

receptors undergo change in diffusion coefficient. This was demonstrated by labeling cells with IgEs with different specificities (dansyl and DNP). Antigen-containing DNP could not cause a change in diffusion of IgE specific for Dansyl. The second observation they made was that cells treated with inhibitors of Src family kinases showed no change in the assembly of signaling complexes or their diffusion characteristics. This result is very similar to what has been observed for TCR microclusters; however, the same may not be true for BCR signaling (Fleire et al., 2006; Tolar et al., 2005).

The data presented here have important implications in understanding signaling via ITAM-containing receptors in the immune system. TCR and BCR under-

go microcluster formation upon encountering antigen and is also the site where antigen accumulates. This paper raises the possibility that transient interactions between antigen receptors and their ligands outside of microclusters that do not cause receptor clustering or immobilization may still generate signals or that microcluster formation succeeds such transient interactions. In the future, progress in imaging technology and computer modeling of microscopy data is likely to illuminate this world of signaling.

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Before They Were Gut Dendritic Cells

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Gut lamina propria dendritic cell (DC) subsets have specialized functions. In this issue of *Immunity*, Varol et al. (2009) and Bogunovic et al. (2009) describe the different blood precursors for CD103⁺ and CD103[−] DC subsets.

How the intestine can tolerate trillions of intestinal bacteria, initiate tolerance toward food antigens, and fight infections is the subject of an intense area of research. Recent advances have highlighted a fundamental role of dendritic cells (DCs) in these functions. In the mouse lamina propria (LP), DCs are characterized by the high expression of CD11c and can be distinguished based on the expression of the integrin CD103, which recognizes E-cadherin. CD103⁺ DCs can drive the differentiation of T regulatory (Treg) cells, the effectors of tolerance, via a mechanism that is mediated by retinoic acid (RA) and TGF- β (Sun et al., 2007). In contrast, CD103[−] DCs can be subdivided into two subsets. One subset expresses CX₃CR1 and drives the development of T helper 17 (Th17) cells (Atarashi et al., 2008). The other

subset expresses the Toll-like receptor 5 (TLR5) and drives the differentiation of both Th17 cells and IgA class-switched B cells (Uematsu et al., 2008). However, it was not clear whether these DCs represented different activation states of the same subset (expressing or not the CD103) or whether they represented two different subsets arising from distinct blood precursors.

In this issue of *Immunity*, two independent reports by Varol et al. (2009) and Bogunovic et al. (2009) describe that the CD103⁺ and CD103[−] DC subsets are further distinguishable based on the expression of CD11b and CX₃CR1 and derive from different bone marrow precursors. By using very elegant model systems based on conditional ablation of DCs and engraftment with defined DC precursors, the two groups demonstrate that a

common macrophage and DC precursor (MDP) can give rise to both CD103⁺ and CD103[−] DCs, whereas pre-DCs or monocytes can differentiate only into CD103⁺ or CD103[−] DCs, respectively (Figure 1). These studies also describe the different requirements of growth factors for the differentiation of the two major DC subsets. Both reports show a common requirement of Flt3L for the differentiation of CD11b⁺CD103⁺ and CD11b[−]CD103⁺ DCs whereas Varol et al. (2009) show that GM-CSF seems to be more involved in the differentiation of CD11b⁺ (mostly CD103[−]) DCs. In contrast, Bogunovic et al. (2009) find that GM-CSF expression is dispensable for CD103[−] DC differentiation, whereas it is required for the differentiation of CD11b⁺CD103⁺ and CD11b[−]CD103⁺ DCs. Bogunovic et al. (2009) also demonstrate a requirement

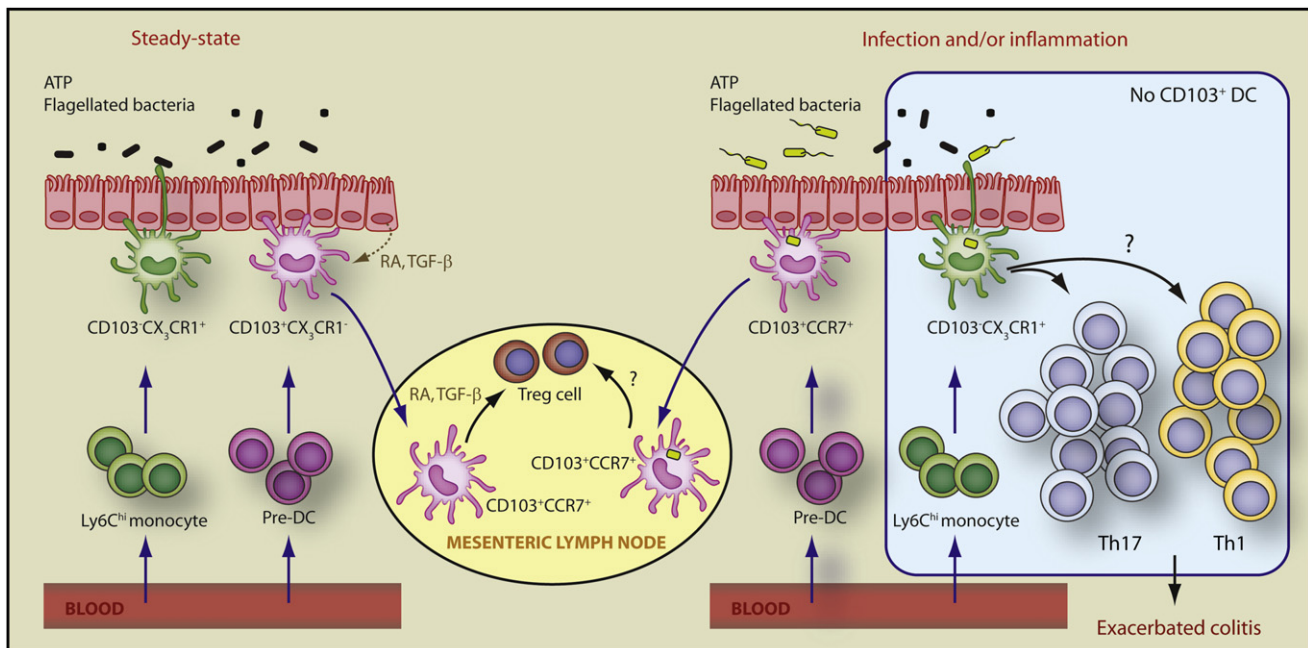


Figure 1. Origin and Function of Lamina Propria DCs

Under steady-state conditions, blood DC precursors give origin to lamina propria (LP) DCs. Ly6C^{hi} monocytes differentiate into CD103⁻CX₃CR1⁺ DCs presumably under the influence of ATP-producing or flagellated bacteria and induce Th17 cell differentiation. In contrast, pre-DCs give rise to CD103⁺ DCs in the presence of RA and TGF-β released by epithelial cells. CD103⁺ DCs are tolerogenic and induce Treg cell differentiation after their migration to the mesenteric lymph node. During infection, even though both CD103⁺CX₃CR1⁻ and CD103⁻CX₃CR1⁺ DCs have taken up *Salmonella*, CD103⁺ DCs are the first to migrate to the MLN. It is not known the function of these DCs, or whether they still induce Treg cells. When CD103⁺ DCs are lacking in mice deleted for DCs and reconstituted only with Ly6C^{hi} monocytes (blue box), mice are more susceptible to DSS colitis. This may be due to the capacity of CX₃CR1⁺ DCs to polarize Th17 cells. Alternatively, and not mutually exclusive, inflammatory monocytes may drive the differentiation of Th1-cell-inducing DCs.

of M-CSFR for the differentiation of the CD103⁻ population. This apparent discrepancy may be due to the different way used by the two groups to phenotypically separate the two cell types. In both cases, the cells were separated based on the high expression of CD11c expression, but whereas Varol et al. (2009) report that they separated the cells based on the expression of CD11b and CX₃CR1, Bogunovic et al. (2009) report that they separated the DCs based on the expression of MHC class II and CD11b. In addition, as shown by Bogunovic et al. (2009), different compartments of either the small or large intestine (i.e., the intestinal epithelial cell fraction, the LP, the serosa, or the muscularis) yielded different DC populations, including a contaminating lymphoid population characterized by the expression of CD103 and the absence of CD11b. Hence, it is likely that the same progenitor may require different growth factors or combination of growth factors for their differentiation depending on the compartment that is analyzed.

The different origin of the DC populations reflects differences in DC function.

When the mesenteric lymph node (MLN) is analyzed, it is clear that again that the DC populations can be distinguished based on CD103 expression; however, whereas the CD103⁺ population expresses CCR7 and seems to be derived from a migratory LP CD103⁺ DC population, the CD103⁻ DC population seems to be a resident nonmigratory cell type. Indeed, only 20% of MLN CD103⁻ DCs express high CX₃CR1, suggesting a different origin than LP CD103⁻ DCs that uniformly express CX₃CR1. This is an intriguing observation because it indicates that under steady-state condition, only CD103⁺ DCs can migrate into the MLN. This population is required for the differentiation of Treg cells (Coombes et al., 2007), and therefore it could be involved in tolerance induction to food antigens. What was unexpected is that CD103⁺CX₃CR1⁻ DCs are also required for the initial transport of *Salmonella* from the intestine to the MLN (Bogunovic et al., 2009). Indeed, it has been shown that DCs can extend protrusions across the epithelial barrier (Rescigno et al., 2001), and CX₃CR1 expression is required

for DC extensions (Niess et al., 2005). Hence it would be more logical to think that CD103⁻CX₃CR1⁺ DCs are the first to migrate to MLNs and to carry ingested microbes. In the model used by Bogunovic et al. (2009), mice are treated with streptomycin and this facilitates *Salmonella* entrance across epithelial cells. Thus, it is likely that CD103⁻ DCs acquire *Salmonella* after their independent crossing across the epithelial barrier. However, it cannot be excluded that CD103⁺ DCs may also extend their protrusions across the epithelial barrier because we have observed that DC extensions in CX₃CR1-GFP and MHC II-GFP mice are only partly overlapping (unpublished observations), suggesting that two populations of DCs may extend protrusions in the lumen. Alternatively, *Salmonella* may be transferred from CD103⁻CX₃CR1⁺ DCs to CD103⁺CX₃CR1⁻ DCs but this has not been demonstrated. Regardless, it appears that CD103⁺ DCs may be the first cells migrating to the draining MLN both under steady-state and under infection conditions. It is not clear what is the

function of these migrating CD103⁺ DCs carrying the *Salmonella* into the MLN given their propensity to drive Treg cell development (Coombes et al., 2007). These cells could be exploited by *Salmonella* for its own spreading in the absence of productive immunity, but this remains to be established.

Varol et al. (2009) have instead focused on the role of monocyte-derived CD103⁺CX₃CR1⁺ DCs. By conditionally ablating mice of DCs and reconstituting them with monocytes that give rise only to CD103⁺CX₃CR1⁺ DCs, they have shown that these DCs are sufficient to restore the internalization of noninvasive *Salmonella* and to present orally administered antigens. However, it remains to be established what is the outcome of this presentation, in terms of T cell activation and polarization. Varol et al. (2009) also show that when mice display only CD103⁺CX₃CR1⁺ DCs, they are more susceptible to DSS-induced colitis. This may be the result of at least two non-mutually exclusive possible scenarios. The first is that CD103⁺ DCs are required to maintain tolerance in the gut because they drive the development of Treg cells that may be involved in preserving tissue integrity or limiting inflammatory damage. In the absence of CD103⁺ DCs, there may be a decrease in Treg cell differentiation and a concomitant increase in the development of colitogenic Th17 cells as indicated by the fact that CD103⁺CX₃CR1⁺ DCs have been shown to drive Th17 cell development in the presence of bacteria-derived ATP (Atarashi et al., 2008). Hence, the balance between CD103⁺ and CD103⁺ DCs is required to preserve gut homeostasis. The second possibility may be the capacity of the local microenvironment to control DC function and to inhibit the development of inflammatory Th1 cell-type of responses (Iliev et al., 2009). Indeed, resident LP DCs are unable to produce IL-12 and to drive Th1 cell-type of responses because they either lack TLR expression (except for TLR5) or they are unresponsive to TLR stimulation (Monteleone et al., 2008). It is possible that CD103⁺CX₃CR1⁺ DCs arising from the transferred monocytes may not have time to be conditioned by the local microenvironment in the absence of CD103⁺ DCs and become "inflammatory" leading to Th1 cell differ-

entiation. Additional studies are required to address this issue.

It remains to be understood whether monocytes are recruited at all during steady-state. Bogunovic et al. (2009) show that in the absence of DC ablation, monocytes are unable to repopulate the LP and to give rise to CD103⁺ DCs. This could be due to the low turn-over of CD103⁺ DCs in the LP because in mice connected by parabiosis, only the CD103⁺ population is replenished by parabiont-derived cells, whereas the CD103⁺ population is not. Alternatively, it could be due to the proliferation of either the monocytes or the differentiated CD103⁺ DCs directly in situ (Jaensson et al., 2008). In contrast, DC ablation could lead to the elimination of both cell types and to the need to reconstitute the DC pools by recruitment of blood precursors.

What is the function of CX₃CR1⁺ DCs? One possibility would be that they are responsible to confer an initial protection toward commensals and pathogenic microorganisms via the induction (or re-stimulation) of Th17 cells. Alternatively, these cells may migrate at later time points after infection and may be responsible for the initiation of Th17 cell differentiation in the draining lymph nodes. Another intriguing possibility is that CD103⁺CX₃CR1⁺ DCs offer only an initial defense whereas the cells responsible for the initiation of a protective immune response toward *Salmonella* are inflammatory monocytes that are recruited after infection. These recruited monocytes may differentiate into an additional inflammatory type of DCs that can initiate Th1 cell immune responses to *Salmonella*.

Another important question that arises from the present studies is whether the progenitor cells are intrinsically committed to become CD103⁺ or CD103⁺ cells and to acquire their mucosal specialized functions or whether these characteristics are extrinsically conferred by the local microenvironment. It has been shown that it is possible to "condition" bone-marrow-derived DCs into the CD103⁺ phenotype of mucosal DCs. In particular, the direct interaction with epithelial cells drives the upregulation of CD103 whereas soluble factors released by epithelial cells, including RA and TGF- β , mediate the acquisition of tolerogenic properties (Figure 1; Iliev et al.,

2009). In this context, Bogunovic et al. (2009) have shown that CD103⁺ DCs are often associated with epithelial cells. In contrast, it is possible that the phenotype of CX₃CR1⁺ DCs is conferred directly by the luminal bacteria as indicated by the fact that bacteria-derived ATP is required to drive Th17-cell-inducing DCs (Atarashi et al., 2008). Hence different environmental conditions may confer the properties to CD103⁺ and CD103⁺ DCs.

In conclusion, at least two different blood precursors differentiate into distinct LP DC subsets in response to specific growth factors. The challenge is to identify the molecular pathways of this differentiation and where it occurs.

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