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REVIEW

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.
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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[1] or by enforcing the host's immune barrier.[2] 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.[3] 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 (T_H) and $CD8^+$ T cells as well as secretory IgA (SIgA) 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 $CD11c^{high}$ MHC class II^{high} population comprises genuine DCs, whereas the $CD11c^{low}$ MHC class II^{low} population is composed of macrophages.[4] Intestinal lamina propria DCs have different origins and functions.[5] 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.[6] 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

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responses and IgA production. Trafficking is carried out by CX3CR1^{hi} 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 CD103$^+$ 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). CX3CR1$^+$ DCs can sample *Salmonella* bacteria by extending long dendrites across the epithelium, which is a CX3CR1-dependent process.[15] In addition to luminal antigen, lamina propria CX3CR1$^+$ 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 CD103$^+$ DCs patrol among enterocytes while extending dendrites toward the lumen, likely using tight junction proteins to penetrate the epithelium.[17] These intraepithelial CD103$^+$ DCs could be recruited into the intestinal epithelium by luminal bacteria to sample bacterial antigens for presentation.

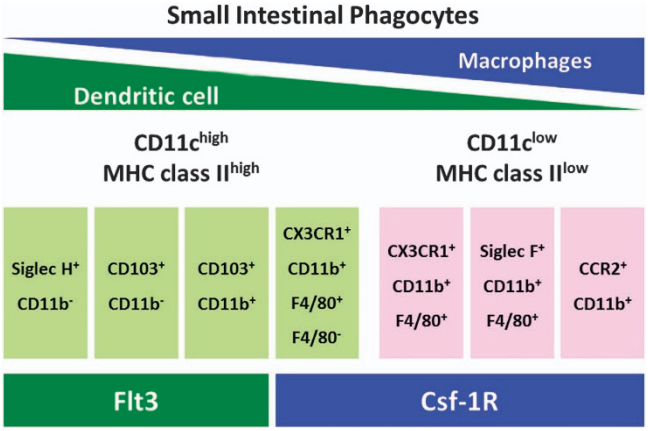

Figure 1 Surface phenotypes in subsets of small intestinal phagocytes. DCs are CD11c^{high} and MHC class II^{high}, whereas macrophages are CD11c^{low} and MHC class II^{low}. Two major DC subset populations are CD103$^+$CD11b$^+$ and CX3CR1$^+$CD11b$^+$. The differentiation of CD103$^+$-expressing DC subsets is dependent on Flt3L, whereas CX3CR1-expressing DCs and macrophages are dependent on CSF-1R.

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 CD103$^+$ DCs can induce regulatory CD4$^+$Foxp3$^+$ T cells via retinoic acid (RA), a metabolic derivative of vitamin A found in food, and TGF-β;[18–20] however, another study showed that compared with DCs, CD11b$^+$F4/80$^+$CD11c$^-$ 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 CD8$^+$ T cells that express IL-10, IL-13, and IL-9. These CD8$^+$ T cells can inhibit pro-inflammatory CD4$^+$ T-cell activation *in vitro* and *in vivo* in intestinal inflammatory disease in an IL-10-dependent manner. Finally, these CD8$^+$ T cells are dispersed at the lamina propria or migrate to the epithelium in a CCR6-dependent manner, and they comprise the regulatory CD8αβ$^+$TCRαβ$^+$ 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 T_H17 cell differentiation.[25] Ivanov *et al.*[26] reported that segmented filamentous bacteria, which are murine commensal bacteria, are sufficient for T_H17 differentiation. This finding suggests that CD103$^+$CD11b$^+$ DCs might interact with segmented filamentous bacteria and generate signals to induce T_H17. CD103$^+$CD8α$^+$ DCs express TLR3, TLR7 and TLR9, and they produce IL-6 and IL-12p40 following TLR ligand stimulation.[26] These DCs induce antigen-specific IgG in serum, a T_H1 response and cytotoxic T lymphocyte (CTL) activity *in vivo*. When stimulated by the TLR5 ligand flagellin, TLR5$^+$ DCs promote the differentiation of antigen-specific T_H17 and T_H1 cells.[27] CX3CR1$^+$ DCs phagocytose and kill intracellular bacteria; however, their

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
	$CD11b^+$	RALDH expression: RA production	IgA class switching	31
		Antigen uptake by extending long dendrites or goblet cell-associated antigen passage (GAP)	Imprinting of lymphocyte gut homing by expression of CCR9	45,46 23
		TLR stimulation: IL-6 production	T_H17 generation	25
$CD103^+$ $CD8^+$ DCs	$CD103^+$ $CD8^+$ $CD11b^{low}$	Expression of TLR3, TLR7, and TLR9 Production of IL-6 and IL-12p40	T_H1 response and CTL activity	26
$CX3CR1^+$ DCs	$CX3CR1^+$ $F4/80^+$ $CD11b^+$	No CCR7 expression: tissue-resident Uptake of circulatory or luminal antigen by extending long dendrites	Generation of regulatory $CD8\alpha\beta^+TCR\alpha\beta^+$ intraepithelial lymphocytes (IELs)	15 16
Tip DCs	TNF-α^+iNOS$^+$ $CD11b^+$	TGF-β APRIL and BAFF production	IgA production	8
$TLR5^+$ DCs	$TLR5^+CD11c^{hi}$ $CD11b^{hi}F4/80^+$ $CD103^+$	IL-6 production RALDH expression: RA production Expression of TLR5 and TLR9	Differentiation of antigen-specific T_H17 and T_H1 cells Generation of IgA–producing cells	27
pDCs	$CD11c^{int}B220^+$ mPDCA1$^+$	Type I IFN receptor expression APRIL and BAFF production	T cell-independent IgA production	28

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	$CD8\alpha^+$ DCs	T_H2 cytokine production IgA antibody responses	35,36
CpG oligonucleotide	Nasal	pDCs	T_H1 cytokine production IgA antibody responses	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 Listeriolysin (LLO) 91-99 loaded BM-DC	Tracheal	*In vitro*-matured bone marrow (BM)-DC	Pulmonary CTL activity	38
pACB-OVA plasmid	Buccal	Langerhans cells	Oral tissue CTL activity	48
Live attenuated influenza or model antigen	Sublingual	Migratory $CD8^-$ DCs Resident $CD8^+$ DCs	Mucosal IgA antibody responses	49,50
Cholera toxin (oral) + Flt3L (intraperitoneal)	Oral	Flt3 ligand expanded DCs	Intestinal IgA antibody responses	51

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.[28] Tip DCs release large amounts of nitric oxide after recognizing commensal bacteria through TLRs.[8] Nitric oxide enhances IgA CSR and production by upregulating TGFβRII 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.[29] 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.[8,27] Langerin-expressing DCs in the MLNs that emerge following transcutaneous vaccination can also induce vaccine antigen-specific

IgA production that is dependent on RA.[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.[31] As shown in several studies, RA is essential for maintaining the intestinal immune environment because it is a determinant for antibody isotype, T_H cell and DC subsets.[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.

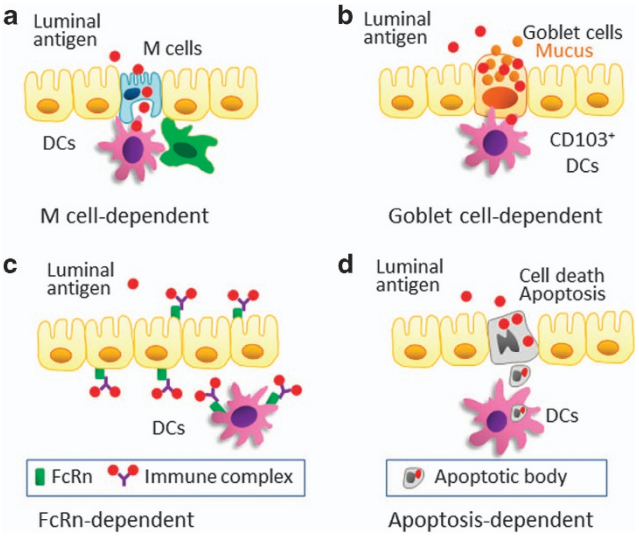

Figure 2 Indirect pathways for antigen uptake by intestinal DCs. These pathways can be classified as M cell-dependent (**a**), goblet cell-dependent (**b**), neonatal Fc receptor (FcRn)-dependent (**c**) and apoptosis-dependent (**d**).

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

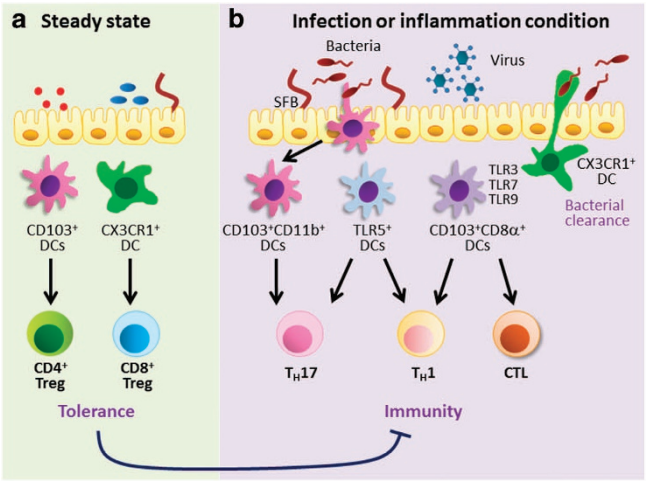

Figure 4 T cell generation by intestinal DCs. (**a**) Under steady-state conditions, lamina propria $CD103^+$ DCs induce $Foxp3^+CD4^+$ Tregs to deliver innocuous antigen. $CX3CR1^+$ DCs can induce IL-10 expressing $CD8^+$ Tregs to both luminal and circulatory antigens. (**b**) During infection or under inflammatory conditions, $CD103^+$ DCs and $TLR5^+$ DCs induce T_H17 cells. $TLR5^+$ DCs and $CD103^+CD8\alpha^+$ DCs can induce T_H1 cells via TLR signaling. $CD103^+CD8\alpha^+$ DCs can induce CTLs.

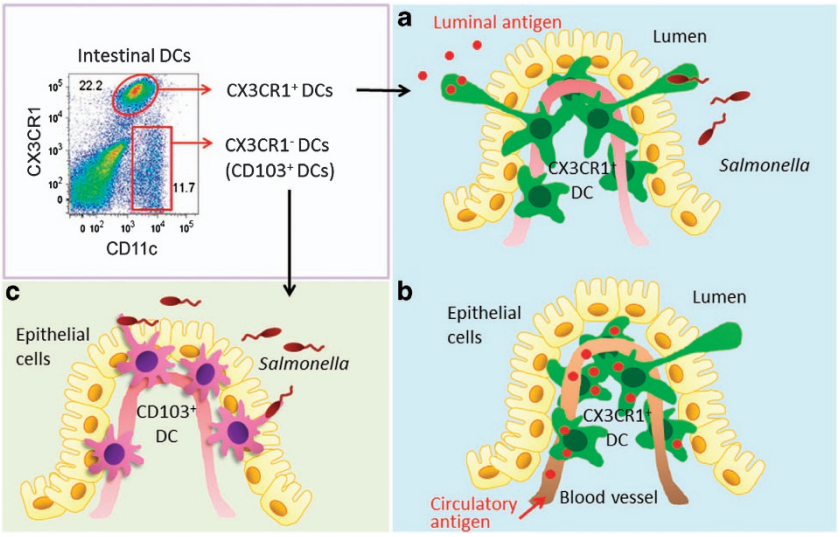

Figure 3 Antigen uptake using intraepithelial dendrites of lamina propria DCs. (**a**) $CX3CR1^+$ DCs can sample *Salmonella* organisms as well as luminal soluble bacterial antigens by extending long dendrites across the epithelium *via* a CX3CR1-dependent mechanism. (**b**) $CX3CR1^+$ DCs facilitate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

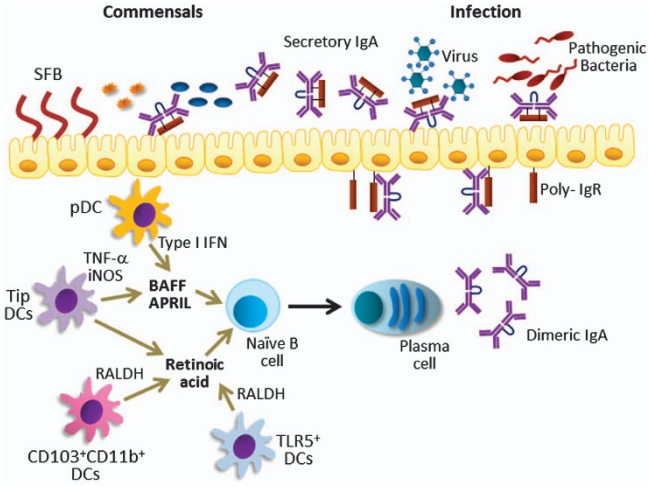

Figure 5 Secretory IgA production by intestinal DCs. Intestinal pDCs and tip DCs induce IgA production by expressing BAFF and APRIL. Intestinal $CD103^+CD11b^+$ DCs, tip DCs and $TLR5^+$ DCs express RALDH2 that is converted into RA and can be used for IgA production.

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 TLR ligand, that are expressed on mucosal DCs (Table 2). Plasmids encoding Flt3 ligand and CpG oligonucleotide selectively target $CD8\alpha^+$ DCs and pDCs, respectively, when administered nasally.[34–36] $CD8\alpha^+$ DCs promote T_H2 cytokine production, whereas pDCs induce T_H1 cytokine production to elicit co-administered antigen-specific IgA antibody responses and cell-mediated immunity. In one study, when soluble antigen plus cholera toxin was applied to intact skin, MLN $langerin^+$ DCs mediated gut IgA production in an RA-dependent manner.[30] Similar to the use oftranscutaneous systemic vaccination to induce gut immunity, systemic immunization with flagellin can recruit $CD103^+$ DCs into MLNs and subsequently induce intestinal IgA antibody responses.[37] Intra-tracheal application of LPS-treated immunodominant CTL epitope-loaded DCs is also a promising strategy for generating CTLs that are protective against respiratory infections caused by intracellular pathogens.[38]

The C-type lectins are a family of calcium-dependent receptors expressed on the surface of innate cells, such as DCs.[33] 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.[39] 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.[30] 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 enancing 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.[40] Additionally, an FcRn-targeted strategy effectively induced HIV-1 antigen-specific immunity to genital infection.[41]

The use of RA as a vaccine adjuvant enhances IgA responses, $CD8^+$ T-cell responses and mucosal protection from viral challenge.[42] 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.[43] 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 propri 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

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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