Dendritic cells in intestinal immune regulation

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Abstract | A breakdown in intestinal homeostasis can result in chronic inflammatory diseases of the gut including inflammatory bowel disease, coeliac disease and allergy. Dendritic cells, through their ability to orchestrate protective immunity and immune tolerance in the host, have a key role in shaping the intestinal immune response. The mechanisms through which dendritic cells can respond to environmental cues in the intestine and select appropriate immune responses have until recently been poorly understood. Here, we review recent work that is beginning to identify factors responsible for intestinal conditioning of dendritic-cell function and the subsequent decision between tolerance and immunity in the intestine.

The gastrointestinal tract represents an important entry site for pathogens. It is also home to a large number and diverse array of commensal bacteria, many of which are beneficial to the host. A key feature of the intestinal immune system is its ability to protect against infection while avoiding the development of destructive inflammatory responses to the normal microbiota. Understanding the mechanisms that control intestinal homeostasis is an active area of research. It is hoped that uncovering the pathways used by the intestinal immune system to prevent immune pathology may direct therapeutic approaches to a broad range of autoimmune and inflammatory conditions. In addition, overcoming default pathways that maintain tolerance in the intestine may be beneficial to the development of oral vaccines.

Of particular interest is the role of populations of dendritic cells (DCs) in the intestine and associated lymphoid tissues. These cells have been implicated both in the maintenance of tolerance towards the commensal microflora, and in the generation of protective immune responses against pathogens. This impressive flexibility in function is probably due to an ability to accurately sense their local environment and use these signals to shape the nature of the ensuing immune response. We are now beginning to understand some of the unique functional properties of populations of intestinal DCs, and also the type of signals that are required for them to mediate these functions. Here, we discuss intestinal DC function in the steady state, the requirements for a shift towards the generation of protective immune responses, and the potential role for DCs in the pathogenesis of inflammatory bowel disease (IBD).

Antigen-presenting cells of the intestine

The intestine and associated lymphoid tissues are home to an extensive network of innate immune cells with antigen-presenting function, including macrophages, conventional CD11chi DCs and plasmacytoid DCs (pDCs)1,2. Adaptations to the intestinal environment that prevent the generation of destructive inflammatory responses have been demonstrated for all of these populations. Nevertheless, these cells also have well described roles in protection against enteric pathogens. The divergent functional properties of different populations of intestinal DCs and macrophages have now begun to be dissected.

Various subpopulations of DCs are present in the organized lymphoid structures of the intestinal immune system, including the Peyer’s patches and mesenteric lymph nodes (MLNs), and throughout the small intestinal and colonic lamina propria (reviewed in Refs 1,3 (BOX 1)). In the steady state, the functional properties of DCs appear to vary according to their anatomical location. The clearest differences are observed between DCs of the intestine and spleen. For example, activated DCs from the Peyer’s patches produce higher levels of interleukin-10 (IL-10) than splenic DCs4. Furthermore, naive CD4+ T cells activated by DCs from the Peyer’s patches produce higher levels of IL-4 and IL-10, indicative of a Th helper 2 (T2)-type phenotype, than those activated by splenic DCs4. Functional differences are also observed between DCs from the Peyer’s patches, from MLNs and from the small intestinal and colonic lamina propria. For example, delayed-typed hypersensitivity responses could be inhibited by the adoptive transfer of DCs from the
these subpopulations. For example, the CD11b+ subset of DCs from the Peyer’s patches has a higher capacity to produce IL-10 and prime T_{H2} cells than the other subsets, whereas CD8α+ and CD11b+CD8α− DCs from the Peyer’s patches were shown to produce IL-12 and drive the production of interferon-γ (IFNγ) by T cells. Similarly, CD103− MLN DCs are superior to their CD103+ (also known as α6-integrin)+ counterparts in promoting IFNγ production by T cells. Again, this could reflect developmental differences between the DC subsets, or minor alterations in their environment. As such, CD11b+ DCs are concentrated largely in the subepithelial dome of the Peyer’s patches, whereas CD8α+ DCs are found in the inter-follicular region.

DCs are also recruited to the intestine during inflammation. Whether these cells represent a separate lineage to those in the steady state, or whether their ability to drive pro-inflammatory responses is a function of their exposure to both pathogens and pro-inflammatory cytokines on arrival in the intestine remains unclear. Alternatively, these cells may simply represent a population of DCs that is already present in the steady state but that becomes more dominant during inflammation.

Intestinal macrophages also display some distinctive characteristics compared with splenic macrophages or those that derive from blood monocytes. Although human intestinal macrophages retain phagocytic and bactericidal activity, they lack CD14 expression, which is required for the Toll-like receptor 4 (TLR4)-mediated recognition of ligands. Accordingly, when cultured with TLR4 ligands, and also with a range of other stimuli, these cells showed an impaired ability to produce pro-inflammatory cytokines. These modifications might contribute to intestinal immune homeostasis by ensuring that contact of intestinal antigen-presenting cells (APCs) with microbial products does not automatically result in the generation of potentially destructive inflammatory responses.

**Box 1 | Dendritic-cell subsets in the intestine**

Dendritic cells (DCs) are often classified into subsets on the basis of cell-surface receptor expression. In the Peyer’s patches, conventional DCs are predominantly of the CD11c+CD11b−CD8α−, CD11c+CD11b+CD8α− and CD11c+CD11b+CD8α+ subtypes, with unique functional properties and anatomical localization described for each subset. DCs from the Peyer’s patches can also be described in terms of their expression of the chemokine receptors CX, C-chemokine receptor 1 (CX, CR1) and CC-chemokine receptor 6 (CCR6). CX, CR1+ DCs were found to be closely associated with the follicle-associated epithelium in the steady state, whereas CCR6+ DCs, which fall largely into the CD11b−CD8α− and CD11b−CD8α+ Peyer’s patch DC populations, were recruited from the subepithelial dome to the follicle-associated epithelium during infection.

Small-intestinal lamina propria DCs have been described to be similar in subset composition to Peyer’s patch DCs, although the presence of conventional CD8α− DCs in the lamina propria appears to be a contentious issue. DCs in the small-intestinal lamina propria were also found to express CX, CR1. In the colon, DCs appear to be concentrated largely within isolated lymphoid follicles, with very few present in the lamina propria under steady-state conditions. A substantial proportion of both colonic and small-intestinal lamina propria DCs express the integrin subunit CD103 (also known as α6-integrin) in the colonic lamina propria to the mesenteric lymph nodes (MLNs) has been studied by cannulating the thoracic duct lymph of mesenteric lymphadenectomized rats. Using this technique, DCs migrating in lymph were found to be better stimulators of mixed leukocyte reaction than DCs in the lamina propria, and therefore probably represent a more mature population. MLNs are also home to populations of CD11c+CD11b+CD8α−, CD11c+CD11b+CD8α− and CD11c+CD11b+CD8α− DCs. They contain both migratory DCs arriving from the intestinal lamina propria in the steady state, and resident DCs that have developed from blood-borne precursors. Expression of the integrin subunit CD103 has been reported by CD11c+ DCs isolated from the MLNs and is likely to mark migratory DCs arriving from the intestine. Consistent with this, CCR7−deficient mice also show a reduced frequency of CD103− DCs in the MLNs. Conversely, CD103+ DCs in the MLNs may arrive as precursors from the blood, as indicated by their expression of the lymph-node homing receptor CCL2 (also known as l-selectin).

An additional population of CD11c−plasmacytoid DCs (pDCs) are also present in the Peyer’s patches and MLNs. pDCs cannot however be detected in the lymph, suggesting they do not participate in migration from the intestine to the MLNs.
and CX<sub>3</sub>CR1<sup>+</sup>Gr1<sup>−/−</sup>Ccr2<sup>−/−</sup> monocytes in the bone marrow<sup>11</sup>. The latter subset has been proposed to migrate to the peripheral tissues under steady-state conditions, and, consistent with this, CX<sub>3</sub>CR1<sup>+</sup>Ccr2<sup>−/−</sup> monocytes in rats give rise to a small proportion of DCs migrating from the intestine in the steady state<sup>15,16</sup>. The reciprocal CX<sub>3</sub>CR1<sup>−/−</sup>Gr1<sup>−/−</sup>Ccr2<sup>−/−</sup> subset is suggested to give rise to DCs in the peripheral tissues under inflammatory conditions, although Gr1<sup>−/−</sup> monocytes may also give rise to steady-state migratory DCs<sup>14,15</sup>. Therefore, the origins of migratory DCs present in the tissues in the steady state and during inflammation remain unclear. CD11<sup>+</sup>LIN<sup>−</sup> CX<sub>3</sub>CR1<sup>+</sup> precursor cells and their monocyctic intermediaries also gave rise to intestinal macrophages<sup>14</sup>.

**Sampling of antigen by intestinal DCs.** DCs can pick up antigen that has been transported across the intestinal epithelium through various different routes. First, specialized M cells (microfold cells) that are present in the follicle-associated epithelium of the Peyer’s patches can transcytose luminal antigen, which is then taken up by nearby DCs. Second, antigen may be transported into the intestinal lamina propria through a mechanism involving the neonatal F<sub>c</sub> receptor for IgG<sup>17,18</sup>. Third, DCs can sample antigen directly from the intestinal lumen by forming tight-junction-like structures with intestinal epithelial cells (IECs)<sup>19</sup> and projecting dendrites through the epithelial-cell layer and into the lumen. It is possible that this process contributes to the sampling of antigen from the commensal microflora, as DC extensions are readily detected under normal conditions<sup>20,21</sup>. Nevertheless, the presence of invasive bacterial species increases the frequency of transepithelial projections, particularly in the terminal ileum<sup>19–21</sup>. The projection of dendrites across the intestinal epithelium is thought to require myeloid differentiation primary-response gene 88 (MyD88)-dependent signalling through Toll-like receptors and the expression of CX<sub>3</sub>CR1<sup>1</sup> abrogates the increase in transepithelial projections that are seen in the terminal ileum during infection, they still occur in more proximal regions of the small intestine<sup>20</sup>. In fact, it has recently been demonstrated that disruption of E-cadherin-mediated DC clustering initiates a programme of DC maturation that is distinct from that driven by microbial products and leads to the generation of tolerogenic DCs<sup>27</sup>. However, the factors involved in triggering the loss of E-cadherin interactions have yet to be defined, and it remains to be established whether this pathway contributes to DC migration in the intestine. Alternatively, constitutive low-level production of pro-inflammatory cytokines might be sufficient to stimulate DC migration<sup>28,29</sup>.

DCs that have migrated to the MLNs in the steady state, or are present in the Peyer’s patches, can interact with B and T cells and initiate responses aimed at maintaining a non-inflammatory state in the intestine. Currently of particular interest is the ability of intestinal DCs to promote the development of forkhead box P3 (FOXP3<sup>−/−</sup>) regulatory T cells (T<sub>reg</sub>) in the periphery<sup>30–34</sup>. It has recently been demonstrated that the gut-associated lymphoid tissue is a preferential site for the peripheral induction of FOXP3<sup>−/−</sup> T<sub>reg</sub> cells<sup>30</sup>. The ability to generate FOXP3<sup>−/−</sup> T<sub>reg</sub> cells from naive T cells might be of particular importance in the intestine, as it could provide a mechanism by which the thymically derived pool of CD4<sup>+</sup>CD25<sup>+</sup>FOXP3<sup>−/−</sup> T<sub>reg</sub> cells could be complemented with FOXP3<sup>−/−</sup> T<sub>reg</sub> cells specific for commensal bacteria or dietary antigens. Alternatively, it is possible that the T<sub>reg</sub>-cell repertoire does not need to be extended per se, but that diversion of naive T cells that are strongly reactive to innocuous antigen to the T<sub>reg</sub>-cell lineage is a useful mechanism to prevent them from inducing pathology at a later stage.

DCs from the lamina propria of the small intestine and from MLNs have been shown to be significantly better than splenic DCs at inducing the expression of FOXP3 in naive T cells in the presence of exogenous transforming growth factor-β (TGF-β)<sup>30,31</sup>. Furthermore, CD103<sup>+</sup> DCs isolated from the MLNs are capable of...
mediating, through TGFβ, the conversion of naive T cells into FOXP3+ T cells in the absence of any exogenous factors. However, this property was not common to all MLN DCs, as CD103- DCs did not promote the expression of FOXP3, and even in the presence of exogenous TGFβ only promoted FOXP3 expression in a small proportion of T cells. This may reflect the idea that CD103- MLN DCs are derived from the intestinal lamina propria, whereas CD103+ DC may not be. Furthermore, it is unclear whether populations of DCs from the Peyer’s patches can also perform this function. In contrast to these reports, Denning et al. suggest that it is the macrophages from the lamina propria of the small intestine and not DCs that induce the differentiation of FOXP3+ Treg cells from naive T cells. However, the site at which this interaction would occur in a physiological setting remains to be clarified.

An important question is what the source of TGFβ for intestinal DC-mediated induction of FOXP3 expression by T cells may be. It is possible that these DCs produce active TGFβ in response to signals in the local environment, or that they mediate the activation of latent TGFβ (REF. 53). In this respect, a recent study has suggested that the expression of αβ integrin by DCs is important for the activation of TGFβ, the accumulation of FOXP3+ Treg cells in the intestine and the prevention of colitis. Furthermore, loss of αβ integrin expression by myeloid cells led to the development of intestinal inflammation, probably through the combined effects of a failure to remove apoptotic cells and a loss of TGFβ activation. Both these studies reported an impaired ability of DCs to promote FOXP3 expression by naive T cells, suggesting that local activation of latent TGFβ by DCs is important for the peripheral induction of FOXP3+ T cells. However, intestinal inflammation could also have resulted from a loss of TGFβ-mediated control of effector T-cell function.

pDCs present in the MLNs might also have a role in the differentiation of regulatory populations of CD4+ T cells. CD8α+ pDCs isolated from the MLNs promoted the differentiation of naive CD4+ T cells into T regulatory 1 (T1) cells with suppressive properties.

DCs also have an important role in dictating the homing potential of recently activated T cells. DCs isolated from the Peyer’s patches, small-intestinal lamina propria and MLNs promote the expression of the gut-homing receptors αβ integrin and CCR9 by CD4+ and CD8+ T cells. In the MLNs the ability to drive the expression of gut-homing receptors by both CD4+ and CD8+ T cells was enriched in the CD103+ DC subset. CCR9 binds to CCL25 produced by epithelial cells of the small intestine and αβ integrin binds to mucosal vascular addressin cell-adhesion molecule 1 (MADCAM1), which is expressed by the vascular endothelium of the gastrointestinal tract. Both MADCAM1 and CCL25 are constitutively expressed, indicating a constitutive migration of T cells into the intestinal lamina propria, which could be attributed to the high antigen load at these sites and the consequent need to attract Treg cells. Consistent with this, DCs could also promote the expression of gut-homing receptors by thymically derived CD4+CD25-FOXP3+ Treg cells.

DCs have also been implicated in class switching to IgA, the predominant isotype at mucosal surfaces. Mechanisms of class switching to IgA are complex and likely to vary depending on the site at which it occurs, the type of B cell, dependence on T cells, and the presence of commensal versus pathogenic species. Nevertheless, a clear role for DCs from the Peyer’s patches in class switching to IgA has been demonstrated. Consistent with this, populations of DCs in the intestine produce various cytokines and other mediators implicated in class switching to IgA, including IL-10, TGFβ, IL-6 and APRIL (a proliferation-inducing ligand).

Role of vitamin A in intestinal DC function. A remarkable aspect of the ability of intestinal DCs to upregulate homing receptors on lymphocytes, to drive the peripheral generation of FOXP3+ Treg cells and to support class switching to IgA is that retinoic acid, the acid form of vitamin A, has an important role in these processes. The observation that DCs from the Peyer’s patches and MLNs used retinoic acid to imprint T cells with gut-homing potential provided a mechanism for this. Consistent with this finding, retinoic acid has also been shown to promote the expression of gut-homing receptors by B cells and by CD4+CD25+ Treg cells.

It has been appreciated for some time that administration of retinoic acid has beneficial effects in autoimmune disease settings. It is possible that some of these effects are attributable to the reported function of retinoic acid in regulating T helper 1- and T helper 2-cell differentiation, such that in vitamin A deficiency the balance is shifted in favour of the generation of T helper 1-type responses. Indeed, the propensity of CD11b+ DCs from the Peyer’s patches to drive T helper 2-cell responses may be mediated by retinoic acid.

Another possible explanation for the beneficial effect of retinoic acid in autoimmune disease comes from a cluster of recent studies demonstrating that retinoic acid enhances the TGFβ-mediated generation of FOXP3+ Treg cells from naive peripheral T cells. Notably, the induction of FOXP3 expression observed in the presence of small-intestinal lamina propria DCs and CD103+ MLN DCs could be inhibited by a retinoic-acid receptor (RAR) antagonist. In addition, culturing splenic or CD103+ MLN DCs with both TGFβ and retinoic acid enhanced the generation of FOXP3+ T cells. Retinoic acid is also an important cofactor for the differentiation of FOXP3+ Treg cells in the presence of intestinal macrophages. Importantly, retinoic acid has a positive effect on the expression of FOXP3 by human CD4+ T cells. Related to this, retinoic acid has also been shown to inhibit the generation of T helper 17 cells. In the presence of TGFβ and IL-6, MLN DCs (through production of retinoic acid) were impaired in their ability to drive IL-17 secretion by T cells. These findings suggest that...
the therapeutic manipulation of the levels of retinoic acid has the potential not only to enhance regulatory pathways, but to directly inhibit the generation of inflammatory CD4+ T-cell populations.

Vitamin A levels are closely linked to the magnitude of IgA responses in the intestine, with vitamin A deficiency leading to reduced levels of IgA56. Consistent with these findings, it has recently been demonstrated that the ability of DCs from the Peyer's patches to promote T-cell-independent class switching to IgA can be attributed to the combined effects of retinoic acid and IL-5 or IL-6 (Ref. 54).

Synthesis of retinoic acid from stored or dietary retinol occurs in a two-step reaction consisting of the oxidation of retinol to retinal, and the subsequent oxidation of retinal to retinoic acid58. The ability of a cell to catalyse this reaction depends on the expression of the appropriate enzymes, with the final step being catalysed by retinal dehydrogenases, such as aldehyde dehydrogenase family 1, subfamily A1 (ALDH1A1) and ALDH1, subfamily A2 (ALDH1A2). Although some of the functional properties of intestinal DCs have been shown to be dependent on retinoic acid, it remains unclear whether the DCs themselves are responsible for its production. In support of this idea, Peyer's patch and MLN DCs have been shown to express Aldh1a1 and Aldh1a2, respectively53. Furthermore, consistent with their functional properties, MLN CD103+ DCs express higher levels of Aldh1a2 than MLN CD103- DCs53. Most importantly, Peyer's patch and MLN DCs could convert retinol to retinoic acid in culture, particularly in the presence of T cells53.
However, similar experiments examining the induction of FOXP3 expression by intestinal lamina propria DCs showed that citral had no effect on FOXP3 expression\(^{36}\). This discrepancy could be explained by a contribution of other cell types to the synthesis of retinoic acid, and by the ability of DCs to store retinoic acid\(^{40}\). In fact, small-intestinal epithelial cells also express ALDH1A1, indicating that they can produce retinoic acid, which can be taken up and transported by DCs\(^{69,60}\).

The functional properties of retinoic acid are mediated through ligation of heterodimers of the RAR and retinoid X receptor families. Accordingly, CD103\(^{+}\) DCs were better than their CD103\(^{-}\) counterparts at inducing the early RAR signalling that led to the expression of gut-homing receptors by T cells\(^{61}\). RARs function as ligand-dependent transcription factors and bind RAR elements (RAREs) or retinoid X response elements (RXREs) in the promoter regions of target genes\(^{62}\). Hundreds of different genes have been suggested to be either direct or indirect targets of receptor-bound retinoic acid, providing an explanation of how it may initiate, augment or inhibit the diverse programmes of differentiation in which it has been implicated.

However, retinoic acid has little effect on FOXP3 expression in isolation, bringing further complexity as to how it enhances the TGF\(\beta\)-mediated induction of FOXP3. In this regard, cooperation between the TGF\(\beta\) and retinoic-acid pathways has been documented, with retinoic-acid increasing expression of TGF\(\beta\) receptor subunits\(^{117}\). Furthermore, a direct interaction between RAR family members and SMAD3 (mothers against decapentaplegic homologue 3) has been proposed, although it remains unclear whether this interaction positively or negatively regulates TGF\(\beta\) signalling\(^{118,119}\).

Retinoic-acid treatment also leads to a reduction in IFN\(\gamma\) production by T cells\(^{62}\). This is consistent with the finding that T cells produce less IFN\(\gamma\) when cultured with CD103\(^{+}\) DCs\(^{5}\). As IFN\(\gamma\) is known to induce SMAD7, which inhibits TGF\(\beta\) signalling, one possible consequence of reduced IFN\(\gamma\) production may be an increase in TGF\(\beta\) signalling and ultimately in the expression of FOXP3.

Retinoic acid may also indirectly enhance the TGF\(\beta\)-mediated induction of FOXP3 through the direct regulation of Il2ra (also known as Cd25). One report has suggested that IL-2 signalling was required for the induction of FOXP3 in the presence of both TGF\(\beta\) and retinoic acid; however, a subsequent study demonstrated that TGF\(\beta\) and retinoic acid could still induce FOXP3 in T cells that lack the IL-2 signalling component Stat5 (signal transducer and activator of transcription 5) or in the presence of blocking antibodies to IL-2 (REFS 31, 57).

Finally, co-stimulation through CD28 impairs TGF\(\beta\)-mediated induction of FOXP3 expression in naive T cells, whereas retinoic acid can overcome this effect\(^{61}\). CD28-induced signals synergize with T-cell receptor signals to activate the transcription factor activator protein 1 (AP1)\(^{64}\). Complexes of nuclear factor of activated T cells (NFAT) and AP1 regulate the expression of genes associated with T-cell activation, including Il2. However, NFAT can also interact with FOXP3 to inhibit the expression of genes that are normally regulated by AP1–NFAT complexes, and induce genes that are important for T\(_{reg}\)-cell function\(^{65}\). Interestingly, ligand-bound RARs can inhibit the transcriptional activity of AP1, perhaps by forming complexes with AP1 subunits\(^{66}\). In doing so, retinoic acid may directly interfere with the negative effects of co-stimulation on FOXP3 induction. Furthermore, retinoic acid may promote the formation of FOXP3–NFAT complexes by limiting competition by AP1. FOXP3 has been proposed to act in an autoregulatory loop by downregulating SMAD7 expression and allowing for enhanced TGF\(\beta\) signalling\(^{67}\).

**Influence of the environment on intestinal DC function.** In light of the unique functional properties described for intestinal DCs, an important question is how they are acquired. Recent evidence suggests that the conditioning of DCs in their local tissue environment, rather than the existence of functionally distinct DC subsets, has an important role in shaping their function (FIG. 2). Communication between the intestinal epithelium and DCs is likely to be integral to this type of conditioning\(^{68}\). IECs show qualitatively distinct responsiveness to commensal and pathogenic bacterial species, and therefore the epithelial-cell layer may act as a sensor for current environmental conditions and instruct nearby DCs accordingly. In fact, following *in vitro* co-culture with an epithelial-cell line, human DCs preferentially induced non-inflammatory T\(_{H2}\)-type responses\(^{69}\), which is consistent with the fact that isolated mouse CD11b\(^{+}\) DCs from the Peyer’s patches also promote the differentiation of T\(_{H2}\) cells\(^{65}\). This effect was mediated in part by production of thymic stromal lymphopoietin (TSLP) by the epithelial cells, which can be increased in response to bacterial stimulation and nuclear factor-\(\kappa\)B (NF-\(\kappa\)B) activation\(^{49,69,70}\). However, these cells behave *in vivo* will be influenced by a wide range of different factors. Nevertheless, an important role for TSLP in dictating the quality of the immune response *in vivo* has recently been suggested; an increased number of CD11b\(^{+}\) and CD11b\(^{-}\)CD80\(^{+}\) DCs expressing IL-12/23p40 are present in the MLNs of mice deficient in the TSLP receptor (TSLPR)\(^{70}\).

TSLP has also been shown to confer human thymic DCs with the ability to induce the differentiation of CD4\(^{+}\)CD25\(^{+}\) thymocytes into FOXP3\(^{+}\)CD4\(^{+}\)CD25\(^{+}\) T\(_{reg}\) cells\(^{71}\). It is therefore possible that the ability of CD103\(^{+}\) MLN DCs or small-intestinal lamina propria DCs to induce FOXP3 is a result of exposure to TSLP *in vivo*. However, TSLP-conditioned human DCs could not induce FOXP3 expression in naive peripheral T cells, and mouse TSLPR-deficient lamina propria DCs were not impaired in their ability to induce FOXP3 expression\(^{69,72}\). It is therefore unlikely that TSLP has a non-redundant role in conditioning DCs for the peripheral generation of mouse FOXP3\(^{+}\) T\(_{reg}\) cells.

Nevertheless, studying the signals that induce the expression of enzymes involved in the production of retinoic acid may be a good starting point to decipher what type of conditioning is necessary to generate DCs capable of promoting FOXP3 expression by T cells. In this respect, activation of the lipid-activated transcription
is thought to be due to a loss of IL-10-mediated control of myeloid cells. A myeloid-cell-specific deletion of STAT3, through which IL-10 signals, led to enhanced TLR-driven production of IL-12/23p40 and the development of chronic IL-12/23p40-driven enterocolitis73,74. Indeed, it is possible that one of the key functions of both IL-10 and TGFβ is to control TLR-mediated activation of epithelial cells and APCs that are continually exposed to components of the commensal microflora. Consistent with this, DCs cultured with IECs produced TGFβ, which inhibited TLR-mediated activation of DCs75. Although some TLR signalling is important for protection from intestinal injury, IL-10 seems to have an important role in inhibiting inflammatory responses induced by commensal bacteria through the MyD88-signalling pathway76,77. As such, IL-10-deficient mice that also lack MyD88 fail to develop intestinal pathology, have reduced levels of IL-12/23p40 in the colon, and generate fewer IFNγ-producing T cells. IL-10 and TGFβ have also been implicated in the conditioning of pDCs in the Peyer’s patches and may inhibit their production of type 1 interferons5. Therefore, many of the unique properties of intestinal APCs appear to be a result of conditioning in their local environment.

Initiation of protective immunity to pathogens

In addition to initiating responses that create an overall tolerant state towards harmless intestinal antigens, intestinal DCs are also implicated in the generation of protective immune responses aimed at the clearance of enteric pathogens. The question of how intestinal DCs mediate these seemingly distinct functional roles is intriguing and remains unresolved. It was initially postulated that the commensal microflora was retained in the gut lumen through the combined actions of secreted IgA, the mucus layer and the tight junctions between epithelial cells, and that only pathogenic species were equipped to cross the epithelial-cell layer and initiate immune responses. Consistent with this, the expression of some TLRs, including TLR5, has been suggested to be restricted to the basolateral surface of IECs, preventing their engagement by luminal bacteria. However, studies have shown that non-invasive Salmonella typhimurium can engage TLR5 expressed by IECs when it was applied to the apical surface of IEC monolayers in vitro5,80. In fact, TLR signalling and NF-κB activation in IECs in the steady state may have an important role in intestinal immune homeostasis5,80,81.

Regardless of the expression patterns of TLRs by IECs, there is also potential for TLRs expressed by DCs to be engaged by commensal species following projection of DC dendrites across the epithelial-cell layer, or following M-cell-mediated translocation of commensal bacteria into the Peyer’s patches and subsequent uptake by DCs. In fact, commensal bacteria within DCs are routinely transported to the MLNs but they do not appear to penetrate any further41. Together, this leaves us with a scenario whereby the commensal microflora can be sensed by IECs, which communicate with resident DCs to limit the generation of destructive immune responses. As a result these DCs become conditioned and thereby initiate appropriate responses upon contact with commensal microflora, such as a result of conditioning in their local environment.
as the differentiation of FOXP3+ Treg cells, Tc1,2 cells and IgA-secreting B cells. We speculate there may be constitutive low-level recruitment of DCs in the steady state from blood precursors that would be capable of driving Tc1,1- or Tc1,7-cell responses. These DCs may act either as sentinels for the presence of pathogenic species, or may constitutively initiate cell-mediated immune responses against the commensal microflora to ensure it is kept under control (FIG. 3). These DCs may escape conditioning by chance encounter of microbial products or other inflammatory stimuli or as a result of a lineage-related lack of appropriate receptor expression. In fact, it is possible that these DCs share their origins with DCs that arrive in the tissues under inflammatory conditions. Nevertheless, under normal circumstances the balance between tolerogenic responses induced by conditioned DCs, and responses induced by unconditioned DCs should be such that no pathology develops. Indeed, IL-23-producing DCs can be found in the terminal ileum of normal mice, where a high bacterial load is present, in the absence of pathology63.

A fundamental difference between the steady state and a state of infection may lie in the greater propensity of pathogens to invade and penetrate beneath the epithelial-cell layer. Invasion of IECs would allow for the activation of cytosolic pattern-recognition receptors and both quantitative and qualitative changes in the secretion of pro-inflammatory cytokines and chemokines. Consistent with this, IECs only produced CXCL8 (CXC-chemokine ligand 8; previously known as IL-8) when confronted with strains of Salmonella spp. that were both invasive and flagellated69. CXCL8 may serve to attract neutrophils to the site of infection, furthering the inflammatory milieu. As a result, the rate of blood-borne DC precursors migrating into the tissues and becoming DCs will increase. These cells will not have been subject to conditioning and can be directly activated by a combination of pathogens that have breached the epithelial-cell barrier and the pro-inflammatory cytokine milieu (FIG. 3).

It is likely that the recruitment of DCs that have not been subject to conditioning, rather than the activation of pre-existing DCs, is essential for the generation of protective immune responses in the gut. Human monocyte-derived DCs conditioned with epithelial-cell supernatants are impaired in their ability to secrete IL-12 and drive Tc1-cell responses following exposure to pathogenic Salmonella spp.60 but can drive Tc1-cell responses if they encounter bacteria before conditioning by IEC-derived factors64. In addition, the CCR6-mediated recruitment of DCs from the subepithelial dome of Pey er’s patches to the follicle-associated epithelium is required for the generation of protective T-cell responses during infection with S. typhimurium.63 This recruitment was necessary despite the constitutive presence of a distinct CXCR1– DC subset in the follicle-associated epithelium. DCs also accumulate in the lamina propria under inflammatory conditions. However, the specific factors that drive this recruitment remain to be determined.

One other possible route for the generation of protective immunity to pathogens may be the uptake of pathogenic species by DCs that are normally resident in the MLNs. In this respect, CD103+ MLN DCs have been shown to produce higher levels of pro-inflammatory cytokines than their intestinal-derived counterparts and drive IFN-γ production by CD4+ T cells63,23. Finally, although conditioned DCs do not regain their ability to drive Tc1-cell responses following exposure to pathogenic species, it remains unclear whether aspects of their ability to induce tolerogenic responses will be impaired. For example, increased production of pro-inflammatory cytokines such as IL-6 by other cells in the microenvironment could impede the generation of FOXP3+ T cells64.

**Intestinal DCs in inflammatory diseases**

IBD is thought to be driven by a dysregulated relationship between the immune system and the commensal microflora that results in a chronic and destructive inflammatory response. Just as DCs have been implicated in maintaining tolerance in the intestine, inappropriate or aberrant DC function may be one factor in the pathogenesis of IBD.

**Protective and pathogenic roles of DCs in intestinal inflammation.** Several studies have drawn comparisons between the phenotype and function of DCs isolated from normal and inflamed intestinal tissue. For example, in Crohn’s disease, intestinal DCs have been shown to express higher levels of TLR2 and TLR4 and produce more IL-6 and IL-12 (REF. 85). Although these changes could be secondary to the ongoing inflammatory response, these results also raise the possibility that changes in DC function may directly contribute to the pathogenesis of IBD. Further support for this hypothesis comes from a T-cell-independent model of colitis in which direct activation of DCs through CD40 leads to the development of intestinal inflammation86.

More recently, mice expressing the diphtheria toxin receptor under the control of the Cdl1c promoter have been used to selectively deplete DCs and thereby study their role in the development of intestinal inflammation. Using this system, DC ablation was shown to ameliorate dextran-sulphate sodium salt (DSS)-induced colitis66. However, if TLR9 ligands were administered before colitis induction, DC ablation actually exacerbated disease64. The authors propose that in the control mice activation of DCs with TLR9 ligands led to the production of IFNβ, which in turn inhibited the production of pro-inflammatory cytokines by macrophages. Consequently, ablation of the DCs reversed this inhibitory effect. However, since pathology in this model of colitis is driven by a breach in the integrity of the epithelial-cell layer, the protective effect of the DCs may also have been mediated through stimulating repair of the epithelial-cell layer, rather than modulation of the immune response.

The role of intestinal APCs in a spontaneous model of colitis has also been investigated. Here, depletion of CD11b+ cells ameliorated colitis in IL-10-deficient mice49. This result is consistent with the idea that a failure to properly condition APCs in the intestines of IL-10-deficient mice results in a propensity for them to contribute to destructive inflammatory responses. Although the effects seen in this study were thought to relate to the depletion
of macrophages, it will be interesting to determine if specific depletion of DCs has a similar effect. In addition, it is possible that the impaired production of TSLP by IECs observed in Crohn’s disease may alter DC conditioning and predispose them to the development of intestinal inflammation. Overall, the way in which a DC is activated or conditioned changes whether it has a protective or pro-inflammatory role in intestinal inflammation.

Figure 3 | Response of intestinal DCs to infection. a | In the steady state, dendritic cells (DCs) resident in the intestine are conditioned by epithelial-cell-derived factors and promote the differentiation of forkhead box P3 (FOXP3)+ regulatory T (Tregs) cells and IgA-secreting B cells (not shown) on migration to the mesenteric lymph nodes. This may occur following sampling of the commensal microflora or in response to self antigens derived from the intestinal epithelium. A small number of DCs may also be recruited that escape conditioning and drive T helper 1 (TH1)- or TH17-type responses. These DCs may share a precursor with other DCs resident in the intestine in the steady state, but encounter bacterial products or other stimuli before conditioning can take place, or they may derive from distinct precursors and be refractory to conditioning. These cells could act as sentinels for the presence of pathogenic species, or mount responses aimed at controlling the commensal microflora. b | In contrast to the commensal microflora, some pathogenic species possess virulence factors that allow them to invade the intestinal epithelium and subvert immune responses to enhance their replication. Invasion of the epithelium leads to activation of cytosolic pattern-recognition receptors and enhanced production of chemokines and pro-inflammatory cytokines. Neutrophils, macrophages and DC precursors are recruited to the site and become activated by a combination of signals from pathogens and pro-inflammatory cytokines and chemokines. Whether these DC precursors also give rise to the populations of DCs present in the steady state remains unclear. Although DCs resident in the tissues before infection may not take on pro-inflammatory functions, it is possible that their ability to promote Tregs-cell differentiation may be impeded. Pathogenic microorganisms may also reach CD103– DCs resident in the mesenteric lymph nodes, which are capable of driving TH1-type responses, probably as a result of not being conditioned in the intestine.
Relationship between IBD susceptibility genes and DC function. Susceptibility to Crohn's disease has been associated with mutations in the gene encoding the cytosolic pattern-recognition receptor nucleotide-binding oligomerization domain 2 (NOD2)\textsuperscript{90}. NOD2 recognizes muramyl dipeptide (MDP), a component of bacterial peptidoglycan, and is expressed by DCs, macrophages and paneth cells. However, neither the mechanism by which NOD2 mutations predispose to Crohn's disease, nor the cell type these mutations primarily affect are currently known. Nevertheless, there is now clear evidence to suggest that NOD2 mutations influence DC and macrophage function, raising the possibility that chronic intestinal inflammation may result from aberrant responses of DCs or macrophages to the intestinal microflora.

Ligand binding by NOD proteins leads to activation of NF-κB and the production of pro-inflammatory cytokines. Mononuclear cells or macrophages taken from patients with Crohn's disease with NOD2 mutations show impaired NF-κB activation and cytokine production following stimulation with MDP, indicating that chronic intestinal inflammation may be associated with the loss of NOD2 function\textsuperscript{91,92}. Signalling through NOD2 has also been shown to enhance the cytokine response to TLR ligation\textsuperscript{93,94,95}. In a recent study, stimulation of NOD2 in DCs enhanced the TLR-mediated induction of IL-23 and IL-1, generating DCs that can promote IL-17 production by T cells\textsuperscript{96}, and DCs from patients with Crohn's disease with NOD2 mutations lacked this IL-17-inducing capacity. One possible explanation for these findings is that impaired responsiveness to the commensal microflora leads to a loss of immune homeostasis, a higher bacterial load and ultimately to the development of intestinal inflammation. Consistent with this, the IL-23–T\textsubscript{H}17-cell axis has been implicated in the generation of protective immunity to extracellular bacterial infection, and both IL-23\textsuperscript{+} DCs and T\textsubscript{H}17 cells can be found in the lamina propria of normal mice\textsuperscript{97,98,99}. Furthermore, NOD2-deficient mice, in which macrophages also show impaired NF-κB activation in response to MDP, are susceptible to oral bacterial infection\textsuperscript{100}.

Conversely, other studies have demonstrated that NOD2 signalling can inhibit TLR2-mediated NF-κB activation and cytokine production in mouse macrophages and DCs\textsuperscript{101,102}. Furthermore, pre-treatment of human monocyte-derived DCs with MDP inhibited cytokine responses to ligands for various TLRs\textsuperscript{103}. Of particular interest was a reduction in IL-12/23p40 production. According to this hypothesis, the impaired responsiveness to MDP observed in monocytes from patients with Crohn's disease with NOD2 mutations would actually result in exacerbated TLR-driven responses to the commensal microflora, and enhanced production of IL-12/23p40, driving intestinal inflammation. In support of this, administration of MDP protected mice from experimental colitis driven by damage to the intestinal epithelium and this protection was associated with a reduced responsiveness to multiple TLR ligands\textsuperscript{104}.

Finally, it remains possible that NOD2 mutations associated with Crohn's disease actually result in enhanced NOD2 signalling in response to MDP. Consistent with this, mouse macrophages expressing a truncated form of the NOD2 protein, similar to that associated with Crohn's disease, actually showed enhanced NF-κB activation and IL-1β production in response to MDP\textsuperscript{105}. However, this was not observed in human cells harbouring the same mutation. Nevertheless, if correct, this result would also be consistent with the notion that mutations in NOD2 predispose to Crohn's disease through the generation of exacerbated inflammatory responses to the commensal microflora.

IL-23 has been implicated in driving intestinal pathology in several animal models, and variants of the gene encoding the IL-23 receptor have been associated with Crohn's disease\textsuperscript{86,104–107}. Therefore, enhanced production of IL-23 by intestinal DCs might be expected to contribute to the pathogenesis of IBD. This could be viewed as somewhat at odds with the idea that impaired generation of T\textsubscript{H}17-cell responses by DCs harbouring NOD2 mutations leads to the development of intestinal inflammation. However, it should be noted that it is unclear whether the pathological role of IL-23 in the intestine is related to the activity of T\textsubscript{H}17 cells. Indeed, IL-23 has direct pro-inflammatory effects on innate immune cells and can also suppress T\textsubscript{reg}-cell differentiation in the intestine\textsuperscript{86,106,108}. Furthermore, T\textsubscript{H}17 cells themselves may also contribute to the healing process in the intestine through the production of IL-22\textsuperscript{[REF. 109]}.

Conclusions
Here, we have discussed an integral role for intestinal DCs in shaping the nature of the immune response in the gut. Depending on the population of DCs and the environmental conditions, these cells are capable of mounting distinct but appropriate immune responses to commensal and pathogenic microbial species, ultimately resulting in the protection of intestinal tissue from damage. In recent years the molecular pathways involved in both the conditioning and functional properties of DCs in the steady state have begun to be resolved. In particular, retinoic acid seems to be integral to several distinct functional properties ascribed to intestinal DCs. We are also beginning to gain a better understanding of how the aberrant function of DCs may contribute to the pathogenesis of IBD. In the future it will be important to understand how the functional properties of tolerogenic steady-state DCs change under inflammatory conditions, and whether this process is similar in infection and in IBD. In addition, a better understanding of the importance of the generation of T\textsubscript{H}11- or T\textsubscript{H}17-cell responses to the commensal microflora for intestinal immune homeostasis is required. In particular, what are the features of the DCs that perform this function, and are they related to those mediating protective immunity to pathogens? In the future, it is hoped that these pathways can be manipulated not only for the prevention of intestinal inflammation, but for the development of better oral vaccination strategies.


This paper deals with the crosstalk between the intestinal epithelium and DCs. Mice with an IEC-specific deletion of IKKε have impaired expression of TSLP, which results in elevated production of IL-12p40 by DCs and failure to mount protective T<sub>h</sub>2-type responses. This effect was in part mediated by TSLP.


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Competing interests statement
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DATABASES
ALDH1A1 | ALDH1A2 | ALDH2 | APRIL | CD11b | CD11c | CD80 | CD86
CCL2 | CCR1 | CCR4 | CCR7 | CXCL13 | ICOS | IFN-γ | IL-10 | IL-12p70 | IL-17A | IL-17B | IL-23p19 | IL-33 | IL-35 | IL-40 | LPS | MAPK
ALL LINKS ARE ACTIVE IN THE ONLINE PDF


Miyazaki, K., et al. Reciprocal developmental programs between epithelial cell and DCs in the regulation of intestinal immune responses. DCs cultured with epithelial-cell supernatants were ‘conditioned’ to produce less IL-12 and promote T<sub>h</sub>2-type responses. This effect was in part mediated by TSLP.


