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Involvement of intestinal dendritic cells in oral tolerance, immunity to pathogens, and inflammatory bowel disease

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Summary: Dendritic cells (DCs) are composed of a family of cells, now recognized to be essential for innate and acquired immunity. DCs at mucosal surfaces have a particular capacity to induce the differentiation of regulatory T cells producing interleukin-10 (IL-10) and transforming growth factor- β (TGF- β) in the steady state (non-infected, non-immunized), yet they retain the capacity to induce effector T cells in response to invasive pathogens. This decision between the induction of active immunity and tolerance will depend on the subpopulation of DC involved and the surface receptors engaged during DC activation and T-cell priming. The local microenvironment will likely play an important role both in defining the DC phenotype and in providing direct signals to responding T cells. Furthermore, DCs in organized mucosal lymphoid tissues preferentially induce the expression of CCR9 and $\alpha 4\beta 7$ on T cells, which results in T-cell homing to the intestinal lamina propria. Finally, DCs may play an important role in the maintenance of abnormal intestinal inflammation either by driving pathogenic T-cell responses in mesenteric lymph nodes or by acting to expand or maintain pathogenic T cells locally at sites of inflammation. In this review, a brief discussion of general issues of DC biology that are pertinent to mucosal immunity is followed by a more in-depth discussion of the phenotype and function of DC populations in the intestine.

Introduction

The fundamental issue in mucosal immunity is how tolerance is induced to commensal bacteria and harmless antigens while responses to pathogenic microorganisms must be generated to prevent overwhelming infection. The balance between these opposing immunological responses is also of fundamental importance for the prevention of abnormal intestinal inflammation. Our laboratory has been addressing the role of dendritic cells (DCs) in the regulation of immune responses in the intestine for the past several years, primarily because it has become clear that DCs are the major antigen-presenting cells (APCs) responsible for priming naïve T cells to a wide variety of infectious and self-antigens. In addition, it has become

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increasingly recognized that DCs are made up of a family of cells, each likely from a separate cell lineage and each with somewhat unique characteristics and contributions to innate and acquired immunity. This review highlights some of the major current views of DC biology and then addresses what is known (and not) about the contribution of DC populations to immune responses in the intestine.

Current views on DC biology

Sensors of infection and induction of immunity

DCs constitute a family of bone marrow-derived APCs that are uniquely capable of inducing the differentiation of naïve T cells. The most commonly held paradigm of DC function is that DCs are present in a less than fully mature (or activated) form within all vascularized tissues, where they efficiently capture or sample foreign and self-antigens. Upon exposure to microbial products or inflammatory signals, DCs mature and migrate to T-cell areas of organized lymphoid tissues, where they activate migrating naïve T cells. Maturation can occur in response to pathogens via interactions between pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs) on DCs, and pathogen-associated molecular patterns (PAMPs) expressed by organisms (1). Maturation may also occur in response to infection or inflammation, via recognition of inflammatory cytokines, e.g. tumor necrosis factor- α (TNF- α) interleukin-1 (IL-1), or type I interferons (IFNs) (2), intracellular compounds, e.g. uric acid (3), and specific immune responses, e.g. via CD40–CD40 ligand (CD40L) interactions (4–7). The DC maturation process is associated with the loss of endocytic and phagocytic receptors, the upregulation of co-stimulatory molecules, the extension of dendrites that increase the surface area available for interaction with T cells, and a shift in chemokine receptor expression (e.g. from CCR6 to CCR7) to promote DC migration to lymphoid tissues. These mature or activated cells are well suited to binding and activating naïve T cells. As a consequence of this unique biology, a primary function of DCs is to detect and to induce immune responses to invading pathogens.

'Steady-state' DCs and the maintenance of self-tolerance

Recent findings, however, complicate the relatively simplistic view of DC maturation and function described above. First, immature DC populations reside not only within peripheral tissues but also within discrete regions of organized secondary lymphoid organs. These lymphoid tissue-resident DCs can process systemically administered or blood-borne soluble

antigens (8), as well as cell-associated foreign or self-antigens, for presentation to T cells, the later by a process termed 'cross-presentation' (9, 10). Second, DCs are not simply stationary cells that are waiting to be activated to mature and to migrate. In fact, the turnover of most DC populations within lymphoid tissues is very rapid (on the order of days), and DCs within all tissues migrate continuously in the absence of inflammation or infection from non-lymphoid and peripheral sites, such as the intestine, to organized lymphoid tissues via the lymph. The DCs that migrate in the 'steady state' are thought to be in an immature or altered state of activation and continuously present self-antigens, most likely acquired from dying apoptotic cells, for the induction and maintenance of self-tolerance (11). In addition, the turnover rate of DCs in tissues and draining lymph nodes is increased substantially by inflammatory cytokines, such as TNF- α , that are induced during systemic or local inflammation or infection. Therefore, a more reasonable view of DC biology is that of continuously migrating immature or partially mature tolerogenic cells, which upon contact with pathogens or inflammatory stimuli become fully activated, resulting in more rapid turnover, migration to draining lymphoid tissues or to T-cell zones within lymphoid tissues, and the induction of effector, rather than tolerogenic T-cell responses.

DC subsets versus the plasticity of DC function

A third complicating factor in understanding DC function is the fact that multiple DC subpopulations have now been identified in mice and humans (12, 13). In the mouse, at least eight different DC and pre-DC populations can be defined (13–15). Three main DC subsets exist in the spleen (CD11c⁺CD11b⁺, subdivided into CD4⁺ or CD4[−], and CD11c⁺CD11b[−]CD8 α ⁺). Mesenteric lymph nodes (MLNs) and Peyer's patches (PPs) contain CD11c⁺CD11b[−]CD8 α ⁺ and CD11c⁺CD11b⁺CD4[−]CD8 α [−] (double-negative) subsets and an additional predominant population CD11c⁺CD11b[−]CD4[−]CD8 α [−] (triple-negative) DCs (15, 16). Furthermore, three subpopulations of the recently characterized plasmacytoid pre-DCs (pDCs) that express intermediate levels of CD11c have been described in lymphoid tissues and blood (14, 17–22).

In humans, most studies of DCs have focused on circulating precursors in the peripheral blood. Human DCs are characterized as human leukocyte antigen (HLA)-DR^{hi} cells that do not express typical markers of T cells, B cells, monocytes, and natural killer (NK) cells [lineage negative (lin[−])]. HLA-DR^{hi}lin[−] cells include three non-overlapping subsets of classical CD11c^{hi} precursor DCs that express CD1b/CD1c, CD16, or

BDCA3, and CD11c⁺ precursor plasmacytoid DCs that express CD123, BDCA2, and BDCA4 (4, 13).

There is considerable functional specialization of murine DC subpopulations, although this is not absolute. The adoptive transfer of antigen-pulsed CD8⁺ DCs to naïve recipient mice preferentially induced T-helper 1 (Th1) responses, which was dependent on DC production of IL-12p40 (thus either IL-12-p70 or IL-23), whereas the transfer of CD8⁻ DCs induced Th2 responses (23, 24), which was dependent to some extent on DC production of IL-10 (25). These and other studies resulted in the original designation of CD8⁺ DCs at 'DC1' cells and CD8⁻ (or CD11b⁺) DCs as 'DC2'. In addition, CD8⁺ but not CD8⁻ DCs can internalize apoptotic cells (26) and are the major cells responsible for T-cell tolerance following intravenous injection of antigen-loaded apoptotic cells (26, 27). CD8⁺ DCs are also responsible for the cross-presentation of self-antigens to CD8⁺ T cells *in vivo* (10, 28, 29).

Studies of human DCs also provide some support for the concept of functional specialization and separate DC1 and DC2 lineages. CD154-activated mature DCs derived from monocytes (myeloid DC) in culture with granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-4 were initially found to preferentially induce Th1 responses *in vitro*, whereas mature plasmacytoid DCs derived upon culture of CD123⁺ blood precursors with IL-3 and CD154-activated preferentially induced Th2 responses (13). Functional specialization is also suggested by the expression of different TLRs on different subpopulations of DCs (1, 30).

Despite evidence for functional specialization, significant plasticity also characterizes the function of DC subsets (5, 6, 13, 31). Recent studies argue that the induction of T-cell differentiation by DCs is directly influenced by the signals DCs receive during antigen uptake, activation, and T-cell priming. Indeed, the ability of DCs to mediate either tolerance or immunization depends on their state of activation, which, in turn, depends on the form of antigen and the presence or absence of activating tissue factors. Furthermore, exposure of DCs to certain cytokines or growth factors, such as IL-10 or transforming growth factor- β (TGF- β), or to certain pathogens or their products, such as *Bordetella pertussis* fimbrial hemagglutinin (FHA) (32), can promote tolerogenic responses via active engagement of regulatory receptors expressed by DCs. In addition, *in vitro* studies have shown that different microbial products can direct DCs to drive either Th1, Th2, or Th3 responses, which is at least partially dependent on the differential production of IL-10 and IL-12 by the DCs (5). This flexibility of DC function may be particularly relevant in the intestine, where DCs are continuously exposed

to a large variety of commensal bacteria, which may promote a regulatory DC phenotype.

Intestinal DCs

The importance of DCs in the induction of mucosal immune responses was initially suggested by studies performed before the availability of specific reagents to identify DCs. They focused on MHC class II-positive cells with the morphology of DCs in PPs (33–45), the lamina propria (LP) of the intestine (33, 34, 43, 46–48), and in the lymph draining the intestine (48–53). They indicated the presence and location of DCs in intestinal tissues and the ability of DCs to act as APCs, primarily through their ability to stimulate a mixed lymphocyte reaction (MLR). Since these early studies, significant progress has been made in understanding the role for DCs in the induction and regulation of mucosal immune responses

PPs and isolated lymphoid follicles

PPs are primary sites for antigen processing and the induction of immune responses, and they are organized into regions according to histology and cell types (Fig. 1). PPs are representative of organized gut-associated lymphoid tissue (GALT) located throughout the intestine, such as isolated lymphoid follicles (ILFs) in the colon (cILF) that are similar in structure to single follicles of PPs. Luminal antigens gain access to the PPs and other GALT via transfer across specialized epithelial cells, known as M (microfold) cells, which are scattered amongst the columnar epithelial cells in the follicle-associated epithelium (FAE) above the PPs (54).

Once antigens gain access to the PPs, DCs play a key role in inducing T- and B-cell responses. Early studies in the mouse demonstrated that PP accessory function, as measured by the proliferation of periodate-treated T cells, was present in a non-B-cell, non-T-cell, Fc-receptor⁻, Ia⁺ 33D1⁺ population with low buoyant density and dendritic morphology (55). In addition, isolated PP DCs were shown to be particularly capable of activating T cells for the support of immunoglobulin A (IgA) production by B cells (56, 57). In the human, early studies identified MHC class II⁺S-100 protein⁺ cells in the PP interfollicular regions (IFR) and subepithelial dome (SED) with cytoplasmic processes that extended into the dome epithelium that were most likely DCs (42, 45, 58). These reports suggested that PP DCs were important APCs for T-cell proliferation and T-cell help for B-cell responses in the PP.

In more recent studies, PP DCs were identified with DC-reactive monoclonal antibodies (16, 18, 59–62). Studies from

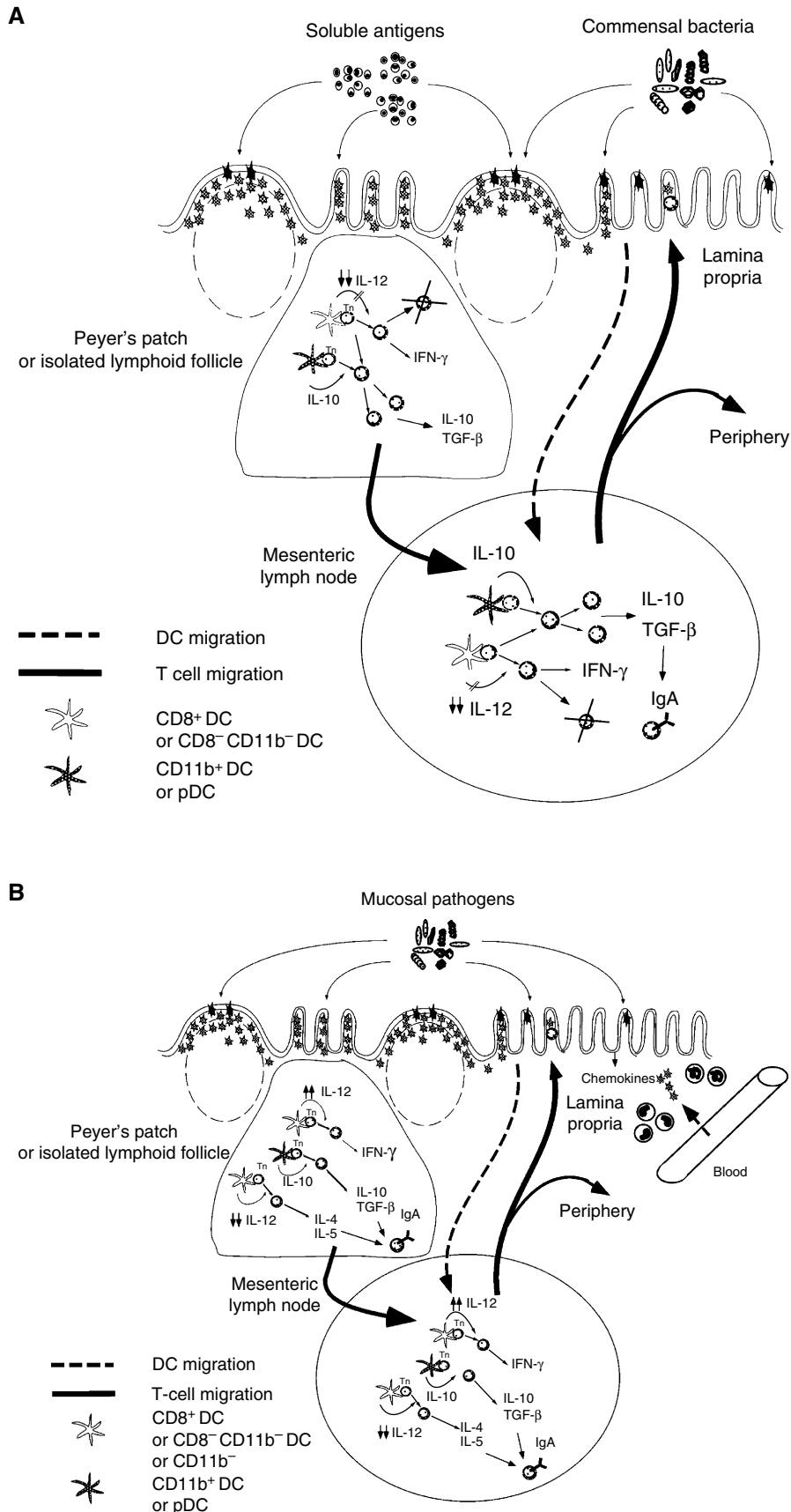


Fig. 1. Theoretical model of dendritic cell (DC) subpopulation involvement in immune regulation in the mouse. (A)

Soluble antigens and commensal organisms are taken up either by DCs in the Peyer's patches (PPs) or colonic isolated lymphoid follicle (cILF) SED following transport by M cells, or in the terminal ileum by direct interaction with DCs that extend dendrites into the intestinal lumen. In the PPs or the mesenteric lymph nodes (MLNs), DC populations induce predominantly regulatory T-cell responses. This response is likely to involve CD11b⁺ DCs or possibly pDCs. Induction of Th1 responses by CD8⁺ or CD8⁻CD11b⁻ DCs may be minimized if preferential processing of commensal organisms by CD11b⁺ DCs occurs in the terminal ileum, and/or by the fact that neither soluble antigens nor CD40 alone (from T cells) is a sufficient stimulus for IL-12 production by CD8⁺ or CD8⁻CD11b⁻ DCs. In addition, CD40 signaling is sufficient to drive IL-10 production from CD11b⁺ DCs, which can further suppress IL-12 production, and the presence of TGF-β in the local microenvironment may prevent IL-12R β2 expression by T cells. Early in the response, under conditions of limiting levels of IL-12, T cells engaged by CD8⁺ or CD8⁻CD11b⁻ DCs may produce some IFN-γ, but then undergo apoptosis or T cells are driven to differentiate into regulatory T cells in the presence of TGF-β and/or IL-10. With commensal bacteria, sIgA is induced which may involve Toll-like receptor (TLR) activation of CD11b⁺ DCs and or B cells, whereas soluble proteins would not result in this activation. (B) Infection by pathogenic microorganisms would result in the activation of DCs via TLRs, but also the recruitment of DCs, macrophages, and neutrophils due to the induction of chemokines by intestinal epithelial cells. This recruitment would allow for additional activating signals (e.g. TNF-α and IL-1-β production by inflammatory cells) to drive full DC activation, and this effect may be more profound on newly recruited cells that have not been exposed to the normally suppressive microenvironment of the intestinal tissues (161). Fully activated DCs would result in effective priming for either Th1 or Th2 responses as well as expansion of regulatory T cells, depending on the specific pathogen-DC interactions, or the production of pathogen-specific environmental factors, or the pathogen load (or dose). CD11b⁺ DCs may be more likely to induce Th2 responses and CD8α⁺ or CD8⁻CD11b⁻ DCs Th1 responses; however, it is not yet clear how plastic these DC responses are in vivo. SED, subepithelial dome.

our laboratory first demonstrated a striking concentration of CD11c⁺ DCs in the SED region just beneath the FAE, and in the IFR (61). In addition, total PP DC populations isolated from mice fed ovalbumin (OVA) stimulated OVA-specific T-cell receptor (TCR)-transgenic T cells (61). Similar findings were reported by Ruedl and colleagues (62). Furthermore, PP DCs were shown to be primarily immature in phenotype and mature to fully immunostimulatory cells upon activation *in vitro* (62). These data established DCs as major APCs in the PP and indicated their capacity to process oral antigen *in vivo*.

Subsequent studies addressed whether PP DCs are unique in their capacity to induce T cells that mediate IgA B-cell switching or oral tolerance. Highly purified PP DCs induced the differentiation of T cells, which upon *in vitro* re-stimulation produced high levels of IL-4 and IL-10 and lower levels of IFN- γ , compared to T cells primed with splenic DCs, which produced predominantly IFN- γ (63). IFN- γ production by T cells primed with PP but not splenic DCs was enhanced by the addition of neutralizing antibody to either TGF- β or IL-10 to the priming cultures (63). Furthermore, PP but not splenic DCs secreted IL-10 after overnight stimulation *in vitro* (63). Similarly, preferential induction of T cells producing IL-4 and IL-6 by PP DCs was reported by Everson and colleagues (64, 65). Finally, PP DCs produce IL-10 in response to activation via the co-stimulatory molecule RANK (receptor activator of nuclear factor κ B), whereas similarly stimulated splenic DCs produce IL-12 (66). Taken together, these studies support the hypothesis that IL-10 and possibly TGF- β are produced by PP DCs upon interaction with naïve T cells and that these cytokines either directly or indirectly drive T cells to differentiate into T cells producing IL-4, IL-10, and TGF- β . Similar findings have been reported for lung DCs (67–69), suggesting that the production of IL-10 and the induction of IL-10-producing T cells appear to be fundamental characteristics of DCs at mucosal sites under steady-state conditions.

Five murine DC populations are present in the PPs. Three express high levels of CD11c and can be defined by their co-expression of CD8 α and CD11b (15). CD11c^{hi} PP DCs were found to be CD8 α ⁺CD4[−]CD11b[−], CD8 α [−]CD4[−]CD11b⁺, or CD8 α [−]CD4[−]CD11b[−]. The latter population, while present in the spleen and peripheral lymph nodes (PLNs), was highly overrepresented in both the PP and the MLN. Following isolation, these three DC subsets expressed similar low levels of MHC class II and co-stimulatory markers, with an enhanced expression upon activation. In addition, all maintained their CD8 α and CD11b expression upon activation, suggesting that one population be not simply a more differentiated or activated form of another population.

In functional assays, PP CD11b⁺ DCs produced low levels of bioactive IL-12p70, similar to the splenic CD11b⁺ DCs upon stimulation with CD40L alone, or with SAC and IFN- γ . However, compared to either the CD8 α ⁺ or CD8 α [−]CD4[−]CD11b[−] PP DCs or CD11b⁺ DCs from the spleen or the PLNs, PP CD11b⁺ DCs produced high levels of IL-10 (15). In addition, CD11b⁺ PP DCs induced naïve T cells to differentiate into cells that produced much higher levels of IL-10 than T cells primed with any other DC subset. By contrast, both CD8 α ⁺ and CD8 α [−]CD4[−]CD11b[−] PP DCs produced IL-12 and little to no IL-10 and induced the differentiation of T cells producing only IFN- γ , similar to the CD8 α ⁺ spleen DCs. These studies identified CD8 α [−]CD4[−]CD11b[−] DCs at mucosal sites of antigen exposure and showed that they were particularly capable of inducing Th1 responses. The studies also showed that the CD11b⁺ DCs from the PP are unique in their ability to induce IL-10-producing T cells and thus may have a role in the induction of regulatory T cells or the induction of IgA B-cell responses. The latter was recently supported by studies, demonstrating that when compared with splenic DCs, freshly isolated PP CD11b⁺ DCs induced higher levels of IgA secretion from naïve B cells in a DC-T cell–B cell co-culture system (70).

CD11c^{hi}CD11b⁺ DCs, such as skin Langerhan's cells, DCs resident in non-lymphoid tissues, and DCs present in the marginal zones of the spleen and the lymph nodes were found to be localized to the SED, whereas CD8 α ⁺ DCs were localized exclusively in the T-cell zone IFRs (16). CD11b[−]CD8 α [−]CD4[−] DCs were present in both SED and IFR and were scattered throughout the B-cell follicle, except the germinal centers. Furthermore, a population of CD8 α [−]CD4[−]CD11b[−] DCs present within the epithelial layer of the FAE expressed high levels of intracellular (rather than surface) MHC class II antigens, indicating an immature phenotype (15). Some of these cells co-associated with UEA⁺ M cells, suggesting that at least some reside in M-cell pockets. Similarly, MHC class II⁺ cells were also identified within the FAE in the rat (47) and human (42). Finally, PP DCs have been shown to express the integrin–ligand mucosal addressin cell adhesion molecule-1 (MAdCAM-1), which preferentially binds the integrin molecule most associated with mucosal homing, α 4 β 7 (71), suggesting that SED DCs express surface molecules that facilitate interactions with mucosal T and B cells.

Recently, CD11c^{int}B220⁺ plasmacytoid pDCs also were identified in the murine PP (18, 22). Using an antibody (120G8), which is reactive with pDCs and when administered *in vivo* depletes IFN- α -producing pDCs, Asselin-Paturel and

colleagues (18) have identified pDCs in the IFR of PPs. In addition, pDCs were isolated from PPs of mice treated with flt-3-ligand, which dramatically and preferentially increases the number of DCs, including pDCs (22). These cells expressed the typical markers of pDCs, including B220 and Ly6C, but also expressed CD8, a marker typically associated with mature plasmacytoid DCs (22). Functional studies showed that the cells produce IFN- α , similar to pDCs from the blood and other tissues. However, these cells were particularly capable of inducing IL-10-producing T cells in mixed lymphocyte assays (22), similar to a CD8⁺ pDC subset described from the spleen (21). In unpublished data from our laboratory, we have identified both CD8 α ⁺ and CD8 α ⁻CD11c^{int}B220⁺, Ly6C⁺ pDCs in the PPs and have confirmed the localization of 120G8⁺ cells in the IFR and SED (Kelsall, unpublished observation).

Similar to studies of mice, PP DCs in the human and macaque have been demonstrated in the IFR, SED, and follicle SED (72). DCs in the IFR and some in the SED expressed DC-SIGN (72), suggesting that these cells may be targets of human immunodeficiency virus (HIV) and other mucosal pathogens that attach to this lectin receptor (73). In addition, M-DC8⁺ cells that most likely represent DCs were also found in the SED region of tonsils and PPs from inflamed tissues (74). The latter produced high levels of TNF- α and may have a role in driving T-cell responses during inflammation.

One additional unique feature of PP DCs has recently been identified. PP and MLN but not splenic or LN DCs can induce the mucosal homing receptor $\alpha 4\beta 7$ as well as the chemokine receptor CCR9 on T cells *in vitro* (75). Upon adoptive transfer to naïve mice, PP-primed CD8⁺ T cells preferentially migrate to mucosal lymphoid tissues. These studies demonstrated, for the first time, that DCs from different tissues have the capacity to imprint T cells with tissue-specific homing receptors.

MLN

In classic studies, 'veiled cells' were described in the thoracic duct lymph from mesenteric lymph-adenectomized rats (48–53). Veiled cells are a heterogeneous population of cells that migrate from all regions of the intestinal tract to draining MLNs, where they are normally trapped (49, 50). These veiled cells process fed antigen (53) or antigen delivered by direct intestinal injection (52, 53), and are capable of stimulating primed T cells *in vitro*, and when transferred to the footpad of an unprimed mouse, prime naïve T cells *in vivo* (53). Although the site of antigen acquisition cannot be precisely determined from these studies, the antigen-loaded DCs

likely came from the intestinal tract, as thoracic duct cells from non-lymphadenectomized rats contain very few DCs (<0.2%), compared with lymphadenectomized rats (8–10%) (51). These migrating DCs have a short half-life in tissues (on the order of 2–3 days), similar to most other DC populations, which is significantly shortened following the induction of TNF- α production by systemically administered lipopolysaccharide (LPS) (51).

In further studies of their ability to stimulate an MLR (48), LP and PP DCs stimulated only a modest MLR, compared to MLN and thoracic duct lymph DCs, which induced a potent MLR. In addition, following overnight culture with GM-CSF, PP and LP DCs had significantly increased potency in the MLR, equivalent to that of lymph or MLN DCs that did not have enhanced accessory activity following culture. These studies thus suggest that PP and LP DCs are less activated than DCs that have migrated to the MLN, and they support the general concept that DCs become more capable of inducing T-cell differentiation after migration from sites of antigen acquisition. Furthermore, the studies provide evidence that under steady-state conditions, DCs constitutively migrate from the intestine to the MLNs, and pathogen products enhance this migration.

Rat intestinal lymph contains at least two DC populations that have typical DC morphology, are MHC class II^{hi}, and express OX62, CD11c, and CD80. One population, CD4⁺OX41 (SIRP α)⁺ DCs, are strong APCs in MLRs, whereas the other population, CD4⁻OX41⁻ DCs are weak APCs (76). Both DC populations are also present in rat MLNs, where CD4⁻ DCs are localized to T-cell zones and CD4⁺ DCs are found outside the T-cell zone in the parafollicular regions. Many MLN CD4⁻ DCs contain cytoplasmic apoptotic DNA, epithelial cell-restricted cytokeratins, and non-specific esterase-positive inclusions, suggesting that the cells carry material from apoptotic enterocytes to T-cell zones of the MLN (77). In addition, CD4⁻ DCs from the rat spleen produce IL-12 after stimulation *in vitro*, whereas the CD4⁺ DCs do not. These studies suggest that rat CD4⁻ DCs are similar to mouse CD8⁺ DCs, whereas rat CD4⁺ DCs resemble mouse CD11b⁺CD4⁺ DCs.

Many of the features of MLN DCs are similar to those of the PP DCs. In the mouse, the MLN contains the same five populations of DCs present in the PP, with an increased proportion of C11b⁻CD8⁻CD⁻ DCs (15). CD8 α ⁺, CD11b⁻CD8⁻CD4⁻, and pDCs have been localized to the T-cell zones (16, 18), whereas CD11b⁺ DCs are located primarily outside the T-cell zones. Whether the population we describe as CD11b⁻CD8⁻CD4⁻ corresponds to a CD8⁻CD11b^{lo}CD205^{int} DC population described in the MLN by others (13) has not

yet been resolved. Interestingly, the CD11b⁺CD8⁺CD4⁺ DCs that we described in the PP do not express CD205 or higher levels of MHC class II molecules than the other subsets following isolation, although both surface antigens could be up-regulated after overnight incubation with CD40L trimer. Thus, the CD8⁺CD11b^{lo}CD205^{int} DCs in the MLNs may represent more 'mature' (at least with regard to MHC class II expression) CD11b⁺CD8⁺CD4⁺ DCs that have migrated from the PP or possibly the intestinal LP under steady-state conditions.

Functional studies have also demonstrated similarities between murine MLN and PP DCs. Similar to PP DCs, MLN DCs from antigen-fed mice produce IL-10 and possibly TGF- β and preferentially stimulate antigen-specific CD4⁺ T cells to produce IL-4, IL-10, and TGF- β (68, 78). Similar results were obtained with both wildtype and μ MT mice, which lack B cells and well-developed PPs, arguing that soluble antigen-loaded MLN DCs do not necessarily originate in the PP (78). An additional feature shared by MLN and PP DCs is the ability to induce the $\alpha 4\beta 7$ integrin on T cells (79, 80).

In humans, DCs in the MLN are HLA-DR^{hi}, large dendriform cells in the T-cell areas that co-express CD40, CD54, CD80, CD83, CD86, and the S-100 protein, but lack CD1 α . These DCs are attached to numerous CD4⁺ T cells and IgD⁺ naive B cells *in vivo* and preferentially formed clusters with IgD⁺IgM⁺ but not IgA⁺ or IgG⁺ B cells *in vitro*. These findings suggest that human MLN DCs may induce T-cell help for primary B-cell responses.

Intestinal LP

DCs are a major APC population in the intestinal LP of the mouse (43), rat (33, 47), and human (46). Irregularly shaped, strong MHC class II-positive LP DC cells have been described primarily in the LP just below the basement membrane. In the rat, DCs have also been described within the epithelium of the intestine (47). There, they formed an organized network that extended dendrites between epithelial cells. Although similar intraepithelial DCs have not been described in the LP-associated epithelium in other species in the steady state, murine small bowel DCs may be induced to migrate into the epithelium and extend their processes into the intestinal lumen to sample bacteria by a well-orchestrated process (81). Recently, DCs were found to be a dominant cell in the LP of the small bowel in mice but rare in the colon, except in cILF (82). In addition, DCs with cell bodies beneath the LP basement membrane, but with dendrites extending into the intestinal lumen have been identified in the terminal ileum of mice (100).

The phenotype of LP DCs is not as well defined as that of PP DCs. Mowat (83) recently detected the same three CD11c^{hi} DC subsets in the LP that had been defined in the PP as well as pDCs. Others found a predominance of CD11b⁺CD8⁺ DCs,

especially in the terminal ileum (82). By contrast, studies by our laboratory show a predominance of CD11b⁺ and fewer CD11b⁺CD8⁺CD4⁺ and no CD8⁺ DCs in the small bowel and colonic LP of mice (Kelsall, unpublished observations). In addition, we have confirmed the concentration of colonic DCs within organized follicles, and the lack of intraepithelial DCs in the steady state in the small bowel and the colon. In humans, one study suggests that DCs are present in the LP and in poorly defined lymphoid aggregates in the small bowel, which do not have the structure of PPs (84). CD11c⁺HLA-DR⁺lin⁺ (CD3⁺CD14⁺CD16⁺CD19⁺CD34⁺) DCs in colonic and rectal biopsies have been reported to be of an immature phenotype but to mature into fully immunostimulatory DCs upon migration out tissue following overnight culture (85). Similarly, CD83⁺ and DC-SIGN⁺ cells have been identified in the LP and may produce IL-12 and IL-18 during intestinal inflammation from Crohn's disease (CD) (86). Finally, DC-SIGN-expressing cells are found diffusely throughout the human and rhesus macaque rectal LP, and some of these cells co-express CCR5 and CD4, suggesting that they could be targets of HIV infection (72).

Functional studies of LP DCs from all species have confirmed that these cells are potent inducers of the MLR (33, 43, 46, 48). The aforementioned DCs in the terminal ileum of mice (but not of the more proximal small intestine) were shown to express IL-12p40 in the steady state, as well as mRNA for p19 (much greater than IL-12p35), suggesting that they constitutively express IL-23 (82). In addition, these cells were associated with bacteria *in vivo*, suggesting that DCs in the terminal ileum may normally process endogenous bacterial microflora. Finally, it has been suggested that DCs from the LP may be particularly capable of inducing oral tolerance (83); however, there are no published studies, to date, of antigen-induced (versus MLR-induced) T-cell responses, specifically using well-defined LP DCs as APCs available to address this possibility.

Antigen handling by intestinal DCs

Fundamental to understanding how mucosal immune responses are induced and regulated is where different types of antigens are processed and presented by DCs to T and B cells. Intestinal DCs are present in several regions where antigens may be processed. From these sites, DCs constitutively traffic to draining lymph nodes in the steady state and are induced to migrate at higher rates following exposure to microorganisms or pro-inflammatory cytokines. Less clear are the mechanisms for the uptake and processing of particular antigens and pathogens by intestinal DCs.

Luminal antigens are transported into the Peyer's patch via specialized epithelial cells, known as M cells, which are scattered among conventional epithelial cells overlying the dome of the PP follicle (87). Compared to absorptive epithelial cells, with which they share a common progenitor cell, M cells have poorly developed brush borders, reduced enzymatic activity, and a thin overlying glycocalyx. These features, possibly combined with a unique cytoskeleton and a pronounced capacity to form endocytic vesicles, facilitate the ability of M cells to transport microorganisms and macromolecules from the mucosal lumen to the SED of the PP. M cells likely do not have APC function due to their limited number and limited contact with T cells in the PP. Rather, DCs in the PP and in particular in the SED are likely to be important for the induction of immune responses to a large number of invading pathogens that gain entry via M-cell uptake. In this regard, it has been shown that DCs in the SED phagocytose orally administered *Salmonella typhimurium* (88, 89) and are the first targets of *Listeria monocytogenes* infection in rats (90). In addition, a population of CD11c⁺CD8⁻CD11b⁻ cells in the SED was shown to take up 0.2 μ m fluorescent latex beads given orally (91). These cells remained in the SED for up to 14 days and rapidly migrated to the IFR following the administration of cholera toxin, suggesting that they may represent a resident population of long-lived cells particularly capable of processing particulate antigens.

Recent studies from our laboratory demonstrate that CD11c⁺CD8⁻CD4⁻CD11b⁻ DCs process antigens from type 1 Reovirus, at least some of which is in the form of apoptotic bodies from infected epithelial cells from the FAE (92). Whether this cross-presentation pathway applies to other organisms shown to induce apoptosis of enterocytes, such as *Salmonella* (93), is not clear. Direct sampling of antigens or organisms by the aforementioned immature intraepithelial DCs in the FAE has not yet been demonstrated; however, such sampling is supported by studies demonstrating the uptake of antigens by MHC class II high, non-B, non-T-cell pocket lymphocytes in the rabbit (94). Finally, antigen uptake by M cells can be enhanced by the presence of specific IgA (95, 96), suggesting that local Ig will direct antigens to PPs. As DCs have been shown to express IgA receptors, which increase their uptake of antigens (97, 98), local antigen-specific IgA may enhance the uptake of luminal antigens by PP (or LP) DCs. Taken together, these findings suggest that DCs in the SED and FAE directly sample antigens and microorganisms transported into the PPs by overlying M cells and from apoptotic epithelial cells of the FAE.

Microorganisms may also gain access to DCs in the LP directly across the normally exclusive intestinal epithelium following epithelial barrier disruption by infection and/or inflammation, as occurs in inflammatory bowel disease (IBD). The DCs identified within the LP epithelium (47) suggest that a population of DCs may be the first APCs to come into contact with luminal antigens that cross the glycocalyx/mucous coat overlying epithelial cells of the LP. These DCs may compete with epithelial cells in the processing and presentation of luminal or self-antigens to $\alpha\beta$ -TCR or $\gamma\delta$ -TCR IEL populations.

What has been shown is that DCs may sample luminal contents by a unique mechanism described by Rescigno and colleagues (81, 99). They showed that DCs were capable of opening tight junctions between enterocytes and of sending dendrites into the intestinal lumen to directly sample bacteria. This sampling process appears to require signals from luminal bacteria, which results not only in DC migration but also in the co-ordinated expression of tight junction proteins by DCs. The latter allows DCs to form adhesive interactions with enterocytes, resulting in the maintenance of the epithelial barrier. This mechanism was also suggested in studies of bacterial uptake *in vivo* using *Salmonella typhimurium* as well as non-pathogenic *Escherichia coli* (81). Recently, bacterial sampling by DC extensions was demonstrated to occur in the steady state in the terminal ileum, indicating that DCs can sample commensal organisms from the lumen (100).

In contrast to these studies demonstrating the uptake of microbial organisms by DCs in the PP and LP, there are few studies demonstrating the direct uptake of soluble antigens by mucosal DCs. Soluble antigens can be transported by a receptor-mediated mechanism from the lumen to the LP by conventional absorptive epithelial cells. While this transport may result in the degradation of proteins into fragments that are non-immunogenic, there may be particular receptors that translocate more intact antigenic fragments. One such receptor is the 'neonatal' Fc receptor for IgG that is present on epithelial cells on both the apical and basolateral membranes, not only during the neonatal period, where it plays a vital role in the absorption of maternal IgG following the ingestion of breast milk, but also during adulthood (101). Antigens transported by this mechanism may be taken up by DCs, because DCs as well as LP macrophages express this receptor (102).

It is clear that following high doses of oral antigen, protein can be found in the peripheral blood, possibly in an 'altered' form that may be particularly tolerogenic (103, 104). Consistent with these studies, very high doses

(100–250 mg) of oral antigen result in simultaneous activation of antigen-specific T cells in multiple sites, including the LP, PPs, and MLNs. In addition, such doses result in peripheral T-cell activation and anergy induction, similar to what has been shown with intravenous antigen administration (105–111). Studies of lower doses of oral antigen (1–60 mg) demonstrated that transferred T cells are activated preferentially in MLNs and PPs (rather than systemic lymphoid tissue), but that such activation occurs with virtually identical kinetics, even at the 1-mg dose (105), suggesting that antigens are simultaneously processed in the PP and LP, with migration of the LP DCs to the MLNs. While none of these studies formally address the initial site of antigen uptake (FAE versus absorptive enterocyte), they suggest that at both high and low doses, antigen is processed by DCs at both sites and that only with very high doses does antigen get distributed via the blood stream for presentation by DCs at systemic sites. Whether specific subsets of DCs preferentially process soluble mucosal antigens is not clear. While following very high oral antigen doses, CD8⁺ DCs in MLNs and PLNs were found to preferentially present antigen/MHC complexes using antibodies specific for MHC class II-peptide complexes (111), targeting of specific antigen to CD8⁺CD205⁺ DCs is particularly effective in inducing systemic tolerance (112, 113).

DC trafficking to the intestine

Chemokines and chemokine receptors that govern the localization and migration of DC populations in the intestine are just beginning to be unraveled. In murine PPs in the steady state, CD11b⁺ DCs appear to be recruited to SED by a number of chemokines expressed constitutively by epithelial cells in the FAE, including CCL9 (114) and possibly CCL20 (16); the chemokines that attract CD8 α ⁺CD4⁺CD11b⁺ DCs to this site have not been identified. Upon activation *in vivo* with a soluble antigen preparation from *Toxoplasma gondii* tachyzoites (STAg), the SED CD11b⁺ and possibly some CD8 α ⁺CD4⁺CD11b⁺ DCs migrated to the PP IFRs (16). This migration correlated with an upregulation upon overnight culture of CCR7, the receptor for CCL7 and CCL8, which are constitutively expressed in the IFR. These studies support the hypothesis that activation of DCs in the SED, as would occur following exposure to organisms entering via M cells, results in DC migration to the IFR, where T-cell priming could occur. By contrast, CD8 α ⁺ DCs constitutively expressed CCR7 and not CCR6 and migrated toward CCL7 but not CCL20 *in vitro*, suggesting that CD8 α ⁺ DCs are resident in the IFR throughout much of their life cycle.

Studies of chemokines responsible for the migration of DCs to the LP in the steady state are lacking, although epithelial cell-expressed CCL25, the ligand for CCR9 and CCR10, is a possible candidate chemokine in the small bowel, and CCL28, the ligand for CCR3 and CCR10, may be an important chemokine in the colon (114–116). During inflammation, however, a large number of inflammatory chemokines, including CCL20/CCR6, CCL2/CCR2, CCL5/CCR5 or CCR1, and CXCL12/CXCR4, are produced by epithelial cells that could attract DCs (115, 117, 118). CCL20 mRNA expression and protein production are upregulated in intestinal epithelial cells *in vitro* by stimulation with LPS, TNF- α or IL-1 α , or infection with enteric bacterial pathogens (119). In addition, an increased expression of CCL20 has been detected in the epithelium of inflamed human colon (119). CCR6 is expressed by more immature DCs and is downregulated following activation, coincident with an upregulation of CCR7 (115, 120). Accordingly, immature DCs may be attracted to mucosal tissues as immature cells, and after activation by inflammatory signals, DCs migrate to the PP or to the MLN for priming naïve or activation of central memory T cells.

Intestinal DCs and the induction of tolerance and immunity to pathogens

The most accepted current model holds that, under steady-state conditions, precursor DCs continuously enter the mucosal LP, PPs, and cILFs, develop into immature DCs, and localize to different regions due to the differential constitutive expression of specific chemokines, such as CCL9 and CCL20, in the FAE of the murine PP (16, 114). After transport of antigens across M cells or epithelial cells, or possibly via the uptake of apoptotic bodies from epithelial cells, DCs migrate from the LP to the MLNs or from the SED of the PPs and cILF to the respective IFRs in the steady state (Fig. 1). This migration is accompanied by an upregulation of chemokine receptors for T-cell zone chemokines, such as CCR7, but low levels of co-stimulatory molecules and cytokines.

Following migration of antigen-loaded 'quiescent' DCs to T-cell zones in the PP, cILF, or MLN, the DCs stimulate T cells to differentiate into regulatory T cells that can mediate bystander tolerance following subsequent antigen encounter. Intestinal stromal cells may promote a suppressive environment that conditions DCs to induce regulatory T cells. While the precise nature of these regulatory cells in relation to the CD25⁺ regulatory T cells originally described by Sakaguchi and colleagues (121) is not clear, the cells seem to suppress primarily via a cytokine-dependent mechanism *in vitro* and

in vivo, involving TGF- β , IL-10, and possibly IL-4 (122, 123). The subset of DCs that induce regulatory T cells is not yet clear, although CD11b⁺ DCs or pDCs (either CD11^{cint}B220⁺ or CD45RB^{hi}B220⁺) in the mouse are potential candidates (14, 15, 22).

In addition to the induction of regulatory T cells by oral antigens, it is clear that very high doses of soluble protein antigen (such as OVA) result in systemic anergy and clonal deletion of T cells (124). How DCs are involved in this process is not yet clear; however, it is likely that after oral administration of high doses of antigens, LP and PP DCs take up antigens, and a certain portion of antigen will gain access to the blood stream for systemic dissemination. The consequence of systemic dissemination is the induction of T-cell anergy or deletion, similar to that, which follows the intravenous injection of antigen. In the mouse, the DCs most likely involved in this process are CD8⁺ DCs in the T-cell zones of the spleen and the LN, although the spleen may be the most important site. The implication that CD8⁺ DCs are involved in this induction is made based on the finding that CD8⁺ DCs are in continuous contact with the majority of T cells and may process antigens for presentation in the absence of activation or migration and thus in the absence of high levels of co-stimulatory molecules and cytokines. In the event of low levels of co-stimulation that may preferentially engage inhibitory co-receptors (125), and the absence of IL-12 (126–128), and possibly IL-18 (129), T cells that may initially produce IFN- γ upon activation are deleted or induced into an anergic state. The most likely fate of anergic T cells is dissemination to peripheral tissues and death (130), although it is possible that some may become regulatory T cells that are poorly proliferative. In support of this possibility, high antigen doses, which when administered once will result in T-cell anergy/deletion, when given repeatedly can also result in the generation of regulatory cells (albeit likely less efficiently than repeated low doses) that can mediate bystander tolerance (131, 132). The induction of regulatory T cells may be more limited following systemic antigen administration, because this may restrict the precursors available for the generation of regulatory T cells at mucosal sites.

In contrast to tolerogenic responses to innocuous antigens, mucosal pathogens induce active local and systemic immunity (Fig. 1). Initial encounter of pathogens with the cellular components of the innate mucosal barrier, epithelial cells, and underlying DCs and macrophages involves the recognition of microbial PAMPs by PRRs, such as TLRs (1, 133–135). TLR signaling of epithelial cells results in the production of pro-inflammatory cytokines and chemokines, such as IL-1, IL-8,

IL-6, TNF- α , IFN- α , CCL5, and CCL20, which can recruit and activate neutrophils, macrophages, and DCs (136). In addition to this indirect mechanism of DC activation by epithelial cell-derived cytokines, DCs may be directly activated by invading pathogens via TLRs and other surface receptors. Such activation results in fully immunogenic DCs with high levels of major histocompatibility complex (MHC), co-stimulatory, and adhesion molecules, as well as cytokines. In the mouse, CD11b⁺ or CD8⁺CD11b⁺CD4⁺ DCs in the SED or the LP are most likely involved in the initial interaction and uptake of invading pathogens, whereas CD8⁺ DCs may be involved in the direct presentation of pathogens that are disseminated or by cross-presenting antigens carried to the IFRs by DCs or macrophages, or exosomes from epithelial cells. Direct activation of pDCs in the PP or LP by viruses will result in type 1 IFN production, which can activate innate defense mechanisms as well as contribute to the activation of non-plasmacytoid DCs. pDCs may also be driven to mature into immunostimulatory DCs by viral pathogens, such as influenza and herpes simplex virus.

The phenotype of the ensuing T-cell response to any mucosal pathogen will ultimately depend on direct and indirect effects of the particular pathogen encountered. This will include the particular subset of DC engaged (which will differ because of the expression of different PRRs), the compilation of signals that the DC receives from direct interaction with the pathogen, as well as from tissue factors present at the site of encounter and in the lymph node during T-cell priming (5, 6, 13, 31).

DCs and IBD

DCs appear to play an important role in the pathogenesis of IBD. Current information suggests that DCs have two major functions. First, studies in mice indicate that DCs are involved in priming abnormal T-cell immune responses to endogenous flora in organized lymphoid tissues, such as PP in the terminal ileum, ILF in the colon, or the MLN. Such DCs may also help maintain T-cell reactivity by the re-stimulation of central memory T cells with these lymphoid tissues. Second, DCs may promote the persistence of the inflammatory T-cell responses by direct interactions with LP T cells within the inflamed tissue. This interaction likely involves DCs that are induced to mature within the tissues but that do not migrate following activation. Locally activated DCs could enhance ongoing T-cell responses by direct cognate interaction and by the production of cytokines, such as IL-12, IL-23, or possibly IL-27, which could expand effector T-cell populations and/or prolong their survival.

DCs in animal models of IBD

Rodent models of colitis have provided strong evidence that the interaction between commensal bacterial flora and the mucosal immune system plays an essential role in the pathogenesis of IBD (137, 138). Four different models of experimental IBD suggest that DCs are instrumental in the establishment and maintenance of inflammation. In the SCID/CD45RB^{hi} transfer model of colitis, in which disease is induced by adoptive transfer of wildtype CD45RB^{hi} T cells into an SCID recipient (137), CD11c⁺ DCs in the MLNs of colitic mice increased in number, and 20–30% expressed high levels of OX40L (CD134L), a marker of DC activation (139). Furthermore, blocking CD134–CD134L interactions prevented the induction of colitis, decreased the proliferation of T cells, and reduced the number of T cells that expressed $\alpha 4\beta 7$ in the MLN. By contrast, CD134L⁺ DCs were not found in the inflamed LP, suggesting that activated MLN and not LP DCs were critical for the onset of colitis through interactions between DC-expressed CD134L and CD134 on T cells. Because the accumulation of CD134L⁺ DCs was prevented by the co-transfer of CD4⁺CD45RB^{low} T cells, it was suggested that regulatory T cells may function, in part, to prevent DC activation (139). How and where CD4⁺CD45RB^{low} T cells may prevent DC maturation is not yet clear; however, one can predict that cytokines, such as IL-10 and TGF- β , which are important for the suppressive function of CD4⁺CD45RB^{low} cells *in vivo* and which can prevent DC maturation, are likely involved.

In a similar model of colitis in which total CD4⁺CD45RB^{hi} T cells are transferred into RAG-1^{-/-} mice, colitic mice can be treated with CD4⁺CD25⁺ T cells. Following transfer after the onset of colitis, CD4⁺CD25⁺ T cells expand not only in the MLNs but also in the inflamed colon in close proximity to CD11c⁺ DCs (140). These data suggest that DCs are important for the activation and expansion of CD25⁺ T cells in both draining lymph nodes and at sites of inflammation and are consistent with a requirement for highly activated DCs for CD4⁺CD25⁺ T-cell expansion (141). In addition, the data suggest that CD4⁺CD25⁺ T cells may act in the lymph node or locally within inflamed tissue to suppress T-cell effector function. A slight variation of this model is one in which total CD4⁺ T cells are transferred to RAG1^{-/-} mice resulting in colitis in some animal facilities. In studies of this model, non-reconstituted, non-colitic RAG1^{-/-} mice have clusters of subepithelial CD11c⁺ DC in the colon, which likely represent poorly formed cILF. Upon reconstitution, CD4⁺ T cells appear in the CD11c⁺ DC clusters, where they expand in number during the early or 'emerging' stages of the disease (142). The degree of expansion within the clusters was proportional to

the severity of the resultant intestinal inflammation, suggesting a role in for DCs within cILF in the initiation (or maintenance) of inflammation (142). Coincident with disease development in this model, DCs in the LP increase in number, display a mature/activated phenotype, and likely produce IL-23 (143), consistent with a role for local activated DCs in the induction or expansion of pathogenic (as well as regulatory) T cells directly within inflamed tissues. Moreover, consistent with these data, in the Gi2 α ^{-/-} model of colitis, we have observed a major increase in the number of activated CD11b⁺ DCs within inflamed LP (Jianping He *et al.*, manuscript in preparation).

In IL-2^{-/-} mice, the inhibition of RANKL–RANK interaction with osteoprotegerin (OPG), a decoy receptor for T-cell-produced RANKL, reduces the spontaneous colitis (144). This effect of OPG may be due to the inhibition of RANK-mediated DC survival. Consistent with a role for RANK–RANKL interactions in DC function in the intestine, PP DCs express RANK and, when stimulated with soluble RANKL, produce IL-10, whereas spleen DCs produced predominantly IL-12. In addition, treatment of mice with soluble RANKL enhanced the sensitivity to oral tolerance induced to soluble OVA. These studies suggest that RANK stimulation can enhance either the tolerogenic capacity of DCs under steady-state conditions or the immunogenic capacity of DCs in inflamed tissue, possibly through effects on DC survival.

Taken together, studies in mice have provided an important experimental support for a central role for DCs in regulating intestinal inflammation in IBD. DCs are likely involved in the induction and expansion of both pathogenic and regulatory T cells within organized lymphoid tissues and at sites of inflammation. What is not at all clear from these studies, however, is how DCs, and in particular different DC populations, are involved in the overall balance of pathogenic versus regulatory cell activation in the pathogenesis of disease in these models. For example, it would be logical to predict that intestinal CD11b⁺ DCs are more involved in regulatory T-cell activation and expansion, given the *in vitro* studies showing the propensity of these DC populations to induce IL-10-producing T cells. However, the finding that CD11b⁺ DCs that express OX-40 L may be directly involved in pathogenic T-cell expansion (139) and that these cells are preferentially increased in number within the inflamed colon (Jianping He *et al.*, manuscript in preparation) is counter to this hypothesis.

DCs in human IBD

Studies of DCs in human IBD have focused on cells in the normal and inflamed colonic LP. Similar to murine models,

early studies pointed to a role for activated DCs at sites of inflammation (145–148). However, the lack of DC-specific markers makes the interpretation of those studies complicated (149, 150). More recently, DCs have been identified with more precision in human gut, and the results of different studies are somewhat controversial (85, 86, 150–155). While some authors have not observed a significant increase in the number or maturation status of LP DCs in IBD (85), others have provided evidence for an increase in both immature and mature DCs within inflamed tissues. On the one hand, CCL20 is overexpressed in the intestinal epithelium associated with human IBD lesions, and this overexpression was reported to correlate with an increase in the number of immature Langerin⁺ DCs *in situ* (151). Furthermore, TNF- α increased the expression of CCL20 in colonic explant cultures from normal patients, suggesting that immature DCs (as well as T cells) expressing CCR6 are attracted to an enhanced expression of CCL20 by epithelial cells in the inflamed intestine (151). On the other hand, others have shown an increase in the number and maturation of DCs within inflamed tissue (86, 150, 152).

In CD, an increase in CD83⁺ LP DCs has been recently documented (86). These cells were found in aggregates in the LP, and their increased presence was accompanied by an increase in IL-12- and IL-18-producing CD83⁺CD80⁺ DC-SIGN⁺ cells that may represent a second population of DCs (86). Another study showed an increased number of lin⁺CD86⁺CD40⁺ DCs in the peripheral blood and in the LP of patients with both CD and ulcerative colitis (UC), consistent with the presence of activated DCs in both the blood and the tissues (152). The significance of the enhanced expression of CD86 and CD40 on blood DCs is not clear, because DCs normally migrate in immature form from the blood to the tissues and after maturation to T-cell zones of lymphoid tissues via the lymph. Thus, while possibly reflecting the cytokine environment in the blood that could result in early maturation, the effect on migration to tissue is unknown. Regarding DC subsets in IBD, our preliminary studies of CD patients demonstrate increases in the number of CD86⁺CD11c⁺ myeloid DCs in peripheral blood and mature fascin^{hi} myeloid DCs in the colonic LP (Leon *et al.*, manuscript in preparation). DCs from IBD tissues also have been shown to express elevated levels of CD40 that are reduced after treatment with anti-TNF- α (150). Finally, M-DC8⁺ monocytes, which may be precursors of DCs with a high potential to secrete TNF- α (153), are also elevated in the colonic LP of CD patients (74), suggesting a novel pathogenic mechanism in CD in addition to the potential initiation and maintenance of the Th1 response.

The role of DCs in the pathogenesis of UC has received less investigative attention, but recent evidence indicates an increase in the number of CD83⁺ and CD86⁺ LP cells, which are most likely DCs and which produce macrophage inhibitory factor (MIF). MIF, in turn, can induce IL-1 and IL-8 production by monocytes and DCs (154), which may contribute to neutrophil recruitment and activation. Moreover, in UC, colonic LP contains numerous basal aggregates composed of lymphocytes and CD80⁺ dendritiform cells that most likely represent activated DCs (155). Because DCs generated *in vitro* from peripheral monocytes from patients with UC showed an increased immunostimulatory capacity (156), it is possible that immature DCs from UC patients are, in general, more sensitive to activation signals or have an accelerated differentiation program in response to factors, such as GM-CSF. Finally, UC is characterized by an increase within LP tissues of EBI-3, one subunit of the IL-12-related cytokine IL-27 (157), which is produced by macrophages and DCs. In addition, EBI-3^{-/-} mice have few NKT cells and are resistant to oxazolone colitis (158), a murine model of UC that is dependent on the presence of NKT cells (159). These data suggest that DC (or macrophage)-derived IL-27 may potentially be important in the pathogenesis of UC through an effect on NKT-cell activation (160).

Conclusions and future directions

While we have learned a great deal about DCs in the intestine over the past 5–10 years, the study of these interesting and important cells is still in its infancy. We now have a more complete understanding of the phenotype, localization, and migration of DCs in mucosal tissues, which allows for the generation of models of how DCs may be involved in oral tolerance, immunity to pathogens, and abnormal intestinal inflammation. More challenging will be to sort out methods to test these models *in vivo*. In particular, one difficult and often daunting prospect is how to make sense of the role of the large number of different subpopulations of DCs in mucosal immune responses. It is partly due to the fact that most studies of DC function to date have relied either on the purification of cells from tissues, which removes them from their complex microenvironment, or on studies of cells generated in culture, which are hard to place into physiological context. In order to make the next leap in understanding the function of DC populations in the intestine, it will likely be necessary to generate novel techniques that allow for the analysis of the phenotype and function of DCs *in vivo*, in well-established animal models of infection and

inflammation. Techniques allowing for the labeling and tracking of subpopulations of DCs [e.g. by two-photon microscopy (100)] and their inducible depletion *in vivo* should help in this regard. In addition, the possibility of analyzing the genome and proteome of very small numbers of cells is becoming

more of a reality, which may allow for more precise analysis of states of DC differentiation and function in animal models. This opportunity may be particularly valuable to study DCs in human tissues and disease states, for which the availability of tissues is a huge impediment.

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