



LUND UNIVERSITY
Faculty of Medicine

LUP

Lund University Publications

Institutional Repository of Lund University

This is an author produced version of a paper published in *Immunobiology*. This paper has been peer-reviewed but does not include the final publisher proof-corrections or journal pagination.

Citation for the published paper:
Emma Persson, Elin Jaensson, William Agace

"The diverse ontogeny and function of murine small intestinal dendritic cell/macrophage subsets."

Immunobiology 2010 Jul 1

<http://dx.doi.org/10.1016/j.imbio.2010.05.013>

Access to the published version may require journal subscription.

Published with permission from: Elsevier

The diverse ontogeny and function of murine small intestinal dendritic cell/macrophage subsets.

Emma K Persson*, Elin Jaensson and William W. Agace

Immunology Section, BMC D-14, Lund University, 221 84 Lund, Sweden.

Short Title: Intestinal dendritic cell subsets

*corresponding author

Tel: 0046-46-2220343

Fax: 0046-46-2224218

E.mail address: emma.persson@med.lu.se

Key Words: CD103, Cx3cr1, dendritic cell, intestine, lamina propria, macrophage

Abbreviations: DC (dendritic cell), LP (lamina propria), PP (peyer's patch), SILT (solitary isolated lymphoid tissues), MLN (mesenteric lymph node), TLR (toll like receptor), MDP (macrophage/DC precursor), CDP (common DC precursor), RA (retinoic acid)

Abstract:

Intestinal dendritic cell and macrophage subsets are believed to play key roles in maintaining intestinal homeostasis in the steady state and in driving protective immune responses in the setting of intestinal infection. This mini-review focuses on recent progress regarding the ontogeny and function of small intestinal lamina propria dendritic cell/macrophage subsets. In particular we discuss recent findings suggesting that small intestinal CD103⁺ dendritic cells and Cx3cr1⁺ cells derive from distinct precursor populations and that CD103⁺ dendritic cells represent the major migratory population of cells with a key role in initiating adaptive immune responses in the draining mesenteric lymph node. In contrast, Cx3cr1⁺ cells appear to represent a tissue resident population, phenotypically indistinguishable from tissue resident macrophages. These latter observations suggest an important division of labour between dendritic cell/macrophage subsets in the regulation of intestinal immune responses in the steady state.

Introduction

The small intestinal mucosa represents the body's largest surface area to the external environment. It is covered by a single layer of epithelial cells, whose primary function is the absorption of nutrients from the intestinal lumen. It is also a major colonisation and entry site for many parasitic, bacterial and viral pathogens. Thus the intestinal immune system must be capable of generating protective immune responses towards these pathogens, while remaining tolerant to harmless food antigens and the resident gut microflora. Intestinal dendritic cells (DCs) and macrophages, with their unique ability to initiate and regulate local innate and adaptive immune responses, are thought to play key roles in these processes.

Intestinal DCs and macrophages are found throughout the gut lamina propria (LP) and within sub-epithelial gut associated lymphoid tissues (GALT), including Peyer's Patches (PP) and solitary isolated lymphoid tissues (SILT). This review will focus on recent findings regarding the ontogeny and unique functions of murine small intestinal LP DC and macrophage subsets in the steady state.

CD103 and Cx3cr1 define phenotypically distinct subsets of LP cells with distinct turnover rates.

Conventional lymphoid tissue DCs are traditionally defined by their co-expression of CD11c and MHCII and have been further sub-grouped based on their expression of an array of markers including CD11b, CD8 α , and CD4 (Merad and Manz, 2009; Miloud et al., 2010; Shortman and Liu, 2002). However the use of these markers alone to define functionally distinct intestinal DC and macrophage subsets is problematic since CD11c is also expressed on tissue macrophages (Hume, 2008) and while CD11b is highly expressed on monocytes and macrophages it is also expressed

on a major subset of DCs in the LP (see below). Moreover, traditional myeloid markers such as F4/80 and SIRP α do not fully discriminate between intestinal mononuclear cell subsets.

We, and others, have recently identified a functionally distinct subset of DCs in the murine small intestinal LP and draining mesenteric lymph nodes (MLN) that express the integrin alpha chain, CD103 (α E) (Annacker et al., 2005; Johansson-Lindbom et al., 2005). CD103⁺ DCs present in LP cell preparations can be divided into CD11b⁺ and CD11b⁻ populations (Bogunovic et al., 2009; Ginhoux et al., 2009; Schulz et al., 2009), however mice lacking SILT and PP (*Id2*^{-/-} mice) display a marked and selective reduction in CD103⁺CD11b⁻ DCs in these preparations indicating that CD103⁺CD11b⁻ cells derive from contaminating SILT (Bogunovic et al., 2009; Ginhoux et al., 2009). Consistent with this possibility, the majority of CD103⁺ PP DCs are CD11b⁻ ((Bogunovic et al., 2009), authors unpublished observation). The ontogeny of CD103⁺CD11b⁻ and CD103⁺CD11b⁺ DCs will be discussed in more detail below. Utilising knockin mice expressing GFP under the control of the fractalkine receptor Cx3cr1 promotor (Cx3cr1^{GFP/+} mice), Niess and colleagues (Niess et al., 2005) identified a major subset of cells within the intestinal LP, that have been described in the literature as DCs (for example, (Bogunovic et al., 2009; Ginhoux et al., 2009; Hapfelmeier et al., 2008; Niess et al., 2005; Vallon-Eberhard et al., 2006; Varol et al., 2009)). Recent data from several laboratories, including our own, have demonstrated that small intestinal Cx3cr1⁺ LP cells and CD103⁺ LP DCs represent phenotypically distinct subsets of cells, and that Cx3cr1⁺ cells display markers associated with tissue resident macrophages (Bogunovic et al., 2009; Schulz et al., 2009; Varol et al., 2009) (Table 1).

In the steady state small intestinal Cx3cr1⁺ LP cells outnumber CD103⁺ DCs approximately 3-4 fold. While these cells are found closely associated with overlying enterocytes, CD103⁺ DCs appear to be located more centrally within the villous core (Bogunovic et al., 2009; Niess et al., 2005; Schulz et al., 2009). Combining Ki67 staining with BrdU pulse chase studies, we found that CD103⁺ LP DCs are primarily a non-dividing population that display a rapid turnover *in vivo*, indicating that this compartment is continually replenished by blood borne precursors (Jaensson et al., 2008). In contrast, Cx3cr1⁺ cells appear to turnover slowly and, in small intestinal transplant experiments, were not replaced in the graft by recipient cells within a 6-day period (Schulz et al., 2009).

Ontogeny of small intestinal CD103⁺ DCs and Cx3cr1⁺ LP cells

CD103⁺ DC and Cx3cr1⁺ LP cell precursors: According to current models, classical DCs, plasmacytoid DCs and monocytes share a common bone marrow progenitor, the macrophage and DC precursor (MDP) (Fogg et al., 2006). MDPs progress in development to common DC precursors (CDP) that can give rise to classical DCs and plasmacytoid DCs but not monocytes. CDP can develop into pre-cDC that are committed to the cDC lineage and have lost the potential to give rise to plasmacytoid DC (Liu et al., 2009). Pre-cDC, in contrast to MDPs and CDPs, are found in blood and can enter lymphoid tissues where they can generate all major steady-state splenic cDC subsets (Geissmann et al., 2010; Liu et al., 2009). Maintenance and division of DCs in lymphoid tissue, in the steady state, is in part dependent on Flt3L (Liu et al., 2009; Waskow et al., 2008). Of note, pre-cDCs are heterogeneous in their expression of CD24 that appears to reflect the presence of

distinct precursors within this population that are already prone to generating specific DC subsets (Naik et al., 2006).

Following up on an earlier observation that adoptively transferred monocytes can generate CD11c⁺ LP cells (Varol et al., 2007), two recent studies have assessed the role of CDPs, pre-DCs and monocytes in the generation of intestinal LP DC subsets (Bogunovic et al., 2009; Varol et al., 2009). While adoptive transfer of MDPs into diphtheria toxin treated CD11c-DTR (diphtheria toxin receptor) mice or wildtype mice gave rise to all LP DC/macrophage subsets, CDP and pre-cDC gave rise only to CD103⁺CD11b⁻ and CD103⁺CD11b⁺ intestinal DCs (Bogunovic et al., 2009; Varol et al., 2009). In contrast, engrafted Ly6C^{hi} monocytes (Bogunovic et al., 2009; Varol et al., 2009) gave rise solely to Cx3cr1⁺ LP cells. Furthermore, in a very elegant set of studies, in which green and red fluorescence Ly6C^{hi} monocytes were co-transferred into CD11c⁺ cell depleted mice, Varol *et al.* demonstrated that individual LP villous structures were primarily repopulated by either red or green Cx3cr1⁺ cells, suggesting that that the Cx3cr1⁺ LP cell compartment is maintained by the seeding of limited numbers of circulating monocytes that undergo local clonal expansion within the LP (Varol et al., 2009). As pointed out by these authors (Varol et al., 2007), a contribution of Ly6C^{lo} monocytes to the Cx3cr1⁺ LP cell compartment cannot be excluded from these studies as Ly6C^{hi} monocytes have been shown to shuttle back to the bone marrow and can give rise to Ly6C^{lo} cells (Varol et al., 2007).

Growth factor requirements: The different ontogeny of small intestinal CD103⁺ DCs and Cx3cr1⁺ LP cells is also reflected in their specific growth factor requirements. Thus while the number of CD103⁺ LP DCs is reduced in *Flt3r* or GM-CSF receptor (*Csf2r*) deficient mice and increases upon exogenous administration of

their ligands, development of Cx3cr1⁺ LP cells is unaffected (Bogunovic et al., 2009; Schulz et al., 2009). In contrast CD103⁻CD11b⁺MHCII⁺ LP cells (which are Cx3cr1⁺) are selectively reduced in M-CSF receptor (*Csf1r*)^{-/-} mice (Bogunovic et al., 2009).

Transcription factor requirements for intestinal CD103⁺ DC subsets: While adoptively transferred pre-cDCs can generate both intestinal CD103⁺CD11b⁻ and CD103⁺CD11b⁺ DC subsets, recent evidence suggests that these populations have distinct transcription factor requirements. Genetic deletion of the transcription factors *Id2*, *Irf8* and *Batf3*, which are required for CD8 α ⁺ splenic DC development (Aliberti et al., 2003; Kusunoki et al., 2003), leads to a selective loss of small intestinal CD103⁺CD11b⁻ DCs (Edelson et al., 2010; Ginhoux et al., 2009). Notably, these transcription factors are also required for the development of CD103⁺CD11b⁻ DCs in extra-intestinal peripheral tissues including the liver, lung and kidney (Edelson et al., 2010; Ginhoux et al., 2009). As with CD8 α ⁺ splenic DCs (den Haan et al., 2000), CD103⁺CD11b⁻ DCs from the skin and lung are important for cross-presentation of cell associated antigen (Bedoui et al., 2009; del Rio et al., 2007). These data suggest that CD103⁺CD11b⁻ DCs found in GALT and extra-intestinal non-lymphoid tissues are developmentally and functionally related to CD8 α ⁺ splenic DCs. The stages and underlying signals regulating the divergence of CD103⁺CD11b⁻ and CD103⁺CD11b⁺ intestinal DC subsets from pre-cDCs still remain to be determined.

Functional specialization of small intestinal CD103⁺ DC and Cx3cr1⁺ LP cells

Antigen sampling: Rescigno and co-workers first visualized dendrites from CD11c⁺ cells extending across the epithelium into the lumen of the small intestine, and suggested that these structures may play an important role in the uptake of luminal antigen and microbes into the LP (Rescigno et al., 2001). The presence of trans-epithelial dendrites was subsequently confirmed utilizing *Cx3cr1*-GFP reporter mice, and shown to mediate the uptake of invasive-defective *Salmonella* (Niess et al., 2005). The number of trans-epithelial dendrites increased in the terminal ileum after *Salmonella* administration, but this response was markedly reduced in *Cx3cr1*-deficient (*Cx3cr1*^{GFP/GFP}) mice (Niess et al., 2005), indicating a role for epithelial derived Cx3c1 in their formation. Notably, *Salmonella* induced trans-epithelial dendrite formation in the proximal ileum and the entry of non-invasive *Aspergillus* species is unaffected in *Cx3cr1*-deficient mice (Chieppa et al., 2006; Vallon-Eberhard et al., 2006). Moreover, the presence of trans-epithelial dendrites appears to be mouse strain dependent (Vallon-Eberhard et al., 2006), challenging the concept that these structures represent a major route for antigen-uptake in the small intestine.

Trans-epithelial dendrites have also been observed in CD11c-GFP and MHC II-GFP reporter mice (Chieppa et al., 2006) however the distribution of dendrites varied somewhat with that of *Cx3cr1*-GFP (Chieppa et al., 2006; Niess et al., 2005). At this point it is unclear whether such differences reflect preferential detection of distinct antigen sampling cell subsets with the different reporter strains or differences in study protocols. Consistent with all studies is that trans-epithelial dendrite formation appears to require local microbial stimulation (Chieppa et al., 2006; Niess et al., 2005; Vallon-Eberhard et al., 2006). Thus the number of trans-epithelial

dendrites increases after introduction of non-invasive *Salmonella*, *Aspergillus* and certain TLR ligands (Chieppa et al., 2006; Niess et al., 2005; Vallon-Eberhard et al., 2006), and is greatly reduced in mice given broad-spectrum antibiotics and in mice lacking the TLR adaptor molecule MyD88 in non-hematopoietic cells (Chieppa et al., 2006). This latter finding suggests that epithelial cell recognition of luminal microbes drives trans-epithelial dendrite formation. In contrast to Cx3cr1⁺ LP cells it is currently unclear whether CD103⁺ DCs can form trans-epithelial dendrites.

With regards to sampling of soluble antigen by CD103⁺ and Cx3cr1⁺ LP subsets, we recently observed efficient accumulation of intra-luminal administered soluble antigen (OVA.Cy-5) in Cx3cr1^{GFP+} LP cells in both Cx3cr1^{+/GFP} and Cx3cr1^{GFP/GFP} (Cx3cr1-deficient) mice demonstrating that these cells take up soluble antigen *in vivo* and that this process does not require Cx3cr1. In the same set of experiments CD103⁺ LP DCs were observed to accumulate OVA-Cy5 but far less efficiently (Schulz et al., 2009). Whether accumulation of soluble antigen in these subsets represents direct uptake of soluble antigen or acquisition from neighbouring cells (i.e. epithelial cells) is currently unclear.

Antigen transport to the draining MLN: DCs continually migrate from the LP to the draining MLN in the steady state in a chemokine receptor (Ccr)7-dependent process (Forster et al., 1999; Jang et al., 2006; Johansson-Lindbom et al., 2005; Milling et al.). CD103⁺ DCs are reduced in the MLN but not LP of Ccr7^{-/-} mice (Jang et al., 2006; Johansson-Lindbom et al., 2005; Worbs et al., 2006), and accumulate with delayed kinetics in the MLN compared to the LP in BrdU pulse chase experiments (Jaensson et al., 2008), indicating that CD103⁺ LP DCs represent a migratory DC population in the LP. Using confocal microscopy to study lymphatic

vessels *ex vivo* and flow cytometry to assess DC subsets in draining MLN-afferent lymph, we recently provided direct evidence that LP derived CD103⁺ DCs make up the major DC population in the murine LP draining lymph (Schulz et al., 2009). Remarkably we failed to detect Cx3cr1^{hi} LP cells in the draining lymph in either the steady state or after oral administration of R848 (TLR7/8 agonist) (Schulz et al., 2009).

Intestinal CD103⁺ DCs and Cx3cr1⁺ LP cells in the initiation of adaptive immune responses: CCR7^{-/-} mice fail to mount T cell responses to soluble luminal antigen in the MLN and are defective in their ability to generate oral tolerance (Johansson-Lindbom et al., 2005; Worbs et al., 2006). Furthermore, following oral ovalbumin (OVA) administration, sorted CD103⁺ but not CD103⁻ MLN DCs induce OVA specific CD4⁺ and CD8⁺ T cell proliferation *in vitro* (Coombes et al., 2007; Jaensson et al., 2008). Together these findings indicate that migratory CD103⁺ DCs play a direct role in the induction of steady state tolerogenic responses to soluble luminal antigen within the MLN. CD103⁺ LP, lymph and MLN DCs have also been shown to contain epithelial fragments in the steady state (Huang et al., 2000; Jang et al., 2006). In addition, following oral administration of *Salmonella*, bacteria are found within CD103⁺CD11b⁺ MLN DCs (Bogunovic et al., 2009; Voedisch et al., 2009) and CD103⁺CD11b⁺ LP DCs are induced to express IL-6, IL-12p40 and IL-12p70 in response to the TLR5 agonist flagellin *in vitro* (Uematsu et al., 2006). These findings indicate that migratory CD103⁺ LP DCs likely contribute to maintaining peripheral tolerance to self-antigen in the steady state and may participate in the initiation of adaptive immune responses in the setting of mucosal infection.

While several reports have compared the ability of LP DC/macrophage subsets to induce naïve T cell differentiation into distinct lineages *in vitro* (Atarashi et al., 2008; Denning et al., 2007; Uematsu et al., 2006), the inefficient migration of naïve T cells to the LP, coupled with the inability of Cx3cr1⁺ LP cells to migrate to the MLN suggest that Cx3cr1⁺ LP cells do not play a major direct role in priming naïve T cells *in vivo*.

Intestinal CD103⁺ DCs display an enhanced ability to induce gut homing receptors on responding T cells and FoxP3⁺ T cell differentiation *in vitro*: TCR transgenic adoptive transfer studies have demonstrated that the MLN is a site of enhanced regulatory T cell (Treg) differentiation (Coombes et al., 2007; Sun et al., 2007), and that T cells primed at this site are induced to express the gut homing receptors CCR9 and $\alpha 4\beta 7$ (Campbell and Butcher, 2002; Johansson-Lindbom et al., 2003; Svensson et al., 2002). The idea that intestinal CD103⁺ DCs are potentially important in this process has arisen from *in vitro* co-culture experiments. In these studies freshly isolated antigen pulsed CD103⁺ MLN and LP DCs displayed an enhanced ability to induce CCR9 and $\alpha 4\beta 7$ on responding T cells and drive naïve T cell differentiation to FoxP3⁺ Tregs (Coombes et al., 2007; Jaensson et al., 2008; Johansson-Lindbom et al., 2005; Sun et al., 2007). This property appears to result, at least in part, from an increased capacity of small intestinal CD103⁺ DCs to generate the vitamin A metabolite retinoic acid (RA). RA itself is sufficient to induce Ccr9 and $\alpha 4\beta 7$ on anti-CD3 antibody activated T cells (Iwata et al., 2004), and synergizes with TGF β to promote FoxP3⁺ Treg differentiation (Mucida et al., 2007). Further, the addition of pan-retinoic acid receptor antagonists inhibits the ability of intestinal CD103⁺ DCs to induce gut homing receptors on responding T cells and enhanced

Treg differentiation *in vitro* (Jaensson et al., 2008; Kang et al., 2007; Mucida et al., 2007; Sun et al., 2007; Svensson et al., 2008). Consistent with these findings, small intestinal CD103⁺ DCs express higher levels of *Aldh1a2*, encoding a key enzyme, (RALDH2) in the conversion of retinal to RA (Coombes et al., 2007), and induce stronger RA-dependent responses in T cells compared with CD103⁻ MLN DCs and CD103⁺ DCs isolated from other tissues (Jaensson et al., 2008; Svensson et al., 2008).

CD103⁺ MLN DCs also express higher levels of tissue plasminogen activator (*Plat*), latent TGFβ binding protein3 (*Ltbp 3*) (Coombes et al., 2007), and integrin β8 subunit (*Itgb8*) mRNA (authors unpublished observation) compared with CD103⁻ MLN DCs, which have all been implicated in the secretion and activation of TGFβ and may thus contribute to their enhanced ability to generate FoxP3⁺ Tregs *in vitro* (Annes et al., 2003; Travis et al., 2007). It is currently unclear whether the expression of these genes is a selective property of intestinal CD103⁺ DCs (as is the case with *Aldh1a2*). However, mice with a cell type-specific deletion of the TGF-β-activating β8 integrin subunit in DCs develop IBD and autoimmunity and display reduced numbers of Tregs in their colon, but not spleen (Travis et al., 2007).

Collectively these results suggest that migratory CD103⁺ DCs have ‘enhanced’ tolerogenic properties in the steady state compared with other intestinal and many non-intestinal DC subsets. Some (if not all) of these properties may be acquired from signals they receive within the intestinal environment.

Imprinting of small intestinal CD103⁺ LP DCs: The ability of small intestinal CD103⁺ LP and MLN DC to metabolize vitamin A appears to underlie many of the unique steady state functions of these cells. Currently there are intense efforts to identify the local ‘imprinting’ factor(s) that induce the Vitamin A metabolizing

property in these cells. Yokota and co-workers recently reported that IL-13, GM-CSF and IL-4 induce *Aldh1a2* expression and aldehyde dehydrogenase activity in splenic DCs *in vitro* and that IL-4 and GM-CSF together induced expression levels similar to that observed in CD103⁺ MLN DCs (Yokota et al., 2009). Similarly, MLN DCs from IL-4R α ^{-/-} mice showed reduced levels of *Aldh1a2* while IL-4 enhanced *Aldh1a2* expression in WT MLN DCs (Elgueta et al., 2008). Nevertheless intestinal DCs from IL-4R α ^{-/-} mice displayed normal aldehyde dehydrogenase activity and while intestinal CD11c⁺ cells in mice deficient in the common beta subunit (Beta-C) of the GM-CSF/IL-3/IL-5 receptor showed reduced aldehyde dehydrogenase activity, these findings may reflect alterations in CD11c⁺ MLN subsets as *Csf2r* (GM-CSFR)^{-/-} mice have reduced numbers of CD103⁺CD11b⁺ intestinal DCs (Bogunovic et al., 2009). Certain TLR ligands have also been shown to induce a modest up-regulation of *Aldh1a2* mRNA and aldehyde dehydrogenase activity in splenic (Guilliams et al., 2010; Manicassamy et al., 2009; Yokota et al., 2009) and MLN DCs (Guilliams et al., 2010), however the aldehyde dehydrogenase activity of CD103⁺ MLN DCs is only moderately decreased in germfree *Myd88*^{-/-}*Trif*^{-/-} double deficient mice suggesting that this signaling pathway plays only a minor role in imprinting intestinal DCs with the ability to metabolize retinol in the steady state *in vivo* (Guilliams et al., 2010). Intriguingly *Aldh1a2* mRNA expression and aldehyde dehydrogenase activity of MLN DCs is dramatically reduced in Vitamin A deficient mice (Yokota et al., 2009). RA alone failed to induce *Aldh1a2* mRNA in splenic and bone marrow derived DCs (Yokota et al., 2009), indicating that RA induction of *Aldh1a2* mRNA in intestinal CD103⁺ DCs may be indirect. One study observed a modest up-regulation of *Aldh1a2* mRNA in BM DCs in response to RA (Iliev et al., 2009a). While it has been suggested from *in vitro* studies that intestinal epithelial cells may provide RA signals

to local DCs (Iliev et al., 2009a), the source of RA that induces the development of Aldh1a2^{hi} CD103⁺ LP DCs *in vivo* remains unclear. Indeed, in contrast to CD103⁺ LP DCs, murine F4/80⁺ (Cx3cr1⁺) LP cells, that are closely associated with the intestinal epithelium, display little aldehyde dehydrogenase activity (Guilliams et al., 2010; Schulz et al., 2009).

Putative functions of small intestinal Cx3cr1⁺ LP cells in the steady state: Our recent findings demonstrating that Cx3cr1⁺ LP cells are poor at priming naïve T cells *in vitro* and do not migrate to the draining MLN suggest that the main function of these cells is to regulate innate and adaptive immune responses within the LP (Schulz et al., 2009). At one end of the scale these cells may simply act to take up and kill any microbes that have made their way to the epithelial surface and into the LP. Consistent with this possibility Cx3cr1⁺ LP cells were recently shown to migrate into the intestinal lumen after capture of *Salmonella* (Arques et al., 2009), thus functioning in a cell-mediated form of immune exclusion. Notably, in this study Cx3cr1⁺ cells were CD11b⁻ and we failed to detect cells with this phenotype in the LP in the steady state (Schulz et al., 2009). It would seem logical, however, given their high numbers and slow turnover rate, that the function of Cx3cr1⁺ cells in intestinal immune responses is more complex. As described above, Cx3cr1⁺ cells appear to be efficient in antigen sampling. Thus, one possibility is that these cells pass on antigen to the migratory populations of antigen presenting cells in the LP. However, this remains to be tested. Given recent reports that resident antigen presenting cells can regulate effector/memory T cell proliferation and cytokine secretion in the periphery (McLachlan et al., 2009; Wakim et al., 2008), another possibility is that Cx3cr1⁺ cells regulate the function of effector T cell subsets subsequent to their entry into the LP.

Accordingly CD11b⁺F4/80⁺ LP cells constitutively produce IL-10 (Denning et al., 2007) and IL-10 production by these cells has been implicated in supporting FoxP3⁺ Treg function within the LP (Denning et al., 2007; Murai et al., 2009). Moreover, IL-10 production by these cells appears to underlie, at least in part, their hypo-responsiveness to TLR stimulation *in vitro* (Denning et al., 2007; Monteleone et al., 2008). While these findings suggest that Cx3cr1⁺ LP cells may contribute to the tolerogenic environment within the small intestine, their role in regulating adaptive immune responses at this site *in vivo* is presently unclear.

Summary

In the past few years, important new insights have been gained regarding phenotype, ontogeny and function of DC/macrophage subsets in the small intestinal LP. There is now strong evidence suggesting that the murine small intestinal LP contains two major subsets of cells, CD103⁺CD11b⁺ migratory DCs and resident Cx3cr1⁺ LP cells. CD103⁺CD11b⁺ DCs and Cx3cr1⁺ LP cells derive from distinct precursors, require distinct growth and transcription factors for their development and show distinct turnover rates in the steady state *in vivo*. Despite these insights, there is still a major lack in our understanding regarding the functional role of these distinct subsets in regulating intestinal immune responses *in vivo* and whether their functions are maintained or altered in the setting of inflammation. Another major knowledge gap is whether phenotypically and functionally similar subsets of cells are found in healthy or inflamed human intestinal LP. In this regard, we have recently demonstrated that CD103⁺ DCs are present in human MLN and, in line with their murine counterparts, induce RA-dependent CCR9 and α 4 β 7 expression on responding CD8 T cells *in vitro* (Jaensson et al., 2008). A recent study also suggested that these cells induce enhanced

Treg differentiation (Iliev et al., 2009b). Filling these gaps will be of fundamental importance for determining the potential of targeting these cell types to regulate mucosal immune responses in humans and to treat human intestinal disease.

References

- 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.
- Annacker, O., Coombes, J.L., Malmstrom, V., Uhlig, H.H., Bourne, T., Johansson-Lindbom, B., Agace, W.W., Parker, C.M., and Powrie, F. 2005. Essential role for CD103 in the T cell-mediated regulation of experimental colitis. *J Exp Med* 202, 1051-1061.
- Annes, J.P., Munger, J.S., and Rifkin, D.B. 2003. Making sense of latent TGFbeta activation. *J Cell Sci* 116, 217-224.
- Arques, J.L., Hautefort, I., Ivory, K., Bertelli, E., Regoli, M., Clare, S., Hinton, J.C., and Nicoletti, C. 2009. Salmonella induces flagellin- and MyD88-dependent migration of bacteria-capturing dendritic cells into the gut lumen. *Gastroenterology* 137, 579-587, 587 e571-572.
- Atarashi, K., Nishimura, J., Shima, T., Umesaki, Y., Yamamoto, M., Onoue, M., Yagita, H., Ishii, N., Evans, R., Honda, K., et al. 2008. ATP drives lamina propria T(H)17 cell differentiation. *Nature* 455, 808-812.
- Bedoui, S., Whitney, P.G., Waithman, J., Eidsmo, L., Wakim, L., Caminschi, I., Allan, R.S., Wojtasiak, M., Shortman, K., Carbone, F.R., et al. 2009. Cross-presentation of viral and self antigens by skin-derived CD103+ dendritic cells. *Nat Immunol* 10, 488-495.
- Bogunovic, M., Ginhoux, F., Helft, J., Shang, L., Hashimoto, D., Greter, M., Liu, K., Jakubzick, C., Ingersoll, M.A., Leboeuf, M., et al. 2009. Origin of the lamina propria dendritic cell network. *Immunity* 31, 513-525.

Campbell, D.J., and Butcher, E.C. 2002. Rapid acquisition of tissue-specific homing phenotypes by CD4(+) T cells activated in cutaneous or mucosal lymphoid tissues. *J Exp Med* 195, 135-141.

Chieppa, M., Rescigno, M., Huang, A.Y., and Germain, R.N. 2006. Dynamic imaging of dendritic cell extension into the small bowel lumen in response to epithelial cell TLR engagement. *J Exp Med* 203, 2841-2852.

Coombes, J.L., Siddiqui, K.R., Arancibia-Carcamo, C.V., Hall, J., Sun, C.M., Belkaid, Y., and Powrie, F. 2007. A functionally specialized population of mucosal CD103+ DCs induces Foxp3+ regulatory T cells via a TGF-beta and retinoic acid-dependent mechanism. *J Exp Med* 204, 1757-1764.

del Rio, M.L., Rodriguez-Barbosa, J.I., Kremmer, E., and Forster, R. 2007. 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* 178, 6861-6866.

den Haan, J.M., Lehar, S.M., and Bevan, M.J. 2000. CD8(+) but not CD8(-) dendritic cells cross-prime cytotoxic T cells in vivo. *J Exp Med* 192, 1685-1696.

Denning, T.L., Wang, Y.C., Patel, S.R., Williams, I.R., and Pulendran, B. 2007. Lamina propria macrophages and dendritic cells differentially induce regulatory and interleukin 17-producing T cell responses. *Nat Immunol* 8, 1086-1094.

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 CD8{alpha}+ conventional dendritic cells. *J Exp Med* Epub ahead of print, PMID 20351058.

Elgueta, R., Sepulveda, F.E., Vilches, F., Vargas, L., Mora, J.R., Bono, M.R., and Roseblatt, M. 2008. Imprinting of CCR9 on CD4 T cells requires IL-4 signaling on mesenteric lymph node dendritic cells. *J Immunol* 180, 6501-6507.

Fogg, D.K., Sibon, C., Miled, C., Jung, S., Aucouturier, P., Littman, D.R., Cumano, A., and Geissmann, F. 2006. A clonogenic bone marrow progenitor specific for macrophages and dendritic cells. *Science* 311, 83-87.

Forster, R., Schubel, A., Breitfeld, D., Kremmer, E., Renner-Muller, I., Wolf, E., and Lipp, M. 1999. CCR7 coordinates the primary immune response by establishing functional microenvironments in secondary lymphoid organs. *Cell* 99, 23-33.

Geissmann, F., Manz, M.G., Jung, S., Sieweke, M.H., Merad, M., and Ley, K. 2010. Development of monocytes, macrophages, and dendritic cells. *Science* 327, 656-661.

Ginhoux, F., Liu, K., Helft, J., Bogunovic, M., Greter, M., Hashimoto, D., Price, J., Yin, N., Bromberg, J., Lira, S.A., et al. 2009. The origin and development of nonlymphoid tissue CD103⁺ DCs. *J Exp Med* 206, 3115-3130.

Guilliams, M., Crozat, K., Henri, S., Tamoutounour, S., Grenot, P., Devilard, E., de Bovis, B., Alexopoulou, L., Dalod, M., and Malissen, B. 2010. Skin-draining lymph nodes contain dermis-derived CD103⁻ dendritic cells that constitutively produce retinoic acid and induce Foxp3⁺ regulatory T cells. *Blood* 115, 1958-1968.

Hapfelmeier, S., Muller, A.J., Stecher, B., Kaiser, P., Barthel, M., Endt, K., Eberhard, M., Robbiani, R., Jacobi, C.A., Heikenwalder, M., et al. 2008. Microbe sampling by mucosal dendritic cells is a discrete, MyD88-independent step in *Delta*inV G S. *Typhimurium colitis*. *J Exp Med* 205, 437-450.

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.

Hume, D.A. 2008. Macrophages as APC and the dendritic cell myth. *J Immunol* 181, 5829-5835.

Iliev, I.D., Mileti, E., Matteoli, G., Chieppa, M., and Rescigno, M. 2009a. Intestinal epithelial cells promote colitis-protective regulatory T-cell differentiation through dendritic cell conditioning. *Mucosal Immunol* 2, 340-350.

Iliev, I.D., Spadoni, I., Mileti, E., Matteoli, G., Sonzogni, A., Sampietro, G.M., Foschi, D., Caprioli, F., Viale, G., and Rescigno, M. 2009b. Human intestinal epithelial cells promote the differentiation of tolerogenic dendritic cells. *Gut* 58, 1481-1489.

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., et al. 2008. Small intestinal CD103⁺ dendritic cells display unique functional properties that are conserved between mice and humans. *J Exp Med* 205, 2139-2149.

Jang, M.H., Sougawa, N., Tanaka, T., Hirata, T., Hiroi, T., Tohya, K., Guo, Z., Umemoto, E., Ebisuno, Y., Yang, B.G., et al. 2006. CCR7 is critically important for migration of dendritic cells in intestinal lamina propria to mesenteric lymph nodes. *J Immunol* 176, 803-810.

Johansson-Lindbom, B., Svensson, M., Pabst, O., Palmqvist, C., Marquez, G., Forster, 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.

Johansson-Lindbom, B., Svensson, M., Wurbel, M.A., Malissen, B., Marquez, G., and Agace, W. 2003. Selective generation of gut tropic T cells in gut-associated lymphoid tissue (GALT): requirement for GALT dendritic cells and adjuvant. *J Exp Med* 198, 963-969.

Kang, S.G., Lim, H.W., Andrisani, O.M., Broxmeyer, H.E., and Kim, C.H. 2007. Vitamin A metabolites induce gut-homing FoxP3⁺ regulatory T cells. *J Immunol* 179, 3724-3733.

Kusunoki, T., Sugai, M., Katakai, T., Omatsu, Y., Iyoda, T., Inaba, K., Nakahata, T., Shimizu, A., and Yokota, Y. 2003. TH2 dominance and defective development of a CD8⁺ dendritic cell subset in Id2-deficient mice. *J Allergy Clin Immunol* 111, 136-142.

Liu, K., Victora, G.D., Schwickert, T.A., Guermonprez, P., Meredith, M.M., Yao, K., Chu, F.F., Randolph, G.J., Rudensky, A.Y., and Nussenzweig, M. 2009. In vivo analysis of dendritic cell development and homeostasis. *Science* 324, 392-397.

Manicassamy, S., Ravindran, R., Deng, J., Oluoch, H., Denning, T.L., Kasturi, S.P., Rosenthal, K.M., Evavold, B.D., and Pulendran, B. 2009. Toll-like receptor 2-dependent induction of vitamin A-metabolizing enzymes in dendritic cells promotes T regulatory responses and inhibits autoimmunity. *Nat Med* 15, 401-409.

McLachlan, J.B., Catron, D.M., Moon, J.J., and Jenkins, M.K. 2009. Dendritic cell antigen presentation drives simultaneous cytokine production by effector and regulatory T cells in inflamed skin. *Immunity* 30, 277-288.

Merad, M., and Manz, M.G. 2009. Dendritic cell homeostasis. *Blood* 113, 3418-3427.

Milling, S., Yrlid, U., Cerovic, V., and MacPherson, G. Subsets of migrating intestinal dendritic cells. *Immunol Rev* 234, 259-267.

Miloud, T., Hammerling, G.J., and Garbi, N. 2010. Review of murine dendritic cells: types, location, and development. *Methods Mol Biol* 595, 21-42.

Monteleone, I., Platt, A.M., Jaensson, E., Agace, W.W., and Mowat, A.M. 2008. IL-10-dependent partial refractoriness to Toll-like receptor stimulation modulates gut mucosal dendritic cell function. *Eur J Immunol* 38, 1533-1547.

Mucida, D., Park, Y., Kim, G., Turovskaya, O., Scott, I., Kronenberg, M., and Cheroutre, H. 2007. Reciprocal TH17 and regulatory T cell differentiation mediated by retinoic acid. *Science* 317, 256-260.

Murai, M., Turovskaya, O., Kim, G., Madan, R., Karp, C.L., Cheroutre, H., and Kronenberg, M. 2009. Interleukin 10 acts on regulatory T cells to maintain expression of the transcription factor Foxp3 and suppressive function in mice with colitis. *Nat Immunol* 10, 1178-1184.

Naik, S.H., Metcalf, D., van Nieuwenhuijze, A., Wicks, I., Wu, L., O'Keeffe, M., and Shortman, K. 2006. Intrasplenic steady-state dendritic cell precursors that are distinct from monocytes. *Nat Immunol* 7, 663-671.

Niess, J.H., Brand, S., Gu, X., Landsman, L., Jung, S., McCormick, B.A., Vyas, J.M., Boes, M., Ploegh, H.L., Fox, J.G., et al. 2005. Cx3cr1-mediated dendritic cell access to the intestinal lumen and bacterial clearance. *Science* 307, 254-258.

Rescigno, M., Urbano, M., Valzasina, B., Francolini, M., Rotta, G., Bonasio, R., Granucci, F., Kraehenbuhl, J.P., and Ricciardi-Castagnoli, P. 2001. Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. *Nat Immunol* 2, 361-367.

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 Liu, Y.J. 2002. Mouse and human dendritic cell subtypes. *Nat Rev Immunol* 2, 151-161.

Sun, C.M., Hall, J.A., Blank, R.B., Bouladoux, N., Oukka, M., Mora, J.R., and Belkaid, Y. 2007. Small intestine lamina propria dendritic cells promote de novo generation of Foxp3 T reg cells via retinoic acid. *J Exp Med* 204, 1775-1785.

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.

Svensson, M., Marsal, J., Ericsson, A., Carramolino, L., Broden, T., Marquez, G., and Agace, W.W. 2002. CCL25 mediates the localization of recently activated CD8alpha(+) lymphocytes to the small-intestinal mucosa. *J Clin Invest* 110, 1113-1121.

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.

Uematsu, S., Jang, M.H., Chevrier, N., Guo, Z., Kumagai, Y., Yamamoto, M., Kato, H., Sougawa, N., Matsui, H., Kuwata, H., et al. 2006. Detection of pathogenic intestinal bacteria by Toll-like receptor 5 on intestinal CD11c+ lamina propria cells. *Nat Immunol* 7, 868-874.

Vallon-Eberhard, A., Landsman, L., Yogev, N., Verrier, B., and Jung, S. 2006. Transepithelial pathogen uptake into the small intestinal lamina propria. *J Immunol* 176, 2465-2469.

Varol, C., Landsman, L., Fogg, D.K., Greenshtein, L., Gildor, B., Margalit, R., Kalchenko, V., Geissmann, F., and Jung, S. 2007. Monocytes give rise to mucosal, but not splenic, conventional dendritic cells. *J Exp Med* 204, 171-180.

Varol, C., Vallon-Eberhard, A., Elinav, E., Aychek, T., Shapira, Y., Luche, H., Fehling, H.J., Hardt, W.D., Shakhar, G., and Jung, S. 2009. Intestinal lamina propria dendritic cell subsets have different origin and functions. *Immunity* 31, 502-512.

Voedisch, S., Koenecke, C., David, S., Herbrand, H., Forster, R., Rhen, M., and Pabst, O. 2009. Mesenteric lymph nodes confine dendritic cell-mediated dissemination of *Salmonella enterica* serovar Typhimurium and limit systemic disease in mice. *Infect Immun* 77, 3170-3180.

Wakim, L.M., Waithman, J., van Rooijen, N., Heath, W.R., and Carbone, F.R. 2008. Dendritic cell-induced memory T cell activation in nonlymphoid tissues. *Science* 319, 198-202.

Waskow, C., Liu, K., Darrasse-Jeze, G., Guermonprez, P., Ginhoux, F., Merad, M., Shengelia, T., Yao, K., and Nussenzweig, M. 2008. The receptor tyrosine kinase Flt3 is required for dendritic cell development in peripheral lymphoid tissues. *Nat Immunol* 9, 676-683.

Worbs, T., Bode, U., Yan, S., Hoffmann, M.W., Hintzen, G., Bernhardt, G., Forster, R., and Pabst, O. 2006. Oral tolerance originates in the intestinal immune system and relies on antigen carriage by dendritic cells. *J Exp Med* 203, 519-527.

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.

Table 1. Phenotypic and functional characteristics of $CD103^+CD11b^+$ and $Cx3cr1^{hi}$ cells in the murine small intestinal LP

		CD103⁺ CD11b⁺	Cx3cr1⁺ LP Cells	References
<i>Location in the small intestine</i>		LP, center of villus	LP, underlying epithelium	(Bogunovic et al., 2009; Jaensson et al., 2008; Niess et al., 2005; Schulz et al., 2009)
<i>Turnover rate in the LP</i>		+++	+	(Jaensson et al., 2008; Schulz et al., 2009; Varol et al., 2009)
<i>Putative precursors</i>		Pre-cDC	Ly6C ^{hi} Monocytes	(Bogunovic et al., 2009; Varol et al., 2009)
<i>In vivo growth factor requirements</i>	<i>Flt3L</i>	+++	-	(Bogunovic et al., 2009; Schulz et al., 2009)
	<i>GM-CSFL</i>	+++	-	(Bogunovic et al., 2009; Schulz et al., 2009)
	<i>M-CSFL</i>	-	++	(Bogunovic et al., 2009)
<i>Trans-epithelial dendrite formation</i>		?	++	(Niess et al., 2005; Vallon-Eberhard et al., 2006)
<i>Migration to draining lymph nodes</i>		+++	-	(Jaensson et al., 2008; Jang et al., 2006; Johansson-Lindbom et al., 2005; Schulz et al., 2009; Worbs et al., 2006)
<i>Efficiency in naïve T cell priming in vitro</i>	<i>CD4⁺ T cells</i>	++	+	(Schulz et al., 2009)
	<i>CD8⁺ T cells</i>	+++	+	(Schulz et al., 2009)
<i>Aldehyde dehydrogenase activity</i>		+++	+	(Guilliams et al., 2010; Schulz et al., 2009)
<i>In vitro induction of gut homing receptors</i>		+++	+	(Jaensson et al., 2008)

