Mucosal dendritic cells shape mucosal immunity

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Dendritic cells (DCs) are key modulators that shape the immune system. In mucosal tissues, DCs act as surveillance systems to sense infection and also function as professional antigen-presenting cells that stimulate the differentiation of naive T and B cells. On the basis of their molecular expression, DCs can be divided into several subsets with unique functions. In this review, we focus on intestinal DC subsets and their function in bridging the innate signaling and adaptive immune systems to maintain the homeostasis of the intestinal immune environment. We also review the current strategies for manipulating mucosal DCs for the development of efficient mucosal vaccines to protect against infectious diseases.

INTRODUCTION

Mucosal tissues contain various lymphoid cells. Of these, accessory cells act as sentinels to sense invading organisms, T cells attack and clear pathogens, and B cells secrete IgA. These cells reside among large numbers of commensal microorganisms that also contribute to host defense through metabolic competition or by enforcing the host’s immune barrier. To maintain homeostasis with commensals on mucosal surfaces, there is a specialized immune system associated with mucosal environments. The intestine—especially the small intestine—strongly drives immune suppression against exogenous antigens. Food antigens can induce oral tolerance by generating inducible regulatory T (Treg) cells. Antigen-presenting cells, such as dendritic cells (DCs) or macrophages, survey the mucosal environment using innate pattern recognition receptors. These cells can adjust and balance the suppressive regulation of commensals or innocuous antigens and protect against pathogens by generating various types of helper T (Th) and CD8⁺ T cells as well as secretory IgA (SlgA) antibodies. Before efficient mucosal vaccines can be developed, it is imperative to achieve an understanding of both the mucosal immune system and the regulatory mechanisms of immune cells. Further, strategies to overcome regulatory mechanisms will be essential. Here, we provide an overview of the intestinal immune systems, focusing on the unique subsets and functional features of intestinal DCs, and consider key biological and technical aspects of mucosal vaccine design. We then summarize the current status of mucosal vaccine development, including strategies involving modulation of mucosal DC activation.

INTESTINAL DC SUBSETS

As DC subsets in the small intestine have been well studied, we closely examined published studies describing lamina propria DCs in the small intestine. Although DCs are frequently characterized as CD11c⁺ major histocompatibility class (MHC) II⁺ cells, this group likely contains macrophages. The CD11cⅤⅤ MHC class IIⅤⅤ population comprises genuine DCs, whereas the CD11cⅠⅠ MHC class IIⅠ population is composed of macrophages. Intestinal lamina propria DCs have different origins and functions. Differentiation of CD103⁺-expressing DC subsets is dependent on the Flt3 ligand, whereas CX3CR1-expressing DCs and macrophages are dependent on CSF-1R (Figure 1). Most DCs can be largely classified as non-migratory DCs, which are tissue-resident, or migratory DCs, which can migrate into draining lymph nodes with sampled antigen and be infiltrated during inflammation. DC migration is tightly controlled by the expression of CCR7. Representative DC subsets and their functions are listed in Table 1; some subsets might overlap by phenotype. Of the DC populations, CD103⁺ has been the best studied. In addition, reports regarding resident CX3CR1⁺ DCs (or phagocytes) have increased recently. In the absence of Myd88 or under conditions of antibiotic-induced dysbiosis, non-invasive bacteria are trafficked to the mesenteric lymph nodes (MLNs) in a CCR7-dependent manner, where they induce both T-cell...
responses and IgA production. Trafficking is carried out by CX3CR1hi phagocytes, which are non-migratory.7 TNF-α/iNOS-producing DCs (Tip DCs) express TNF and inducible nitric oxide synthase (iNOS) and release large amounts of nitric oxide after recognizing commensal bacteria through toll-like receptors (TLRs).8 The detailed functions of each subset will be discussed later (Table 2).

ANTIGEN UPTAKE
The antigen uptake process may be the first step during the process in which DCs link the innate and adaptive immune systems. DCs can uptake antigens by both direct and indirect pathways. In turn, the indirect pathway can be subdivided into a number of categories: M cell-dependent (Figure 2a), goblet cell-dependent (Figure 2b), neonatal Fc receptor (FcRn)-dependent (Figure 2c) and apoptosis-dependent (Figure 2d). The M cell-dependent pathway is involved in antigen entry into specialized M cells in the follicle-associated epithelium of Peyer’s patches (Figure 2a).9 M cells transcytose luminal antigen and enteric bacteria inside the subepithelial dome. Underlying DCs and macrophages can capture the antigen delivered by M cells. M cells also exit the villous epithelium and contribute to antigen sampling in the lamina propria.10 Villous M cells are induced under inflammatory conditions.11 FcRn mediates the bidirectional transport of IgG, resulting in transport into the lumen and trafficking back to the lamina propria of antigen–antibody immune complexes (Figure 2c).12 Antigens associated with apoptotic epithelial cells can be taken up by DCs either at steady state or after microbial infection (Figure 2d).13 Goblet cells function as passages that deliver low-molecular-weight soluble antigens from the intestinal lumen to underlying CD103þ DCs in the lamina propria, a process termed goblet cell-associated antigen passage (GAP) (Figure 2b).14 DCs can also extend dendrites between epithelial cells to directly sample antigens from the intestinal lumen (Figure 3). CD3+CD11cþ DCs can sample Salmonella bacteria by extending long dendrites across the epithelium, which is a CD3CR1-dependent process.15 In addition to luminal antigen, lamina propria CD3CR1þ DCs facilitate the surveillance of circulatory antigens and act as a conduit for the processing of self- and intestinally-absorbed antigens.16 One recent report showed that CD103þ DCs patrol among enterocytes while extending dendrites toward the lumen, likely using tight junction proteins to penetrate the epithelium.17 These intraepithelial CD103þ DCs could be recruited into the intestinal epithelium by luminal bacteria to sample bacterial antigens for presentation.

T-CELL IMMUNITY BY INTESTINAL DCs
When DC-sampled antigen undergoes maturation, antigen processing and presentation process occur simultaneously. Differentiation of T cell subsets as instructed by intestinal DCs is summarized in Figure 4. Several studies have reported that CD103þ DCs can induce regulatory CD4þFoxp3þ T cells via retinoic acid (RA), a metabolic derivative of vitamin A found in food, and TGF-β18-20 however, another study showed that compared with DCs, CD11bþF4/80þCD11c+ macrophages in the lamina propria are more potent inducers of Treg cells.21 Intestinal CX3CR1+ macrophages support the expansion of Treg cells by means of IL-10 production to harness immune tolerance.22 CX3CR1+ DCs can sample and process both circulatory and luminal antigens.16 Cross-presentation by resident CX3CR1+ DCs induces differentiation into CD8þ T cells that express IL-10, IL-13, and IL-9. These CD8þ T cells can inhibit pro-inflammatory CD4þ T-cell activation in vitro and in vivo in intestinal inflammatory disease in an IL-10-dependent manner. Finally, these CD8þ T cells are dispersed at the lamina propria or migrate to the epithelium in a CCR6-dependent manner, and they comprise the regulatory CD8αβ+TCRβþ T-cell population. Therefore, both CD103+ and CX3CR1+ DCs induce two arms of regulatory CD4+ and CD8+ T cells to maintain intestinal immune homeostasis at a steady state (Figure 4).

CD103+CD11b+ DCs are the primary migratory DC population within the small intestinal lamina propria and can be infiltrated under inflammatory conditions.23,24 CD103+CD11b+ DCs produce IL-6 upon TLR stimulation and subsequently induce TH17 cell differentiation.25 Ivanov et al.26 reported that segmented filamentous bacteria, which are murine commensal bacteria, are sufficient for TH17 differentiation. This finding suggests that CD103+CD11b+ DCs might interact with segmented filamentous bacteria and generate signals to induce TH17. CD103+CD8αþ DCs express TLR3, TLR7 and TLR9, and they produce IL-6 and IL-12p40 following TLR ligand stimulation.27 These DCs induce antigen-specific IgG in serum, a TH1 response and cytotoxic T lymphocyte (CTL) activity in vivo. When stimulated by the TLR5 ligand flagellin, TLR5+ DCs promote the differentiation of antigen-specific TH17 and TH1 cells.28 CX3CR1+ DCs phagocytose and kill intracellular bacteria; however, their
in vivo function remains unclear under inflammatory conditions or during infection.

SECRETORY IgA PRODUCTION BY INTESTINAL DCs
A unique feature of the mucosal immune system is the local production and secretion of dimeric or multimeric IgA from B cells. IgA class switching occurs in gut-associated lymphoid tissues, including Peyer’s patches, MLNs and isolated lymphoid follicles in the lamina propria. SIgA within the mucosal fluid constitutes the first barrier against pathogen infection and forms a barrier between invading and commensal microorganisms (Figure 5). Mucosal DCs support B-cell activation, IgA isotype class-switch DNA recombination (CSR) and differentiation into IgA-secreting plasma cells with the assistance of T cells or by means of a T cell-independent pathway that expresses B cell-activating factor. The latter belongs to the TNF family (BAFF), and as does a proliferation-inducing ligand (APRIL). Intestinal plasmacytoid DCs (pDCs) induce IgA production by expressing BAFF and APRIL. Tip DCs release large amounts of nitric oxide after recognizing commensal bacteria through TLRs. Nitric oxide enhances IgA CSR and production by upregulating TGF-βRII expression on B cells and by inducing the expression of BAFF and APRIL in DCs through unknown mechanisms. In the intestinal environment, RA and TGF-β enforce efficient IgA class switching. In fact, retinaldehyde dehydrogenase type 2 (RALDH2) expressed DCs, but not all mucosal DCs can induce IgA CSR. Intestinal CD103⁺CD11b⁺ DCs, Tip DCs and TLR5⁺ DCs express RALDH and convert it into RA. In turn, RA can be used for IgA production. Langerin-expressing DCs in the MLNs that emerge following transcutaneous vaccination can also induce vaccine antigen-specific

Table 1 Representative DC subsets in the small intestine

| Name | Phenotype | Characteristic features | Functions | References |
|------|-----------|-------------------------|-----------|------------|
| CD103⁺ DCs | CD103⁺ | CCR7 expression: migration into LNs | CD4⁺Foxp3⁺ Treg generation | 18-20,44 |
| CD11b⁺ DCs | CD11b⁺ | RALDH expression: RA production | IgA class switching | 31 |
| CD103⁺ CD103⁺ CD8⁺ DCs | | Antigen uptake by extending long dendrites or goblet cell-associated antigen passage (GAP) | Imprinting of lymphocyte gut homing by expression of CCR9 | 45,46 |
| CD8⁺ DCs | CD11b⁺ | Expression of TLR3, TLR7, and TLR9 | T<sub>H</sub>17 generation | 23 |
| CX3CR1⁺ DCs | CX3CR1⁺ | No CCR7 expression: tissue-resident | T<sub>H</sub>1 response and CTL activity | 26 |
| DCs | F4/80⁺ CD11b⁺ | Uptake of circulatory or luminal antigen by extending long dendrites | | |
| Tip DCs | TFN-α⁺iNOS⁺ | TGF-β | IgA production | 8 |
| CD11b⁺ | | APRIL and BAFF production | | |
| TLR5⁺ DCs | TLR5⁺ CD11c⁺ | IL-6 production | Differentiation of antigen-specific T<sub>H</sub>17 and T<sub>H</sub>1 cells | 27 |
| DCs | CD11b⁺F4/80⁺ | RALDH expression: RA production | | |
| CD103⁺ | | Expression of TLR5 and TLR9 | | |
| pDCs | CD11c⁺mPDCA1⁺ | Type I IFN receptor expression | | |

Table 2 Mucosal vaccines that target mucosal DC subsets

| Adjuvant or vaccine type | Route | Target DC subset(s) and effect | Immunity | References |
|-------------------------|-------|-------------------------------|----------|------------|
| Flt3 ligand-encoded plasmid | Nasal | CDB2⁺ DCs | T<sub>H</sub>2 cytokine production | 35,36 |
| CpG oligonucleotide | Nasal | pDCs | T<sub>H</sub>1 cytokine production | 34,36 |
| Plasmodium antigen conjugated to flagellin | Nasal | TLR5⁺ DCs | Mucosal IgA antibody responses | 47 |
| Flagellin + model antigen | Systemic | MLN CD103⁺ DCs | Intestinal IgA antibody responses | 37 |
| Cholera toxin + soluble antigen | Transcutaneous | Langerin⁺ DCs (MLN) | Intestinal IgA antibody responses | 30 |
| LPS-treated | Tracheal | in vitro-matured bone marrow (BM)-DC | Pulmonary CTL activity | 38 |
| Listeriolysin (LLO) 91-99 loaded BM-DC | Buccal | Langerhans cells | Oral tissue CTL activity | 48 |
| pACB-OVA plasmid | Sublingual | Migratory CDB⁻ DCs | Mucosal IgA antibody responses | 49,50 |
| Live attenuated influenza or model antigen | Sublingual | Resident CDB⁺ DCs | | |
| Cholera toxin (oral) + Flt3L (intraperitoneal) | Oral | Flt3 ligand expanded DCs | Intestinal IgA antibody responses | 51 |
IgA production that is dependent on RA.\textsuperscript{30} Moreover, intestinal DCs imprint gut homing of IgA-secreting plasma cells via RA, which induces the expression of gut homing receptors, such as \( \alpha_4\beta_7 \) integrin and CCR9, on lymphocytes.\textsuperscript{31} As shown in several studies, RA is essential for maintaining the intestinal immune environment because it is a determinant for antibody isotype, \( \text{T}_H \) cell and DC subsets.\textsuperscript{32,33} The main goal of a mucosal vaccine is to elicit vaccine antigen-specific IgA production in the mucosal tissue of the infection route.

**MUCOSAL VACCINATION VIA THE MODULATION OF MUCOSAL DCs**

Mucosal immune responses are most efficiently induced by the administration of vaccines onto mucosal surfaces, whereas vaccines injected deep into skin tissue (subcutaneously) or muscle (intramuscularly) are usually poor inducers of mucosal immunity and are therefore less effective against infection at mucosal surfaces. Mucosal vaccines given at mucosal surfaces must overcome the same physical host defense challenges as

![Figure 2](image2.png) **Figure 2** Indirect pathways for antigen uptake by intestinal DCs. These pathways can be classified as M cell-dependent (a), goblet cell-dependent (b), neonatal Fc receptor (FcRn)-dependent (c) and apoptosis-dependent (d).

![Figure 3](image3.png) **Figure 3** Antigen uptake using intraepithelial dendrites of lamina propria DCs. (a) CX3CR1\(^+\) DCs can sample Salmonella organisms as well as luminal soluble bacterial antigens by extending long dendrites across the epithelium via a CX3CR1-dependent mechanism. (b) CX3CR1\(^+\) DCs facilitate the surveillance of circulatory antigens. (c) Intraepithelial CD103\(^+\) DCs can be recruited into the intestinal epithelium by luminal bacteria to sample bacterial antigens.

![Figure 4](image4.png) **Figure 4** T cell generation by intestinal DCs. (a) Under steady-state conditions, lamina propria CD103\(^+\) DCs induce Foxp3\(^+\)CD4\(^+\) Tregs to deliver innocuous antigen. CX3CR1\(^+\) DCs can induce IL-10 expressing CD8\(^+\) Tregs to both luminal and circulatory antigens. (b) During infection or under inflammatory conditions, CD103\(^+\) DCs and TLR5\(^+\) DCs induce \( \text{T}_H\)17 cells. TLR5\(^+\) DCs and CD103\(^+\)CD8\(^α\) DCs can induce \( \text{T}_H\)1 cells via TLR signaling. CD103\(^+\)CD8\(^α\) DCs can induce CTLs.
The C-type lectins are a family of calcium-dependent receptors expressed on the surface of innate cells, such as DCs. DC-targeted delivery strategies have utilized the well-characterized DC receptor DEC-205 (CD205) and langerin (CD207). By reinforcing the immunizing functions of mature DCs, antibody-mediated antigen targeting via DEC-205, a C-type lectin receptor, increases the efficiency of vaccination for inducing T-cell immunity. Without a strong stimulus through innate pattern recognition receptors, DC-captured soluble antigen induces Treg cells to maintain immune tolerance. To induce protective immunity by vaccination, it is necessary to boost the host's innate stimulus. One study found that when vaccine antigen is delivered with a strong adjuvant (that is, cholera toxin transcutaneously injected into intact skin), emergent langerin-expressing mucosal DCs in the MLNs could modulate intestinal IgA responses. Although the targeting of antigen-presenting cells is not unique to mucosal vaccination strategies, it could help potentiate stronger immune responses to antigens that are delivered mucosally.

Another promising approach for mucosal vaccine development involves enhancing antigen uptake by antigen sampling via specialized mechanisms in addition to DCs. It is possible that antigen uptake may need to occur via more than one pathway. To date, studies have used M cell-targeted delivery and lectin targeting strategies on M cells or directly on DCs. The uptake of particulate antigen by M cells or adhesion to specific M-cell receptors mimics the entry of pathogens into these cells and enhances antigen uptake. This phenomenon was illustrated by the promising findings of a study in which targeting of an M cell-specific carbohydrate moiety using NKM 16-2-4 successfully induced IgA responses. Additionally, an FcRn-targeted strategy effectively induced HIV-1 antigen-specific immunity to genital infection.

The use of RA as a vaccine adjuvant enhances IgA responses, CD8+ T-cell responses and mucosal protection from viral challenge. To improve phagocytic antigen uptake, particulate delivery systems based on synthetic or natural polymers offer opportunities to control the methods, timing and amount of antigens delivered. These polymers include chitosan, PLGA microparticles, liposomes, immune stimulating complex, nanocapsules and nanoparticles.

**CONCLUSION AND FUTURE PERSPECTIVES**

In this review, we focused on the integral role of DCs in shaping the unique mucosal immune system, especially the lamina propria of the small intestine. Depending on the DC subset and environmental conditions, DCs can elicit differential but appropriate immune responses to commensal and pathogenic microbial species, resulting in protection against infectious disease. Knowledge about the novel mucosal immune system has been largely accumulated within the last decade since the advent of advanced experimental techniques for mucosal tissues, the increased availability of germ-free mice and the development advanced techniques for metagenomic analysis of commensals. Recent findings indicate that mucosal DCs and immune effectors may function together to prevent microbial pathogens: They are diluted in mucosal secretions, captured in a sticky barrier of mucus, attacked by degradation enzymes and excluded by tight epithelial barriers. Therefore, relatively large doses of vaccine are required for mucosal delivery. Vaccine formulations for targeted delivery must have an effective mucosal adjuvant to surmount tolerance and mimic infectious conditions. For several reasons, live attenuated mucosal vaccines are likely to be more effective than injected vaccines. First, because they are naturally particulate, antigens will selectively adhere to M-cell mucosal surfaces, enabling efficient uptake. Second, via the use of pattern molecules, they should efficiently stimulate innate signals on innate effector cells (especially DCs). Third, they will elicit appropriate adaptive immune responses for clearance of the target pathogen. Another DC-targeted vaccine strategy is the use of appropriate adjuvants, such as DC growth factor or RALDH2 that is converted into RA and can be used for IgA production.
and control infectious diseases. Therefore, the current challenge is to apply this knowledge to vaccine design and to carry out collaborative, comparative, and integrated studies for vaccine development. Much work will be required to modulate mucosal DCs before strategies can be implemented to exploit the full potential of mucosal vaccines.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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