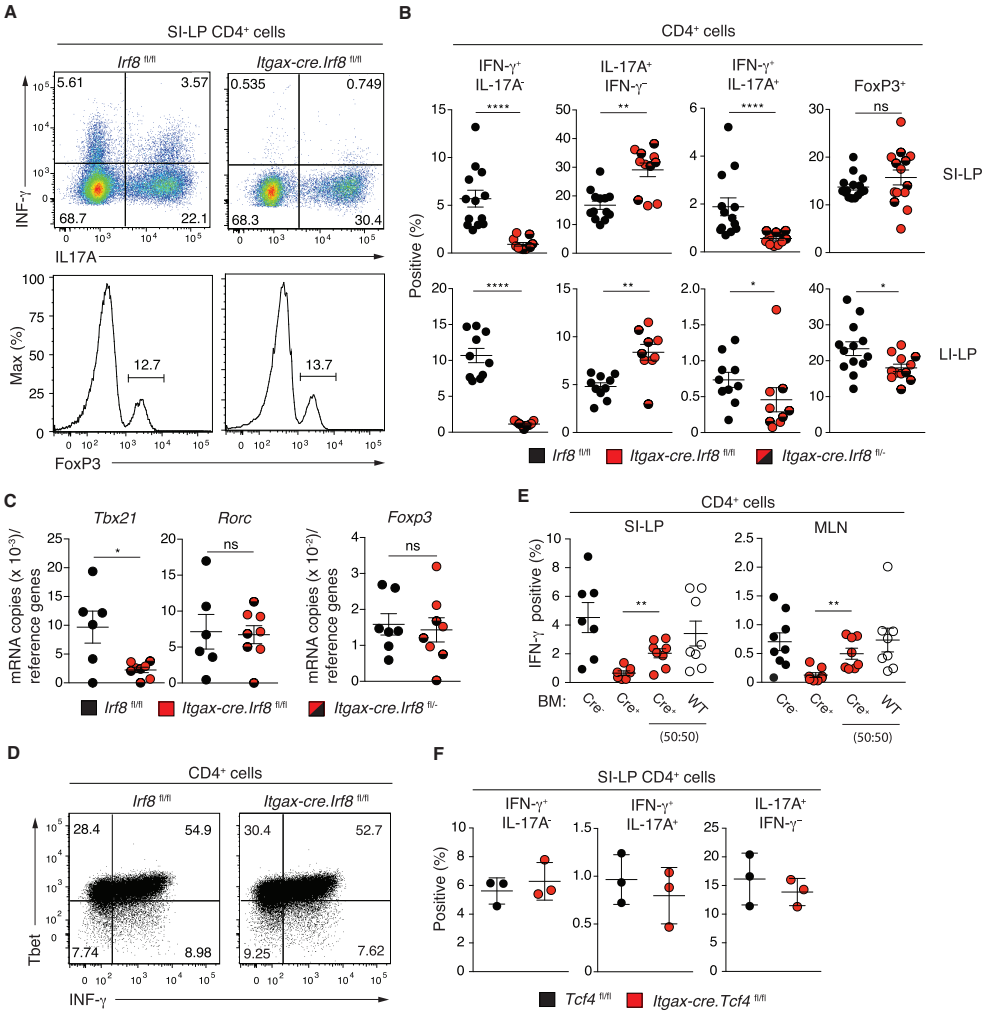

Figure 6. *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ Mice Fail to Mount Mucosal Th1 Cell Responses

Representative flow cytometry plots (A) and percentage (B) of Treg and cytokine producing cells within (A) and (B) SI-LP and (B) LI-LP CD4$^+$ T cells of *Irf8*$^{fl/fl}$ and *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ mice.

(C) Relative expression of indicated transcription factors in sorted SI-LP CD4$^+$ T cells from *Irf8*$^{fl/fl}$ and *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ mice.

(D) Representative flow cytometry plots of Tbet and IFN-γ expression by splenic CD4$^+$ T cells from indicated mice after 4.5 days culture under Th1 cell polarizing conditions.

(E) Percentage of IFN-γ$^+$IL17$^-$ cells within the indicated BM derived CD4$^+$ SI-LP (left panel) or CD4$^+$ MLN (right panel) population in single and mixed BM chimeras.

(F) Percentage of cytokine producing CD4$^+$ T cells in the SI-LP of *Tcf4*$^{fl/fl}$ and *Itgax-cre.Tcf4*$^{fl/fl}$ mice. (A–E) Results are from 2–5 independent experiments or (F) from one representative experiment of two performed. Each dot represents one mouse. Error bars represent mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, ns, not significant. See also Figure S6.

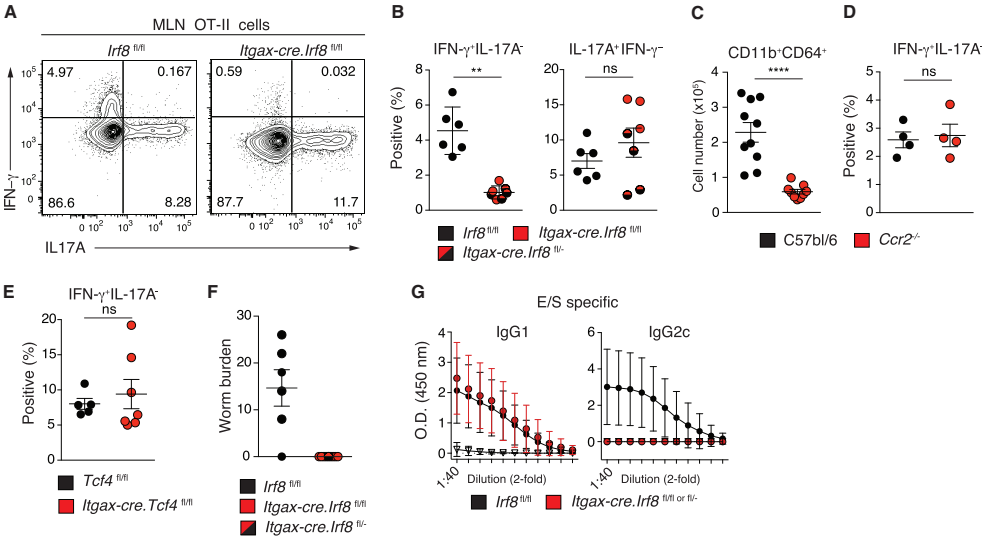

Figure 7. *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ Mice Fail to Prime Th1 Cells in the MLN

(A) Representative flow cytometry plots and (B) percentage of IFN-γ and IL-17 producing OT-II cells in the MLN of indicated mice 4 days after i.p. immunization with OVA, αCD40, and LPS.

(C) Total number of CD11b$^+$CD64$^+$ cells and (D) and (E) percentage of IFNγ$^+$IL17$^-$ producing OT-II cells in the MLN of (C) and (D) *Ccr2*$^{-/-}$ and control C57BL/6 mice or (E) *Tcf4*$^{fl/fl}$ and *Itgax-cre.Tcf4*$^{fl/fl}$ mice 4 days after i.p. immunization with OVA, αCD40, and LPS.

(F) Worm burden and (G) amounts of E/S specific serum IgG1 and IgG2c in *Irf8*$^{fl/fl}$ and *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ mice 35 days after oral administration of *T.muris* eggs (infective dose approximately 20), (G) opened triangles represent uninfected control mice. Results are from (D) one representative experiment of two performed and (A–C, E–G) 2 independent experiments. (B–F) Each dot represents one mouse or (G) a mean value of data pooled from 7-8 mice (red and black circles) or 3 mice (opened triangles). (B–F) Error bars represent mean ± SEM or (G) ± SD. $^{**}p < 0.01$, $^{****}p < 0.0001$, ns, not significant.

DC-centric therapies in the treatment of inflammatory bowel disease.

EXPERIMENTAL PROCEDURES

Mice

Mice were bred and maintained at the Biomedical Center (BMC), Lund University, or Clinical Research Center, Malmö. Animal experiments were performed in accordance with the Lund/Malmö Animal Ethics Committee. For information on the mouse strains, see Supplemental Information.

Cell Isolation

SI-IEL isolation was performed as previously described (Svensson et al., 2002). The SI-LP, LI-LP cell isolation was performed as described previously (Schulz et al., 2009) with minor changes regarding used enzymes: collagenase A and VIII was replaced with Liberase TM (0.3 WuenschU/ml, Roche). For analysis of SI endothelial cells, SI-LP suspensions were stained directly after enzymatic digestion. For generation of lung cell suspensions, perfused lungs were cut into small pieces and digested for 40 min at 37°C in RPMI 1640 (GIBCO, Invitrogen) with Liberase TM (0.3 WuenschU/ml) and DNase I (30 μg/ml, Roche) while shaking and the resulting cell suspension was filtered through a 100 μm strainer (Fisher Scientific) prior to staining. For analysis of MLN and splenic T cells, organs were mashed through a 70 μm cell strainer and red blood cells were lysed using ACK lysing buffer. Naive T cells were purified from splenic cell suspensions using EasySep™ mouse naive CD8$^+$ or CD4$^+$ T Cell Isolation Kit (StemCell Technologies). For cytokine analysis, T cells were first enriched using αCD4 conjugated MACS beads (Miltenyi). For analysis of MLN, PP, and splenic DCs, organs were cut into small pieces and enzymatically digested with Collagenase IV (0.5 mg/ml, Sigma-Aldrich) and DNase I (12.5 μg/ml, Sigma Aldrich) for 40 min at 37°C while shaking and filtered prior to analysis. For DC purification, αCD11c conjugated MACS beads (Miltenyi) were used to enrich for pDC and DCs prior to cell sorting.

Flow Cytometry

Flow cytometry was performed according to standard procedures. Dead cells identified as propidium iodide$^+$ by fixable Viability Dye eFluor®450 (eBioScience), or by Red or Aqua LIVE/DEAD Fixable Dead Cell Staining Kit (Life Technologies) and cell aggregates (identified on FSC-A versus FSC-W scatterplots) were excluded from analyses. Intracellular staining was performed using the FoxP3 Fixation/Permeabilization Kit (eBioscience) according to the manufacturer's instructions. Data were acquired on a FACSAriaII or LSRII (BD Biosciences) and analyzed using FlowJo software (Tree Star). Sorting was performed on a FACSAriaII or on a MoFlow®Astrios (Beckman Coulter).

Adoptive Transfers

Bone marrow (BM) chimeras were generated by i.v. injection of BM (2 × 10^6) cells into irradiated (900 rad) recipients. Analysis of BM chimeras was performed 8 weeks after cell transfer. For T cell transfers, naive OT-I or OT-II cells were labeled with CellTrace Violet (Life Technologies) according to the manufacturer's instructions, and injected i.v. (1–3 × 10^6 cells/mouse) into recipient mice. For immunization protocols, see Supplemental Information.

Cell Culture

SI-LP, LI-LP, and MLN cells were re-stimulated in vitro essentially as described previously (Persson et al., 2013), except SI-LP and LI-LP were stimulated with

0.25 μg/ml PMA and 0.5 μg/ml ionomycin (Sigma). For Th1 cell polarization and pDC stimulation protocols, see Supplemental Information.

Statistical Analysis

Statistical significance was estimated by using GraphPad Prism software (GraphPad), with Mann-Whitney U test or two-way ANOVA where applicable.

SUPPLEMENTAL INFORMATION

Supplemental Information includes six figures and Supplemental Experimental Procedures and can be found with this article online at http://dx.doi.org/10.1016/j.immuni.2016.02.008.

AUTHORS CONTRIBUTIONS

K.M.L. and W.W.A. conceived of and designed the study. K.M.L., T.J., E.K.P., A.R., K.M.S., L.P., L.R., and K.K. (Lund University) performed experiments. M.S.-F. and M.D. performed the *T. muris* infections. M.A.T. and F.M.-G. supported and performed the analysis of *Itgax-cre.Itgb8*$^{fl/fl}$ mice. K.K. (Copenhagen University), J.B.H. and A.R. supported and performed the microbiota analysis. B.N.L. generated and provided the *Irf8*$^{fl/fl}$ mice. K.M.L., T.J., E.K.P., K.K. (Lund University) and W.W.A. were involved in critical discussions throughout. K.M.L. and W.W.A. wrote the paper.

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REFERENCES

Agace, W.W. (2008). T-cell recruitment to the intestinal mucosa. Trends Immunol. *29*, 514–522.

Aliberti, J., Schulz, O., Pennington, D.J., Tsujimura, H., Reis e Sousa, C., Ozato, K., and Sher, A. (2003). Essential role for ICSBP in the in vivo development of murine CD8alpha + dendritic cells. Blood *101*, 305–310.

Bain, C.C., Bravo-Blas, A., Scott, C.L., Gomez Perdiguero, E., Geissmann, F., Henri, S., Malissen, B., Osborne, L.C., Artis, D., and Mowat, A.M. (2014). Constant replenishment from circulating monocytes maintains the macrophage pool in the intestine of adult mice. Nat. Immunol. *15*, 929–937.

Bancroft, A.J., Else, K.J., and Grencis, R.K. (1994). Low-level infection with Trichuris muris significantly affects the polarization of the CD4 response. Eur. J. Immunol. *24*, 3113–3118.

Caton, M.L., Smith-Raska, M.R., and Reizis, B. (2007). Notch-RBP-J signaling controls the homeostasis of CD8- dendritic cells in the spleen. J. Exp. Med. *204*, 1653–1664.

Cauley, L.S., and Lefrançois, L. (2013). Guarding the perimeter: protection of the mucosa by tissue-resident memory T cells. Mucosal Immunol. *6*, 14–23.

Cerovic, V., Houston, S.A., Scott, C.L., Aumeunier, A., Yrlid, U., Mowat, A.M., and Milling, S.W. (2013). Intestinal CD103(-) dendritic cells migrate in lymph and prime effector T cells. Mucosal Immunol. *6*, 104–113.

Cheroutre, H., and Madakamutil, L. (2004). Acquired and natural memory T cells join forces at the mucosal front line. Nat. Rev. Immunol. *4*, 290–300.

Cheroutre, H., Lambolez, F., and Mucida, D. (2011). The light and dark sides of intestinal intraepithelial lymphocytes. Nat. Rev. Immunol. *11*, 445–456.

Cisse, B., Caton, M.L., Lehner, M., Maeda, T., Scheu, S., Locksley, R., Holmberg, D., Zweier, C., den Hollander, N.S., Kant, S.G., et al. (2008). Transcription factor E2-2 is an essential and specific regulator of plasmacytoid dendritic cell development. Cell *135*, 37–48.

Cortez, V.S., Cervantes-Barragan, L., Song, C., Gilfillan, S., McDonald, K.G., Tussiwand, R., Edelson, B.T., Murakami, Y., Murphy, K.M., Newberry, R.D., et al. (2014). CRTAM controls residency of gut CD4+CD8+ T cells in the steady state and maintenance of gut CD4+ Th17 during parasitic infection. J. Exp. Med. *211*, 623–633.

Denning, T.L., Norris, B.A., Medina-Contreras, O., Manicassamy, S., Geem, D., Madan, R., Karp, C.L., and Pulendran, B. (2011). Functional specializations of intestinal dendritic cell and macrophage subsets that control Th17 and regulatory T cell responses are dependent on the T cell/APC ratio, source of mouse strain, and regional localization. J. Immunol. *187*, 733–747.

Edelson, B.T., Kc, W., Juang, R., Kohyama, M., Benoit, L.A., Klekotka, P.A., Moon, C., Albring, J.C., Ise, W., Michael, D.G., et al. (2010). Peripheral CD103+ dendritic cells form a unified subset developmentally related to CD8alpha+ conventional dendritic cells. J. Exp. Med. *207*, 823–836.

Eickhoff, S., Brewitz, A., Gerner, M.Y., Klauschen, F., Komander, K., Hemmi, H., Garbi, N., Kaisho, T., Germain, R.N., and Kastenmüller, W. (2015). Robust Anti-viral Immunity Requires Multiple Distinct T Cell-Dendritic Cell Interactions. Cell *162*, 1322–1337.

El-Asady, R., Yuan, R., Liu, K., Wang, D., Gress, R.E., Lucas, P.J., Drachenberg, C.B., and Hadley, G.A. (2005). TGF-beta-dependent CD103 expression by CD8(+) T cells promotes selective destruction of the host intestinal epithelium during graft-versus-host disease. J. Exp. Med. *201*, 1647–1657.

Everts, B., Tussiwand, R., Dreesen, L., Fairfax, K.C., Huang, S.C., Smith, A.M., O'Neill, C.M., Lam, W.Y., Edelson, B.T., Urban, J.F., Jr., et al. (2016). Migratory CD103+ dendritic cells suppress helminth-driven type 2 immunity through constitutive expression of IL-12. J. Exp. Med. *213*, 35–51.

Gao, Y., Nish, S.A., Jiang, R., Hou, L., Licona-Limón, P., Weinstein, J.S., Zhao, H., and Medzhitov, R. (2013). Control of T helper 2 responses by transcription factor IRF4-dependent dendritic cells. Immunity *39*, 722–732.

Grajales-Reyes, G.E., Iwata, A., Albring, J., Wu, X., Tussiwand, R., Kc, W., Kretzer, N.M., Briseño, C.G., Durai, V., Bagadia, P., et al. (2015). Batf3 maintains autoactivation of Irf8 for commitment of a CD8α(+) conventional DC clonogenic progenitor. Nat. Immunol. *16*, 708–717.

Guy-Grand, D., Vassalli, P., Eberl, G., Pereira, P., Burlen-Defranoux, O., Lemaitre, F., Di Santo, J.P., Freitas, A.A., Cumano, A., and Bandeira, A. (2013). Origin, trafficking, and intraepithelial fate of gut-tropic T cells. J. Exp. Med. *210*, 1839–1854.

Heiss, K., Jänner, N., Mähnss, B., Schumacher, V., Koch-Nolte, F., Haag, F., and Mittrücker, H.W. (2008). High sensitivity of intestinal CD8+ T cells to nucleotides indicates P2X7 as a regulator for intestinal T cell responses. J. Immunol. *181*, 3861–3869.

Holtschke, T., Löhler, J., Kanno, Y., Fehr, T., Giese, N., Rosenbauer, F., Lou, J., Knobeloch, K.P., Gabriele, L., Waring, J.F., et al. (1996). Immunodeficiency and chronic myelogenous leukemia-like syndrome in mice with a targeted mutation of the ICSBP gene. Cell *87*, 307–317.

Hor, J.L., Whitney, P.G., Zaid, A., Brooks, A.G., Heath, W.R., and Mueller, S.N. (2015). Spatiotemporally Distinct Interactions with Dendritic Cell Subsets Facilitates CD4+ and CD8+ T Cell Activation to Localized Viral Infection. Immunity *43*, 554–565.

Huang, F.P., Platt, N., Wykes, M., Major, J.R., Powell, T.J., Jenkins, C.D., and MacPherson, G.G. (2000). A discrete subpopulation of dendritic cells

transports apoptotic intestinal epithelial cells to T cell areas of mesenteric lymph nodes. J. Exp. Med. *191*, 435–444.

Huleatt, J.W., and Lefrançois, L. (1995). Antigen-driven induction of CD11c on intestinal intraepithelial lymphocytes and CD8+ T cells in vivo. J. Immunol. *154*, 5684–5693.

Iwata, M., Hirakiyama, A., Eshima, Y., Kagechika, H., Kato, C., and Song, S.Y. (2004). Retinoic acid imprints gut-homing specificity on T cells. Immunity *21*, 527–538.

Jaensson, E., Uronen-Hansson, H., Pabst, O., Eksteen, B., Tian, J., Coombes, J.L., Berg, P.L., Davidsson, T., Powrie, F., Johansson-Lindbom, B., and Agace, W.W. (2008). Small intestinal CD103+ dendritic cells display unique functional properties that are conserved between mice and humans. J. Exp. Med. *205*, 2139–2149.

Jaensson-Gyllenbäck, E., Kotarsky, K., Zapata, F., Persson, E.K., Gundersen, T.E., Blomhoff, R., and Agace, W.W. (2011). Bile retinoids imprint intestinal CD103+ dendritic cells with the ability to generate gut-tropic T cells. Mucosal Immunol. *4*, 438–447.

Johansson-Lindbom, B., Svensson, M., Pabst, O., Palmqvist, C., Marquez, G., Förster, R., and Agace, W.W. (2005). Functional specialization of gut CD103+ dendritic cells in the regulation of tissue-selective T cell homing. J. Exp. Med. *202*, 1063–1073.

Kumagai, Y., Takeuchi, O., Kato, H., Kumar, H., Matsui, K., Morii, E., Aozasa, K., Kawai, T., and Akira, S. (2007). Alveolar macrophages are the primary interferon-alpha producer in pulmonary infection with RNA viruses. Immunity *27*, 240–252.

Kurotaki, D., Osato, N., Nishiyama, A., Yamamoto, M., Ban, T., Sato, H., Nakabayashi, J., Umehara, M., Miyake, N., Matsumoto, N., et al. (2013). Essential role of the IRF8-KLF4 transcription factor cascade in murine monocyte differentiation. Blood *121*, 1839–1849.

Lewis, K.L., Caton, M.L., Bogunovic, M., Greter, M., Grajkowska, L.T., Ng, D., Klinakis, A., Charo, I.F., Jung, S., Gommerman, J.L., et al. (2011). Notch2 receptor signaling controls functional differentiation of dendritic cells in the spleen and intestine. Immunity *35*, 780–791.

Melton, A.C., Bailey-Bucktrout, S.L., Travis, M.A., Fife, B.T., Bluestone, J.A., and Sheppard, D. (2010). Expression of αvβ8 integrin on dendritic cells regulates Th17 cell development and experimental autoimmune encephalomyelitis in mice. J. Clin. Invest. *120*, 4436–4444.

Morrissey, P.J., Charrier, K., Horovitz, D.A., Fletcher, F.A., and Watson, J.D. (1995). Analysis of the intra-epithelial lymphocyte compartment in SCID mice that received co-isogenic CD4+ T cells. Evidence that mature post-thymic CD4+ T cells can be induced to express CD8 alpha in vivo. J. Immunol. *154*, 2678–2686.

Mowat, A.M., and Agace, W.W. (2014). Regional specialization within the intestinal immune system. Nat. Rev. Immunol. *14*, 667–685.

Mucida, D., Husain, M.M., Muroi, S., van Wijk, F., Shinnakasu, R., Naoe, Y., Reis, B.S., Huang, Y., Lambolez, F., Docherty, M., et al. (2013). Transcriptional reprogramming of mature $CD4^+$ helper T cells generates distinct MHC class II-restricted cytotoxic T lymphocytes. Nat. Immunol. *14*, 281–289.

Persson, E.K., Uronen-Hansson, H., Semmrich, M., Rivollier, A., Hägerbrand, K., Marsal, J., Gudjonsson, S., Håkansson, U., Reizis, B., Kotarsky, K., and Agace, W.W. (2013). IRF4 transcription-factor-dependent CD103(+) CD11b(+) dendritic cells drive mucosal T helper 17 cell differentiation. Immunity *38*, 958–969.

Pino-Lagos, K., Guo, Y., Brown, C., Alexander, M.P., Elgueta, R., Bennett, K.A., De Vries, V., Nowak, E., Blomhoff, R., Sockanathan, S., et al. (2011). A retinoic acid-dependent checkpoint in the development of CD4+ T cell-mediated immunity. J. Exp. Med. *208*, 1767–1775.

Reimann, J., and Rudolphi, A. (1995). Co-expression of CD8 alpha in CD4+ T cell receptor alpha beta + T cells migrating into the murine small intestine epithelial layer. Eur. J. Immunol. *25*, 1580–1588.

Reis, B.S., Rogoz, A., Costa-Pinto, F.A., Taniuchi, I., and Mucida, D. (2013). Mutual expression of the transcription factors Runx3 and ThPOK regulates intestinal $CD4^+$ T cell immunity. Nat. Immunol. *14*, 271–280.

Sancho, D., Joffre, O.P., Keller, A.M., Rogers, N.C., Martínez, D., Hernanz-Falcón, P., Rosewell, I., and Reis e Sousa, C. (2009). Identification of a dendritic cell receptor that couples sensing of necrosis to immunity. Nature *458*, 899–903.

Schiavoni, G., Mattei, F., Sestili, P., Borghi, P., Venditti, M., Morse, H.C., 3rd, Belardelli, F., and Gabriele, L. (2002). ICSBP is essential for the development of mouse type I interferon-producing cells and for the generation and activation of CD8alpha(+) dendritic cells. J. Exp. Med. *196*, 1415–1425.

Schlitzer, A., McGovern, N., Teo, P., Zelante, T., Atarashi, K., Low, D., Ho, A.W., See, P., Shin, A., Wasan, P.S., et al. (2013). IRF4 transcription factor-dependent CD11b+ dendritic cells in human and mouse control mucosal IL-17 cytokine responses. Immunity *38*, 970–983.

Schulz, O., Jaensson, E., Persson, E.K., Liu, X., Worbs, T., Agace, W.W., and Pabst, O. (2009). Intestinal CD103+, but not CX3CR1+, antigen sampling cells migrate in lymph and serve classical dendritic cell functions. J. Exp. Med. *206*, 3101–3114.

Shortman, K., and Heath, W.R. (2010). The CD8+ dendritic cell subset. Immunol. Rev. *234*, 18–31.

Svensson, M., Marsal, J., Ericsson, A., Carramolino, L., Brodén, T., Márquez, G., and Agace, W.W. (2002). CCL25 mediates the localization of recently activated CD8alphabeta(+) lymphocytes to the small-intestinal mucosa. J. Clin. Invest. *110*, 1113–1121.

Svensson, M., Johansson-Lindbom, B., Zapata, F., Jaensson, E., Austenaa, L.M., Blomhoff, R., and Agace, W.W. (2008). Retinoic acid receptor signaling levels and antigen dose regulate gut homing receptor expression on CD8+ T cells. Mucosal Immunol. *1*, 38–48.

Tailor, P., Tamura, T., Morse, H.C., 3rd, and Ozato, K. (2008). The BXH2 mutation in IRF8 differentially impairs dendritic cell subset development in the mouse. Blood *111*, 1942–1945.

Travis, M.A., and Sheppard, D. (2014). TGF-β activation and function in immunity. Annu. Rev. Immunol. *32*, 51–82.

Travis, M.A., Reizis, B., Melton, A.C., Masteller, E., Tang, Q., Proctor, J.M., Wang, Y., Bernstein, X., Huang, X., Reichardt, L.F., et al. (2007). Loss of integrin alpha(v)beta8 on dendritic cells causes autoimmunity and colitis in mice. Nature *449*, 361–365.

Turner, D.L., and Farber, D.L. (2014). Mucosal resident memory CD4 T cells in protection and immunopathology. Front. Immunol. *5*, 331.

Tussiwand, R., Lee, W.L., Murphy, T.L., Mashayekhi, M., Kc, W., Albring, J.C., Satpathy, A.T., Rotondo, J.A., Edelson, B.T., Kretzer, N.M., et al. (2012). Compensatory dendritic cell development mediated by BATF-IRF interactions. Nature *490*, 502–507.

Welty, N.E., Staley, C., Ghilardi, N., Sadowsky, M.J., Igyártó, B.Z., and Kaplan, D.H. (2013). Intestinal lamina propria dendritic cells maintain T cell homeostasis but do not affect commensalism. J. Exp. Med. *210*, 2011–2024.

Yokota, A., Takeuchi, H., Maeda, N., Ohoka, Y., Kato, C., Song, S.Y., and Iwata, M. (2009). GM-CSF and IL-4 synergistically trigger dendritic cells to acquire retinoic acid-producing capacity. Int. Immunol. *21*, 361–377.

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Supplemental Information

IRF8 Transcription-Factor-Dependent Classical Dendritic Cells Are Essential for Intestinal T Cell Homeostasis

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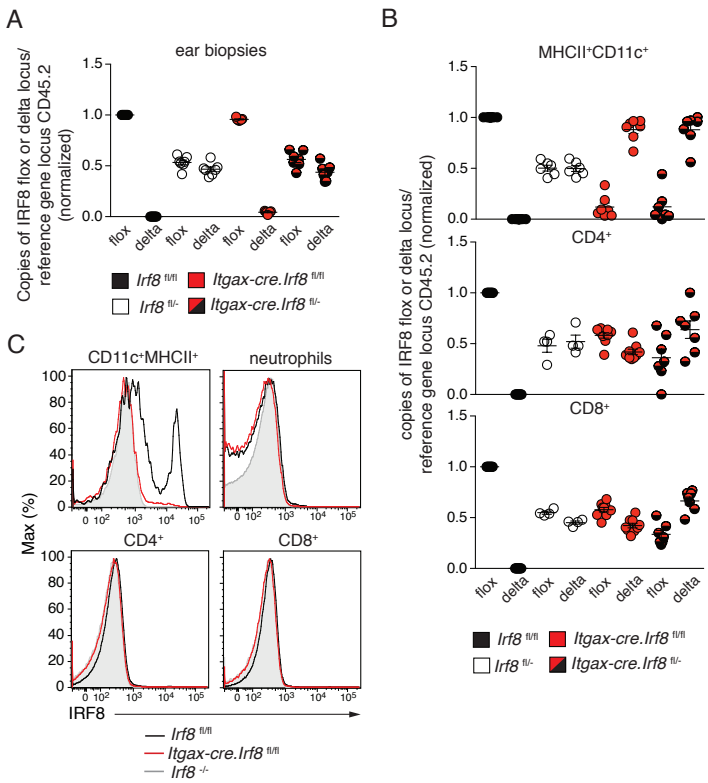

Figure S1. Genotypic characterization of *Itgax-cre.Irf8*$^{fl/-}$, *Itgax-cre. Irf8*$^{fl/fl}$, *Irf8*$^{fl/-}$ and *Irf8*$^{fl/fl}$ mice, related to Figure 1.

(**A** and **B**) Quantitative genotyping of (**A**) ear biopsy and (**B**) sorted splenic CD11c^{+}MHCII^{+} DCs and CD4^{+}TCRβ $^{+}$ and CD8^{+} TCRβ $^{+}$ T cells. Delta, deleted allele. Each dot represents results from an individual mouse. Bars, mean (SEM). (**C**) Representative intracellular IRF8 staining in neutrophils (CD11b^{+}Ly6G^{+}SSC^{int}), splenic CD11c^{+}MHCII^{+} DCs, and CD4^{+}TCRβ $^{+}$ and CD8^{+} TCRβ $^{+}$ T cells-from indicated mice. Data are representative plots from 2-3 independent experiments.

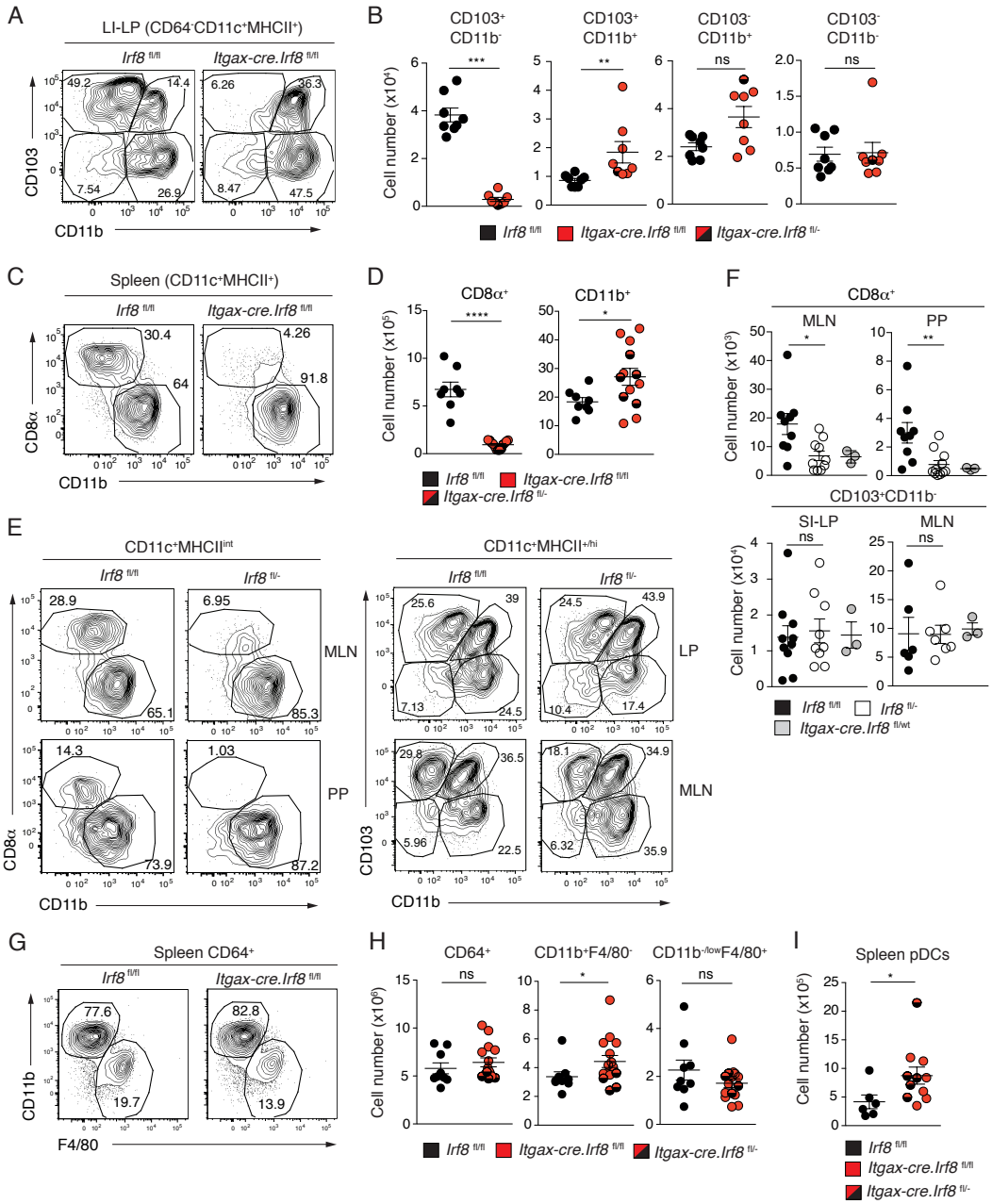

Figure S2. Mononuclear phagocyte composition in *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ and *Irf8*$^{fl/fl}$ mice and effect of *Irf8* haplo-insufficiency on intestinal DC subset composition, related to Figure 1. (**A** and **C**) Representative flow cytometry plots and (**B** and **D**) total number of (**A** and **B**) colonic and (**C** and **D**) splenic DC subsets in indicated mice. (**E** and **F**) LN resident CD8α but not migratory CD103^{+}CD11b^{-} DCs are reduced in mice expressing one allele of *Irf8*. (**E**) Representative flow cytometry plots and (**F**) total number of MLN, PP and SI-LP DC subsets in indicated mice. (**G**) Representative flow cytometry plots and (**H**) total number of splenic myeloid subsets in indicated mice. (**I**) Total number of splenic pDCs in indicated mice. (**A-I**) Data are pooled from 2-4 independent experiments. Each dot represents one mouse. Bars, mean (± SEM). *p<0.05, ** p<0.01, ***p<0.001, ns, not significant.

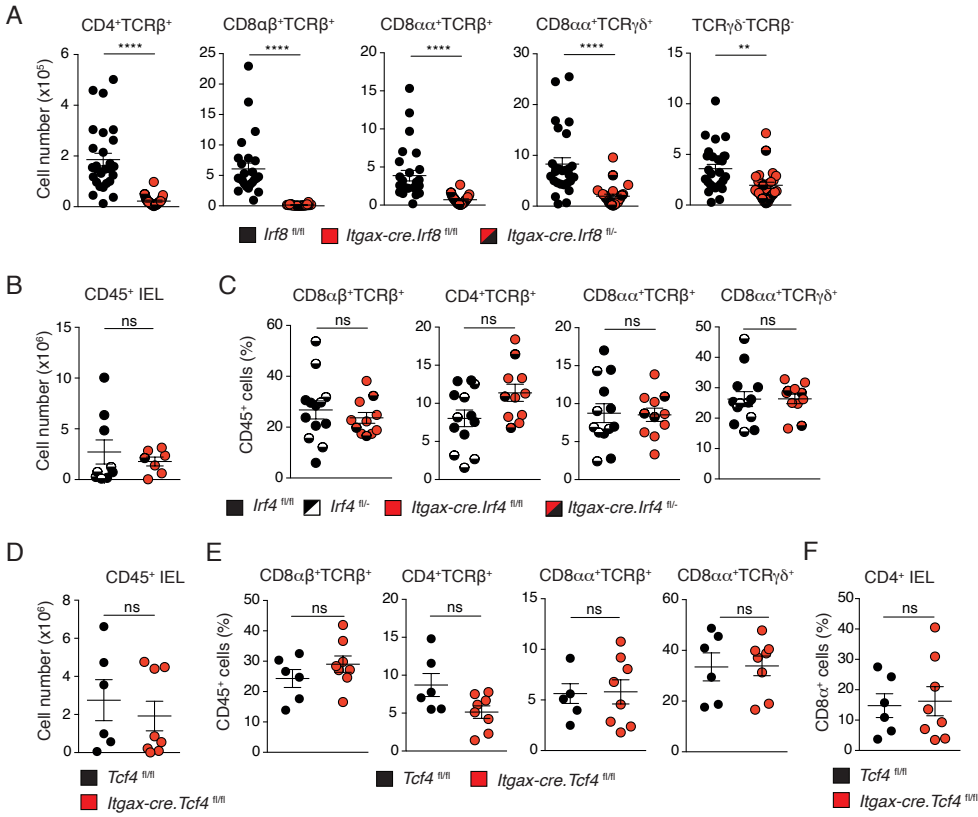

Figure S3. IEL composition in *Itgax-cre.Irf8*$^{fl/f\ or\ fl/-}$, *Itgax-cre.Irf4*$^{fl/fl\ or\ fl/-}$ and *Itgax-cre.Tcf4*$^{fl/fl}$ mice, related to Figure 2.

(**A**) Total number of SI-IEL subsets in *Irf8*$^{fl/fl}$ and *Itgax-cre.Irf8*$^{fl/fl\ and\ fl/-}$ mice. (**B** and **D**) Total number of CD45^{+} SI-IEL and (**C** and **E**) proportion of SI-IEL subsets within CD45^{+} IEL in indicated mice. (**F**) Proportion of CD4^{+} IEL expressing CD8α in *Itgax-cre.Tcf4*$^{fl/fl}$ mice. Results are pooled from 3-9 independent experiments. Each dot represents one mouse. Bars, mean (± SEM). ** p<0.01, ****p<0.0001, ns, not significant.

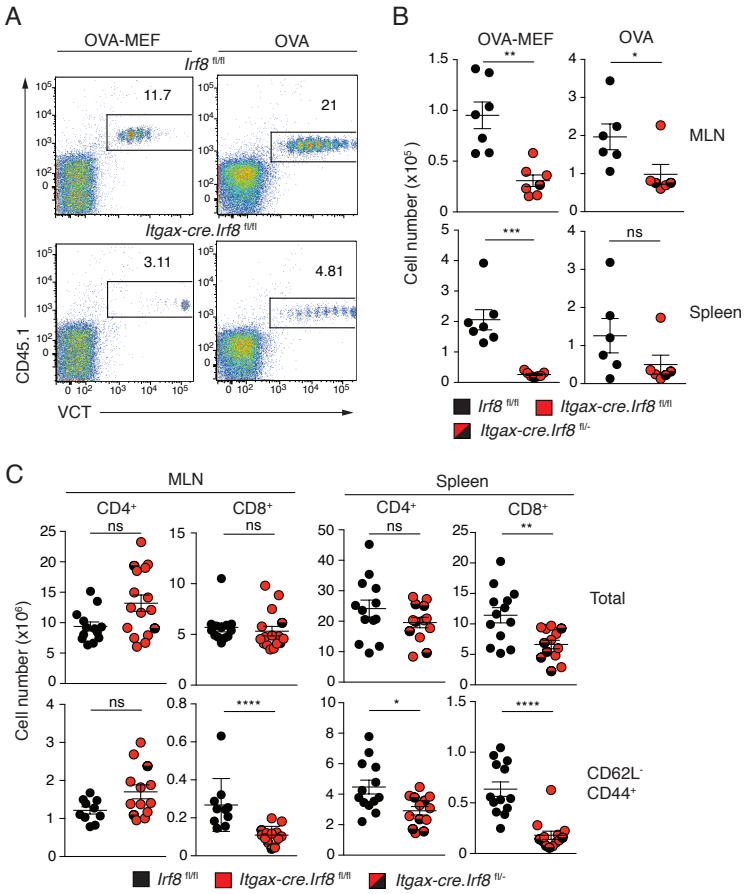

Figure S4. *Itgax-cre.Irf8*$^{fl/fl}$ mice display defects in cross-presentation of soluble and cell associated antigen, related to Figure 3.

(**A**) Representative flow cytometry plots and (**B**) total number of OT-I cells in the MLN and spleen of *Irf8*^{flfl} and *Itgax-cre.Irf8*$^{flfl\ or\ fl/-}$ mice 3 days after i.p. immunization with heat shocked OVA-MEF or soluble OVA. (**C**) Total number of $CD4^+$ and $CD8^+$ T cells and effector $CD62L^-$ $CD44^+$ T cells in the MLN and spleen of *Irf8*^{flfl} and *Itgax-cre.Irf8*$^{flfl\ or\ fl/-}$ mice. Data are pooled from 2-5 independent experiments. Each dot represents one mouse. Bars, mean (± SEM). *p<0.05, ** p<0.01, ***p<0.001, ns, not significant.

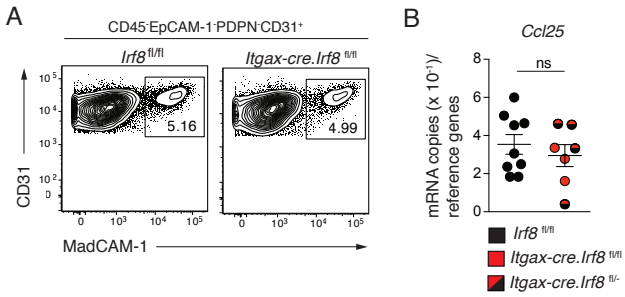

Figure S5. Small intestinal expression of MadCAM-1 and CCL25 is not altered in *Itgax-cre.Irf8*$^{fl/fl}$ mice, related to Figure 4.
(**A**) Representative plots of MadCAM-1 expression on SI-LP (jejunal) vascular endothelial cells and (**B**) *Ccl25* mRNA levels in SI (jejunal) tissue from indicated mice. Data are from 2-3 independent experiments. (**B**) Each dot represents one mouse. Bars, mean (± SEM). ns, not significant.

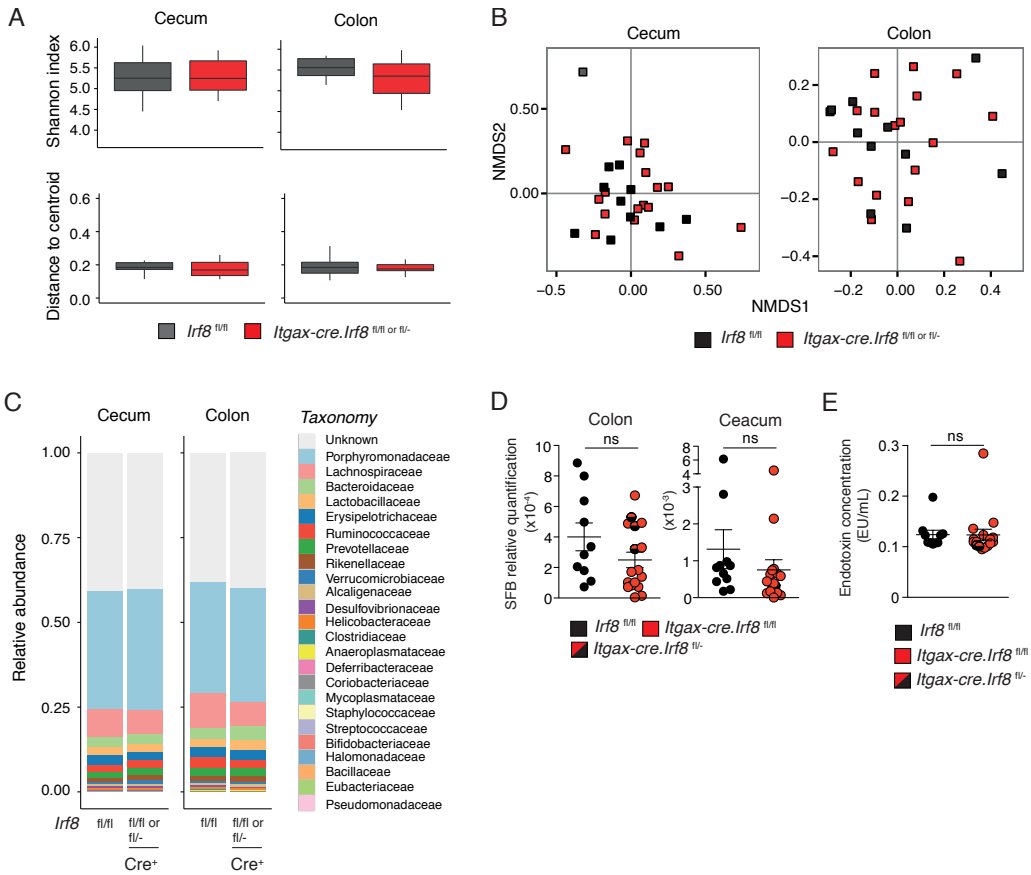

Figure S6. Colonic and cecal microbiota composition appears similar between *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ and *Irf8*$^{fl/fl}$ mice, related to Figure 6.

(**A-C**) Microbial analysis was performed using 16S rDNA amplicon sequencing on 11 *Irf8*$^{fl/fl}$ and 17 *Itgax-cre.Irf8*$^{fl/fl\ or\ fl/-}$ 12-16 w old female mice, cohoused as littermates. (**A**) Alpha and beta diversity of cecal and colonic microbiota samples. Top: The median alpha diversity based on Shannon index of unfiltered microbiota data. Bottom: Median beta diversity based on Sørensen index. The upper and lower whiskers correspond to the 25th and 75th percentiles. (**B**)!Non-metric Multi-Dimensional Scaling (NMDS) plot using Bray-Curtis dissimilarity indices. (**C**) Taxa summary plots at family level. "Unknown" refers to OTUs that we were unable to classify. Data represent mean relative abundance. (**D**) Levels of segmented filamentous bacterial (SFB) DNA in cecal and colonic samples and (**E**) serum endotoxin levels in *Irf8*$^{fl/fl}$ and *Itgax-cre.Irf8*$^{fl/fl\ and\ fl/-}$ mice. Each point represents an individual mouse. Bars, mean (± SEM).

Supplemental Experimental Procedures

Mice

The following mouse strains were used during the course of this study: *Itgax-cre (Itgax-Cre*) (Caton et al., 2007), C57BL/6-Tg(Tcra Tcrb)425Cbn/J (OT-II), C57BL/6-Tg(Tcra Tcrb)1100Mjb/J (OT-I), C57BL/6, C57BL/6 (CD45.1), C57BL/6 (CD45.1/CD45.2), B6.129S1-*Irf4*^{tm1Rdf}/J (Klein et al., 2006), *Tcf4*$^{fl/fl}$ (Bergqvist et al., 2000), *Ccr2*$^{-/-}$ (Saederup et al., 2010), *Cd4-Cre* (Lee et al., 2001), *dnRara*$^{lsl/lsr}$ (Rajaii et al., 2008) and *Rag*1$^{-/-}$ mice. For generation of *Irf8*$^{fl/fl}$ mice an *Irf8* ES cell clone with conditional potential (clone EPD0246_7_C11, KOMP repository) was injected into blastocysts. Germ line transmitting offspring (*Irf8*$^{lacZ/lacZ}$ mice) were crossed to FLPe recombinase mice to generate *Irf8* conditional KO mice (*Irf8*$^{fl/fl}$). *Itgax-cre.Itgb8*$^{fl/fl}$ mice (Travis et al., 2007) were maintained at the University of Manchester, UK.

Mouse genotyping

Presence of a wildtype (wt) or flox *Irf8* allele was identified in ear biopsies with the following primers: *irf8* fw 5`GCCCCTGCTTTGAATTTCTGGCCT 3`, *irf8* wt_rev 5` ACATGTGTATCCACCGGTCTCTCC 3`, and *irf8* flox_rev 5` GCACCGTCAAAGACTTTCCGCC 3`resulting in a 370 bp band for the flox allele and a 200 bp band for the wt allele. Presence of a deleted or flox *Irf8* allele was identified with the following primers; *irf8* fw 5`CAAAAAAGCAGGCTGGCGCCG 3`, *irf8* flox_rev 5`GCACCGTCAAAGACTTTCCGCC 3`, *irf8* delta_rev 5`CCCTTTGAACTGATGGCGAGCTC 3` resulting in an approximate 200 bp band for the flox allele and a 170 bp band for the deleted allele.

Quantitative analysis of the *Irf8* floxed and deleted allele in *Itgax-cre.Irf8*$^{fl/fl}$, *Itgax-cre.Irf8*$^{fl/-}$, *Irf8*$^{fl/-}$ and *Irf8*$^{fl/fl}$ mice

Quantitative analysis of the *Irf8* flox and deleted allele in ear biopsies or sorted cell populations was performed as previously described (Persson et al., 2013) using the flox and delta primers described above. The amount of flox or deleted (delta) allele was calculated as a delta or flox locus ratio to the reference gene locus CD45.2 (CD45.2_fw 5' GCCTGTATCTAAACCTGAGT 3' and CD45.2_rev 5' TTCCCTAGTGGAATTTTACATTA 3'). Data was stratified by assuming that the same numbers of alleles relative to CD45.2 were present in all investigated samples. Such analysis gave the expected ratio of *Irf8* flox and deleted bands in ear biopsies of *Itgax-cre.Irf8*$^{fl/fl}$, *Itgax-cre.Irf8*$^{fl/-}$, *Irf8*$^{fl/-}$ and *Irf8*$^{fl/fl}$ mice (**Figure S1A**). While both alleles were detected at similar levels in cells sorted from *Irf8*$^{fl/-}$ mice only the *Irf8* deleted allele was detected in sorted splenic DCs from *Itgax-cre.Irf8*$^{fl/fl}$ and *Itgax-cre.Irf8*$^{fl/-}$ mice (**Figure S1B**). Both *Irf8* flox and deleted alleles were detected in CD4^{+} and CD8^{+} splenocytes from *Itgax-cre.Irf8*$^{fl/fl}$ and *Itgax-cre.Irf8*$^{fl/-}$ mice (**Figure S1B)** indicating, similar to our previous findings in *Itgax-cre.Irf4*$^{fl/fl}$ mice (Persson et al., 2013), that CD11c driven Cre recombinase can drive a degree of off target deletion of the *Irf8* floxed allele in hematopoietic cells.

Antibodies and reagents

The following mAbs and reagents were used for during the study: anti-NK1.1 (PK 136), anti-CD19 (6D5 or 1D3), anti-CD3e (17A2 or 145-2C11), anti-MHC II(IA/I-E) (M5/114.15.2), anti-CD11b (M1/70), anti-CD8α (53-6.7), anti-Ly6C (HK1.4 or AL-21), anti-Ly6G (1A8), anti-CD45.2 (104), anti-B220 (RA3-6B2), anti-BST2 (927 or

eBio927), anti-CD45.1 (A20), anti-TCRβ (H57-597), anti-TCRγδ (GL3 or eBioGL3), anti-CD8β (53-5.8 or YST156.7.7 or eBioH35-17.2), anti-CD4 (RM4-5 or GK1.5 or eBioGK1.5), anti-MadCAM-1 (MECA-1), anti-gp38 (8.1.1), anti-Ter119 (TER-119), anti-CD44 (IM7), anti-Vα2 (B20.1), anti-TCR Vβ5.1,5.2 (MR 9-4), anti-IFNγ (XMG 1.2), anti-Siglec H (551), anti-CD11c (N418), anti-IRF8 (V3GYWCH), anti-CD31 (390), anti-EpCAM-1 (G8.8), anti-CCR9 (eBioCW-1.2), anti-α4β7 (DATK32), anti-IL17A (TC11-18-10.1), anti-FoxP3 (FJK-165), anti-T-bet (eBio4B10), anti-CD103 (M290), anti-Siglec F (E50-2440), anti-CD64 (X54-5/7.1), anti-CD62L (MEL-14), anti-2B4 (2B4), anti-Granzyme A (GzA-3G8.5) all from eBioscience, BioLegend or BD Biosciences. Propidium iodide and fluorescently conjugated Streptavidin were purchased from Invitrogen and BD Biosciences.

Preparation of OVA-MEsF for immunization

H-2^{bm1} MEFs expressing truncated non-secreted OVA (OVA-MEF, (Sancho et al., 2009)) were prepared as described previously (Chen et al., 2007). Briefly, harvested OVA-MEFs were suspended in PBS at a concentration 5x10^7/ml and heat shocked for 10 min at 45°C. Treated cells were subsequently incubated for 5h at 37°C prior to immunization.

Immunizations

OT-I recipients were immunized by oral gavage with OVA (5mg, Grade V, Sigma-Aldrich) or OVA (50 mg) and R848 (20 μg, InvivoGen) or i.p. with heat shocked OVA- MEFs (10^7 cells/mouse) or OVA (0.1mg/mouse, Grade VI, Sigma-Aldrich). OT-II recipients were immunized by oral gavage with OVA (50 mg) or OVA (50 mg) and R848 (20 μg). At day 1 after immunization recipient mice were injected with

FTY720 (20 μg/mouse; Cayman) in saline i.p. OT-I recipients were sacrificed 3 days after immunization while OT-II cell recipients received an additional FTY720 injection day 3 and were sacrificed 4 day after immunization. For T cell homing experiments FTY720 was omitted and animals were sacrificed 4 days after immunization. For cytokine analysis studies OT-II cell recipients were immunized i.p. with OVA (0.5mg, Grade VI, Sigma-Aldrich), αCD40 (25 μg, BioLegend) and LPS (20 μg, Sigma-Aldrich), treated with FTY720 as above, and sacrificed 4 day after immunization.

***In vitro* cultures**

For *in vitro* Th1 cell polarization, FACS-sorted naive ($CD4^+CD62L^+CD25^-CD44^{lo}$) T cells were cultured in αCD3-coated (clone 145-2C1, BioXCell, 5 ug/mL) round bottom 96 well plates (0.25 $x10^6$ cells /ml) in the presence of αCD28 (1ug/mL, clone 37.51, Biolegend), IL-2 (20 ng/mL, bio-techne), IL-12 (20 ng/mL, bio-techne), and αIL-4 (10ug/mL clone 11B11, BioXCell) for 4.5 d prior to re-stimulation and intracellular cytokine analysis. For splenic $CD11c^+$ cell cultures, MACS-enriched $CD11c^+$ cells were suspended in RPMI 1640 containing with 10% FCS (Sigma Aldrich), 1 mM sodium pyruvate (Gibco), 10 mM HEPES, 100 U/ml Penicillin and 100 μg/ml Streptomycin, 50 μg/ml Gentamycin (Gibco) (R10 medium) and plated ($2.5x10^5$ cells/well) in 96 well U-bottom plates in the presence/absence of CpG ODN1585 (5 μg/ml, InvivoGen) for 42 h at 37°C. IFNα levels in culture supernatants was assessed using the VeriKine™ Mouse IFNα ELISA Kit (PBL Assay science) according to manufacturers instructions.

Real-Time PCR

For analysis of sorted cells, total RNA was isolated from 1-1.5x10^4 sorted cells using Absolutely RNA Nanoprep Kit (Agilent) or Quick-RNA MicroPrep kit (Zymo Research). cDNA was generated and amplified using the Ovation® PicoSL WTA Systems V2 (Nugen, San Carlos, US). Quantitative PCR was performed on a MyiQ Single Color-Real-Time PCR detection System (Bio-Rad), using KAPA SYBR FAST detection reagent (Kapabiosystems). For analysis of intestinal tissues, total RNA was isolated using the RNeasy Mini Kit (Agilent). cDNA was generated using Superscript III First-Strand Kit (Invitrogen) with both random hexamers and pT-primer present and used as a template for quantitative real-time PCR. Quantitative PCR was performed on a MyiQ Single-Color Real-Time PCR Detection System, using SsoFAST EvaGreen SuperMix (Bio-Rad). The expression of all genes was normalized to the two house-keeping genes beta-actin and GAPDH.

Immunohistochemistry

Frozen sections (7-8 μm) of SI tissue (6-12 cm from the pylorus) were fixed and blocked with 10% donkey serum. Endogenous peroxidase and biotin activities were quenched with 0.1 % H_20_2 (vol/vol) in PBS and Avidin-Biotin blocking kit (Vector Laboratories), respectively. TCRγδ was detected using FITC conjugated anti-TCRγδ (GL3, BD Biosciences) and the signal was amplified with Alexa-488 signal amplification kit (Molecular Probes). CD8β was detected using biotinylated anti-CD8β (clone H35-17.2, eBioscience) and signal was amplified with biotinyl-tyramide signal amplification kit (PerkinElmer Life Science) and Alexa Fluor 555-conjugated streptavidin (Invitrogen). Nuclei were counterstained with DAPI (Invitrogen). Images

were acquired with LSM 750 confocal microscope and analyzed using ZEN software (both from Carl Zeiss MicroImaging Inc).

Aldehyde dehydrogenase activity

Aldehyde dehydrogenase activity of MACS-enriched MLN $CD11c^+$ cells was determined using the ALDEFLOUR staining kit (StemCell Technologies) according to the manufacturer's instructions and as described previously (Schulz et al., 2009)

Microbiota analysis

Cecal and colonic samples were collected after sacrifice and immediately frozen in liquid nitrogen. Microbial DNA was extracted using a NucleoSpin soil kit (Macherey-Nagel) according to manufacturer's instructions. PCR-based library formation, sequencing (Illumina MiSeq), taxonomy assignment and subsequent data analysis were performed as previously described (Holm et al., 2015). The dataset contained an average of 33,296 and 28,404 sequences per sample before and after filtering, respectively. Assessment of significant differences was performed with the adonis test, using Bray Curtis distance, and metagenomeSeq (Paulson et al., 2013) for the effect on specific OTUs or phylotypes. All sequence data is available from the European Nucleotide Archive (ENA) with study accession number: PRJEB10856. Quantitation of segmented filamentous bacteria (SFB) was performed using qPCR reactions run on 75ng of DNA using SFB-specific primers (SFB736F: 5'-GAC GCT GAG GCA TGA GAG CAT-3'; SFB844R: 5'-GAC GGC ACG GAT TGT TAT TCA-3') (Bouskra et al., 2008) and normalized to the total amount of bacterial DNA using total eubacteria-specific primers (27F 5'TCCTACGGGAGGCAGCAG T3'; 343R 5' GACTACCAG GGTATCTAATCCTGTT 3') (Marathe et al., 2012).

Trichuris muris infection

Mice received approximately 20 infective *T. muris* (strain E) eggs by oral gavage and worm burden and *T. muris*-specific serum IgG1 and IgG2a assessed 35 d post infection. To quantify worm burden, the cecum and proximal colon were removed, cut longitudinally, and worms collected and counted under a microscope. For analysis of *T. muris*-specific serum IgG1 and IgG2a levels, ELISA plates were coated over night with *T. muris* antigen, prepared as previously described (Wakelin, 1967). After addition of serum for 2 h at room temperature, plates were washed and incubated with anti-mouse IgG1 (RMG1-1) and IgG2a biotin-conjugated antibodies (RMG2a-62; BioLegend) followed by streptavidin-conjugated horseradish peroxidase (BioLegend) and tetramethylbenzidine (BD Bioscience). The reaction was stopped by addition of H_2SO_4 (1M), and absorbance measured at 450 nm on a SPECTROstar Nano (BMG LABTECH).

Primer Sequences, Related to Experimental Procedures

Gene	fw primer 5'-3'	rev primer 5'-3'
Cd244	CAGTTGCCACAGCAGACTTTCC	GCCATGGGTTTCCAACCTCC
Gzma	GGGAGATTTGGCAATAAGTCAG	TCGCAAAATACCATCACATAGC
Gzmb	AGATCCTCCTGCTACTGCTGAC	GCCCCCAAAGTGACATTTATTA
Itgae	GCTCCAAAAAGATTCTCCCCA	CATTGCAGTTCTTTCCAGACCC
Runx3	ACCGAGCCATCAAGGTCACTG	ATGCGCAGGTCTCCAAAGCGG
Tbx21	CATGCCAGGGAACCGCTTAT	TTGGAAGCCCCCTTGTTGTT
Cd8a	GAAAGTGAACTCTACTACTACC	GCGAAGTCCAATCCGGTCCCCT
Actb	CCGGGACCTGACAGACTA	GTTTCATGGATGCCACAGGAT
Gapdh	CCTGCACCACCAACTGCTTA	TCATACTTGGCAGGTTTCTCCA
Ccl25	AGGCACCAGCTCTCAGGACC	GCGGAATTCGTCTTCAAAGGCA CCTTGGGCATGG
Foxp3	GAGAGAAGTGGTGCAGTCTCT	AGCAAGAGCTCTTGTCCATTG
Rorc	GCAGATCTAAGGGCTGAGGCAC	GCAAGGGATCACTTCAATTTGT GTT
Itgb8	CGCCATACATTAGTATCCACCC	CTTCAGGGGTGTCTATGTTTCC
P2x7r	CACATACTCCAGTGCTTTCTGC	GAACTTCTTGGCCTTTGACAACTT
Itga4	GCACAGCCACGGGTCGAA	GCATGTCTTCCCACAAGGCTC
Ccr9	AAGCTGATTGGCGTCCGATCCA	GCGGAATTCATTATTTTTCTTACAG AAGAAGCTA

Supplemental references

Bergqvist, I., Eriksson, M., Saarikettu, J., Eriksson, B., Corneliussen, B., Grundstrom, T., and Holmberg, D. (2000). The basic helix-loop-helix transcription factor E2-2 is involved in T lymphocyte development. Eur J Immunol *30*, 2857-2863.

Bouskra, D., Brezillon, C., Berard, M., Werts, C., Varona, R., Boneca, I.G., and Eberl, G. (2008). Lymphoid tissue genesis induced by commensals through NOD1 regulates intestinal homeostasis. Nature *456*, 507-510.

Chen, C.J., Kono, H., Golenbock, D., Reed, G., Akira, S., and Rock, K.L. (2007). Identification of a key pathway required for the sterile inflammatory response triggered by dying cells. Nat Med *13*, 851-856.

Holm, J.B., Sorobetea, D., Kiilerich, P., Ramayo-Caldas, Y., Estelle, J., Ma, T., Madsen, L., Kristiansen, K., and Svensson-Frej, M. (2015). Chronic Trichuris muris Infection Decreases Diversity of the Intestinal Microbiota and Concomitantly Increases the Abundance of Lactobacilli. PLoS One *10*, e0125495.

Klein, U., Casola, S., Cattoretti, G., Shen, Q., Lia, M., Mo, T., Ludwig, T., Rajewsky, K., and Dalla-Favera, R. (2006). Transcription factor IRF4 controls plasma cell differentiation and class-switch recombination. Nat Immunol *7*, 773-782.

Lee, P.P., Fitzpatrick, D.R., Beard, C., Jessup, H.K., Lehar, S., Makar, K.W., Perez-Melgosa, M., Sweetser, M.T., Schlissel, M.S., Nguyen, S*., et al.* (2001). A critical role for Dnmt1 and DNA methylation in T cell development, function, and survival. Immunity *15*, 763-774.

Marathe, N., Shetty, S., Lanjekar, V., Ranade, D., and Shouche, Y. (2012). Changes in human gut flora with age: an Indian familial study. BMC Microbiol *12*, 222.

Paulson, J.N., Stine, O.C., Bravo, H.C., and Pop, M. (2013). Differential abundance analysis for microbial marker-gene surveys. Nat Methods *10*, 1200-1202.

Saederup, N., Cardona, A.E., Croft, K., Mizutani, M., Cotleur, A.C., Tsou, C.L., Ransohoff, R.M., and Charo, I.F. (2010). Selective chemokine receptor usage by central nervous system myeloid cells in CCR2-red fluorescent protein knock-in mice. PLoS One *5*, e13693.

Wakelin, D. (1967). Acquired immunity to Trichuris muris in the albino laboratory mouse. Parasitology *57*, 515-524.

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Different populations of CD11b$^+$ dendritic cells drive Th2 responses in the small intestine and colon

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T-helper 2 (Th2) cell responses defend against parasites. Although dendritic cells (DCs) are vital for the induction of T-cell responses, the DC subpopulations that induce Th2 cells in the intestine are unidentified. Here we show that intestinal Th2 responses against *Trichuris muris* worms and *Schistosoma mansoni* eggs do not develop in mice with IRF-4-deficient DCs (IRF-4$^{f/f}$ CD11c-cre). Adoptive transfer of conventional DCs, in particular CD11b-expressing DCs from the intestine, is sufficient to prime *S. mansoni*-specific Th2 responses. Surprisingly, transferred IRF-4-deficient DCs also effectively prime *S. mansoni*-specific Th2 responses. Egg antigens do not induce the expression of IRF-4-related genes. Instead, IRF-4$^{f/f}$ CD11c-cre mice have fewer CD11b$^+$ migrating DCs and fewer DCs carrying parasite antigens to the lymph nodes. Furthermore, CD11b$^+$CD103$^+$ DCs induce Th2 responses in the small intestine, whereas CD11b$^+$CD103$^-$ DCs perform this role in the colon, revealing a specific functional heterogeneity among intestinal DCs in inducing Th2 responses.

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Type 2 immunity, the typical response against parasitic or allergic stimuli, can protect against parasites or exacerbate allergic conditions[1]. Approximately, a third of the world's population are infected with parasitic worms, most of which affect the gastrointestinal tract[2]. Many of these infections develop into chronic pathologies and are an enormous global health burden. There is insufficient knowledge about how worm infections, which can selectively infect either the small intestine or the colon, are controlled by the intestinal immune system. Helminth parasite infections in both the small intestine and colon induce potent T-helper 2 (Th2) responses that can control parasite burden or lead to chronic pathologies[3].

Schistosoma mansoni eggs and their soluble egg antigens (schistosome egg antigen, SEA) induce potent Th2 and interferon (IFN)-γ responses, both during infection with live parasites[4,5] and in experimental models in which eggs or SEA are injected into tissues[6,7]. During the natural parasite infection, a proportion of the eggs released by intravascular adult worms become lodged in the intestinal wall and the liver, where they induce strong type 2 immune responses. These eggs are central to immunopathology associated with this infection, as they induce granulomatous inflammation and tissue fibrosis, which can lead to severe organ damage[5].

Both non-professional antigen-presenting cells, such as basophils[8] and monocyte-derived dendritic cells (DCs)[9], and conventional DCs[10,11] have been shown to have functions in the induction or maintenance of Th2 responses. However, the cells that are sufficient to induce Th2 responses in the intestine have not been clearly identified.

In the small intestine and colon, four different populations of conventional DCs can be identified, categorized by their differential expression of the integrins CD11b and CD103 (refs 12–14). These populations are present at different frequencies in the small intestine and colon[15,16], and migrate via intestinal-draining lymphatics to the mesenteric lymph nodes (MLN) to initiate T-cell responses[14]. Studies have indicated that intestinal DC populations are specialized to induce different facets of the T-cell response. For example, transcription factor IFN regulatory factor (IRF)-8-dependent intestinal $CD11b^-CD103^+$ (CD103 single-positive (SP)) DCs have a predominant function in cross-presentation to $CD8^+$ T cells and induction of intestinal Th1 responses[17,18], and IRF-4-dependent $CD11b^+CD103^+$ (double-positive (DP)) DCs seem to drive Th17 cell differentiation in intestine-draining MLNs[13,19]. Although the function of these populations in intestinal Th2 responses is unclear, studies have demonstrated that IRF-4 expression by $CD11c^+$ cells is crucial for the development of Th2 responses[20,21]. In the intestine, IRF-4 is predominantly expressed by $CD11b^+CD103^-$ (CD11b SP) DCs and DP DCs, and IRF-4 deficiency in $CD11c^+$ cells results in fewer small intestinal DP DCs, as well as the absence of DP DCs and fewer CD11b SP DCs in the draining MLNs[13].

To investigate how IRF-4-expressing DCs drive intestinal Th2 responses, we use two models of human parasite infection that drive Th2 responses in the gastrointestinal tract. We address the induction of Th2 responses *in vivo* by experimental immunization with *S. mansoni* eggs and validate our findings during live infection with the intestinal parasite *Trichuris muris*. We find that CD11b-expressing DCs are specializd to drive antigen-specific Th2 responses. Furthermore, different populations of $CD11b^+$ $IRF\text{-}4^+$ DCs induce Th2 responses in the small intestine and colon. DP DCs from the small intestine are the only population sufficient to drive antigen-specific Th2 responses in the small intestine-draining lymph nodes and CD11b SP DCs fulfil this function in colon-draining lymph nodes. We thus demonstrate that different DC populations have distinct functions in separate regions of the intestine, which is important for understanding how intestinal immune responses are controlled, and offers the opportunity to develop more precise therapeutic targets.

Results

Intestinal Th2 responses require IRF-4-positive $CD11c^+$ cells. To identify the cellular mechanisms central to the induction of Th2 responses in the intestine we developed a novel method of experimental delivery of *S. mansoni* eggs directly into intestinal tissue. Eggs were injected directly into sites where they become trapped during live infection, thus providing a refined and relevant method to investigate the Th2 responses generated against trapped and penetrating eggs in the intestine (Supplementary Fig. 1a,b). The method also allowed precise temporal control of the induction of Th2 responses against *S. mansoni* eggs in the gastrointestinal tract *in vivo*, which has not been previously possible. We found that the injection of 1,000 *S. mansoni* eggs into the subserosal tissue of the small intestine was sufficient to induce antigen-specific Th2 and IFN-γ responses in the MLNs, with the key Th2 cytokines interleukin (IL)-4, IL-5 and IL-13 induced in *in vitro* total MLN cell cultures, specifically after the restimulation with SEA 5 days after *in vivo* immunization (Fig. 1a and Supplementary Fig. 1c–e). Consistent with published findings[22], we observed no antigen-specific induction of Th17 cytokines (Supplementary Fig. 1d). Intracellular flow cytometric staining after phorbol 12-myristate 13-acetate (PMA)/ionomycin stimulation confirmed that these cytokines were produced by CD4 T cells that produced IFN-γ or had differentiated into Th2 cells (Fig. 1b and Supplementary Fig. 1f,g). To determine whether intestinal egg injection could also be used as a model of colonic Th2 induction, eggs were injected either in the small intestine or colon and the small intestine-draining MLNs (sMLNs) and colon-draining MLNs (cMLNs)[16] were harvested 5 days after immunization. Analysis of restimulated individual lymph nodes revealed increased concentrations of antigen-specific cytokines, compared with analysis of pooled MLNs (Fig. 1a). These responses were only observed in the sMLNs or cMLNs draining the respective injection sites (Fig. 1c). Thus, intestinal *S. mansoni* egg injections can be used as an experimental model to further investigate the mechanisms of Th2 induction in both tissues.

Many aspects of type 2 immunity are controlled by the transcription factor IRF-4, which controls the development of Th2 cells[23], alternatively activated macrophages[24] and CD11b-expressing DCs[25]. However, little is known about how IRF-4 regulates the induction of Th2 responses in the intestine. To determine what impact the expression of IRF-4 by antigen-presenting cells had in driving intestinal Th2 responses in the small intestine and colon, we used the $IRF\text{-}4^{f/f}$ CD11c-cre mouse model that allows targeted deletion of IRF-4 on all $CD11c^+$ cells[13], including intestinal macrophages and conventional DCs. It has been reported that CD11c-cre expression in these mice does not affect DC frequencies[26,27]. Subserosal injection of *S. mansoni* eggs into the bone marrow (BM) chimeric mice, generated by lethal irradiation and reconstitution of C57BL/6.SJL mice with BM from $IRF\text{-}4^{f/f}$ CD11c-cre-positive (cre+) or $IRF\text{-}4^{f/f}$ cre-negative (cre−) mice (Supplementary Fig. 2a–c), resulted in dramatically impaired Th2 responses in the MLNs of cre+ chimeras, accompanied by an elevated IFN-γ response (Fig. 1d). The loss of IRF-4-dependent cells in these animals did not affect the total number of cells in the small intestine (Supplementary Fig. 2c). The impaired Th2 responses were evident in both the small intestine and colon, demonstrating a central requirement for $IRF\text{-}4^+$ $CD11c^+$ cells for Th2 induction in both organs. When we assessed the expression of IRF-4 by DCs and macrophages we observed that a high percentage of DCs

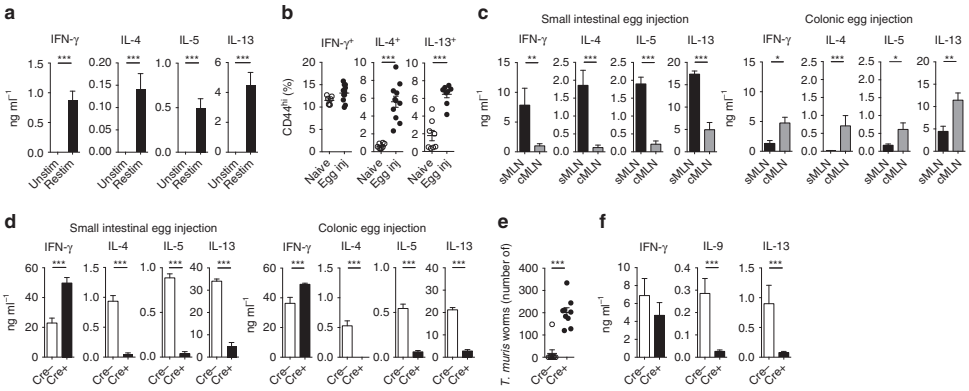


Figure 1 | Intestinal Th2 responses to *S. mansoni* eggs and *T. muris* worms are dependent on IRF-4$^+$ CD11c$^+$ cells. (**a**) One thousand *S. mansoni* eggs were injected into the subserosal layer of the small intestine and resulting T-cell responses were analysed after 5 days by restimulating MLN cells for an additional 3 days in the presence of SEA. Cytokines were measured from supernatants of restimulation cultures of unstimulated (Unstim) and restimulated (Restim) MLN cells ($n=9$ mice, in three independent experiments, mean ± s.e.m., Mann–Whitney *U*-tests, $^{***}P\leq0.001$). (**b**) Five days after immunization with *S. mansoni* eggs (Egg inj), MLNs were harvested, CD44^{hi} CD4 T cells identified by flow cytometry and levels of IFN-γ, IL-4 and IL-13 cytokine production measured after PMA/ionomycin stimulation and compared with cells harvested from naive animals ($n=10$ mice per group, in three independent experiments, mean ± s.e.m., Mann–Whitney *U*-tests, $^{***}P\leq0.001$). (**c**) Cytokine responses of individually harvested small intestinal (sMLN) and colonic (cMLN) draining lymph node restimulation cultures 5 days after small intestinal (left panel) or colonic (right panel) egg injection ($n=9$ mice per group, in three independent experiments, mean ± s.e.m., Mann–Whitney *U*-tests, $^{*}P\leq0.05$, $^{**}P\leq0.01$ and $^{***}P\leq0.001$). (**d**) Cytokine responses of restimulated MLN cells from small intestinal (left panel) or colonic (right panel) egg injected IRF-4$^{f/f}$ CD11c-cre + or littermate IRF-4$^{f/f}$ cre − BM chimeric mice ($n=6$ mice per group, in two independent experiments, mean ± s.e.m., Mann–Whitney *U*-tests, $^{***}P\leq0.001$). (**e**) Worm burden in cre + or littermate cre − mice following *T. muris* infection. Mice were infected with ~300 infectious eggs by oral gavage and worms in the colon quantified at 35 days post infection ($n=8$–9 mice per group, in three independent experiments, mean ± s.e.m., Mann–Whitney *U*-tests, $^{***}P\leq0.001$). (**f**) Secreted cytokines after *T. muris* E/S antigen-specific restimulation of MLN cells 35 days after *T. muris* infection ($n=9$ mice per group, in three independent experiments, mean ± s.e.m., Mann–Whitney *U*-tests, $^{***}P\leq0.001$).

expressed IRF-4, whereas only a few macrophages were IRF-4$^+$ (Supplementary Fig. 2d–g). Although macrophages expressed lower levels of IRF-4, a role for IRF-4 in these cells cannot be excluded. However, consistent with previous published work[14,18,28], we found that macrophages were absent from thoracic duct lymph of mesenteric lymphadenectomized (MLNx) mice (Supplementary Fig. 3c) and are therefore unable to prime T-cell responses in the MLNs, excluding the possibility that IRF-4$^+$ macrophages are necessary to prime Th2 responses. To further address whether IRF-4 in DCs was necessary for Th2 induction, we performed DC transfer experiments.

To verify that IRF-4$^+$ DCs were also necessary for establishing a physiological type 2 immune response against live parasites, cre + and cre − mice were infected with ~250–300 eggs from the nematode *T. muris* by oral gavage. Under these conditions, *T. muris* infection evokes a strong Th2 response in the colon of C57BL/6 mice that mediates expulsion within 35 days[29]. We observed that cre + mice did not effectively clear adult worms by 35 days post infection, suggesting an inefficient type 2 immune response (Fig. 1e). Indeed Th2 responses were markedly decreased in cre + mice and we observed reduced production of IL-9 and IL-13 in *in vitro* MLN restimulation cultures 35 days post infection (Fig. 1f).

Thus, Th2 responses to parasite antigen present in the small intestine or colon require IRF-4-expressing CD11c$^+$ cells for their induction in the respective draining lymph nodes.

Lymph DCs prime responses to *S. mansoni* eggs. To determine which migratory cell populations were responsible for transporting parasite antigen from the periphery to the draining lymph nodes, AlexaFluor660 (AF660)-labelled SEA was injected into the intestinal serosa. To directly assess migrating cell populations, thoracic duct lymph was collected from MLNx mice for 18 h after SEA-AF660 injection, using previously described techniques[14]. We observed that among all lymph migrating cells, B cells and conventional DCs labelled positive for Alexa660-labelled SEA (Fig. 2a and Supplementary Fig. 3a). DCs were the most efficient population to transport SEA-AF660 from the intestine, representing the highest proportion of AF660-labelled cells. To determine which cells were capable of inducing SEA-specific immune responses *in vivo*, fluorescence-activated cell sorting (FACS)-purified donor cells from egg-injected animals were transferred under the MLN capsule of wild-type recipient mice. We have previously used this technique to assess DC functions *in vivo*[18], inspired by an elegant study examining migration of transferred DCs into recipient lymph nodes[30].

Intranodal DC transfer allowed the direct assessment of the *in vivo* priming capabilities of the transferred cells in their physiological location. Five days after cell transfer, the injected MLNs were harvested and restimulated with SEA *in vitro* to test for antigen-specific immune responses. DCs from egg-injected donors were the only cells able to induce antigen-specific immune responses upon cell transfer, whereas B cells—despite carrying antigen—could not drive antigen-specific immune responses after transfer (Fig. 2b). In agreement with previous experiments using BM-derived DCs *in vivo*[6,31] or splenic DCs *in vitro*[32], SEA-specific Th2 induction by intestinal lymph DCs required major histocompatibility complex II (MHCII) expression (Fig. 2c and Supplementary Fig. 3b), but was independent of their ability to produce IL-4 (Supplementary Fig. 3e).

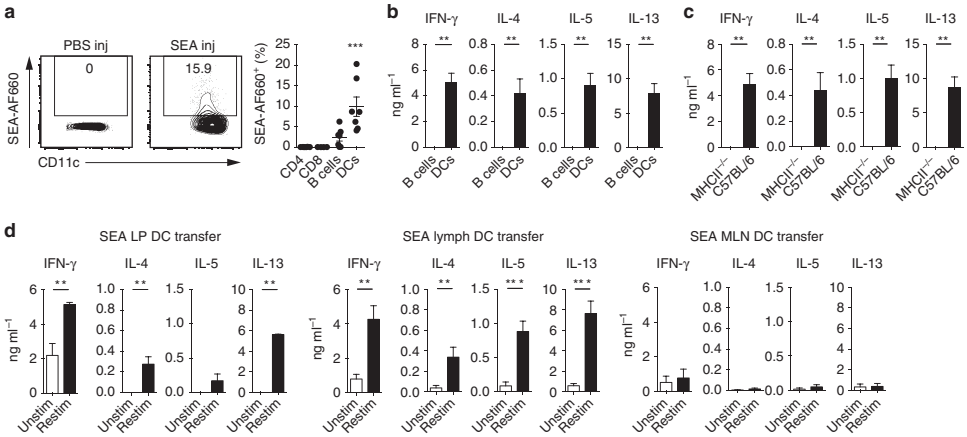


Figure 2 | Conventional DCs drive immune responses against SEA in the MLN. (**a**) Transport of parasite antigen was assessed by injecting AF660-labelled SEA (SEA-AF660) into the small intestine of MLNx mice and measuring the percentage of fluorescent cells in intestinal draining lymph 18 h after injection. Representative FACS plots of SEA-transporting DCs from PBS (PBS inj) and SEA (SEA inj) injected mice (left panel), and percentage of SEA-transporting lymph migrating CD4 and CD8 T cells, B cells and DCs (DCs) (right panel) are shown ($n=7$ mice in three independent experiments, mean ± s.e.m., Kruskal–Wallis test, $^{***}P\leq0.001$). (**b**) Fifty thousand MHCII^{hi} CD64$^-$ B220$^-$ CD11c^{hi} DCs and 100,000 B cells were purified from the lymph of egg injected MLNx donor mice 18 h after injection and delivered under the MLN capsule of wild type recipient mice. After 5 days, antigen specific T-cell responses were measured in the injected MLNs by SEA restimulation for 3 days and subsequent cytokine measurement by ELISA ($n=6$ mice per group, in three independent experiments, mean ± s.e.m., Mann–Whitney *U*-tests, $^{**}P\leq0.01$). (**c**) Similar to **b**, CD11c^{hi} CD64$^-$ B220$^-$ DCs were purified from the lymph of egg-injected C57BL/6 or MHCII$^{-/-}$ MLNx donor mice, transferred into the MLNs of recipient animals and antigen specific T-cell responses measured after *in vitro* restimulation with SEA ($n=6$ mice per group, in three independent experiments, mean ± s.e.m., Mann–Whitney *U*-tests, $^{**}P\leq0.01$). (**d**) Fifty thousand DCs were purified from the small intestinal LP (left panel), the lymph of MLNx mice (middle panel) or the MLNs (right panel) of C57BL/6 mice and loaded with SEA for 18 h *in vitro*. Unbound antigen was washed off and cells transferred under the MLN capsule of wild-type recipient animals. T-cell responses in the injected MLNs were measured 5 days after cell transfer by cytokine analysis of *in vitro* restimulation cultures with (Restim) or without (Unstim) SEA ($n=10$ mice per group, in three independent experiments, mean ± s.e.m., Mann–Whitney *U*-tests, $^{**}P\leq0.01$ and $^{***}P\leq0.001$).

Antigen-specific immune responses could also be induced by DCs incubated with SEA *in vitro*. FACS-purified DCs from the intestinal lamina propria (LP), the lymph, and the MLNs of wild-type C57BL/6 animals were cultured with SEA for 18 h and transferred into recipient animals. Transferred LP-derived and lymph DCs induced antigen-specific immune responses, measured in *in vitro* restimulation cultures. However, SEA-loaded MLN DCs did not induce any antigen-specific responses after transfer (Fig. 2d).

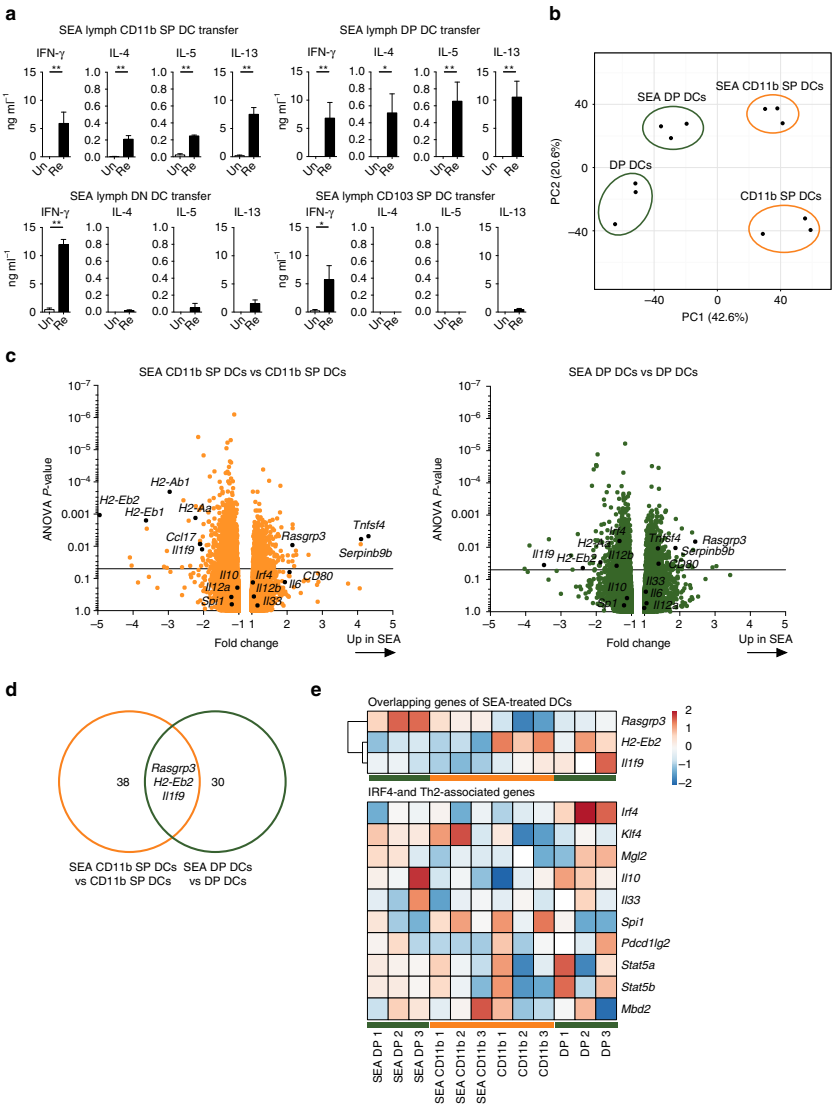
Thus, conventional DCs are sufficient to drive *S. mansoni* egg antigen-specific immune responses in the intestinal draining lymph nodes, and process and present egg antigens to CD4 T cells in a MHCII-dependent manner.

SEA treatment of DCs does not affect IRF-4-related genes. To identify the intestinal DC populations driving egg antigen-specific immune responses, lymph DCs were separated by their expression of CD11b and CD103 (Supplementary Fig. 3c). Thirty thousand cells of each population were isolated from wild-type C57BL/6 MLNx lymph, incubated with SEA *in vitro* and transferred under the MLN capsule of wild-type recipient mice. Both, CD11b$^+$CD103$^-$ SP (CD11b single-positive (SP)) and CD11b$^+$CD103$^+$ double-positive (DP) DCs could induce antigen-specific Th2 and IFN-γ responses after transfer, whereas CD11b$^-$CD103$^-$ double-negative (DN) and CD11b$^-$CD103$^+$ (CD103 SP) DCs could only induce antigen-specific IFN-γ responses (Fig. 3a). Thus, only CD11b-expressing DC populations were specialized to induce Th2 responses, whereas all DC populations could induce antigen-specific IFN-γ.

To understand the underlying molecular mechanisms that selectively enabled CD11b SP and DP DCs to prime antigen-specific Th2 responses, we performed microarray analysis and compared the gene expression profiles of sorted CD11b SP and DP DCs after *in vitro* incubation with or without SEA. Five thousand and eighteen significant loci of coding and non-coding gene elements were identified as differentially expressed between any of the four conditions (CD11b SP DCs/DP DCs, SEA-treated/untreated). Principal component analysis revealed clustering of replicate samples and a clear separation between the cell populations and between treatments (Fig. 3b). Several of the genes affected by SEA treatment have been shown to be involved in antigen presentation and T-cell differentiation, and 41 differentially expressed genes changed their expression levels more than 2-fold after SEA treatment of CD11b SP DCs. The highest fold changes were observed in downregulated MHCII-related genes, *Ccl17* and *Il1f9*, which encodes the proinflammatory cytokine IL-36γ. Costimulatory molecules such as *Cd80* and *Tnfsf4*, which encodes OX40L, and *Rasgrp3* and *Serpinb9b* were upregulated by SEA treatment of CD11b SP DCs (Fig. 3c). Thirty-three genes were differentially expressed after SEA treatment of DP DCs and a downregulation of MHCII-related genes and *Il1f9*, and an upregulation of *Rasgrp3* and *Serpinb9b* was again observed (Fig. 3c). We observed limited overlap between the differentially expressed genes with high fold

change (absolute fold change $< -2; > 2$ and analysis of variance P-value < 0.05) after SEA treatment of CD11b SP and DP DCs, which were *Rasgrp3, H2-Eb2* and *Il1f9* (Fig. 3d). However, none of these genes have previously been associated with Th2 cell polarization. Strikingly, IRF-4-associated genes did not change their expression profile upon treatment with SEA (Fig. 3e), despite the fact that IRF-4 expression by CD11c$^+$ cells was required for the induction of Th2 responses against *S. mansoni* eggs and *T. muris* worms.

IRF-4 affects the migration of intestinal DCs. Our observation that egg antigens did not induce the expression of IRF-4-associated genes in DCs suggested that the defect of Th2 responses observed in IRF-4$^{f/f}$ CD11c-cre+ mice was not due to the defective induction of these genes. We therefore investigated whether other mechanisms were involved and assessed whether IRF-4 deletion in CD11c$^+$ cells affected the number of each of the DC populations in the intestine. Consistent with reports in IRF-4$^{f/f}$ CD11c-cre+ mice[13], cre+ BM chimeras showed a



decrease in the DP DC population in the small intestine (Fig. 4a and Supplementary Fig. 4a). In the colon, a general decrease of DCs, again most strikingly observed in DP DCs, was observed (Fig. 4b). However, this developmental defect did not impair intestinal DC function. Antigen uptake, visualized by the injection of Alexa660-labelled SEA, was not affected in cre+ mice and comparable numbers of intestinal LP DCs labelled positive for SEA-AF660 18 h after injection in the small intestine and colon (Fig. 4c). Furthermore, transferred SEA-loaded DP DCs from the small intestinal LP of cre+ animals induced similar antigen-specific cytokine responses to C57BL/6 small intestinal LP DCs after transfer into wild-type recipient animals (Fig. 4d). Thus, IRF-4 deficiency in CD11c$^+$ cells influenced the development of intestinal LP DCs, in particular the DP DC population, and the numbers of CD11b-expressing DCs in the MLN, but did not inhibit the ability of the remaining intestinal DCs to drive Th2 cell differentiation.

To investigate whether the IRF-4-dependent reduction of LP DCs was reflected in the draining lymph nodes, we analysed DC populations in the small intestinal sMLNs and the colonic cMLNs. We observed a 50% reduction of migratory CD11b SP DCs and a near absence of migratory DP DCs in the sMLNs, as previously observed[13]. Reciprocally, the percentage of migratory CD103 SP DCs increased dramatically, but total numbers of CD103 SP DCs were not affected by the deletion of IRF-4 (Fig. 4e and Supplementary Fig. 4b). Migratory DC populations were affected to a similar extent in the cMLNs, where numbers of CD11b SP were reduced by half, DP DCs became almost absent and the numbers of CD103 SP DCs remained unaffected by the deletion of IRF-4 (Fig. 4f). Similar to LP DCs, the T-cell priming capabilities of these migratory MLN DC populations were not affected by the deletion of IRF-4. In *in vitro* co-cultures ovalbumin (OVA), pulsed migratory DC populations from the sMLNs and cMLNs of cre+ animals drove equivalent proliferation of OVA-specific OT-II CD4$^+$ T cells compared with their cre− counterparts (Fig. 4g and Supplementary Fig. 4c). In contrast, antigen delivery to the MLNs was strongly affected *in vivo*. Eighteen hours after AF660-SEA injection into the small intestinal or colonic LP, the number of AF660-SEA$^+$ DCs was greatly reduced in the sMLNs and cMLNs of cre+ animals (Fig. 4h). Thus, IRF-4 deficiency in CD11c$^+$ cells did not influence the capacity of DCs to prime T cells and drive Th2 differentiation, consistent with our conclusions from gene expression analysis. Rather, the striking loss of migratory CD11b SP and DP DCs from the draining lymph nodes, combined with the decrease in the amount of transported parasite antigen, were the probable cause of the inadequate intestinal Th2 responses observed in IRF-4$^{f/f}$ CD11c-cre+ animals.

Distinct DCs drive Th2 response in small intestine and colon. As the composition of CD11b-expressing DC populations varies between the small intestine and colon[15], we assessed whether tissue-specific roles could be attributed to CD11b SP and DP DCs in priming intestinal Th2 responses. To directly assess the migration of DCs from the small intestine to the sMLNs, where priming occurs, we collected DCs from thoracic duct lymph from small intestinal MLNx (sMLNx) mice[16]. We observed that DP DCs, the most abundant DC population in the small intestine, migrated at an increased frequency, compared with PBS-injected controls, after the injection of *S. mansoni* eggs into the small intestine (Fig. 5a and Supplementary Fig. 5a,c). DP DCs were also the predominant population to transport small intestinally injected SEA-AF660 in sMLNx lymph (Fig. 5b). Importantly, the transfer of FACS-purified sMLNx lymph DC populations from egg-injected donor mice into recipient animals revealed that DP DCs were the only population sufficient to drive antigen-specific immune responses after transfer (Fig. 5c). This was confirmed after SEA loading of small intestinal LP DC populations, to ensure that any differences in antigen availability did not influence the results. Again, DP DCs were the most efficient population to prime antigen-specific Th2 responses against egg antigens. Similar to our previous observations, IFN-γ responses could be induced by all DC populations (Fig. 5d).

Thus, DP DCs specialize in transporting and presenting *S. mansoni* egg antigens from the small intestine and prime antigen-specific Th2 cells in the draining lymph nodes.

Examination of DC populations migrating from the colon revealed a different picture. In contrast to the small intestine, CD11b SP DCs migrated in increased frequency in colon-draining cMLNx lymph after the injection of *S. mansoni* eggs into the colon (Fig. 5e and Supplementary Fig. 5b,d). As well as being the predominant DC population within the colonic LP[15], CD11b SP DCs were the only population to carry fluorescently labelled SEA-AF660 in the lymph after colonic injection (Fig. 5f). Furthermore, colonic CD11b SP LP DCs were the most efficient at inducing Th2 responses against *in vitro*-loaded SEA after transfer (Fig. 5g).

Thus, in contrast to the small intestine, CD11b SP DCs were responsible for transporting and presenting *S. mansoni* egg antigens from the colon and were the most efficient population for priming antigen-specific Th2 cell in the colon-draining lymph nodes.

Figure 3 | CD11b$^+$ DCs drive antigen-specific Th2 responses but SEA does not alter IRF-4-related gene expression. (**a**) Thirty thousand cells of each of the four intestinal DC populations, distinguished by their expression of CD11b and CD103, were purified from the MLNx C57BL/6 animals and incubated with SEA for 18 h *in vitro*. Unbound antigen was washed off and cells transferred under the MLN capsule of wild-type recipient animals. T-cell responses in the injected MLNs were measured 5 days after cell transfer by cytokine analysis of *in vitro* restimulation cultures with (Re) or without (Un) SEA ($n=6$ mice per group, in three independent experiments, mean ± s.e.m., Mann–Whitney *U*-tests, $^{**}P\leq0.01$ and $^{***}P\leq0.001$). (**b**) The gene expression profiles of SEA-treated or -untreated CD11b$^+$CD103$^-$ single-positive (CD11b SP) and CD11b$^+$CD103$^+$ double-positive (DP) DCs were analysed by microarray analysis. Principle component analysis of the 5,018 significant loci of coding and non-coding gene elements identified to be differentially expressed between any of the four conditions ($n=3$ samples per condition, unpaired one-way (single factor) analysis of variance (ANOVA) for each pair of condition groups, ANOVA *P*-value (condition pair) <0.05). (**c**) All gene loci from CD11b SP (left panel) and DP DCs (right panel) were compared between SEA-treated and -untreated cells and the absolute fold change and ANOVA *P*-value visualized using volcano plots. Coding genes of interests are highlighted ($n=3$ samples per condition, unpaired one-way (single factor) ANOVA for each pair of condition groups). (**d**) All coding genes that were found to be differentially expressed within each condition pair with absolute fold change $<-2;>2$ and ANOVA *P*-value <0.05 were selected and summarized. Genes that changed in both CD11b SP and DP DCs are highlighted ($n=3$ samples per condition, unpaired one-way (single factor) ANOVA for each pair of condition groups; ANOVA *P*-value (condition pair) <0.05). (**e**) The relative expression intensities of the overlapping genes from **d** and of IRF-4- and Th2-associated genes determined from the literature are shown for each individual sample (changes of overlapping genes are significant, whereas changes of IRF-4- and Th2-associated genes are not significant) ($n=3$ samples per condition, unpaired one-way (single factor) ANOVA for each pair of condition groups for the two condition groups; ANOVA *P*-value (condition pair) <0.05).

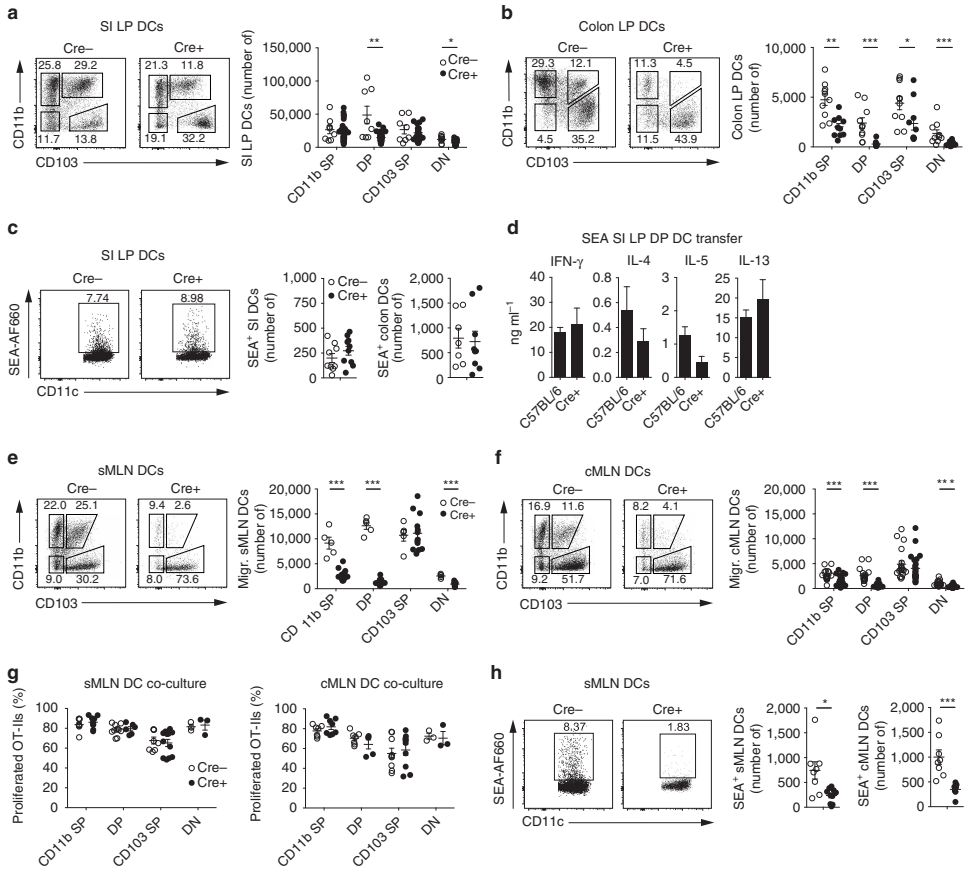


Figure 4 | IRF-4 deficiency alters DC population composition to reduce antigen availability in the MLNs. (**a**) Representative percentages and total numbers of CD11b and CD103-expressing DC populations from the small intestines of IRF-$4^{f/f}$ CD11c-cre+ or littermate IRF-$4^{f/f}$ cre− bone-marrow (BM) chimeras ($n=8$ mice per group, in three independent experiments, mean ± s.e.m., Mann–Whitney *U*-tests, $^{*}P\leq0.05$ and $^{**}P\leq0.01$). (**b**) Representative percentages and total numbers of DC populations from the colon of cre+ and cre− BM chimeras ($n=8$ mice per group, in three independent experiments, mean ± s.e.m., Mann–Whitney *U*-tests, $^{*}P\leq0.05$, $^{**}P\leq0.01$ and $^{***}P\leq0.001$). (**c**) Uptake of AF660 labelled SEA (SEA-AF660) by intestinal DCs from cre+ and cre− BM chimeras 18 h after injection into the small intestine or colon. Representative FACS plots from the small intestine (left panel) and total numbers of SEA-AF660^{+} small intestinal (middle panel) and colonic (right panel) DCs are shown ($n=7$–10 mice per group, in three independent experiments, mean ± s.e.m., Mann–Whitney *U*-tests (not significant)). (**d**) Thirty-thousand CD11b^{+}CD103^{+} double-positive (DP) DCs from the small intestine of C57BL/6 or cre+ BM chimeras were incubated with SEA for 18 h *in vitro* and transferred under the MLN capsule of wild-type recipient animals. Antigen specific T-cell responses in the injected MLNs were measured 5 days after cell transfer by cytokine analysis of *in vitro* SEA restimulation cultures ($n=6$ mice per group, in three independent experiments, mean ± s.e.m., Mann–Whitney *U*-tests (P(IFN-γ) = 0.59, P(IL-4) = 0.82, P(IL-5) = 0.09 and P(IL-13) = 0.50). (**e**) Representative percentages and total numbers of DC populations from the sMLNs of cre+ and cre− BM chimeras ($n=5$–12 mice per group, in three independent experiments, mean ± s.e.m., Mann–Whitney *U*-tests, $^{***}P\leq0.001$). (**f**) Representative percentages and total numbers of DC populations from the colonic draining MLNs (cMLNs) of cre+ and cre− BM chimeras ($n=12$ mice per group, in three independent experiments, mean ± s.e.m., Mann–Whitney *U*-tests, $^{***}P\leq0.001$). (**g**) Six thousand sMLN (left panel) or 3,000 cMLN (right panel) DC populations from cre+ and cre− BM chimeras were pulsed with OVA and co-cultured with CFSE-labelled OT-II MLN cells for 3 days. *In vitro* OT-II CD4 T-cell proliferation was assessed by CFSE dilution and compared between cre+ and cre− DC populations ($n=3$–16 duplicate cocultures per group, in three independent experiments, mean ± s.e.m., Mann–Whitney *U*-tests (not significant)). (**h**) Transport of SEA-AF660 by cre+ and cre− migratory DCs to the draining lymph nodes 18 h after injection into the small intestine or colon. Representative FACS plots from the sMLN (left panel) and total numbers of SEA-AF660^{+} migratory sMLN (middle panel) and cMLN (right panel) DCs are shown ($n=8$ mice per group, in three independent experiments, mean ± s.e.m., Mann–Whitney *U*-tests, $^{*}P\leq0.05$ and $^{***}P\leq0.001$).

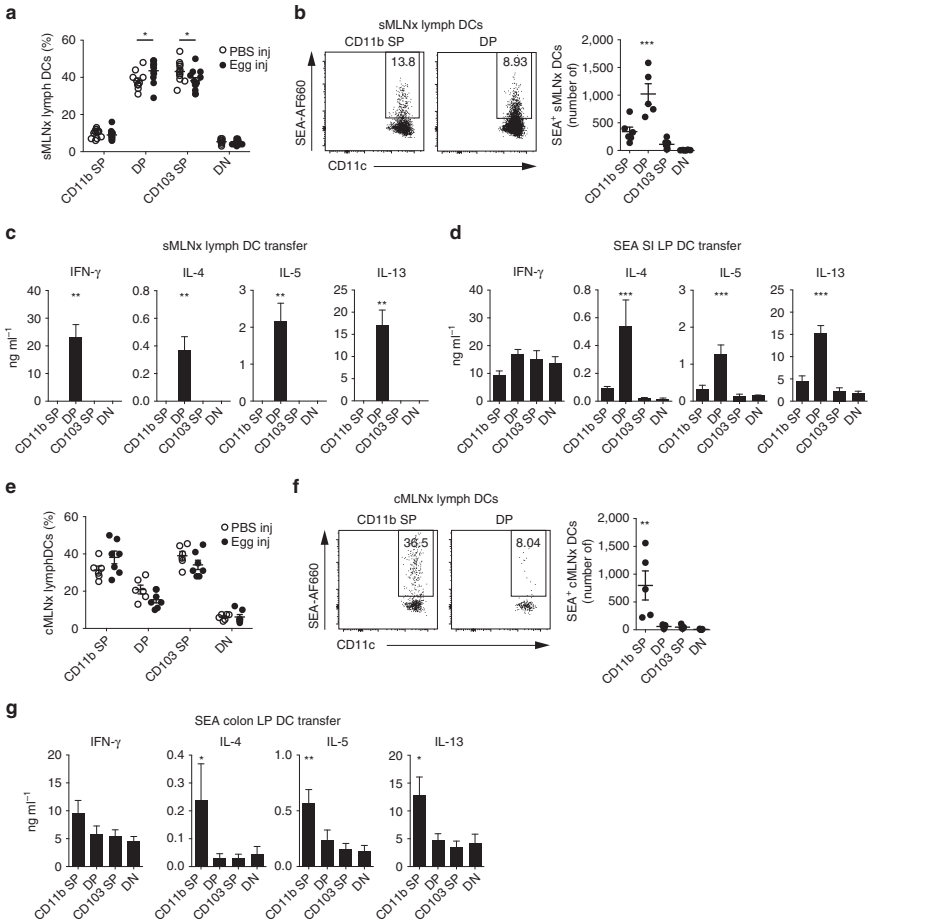


Figure 5 | Th2 responses in the small intestine and colon are driven by distinct CD11b$^+$ DCs. (**a**) Frequency of lymph migrating DC populations 18 h after the injection of PBS (PBS inj) or *S. mansoni* eggs (Egg inj) into the small intestine of sMLNx C57BL/6 animals ($n=10$–11 mice per group, in three independent experiments, mean ± s.e.m., Mann–Whitney *U* tests (P(CD11b SP) = 0.25, P(DP) = 0.02, P(CD103 SP) = 0.04 and P(DN) = 0.14). (**b**) Transport of AF660-labelled SEA (SEA-AF660) by lymph-migrating DC populations 18 h after small intestinal injection into sMLNx animals. Representative FACS plots from CD11b$^+$CD103$^-$ single-positive (CD11b SP) and CD11b$^+$CD103$^+$ double-positive DCs (left panel) and total numbers of SEA-AF660$^+$ DC populations (right panel) are shown ($n=5$ mice per group in two independent experiments, mean ± s.e.m., Kruskal–Wallis test, $^{***}P\leq0.001$). (**c**) Thirty-thousand DCs from each population were isolated from the lymph of small intestinal egg-injected sMLNx mice and transferred under the MLN capsule of wild-type recipient animals. Antigen-specific T-cell responses in the injected MLNs were measured 5 days after cell transfer by cytokine analysis of *in vitro* SEA restimulation cultures ($n=6$ mice per group, in three independent experiments, mean ± s.e.m., Kruskal–Wallis test, $^{**}P\leq0.01$). (**d**) Thirty-thousand DCs from each population were isolated from the small intestine of C57BL/6 mice, incubated with SEA *in vitro* and transferred under the MLN capsule of wild-type recipient animals. Antigen-specific immune responses were analysed as in **c** ($n=6$ mice per group, in three independent experiments, mean ± s.e.m., Kruskal–Wallis test, $^{***}P\leq0.001$). (**e**) Frequency of lymph-migrating DC populations 18 h after the injection of PBS or *S. mansoni* eggs into the colon of colonic MLNx (cMLNx) C57BL/6 animals ($n=6$–7 mice per group, in three independent experiments, mean ± s.e.m., Mann–Whitney *U*-tests (P(CD11b SP) = 0.17, P(DP) = 0.17, P(CD103 SP) = 0.22 and P(DN) = 0.71). (**f**) Transport of SEA-AF660 by lymph-migrating DC populations 18 h after injection into the colon of cMLNx animals. Representative FACS plots from CD11b SP and DP DCs (left panel), and total numbers of SEA-AF660$^+$ DC populations (right panel) are shown ($n=5$ mice per group, in two independent experiments, mean ± s.e.m., Kruskal–Wallis test, $^{**}P\leq0.01$). (**g**) Thirty-thousand DCs from each population were isolated from the colon of C57BL/6 mice, incubated with SEA *in vitro* and transferred under the MLN capsule of wild-type recipient animals. Antigen-specific immune responses in the injected MLNs were measured 5 days after cell transfer by cytokine analysis of *in vitro* SEA restimulation cultures ($n=6$ mice per group, in three independent experiments, mean ± s.e.m., Kruskal–Wallis test, $^{*}P\leq0.05$ and $^{**}P\leq0.01$).

Discussion

Many cell types have been implicated in inducing type 2 immune responses against *S. mansoni* egg antigens, including monocyte-derived DCs[7], conventional DCs[11] and basophils[33]. In this context, the intestine is an important tissue, being heavily affected by penetrating *S. mansoni* eggs during live infection, and the target of many Th2-inducing helminth parasite infections[34]. To investigate which cell populations are sufficient to induce intestinal Th2 responses, we establish an experimental immunization procedure for controlled delivery of eggs into intestinal subserosal tissue. We observe that egg antigen-specific CD4 T-cell responses are induced in the draining MLNs (Fig. 1), which, as previously reported[11,35,36], produced both IFN-γ- and the Th2-associated cytokines IL-4, IL-5 and IL-13. *S. mansoni* eggs induce antigen-specific immune responses in both the small intestinal and colonic draining lymph nodes after injection into the respective tissues. IRF-4-expressing $CD11c^+$ cells are critical for the induction of Th2 responses both in the small intestine and colon (Fig. 1), as Th2 immune responses did not develop in IRF-$4^{f/f}$ CD11c-cre+ mice, similar to previous reports in the lung[21] and the small intestine during *Nippostrongylus brasiliensis* infection[20].

As CD11c is expressed by monocyte-derived DCs, conventional DCs and macrophages in the intestine[37], we address which specific cell population transports egg antigen from the intestine to the draining lymph nodes and is sufficient to drive antigen-specific immune responses. Injection of fluorescently labelled SEA enables identification of B cells and conventional DCs transporting egg antigens to the MLN. Transfer of cells directly under the MLN capsule allows for delivery of cells by their physiological route of entry and to assess their priming capabilities *in vivo*[18,30]. MHCII-expressing DCs, but not B cells, from egg-injected donor mice are sufficient to induce egg antigen-specific immune responses in the recipient MLN (Fig. 2). Furthermore, both intestinal LP DCs and lymph DCs isolated from wild-type C57BL/6 mice can present SEA after *in vitro* incubation. Thus, our data indicate that conventional DCs transport parasite antigen from the intestine to the draining lymph nodes and are sufficient to directly prime antigen-specific immune responses. Further analysis of the four intestinal DC populations that can be defined by the expression of CD11b and CD103 (refs 12–14) reveals that SEA-loaded $CD11b^+CD103^-$ single-positive (CD11b SP) and $CD11b^+CD103^+$ double-positive (DP) DCs are specialized to prime antigen-specific Th2 and IFN-γ responses upon transfer, whereas $CD11b^-CD103^-$ double-negative (DN) and $CD11b^-CD103^+$ single-positive (CD103 SP) DCs can only induce IFN-γ responses (Fig. 3). This observation is in line with previous findings that demonstrate that CD11b-expressing DCs from the spleen, lung and skin induce Th2 responses[9,31,38–40]. In contrast, CD103 SP DCs have been shown to negatively regulate Th2 responses, by their constitutive expression of IL-12 (ref. 41). The balance between the different DC populations is therefore critical for determining the outcome of the T-cell response.

Microarray analysis of the Th2-priming CD11b-expessing DCs reveals that the expression levels of genes involved in antigen presentation and T-cell differentiation are influenced by incubating CD11b SP and DP DCs with SEA. For example, *CD80* and *Tnfsf4*, which encodes OX40L, are upregulated by SEA. These costimulatory markers are required for T-cell differentiation and OX40L has been previously been shown to be induced by SEA[42]. Furthermore, the downregulation of MHCII-related genes, proinflammatory mediators such as IL-36γ, encoded by *Il1f9*, and the chemokine CCL17, which has been shown to recruit proinflammatory Th2 cells during allergy[43] could suggest that SEA dampens proinflammatory responses, as previously observed *in vitro* and *in vivo*[31,32,44].

We anticipated that our expression analysis would reveal genes induced in both CD11b-expressing DC populations that contribute to their common ability to prime Th2 responses. However, we observe little overlap and only three genes are differentially expressed in SEA-cultured CD11b SP and DP DCs. *Rasgrp3* is upregulated, whereas *H2-Eb2* and *Il1f9* are downregulated upon SEA incubation in both populations. RAS activators, such as RasGRP3, provide a key link between cell surface receptors and RAS activation, and RasGRP3 has been shown to control CCR9-dependent entry of early thymic progenitors in the thymus[45], B-cell receptor signalling[46] and the production of Toll-like receptor (TLR)-triggered proinflammatory cytokines in macrophages[47]. Together with the downregulation of *H2-Eb2* and *Il1f9*, a dampening of proinflammatory responses is thus observed in these SEA-cultured DCs. However, no direct involvement of these genes in type 2 immune response has previously been reported and comparison with data sets from Th2-impaired $Mbd2^{-/-}$ bone-marrow DCs[35] reveal no commonality. Surprisingly, the expression of IRF-4 and its binding partner PU.1, encoded by *Spi1*, are not upregulated by SEA, despite the fact that IRF-4 expression by $CD11c^+$ cells is required to drive Th2 polarization. These genes have been shown to promote the expression of OX40L[48], IL-10 and IL-33 (ref. 21), which influence Th2 polarization, but are not upregulated in DCs after SEA treatment. Other genes that have been implicated in polarizing and enhancing Th2 responses, such as *Mgl2* (refs 20,49), *Pdcd1lg2* (refs 50,51), which encodes PDL2, *Stat5a/b*[52] and *Mbd2* (ref. 35) are also not upregulated by SEA (Fig. 3), leading us to conclude that *S. mansoni* egg antigens induce the ability to drive Th2 polarization in intestinal DCs by mechanisms that may not be revealed by gene expression analysis.

A key element of our work is that we have discovered that IRF-4 deficiency does not directly interfere with intestinal DC ability to induce T-cell priming or drive Th2 differentiation (Fig. 4), despite being previously suggested in the literature[21,53]. Rather, IRF-4 deficiency limits the number of intestine-derived CD11b-expressing DCs in the draining lymph nodes, consistent with previous observations in the intestine[13] and the skin[12]. Impaired survival[12,13] and lack of migration[54] have both been suggested to cause this pronounced IRF-4-dependent decrease in CD11b-expressing DCs. As a result, limited amounts of egg antigen are present in the MLNs of IRF-$4^{f/f}$ CD11c-cre+ mice, which probably causes the impaired Th2 responses in these mice (Fig. 4). Thus, IRF-4 does not directly control the ability of intestinal DCs to polarize Th2 cells, but rather affects the number of CD11b-expressing DCs, probably by influencing their differentiation and survival, and thus the amount of presented antigen in the draining lymph nodes.

As the composition of CD11b-expressing DCs varies along the gastrointestinal tract, we assessed whether CD11b SP and DP DC populations play tissue-specific roles. We and others have observed that DP DCs are the most abundant DCs in the small intestine, whereas CD11b SP DCs are more frequent in the colon[15,16]. These differences can be observed within the lamina propria (LP), as well as in tissue-specific draining lymph, and the respective draining lymph nodes[16]. We observe that after small intestinal egg injection DP DCs are present at increased frequency within lymph, suggesting increased migration (Fig. 5). It is well established that TLR activation can lead to DC migration in the intestine, which has been shown for R848 (ref. 55), a TLR7/8 agonist, and soluble flagellin[3,56], which activates TLR5. DCs do not require MyD88-mediated TLR signalling to induce Th2 responses against *S. mansoni* eggs[57], but the effects of *S. mansoni* eggs on DC migration have not previously been addressed. Diverse pathogen-associated molecular patterns, such as Omega-1, have been identified in *S. mansoni* eggs and SEA[58,59],

indicating that a range of parasite molecules may influence DC migration.

As well as displaying increased migration, DP DCs are the most numerous population to carry fluorescently labelled SEA from the small intestine to the draining lymph nodes. Transfer of purified DC populations from egg-injected donor mice reveals that DP DCs are also the only DCs capable of inducing antigen-specific immune responses in recipient animals (Fig. 5). This is likely to be both due to their ability to capture egg antigen but also an intrinsic specialisation for inducing Th2 responses, which we verified by transfer of *in vitro* SEA-loaded small intestinal LP DCs. In this system, all populations can induce antigen-specific IFN-γ responses but only DP DCs induce strong Th2 responses, suggesting that DP DCs from the small intestine are either specialized to process and present specific Th2-inducing antigens within SEA or, more probably, can generate specific signals that drive the differentiation of Th2 cells. In contrast to the small intestine, CD11b SP DCs migrate in increased frequency in lymph of egg-injected animals after colonic egg injection, transport fluorescently labelled SEA from the colon and are the predominant population to induce Th2 responses when loaded with SEA (Fig. 5). Unexpectedly therefore, the Th2-inducing DC populations in the small intestine and colon are different, revealing unappreciated complexity in the functional specialisations of DCs in different tissues.

We thus demonstrate that specific populations of intestinal CD11b-expressing DCs are specialized to prime Th2 cells in the small intestine and colon, and speculate that this capacity extends beyond antigens from *S. mansoni* eggs and may also be relevant for other parasitic antigens or intestinal food allergens. The differences in the functions of CD11b-expressing DC populations between the small intestine and colon also provide clear evidence that the induction of immune responses in these tissues is controlled differently. This idea is supported by recent findings that have demonstrated that oral tolerance in the small intestine and colonic tolerance are driven by distinct populations of tissue-specific DCs[60]. Beyond advancing our understanding of the immunological differences between these tissues, this raises the possibility that diseases in the small intestine and colon could also be influenced by distinct populations of DCs. Many important infections and inflammatory conditions (for example, parasite infections, Crohn's disease, ulcerative colitis and celiac disease) selectively affect the small intestine or colon and DCs have been shown to contribute to disease induction or progression. Delineation of the roles of specific DC populations in these conditions could thus reveal novel pathways that may be precisely and independently targeted to beneficially modify the involved protective or pathogenic immune responses. Thus, our identification of the tissue-specific DC populations that induce Th2 responses against *S. mansoni* eggs in the intestine reveals novel insight into the induction of intestinal type 2 immune responses. It also impacts our understanding of intestinal immune responses in general, by demonstrating that different tissue-specific DC populations are responsible for driving similar responses in anatomically distinct intestinal locations.

Methods

Mice. C57BL/6 (C57BL/6JOlaHsd) were ordered from Envigo and C57BL/6.SJL, IL-4$^{-/-}$, OT-II, IRF-4$^{f/f}$ CD11c-cre and IRF-4$^{f/f}$ cre− mice (all on C57BL/6 background) were bred and housed under specific pathogen free conditions at the University of Glasgow, UK, or at Lund University, Sweden. Age- and gender-matched adult animals were used in each individual experiment, which were approved by the University of Glasgow Animal Welfare Ethical Review Board and the Malmö/Lund Ethical board for Animal research and performed under licenses issued by the UK Home Office and the Swedish Board of Agriculture. IRF-4$^{f/f}$ CD11c-cre and IRF-4$^{f/f}$ cre− bone-marrow (BM) chimeras were created by lethally irradiating 6-week-old C57BL/6.SJL recipients with 10 gray using a Small Animal Radiation Research Platform (Xstrahl) and reconstituted with 2–4 × 10^6 IRF-4$^{f/f}$ CD11c-cre+ or IRF-4$^{f/f}$ cre− BM cells. Experiments with irradiated animals were performed 8–10 weeks after irradiation.

Surgical procedures. All surgical procedures were carried out under aseptic conditions and inhalation anaesthesia with Isoflurane (Abbot Animal Health). For egg injections, 1,000 freeze/thawed *S. mansoni* eggs were resuspended in 20 µl DPBS (Life Technologies) and injected into the intestinal LP of anaesthetized mice. *S. mansoni* eggs for these studies were isolated under sterile conditions from the livers of infected C57BL/6 mice before cryopreservation and SEA was prepared by homogenization and ultracentrifugation of eggs, and concentrated by vacuum dialysis to 1 mg ml^{-1} in DPBS[61]. For subcapsular injections, 6-week-old male mice were fed 0.2 ml olive oil to visualize the MLN capsule and MLNs were accessed by laparotomy. Cells were resuspended in 5 µl DPBS and injected under the MLN capsule. MLNx was performed on 6-week-old male mice by laparotomy and blunt dissection of the small intestinal or colonic draining lymph nodes. After 6 weeks, MLNx mice were fed 0.2 ml olive oil to visualize the lymphatics and the thoracic lymph duct was accessed by laparotomy and cannulated by the insertion of a polyurethane medical grade intravascular tube (2Fr; Linton Instrumentation). Lymph was collected for 18 h on ice in DPBS supplemented with 20 U ml^{-1} of heparin sodium (Wockhardt UK).

Infection models. Mice were infected with ~250–300 infective *T. muris* eggs from the E (Edinburgh) isolate by oral gavage. Adult worms were isolated from the colons of infected C57BL/6 mice. For the preparation of eggs and parasite E/S antigens worms were cultured in sterile RPMI 1640 supplemented with 500 U ml^{-1} penicillin and 500 µg ml^{-1} streptomycin (all Thermo-Fisher Scientific) and incubated at 37 °C for 24 h. Eggs were collected by centrifugation and parasite antigens concentrated using centriprep-centrifugal columns with 10,000 NMWL (Merck-Millipore) and dialysed to DPBS using Amicon Ultracel-3 K Centrifugal Filters with 3000 NMWL (Merck-Millipore) to a final concentration of 1 mg ml^{-1}. To assess worm burden, colons were isolated and frozen at −20 °C; tissues were subsequently thawed and worms scraped free from the tissue and counted under a microscope.

Cell isolation. MLNs were digested using RPMI 1640 (Life Technologies) supplemented with 8 U ml^{-1} Liberase and 10 µg ml^{-1} DNase (all Sigma-Aldrich) for 45 min at 37 °C in a shaking incubator and single-cell suspensions were prepared using a 40 µm cell strainer (Greiner Bio One). Intestines were excised, cleaned and cut into 0.5 cm segments. Segments were washed in HBSS (Life Technologies) supplemented with 2 mM EDTA (Sigma-Aldrich) twice for 15 min at 37 °C in a shaking incubator. Small intestinal segments were digested in RPMI 1640 supplemented with 1 mg ml^{-1} Collagenase VIII (Sigma-Aldrich) and 10% FCS for 15 min at 37 °C in a shaking incubator. Colons were digested with RPMI supplemented with 0.425 mg ml^{-1} Collagenase V (Sigma-Aldrich), 0.425 mg ml^{-1} Collagenase D (Roche), 1 mg ml^{-1} Dispase (Gibco), 30 µg ml^{-1} DNase (Roche) and 10% FCS for 40 min at 37 °C in a shaking incubator. Single-cell suspensions were prepared using a 100 and 40 µm cell strainer (Corning). Lymph cells were passed through a 40 µm cell strainer (Greiner Bio One) and stained directly.

***In vitro* restimulation cultures and cytokine measurement.** MLN cells (1 × 10^6) were cultured in X-vivo 15 media (Lonza) supplemented with 1% L-glutamine (Invitrogen), 0.1% 2-mercaptoethanol (Sigma-Aldrich) and 15 µg ml^{-1} SEA in round bottom 96-well plates (Corning) at 37 °C and 5% CO_2. Supernatants were collected after three days and cytokines detected using the IL-4 (88-7044-77), IL-5 (88-7054-77), IL-13 (88-7137-77), IL-17 (88-7371-77) and IFN-γ (88-7341-77) 'ready-set-go' ELISA kits (eBioscience) following the manufacturer's instructions. For *T. muris* E/S-antigen-specific restimulation of total MLN cells, 0.5 × 10^6 cells were cultured with 50 µg ml^{-1} E/S antigens for 48 h at 37 °C and 5% CO_2. Supernatants were collected and frozen at −20 °C before cytokine analysis. Cytokine concentrations were determined by cytometric bead array kit (BD Biosciences) according to the manufacturer's instructions. Samples were acquired on a BD LSR II flow cytometer (BD Biosciences) and data analysed with FCAP array v3.0 software.

Antibodies for flow cytometric analysis and cell sorting. Mouse tissue cell surface markers and intracellular cytokines were stained using combinations of fluorescently labelled primary antibodies at a dilution of 1:200. These included anti-CD4 (clones GK1.5 and RM4-5), anti-CD8a (53–6.7), anti-CD44 (IM7), anti-CD45R/B220 (RA3-6B2), anti-CD11c (N418), anti-I-A/I-E (M5/114.15.2), anti-CD11b (M1/70), anti-CD103 (2E7), anti-CD64 (X54-5/7.1), anti-Ly6C (HK1.4), anti-TCR Vα2 (B20.1), anti-IL4 (11B11), anti-CD45 (30-F11), anti-CD45.1 (A20) and anti-CD45.2 (104) purchased from Biolegend, and anti-IFNγ (XMG1.2), anti-IRF-4 (3E4), anti-GATA3 (TWAJ) and anti-IL13 (eBio13A) from eBioscience. SEA was fluorescently labelled using the Microscale Antibody Labelling Kit (Life Technologies) following the manufacturer's instructions. For intracellular transcription factor staining, cells were fixed and permeabilized using the eBioscience Foxp3/Transcription Factor Staining Buffer

Set and intracellular staining was performed following the manufacturer's instructions. Cells were analysed using a LSRII flow cytometer running FACSDiva Software (BD Bioscience) and analysed using FlowJo Software (Tree Star). 7AAD (Biolegend) or Fixable Viability Dye eFluor780 (eBioscience) were used to exclude dead cells from analysis. For cell sorting, DCs were gated on by selecting live $CD45^+$ $CD45R/B220^-$ $CD64^-$ $Ly6C^-$ $CD11c^{hi}$ $MHCII^{hi}$ single cells and individual CD11b/CD103-expressing populations were sorted using an AriaIII cell sorter (BD Bioscience). Cells undergoing subsequent antigen loading were incubated in supplemented (as above) *X-vivo* 15 media (Lonza) with 15 μg ml^{-1} SEA for 18 h or 2 mg ml^{-1} OVA protein (Sigma-Aldrich) for 2 h at 37 °C and 5% CO_2.

***In vitro* cell stimulation.** For intracellular staining experiments 2×10^6 MLN cells were incubated in RPMI 1640 supplemented with 2.5 ng ml^{-1} PMA (Sigma-Aldrich), 1 μg ml^{-1} ionomycin (Invitrogen), 0.5% GolgiStop (BD Bioscience) and 10% FCS for 4 h at 37 °C, after which cell surface markers were stained. Cells were fixed and permeabilized using the eBioscience Foxp3/Transcription Factor Staining Buffer Set (eBioscience) and intracellular staining was performed following the manufacturer's instructions.

***In vitro* co-cultures.** For *in vitro* OT-II co-cultures 2×10^5 OT-II MLN cells were labelled with CFSE (eBioscience) at a dilution of 1:1,000 and cocultured with 6,000 sMLN or 3,000 cMLN FACS-sorted DCs from each population. Each population had been pulsed with 2 mg ml^{-1} of OVA (Worthington, Lakewood) for 2 h at 37 °C and 5% CO_2 and then extensively washed. After 3 days of co-culture, cells were stained for flow cytometry and CFSE dilution was assessed to quantify cell proliferation.

Microarray analysis. Total RNA from SEA-incubated lymph DC populations (as above) was isolated with the RNeasy micro kit (Qiagen) and prepared for microarray analysis at Hologic Ltd using the Affymetrix Mouse Transcriptome Pico Assay 1.0. RNA samples were applied to a Mouse Transcriptome Array 1.0 (Affymetrix). Scanned CEL files were background corrected, normalized and summarized by using the Affymetrix Expression Console Software 1.4. Differential gene expression was analysed using the Affymetrix Transcription Analysis Console Software 3.0 and visualized using Prism 6 Software (GraphPad). Principal component analysis analysis and heatmap visualization were conducted using ClustVis[62].

Statistical analysis. Based on analyses of preliminary experiments, group sizes were chosen to ensure that a twofold difference between means, where the common standard deviation was less than or equal to half of the smaller mean, could be detected with a power of at least 80%. Prism 6 Software (GraphPad) was used to calculate the s.e.m. and statistical differences between groups were calculated using Mann–Whitney *U*-tests and Kruskal–Wallis tests, where appropriate, with $P < 0.05$ being considered as significant.

Data availability. Microarray gene expression data are available from the Gene Expression Omnibus, accession number GSE91381. All other relevant data are available from the authors.

References

1. Licona-Limón, P., Kim, L. K., Palm, N. W. & Flavell, R. A. TH2, allergy and group 2 innate lymphoid cells. *Nat. Immunol.* **14,** 536–542 (2013).
2. Hotez, P. J. *et al.* Helminth infections: the great neglected tropical diseases. *J. Clin. Invest.* **118,** 1311–1321 (2008).
3. Grencis, R. K. Immunity to helminths: resistance, regulation, and susceptibility to gastrointestinal nematodes. *Annu. Rev. Immunol.* **33,** 201–225 (2015).
4. Pearce, E. J. *et al.* Th2 response polarization during infection with the helminth parasite *Schistosoma mansoni*. *Immunol. Rev.* **201,** 117–126 (2004).
5. Pearce, E. J. & MacDonald, A. S. The immunobiology of schistosomiasis. *Nat. Rev. Immunol.* **2,** 499–511 (2002).
6. MacDonald, A. S., Straw, A. D., Dalton, N. M. & Pearce, E. J. Cutting edge: Th2 response induction by dendritic cells: a role for CD40. *J. Immunol.* **168,** 537–540 (2002).
7. Klaver, E. J., Kuijk, L. M., Lindhorst, T. K., Cummings, R. D. & van Die, I. *Schistosoma mansoni* soluble egg antigens induce expression of the negative regulators SOCS1 and SHP1 in human dendritic cells via interaction with the mannose receptor. *PLoS ONE* **10,** e0124089 (2015).
8. Yoshimoto, T. Basophils as T(h)2-inducing antigen-presenting cells. *Int. Immunol.* **22,** 543–550 (2010).
9. Plantinga, M. *et al.* Conventional and monocyte-derived $CD11b(^+)$ dendritic cells initiate and maintain T helper 2 cell-mediated immunity to house dust mite allergen. *Immunity* **38,** 322–335 (2013).
10. Connor, L. M., Tang, S. C., Camberis, M., Le Gros, G. & Ronchese, F. Helminth-conditioned dendritic cells prime $CD4^+$ T cells to IL-4 production *in vivo*. *J. Immunol.* **193,** 2709–2717 (2014).
11. Phythian-Adams, A. T. *et al.* CD11c depletion severely disrupts Th2 induction and development *in vivo*. *J. Exp. Med.* **207,** 2089–2096 (2010).
12. Schlitzer, A. *et al.* IRF4 transcription factor-dependent $CD11b^+$ dendritic cells in human and mouse control mucosal IL-17 cytokine responses. *Immunity* **38,** 970–983 (2013).
13. Persson, E. K. *et al.* IRF4 transcription-factor-dependent $CD103^+CD11b^+$ dendritic cells drive mucosal T helper 17 cell differentiation. *Immunity* **38,** 958–969 (2013).
14. Cerovic, V. *et al.* Intestinal $CD103^{(-)}$ dendritic cells migrate in lymph and prime effector T cells. *Mucosal Immunol.* **6,** 104–113 (2013).
15. Denning, T. L. *et al.* Functional specializations of intestinal dendritic cell and macrophage subsets that control Th17 and regulatory T cell responses are dependent on the T cell/APC ratio, source of mouse strain, and regional localization. *J. Immunol.* **187,** 733–747 (2011).
16. Houston, S. A. *et al.* The lymph nodes draining the small intestine and colon are anatomically separate and immunologically distinct. *Mucosal Immunol.* **9,** 468–478 (2016).
17. Luda, K. M. *et al.* IRF8 transcription-factor-dependent classical dendritic cells are essential for intestinal T cell homeostasis. *Immunity* **44,** 860–874 (2016).
18. Cerovic, V. *et al.* Lymph-borne $CD8\alpha^+$ dendritic cells are uniquely able to cross-prime $CD8^+$ T cells with antigen acquired from intestinal epithelial cells. *Mucosal Immunol.* **8,** 38–48 (2015).
19. Scott, C. L. *et al.* $CCR2(^+)CD103^{(-)}$ intestinal dendritic cells develop from DC-committed precursors and induce interleukin-17 production by T cells. *Mucosal Immunol.* **8,** 327–339 (2015).
20. Gao, Y. *et al.* Control of T helper 2 responses by transcription factor IRF4-dependent dendritic cells. *Immunity* **39,** 722–732 (2013).
21. Williams, J. W. *et al.* Transcription factor IRF4 drives dendritic cells to promote Th2 differentiation. *Nat. Commun.* **4,** 2990 (2013).
22. Larkin, B. M. *et al.* Induction and regulation of pathogenic Th17 cell responses in schistosomiasis. *Semin. Immunopathol.* **34,** 873–888 (2012).
23. Lohoff, M. *et al.* Dysregulated T helper cell differentiation in the absence of interferon regulatory factor 4. *Proc. Natl Acad. Sci. USA* **99,** 11808–11812 (2002).
24. Satoh, T. *et al.* The Jmjd3-Irf4 axis regulates M2 macrophage polarization and host responses against helminth infection. *Nat. Immunol.* **11,** 936–944 (2010).
25. Suzuki, S. *et al.* Critical roles of interferon regulatory factor 4 in CD11bhighCD8alpha- dendritic cell development. *Proc. Natl Acad. Sci. USA* **101,** 8981–8986 (2004).
26. Caton, M. L., Smith-Raska, M. R. & Reizis, B. Notch-RBP-J signaling controls the homeostasis of CD8- dendritic cells in the spleen. *J. Exp. Med.* **204,** 1653–1664 (2007).
27. Varol, C. *et al.* Intestinal lamina propria dendritic cell subsets have different origin and functions. *Immunity* **31,** 502–512 (2009).
28. Schulz, O. *et al.* Intestinal $CD103^+$, but not $CX3CR1^+$, antigen sampling cells migrate in lymph and serve classical dendritic cell functions. *J. Exp. Med.* **206,** 3101–3114 (2009).
29. Cliffe, L. J. & Grencis, R. K. The *Trichuris muris* system: a paradigm of resistance and susceptibility to intestinal nematode infection. *Adv. Parasitol.* **57,** 255–307 (2004).
30. Braun, A. *et al.* Afferent lymph-derived T cells and DCs use different chemokine receptor CCR7-dependent routes for entry into the lymph node and intranodal migration. *Nat. Immunol.* **12,** 879–887 (2011).
31. MacDonald, A. S., Straw, A. D., Bauman, B. & Pearce, E. J. CD8- dendritic cell activation status plays an integral role in influencing Th2 response development. *J. Immunol.* **167,** 1982–1988 (2001).
32. Jankovic, D., Kullberg, M. C., Caspar, P. & Sher, A. Parasite-induced Th2 polarization is associated with down-regulated dendritic cell responsiveness to Th1 stimuli and a transient delay in T lymphocyte cycling. *J. Immunol.* **173,** 2419–2427 (2004).
33. Perrigoue, J. G. *et al.* MHC class II-dependent basophil-$CD4^+$ T cell interactions promote T(H)2 cytokine-dependent immunity. *Nat. Immunol.* **10,** 697–705 (2009).
34. Grencis, R. K., Humphreys, N. E. & Bancroft, A. J. Immunity to gastrointestinal nematodes: mechanisms and myths. *Immunol. Rev.* **260,** 183–205 (2014).
35. Cook, P. C. *et al.* A dominant role for the methyl-CpG-binding protein Mbd2 in controlling Th2 induction by dendritic cells. *Nat. Commun.* **6,** 1–11 (2015).
36. MacDonald, A. S. & Maizels, R. M. Alarming dendritic cells for Th2 induction. *J. Exp. Med.* **205,** 13–17 (2008).
37. Cerovic, V., Bain, C. C., Mowat, A. M. & Milling, S. W. F. Intestinal macrophages and dendritic cells: what's the difference? *Trends Immunol.* **35,** 270–277 (2014).
38. Connor, L. M. *et al.* Th2 responses are primed by skin dendritic cells with distinct transcriptional profiles. *J. Exp. Med.* **214,** 125–142 (2016).
39. Maldonado-López, R. *et al.* $CD8alpha^+$ and $CD8alpha^-$ subclasses of dendritic cells direct the development of distinct T helper cells *in vivo*. *J. Exp. Med.* **189,** 587–592 (1999).

40. Pulendran, B. *et al.* Distinct dendritic cell subsets differentially regulate the class of immune response *in vivo*. *Proc. Natl Acad. Sci. USA* **96,** 1036–1041 (1999).
41. Everts, B. *et al.* Migratory CD103$^+$ dendritic cells suppress helminth-driven type 2 immunity through constitutive expression of IL-12. *J. Exp. Med.* **213,** 35–51 (2016).
42. Jenkins, S. J., Perona-Wright, G., Worsley, A. G. F., Ishii, N. & MacDonald, A. S. Dendritic cell expression of OX40 ligand acts as a costimulatory, not polarizing, signal for optimal Th2 priming and memory induction *in vivo*. *J. Immunol.* **179,** 3515–3523 (2007).
43. Lambrecht, B. N. & Hammad, H. Lung dendritic cells in respiratory viral infection and asthma: from protection to immunopathology. *Annu. Rev. Immunol.* **30,** 243–270 (2012).
44. van Liempt, E. *et al. Schistosoma mansoni* soluble egg antigens are internalized by human dendritic cells through multiple C-type lectins and suppress TLR-induced dendritic cell activation. *Mol. Immunol.* **44,** 2605–2615 (2007).
45. Golec, D. P., Henao Caviedes, L. M. & Baldwin, T. A. RasGRP1 and RasGRP3 are required for efficient generation of early thymic progenitors. *J. Immunol.* **197,** 1743–1753 (2016).
46. Coughlin, J. J., Stang, S. L., Dower, N. A. & Stone, J. C. RasGRP1 and RasGRP3 regulate B cell proliferation by facilitating B cell receptor-Ras signaling. *J. Immunol.* **175,** 7179–7184 (2005).
47. Tang, S. *et al.* RasGRP3 limits Toll-like receptor-triggered inflammatory response in macrophages by activating Rap1 small GTPase. *Nat. Commun.* **5,** 4657 (2014).
48. Yashiro, T., Hara, M., Ogawa, H., Okumura, K. & Nishiyama, C. Critical role of transcription factor PU.1 in the function of the OX40L/TNFSF4 promoter in dendritic cells. *Sci. Rep.* **6,** 34825 (2016).
49. Kumamoto, Y. *et al.* CD301b$^+$ dermal dendritic cells drive T helper 2 cell-mediated immunity. *Immunity* **39,** 733–743 (2013).
50. van Rijt, L. S. *et al. In vivo* depletion of lung CD11c$^+$ dendritic cells during allergen challenge abrogates the characteristic features of asthma. *J. Exp. Med.* **201,** 981–991 (2005).
51. Lewkowich, I. P. *et al.* PD-L2 modulates asthma severity by directly decreasing dendritic cell IL-12 production. *Mucosal Immunol.* **6,** 728–739 (2013).
52. Bell, B. D. *et al.* The transcription factor STAT5 is critical in dendritic cells for the development of TH2 but not TH1 responses. *Nat. Immunol.* **14,** 364–371 (2013).
53. Vander Lugt, B. *et al.* Transcriptional programming of dendritic cells for enhanced MHC class II antigen presentation. *Nat. Immunol.* **15,** 161–167 (2014).
54. Bajaña, S., Roach, K., Turner, S., Paul, J. & Kovats, S. IRF4 promotes cutaneous dendritic cell migration to lymph nodes during homeostasis and inflammation. *J. Immunol.* **189,** 3368–3377 (2012).
55. Yrlid, U. *et al.* Regulation of intestinal dendritic cell migration and activation by plasmacytoid dendritic cells, TNF-alpha and type 1 IFNs after feeding a TLR7/8 ligand. *J. Immunol.* **176,** 5205–5212 (2006).
56. Flores-Langarica, A. *et al.* Systemic flagellin immunization stimulates mucosal CD103$^+$ dendritic cells and drives Foxp3$^+$ regulatory T cell and IgA responses in the mesenteric lymph node. *J. Immunol.* **189,** 5745–5754 (2012).
57. Kane, C. M., Jung, E. & Pearce, E. J. *Schistosoma mansoni* egg antigen-mediated modulation of Toll-like receptor (TLR)-induced activation occurs independently of TLR2, TLR4, and MyD88. *Infect. Immun.* **76,** 5754–5759 (2008).
58. Everts, B. *et al.* Omega-1, a glycoprotein secreted by *Schistosoma mansoni* eggs, drives Th2 responses. *J. Exp. Med.* **206,** 1673–1680 (2009).
59. Steinfelder, S. *et al.* The major component in schistosome eggs responsible for conditioning dendritic cells for Th2 polarization is a T2 ribonuclease (omega-1). *J. Exp. Med.* **206,** 1681–1690 (2009).
60. Veenbergen, S. *et al.* Colonic tolerance develops in the iliac lymph nodes and can be established independent of CD103(+) dendritic cells. *Mucosal Immunol.* **9,** 894–906 (2016).
61. Dalton, J. P., Day, S. R., Drew, A. C. & Brindley, P. J. A method for the isolation of schistosome eggs and miracidia free of contaminating host tissues. *Parasitology* **115**(Pt 1): 29–32 (1997).
62. Metsalu, T. & Vilo, J. ClustVis: a web tool for visualizing clustering of multivariate data using principal component analysis and heatmap. *Nucleic Acids Res.* **43,** W566–W570 (2015).

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Author contributions

J.U.M. and M.D. performed the experiments. W.W.A., A.S.M. and M.S.-F. provided reagents and with S.W.M. helped direct the study. J.U.M. conceived the project with S.W.M. and A.S.M., and wrote the manuscript with S.W.M.

Additional information

Supplementary Information accompanies this paper at http://www.nature.com/naturecommunications

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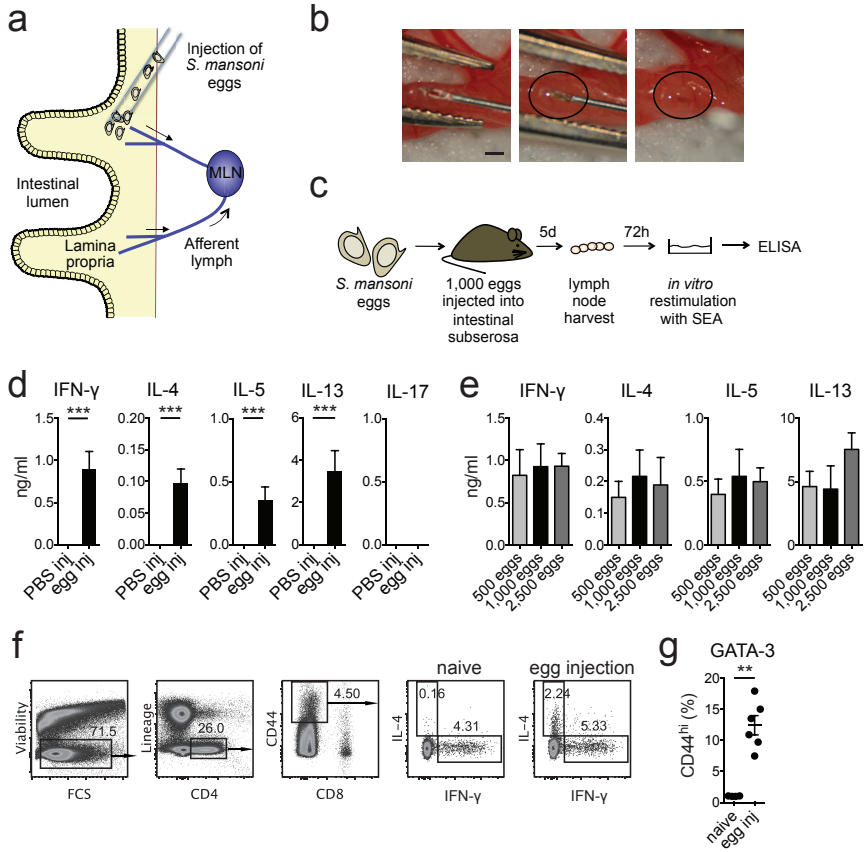
Supplemental information

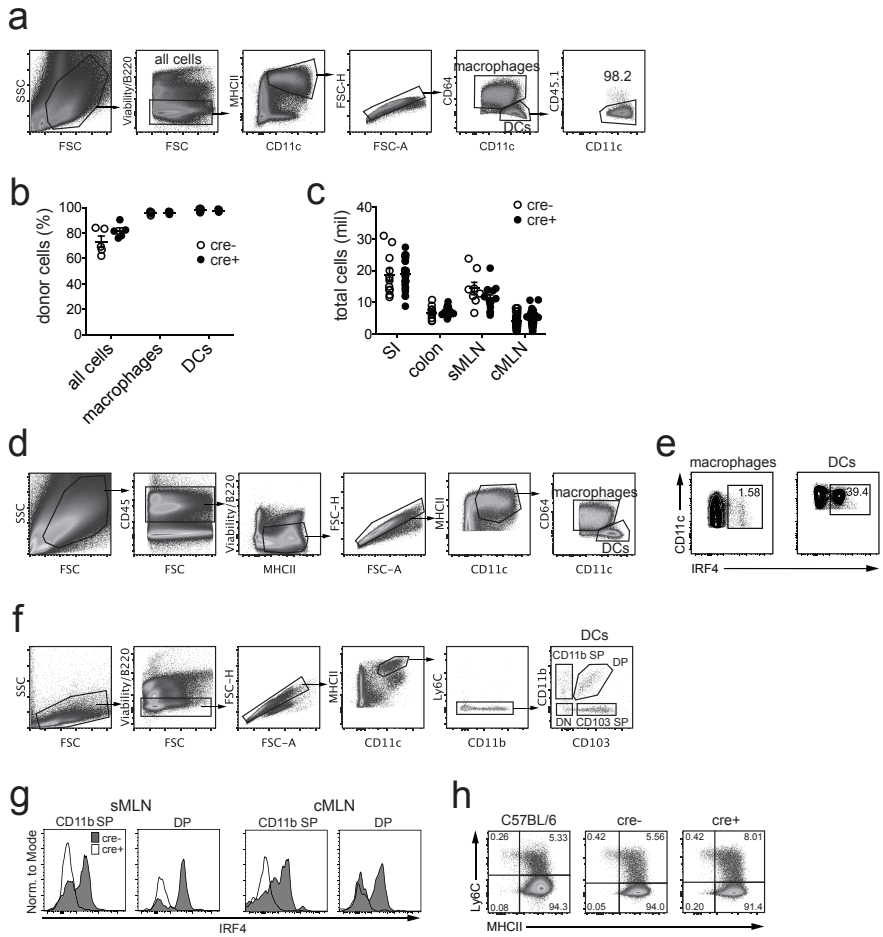
Different populations of CD11b^{+} dendritic cells drive Th2 responses in the small intestine and colon

Johannes U. Mayer, Mimoza Demiri, William W. Agace, Andrew S. MacDonald, Marcus Svensson-Frej, and Simon W. Milling



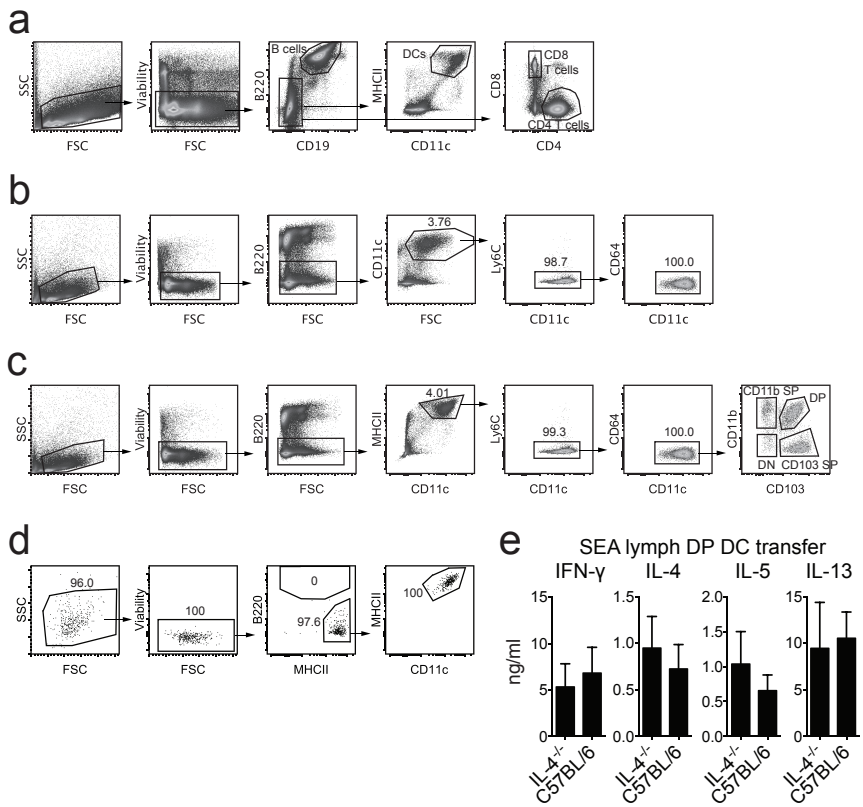
Supplementary Figure 1. Intestinal Th2 responses to *Schistosoma mansoni* eggs induce antigen specific IFN-γ and Th2 responses. (**a**) Schematic illustrating the injection of *S. mansoni* eggs into the intestine. (**b**) Photographs taken during the injection procedure into the murine small intestine. Insertion of the syringe into the subserosal layer (left panel), injection of the eggs (middle panel) and deposited eggs after the removal of the syringe (right panel) are shown. Scale bar corresponds to 1 mm. (**c**) Schematic illustrating the experimental set up for immunization. 1,000 *S. mansoni* eggs were injected into the subserosal layer of the intestine and resulting T cell responses were analyzed after 5 days by restimulating mesenteric lymph node (MLN) cells for an additional 3 days in the presence of schistosome egg antigen (SEA) and measuring the released cytokines by ELISA. (**d**) Cytokine responses of restimulated MLN cells from PBS injected (PBS inj) and egg injected (egg inj) mice (n=9 mice per group, in three independent experiments, mean ± SEM, Mann-Whitney *U* tests, ***P≤0.001). (**e**) Cytokine responses of restimulated MLN cells from egg injected mice, where 500, 1,000 or 2,500 eggs were injected (n=4-6 mice per group, in two

independent experiments, mean ± SEM, Kruskal Wallis tests (not significant)). (**f**) Corresponds to data shown in Fig. 1b. Representative gating strategy of MLN cells harvested from egg injected animals five days after immunization. $CD44^{hi}$ CD4 T cells were identified by flow cytometry and levels of IFN-γ, IL-4 and IL-13 cytokine production measured after PMA/ionomycin stimulation and compared to cells harvested from naive animals. (**g**) Five days after immunization with *S. mansoni* eggs (egg inj), MLNs were harvested, $CD44^{hi}$ CD4 T cells identified as in (**f**) and levels of the Th2 associated transcription factor GATA-3 measured by flow cytometry and compared to cells harvested from naive animals (n=6 mice per group, in two independent experiments, mean ± SEM, Mann-Whitney *U* tests, **P≤0.01).

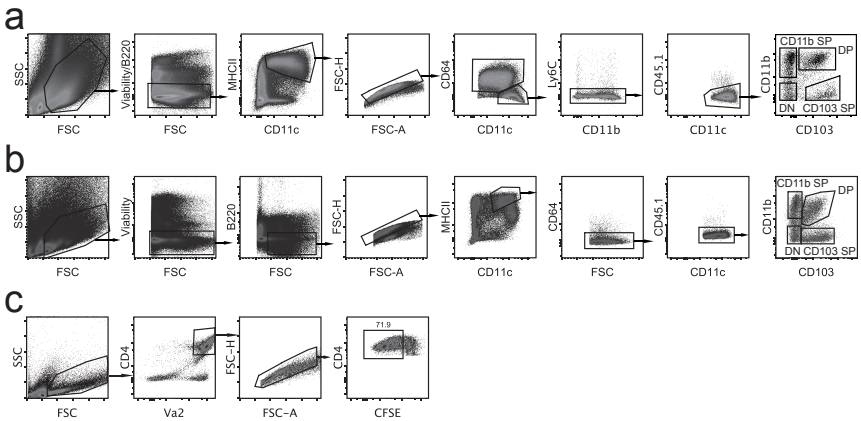


Supplementary Figure 2. Intestinal DCs and macrophages are completely reconstituted in IRF4$^{f/f}$ CD11c-cre positive and cre-negative bone marrow chimeric mice. (**a**) Representative gating strategy identifying small intestinal macrophages and dendritic cells (DC) from IRF4$^{f/f}$ cre-negative (cre-) bone marrow (BM) chimeric mice. (**b**) The percentage of reconstitution was measured in IRF4$^{f/f}$ CD11c-cre positive (cre+) or littermate cre- BM chimeric mice. Live cells, intestinal macrophages and DCs, as gated in (**a**), were analyzed (n=5 mice per group, in two independent experiments, mean ± SEM, Mann-Whitney *U* tests (not significant)). (**c**) Total cell numbers of enzymatically digested small intestines, colons and their respective draining lymph nodes were compared between cre+ or littermate cre- BM chimeras (n=8-20 mice per group, in more than three independent experiments, mean ± SEM, Mann-Whitney *U* tests (not significant)). (**d**) Corresponds to data shown in Fig. 2d. Representative gating strategy identifying small intestinal macrophages and DCs from C57BL/6 mice. (**e**) IRF4 expression by intestinal macrophages and DCs, as gated in (**d**), analyzed by flow cytometry in

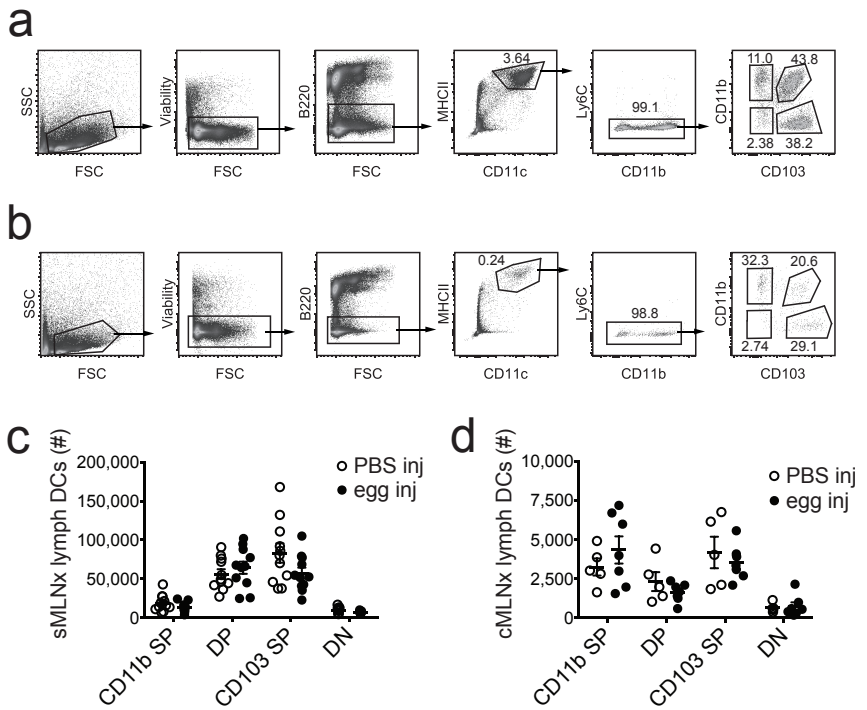
C57BL/6 mice, and gated on the appropriate isotype control. (**f**) Representative gating strategy of enzymatically digested small intestinal mesenteric lymph node (sMLN) cells of cre- animals to identify migratory DCs and their CD11b and CD103 expressing populations. (**g**) Representative IRF4 expression of sMLN and colonic MLN (cMLN) $CD11b^{+}CD103^{-}$ single positive (CD11b SP) and $CD11b^{+}CD103^{+}$ double positive (DP) DCs from cre+ and cre- animals, as gated in (**f**). (**h**) Representative FACS plots depicting $Ly6C^{hi}$ monocytes and mature $Ly6C^{-}$ $MHCII^{+}$ macrophages in C57BL/6 mice and cre+ and cre- BM chimeras.



Supplementary Figure 3. Lymph migrating DCs can be identified in intestinal draining lymph and can induce Th2 responses independent of their expression of IL-4. (**a**) Corresponds to data shown in Fig. 2a,b. Representative gating strategy of lymph migrating CD4 and CD8 T cells, B cells and dendritic cells (DCs) in intestinal draining lymph collected from mesenteric lymphadenectomized (MLNx) C57BL/6 animals for 18 hours. (**b**) Corresponds to data shown in Fig. 2c. Representative gating strategy of lymph migrating CD11c^{hi} CD64^{-} B220^{-} DCs from MHCII$^{-/-}$ MLNx mice. (**c**) Corresponds to data shown in Fig. 2d and 3a. Representative gating strategy of lymph migrating MHCII^{hi} CD64^{-} B220^{-} CD11c^{hi} DCs from MLNx mice, identifying the four CD11b and CD103 expressing DC populations. (**d**) Representative sort purity of lymph migrating MHCII^{hi} B220^{-} CD11c^{hi} DCs from MLNx mice. (**e**) 30,000 DP DCs from the lymph of C57BL/6 or IL-4$^{-/-}$ MLNx mice were incubated with schistosome egg antigen (SEA) for 18 hours *in vitro* and transferred under the mesenteric lymph node (MLN) capsule of wild type recipient animals. Antigen specific T cell responses in the injected MLNs were measured 5 days after cell transfer by cytokine analysis of *in vitro* SEA restimulation cultures (n=6 mice per group, in three independent experiments, mean ± SEM, Mann-Whitney *U* tests (not significant)).



Supplementary Figure 4. CD11b and CD103 expressing DC populations can be identified in the intestine and lymph nodes of IRF4$^{f/f}$ CD11c-cre positive and cre-negative bone marrow chimeric mice. (**a**) Corresponds to data shown in Fig. 4a-d. Representative gating strategy for enzymatically digested small intestinal lamina propria cells of IRF4$^{f/f}$ cre-negative (cre-) bone marrow (BM) chimeric mice to identify CD11b and CD103 expressing dendritic cell (DC) populations. Equivalent analysis was performed in IRF4$^{f/f}$ CD11c-cre positive (cre+) animals and the colon. (**b**) Corresponds to data shown in Fig. 4e,f,h. Representative gating strategy of enzymatically digested small intestinal mesenteric lymph node (sMLN) cells of cre- chimeric mice to identify migratory DC populations. Equivalent analysis was performed in cre+ animals and in the colon draining colonic MLNs. (**c**) Corresponds to data shown in Fig. 4g. Representative gating strategy identifying proliferated OT-II CD4 T cells, by their diluted concentration of CFSE, after 3 days of coculture with OVA-pulsed cre+ or cre- MLN DC populations.



Supplementary Figure 5. CD11b and CD103 expressing dendritic cell populations can be identified in small intestinal and colonic draining lymph. (**a**) Corresponds to data shown in Fig. 5a-c. Representative gating strategy of lymph migrating dendritic cell (DC) populations from small intestinal mesenteric lymphadenectomized (sMLNx) C57BL/6 animals that specifically drain from the small intestine. (**b**) Corresponds to data shown in Fig. 5e,f. Representative gating strategy of colon draining lymph migrating DC populations from colonic mesenteric lymphadenectomized (cMLNx) mice. (**c**) Total number of lymph migrating DC populations, as gated in (**a**), 18 hours after the injection of PBS (PBS inj) or *Schistosoma mansoni* eggs (egg inj) into the small intestine of sMLNx animals (n=10-11 mice per group, in three independent experiments, mean ± SEM, Mann-Whitney *U* tests (not significant)). (**d**) Total number of lymph migrating DC populations, as gated in (**b**), 18 hours after the injection of PBS or *S. mansoni* eggs into the colon of cMLNx animals (n=6-7 mice per group, in three independent experiments, mean ± SEM, Mann-Whitney *U* tests (not significant)).

Paper III

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ORIGINAL ARTICLE

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Distinct DC subsets regulate adaptive Th1 and 2 responses during *Trichuris muris* infection

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Summary

Low- and high-dose infections with the murine large intestinal nematode *Trichuris muris* are associated with induction of adaptive Th1 and Th2 responses, respectively, in mesenteric lymph nodes (MLN). Classical dendritic cells (cDC) accumulate in the large intestinal mucosa and MLN upon *T. muris* infection, yet their role in driving adaptive responses to infection remains largely unknown. We performed low- and high-dose *T. muris* infections of mice deficient in defined cDC subsets to investigate their role in induction of adaptive immune responses. Mice lacking IRF4-dependent cDC failed to clear a high-dose infection and displayed impaired Th2 responses. Conversely, mice lacking IRF8-dependent cDC cleared a low-dose infection and displayed an impaired Th1 response while increased production of Th2 cytokines. Finally, mice lacking both IRF4- and IRF8-dependent cDC were able to generate a Th2 response and clear a low-dose infection. Collectively, these results suggest that IRF4- and IRF8-dependent cDC act antagonistically during *T. muris* infection, and demonstrate that intestinal Th2 responses can be generated towards *T. muris* in the absence of IRF4-dependent cDC.

KEYWORDS
dendritic cells, infection, intestine, mesenteric lymph nodes, parasite, T helper cells

1 | BACKGROUND

Trichuris muris (T. muris) is the murine counterpart to the human parasitic nematode *T. trichiura* that infects almost 500 million people in tropical regions.[1] *T. muris* larvae invade the caecal epithelium upon hatching and give rise to acute or chronic infections depending on the nature of the adaptive immune response. Hence, chronic infections, defined by the presence of worms at day 35 post-infection (p.i.), are tightly associated with Th1 responses, while clearance of infection prior to the worms reaching fecundity is dependent on generation of a Th2 response.[2] This balance, and the subsequent outcome of the infection, is influenced by differences in genetic background, infection dose and gender.[3] This is particularly evident in C57BL/6 mice, where a high-dose infection stimulates a Th2 response and clearance, while a low-dose infection results in a Th1 response and chronicity.[2] Production of the Th1-associated cytokines IL-12 and IFN-γ is critical for establishment of chronic *T. muris* infection. Thus, neutralization of IFN-γ in normally susceptible AKR mice results in expulsion of *T. muris* worms,[4] while resistant mice become susceptible and display increased worm burden upon IL-12 administration.[5]

Classical dendritic cells (cDC) play a central role in the induction and regulation of adaptive T helper cell responses. The intestinal mucosa contains several cDC subsets that are subdivided based on their expression of the integrins CD11b and CD103 into CD103+ CD11b− (CD103 SP), CD103+ CD11b+ (DP) and CD103-CD11b+ (CD11b SP) cDC.[6] The development of intestinal CD103 SP cDC is dependent on the transcription factors IRF8, BATF3 and ID2,[7–10] while lack of IRF4 or Notch2 results in a loss of DP cDC and reduced numbers of CD11b SP cDC in intestinal-draining MLN.[11–14] We have previously demonstrated that mice lacking IRF8-dependent cDC have dramatically reduced numbers of intestinal Th1 cells and fail to support Th1 responses to immunized protein antigen in MLN.[7] In contrast, mice deficient in IRF4-dependent cDC have reduced numbers of intestinal Th17 cells[11] and fail to mount Th2

responses to helminth infection, or immunization with allergens or parasite-derived antigens, in the respiratory tract, skin and intestinal mucosa.[15–18] While cDC accumulate in the intestinal mucosa[19] and MLN of *T. muris*-infected mice,[20] the role of intestinal cDC subsets in the induction of T cell responses, and the interplay between these subsets in determining infection outcome, remains limited. In this study, we assessed the role of MLN cDC subsets in high- and low-dose *T. muris* infection and the potential synergistic or antagonistic role these subsets play during infection.

2 | MATERIALS AND METHODS

2.1 | Mice

C57BL/6, CD11c-cre.*IRF4*$^{fl/-\text{ or }fl/fl}$,[11] CD11c-cre.*IRF8*$^{fl/-\text{ or }fl/fl}$[7] and CD11c-cre.*IRF4*$^{fl/-\text{ or }fl/fl}$.*IRF8*$^{fl/fl}$ were generated, bred and maintained at the Biomedical Center (BMC), Lund University, or at the quarantine at Medicon Village, Lund University, as previously described.[7,11] Cre-negative *IRF4*$^{fl/fl}$ or *IRF4*$^{fl/-}$ and *IRF8*$^{fl/fl}$ littermates were used as controls. Cre-expressing litters with either flox/flox or flox/- genotype displayed the same phenotype and were combined for all experiments. For generation of bone marrow (BM) chimeras, irradiated recipient mice (CD45.1+; 900 cGy) were reconstituted with BM cells (~1 × 10^6 cells) from indicated donor mice (CD45.2+) by i.v. injection within 24 hours of irradiation. Infection with *T. muris* eggs was performed 7-8 weeks after reconstitution. The donor cells constituted >95% of the cells within the DC compartment in both groups of chimeric mice (Cre- mice: average 97,5% ± 2,1% [n = 7]; Cre+ mice: average 99,8% ± 0,2% [n = 5]) (Fig. S2). All experimental procedures were performed in strict accordance with ethical permission granted by the Malmö/Lund Animal Ethics Committee.

2.2 | *Trichuris muris*

Mice were infected by oral gavage with approximately 30-40 or 250-300 infective *T. muris* eggs (E isolate) for low- and high-dose infections, respectively. Parasite-derived excretory/secretory (E/S) antigens were collected from secreting worms after 4 hours.[21] To assess worm burden, large intestinal and caecal tissue were carefully scraped and worms counted under a microscope.

2.3 | Cell isolation, in vitro stimulations and cytokine analyses

MLN cells were isolated according to standard procedure.[7] For E/S antigen re-stimulation, ~1 × 10^6 MLN cells were suspended in 100 μL RPMI1640 supplemented with 10% foetal calf serum (Sigma-Aldrich, Stockholm, Sweden), 1 mM sodium pyruvate (Gibco), 10 mM HEPES, 100 U/mL penicillin and 100 μg/mL streptomycin, and 50 μM 2-mercaptoethanol (Gibco)), and cultured with 50 μg/mL E/S antigens for 48 hours at 37°C, 5% CO_2, in a 96-well U-bottomed plate. Cell-free supernatants were subjected to cytokine analysis using cytometric bead arrays (BD Biosciences), according to manufacturer's instructions. Samples were acquired on a BD Accuri C6 Flow cytometer Sampler (BD Biosciences) and data analysed with FCAP Array v3.0 (SoftFlow Inc.).

For analysis of intracellular cytokines, MLN cells were stimulated in a 96-well plate with PMA (Sigma-Aldrich; 50 ng/mL) and ionomycin (Sigma-Aldrich; 500 ng/mL). Brefeldin A (Sigma-Aldrich; 10 μg/mL) and monensin (Sigma-Aldrich; 2 μg/mL) were added after 1 hour of culture, and the cells harvested three hours later, followed by flow cytometry analysis.

2.4 | Flow cytometry

Cells were stained for flow cytometry analysis of surface marker expression and intracellular cytokine production according to standard procedures. Briefly, cells were surface-stained with indicated antibodies (Table S1) at 4°C for 20 minutes. Intracellular staining was performed on fixed and permeabilized cells at room temperature for 45 minutes, according to manufacturer's instructions (FoxP3 Transcription factor staining buffer set; eBioscience). Dead cells were excluded by staining with propidium iodide or Violet Live/Dead (Life Technologies), according to manufacturer's instructions. Data were acquired on a BD LSR II flow cytometer and analysed using FlowJo software v9.6.2 (Tree Star).

2.5 | Statistical analyses

Statistical analyses were performed using GraphPad Prism software (GraphPad); significance was determined with Mann-Whitney U test or Kruskal-Wallis test followed by Dunns's post-test (as indicated in Figure legends).

3 | RESULTS

3.1 | Investigation of MLN cDC composition during *Trichuris muris* infection

To investigate the MLN cDC subset composition during acute and chronic *T. muris* infection, C57BL/6 mice were infected with a high (250-300) and low (30-40) egg dose, respectively. Analysis of the cDC subset composition in the MLN was performed at day 7 p.i., as previous publications have demonstrated that *T. muris*-specific cytokine responses can be detected in the MLN from approximately day 10 p.i.[22,23] cDC in the MLN can be subdivided into MHC-II^{hi} and MHC-II^{int} cells, with the former representing intestinal-derived migratory cDC, and the latter lymph node-resident cDC.[24] Migratory MHC-II^{hi} MLN cDC consist of CD103 SP, DP and CD11b SP cDC, while resident MHC-II^{int} MLN cDC can be subdivided into CD11b+ and CD11b- cells (gating strategy in Fig. S1;[11]). Both high- and low-dose *T. muris* infection led to a significant increase in MLN cellularity at day 7 p.i. (Figure 1A). Total cDC (CD11c+ MHC-II+ CD64-) numbers also increased in the MLN during high, but not low-dose infection (Figure 1B), which included an increase in both MHC-II^{hi} and MHC-II^{int} cDC (Figure 1B). After high-dose infection, there was a significant increase in the numbers of DP and CD11b SP, but not

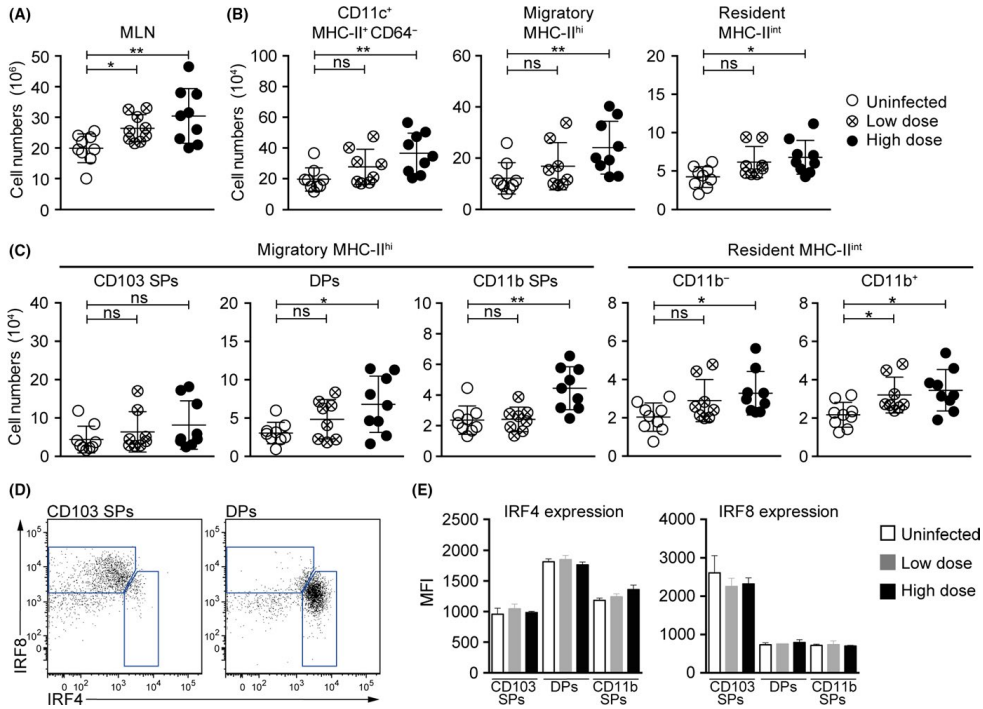

FIGURE 1 Characterization of MLN cDC subset composition during *Trichuris muris* infection. Analysis of cDC subsets in the MLN, 7 d after high (filled circles) or low-dose (crossed circles) infection with *T. muris*, and uninfected controls (open circles). (A-C) Total number of (A) MLN cells, (B) total CD11c+ MHC-II+ CD64- cDC, and migratory and resident cDC, and (C) cDC subsets, identified as described in Fig. S1. Graphs depict mean ± standard deviation (SD). Data are from three independent experiments (n = 8-9). (D and E) Flow cytometry analysis of intracellular IRF4 and IRF8 expression by MLN cDC subsets. (D) Representative flow cytometry-plots of IRF4 and IRF8 expression on CD103 SP and DP MLN cDC isolated from uninfected mice, gated as described in Fig. S1. (E) Quantification of the mean fluorescence intensity (MFI) of IRF4 and IRF8 in uninfected, and low- or high-dose-infected mice, respectively. The graphs depict the mean ± SD MFI from one representative experiment of two performed (n = 3 for each group and condition). $^{**}P < .01$, $^{*}P < .05$, ns—not significant. (Kruskal-Wallis, followed by Dunns's multiple comparisons test)

CD103 SP MHC-II^{hi} cDC, as well as CD11b+ and CD11b- MHC-II^{int} cDC (Figure 1C). Taken together, these data suggest that cDC accumulate in MLN at day 7 p.i. preferentially during high-, but not low-, dose infection with *T. muris*.

To investigate whether IRF4 and IRF8 expression was altered in cDC during infection, we analysed intracellular expression of IRF4 and IRF8 in MLN cDC subsets at day 7 after high- and low-dose *T. muris* infection. Consistent with previous reports,[25] IRF4 and IRF8 were expressed selectively by DP and CD103 SP cDC, respectively, in uninfected mice (Figure 1D,E), while CD11b SP cDC expressed low levels of IRF4 but not IRF8 (Figure 1E). Furthermore, we detected no alterations in IRF4 and IRF8 expression levels following high or low-dose infection (Figure 1E). Therefore, we conclude that MLN cDC subsets maintain their selective pattern of IRF4 and IRF8 expression during *T. muris* infection.

3.2 | IRF4-dependent cDC are critical for induction of adaptive Th2 responses during *Trichuris muris* infection

Mice with a selective deletion of IRF4 in the CD11c-compartment (CD11c-cre.*IRF4*$^{fl/-\text{ or } fl/fl}$ mice) have a strong reduction of migratory DP cDC and a twofold reduction of migratory CD11b SP cDC in the MLN.[11] We have recently shown that CD11c-cre.*IRF4*$^{fl/fl}$ mice infected with a high-dose of *T. muris* become chronically infected and display reduced Th2 responses at day 35 p.i.[18] To further examine the role of IRF4-dependent cDC in response to *T. muris* infection at a time point when the infection is still ongoing in both mouse strains and coinciding with the peak cytokine response, CD11c-cre.*IRF4*$^{fl/-\text{ or } fl/fl}$ and *IRF4*$^{fl/-\text{ or } fl/fl}$ control mice were infected with a high egg dose followed by analysis at day 21 p.i. Interestingly, total

MLN cellularity, total numbers of cDC and total numbers of MHC-II^{hi} and MHC-II^{int} cDC were higher in CD11c-cre.*IRF4*$^{fl/-\text{ or } fl/fl}$ mice compared with control mice (Figure 2A). As previously observed in the steady state,[11] migratory DP cDC were absent in the MLN of *T. muris*-infected CD11c-cre.*IRF4*$^{fl/-\text{ or } fl/fl}$ mice (Figure 2B and C). The increase in MHC-II^{hi} cDC in the MLN of CD11c-cre.*IRF4*$^{fl/-\text{ or } fl/fl}$ mice was due to an enhanced accumulation of migratory CD11b SP and CD103 SP cDC (Figure 2C). In addition, both CD11b^{+} and

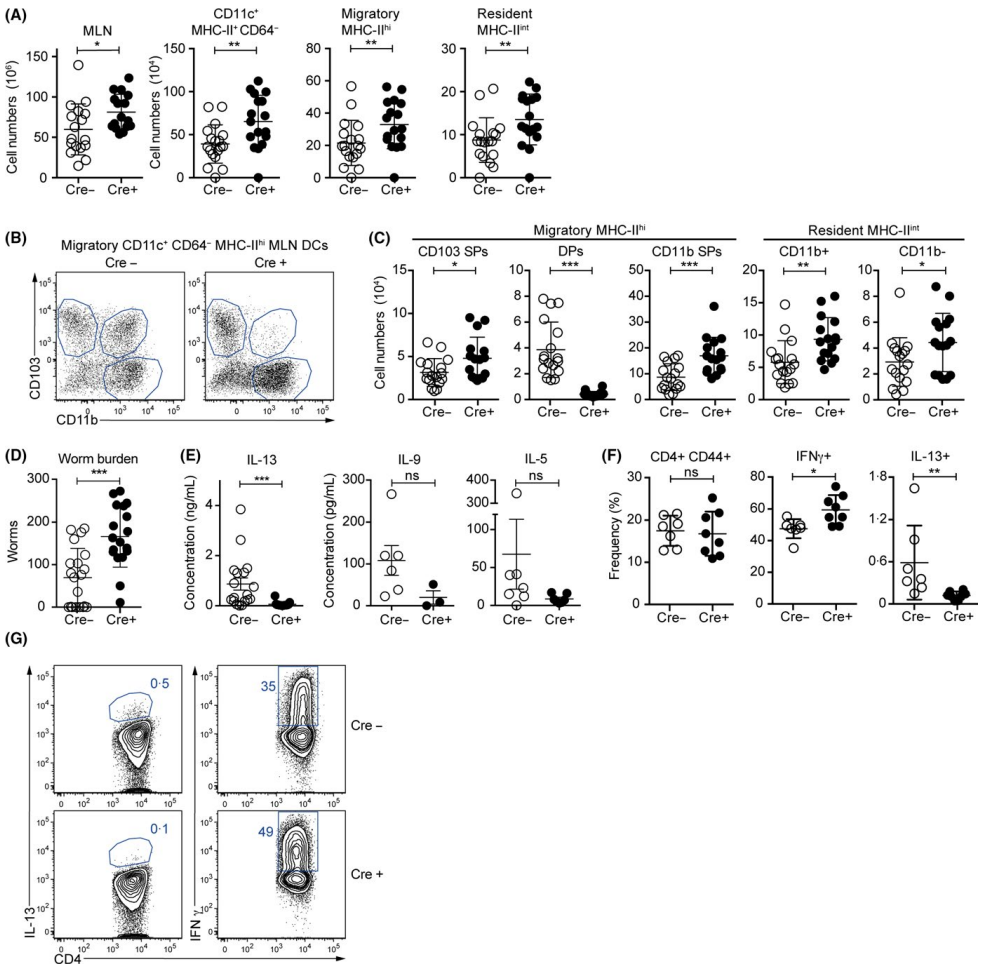

FIGURE 2 IRF4-dependent cDC are critical for induction of Th2 cells and worm expulsion following high-dose infection with *Trichuris muris*. (A-C) Analysis of MLN cDC subsets in *IRF4*$^{fl/-\text{ or } fl/fl}$ (cre-; open circles) and CD11c-cre.*IRF4*$^{fl/-\text{ or } fl/fl}$ (cre+; filled circles) mice at day 21 after *T. muris* infection. (A) Total numbers of MLN cells, CD11c+ MHC-II+ CD64- cDC, and migratory and resident cDC. (B) Representative flow cytometry analysis of migratory MLN cDC subsets in infected mice. (C) Quantification of cDC subsets in the MLN of infected mice. Graphs depict mean ± SD and are pooled from three individual experiments (n = 16-17). (D) Large intestinal worm burden at day 21 p.i. (n = 16-17). (E-G) Cytokine production by MLN cells isolated from infected mice at day 21 p.i. after in vitro re-stimulation with (E) *T. muris*-derived antigens and (F-G) PMA and ionomycin. (E) Graphs depict the mean ± SD level of indicated cytokines in the culture supernatants and are from 1 to 3 experiments (n = 3-18). (F) Frequencies and (G) representative flow cytometry-plots of IL-13+ and IFN-γ+ cells, pregated on TCRb+ CD4+ and CD44+ cells in the MLN of infected mice. Graphs depict the mean ± SD from two independent experiments (n = 7-8). ***P < .001, **P < .01, *P < .05, ns—not significant (Mann-Whitney U test)

CD11b^{-} MHC-II^{int} cDC subsets were increased in CD11c-cre.*IRF4*$^{fl/- \text{ or } fl/fl}$ mice (Figure 2C). Furthermore, CD11c-cre.*IRF4*$^{fl/- \text{ or } fl/fl}$ mice retained a significantly higher number of worms compared to control mice (Figure 2D). Given the time point of analysis (day 21 p.i.), these results suggest ongoing but not completed worm expulsion in control, but not CD11c-cre.*IRF4*$^{fl/- \text{ or } fl/fl}$ mice. In line with these results, we observed reduced IL-13 production by MLN cells from CD11c-cre.*IRF4*$^{fl/- \text{ or } fl/fl}$ mice after re-stimulation with *T. muris*-derived E/S antigens, with similar trends for IL-9 and IL-5 (Figure 2E). Furthermore, while the frequency of CD44+ cells in the CD4+ MLN T cell population did not differ between the two groups (Figure 2F), CD11c-cre.*IRF4*$^{fl/- \text{ or } fl/fl}$ mice contained a significantly decreased proportion of IL-13-producing CD4+ CD44+ T cells and an increased frequency of IFN-γ+ CD4+ CD44+ T cells after polyclonal re-stimulation (Figure 2F,G). Taken together, these results demonstrate that CD11c-cre.*IRF4*$^{fl/- \text{ or } fl/fl}$ mice have impaired capacity to mediate worm expulsion and induce Th2 cells in the MLN following high-dose *T. muris* infection. Furthermore, the increased number of IFN-γ-producing CD4+ cells in CD11c-cre.*IRF4*$^{fl/- \text{ or } fl/fl}$ mice may indicate that IRF4-dependent cDC, potentially through their ability to drive Th2 responses, curtail the Th1 response to *T. muris* infection.

3.3 | CD11c-cre.*IRF8*$^{fl/- \text{ or } fl/fl}$ mice infected with a low dose of *Trichuris muris* eggs expel worms and display an increased Th2 response

We recently demonstrated that mice that lack IRF8-dependent cDC, in contrast to wild-type control mice, are able to clear a low-dose *T. muris* infection.[7] Given the importance of Th1 cells for the establishment of chronic *T. muris* infection,[2,5] we therefore assessed the generation of Th1 cells in CD11c-cre.*IRF8*$^{fl/- \text{ or } fl/fl}$ mice following low-dose infection. MLN cellularity, total numbers of cDC, and MHC-II^{hi} and MHC-II^{int} cDC, did not differ between CD11c-cre.*IRF8*$^{fl/- \text{ or } fl/fl}$ and control *IRF8*$^{fl/fl}$ mice at day 18 p.i. (Figure 3A). Consistent with previous results in the steady state,[7] infected CD11c-cre.*IRF8*$^{fl/- \text{ or } fl/fl}$ mice completely lacked migratory CD103 SP cDC and had an increased number of resident CD11b+ cDC in the MLN (Figure 3B,C), while the total numbers of migratory DP and CD11b SP cDC, and resident CD11b- cDC, remained unchanged (Figure 3C). Furthermore, while worms were still present in control mice, CD11c-cre.*IRF8*$^{fl/- \text{ or } fl/fl}$ mice showed near complete worm expulsion already by day 18 p.i. (Figure 3D), consistent with our previous analysis at day 35 p.i.[7] To determine whether CD11c-cre.*IRF8*$^{fl/- \text{ or } fl/fl}$ mice display an altered immune response to *T. muris* infection, we assessed production of Th1 and Th2 cytokines by MLN cells. The numbers of IFN-γ-producing CD4+ CD44+ T cells after polyclonal stimulation at day 18 and 35 p.i. were dramatically reduced in CD11c-cre.*IRF8*$^{fl/fl}$ mice compared with *IRF8*$^{fl/fl}$ mice (Figure 3E). Strikingly, low-dose infection induced a strong Th2 response in CD11c-cre.*IRF8*$^{fl/fl}$, but not in control *IRF8*$^{fl/fl}$ mice, as evidenced by marked elevation in IL-4, IL-5, IL-9 and IL-13 levels in MLN cell suspensions re-stimulated with E/S antigens (Figure 3F). Collectively, these results suggest that IRF8-dependent cDC may serve to limit *T. muris*-specific Th2 responses during low-dose infection, and that their absence results in Th2-mediated worm expulsion.

3.4 | CD11c-cre.*IRF4*$^{fl/fl}$.*IRF8*$^{fl/fl}$ BM chimeric mice infected with a low dose of *Trichuris muris* expel worms and display an elevated Th2 response

Given the key role of IRF4- and IRF8-dependent cDC in driving adaptive Th2 and Th1 responses to *T. muris*, respectively, and our results suggesting that these subsets antagonize one another's function during *T. muris* infection, we next assessed how mice lacking both IRF4 and IRF8 in their CD11c-compartment would respond to a low-dose *T. muris* infection. In preliminary experiments, we first examined the MLN cDC composition of CD11c-cre.*IRF4*$^{fl/- \text{ or } fl/fl}$.*IRF8*$^{fl/fl}$ and littermate control (*IRF4*$^{fl/- \text{ or } fl/fl}$.*IRF8*$^{fl/fl}$) mice. The total number of MLN cells was higher in uninfected CD11c-cre.*IRF4*$^{fl/- \text{ or } fl/fl}$.*IRF8*$^{fl/fl}$ mice compared with littermate controls; however, no differences in MLN cell number were observed at day 21 p.i. (Fig. S2A,B), while total cDC numbers were similar between CD11c-cre.*IRF4*$^{fl/- \text{ or } fl/fl}$.*IRF8*$^{fl/fl}$ and littermate controls in steady state and at day 21 p.i (Fig. S2A,B). In contrast, migratory MHC-II^{hi} MLN cDC were strongly decreased and lymph node-resident MHC-II^{int} cDC increased in both infected and uninfected CD11c-cre.*IRF4*$^{fl/- \text{ or } fl/fl}$.*IRF8*$^{fl/fl}$ mice (Fig. S2B,C). A decrease in migratory cDC numbers was observed for both CD103 SP and DP cDC, while CD11b SP cDC were increased in uninfected mice but reduced after infection (Fig. S2D,E). Within the lymph node-resident MHC-II^{int} cDC population, there was an increased number of CD11b+ cDC (Fig. S2B,E). Strikingly, despite the absence of both IRF4- and IRF8-dependent cDC, CD11c-cre.*IRF4*$^{fl/- \text{ or } fl/fl}$.*IRF8*$^{fl/fl}$ mice had expelled *T. muris* larvae already at day 21 p.i. (Fig. S2F).

As insufficient numbers of CD11c-cre.*IRF4*$^{fl/fl}$.*IRF8*$^{fl/fl}$ mice were available for further analyses, due to major breeding issues, and to further extend the preliminary analyses in CD11c-cre.*IRF4*$^{fl/- \text{ or } fl/fl}$.*IRF8*$^{fl/fl}$ mice, we next assessed the nature of adaptive immune responses to *T. muris* infection in the absence of IRF4- and IRF8-dependent cDC, by generating BM chimeric CD11c-cre.*IRF4*$^{fl/fl}$.*IRF8*$^{fl/fl}$ and control *IRF4*$^{fl/fl}$.*IRF8*$^{fl/fl}$ mice. While the total number of MLN cells and cDC was similar in both groups of BM chimeric mice upon analysis at day 21 p.i. (Figure 4A,B), CD11c-cre.*IRF4*$^{fl/fl}$.*IRF8*$^{fl/fl}$ mice had a marked decrease in the number of migratory cDC (Figure 4C), consistent with the observation in infected and uninfected CD11c-cre.*IRF4*$^{fl/- \text{ or } fl/fl}$.*IRF8*$^{fl/fl}$ mice (Fig. S2C). Furthermore, while the total number of resident cDC was similar between the groups (Figure 4C), the proportion of CD11b+ cells within this population was increased in CD11c-cre.*IRF4*$^{fl/fl}$.*IRF8*$^{fl/fl}$ BM chimeric mice (Figure 4D). Moreover, chimeric CD11c-cre.*IRF4*$^{fl/fl}$.*IRF8*$^{fl/fl}$ mice, but not control *IRF4*$^{fl/fl}$.*IRF8*$^{fl/fl}$ BM chimeras, were capable of mediating worm expulsion by day 21 p.i. (Figure 4E). To assess the nature of the immune response to infection, we analysed Th1 and Th2 cytokine production by MLN cells. In contrast to control BM chimeras, E/S antigen-stimulation of MLN cells from CD11c-cre.*IRF4*$^{fl/fl}$.*IRF8*$^{fl/fl}$ BM chimeras resulted in Th2 cytokine production (Figure 4F). In contrast, IFN-γ-producing CD4+ CD44+ T cells were readily detected in the MLN of *IRF4*$^{fl/fl}$.*IRF8*$^{fl/fl}$, but

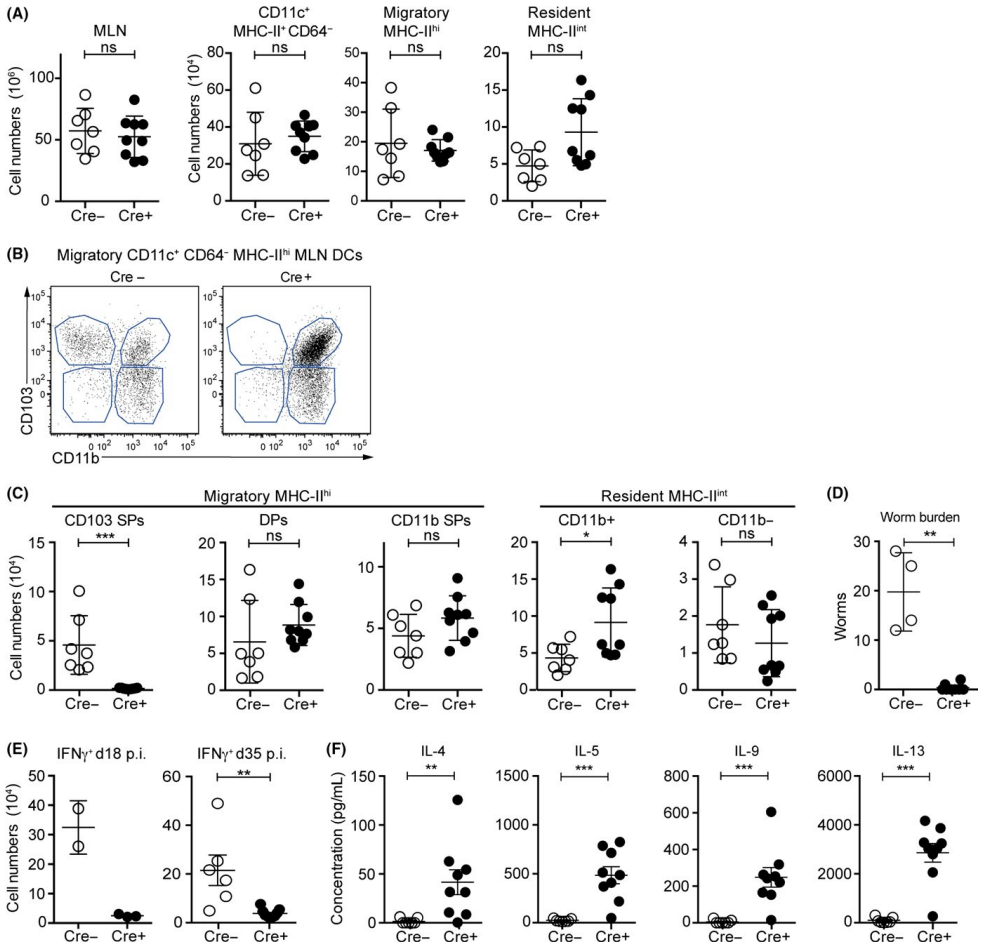

FIGURE 3 Mice that lack IRF8-dependent cDC fail to induce Th1 cells and mediate chronic infection following low-dose infection with *Trichuris muris* (A-C) Analysis of MLN cDC subsets in *IRF8*$^{fl/fl}$ (cre-; open circles) and CD11c-cre.*IRF8*$^{fl/-\text{ or }fl/fl}$ (cre+; filled circles) mice at day 18 after low-dose *T. muris* infection. (A) Total numbers of MLN cells, CD11c+ MHC-II+ CD64- cDC, and migratory and resident cDC, respectively. (B) Representative flow cytometry analysis of migratory MLN cDC subsets of infected mice. (C) Quantification of cDC subsets in the MLN of infected mice. Graphs depict mean ± SD and are pooled from two individual experiments (n = 4-8). (D) Large intestinal worm burden at day 18 p.i., pooled from two independent experiments (n = 4-8). (E and F) Analysis of cytokine production in the MLN of infected mice, following in vitro re-stimulation with (E) PMA and ionomycin (F) or *T. muris*-derived E/S antigens. (E) Mean ± SD number of IFN-γ+ T cells, pregated on CD4+ CD44+ cells, in the MLN of infected mice at day 18 and 35 p.i. Data are from one (day 18 p.i.; n = 2-3) and two (day 35p.i.; n = 6-9) experiments. (F) Graphs depict the mean ± SD level of indicated cytokines in the culture supernatants, assessed at day 18p.i., from two independent experiments (n = 7-9), ***P < .001, **P < .01, ns—not significant (Mann-Whitney U test)

not in CD11c-cre.*IRF4*$^{fl/fl}$.*IRF8*$^{fl/fl}$ BM chimeras (Figure 4G). Thus, despite previous studies suggesting a critical role for IRF4-dependent cDC in the induction of Th2 responses, additional APC subsets appear capable of driving Th2 immunity to *T. muris* infection in mice with a combined deficiency in IRF4- and IRF8-dependent cDC.

4 | DISCUSSION

Adaptive immune responses to infection with the large intestinal nematode *T. muris* can be polarized into either Th1 or Th2 responses, depending on the infection dose and genetic background of the mice.[26]

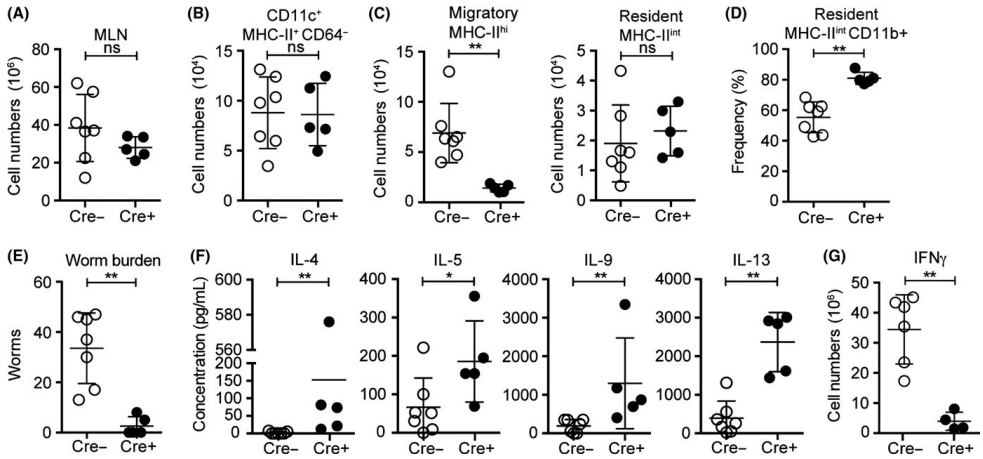

FIGURE 4 CD11c-cre.*IRF4*$^{fl/fl}$.*IRF8*$^{fl/fl}$ BM chimeric mice induce a Th2 response and expel worms upon low-dose infection with *Trichuris muris*. Low-dose *T. muris* infection of *IRF4*$^{fl/fl}$.*IRF8*$^{fl/fl}$ (cre-; open circles) and CD11c-cre. *IRF4*$^{fl/fl}$.*IRF8*$^{fl/fl}$ BM chimeric mice (cre+; filled circles). Analysis of (A) total MLN cells, (B) CD11c+ MHC-II+ CD64- MLN cDC, (C) migratory and resident MLN cDC and (D) CD11b+ resident MLN cDC at day 21 after low-dose *T. muris* infection. (E) Large intestinal worm burden at day 21 p.i. (F) Production of indicated cytokines from single-cell suspensions of MLN of cre- and cre+ BM chimeric mice after in vitro re-stimulation with *T. muris*-derived E/S antigens. (G) Number of IFN-γ+ T cells, pregated on CD4+ CD44+ MLN cells, from infected cre- and cre+ BM chimeric mice following re-stimulation with PMA and ionomycin. Data represent mean ± SD, pooled from two independent experiments (n = 5-7). ** $P < .01$, * $P < .05$, ns—not significant (Mann-Whitney U test)

However, despite its widespread use as a model to study intestinal adaptive immune responses, the role of cDC in induction of Th1 and Th2 responses to *T. muris* infection remains incompletely characterized.[19,20,27] Consistent with previous studies,[7,18] we demonstrate here that induction of Th1 and Th2 responses during low- and high-dose infection with *T. muris* is mediated by IRF8- and IRF4-dependent cDC, respectively. Interestingly, the absence of IRF8-dependent cDC, and the associated failure to induce Th1 cells, during low-dose infection resulted in a dramatic increase in Th2 cytokines and worm expulsion. Finally, we show that Th2 responses to *T. muris* can develop in the absence of IRF4-dependent cDC, when IRF8-dependent cDC and associated Th1 responses are not present. Collectively, these results suggest a complex and antagonistic interplay between cDC subsets in the generation and regulation of *T. muris*-specific Th cell responses.

We have previously demonstrated that mice deficient in IRF8-dependent cDC clear low-dose *T. muris* infection prior to day 35 p.i, and that this is associated with an inability to generate *T. muris*-specific IgG2c.[7] Switching to IgG2c,[28] as well as establishment of chronic *T. muris* infection,[2,5] is associated with IFN-γ-producing CD4+ T cells, and we demonstrate here that in the absence of IRF8-dependent cDC, *T. muris*-specific IFN-γ-responses are markedly reduced. Such findings are consistent with our prior observations that IRF8-dependent cDC are essential for the establishment/maintenance of intestinal Th1 cells during homoeostasis.[7] Strikingly, Th2 responses were dramatically increased in the absence of IRF8-dependent cDC, suggesting that in addition to generating Th1 responses, IRF8-dependent cDC may serve to antagonize Th2 responses during *T. muris* infection. A plausible mechanism by which IRF8-dependent cDC counteract *T. muris*-specific Th2 responses could be through their constitutive production of IL-12, as recently suggested during *Heligmosomoides polygyrus* and *Schistosoma mansoni* infection.[29] IL-12 is known to play a critical role in establishing chronic *T. muris* infection in an IFN-γ-dependent manner;[5] however, whether IL-12 can also impact directly on Th2 responses during *T. muris* infection is yet to be investigated. Interestingly, mice that lack the TGFβ-activating integrin αvβ8 selectively in the CD11c-compartment display enhanced Th2 responses following low-dose *T. muris* infection compared to wild-type mice, resulting in worm expulsion.[30] Furthermore, we have recently demonstrated that αvβ8 is selectively expressed by CD103 SP cDC.[7] Thus, an alternative possibility for why an absence of IRF8-dependent DCs results in enhanced Th2 responses is that these cells selectively generate the active form of TGFβ, which may be involved in negatively regulating Th2 responses. Finally, although less dramatic, our results also indicated a potential role of IRF4-dependent cDC in antagonizing Th1 responses, as IFN-γ was increased in the absence of these cells, associated with failure to clear the infection. Together, these results suggest an important interplay between cDC subsets in the regulation of adaptive T helper cell responses during *T. muris* infection.

We recently demonstrated that IRF4-dependent cDC play a critical role in production of Th2 cytokines during high-dose *T. muris* infection, and that mice deficient in IRF4-dependent cDC become chronically infected.[18] Consistent with these results, we show here that mice lacking IRF4-dependent cDC failed to induce Th2 cells after high-dose infection and displayed delayed worm expulsion. Given these results,

surprisingly, mice with a combined deficiency in both IRF4- and IRF8-dependent cDC, which as a consequence have a strong reduction in migratory MLN cDC, responded to low-dose *T. muris* infection by production of Th2 cytokines and expulsion of the larvae. It remains unclear how Th2 responses can be generated in the absence of migratory IRF4-dependent cDC. One possibility is that parasite-derived antigens reach the MLN via the lymphatic drainage in the absence of migratory cDC to be taken up and presented by the remaining cDC in the MLN. The dominating cDC subsets in the MLN of infected CD11c-cre.*IRF4*$^{fl/fl}$.*IRF8*$^{fl/fl}$ mice consisted of resident CD11b+ MHC-II^{int} cDC. While the role of these cells in the generation of Th2 responses has not been investigated, it is plausible that they are capable of driving Th2 responses in the absence of IRF8-dependent cDC. Furthermore, although strongly reduced in number, we did observe a population of residual migratory cDC in the MLN of *T. muris*-infected CD11c-cre.*IRF4*$^{fl/-\ or\ fl/fl}$.*IRF8*$^{fl/fl}$ mice. Thus, we cannot rule out that these cells may contribute to the Th2 responses detected in the MLN of CD11c-cre.*IRF4*$^{fl/fl}$.*IRF8*$^{fl/fl}$ mice. Alternatively, while cDC are the critical antigen-presenting cells during induction of Th2 responses under normal conditions,[31,32] other cells may compensate for the lack of migratory IRF4- and IRF8-dependent cDC. In this respect, a variety of innate immune cell types have previously been suggested to present antigen to induce Th2 responses, including basophils,[33–35] eosinophils[36–39] and ILCs.[40,41] While these cells are preferentially thought to play accessory roles during the initial phase of Th2 responses, it is plausible that they take more active part in antigen presentation in the absence of migratory cDC, in particular IRF8-dependent cDC. Together, our data demonstrate that although IRF4-dependent cDC are considered as the major Th2-inducing antigen-presenting population, Th2 responses can be induced in the absence of these cells, and the mere lack of antagonizing IRF8-dependent cDC seems to facilitate a shift towards a Th2 response, by a yet unknown mechanism.

The infection dose is an important determinant during polarization of adaptive Th1 and Th2 responses to *T. muris* infection.[3] We demonstrate here that induction of these responses is driven by different cDC subsets; however, how distinct DC subsets can regulate the induction of adaptive responses depending on the dose of infection remains unclear. Several possibilities can be envisaged; for example, the functionality of DC subsets may be regulated differently by their capacity to respond to environmental factors that are produced dose-dependently during infection. Infection with parasitic nematodes is known to cause substantial damage to the affected tissue, resulting in the release of endogenous danger-associated signals. High-dose infection with *T. muris* is likely to result in increased damage to the caecal epithelium compared to low-dose infection and, as a consequence, increased release of epithelial-derived factors, several of which are known to promote Th2 responses during *T. muris* infection, including the cytokines IL-25, IL-33 and TSLP.[42–44] Interestingly, TSLP was recently suggested to regulate production of IL-12 by BM-derived DC;[45] it is therefore tempting to speculate that increased production of TSLP, or other epithelial-derived factors, during high-dose *T. muris* infection may limit production of IL-12 by IRF8-dependent cDC to allow development of a Th2 response. Epithelial-derived factors secreted during high- vs low-dose infection may also result in differential localization of cDC subsets and with this an altered exposure to antigen. Consistently, CD103+ DC localize to the infected epithelium in response to epithelial-derived chemokines during *T. muris* infection in resistant, but not susceptible mice.[19] Moreover, high-dose infection is also associated with increased release of *T. muris*-derived antigens that may directly affect the capacity of antigen-presenting cells to promote adaptive responses, as previously shown for antigens released during *H. polygyrus*[46–49] and *S. mansoni* infection.[50,51] Alternatively, the increased egg load during high-dose infection may result in hatching of larvae more distally compared to during low-dose infection, and therefore target cDC subsets differently as the cDC subset composition varies between intestinal regions[6]. Finally, cDC subsets may differ in their capacity to transport and present antigens in draining lymph nodes during high- and low-dose infection. We recently demonstrated that induction of Th2 responses to *S. mansoni* egg antigens injected directly into the intestinal serosa is mediated selectively by IRF4-dependent cDC and that this depended on their selective capacity to transport and present the antigen in the MLN.[18] Taken together, while we have shown that distinct cDC subsets induce adaptive responses during *T. muris* infection depending on the dose of infection, the underlying mechanism remains to be determined.

In conclusion, we demonstrate herein that IRF4- and IRF8-dependent cDC induce adaptive Th2 and Th1 responses, respectively, during high- and low-dose *T. muris* infection, in line with previous studies.[15–18] Importantly, we also demonstrate an antagonistic interplay between IRF4- and IRF8-dependent cDC subsets in the regulation of T helper cell responses, and finally, that IRF4-dependent cDC can be redundant for induction of Th2 responses, at least in the concurrent absence of IRF8-dependent cDC.

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CONFLICT OF INTEREST

The authors declare no conflict of interests.

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REFERENCES

1. Pullan RL, Smith JL, Jasrasaria R, Brooker SJ. Global numbers of infection and disease burden of soil transmitted helminth infections in 2010. *Parasit Vectors*. 2014;7:37.

2. Bancroft AJ, Else KJ, Grencis RK. Low-level infection with Trichuris muris significantly affects the polarization of the CD4 response. *Eur J Immunol*. 1994;24:3113-3118.
3. Hurst RJM, Else KJ. Trichuris muris research revisited: a journey through time. *Parasitology*. 2013;140:1325-1339.
4. Else KJ. Cytokine-mediated regulation of chronic intestinal helminth infection. *J Exp Med*. 1994;179:347-351.
5. Bancroft AJ, Else KJ, Sypek JP, Grencis RK. Interleukin-12 promotes a chronic intestinal nematode infection. *Eur J Immunol*. 1997;27:866-870.
6. Joeris T, Müller-Luda K, Agace WW, Mowat AM. Diversity and functions of intestinal mononuclear phagocytes. *Mucosal Immunol*. 2017;10:845-864.
7. Luda KM, Joeris T, Persson EK, et al. IRF8 Transcription-Factor-Dependent Classical Dendritic Cells Are Essential for Intestinal T Cell Homeostasis. *Immunity*. 2016;44:860-874.
8. Edelson BT, KC Wumesh, Juang R, et al. Peripheral CD103+ dendritic cells form a unified subset developmentally related to CD8alpha+ conventional dendritic cells. *J Exp Med* 2010;207:823-836.
9. Ginhoux F, Liu K, Helft J, et al. The origin and development of nonlymphoid tissue CD103+ DCs. *J Exp Med*. 2009;206:3115-3130.
10. Bogunovic M, Ginhoux F, Helft J, et al. Origin of the Lamina Propria Dendritic Cell Network. *Immunity*. 2009;31:513-525.
11. Persson EK, Uronen-Hansson H, Semmrich M, et al. IRF4 Transcription-Factor-Dependent CD103+ CD11b+ Dendritic Cells Drive Mucosal T Helper 17 Cell Differentiation. *Immunity*. 2013;38:958-969.
12. Schlitzer A, McGovern N, Teo P, et al. IRF4 Transcription Factor-Dependent CD11b+ Dendritic Cells in Human and Mouse Control Mucosal IL-17 Cytokine Responses. *Immunity*. 2013;38:970-983.
13. Satpathy AT, Briseño CG, Lee JS, et al. Notch2-dependent classical dendritic cells orchestrate intestinal immunity against attaching and effacing bacterial pathogens. *Nat Immunol*. 2013;14:937-948.
14. Lewis KL, Caton ML, Bogunovic M, et al. Notch2 receptor signaling controls functional differentiation of dendritic cells in the spleen and intestine. *Immunity*. 2011;35:780-791.
15. Williams JW, Tjota MY, Clay BS, et al. Transcription factor IRF4 drives dendritic cells to promote Th2 differentiation. *Nat Commun*. 2013;4:2990.
16. Gao Y, Nish SA, Jiang R, et al. Control of T helper 2 responses by transcription factor IRF4-dependent dendritic cells. *Immunity*. 2013;39:722-732.
17. Kumamoto Y, Linehan M, Weinstein JS, Laidlaw BJ, Craft JE, Iwasaki A. CD301b+ dermal dendritic cells drive T helper 2 cell-mediated immunity. *Immunity*. 2013;39:733-743.
18. Mayer JU, Demiri M, Agace WW, MacDonald AS, Svensson-Frej M, Milling SW. Different populations of CD11b+ dendritic cells drive Th2 responses in the small intestine and colon. *Nat Commun*. 2017;8:15820.
19. Cruickshank SM, Deschoolmeester ML, Svensson M, et al. Rapid dendritic cell mobilization to the large intestinal epithelium is associated with resistance to Trichuris muris infection. *J Immunol*. 2009;182:3055-3062.
20. Koyama K. Dendritic cell expansion occurs in mesenteric lymph nodes of B10.BR mice infected with the murine nematode parasite Trichuris muris. *Parasitol Res*. 2005;97:186-190.
21. Sorobetea D, Holm JB, Henningsson H, Kristiansen K, Svensson-Frej M. Acute infection with the intestinal parasite Trichuris muris has long-term consequences on mucosal mast cell homeostasis and epithelial integrity. *Eur J Immunol*. 2017;47:257-268.
22. deSchoolmeester ML, Little MC, Rollins BJ, Else KJ. Absence of CC chemokine ligand 2 results in an altered Th1/Th2 cytokine balance and failure to expel Trichuris muris infection. *J Immunol*. 2003;170:4693-4700.
23. Svensson M, Bell L, Little MC, Deschoolmeester M, Locksley RM, Else KJ. Accumulation of eosinophils in intestine-draining mesenteric lymph nodes occurs after Trichuris muris infection. *Parasite Immunol*. 2011;33:1-11.
24. Hägerbrand K, Westlund J, Yrlid U, Agace W, Johansson-Lindbom B. MyD88 Signaling Regulates Steady-State Migration of Intestinal CD103 $^{+}$ Dendritic Cells Independently of TNF-α and the Gut Microbiota. *J Immunol*. 2015;195:2888-2899.
25. Vander Lugt B, Khan AA, Hackney JA, et al. Transcriptional programming of dendritic cells for enhanced MHC class II antigen presentation-Supplemental. *Nat Immunol* 2014;15:161-167.
26. Cliffe LJ, Grencis RK. The Trichuris muris system: a paradigm of resistance and susceptibility to intestinal nematode infection. *Adv Parasitol*. 2004;57:255-307.
27. Bowcutt R, Bramhall M, Logunova L, et al. A role for the pattern recognition receptor Nod2 in promoting recruitment of CD103(+) dendritic cells to the colon in response to Trichuris muris infection. *Mucosal Immunol*. 2014;7:1094-1105.
28. Koyama K, Tamauchi H, Tomita M, Kitajima T, Ito Y. B-cell activation in the mesenteric lymph nodes of resistant BALB/c mice infected with the murine nematode parasite Trichuris muris. *Parasitol Res*. 1999;85:194-199.
29. Everts B, Tussiwand R, Dreesen L, et al. Migratory CD103+ dendritic cells suppress helminth-driven type 2 immunity through constitutive expression of IL-12. *J Exp Med* 2016;213:35-51.
30. Worthington JJ, Klementowicz JE, Rahman S, et al. Loss of the TGFβ-activating integrin αvβ8 on dendritic cells protects mice from chronic intestinal parasitic infection via control of type 2 immunity. *PLoS Pathog* 2013;9:e1003675.
31. Phythian-Adams AT, Cook PC, Lundie RJ, et al. CD11c depletion severely disrupts Th2 induction and development in vivo. *J Exp Med* 2010;207:2089-2096.
32. Lundie RJ, Webb LM, Marley AK, et al. A central role for hepatic conventional dendritic cells in supporting Th2 responses during helminth infection. *Immunol Cell Biol*. 2016;94:400-410.
33. Perrigoue JG, Saenz SA, Siracusa MC, et al. MHC class II–dependent basophil–CD4+ T cell interactions promote TH2 cytokine–dependent immunity. *Nat Immunol*. 2009;10:697-705.
34. Sokol CL, Chu N-Q, Yu S, Nish SA, Laufer TM, Medzhitov R. Basophils function as antigen-presenting cells for an allergen-induced T helper type 2 response. *Nat Immunol*. 2009;10:713-720.
35. Yoshimoto , et al. T. Basophils contribute to Th2-IgE responses in vivo via IL-4 production and presentation of peptide-MHC class II complexes to CD4+ T cells. *Nat Immunol*. 2009;10:706-712.
36. Mawhorter SD, Pearlman E, Kazura JW, Boom WH. Class II major histocompatibility complex molecule expression on murine eosinophils activated in vivo by Brugia malayi. *Infect Immun*. 1993;61:5410-5412.
37. Del Pozo V, De Andres B, Martin E, et al. Eosinophil as antigen-presenting cell: activation of T cell clones and T cell hybridoma by eosinophils after antigen processing. *Eur J Immunol*. 1992;22:1919-1925.
38. Padigel UM, Lee JJ, Nolan TJ, Schad GA, Abraham D. Eosinophils can function as antigen-presenting cells to induce primary and secondary immune responses to Strongyloides stercoralis. *Infect Immun*. 2006;74:3232-3238.
39. Duez C, Dakhama A, Tomkinson A, et al. Migration and accumulation of eosinophils toward regional lymph nodes after airway allergen challenge. *J Allergy Clin Immunol*. 2004;114:820-825.
40. Mirchandani AS, Besnard AG, Yip E, et al. Type 2 innate lymphoid cells drive CD4+ Th2 cell responses. *J Immunol*. 2014;192:2442-2448.
41. Oliphant CJ, Hwang YY, Walker JA, et al. MHCII-mediated dialog between group 2 innate lymphoid cells and CD4+ T cells potentiates type 2 immunity and promotes parasitic helminth expulsion. *Immunity*. 2014;41:283-295.
42. Massacand JC, Stettler RC, Meier R, et al. Helminth products bypass the need for TSLP in Th2 immune responses by

directly modulating dendritic cell function. *Proc Natl Acad Sci U S A*. 2009;106:13968-13973.
43. Humphreys NE, Xu D, Hepworth MR, Liew FY, Grencis RK. IL-33, a Potent Inducer of Adaptive Immunity to Intestinal Nematodes. *J Immunol*. 2008;180:2443-2449.
44. Owyang AM, Zaph C, Wilson EH, et al. Interleukin 25 regulates type 2 cytokine-dependent immunity and limits chronic inflammation in the gastrointestinal tract. *J Exp Med*. 2006;203:843-849.
45. Taylor BC, Zaph C, Troy AE, et al. TSLP regulates intestinal immunity and inflammation in mouse models of helminth infection and colitis. *J Exp Med*. 2009;206:655-667.
46. Smith KA, Filbey KJ, Reynolds LA, et al. Low-level regulatory T-cell activity is essential for functional type-2 effector immunity to expel gastrointestinal helminths. *Mucosal Immunol*. 2015;9:428-443.
47. Setiawan T, Metwali A, Blum AM, et al. Heligmosomoides polygyrus promotes regulatory T-cell cytokine production in the murine normal distal intestine. *Infect Immun*. 2007;75:4655-4663.
48. Finney CAM, Taylor MD, Wilson MS, Maizels RM. Expansion and activation of CD4+ CD25+ regulatory T cells in Heligmosomoides polygyrus infection. *Eur J Immunol*. 2007;37:1874-1886.
49. Rausch S, Huehn J, Kirchhoff D, et al. Functional analysis of effector and regulatory T cells in a parasitic nematode infection. *Infect Immun*. 2008;76:1908-1919.
50. Zaccone P, Burton O, Miller N, Jones FM, Dunne DW, Cooke A. Schistosoma mansoni egg antigens induce Treg that participate in diabetes prevention in NOD mice. *Eur J Immunol*. 2009;39:1098-1107.
51. Ritter M, Gross O, Kays S, et al. Schistosoma mansoni triggers Dectin-2, which activates the Nlrp3 inflammasome and alters adaptive immune responses. *Proc Natl Acad Sci*. 2010;107:20459-20464.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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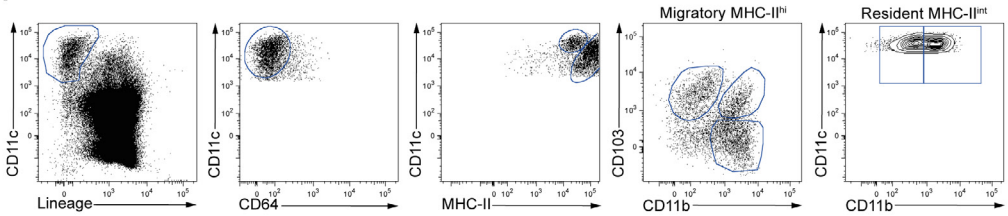
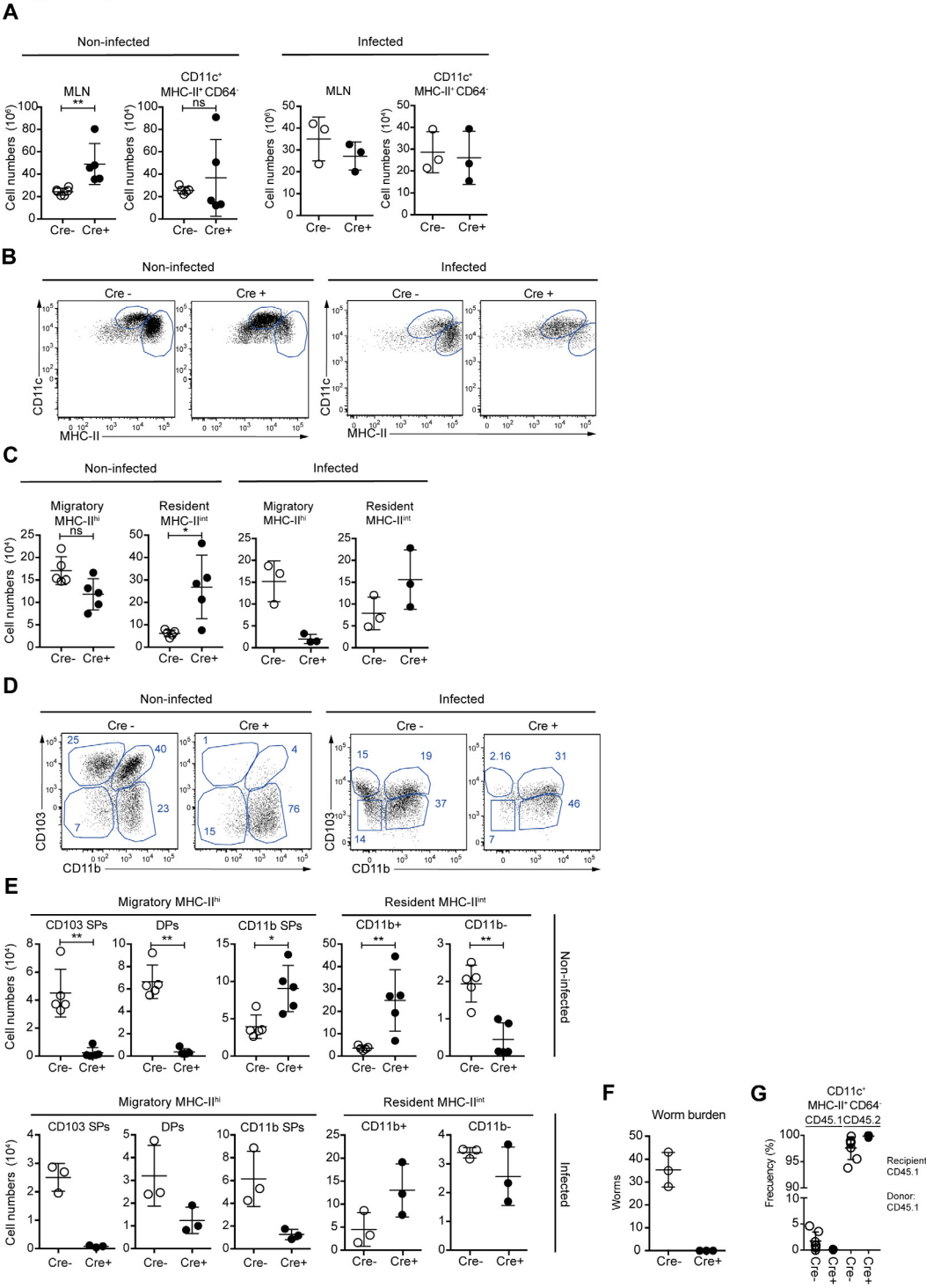
Supporting information S1

A



Supporting information S2



Supporting information Tabel S3

Target	Fluorochrome	Clone	Provider
IL-13	PerCPeF710	eBio13A	eBioscience
IFNγ	PE-Cy7	XMG1.2	BioLegend
CD4	APC	RM4-5	eBioscience
CD4	PB	GK1.5	BioLegend
CD4	PE-CF594	RM4-5	BD Biosciences
CD8a	BV510	53-6.7	BioLegend
CD8a	APC-eF780	53-6.7	eBioscience
CD8b	PE	eBioH35-17.2	eBioscience
CD44	A700	IM7	BioLegend
CD44	PE	IM7	BioLegend
CD45	BV510	30-F11	BD Biosciences
CD45.1	FITC	A20	BioLegend
CD45.2	A700	104	BioLegend
CD64	A647	X54-5/7.1	BD Biosciences
TCR-B	APC-eF780	H57-597	eBioscience
CD103	PE	M290	BD Biosciences
CD103	BV421	M290	BD Biosciences
CD11c	PE-Cy7	N418	eBioscience
CD3	A700	17A2	BioLegend
CD19	A700	6D5	BioLegend
Nk1.1	A700	PK136	BioLegend
Ter119	A700	TER-119	BioLegend
B220	A700	RA3-6B2	eBioscience
MHC-II	PB	M5/114.15.2	BioLegend
MHC-II	BV510	M5/114.15.2	BioLegend
CD11b	APC-eF710	M1/70	eBioscience
CD11b	BV510	M1/70	BioLegend
IRF4	PE	3E4	eBioscience
IRF8	PerCPeF710	V3GYWCH	eBioscience

Isotype controls

Host	Fluorochrome	Clone	Provider
Rat IgG1 k	PerCPeF710	eBRG1 k	eBioscience
Mouse IgG1	PE-Cy7	P3	eBioscience
Rat IgG1 k	PE	eBRG1	eBioscience