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REVIEW

Enemy at the gates: dendritic cells and immunity to mucosal pathogens

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Dendritic cells (DC) are diverse and specialized hematopoietic cells serving as an essential bridge between innate and adaptive immunity. DC exist in all lymphoid and nonlymphoid organs and are amongst the first responders to infection at epithelial surfaces including mucosal tissues. DC of the lung, gut, and vaginal mucosa display different phenotypes and functions reflecting each unique tissue and, in contrast to their counterparts in spleen and lymph nodes, are constantly exposed to both harmful and benign factors of their environments. Mucosal DC recognize and respond to pathogens through engagement of pattern recognition receptors, and activated DC migrate to draining lymph nodes to induce adaptive immune responses. The specialized function of DC aids in the induction of immunity and pathogen control at the mucosa. Such specialization includes the potent antiviral interferon response of plasmacytoid DC to viral nucleic acids, the ability of mucosal DC to capture organisms in the gut lumen, the capacity of DC to cross-present antigen from other infected cells, and the ability of mucosal DC to initiate IgA class switching in B cells. DC plasticity is also critical in the immune response to mucosal pathogens, as DC can respond to the microenvironment and sense pathogen-induced stress in neighboring epithelial cells. Finally, DC interact with each other through crosstalk to promote antigen presentation and T-cell immunity. Together, these processes condition mucosal DC for the induction of a tailored immune response to pathogens.

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Introduction

Dendritic cells (DC) comprise a diverse hematopoietic cell population marked by phenotypic heterogeneity and functional plasticity, which allows for expansive recognition and tailored reaction to pathogens. DC reside at low frequency in nonlymphoid and lymphoid tissues and serve to induce adaptive immune responses to pathogens. Based on early conceptions of their developmental lineages, DC are categorized as conventional DC (cDC), often termed as myeloid DC, or plasmacytoid DC (pDC) [1]. DC and their hematopoietic progenitors develop in the bone marrow, progressing from a macrophage and DC precursor capable of giving rise to both lineages to a common DC progenitor generating cDC and pDC but not monocytes/macrophages [2-6]. It is clear that DC play a central role in many aspects of immunology beyond the generation of immunity to pathogens, including maintenance of tolerance and control of allergy [7, 8]. In addition, approaches are being developed to manipulate DC for such diverse clinical applications as induction of transplant tolerance, development of vaccines for infectious diseases and cancer, and therapy for autoimmune diseases [9-13]. In this review, we will focus on DC in immunity to mucosal pathogens, which itself is a burgeoning field of research [14-16]. We will first describe the anatomy of DC subsets in the different mucosal compartments and their expression of pathogen receptors and

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Abbreviations: DC (dendritic cell); LC (langerhans cell); LP (lamina propria); MALT (mucosal-associated lymphoid tissue); CTL (cytotoxic T lymphocyte); HSV (herpes simplex virus); LPS (lipopolysaccharide); NLR (nucleotide-binding domain and leucine-rich repeat containing); PRR (pattern recognition receptor); TLR (Toll-like receptor); Treg (regulatory T cell); TSLP (thymic stromal lymphopoietin)

will then discuss the nature of the DC response to mucosal pathogens. In this discussion, we will highlight the role of DC trafficking and the importance of DC specialization, plasticity and crosstalk in shaping the mucosal immune response.

Attributes of mucosal DC

Mucosal tissues are compartmentalized into the lumen, or external environment, the epithelial barrier, and the parenchyma or nonlymphoid connective tissues, collectively known as the lamina propria (LP). Within the mucosa, DC are continually replenished through the influx of committed DC, as well as the local differentiation of DC precursors and immigrating monocytes. Originally thought to occur exclusively during inflammatory conditions, monocytes inherently differentiate into DC within mucosal tissues, further defining the mechanisms of homeostatic DC maintenance [17]. This was demonstrated when adoptively transferred blood $CCR2^{-}$ CX_3CR1^{hi} 'noninflammatory' monocytes were found to differentiate into intestinal lymphoid tissue-resident DC in the absence of inflammatory mediation, an event replicated at the lung and vaginal mucosa [18-20]. During steady-state conditions, monocytes immigrating from the blood undergo a progressive morphological and functional differentiation, gaining dendriform structure and DC phenotype as they travel into the LP, establishing mucosal monocyte-derived DC. In contrast, the more conventionally recognized 'inflammatory' monocyte-derived DC are generated rapidly from $CCR2^{+}$ CX_3CR1^{low} monocyte precursors during infection in a granulocyte-macrophage colony-stimulating factor-dependant manner [17].

Mucosal tissue is characterized as either type I, consisting of a simple single-cell epithelial layer, or type II, which is covered by stratified squamous epithelium. Physiologically, type I mucosa serves as a region of absorption, exchange, and respiration, covering the intestine, lung, and uterus, and is supported by mucosal-associated lymphoid tissues (MALT). MALT consist of organized secondary lymphoid structures, such as Peyer's patches and isolated lymphoid follicles, which reside directly beneath the epithelial cells providing a site of immune induction. In contrast, type II mucosal surfaces establish a physical barrier for protection as found at the oral, corneal, and lower vaginal mucosa, and are monitored by regional lymph nodes instead of MALT structures. Furthermore, organized lymphoid structures may be generated on inflammatory insult, as illustrated by the development of inducible bronchus-associated lymphoid tissue in mice following influenza virus infection [21]. Mucosal DC have evolved exquisite adaptations to the anatomic microenvironment in which they reside. DC populations of type II mucosal surfaces bear a striking resemblance to their relatives in the skin, with Langerhans cells (LC) residing at both locations [22]. Within type II mucosal tissues, LC intercalate with the epithelial layer as submucosal DC and reside beneath the basement membrane. Generally speaking, DC residing in type II mucosal tissue serve to protect the host through the induction of inflammatory responses, whereas DC within type I mucosa must maintain the immunological balance between tolerance and inflammation and may therefore serve a more regulatory function [23]. However, within the different compartments of the mucosal tissues, DC have different specializations and functions, as described below.

DC of the respiratory tract

The lung samples ~10 000 liters of air every day, and is continually challenged with exposure to both innocuous and detrimental particulate matter. Unique to the lung mucosa, the contiguous DC network of the upper and lower respiratory tracts differ in their functional response to antigen as a result of the normally sterile nature of the bronchioles and alveoli in the lower tract. Similar to other mucosal cDC subsets in the mouse, the species for which most is known about mucosal immunity, respiratory cDC are phenotypically defined by the presence of the α-integrin CD11c and major histocompatability complex (MHC) class II, and show variable expression of CD11b and CD8α. pDC expressing $CD11c^{Int}$, $Gr\text{-}1^{+}$, $B220^{+}$, and the bone marrow stromal cell antigen 2 (BST-2, PDCA-1, 120G8) have been found to reside in the interalveolar interstitium [24]. Two major subsets of cDC in mouse lung have been defined based on CD103 ($\alpha E\beta_7$) and CD11b expression. Employing parabiotic mice in which the circulatory systems of two animals are surgically linked, studies have suggested that $CD103^{+}CD11b^{lo}CX_3CR1^{-}$ and $CD103^{-}CD11b^{hi}CX_3CR1^{+}$ cDC in the resting lung are derived from $Ly6C^{hi}CCR2^{hi}$ and $Ly6C^{lo}CCR2^{lo}$ blood-borne monocytes, respectively, under steady-state conditions [25]. Consistent with this finding, the selective depletion of DC at the mucosa from CD11c-DTR transgenic mice, which express diphtheria toxin under control of the CD11c promoter, followed by reconstitution of $Ly6C^{+}$ monocytes reconstituted the cDC population in the lung and intestinal LP, but not cDC in spleen [20].

Confocal microscopic analysis of rat trachea revealed that under homeostatic conditions roughly 20% of resident DC interdigitate within the resting epithelium, with the remaining 80% dwelling within the subepithelial connective tissues [26]. It is likely that these cells repre-

sent a developmental continuum, with recently emigrated monocyte-derived DC of the LP undergoing further differentiation while traveling into the epithelium, as seen through slight increases in MHC-II expression, size, and dendrite expression, along with a minor decrease in endocytic activity [26]. Steady-state pulmonary DC replacement rates are differentiated by both anatomic location and DC phenotype, with upper airway DC expressing moderate levels of CD11c and undergoing rapid turnover, with greater than 80% DC exchange in 18-36 h. In contrast, DC populations of the lung parenchyma express high levels of CD11c and have reduced rates of replacement of roughly ~12% exchange in 9 days [27, 28]. Although the mechanisms of DC precursor recruitment to the lung mucosa are incompletely defined, CCR1 and CCR5 have been implicated under both homeostatic and pathogen-induced conditions [29, 30].

Studies utilizing carboxyfluorescein succinimidyl ester or fluorescein isothiocyanate-conjugated ovalbumin delivery to the trachea in mice under steady-state conditions have shown that DC maintain a constitutive CCR7-mediated migration from the mucosal tissues to the draining lymph nodes (constituting roughly 2% of total cells) where they help establish antigen-specific tolerance among naïve T lymphocyte populations [27, 31, 32]. To maintain homeostatic tolerance to innocuous environmental factors, $CD11b^{+}ICOSL^{+}$ cDC present in lung-draining mediastinal lymph nodes are responsible for the induction of regulatory T cells that inhibit inflammation in an IL-10-dependant manner. Furthermore, epithelial cell production of thymic stromal lymphopoietin (TSLP) and TGF-β condition respiratory DC toward tolerance, emphasizing an antiinflammatory local environment until pathogenic invasion [33].

Recently, a novel mechanism for immunological surveillance by DC of the lung mucosa was proposed in a model of pulmonary embolism as an evolutionary means of detecting large complex antigens such as parasitic worms or eggs. This study utilized intravenous delivery of large particulate antigen, represented by ovalbumin-coated Sepharose beads. The large embolic antigens trapped in the lung vascular bed induced cDC-mediated $CD4^{+}$ and $CD8^{+}$ T-cell responses in the peribronchial lymph node, leading to the formation of short-lived T cell/DC granulomas responsible for antigenic clearance [34]. Furthermore, T-cell-mediated immunity to embolic antigen was severely inhibited following DC ablation, but restored on adoptive transfer of $Ly6C^{hi}$ monocytes, implicating monocyte-derived DC in clearance [34].

DC of the gastrointestinal tract

The gastrointestinal tracts of rodents and humans are colonized with an estimated 500-1 000 individual species of microflora accounting for roughly 10^{14} organisms [35]. Regulation of immunological balance in the gastrointestinal mucosa involves DC-mediated tolerance to ingested environmental and food antigens, as well as beneficial resident bacteria, together with the need of recognizing and responding to pathogenic threats. Failure to maintain this balance may lead to the development of allergic and chronic inflammatory diseases, such as Crohn's disease or irritable bowel syndrome, or conversely, systemic infection. Under homeostatic conditions, intestinal LP DC induce self- and microbial-oral tolerance through the presentation of apoptotic epithelial cells in the absence of co-stimulatory signals, and were shown to constitutively produce IL-10 and IFN-β but not IL-12 [36-39]. Within the gastrointestinal lymphoid tissue of the small and large intestine, DC are found in a contiguous network within and interspersed beneath the epithelial layer, congregated within isolated lymphoid follicles and compartmentalized within the highly specialized Peyer's patches of the LP. Intestinal DC work in concert with a class of epithelial cells specialized for antigen transport termed as M-cells that reside above the organized lymphoid structures of the gut within the follicle-associated epithelium. M-cells transport luminal antigens, including viruses and bacteria, for evaluation by the underlying DC resident within the Peyer's patches of the small intestine, and isolated lymphoid follicles of the small and large intestine [40].

The majority of LP DC are $CD11b^{+}CD8\alpha^{-}$, with minor populations of $CD11b^{-}CD8\alpha^{+}$, $CD11b^{-}CD8\alpha^{-}$, and pDC also being present [36, 39]. During homeostatic conditions, most DC reside in the subepithelial layer with fewer DC localizing to the follicle-associated epithelium. Recently, two elegant studies employing diphtheria toxin-mediated depletion of $CD11c^{+}$ cells coupled with reconstitution of DC precursors demonstrated the origins of homeostatic LP DC [41, 42]. Of the two main DC subsets in the LP, $CD103^{+}CX_3CR1^{-}$ DC were found to arise from pre-cDC precursors via a Flt3 ligand-mediated pathway, whereas $CD103^{-}CX_3CR1^{+}$ ($CD11b^{+}CD14^{+}$) DC originated from macrophage and DC precursor-derived $Ly6c^{hi}$ monocytes under the control of granulocyte-macrophage colony-stimulating factor [41, 42]. CX_3CR1^{+} LP DC underwent massive clonal expansion in the LP layer during cellular reconstitution with $Ly6c^{hi}$ monocytes [42]. Interestingly, LP resident $CD103^{+}CX_3CR1^{-}$ DC sustain rapid homeostatic turnover rates, and have been found to emigrate from the intestinal mucosa to the mesenteric lymph nodes following exposure to pathogenic *Salmonella typhimurium* antigen, Toll-like receptor 7/8 (TLR7/8) or lipopolysaccharide (LPS) stimulation via CCR7-medi-

ated recruitment, whereas long-tenured $CD103^{-}CX_3CR1^{+}$ DC of the LP failed to deliver antigen to mesenteric lymph nodes [41, 43]. In addition, $CD103^{-}CX_3CR1^{+}$ LP DC have limited capacity to stimulate T-cell responses *in vitro* or *in vivo* compared to $CD103^{+}CX_3CR1^{-}$ LP DC, suggesting cooperative mechanisms between DC subsets to facilitate antigen capture and immune induction, respectively [43]. Examination of the mouse colon revealed that all $CD8\alpha^{+}$ and some $CD11b^{+}$ cDC express $CD103^{+}$, and they were found to preferentially induce CCR9 expression on naïve T cells, necessary for gastrointestinal recruitment [44]. Importantly, specific subsets of DC in the gut mucosa have the capacity to extend processes between epithelial cell tight junctions and sample enteric organisms [45]. This intriguing specialization is discussed in detail below.

DC of the reproductive tract

The vaginal mucosa undergoes hormonally induced cyclic changes, necessitating a unique system of cellular regeneration and maintenance. Within the mouse, vaginal epithelial-lining cDC and LC express combinations of $F4/80^{+}$ and $CD205^{+}$, but show little to no CD11b and CD207 expression, whereas the submucosal cDC of the female reproductive tract express combinations of $CD11b^{+}$, $CD8\alpha^{+}$, and $F4/80^{+}$ similar to those of the lung and gut [46, 47]. Unlike skin-resident LC, vaginal epithelial DC are repopulated through nonmonocytic bone marrow-derived progenitors, likely common DC progenitors and pre-cDC, with a half-life of 13 days under homeostatic conditions [47]. Furthermore, recruited $Ly6C^{hi}$ or $Gr\text{-}1^{+}$ monocytes rapidly differentiate into epithelial cDC as well as epidermal vaginal LC expressing a constitutively activated phenotype following vaginal herpes simplex virus (HSV)-2 infection [47, 48].

DC inhabiting the ectocervix and vagina, the type II mucosal tissues serving as physical barriers for protection during coitus, resemble dermal LC and likely function as dominant promoters of T_H1/T_H2-based immunity against invading sexually transmitted pathogens. In contrast, examination of human decidual endometrium has shown that the cDC of the upper genital tract, the type I mucosa of the ovaries, uterus, and endocervix, fail to produce IL-12p70 on exposure to LPS or *Staphylococcus aureus*, instead inducing IL-4 production from T cells [49]. Although the ability of decidual DC to induce a T_H2-biased T-cell response likely plays a role in establishing tolerance toward the semiallogeneic fetus during pregnancy, these mechanisms are not fully understood.

Following vaginal HSV-2 infection in mice, $CD11b^{+}$ submucosal DC, but not LC, were capable of migrating to draining lymph nodes, where they initiated antiviral T_H1-biased cellular immunity, a mechanism dependant on MyD88 signaling [46, 50]. $CD11b^{+}$ submucosal DC, but not vaginal LC or $CD8\alpha^{+}$ lymph node-resident DC, were shown to control mucosal HSV-2 infection by inducing T_H1 T-cell-mediated antiviral immunity [46]. In response to intravaginal HSV-2 infection, pDC are rapidly recruited to the vaginal mucosa where they provide protection from lethal challenge through the production of copious amounts of IFN-α initiated by TLR9-based recognition of viral CpG oligodeoxynucleotides [51, 52]. In support of these findings, human herpetic lesions contain vast infiltration of uninfected pDC, which readily associated with T and NK cells *in vivo* and stimulated autologous T-cell proliferation *in vitro* [53]. Rapid infiltration of pDC into vaginal mucosa has also been described within 1 day of vaginal exposure of *Rhesus macaques* to simian immunodeficiency virus [54]. Paradoxically, recruited and activated pDC produced inflammatory chemokines CCL3 and CCL4, which attract $CCR5^{+}CD4^{+}$ T cells to the mucosa, providing targets for infection and fueling virus expansion [54]. pDC are known to be depleted from blood and lymph nodes including mesenteric lymph nodes in established simian immunodeficiency virus infection [55, 56], but the effect of established infection on mucosal DC has not been determined.

Pathogen recognition by DC

DC detect biological threats through the interaction of germ-line-encoded pattern recognition receptors (PRR), with pathogen-associated molecular patterns (PAMP) representing invariant structures present across species of viruses, bacteria, parasites, and fungi. Combinations of PAMP expressed within individual pathogens along with overlapping molecular signaling pathways of PRR allows for a uniquely qualitative host response. The cellular localization of PRR types or families at the cell membrane, within intracellular compartments such as the endosome or lysosome, or in the cytoplasm enhances the perception of pathogens by DC, as seen during the phagocytosis of extracellular bacteria, detection of intracellular viral replication, or the crosstalk between DC and infected epithelial cells. The field of innate immune recognition via PRR is a rapidly expanding field of active exploration [57], and here we will discuss just the salient features as they relate to DC. The predominant classes of PRR utilized by DC consist of the TLR, retinoic acid-inducible gene-I (RIG-I)-like receptors, C-type lectin receptors, and nucleotide oligomerization domain (NOD)-like receptors, with other yet to be defined molecules likely in existence [58].

Toll-like receptors

The mammalian TLR family consists of 13 highly conserved type 1 transmembrane proteins, which require homo- or heterodimerization of receptors following ligand recognition to provide the structural conformation necessary for downstream signaling. Cellular distribution of TLR is confined to either the cell surface, represented by TLR1, TLR2, TLR4, TLR5, TLR6, and TLR11, or within intracellular vesicles such as the endosome, lysosome, and endoplasmic reticulum, represented by TLR3, TLR7, TLR8, and TLR9. The localization of TLR dictates their pathogen recognition capabilities, as TLR expressed on the cell surface detect microbial membrane components, whereas intracellular TLR will recognize microbial nucleic acid species. Restricted TLR expression within DC subsets helps dictate their pathogen recognition profiles, exemplified by TLR9 expression in pDC compared with TLR4 or TLR2 and TLR4 expression in cDC of mice and humans, respectively [59, 60]. $CD8\alpha^+$cDC preferentially express TLR3, but are selectively absent of TLR5 and TLR7 [61]. Phagocytosis of virally infected target cells activates $CD8\alpha^+$ cDC through the interaction of endosomally contained TLR3 with the dsRNA intermediate of viral infection, an event that leads to the enhancement of antigen cross-presentation to $CD8^+$ cytotoxic T lymphocytes (CTL) [62]. $CD11c^+CD11b^+$ DC of the intestinal LP detect invasive pathogenic bacteria through TLR5-flagellin interactions, leading to adaptive inflammatory T_H1 and T_H17 T-cell responses and inducing the differentiation of immunoglobulin A (IgA)-producing plasma cells through a retinoic acid-dependant, GALT-independent mechanism [63].

pDC contain elevated expression of endosomal TLR7 capable of recognizing genomic RNA or ssRNA intermediates from viral pathogens such as influenza virus, vesicular stomatitis virus and HSV [64]. TLR7-mediated detection of ssRNA species in pDC is not dependent on viral replication, as genomic RNA is sufficient for activation. IFN-α/β production was abrogated in pDC deficient of TLR7, following exposure to influenza virus or vesicular stomatitis virus [65, 66]. Interestingly, although influenza virus can be detected by either TLR7 or RIG-I (described below), mice deficient for TLR7 signaling fail to develop protective influenza-specific immunity following vaccination with inactivated virus [67]. These findings suggest that the TLR pathway is the preferential method of influenza detection, and conceivably the dominant mechanism for the induction of adaptive antiviral immunity within pDC [68]. Notably, IRF7, the master regulator for induction of type I IFN production through TLR7 and TLR9 signaling, is highly expressed within the cytosol of pDC, potentially accounting for the speed and magnitude of IFN-α/β production from pDC [69]. An additional, but by no means exclusive mechanism for the heightened IFN-α/β response of pDC is their ability to sequester TLR signaling complexes, as illustrated by the retention of CpG DNA-TLR9 within the endosomal vesicles for extended periods of time over that seen in cDC, allowing for continual TLR activation and IRF signaling [70]. pDC recognition of dsDNA genomic intermediates of replicating DNA viruses via TLR9 accounts for the detection of HSV and murine cytomegalovirus [51, 71-73]. Interestingly, TLR9-mediated recognition of the malarial byproduct hemozoin derived from *Plasmodium falciparum* and subsequent production of IL-12 and IFN-γ by DC have been implicated in the proinflammatory cytokinemia associated with acute disease and related sepsis by enhancing TLR expression- and 'priming'-associated signaling pathways leading to hyperresponsiveness to further bacterial or parasitic TLR activation [74, 75].

RIG-I-like receptors

The RIG-I-like receptor family consisting of RIG-I, melanoma differentiation-associated gene 5 (MDA5), and laboratory of genetics and physiology 2 (LGP2) receptors detect cytoplasmic viral RNA through a TLR-independent mechanism [76]. Utilizing knockout mice, RIG-I was shown to respond to ssRNA viruses including influenza virus, vesicular stomatitis virus, paramyxoviruses, and Japanese encephalitis virus, whereas MDA5 was found to recognize an alternative array of RNA viruses such as picornaviruses. Differential recognition of RNA viruses by RIG-1 and MDA5 suggests that these receptors recognize different structures of the viral RNA. In contrast to other known PRR, the RIG-I family member LGP2 may serve a regulatory function [77]. RIG-I is essential for induction of type I IFN after infection with RNA viruses of cDC but not pDC, the latter instead using the TLR system for viral detection [68].

C-type lectin receptors

The C-type lectin receptor molecules bind the carbohydrate moiety of glycoproteins and serve as adhesion molecules for microbes such as viruses, bacteria, parasites, or fungi, as well as endothelial cells, neutrophils, and T cells to name a few. C-type lectin receptor binding allows for pathogen internalization, degradation, and antigen presentation with associated cellular activation [78]. Similar to TLR, distinct DC subsets express various C-type lectins with receptors such as BDCA2 expressed by human pDC, langerin (CD207) by LC and dermal DC, and DNGR1 (CLEC9A) by $BDCA3^+/CD8\alpha^+$ DC. DC-SIGN (CD209), dectin 1 (CLEC7A), and CD205 are displayed broadly on cDC [78].

NOD-like receptors

The NOD-like receptor family of PRR known as NLR (for nucleotide-binding domain and leucine-rich repeat containing) is comprised of three subfamilies distinguished by unique N-terminal protein interaction domains, recognizing intracellular microbial components and stress signals [79]. As a large PRR family, with at least 23 human and 34 murine genes identified, NLR recognize structural components of Gram-negative and Gram-positive bacteria, bacterial toxins, and microbial RNA within the cytosol. Although the expression of NLR in DC is mostly unknown, studies have identified NOD1/2 expression in human DC, and high expression of NALP1 within LC of the mucosal surfaces and skin.

The critical role of DC migration in immunity

DC recognition of pathogens via PRR at the mucosa initiates a highly coordinated temporal cascade of maturation and events, resulting in DC mobilization, interstitial migration, entry into the afferent lymphatics, and transit via the lymph to secondary lymph nodes [80]. During early activation, DC locomotion and antigen presentation are linked in time and space, as the MHC-II invariant chain CD74, shared by both processes, transiently sequesters myosin II in the endosomal compartment while loading MCH-II-peptide complexes, temporarily inhibiting DC motility [81]. Discontinuous locomotion by DC may serve to coordinate antigen capture and processing with presentation by immature DC, allowing them to maximize antigen uptake and directional targeting while processing antigen, and subsequently increasing velocity on MHC-II-antigen expression [82]. The sequential activation and maturation process within DC occurs en route from the mucosa to the draining lymph nodes, resulting in CCR7-directed transport to peripheral lymphatic endothelial cells as well as lymph node stroma cells expressing CCL19 and CCL21 [83, 84]. Submucosal DC are found to upregulate co-stimulatory markers and assume dendriform morphology while trafficking to the draining lymph nodes following respiratory challenge with heat-killed *Moraxella catarrhalis* [26]. Additionally, T cell stimulatory ability is found in peribronchial lymph node populations 24 h following bacterial challenge, but is absent 2 h post-infection [26]. This compartmentalization of functions protects mucosal tissues, particularly those seen in the airway, from T-cell-mediated damage that could ensue from continuously responding to ubiquitous nonpathogenic antigens present in ambient air.

Inflammation-mediated recruitment of DC and monocyte-derived DC at the mucosa can increase more than 100-fold in response to infection over homeostatic levels in naïve mice. Antigen-bearing airway mucosal DC were found to migrate from the lung to peribronchial lymph nodes in as little as 30 min following cessation of a 1-h exposure to a mixture of *Moraxella catarrhalis* and ovalbumin in the rat [26]. Following respiratory influenza virus infection, migration of pulmonary DC to the peribronchial lymph nodes increases dramatically, with recently immigrated DC populations attaining a maximum of 18% of total lymph node cells at 18 h post-infection [27]. Interestingly, the advanced emigration of pulmonary DC to the peribronchial lymph nodes ceases by 48 h post-infection in the presence of ongoing influenza replication, gradually returning to pre-infection levels, with pulmonary DC migration becoming refractory to subsequent influenza challenge [27]. Although pulmonary DC populations seem to be refractory to homologous viral challenge, the number of $CD11c^+$ cells in the peribronchial lymph nodes continually increased over this period, suggesting DC recruitment from additional sources [27]. In contrast, analysis of lymph cells collected from the thoracic duct of rats following the surgical removal of the intestinal draining lymph nodes failed to detect migratory pDC during either steady-state or TLR7/8-induced inflammatory conditions [85].

Elegant studies by Lee *et al*. [86] have recently demonstrated that following vaginal HSV-1 infection of mice, migratory $CD8\alpha^+$ and $CD8\alpha^-$ submucosal DC are both capable of cytotoxic T-cell priming and T_H1-induction in the draining lymph node from $CD8^+$ and $CD4^+$ T cells, respectively, an effect that was mediated predominantly by the $CD8\alpha^-$ submucosal DC population identified through cell sorting of antigen-carrying, labeled occupants of the lymph node. Interestingly, on needle infection with HSV, antigen became lymph borne and was presented in the draining lymph node by resident DC to $CD8^+$ and $CD4^+$ T cells, illustrating the variation in antigen capture and presentation mechanisms on parenteral or mucosal infection [86].

In addition to migration of DC themselves, it is important to note that mucosal DC imprint specific homing patterns on lymphocytes [23]. Studies have demonstrated that DC of the intestine induce the expression of the mucosal homing receptors $\alpha_4\beta_7$ and CCR9, specific for the gut-associated chemokines MAdCAM-1 and TECK/CCL25, respectively, on T and B cells through the production of retinoic acid (converted from retinol or vitamin A) [87-90]. Imprinting of T cells to home to the intestinal mucosa was mediated by $CD103^+$ cDC, both with and without $CD11b^+$ and $CD8\alpha^+$, but not their $CD103^-$ counterparts [44]. In contrast, cDC populations of the spleen and cervical lymph nodes were incapable of inducing $\alpha_4\beta_7$ and CCR9 on lymphocyte populations,

and DC from systemic lymph nodes created $CD8^+$ T cells which migrated to inflamed skin [87].

DC specialization in pathogen control and adaptive immunity

The antiviral response of pDC

The induction and maintenance of immunity is greatly impacted by the specialization of DC subsets [91]. There is no more clearly defined example of DC specialization than that of the pDC population. Compared to cDC, pDC appear to function only indirectly in induction of adaptive responses, as discussed below, and rather have a primary function of establishing a state of inflammatory preparation and defense against invading pathogens [92]. Unmatched in their ability to produce copious amounts of type I IFN on activation, pDC have evolved multiple physiological mechanisms to activate innate immunity to viral infection. On endosomal capture of Newcastle disease virus, a paramyxovirus, pDC activation and type I IFN production is mediated via TLR7 in a replication-independent manner that is strongly supported by type I IFN positive feedback [93] (Figure 1). Additionally, through the diphtheria toxin-mediated elimination of cDC, it was shown that pDC are capable of priming naïve $CD4^+$ but not $CD8^+$ T-cell responses in the lymph nodes of mice [94]. This study suggests that pDC either develop compensatory T cell priming mechanisms in the absence of cDC, or alternatively, that the presence of cDC masks the inherent, albeit low, priming capacity of pDC.

Sampling of luminal organisms by gut DC

Gut DC have evolved a fascinating specialization, which allows them to sample environmental antigens without disrupting the physical barrier of the epithelium of type I mucosal surfaces. DC residing in the intraepithelial layer and LP actively sample their microenvironment by traversing their dendrites through the simple epithelial layer into the lumen through the formation of tight junctions with adjacent epithelial cells. This process requires the presence of CX_3CR1 on DC [26, 45, 95] (Figure 1). Transepithelial dendrite formation allows for sampling of commensal and pathogenic bacteria alike, as seen through capture of enteroinvasive *Salmonella typhimurium* and commensal *Escherichia coli* species [95]. These monocyte-derived $CD103^-CX_3CR1^+$ LP DC demonstrated robust TNF-α^+ production in an innate colitis model, suggesting a propensity for inflammatory reactions [41, 42]. Although the precise roles of CX_3CR1^+ and CX_3CR1^- DC during mucosal infection have yet to be addressed, luminal-sampling $CX_3CR1^+CD103^-$ DC may capture and transport unattainable antigen to neighboring $CD103^+CX_3CR1^-$ LP DC capable of emigrating to peripheral lymph nodes for immune induction. Many pathogens including *Helicobacter pylori*, *Listeria monocytogenes*, and *Brucella abortus* have developed mechanisms to subvert the environmental sampling by DC to gain entry to the host through cellular translocation or by infecting the DC itself [96-98]. The capacity of DC to breach the epithelial layer and sample environmental organisms may not be unique to the gut, as it has recently been reported that activated LC in skin can penetrate keratinocyte tight junctions and capture antigens under the keratinized stratum corneum [99].

DC cross-presentation

Another clear example of DC specialization is the phenomenon of cross-presentation, which is the capacity to present pathogen-derived antigenic peptides to $CD8^+$ T cells via MHC-I in the absence of direct infection. Although both $CD8\alpha^+$ and $CD8\alpha^-$ cDC subsets are capable of cross-presenting antigen to $CD8^+$ T cells following activation via the Fcγ receptor, $CD8\alpha^+$ cDC are vastly superior in facilitating cross-presentation of antigen expressed in a cell-associated, soluble, particulate-bound, or bacterially contained form, suggesting that only $CD8\alpha^+$ cDC possess the cellular machinery necessary to cross-present antigen without Fcγ receptor association [100-103]. Furthermore, $CD8\alpha^+$ cDC seem to be solely responsible for cross-presenting antigen obtained from apoptotic host cells [101, 102, 104]. Recent studies have reported the discovery of a novel C-type lectin receptor, CLEC9A, which is capable of mediating endocytosis, but not phagocytosis [105] (Figure 1). CLEC9A is restricted to high- and low-level expression in $CD8\alpha^+$ DC and pDC in mice, respectively, and detectable expression in blood $BDCA3^+$ DC and a small subset of $CD14^+CD16^-$ monocytes in humans, but is absent from other hematopoietic cells [105]. $CD8\alpha^+$ DC have been shown to utilize the CLEC9A receptor in a novel immunological surveillance mechanism to recognize preformed signals exposed on necrotic cells, allowing for cross-presentation of dead-cell-associated antigens via SYK kinase signaling [106, 107].

DC induction of IgA class switching

IgA, the most abundant antibody of the body, provides protection through neutralizing toxins and inhibiting pathogenic infection, as well as blocking commensal bacteria from invading the systemic organs [108]. Mucosal DC are specialized to induce IgA class switching in B cells through their production of the cytokines IL-10, TGF-β, and IL-6, induction of $CD4^+$ T cells to secrete

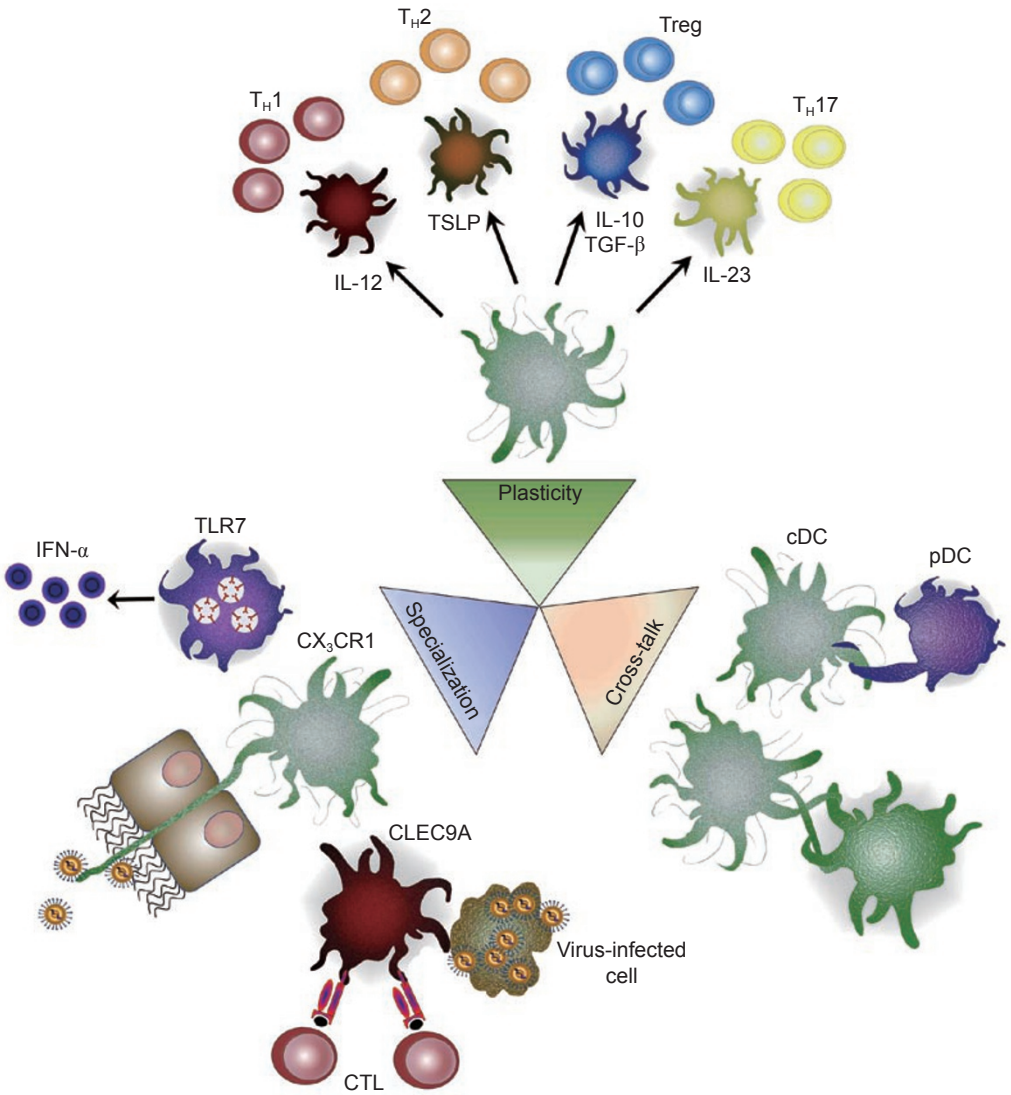

Figure 1 Functional interplay between DC. The DC response to pathogens involves a complex interplay between DC specialization, plasticity, and crosstalk at the mucosa and associated draining lymph nodes. DC specialization is predetermined through the inherent mechanical capabilities of unique DC subsets. This is exemplified by pDC that produce copious amounts of IFN-α on exposure of endosomal TLR7 to viral ssRNA, mucosal DC expressing CX_3CR1 that actively sample the contents of the lumen through transepithelial dendrite extensions, and $CD8\alpha^+$ DC expressing the CLEC9A receptor that are uniquely suited for antigen cross-presentation to CTL. DC plasticity is influenced by the cytokine and chemokine constitution of the mucosal environment to which the DC is exposed. The default state licensing mucosal DC for the induction of tolerance is achieved through the constitutive production of TSLP by epithelial cells and predilection toward IL-10 production of neighboring local DC, leading to the promotion of T_H2 and Treg responses, respectively. Following PRR signaling, production of IL-12 and IL-23 licenses mucosal DC to promote T_H1- and T_H17-mediated adaptive responses from lymphocytes present in the associated lymphatic tissue. The recently appreciated phenomenon of DC crosstalk occurs as DC physically interact with neighboring cells to promote antigen presentation and pro- or antiinflammatory states. Together, these processes culminate to condition mucosal DC for the induction of situation-specific, tailored immune responses to pathogens.

IL-4 and IL-10, and their direct presentation of intact protein antigen via FcγRIIB receptor-mediated immune complexes to B cells in the secondary lymphoid tissues [109, 110]. Evidence suggests that IL-12 and type I IFN secreted from cDC and pDC, respectively, act to differentiate memory B cells into plasmablasts, while IL-6 with or without additional cytokines, completes the differentiation process into immunoglobulin-secreting plas-

ma cells. Peyer's patch DC are sufficient to induce IgA class switching and the expression of gut-homing receptors by B cells through the production of cytokines IL-5 and IL-6 and retinoic acid, respectively, in the absence of T cell help [90]. DC-mediated class switching within B cells is dependent on BAFF and APRIL signaling. Utilizing diphtheria toxin-mediated elimination of DC in a $CD11c^+$-DTR transgenic mouse model, cDC were shown to be essential for the induction of $CD4^+$ T cell help and antigen-specific intestinal IgA secretion following oral or nasal immunization [111]. Influenza virus activated pDC from human blood promote plasma cell differentiation and activation through the production of IFN-α/β and IL-6 [112]. Similarly, T-cell-dependant mucosal IgA responses following intranasal exposure to virus-like particles containing ssRNA were mediated by DC through TLR7 signaling and the induction of BAFF and APRIL, leading to Th cell activation resulting in TGF-β secretion and the delivery of CD40-CD40L cognate signaling to B cells [113].

DC plasticity

Decades of work has established cofactors, such as type I IFN, TSLP, IL-10, IFN-γ, and IL-15, that are capable of conditioning DC to perform multiple functions including the polarization of T-cell responses [114, 115]. This capacity of DC to respond to the microenvironment may allow the cells to impact the immune response in the absence of infection or even the direct interaction with a pathogen. At sites of the mucosa, for example, this occurs through intimate association with neighboring epithelial cells that constitutively secrete TSLP and other factors, which in turn leads to DC production of IL-10, TGF-β, or IL-6, but not IL-12, and the capacity to induce a T_H2/T_H3-polarized response [116, 117]. Alternatively, mucosal epithelial cells that are undergoing pathogen-induced stress, including PRR signaling, provide inflammatory conditioning of mucosal DC to induce T_H1 and T_H17 responses [50] (Figure 1). TLR-mediated signaling within both epithelial cells and DC was found to be necessary for the induction of antiviral effector T cells during vaginal HSV-2 infection, implicating epithelial cell interactions as a necessary factor for DC conditioning [50].

Current notions of DC activation stress that a combination of cytokine- and pathogen-mediated signaling are not only necessary for optimal DC activation but also endow further specialization on individual DC subtypes. Lymph node and splenic DC rendered insensitive to LPS stimulation failed to respond to LPS-induced cytokine production from nonhematopoietic radio-resistant tissues *in vivo*, despite pronounced local and systemic inflammation [118]. By contrast, several models have shown that PRR-refractory DC are matured both directly and indirectly by other hematopoietic cells, albeit with deficiencies in proinflammatory cytokine production and polyfunctional T_H1 $CD4^+$ and $CD8^+$ T lymphocyte induction, via stimulation with PAMP [119, 120]. Notably, although the phenotype of DC in various activation states may appear to be the same, insufficiently activated, but mature DC, which are not immunogenic, fail to produce inflammatory cytokines necessary for effective lymphocyte immune induction. Thus, although direct PAMP recognition by DC is the prominent mechanism by which DC activation and subsequent T_H1 $CD4^+$ and $CD8^+$ T lymphocyte polarization occurs, a yet undefined role of leukocyte-DC and DC-DC crosstalk, potentially due to qualitative paracrine signaling or membrane exchange, may act as a sufficient substitute [58].

DC crosstalk

The importance of crosstalk between DC subsets is becoming increasingly evident, as cooperative interactions serve to compensate for inherent deficits in DC subset function while enhancing the overall quality and magnitude of immunity (Figure 1). Synergistic effects of crosstalk between cDC and pDC populations following synthetic or bacterial-derived CpG DNA exposure result in increased IL-12 production from cDC during lethal *Listeria monocytogenes* infection [121]. DC crosstalk in this model was facilitated by cDC-produced IL-15-mediated upregulation of CD40, enabling stimulation by pDC-supplied CD40L [121]. Immunization utilizing a combination of cDC and pDC provided superior immunity and protection over either DC population alone in a mouse tumor model, dependent on MHC-I expression in cDC but not pDC, suggesting that synergism augments antigen presentation [122]. Selective depletion of pDC, which are themselves inherently limited in CTL-priming capacity during cutaneous HSV infection, nevertheless resulted in an impaired ability of lymph node-resident cDC to promote antiviral CTL responses in the mouse [86, 94, 123]. Adoptive transfer of pDC precursors from wild-type, but not CXCR3-deficient, mice to pDC-depleted mice restored CTL induction by impaired lymph node DC in a CD2- and CD40L-dependent, IFN-α-assisted manner [123]. Similarly, migrant airway-derived cDC from the lung have been shown to transport viral antigen to mediastinal lymph nodes for presentation to T cells, as well as share captured antigens with resident blood-derived $CD8\alpha^+$ DC capable of further cross-presenting viral peptides to $CD8^+$ T cells [124]. *In vitro* examination of human blood DC exposed to whole bacteria found that

activated cDC, having phagocytosed pathogen, stimulated resting pDC to upregulate maturation markers and antigen presentation capacity without increasing cytokine production [125]. It is proposed that crosstalk between pathogen-responsive and refractory DC subtypes may allow for licensing of the resting DC population to become antigen-presenters, allowing for amplification of the qualitative immune responses dictated by the leading (PRR-activated) DC population [125].

Cooperative DC crosstalk plays a vital role in $CD4^+$ T cell priming, as lymph node-resident DC are mandatory to initiate activation and trapping of cognate T cells, whereas migratory DC are necessary to induce proliferation of the $CD4^+$ T-cell compartment [126]. Furthermore, evidence of crosstalk between skin and gastrointestinal DC populations was illustrated in the murine model [127]. This study proposed that immunogenic $langerin^+$ DC occupying the mesenteric lymph node following transcutaneous immunization were derived from bone marrow precursors, and had rapidly differentiated in or migrated to the gut-draining lymph nodes [127]. $Langerin^+$ DC, displaying the unusual $CD205^+CD8\alpha^-CD11b^+$ phenotype and expressing the gut-homing receptors $CD103^+$, $CCR9^+$, and $\alpha_4\beta_7$ facilitated the induction of intestinal IgA responses in a retinoic acid-dependant manner within the mesenteric lymph node [127]. The different localization and interactions of migratory and lymph node-resident DC with conduits, endothelial cells, T cells, and within the regions of the lymph node, in conjunction with the conditioning gained from PRR-mediated pathogen recognition suggest that DC crosstalk plays an essential role in providing redundant induction mechanisms and qualitative tuning of adaptive immunity.

Conclusions

While DC in mucosal surfaces have many attributes of DC in other tissues, including the expression of pathogen-sensing receptors and the capacity to mature and migrate to draining lymph nodes, they are adapted to the highly specialized environment of the different mucosal compartments. Importantly, DC within the mucosa and particularly the gut mucosa are constantly exposed to both harmful and innocuous organisms and maintain a balance between inflammatory protection from infection and tolerance to commensal flora. DC specialization and plasticity allow the cells to function within the mucosal environment and to sense pathogen-derived stress of neighboring cells such as epithelial cells to instruct the immune response to pathogens. The interaction between DC subsets is also a fundamental process in establishing the adaptive immune response. Collectively this interplay between DC specialization, plasticity, and crosstalk shapes the immune response to mucosal pathogens.

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