

Review Series: Immune Responses in the Host-Environment Interface - Understanding the Mechanisms of Allergic Sensitization

Mucosal Immunity and the Onset of Allergic Disease

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ABSTRACT

Mucosal barriers encounter an environment that is rich in pathogens that possess mechanisms for invading mucosal tissues. These barriers also encounter innocuous antigens, such as foods, airborne antigens, and microbiota. The mucosa has developed a sophisticated immune system that can mount robust immune responses against pathogenic antigens, while maintaining mucosal tolerance against non-pathogenic antigens. Accumulating evidence indicates that the mucosal epithelium, dendritic cells, and a subtype of T cells with regulatory properties play important roles in the development and maintenance of mucosal tolerance. Moreover, the microbiota also contribute to regulating the mucosal immune system. A failure to develop or the breakdown of mucosal tolerance can result in allergic diseases, such as food allergy and asthma. By taking advantage of the unique characteristics of the mucosal immune system, strategies that induce regulatory cells *in vivo* and, thereby, reconstitute mucosal tolerance may be used to develop novel therapies that are suitable for treating or preventing of allergic diseases.

KEY WORDS

asthma, dendritic cells, epithelial cells, food allergy, regulatory T cells

INTRODUCTION

Because most pathogens enter the body through mucosal surfaces, mucosal immune responses are required to ward off infection. These mucosal surfaces are colonized by vast numbers of microbes, collectively referred to as the microbiota. The microbiota has been found to have profound effects on a host's immune system without provoking deleterious immune responses.¹ In addition, mucosal surfaces are exposed to a wide variety of innocuous environmental antigens, such as food in the intestine and aeroantigens in the respiratory tracts. These innocuous antigens can induce immune tolerance or hyporesponsiveness rather than active immunity.

Mucosal tolerance prevents harmful inflammatory responses, for example to food antigens, and plays a role in establishing homeostasis between the microbiota and the host at mucosal membranes. Thus, mucosal tolerance is not merely a lack of immune re-

sponse; rather, it is a specific immunological response. A failure to develop or the breakdown of oral tolerance can result in food allergy.² This review will describe recent findings that have contributed to our understanding of the mechanisms of oral tolerance and the pathophysiology of food allergy.

ORAL TOLERANCE IS PRIMARILY MEDIATED BY REGULATORY T CELLS

In healthy adults, certain food proteins can be detected in the blood soon after eating.³ These ingested food proteins are not always ignored by the immune system, because food antigen can simultaneously induce the expression of an activation marker, CD69, and behavioral changes in T cells in both local and peripheral lymphoid tissues, depending on the antigen doses.^{4,5}

Weiner *et al.*⁶ demonstrated that the dose of a fed antigen affected the mechanism underlying oral tolerance induction. Repeated exposure to low doses of

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antigen induced active suppression by several subsets of regulatory T cells (Tregs), whereas exposure to high antigen doses resulted in clonal deletion and/or anergy. We previously showed that active suppression by Tregs and clonal deletion and/or anergy were not mutually exclusive and that they may occur simultaneously after oral tolerance induction in Ag-specific T cell receptor transgenic mice.⁷

Using an adoptive transfer system of Ag-specific T cells, however, it has been demonstrated that Foxp3⁺ Tregs could be induced *de novo* from conventional naïve CD25-CD4⁺ T cells after tolerogenic Ag administration via the oral route in a dose-dependent fashion.⁸ Moreover, there is little direct evidence that clonal deletion and/or anergy contributes to oral tolerance in the presence of a polyclonal T-cell repertoire. These findings suggest that Tregs play a pivotal role in the development of oral tolerance.

A number of different Tregs have been implicated in oral tolerance, including IL-10-producing T-regulatory type 1 cells (Tr1 cells) and TGF- β -producing Th3 T cells, in addition to Foxp3⁺ Tregs. Two main types of Foxp3⁺ Tregs have been identified. Natural Tregs (nTregs) are selected in the thymus as a consequence of their reactivity to self, whereas induced or adaptive Tregs (iTregs or aTregs) develop in the peripheral immune system under various conditions.⁹ Induction of the Foxp3⁺ peripherally derived Tregs (pTregs) from naïve T cells requires TGF- β , which is produced by various cell types, including Th3 CD4⁺ T cells. Although nTregs, also referred to as thymus-derived Treg cells (tTregs), are stable *in vivo*, iTregs can differentiate into other types of effector T cells under inflammatory conditions.¹⁰ In a mouse model in which iTregs but not nTregs could be generated, oral tolerance could not be induced, suggesting that iTregs were essential for the development of oral tolerance.^{10,11}

The common characteristics of Tregs are their expression of one or more anti-inflammatory cytokines, such as IL-10, TGF- β , or IL-35, and/or inhibitory surface molecules, such as cytotoxic T-lymphocyte antigen 4 (CTLA4), lymphocyte-activation gene-3 (LAG-3), glucocorticoid-induced tumor necrosis factor receptor (GITR), CD39, CD73, or Nrp-1 (neurophilin 1).¹²⁻¹⁵

Oral tolerance is abolished in lymphotoxin-deficient mice that lack both lymph nodes and Peyer's patches (PPs), but can be readily induced in mice without PPs but with mesenteric lymph nodes (MLNs).¹⁶ The initiation of oral tolerance is impeded by mesenteric lymphadenectomy.¹⁷ MLNs appear to be critical for inducing oral tolerance.

In contrast, it has been shown that injecting antigen directly into the portal vein that drains blood from the intestine to the liver induces antigen-specific tolerance.¹⁸ Conversely, diverting the blood flow away from the liver by portocaval shunting prevents

the induction of oral tolerance,^{19,20} indicating the inherent tolerogenicity of the liver. Kupffer cells, conventional liver dendritic cells (DCs), and liver sinusoidal endothelial cells, which efficiently sample circulating antigen and act as non-professional antigen-presenting cells, have been shown to induce tolerance rather than active immunity.¹⁸ Moreover, plasmacytoid DCs (pDCs), which have been implicated in inducing systemic tolerance, are particularly abundant in the liver.²¹ The relative contributions of MLNs, the liver, and other sites to oral tolerance induction remain unknown (Fig. 1).

A fraction of Tregs that expand in the lamina propria (LP) may exit this tissue and enter the circulation either via the draining lymphatics, or directly via the blood stream. Those iTregs that emigrate from the LP may act more generally, either by directly inhibiting systemic effector T cells or by promoting the generation of additional iTregs in systemic tissues by a process of infectious tolerance.

INTESTINAL DENDRITIC CELLS ORGANIZE INDUCTION AND EXPANSION OF iTregs

Two types of conventional CD11c⁺DCs (cDCs), such as CD103 ($\alpha\text{E}\beta\text{7}$)⁺DCs and CD103⁻CX3CR1⁺ DCs have been identified in the LP (2). Mucosal DCs can capture antigen through at least four different routes. CD103⁻CX3CR1⁺ DCs directly sample antigens by extending transepithelial dendrites between enterocytes. CD103⁺DCs can capture antigens that are transported by paracellular diffusion across enterocytes that line the intestinal villus, either by a transcellular transport mechanism, transcytosis by microfold cells (M cells), or by goblet-cell-associated antigen passage.²² To a certain extent, the routes of uptake depend on the nature of the particular antigen. Orally administered inert particles or nonpathogenic bacteria appear to preferentially enter into gut associated lymphoid tissue (GALT) by M-cell-mediated transcytosis, whereas soluble antigens are taken up by DCs in the LP and, to a lesser extent, the GALT.²³ Antigenic material may also reach the LP within exosomes, which are formed by fusing major histocompatibility complex class II loading compartments with endosomes that contain only partially degraded proteins in enterocytes.²³

The differentiation of CD103⁺DCs depends upon retinoic acid (RA) and TGF- β produced by intestinal epithelial cells.²⁴ After acquiring antigen, CD103⁺DCs migrate from the LP to the MLNs in a CCR7-dependent manner.²⁵ CD103⁺DCs express the enzyme retinaldehyde dehydrogenase (RALDH) 2, which converts the vitamin A metabolite retinal to RA. In concert with TGF- β , which is also produced by CD103⁺DCs and is activated by the integrin $\alpha\text{v}\beta\text{s}$ on these DCs, RA can promote the differentiation of naïve T cells within the NLNs into Foxp3⁺ iTregs.^{14,26-29} CD103⁺DCs use RA to imprint naïve T

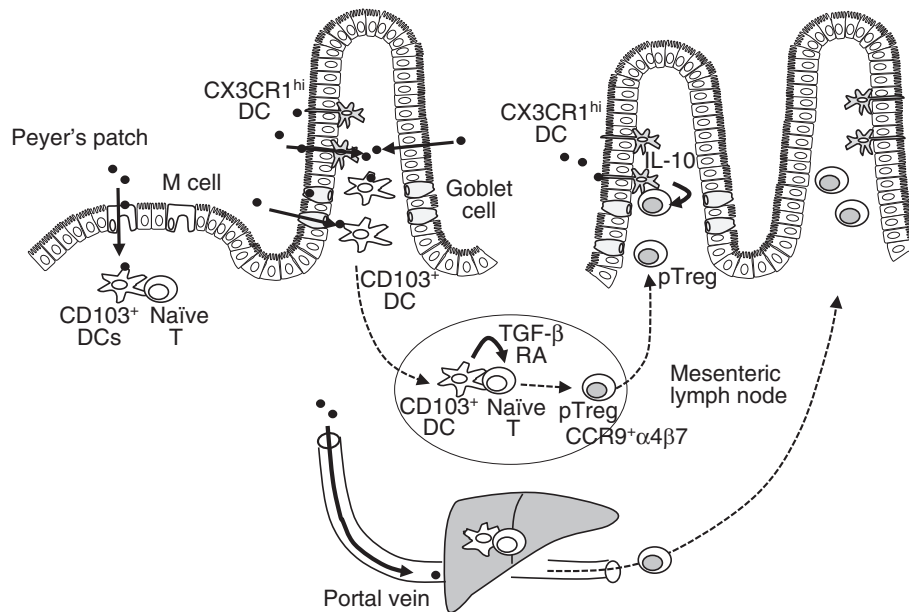


Fig. 1 Roles of dendritic cells and mesenteric lymph nodes in oral tolerance. CX3CR1⁺ cDCs sample antigens by extending dendrites. CD103⁺DCs capture antigens that are transported by paracellular diffusion across enterocytes, either by M cell-mediated transcytosis, or by goblet-cell-associated antigen passage. Antigen-loaded CD103⁺ cDCs migrate to the mesenteric lymph nodes and then induce the differentiation of iTregs. CX3CR1⁺ cells produce IL-10 and facilitate the expansion of iTregs in the lamina propria. Antigen transported into the lamina propria may reach the liver via portal vein. Kupffer cells, cDCs, and sinusoidal endothelial cells act as non-professional antigen-presenting cells to induce tolerance.

cells with the gut homing markers integrin $\alpha\beta 7$ and chemokine receptor CCR9. Further contributing to their tolerogenic capabilities, CD103⁺DCs also express the enzyme indoleamine 2,3-dioxygenase (IDO), which inhibits the generation of T-effector cells by metabolizing tryptophan to kynurenine.³⁰ Inhibition of IDO was shown to abrogate the development of Foxp3⁺iTregs *in vitro* and prevent the development of oral tolerance *in vivo*.³¹ Removing vitamin A and/or tryptophan from the diet also hinders the tolerogenic effects of CD103⁺DC by reducing their ability to induce iTregs and imprint gut homing receptors on lymphocytes.³⁰ Thus, CD103⁺DCs are essential for oral tolerance induction.

In contrast, CD103⁻CX3CR1⁺CD11c⁺DCs are functionally closer to macrophages than they are to cDCs, as they do not migrate from the LP to MLNs and cannot present antigen to naïve T cells under steady state conditions.³² CD103⁻CX3CR1⁺ cells are efficient at sampling antigens by extending transepithelial dendrites and passing these antigens to neighboring migratory CD103⁺DCs for subsequent transport and presentation.²³ CD103⁻CX3CR1⁺ cells have been shown to produce IL-10 and locally facilitate the expansion of Foxp3⁺ iTregs in the LP.³³ CD103⁻CX3CR1⁺ cells are necessary for maintaining local tol-

erance induction, as tolerance is abrogated in CXCR1-deficient mice.³³

The transepithelial dendrites extended by CX3CR1⁺ cells in the LP have been shown to interact with bacteria in the lumen; thus, they may also play a specific role in inducing tolerance to commensal bacteria. Recently, CX3CR1⁺CD11b⁺CD11c⁺ cells were further subdivided into two subsets based on their expression levels of CX3CR1.³⁴ CX3CR1^{int}CD11b⁺CD11c⁺ cells could promote the development of Th17 cells, whereas CX3CR1^{high}CD11b⁺CD11c⁺ cells suppressed CD4⁺ T-cell proliferation in a cell-cell contact-dependent manner, and contributed to preventing intestinal inflammation.

pDCs can produce high levels of type I interferons, and can also activate Foxp3⁺ iTreg as well as IL10⁺ Tr1 cells. A substantial proportion of MLN DCs produce CCL22, which attracts iTregs via CCR4, and are located in juxtaposition to iTregs.³⁵ The interaction with iTregs via CTLA-4/B7 may make DCs constitutively express functional IDO. Mice that were genetically deficient in CCR4 showed markedly reduced IDO expression in mesenteric lymph node DCs.

INTESTINAL MUCOSA AND MESENTERIC LYMPH NODES ENGENDER A TOLEROGENIC MILIEU

The acquisition of the unique tolerogenic properties of CD103⁺DCs likely requires a specific milieu of local conditioning factors provided by intestinal epithelial cells (IECs), commensal bacteria, and dietary constituents. IEC-derived factors may include TGF- β , RA, IL-10, vasoactive intestinal peptide (VIP), thymic stromal lymphopoietin (TSLP), and hepatocyte growth factor (HGF).^{24,36-38} The sources of RA in the intestine may include RA produced by epithelial cells, which metabolize retinoids taken up from the diet.³⁹ Intestinal epithelial cell-derived RA induces DCs to express RALDH, and thereby intestinal DCs are capable of metabolizing food-derived vitamin A to produce RA by themselves.³⁸ Independent of tolerogenic DCs, epithelial cells express MHC class II molecules, but not conventional co-stimulatory molecules; thus, that they may act as nonprofessional APCs to induce T cell anergy.⁴⁰

Stromal cells from mesenteric lymph nodes also express higher levels of RA-producing enzymes than do peripheral lymph node stromal cells.⁴¹ Stromal cells from mesenteric lymph nodes, but not from skin-draining lymph nodes, support the generation of Foxp3⁺ Tregs. This suggests that tissue-specific differences in stroma may also aid in generating a uniquely tolerogenic milieu.

CD8⁺ T CELLS AND $\gamma\delta$ T CELLS ALSO FUNCTION AS REGULATORY CELLS IN MUCOSAL IMMUNITY

Accumulating evidence has shown that subsets of CD8⁺ T-cells function as regulatory cells either directly by killing immune cells or indirectly by coopting other cells to produce suppressive molecules, such as TGF- β and IL-10.^{42,43} Although LP CD8⁺ T cells and intestinal epithelial cell-activated CD103⁺ CD8⁺ T cells exhibit regulatory activities *in vitro* and, thus, might play an active role in oral tolerance,^{44,45} the role of these CD8⁺ T cells in controlling allergic reactions in the gastrointestinal mucosa remains uncertain. We have demonstrated that systemic immunization with allergen induced regulatory CD8⁺ T cells that could inhibit the development of allergic diarrhea and that these CD8⁺ T cells may have exerted a regulatory function through both IL-10-dependent and -independent mechanisms.⁴⁶ These findings suggest the possibility that manipulating CD8⁺ regulatory T cells may expand our therapeutic strategies for food allergy.

In a food allergy mouse model, $\gamma\delta$ TCR blockade prior to inducing sensitization resulted in higher antigen-specific IgE levels and increased mast cell degranulation. These findings suggest a regulatory role for $\gamma\delta$ T cells in the development of allergic sensitiza-

tion.⁴⁷ A possible role for natural killer T (NKT) cells in oral tolerance is controversial and the outcomes may depend on the experimental models used. NKT cell-deficient mice showed impaired oral tolerance due to a failure to induce IL-10 and TGF- β producing CD25⁺CD4⁺ Tregs.⁴⁸ Oral tolerance was normally induced in J α 281^{-/-} mice that lacked V α 14⁺ NKT cells, suggesting that NKT cells are dispensable for inducing oral tolerance.⁴⁹

REGULATORY B CELLS CAN INDUCE Tregs

Emerging evidence suggests that regulatory types of B cells (Bregs) that are generated under inflammatory conditions are capable of inducing tolerance. Bregs may comprise several subpopulations that exhibit different mechanisms of immunomodulation, including the production of anti-inflammatory cytokines (IL-10 or TGF- β 1), functioning as inhibitory antigen presenting cells, and inducing or recruiting Tregs.⁵⁰ Mouse IL-10-producing B cells (designated B10 cells) display the CD1d^{hi}CD5⁺CD19^{hi} phenotype and suppress allergic inflammation through their induction of iTreg cells.⁵¹ B cells isolated from Peyer's patches can generate iTregs that express CTLA4, ICOS, OX40, PD-1, and TNF-RII, and produce lower amounts of IL-2 and higher amounts of IL-10.⁵² B cells in Peyer's patches may play an important role in oral tolerance as regulatory cells.

In humans, B10 cells have been identified within the CD24^{hi}CD27⁺ B cell compartment and negatively regulate monocyte cytokine production in an IL-10-dependent manner.⁵³ Although B10 cells represent a small fraction of blood B cells in healthy subjects, they are increased in the blood of patients with inflammatory disorders.⁵³ Recent evidence has implicated regulatory B cells in the development of tolerance to food allergens in humans; however, it remains unknown how these cells are related to human B10 cells.^{54,55}

IgA PROTECTS AGAINST SENSITIZATION OR TRIGGERING OF ALLERGIC REACTIONS BY ORAL ANTIGENS

Foxp3⁺CD4⁺ Treg cells in Peyer's patches can differentiate into follicular B helper T cells (T_{fh} cells), which then participate in immunoglobulin A responses against intestinal antigens.⁵⁶ Secretory IgA (sIgA), a prototypic mucosal immunoglobulin, prevents the uptake of antigens from the gut lumen and also secretes back out antigens that have penetrated the epithelial barrier into the lumen by forming sIgA-antigen complexes. IgA deficiency in children has been reported to be associated with an increased frequency of food allergy.⁵⁷ Thus, IgA has been hypothesized to play a protective role in the context of food allergy.

However, Strait *et al.* found that systemic transfer

of antigen-specific IgA was also protective against anaphylaxis that was triggered by the oral route.⁵⁸ However, this was not dependent on IgA secretion into the lumen, and IgA had no protective effect when it was administered in conjunction with the antigen intraluminally, suggesting that IgA has protective effects at the systemic level but do not rule out a protective role for IgA at the mucosal surface.

In contrast to the immune exclusion by sIgA, sIgA-antigen complexes are transported by M cells from the intestinal lumen to subepithelial DCs, which recognize and internalize these complexes through the C-type lectin DC-SIGN on DCs.⁵⁹ Antigen presented in conjunction with IgA generates a different type of immune response than that when antigen is presented alone. Antigen-specific and total IgA levels in maternal milk were found to be significantly lower in mothers whose children developed cow's milk allergy, indicating that IgA may play a critical role in preventing early sensitization to food allergens.⁶⁰

Sublingual immunotherapy with peanut antigens for treating peanut allergy in humans resulted in increased peanut-specific IgA levels in saliva, and these IgA levels were correlated with the degree of clinical protection.⁶¹ These results support the concept that antigen-specific IgA is protective against primary sensitization or triggering of allergic reactions by food antigens.

COMMENSAL BACTERIA AFFECT THE DEVELOPMENT AND MAINTENANCE OF ORAL TOLERANCE

The intestinal microbiota is beneficial for a host, as they aid in digestion, provide protection against pathogens, and induce and maintain immune system suitability. Conversely, the host can actively control the steady state composition of the microbiota by secreting antimicrobial peptides and immunoglobulins, which highlights the state of a highly active dynamic symbiosis.⁶² The microbiota tightly controls constitutive cytokine production by lymphocytes, as germ-free mice exhibit considerable deficiencies in basal cytokine production.^{63,64} Specific commensals can shape the gut-associated immune system. For example, segmented filamentous bacteria (SFBs) and *Clostridium* spp. regulate the prevalence of distinct helper and regulatory T cell populations.^{65,66} Commensal bacteria, such as *Bacteroides* and *Bifidobacteria* spp., can directly induce monocyte-derived DCs to acquire a tolerogenic phenotype.⁶⁷ The microbiota can also directly contribute to the expansion of LP resident CX3CR1⁺ cells, which is associated with local expansion of Treg cells.⁶⁸ Microbiota-derived products can also control the activation status of liver resident DCs and increase the activation threshold required for inducing of immune responses.⁶⁹ Thus, it is conceivable that the composition of microbiota might affect oral tolerance.

There are controversial reports on the capacity to induce oral tolerance in germ-free mice that are devoid of live intestinal bacteria.^{70,71} In the absence of a microbiota, the CD4⁺ T cell population is diminished and disproportionately affects the production of Th1 and Th17 cells, although Treg frequencies are maintained or increased in the small intestine.⁶³ Colonization of germ-free mice with complex microbiota orchestrates a broad spectrum of Th1, Th17 and Treg responses.⁷² Oral tolerance can be restored by reconstituting germ free mice with *Bifidobacterium infantis*, a dominant commensal bacterium or by feeding these mice with LPS.^{73,74} Although LPS has been associated with the acquisition of oral tolerance, the precise molecular mechanisms underlying this phenomenon and the targets of microbe-derived signals remains incompletely understood. Moreover, in a mouse model, feeding them with *Clostridium leptum* increased the number of Tregs in the spleens, and mediastinal lymph nodes, and attenuated allergen-induced airway hyperresponsiveness and inflammation by inhibiting inflammatory cytokine production, while enhancing IL-10 and TGF- β 1 production in the lungs.⁷⁵

After the hygiene hypothesis was first proposed, some epidemiological data suggested that asthma and atopic diseases were associated with childhood antibiotic use and an altered intestinal microbiota and that the compositions of the intestinal microbiota differed between allergic and nonallergic infants.^{76,77} A birth cohort study that followed high-risk children from birth to 6 years of age demonstrated that the bacterial diversity in the intestinal microbiota was inversely associated with the risk of allergic sensitization to foods and aeroallergens, peripheral blood eosinophilia, and allergic rhinitis.⁷⁸ Other studies have identified specific differences in the microbiota of allergic and nonallergic children.^{79,80}

To date, no definitive subset of the microbiota has been identified that is associated with atopy, aside from limited evidence that *Clostridium difficile* may be overrepresented in some atopic infants.⁸¹ This suggests that although intestinal microbial colonization is required for proper immune function development, certain types of microbes may play a significant role in skewing immune responses toward allergic sensitization, possibly by creating an environment that enhances the development of allergic disease.

ORAL ANTIGEN SENSITIZATION AND THE DEVELOPMENT OF FOOD ALLERGY

Food allergies develop in genetically predisposed individuals, presumably when oral tolerance fails to develop normally or when it breaks down. The mechanisms underlying allergic sensitization to food antigens remain elusive. Roth-Walter *et al.* reported that the soluble whey proteins α - and β -lactalbumin were readily transcytosed through enterocytes, whereas

insoluble casein in micelles and heat-aggregated α - and β -lactalbumin were preferentially taken up into Peyer's patches.⁸² The delivery of food antigens into the Peyer's patches may have resulted in a more robust sensitization. However, if these antigens were unable to traffic across the villus enterocytes, they were unable to elicit anaphylactic symptoms upon oral challenge. These results suggest that for the development of food allergy, both antigen sensitization established by antigen uptake via Peyer's patches and exposure to ample amounts of soluble antigens that are transferred across the epithelial barrier are indispensable.

Human intestinal epithelial cells constitutively express the low affinity IgE receptor CD23 and, thereby, transport IgE from both the basal-to-apical and apical-to-basal directions.⁸³ Antigen-IgE complexes were preferentially transported in the apical-to-basal direction and were released into the basolateral media in a form that could stimulate Fc ϵ RI receptor.⁸³ Therefore, it is possible that the presence of antigen-specific IgE can increase the amounts of transported antigens across the epithelium.

PULMONARY DENDRITIC CELLS DICTATE MUCOSAL TOLERANCE AND Th2 IMMUNITY

A network of CD11b⁻ cDCs that express CD103, langerin, and increased lymphotactin receptor XCR1, is found in the epithelial layer of the conducting airways. These cells protrude cellular extensions between the basolateral spaces of basal epithelial cells. Immediately below the basement membrane, the lamina propria contains CD11b^{hi} cDCs that express SIRP α , and increased levels of the fractalkine receptor CX3CR1. The conducting airways also contain CD11c^{dim} pDCs that express Siglec-H and bone marrow stromal antigen-2 (BST2).⁸⁴

Lung parenchymal DCs are found in the alveolar septa. These can also be subdivided into CD11b⁺ and CD11b⁻ cDCs, and include a subpopulation of pDCs. Some DCs, primarily CX3CR1⁺CD11b⁺ cells, also patrol the vessel walls of the pulmonary arterial vasculature and can capture injected embolic material.⁸⁴

During inflammation, additional CD11b⁺ monocyte-derived DCs that express Ly6C and Fc ϵ RI are recruited to the lungs. In human bronchoalveolar lavage (BAL) fluid, three types of DC populations have been identified; CD11c⁺CD1c⁺ myeloid (m)DC type 1, CD11c⁺BDCA-3 (CD141)⁺ mDC type 2, and CD11c⁺BDCA-2⁺ pDCs. Human mDC1s correspond to the mouse CD11b⁺CD103⁻ cDC subset, mDC2s correspond to CD11b⁻CD103⁺ langerin⁺ cDCs, and human pDCs correspond to murine pDCs.⁸⁴

Mouse intraepithelial lung CD103⁺CD11b⁻ cDCs and human monocyte-derived DCs form tight junctions with neighboring airway epithelial cells to protect the epithelial barrier function during the proc-

esses involved with antigen uptake, while allowing the exchange of inhaled particles. The transepithelial transport of inhaled antigens by CD103⁺CD11b⁻ cDCs is followed by antigen uptake by more deeply situated CD11b⁺ cDCs. These lung DCs orchestrate the immune responses to pathogens, commensal bacteria and innocuous inhaled antigens.⁸⁵ Whether a specific subset of DCs presents inhaled antigens in a tolerogenic manner or induces Th2-biased immune responses remains unknown.

Pulmonary DCs survey the airways by continuously migrating from the lungs to the T cell paracortex of the draining mediastinal and peribronchial lymph nodes in a CCR7-dependent way.^{86,87} Those DCs that concomitantly acquire a semi-mature phenotype by receiving subtle maturation signals, presumably from the local microbiota, are thought to induce Tregs that control pulmonary tolerance and homeostasis.^{11,88-90} Quiescent pulmonary stromal cells promote TGF- β -dependent differentiation of tolerogenic DCs, which promote the differentiation of Tregs in vitro.⁹¹

Three pDCs subsets can be identified based on their expressions of CD8 α and CD8 β , and exhibit differences in their functional capacities to prime naïve CD4⁺ T cells. CD8 α β ⁻ pDCs are immunogenic, whereas CD8 α β ⁻ and CD8 α β ⁺ pDCs exhibit tolerogenic properties to support the conversion of naïve CD4⁺ T cells into Foxp3⁺ Treg cells.⁹² Expression of RALDH1, 2 and 3 by tolerogenic lung pDCs and the subsequent production of RA are critical for inducing Treg cells.^{14,92} The steady state co-existence of cDCs with pDCs is thought to promote tolerance to inhaled antigens.

When there is concomitant exposure to innocuous antigens with inhaled environmental adjuvants, such as cigarette smoke, fine particulate matter, or bacterial contaminants, lung DCs acquire appropriate maturation stimuli, and subsequently release cytokines and express costimulatory molecules that govern acquired immunity. A subset of c-kit⁺ Fc ϵ RI⁺ DCs or SIRP α ⁺CD11b⁺ DCs has been shown to effectively prime Th2-biased immunity.⁹³⁻⁹⁶ It was recently shown that CD11b⁺ cDCs, but not CD103⁺ cDCs, were primarily responsible for inducing Th2 immunity in HDM-specific T cells and that monocyte-derived DCs were also sufficient to induce Th2 cell-mediated immunity, but only when high-dose HDM was administered.⁹⁷ Moreover, in allergen-challenged mice, CD11b⁺ DCs were a prominent source of the chemokines CCL17 and CCL22, and thereby recruited CCR4⁺ Th2 cells to the lungs.^{98,99} Although any lung DCs appear to have the capacity to induce Th2 effector responses, as well as Th1, or Th17 responses, depending on the type and strength of the provoking stimulus, a subset of DCs may play critical roles during the priming and maintenance of Th2 immunity and antigen sensitization.

PULMONARY EPITHELIUM INITIATES ANTIGEN SENSITIZATION

The pulmonary epithelium provides a barrier between the external inhaled environment and the internal tissues. Besides providing a physical barrier, the pulmonary epithelium is immunologically active and is pivotal in the development of immune responses in the lung. Pulmonary epithelial cells can secrete a wide range of cytokines and chemokines in response to danger signals and, thereby, direct immune responses and recruit cells of the innate and adaptive immune system.

Epithelial cells sense microbes through pattern recognition receptors (PRRs) that include TLRs, NOD-like receptors, protease-activated receptors (PARs), and C-type lectin receptors, which recognize pathogen-associated molecular patterns from viruses, bacteria, fungi, and multicellular parasites.¹⁰⁰ Although PRR-triggered immune responses provide a critical antimicrobial monitoring system, it is now clear that many allergens have the potential to modify the epithelial barrier through PRRs. CD23 ligation can activate pro-inflammatory pathways in human intestinal epithelial cells and result in the release of chemokines, including CCL20.¹⁰¹

Bronchial epithelial cells first attract immature DCs or their precursors through their release of CCL2 or CCL20 chemokines and subsequently instruct them to induce Th2 immunity to allergens via the release of innate pro-Th2 cytokines, such as GM-CSF, thymic stromal lymphopoietin (TSLP), and IL-25 and -33.^{102,103} IL-33 and TSLP can drive allergic inflammation, even in the absence of IL-4, and promote Th2 cell differentiation by programming the function of DCs.¹⁰⁴⁻¹⁰⁶ IL-25 exacerbates airway hyperreactivity in the absence of Th2 cytokines.¹⁰⁷

House dust mite antigen triggers epithelial cells to produce IL-1 α in a TLR4-dependent manner.¹⁰⁸ The IL-1 α subsequently acts in an autocrine manner on the lung epithelial cells, which results in their secretion of proinflammatory chemokines, GM-CSF, and IL-33. Together, these mediators recruit and activate inflammatory DCs that induce adaptive Th2 immunity to this antigen. TSLP becomes important only when high doses of antigen are administered.

APPLICATION OF MUCOSAL IMMUNITY TO IMMUNOTHERAPY FOR ALLERGIC DISEASES

Mucosal immunotherapy is more advantageous compared with subcutaneous immunotherapy from a production and regulatory perspective; antigens that are used for mucosal immunotherapy do not require extensive purification to remove bacterial contaminants, as the mucosal surface is already colonized by bacteria. The procedure for antigen administration is relatively easy. Under normal conditions, however, after

their ingestion, proteins or carbohydrates are exposed to changing pH levels and digestive enzymes during gastrointestinal transit, resulting in antigen degradation.¹⁰⁹

The capacity of transcytosis through the intestinal epithelial layer depends on particle size; a particle size <1 μ m is optimal for M-cell absorption.¹¹⁰ Transcytosis also depends on the charge and hydrophilicity of the particle carrier. Once bound to the apical side of M cells, macromolecules are endocytosed via clathrin-coated vesicles, and thereby reach the lysosomal compartment, from which they are exocytosed to a basolateral site to be taken up by DCs. To overcome the degrading conditions in the gastrointestinal tract, encapsulating antigens into particle carriers, such as liposomes may enable intact antigens to reach the intestinal epithelial layer.¹¹¹ Furthermore, modifying particles by special targeting materials can direct particle binding to specific cells and enhance the particle uptake capacity via intestinal cells and DCs.

Several studies have demonstrated that mannosylated-liposomes could enhance both Ag presentation and T cell stimulation by targeting mannose receptors on APCs.¹¹²⁻¹¹⁴ The combination of a dimannosyl ligand for liposomal vesicle targeting and Toll-like receptor (TLR)-2 ligand Pam2CAG for immune stimulation promoted binding to APCs and stimulating cellular immune responses.¹¹⁵ Subcutaneous or intraperitoneal delivery of oligomannose-coated liposomes induced only systemic immune responses, whereas intranasal delivery induced mucosal immunity as well as systemic immunity.¹¹⁶ Therefore, the modifications used for antigen particle carriers and the route of administration are important determinants for the effects of immunotherapy.

Zhou *et al.* reported that sugar modified antigen binding to the innate immune receptor SIGN-R1 (murine homolog of human DC-SIGN) could reduce the anaphylactic response to food allergen by inducing DCs to release IL-10 and to generate Tr1 cells.¹¹⁷ Based on their findings, we have demonstrated that intranasal immunization with Ag encased in oligomannose coated liposomes may be an effective immunotherapy for food allergies, as it induced a subset of regulatory CD8⁺ T cells as well as CD4⁺CD25⁺Foxp3⁺ T cells and modulated humoral immune responses, even in allergen-sensitized mice.¹¹⁸

CONCLUSION

We have focused primarily on DCs and various regulatory cells that may participate in the induction of oral tolerance and the mechanisms that have been elucidated primarily in animal models. Understanding the mechanisms involved in oral tolerance is crucial for the development of novel therapeutic strategies to prevent or reverse hypersensitivity reactions to foods and aeroantigens.

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