The role of dendritic cells in food allergy

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In recent years, our understanding of the initiation of T_H2-type immunity has increased significantly, yet the mechanism behind the induction of T_H2 responses and allergic sensitization to food antigens largely remains an enigma. Dendritic cells (DCs) were first described almost 4 decades ago and have since been recognized as the most important antigen-presenting cells and crucial in the induction of T-cell differentiation. Here we discuss our current knowledge of the role of DCs in food allergy. In both murine models and allergic patients, characteristics of DCs have been identified that might play a role in sensitization to food and enhance susceptibility to food allergy. In addition, it has now been shown that several allergens, including some from foods, can directly activate DCs to induce T_H2 skewing. Other cell types with innate immune functions, such as epithelial cells and basophils, might also be involved in sensing of food allergens in human subjects, and interaction of DCs with these cells might facilitate sensitization. DCs appear to play an important role in allergen-specific immunotherapy and could be an attractive target for tolerance induction in patients with food allergy. Further characterization of differences in DC responses between human food-allergic and nonallergic subjects is necessary to gain a better insight into the role of DCs in sensitization and tolerance to food allergens. (J Allergy Clin Immunol 2012;129:921-8.)

Key words: *Dendritic cells, food allergy, innate immunity, T_H2 differentiation, T_H2 adjuvant, sensitization, tolerance, allergen-specific immunotherapy, Toll-like receptors, C-type lectin receptors*

Abbreviations used

APC:	Antigen-presenting cell
CLR:	C-type lectin receptor
CT:	Cholera toxin
DC:	Dendritic cell
DC-SIGN:	Dendritic cell–specific intercellular adhesion molecule 3–grabbing nonintegrin
FoxP3:	Forkhead box protein 3
HDM:	House dust mite
HKE:	Heat-killed *Escherichia coli*
IEC:	Intestinal epithelial cell
iNKT:	Invariant natural killer T
LP:	Lamina propria
mDC:	Myeloid dendritic cell
MLN:	Mesenteric lymph node
MoDC:	Monocyte-derived dendritic cell
OIT:	Oral allergen immunotherapy
OX40L:	OX40 ligand
pDC:	Plasmacytoid dendritic cell
PRR:	Pattern-recognition receptor
RA:	Retinoic acid
SIT:	Allergen-specific immunotherapy
SLIT:	Sublingual allergen immunotherapy
TIM:	T-cell immunoglobulin and mucin domain-containing protein
TLR:	Toll-like receptor
Treg:	Regulatory T
TSLP:	Thymic stromal lymphopoietin

Allergy to food has become a serious health concern in the Western world. Recent studies in the United States estimate that food allergy affects 4% to 8% of children and 3% to 4% of adults, and its prevalence appears to be on the increase.[1-3] Research conducted in the past few decades has led to the identification of hundreds of food allergens clustered into dozens of protein families,[4] as well as a growing appreciation of the aspects of the effector phase of the allergic response and their contribution to clinical symptoms.[5] However, the immunologic mechanisms driving sensitization to food constituents are still poorly understood.

Dendritic cells (DCs) were first described in 1973 by Nobel Prize winner Ralph Steinman and his advisor Zanvil Cohn. When observing adherent cells derived from murine spleens, they noticed a large stellate cell with distinct properties from mononuclear phagocytes, granulocytes, and lymphocytes. The cytoplasm of these cells was arranged in processes of varying sizes and shapes, leading Steinman to coin the term "dendritic cells."[6] The morphology of DCs results in a very large contact surface to their surroundings compared with overall cell volume, which facilitates their function as the most important antigen-presenting cells (APCs) in the immune system. DCs present antigen to $CD4^+$ and $CD8^+$ T cells and induce differentiation of these T cells into their various subtypes. Allergy is driven by allergen-specific $CD4^+$ T_H2 cells, which produce the cytokines IL-4, IL-5, and IL-13. IL-4 plays a major role in driving T_H2 differentiation and induces IgE production by allergen-specific B cells,[7] which binds to the high-affinity IgE receptor (FcεRI) on mast cells and basophils. These effector cells play key roles in the immediate clinical symptoms upon re-exposure to allergen. IL-13 functions as an effector molecule that mediates eosinophilic inflammation, airway hyperresponsiveness, and mucus hypersecretion.[7] IL-5 is the major eosinophil-active cytokine, inducing eosinophil proliferation and differentiation and acting as a costimulator for eosinophil activation.[8]

In recent years, it has become clear that DCs act as messengers between the innate and adaptive immune systems. Innate sensing of allergens by DCs and other cells with innate immune functions, such as granulocytes and epithelial cells, as well as interaction

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between these cell types, is therefore likely to have a profound influence on the resulting adaptive immune response.[9,10] This review will focus on the involvement of DCs in sensitization and tolerance to food proteins.

DCs IN ORAL TOLERANCE

It is necessary to know how DCs function upon exposure to food under physiologic circumstances to obtain a better understanding of the aberrant immune responses that give rise to food allergy and the role of DCs in these responses. The main route of exposure to dietary constituents is through the mucosal surface of the gastrointestinal tract, which comprises the largest surface area of the body that is in contact with the external environment. The intestinal mucosa is exposed to a variety of harmful invasive pathogens, as well as harmless antigens, such as food proteins and commensal bacteria. The gastrointestinal mucosal barrier consists of physiologic components, such as a thick mucus layer, enzymes, bile salts, and extremes of pH, to protect the host against ingested pathogens. This allows for the local immunologic microenvironment to be immunosuppressive, which prevents the development of deleterious immune responses against food constituents and facilitates the uptake of nutrients. Interestingly, once a tolerogenic response to a dietary food protein has developed, immune responses to this antigen are suppressed in the intestinal tissue, as well as in the periphery. This phenomenon is known as oral tolerance.[11,12]

The development of oral tolerance has been shown to depend on multiple mechanisms, including T-cell clonal anergy and deletion, as well as the generation of antigen-specific regulatory T (Treg) cells.[13-15] Gut-associated DCs play a key role in the induction of Treg cells. These DCs sample antigen, either directly from the gut lumen or through specialized microfold (M) cells, and present antigen to naive T cells in the Peyer patches or the gut-draining mesenteric lymph nodes (MLNs).[16,17] Resident DCs in the intestinal mucosa, which drive Treg cell differentiation, are characterized by the expression of CD11c and CD103. These DCs express the enzyme retinaldehyde dehydrogenase 2, which converts the vitamin A metabolite retinal into retinoic acid (RA). In the presence of TGF-β, which is also produced by this DC subset, RA promotes conversion of naive T cells into Treg cells, which express the transcription factor forkhead box protein 3 (FoxP3).[18,19] Moreover, RA produced by gut-associated DCs induces the integrin $\alpha_4\beta_7$ and the chemokine receptor CCR9 in naive T cells, which are involved in gut homing.[20] Recently, 2 studies have shown conclusively that $Foxp3^+$ Treg cells expressing $\alpha_4\beta_7$ integrin and CCR9 are required for oral tolerance. These studies used DEREG mice, which express diphtheria toxin receptor under the control of the Foxp3 promoter. In these mice $Foxp3^+$ Treg cells can be depleted by injection with diphtheria toxin, and this treatment abrogated oral tolerance.[21,22] In addition to producing RA, mucosal DCs also contribute to a tolerogenic environment by releasing the immunosuppressive cytokine IL-10 and inducing production of this cytokine in responding T cells.[23,24]

The capacity to promote Treg cell differentiation is imprinted on gut-associated DCs by intestinal epithelial cells (IECs), which also express retinaldehyde dehydrogenase. Differentiation of Treg cell–promoting DCs is dependent on TGF-β and RA produced by IECs.[25] Interestingly, it was recently shown that the role of RA in $CD4^+$ T-cell differentiation goes beyond induction of Treg cells because pathogen-specific proinflammatory T-cell responses depend on RA signaling as well.[26] Hence regulation of T-cell differentiation by RA appears to depend on accompanying signals that dictate whether a response ultimately becomes regulatory in nature or inflammatory.

INNATE SENSING OF ALLERGENS BY DCs

The gastrointestinal immune system is exposed to an enormous variety of dietary constituents, yet only a small number of these

GLOSSARY

DENDRITIC CELLS (DCs): APCs that capture antigen in the periphery, traffic to draining lymph nodes, and process and present antigen to T lymphocytes through MHC–T-cell receptor interactions. DCs can respond to exogenous stimuli by upregulating costimulatory molecules (eg, CD40, CD80, and CD86) and cytokine secretion (eg, IL-10 and IL-12), which can influence the response of the responder T cell. In human subjects peripheral blood DCs are often broadly divided into mDCs, which originate from myeloid progenitor cells, and pDCs, which are derived from lymphoid progenitors. mDCs express the surface marker CD11c, as well as TLRs, such as the extracellular TLR2 and TLR4, which recognize a wide range of pathogen-derived molecules, such as zymosan and LPS. Ligation of these receptors induces production of proinflammatory cytokines, such as TNF, IL-1, IL-6, and IL-12. pDCs express CD123 and the intracellular TLR7 and TLR9, which detect single-stranded RNA and CpG DNA motifs, respectively. These cells produce large amounts of type I interferon (IFN-α and IFN-β) and are critical in the antiviral immune response. Human MoDCs are DC-like cells generated by culturing $CD14^+$ monocytes in the presence of GM-CSF and IL-4. In mice numerous DC subsets have been defined, which are distinguished by the expression of surface markers, such as CD11c, CD11b, CD4, and CD8α, and their presence in the various lymph nodes and other lymphoid tissues.

PATTERN-RECOGNITION RECEPTORS (PRRs): Innate recognition of microorganisms occurs through PRRs, of which many different types have been described. These include TLR, NOD1, and NOD2 (containing a nucleotide-binding oligomerization domain, which gives them their name), and a number of CLRs, such as dectin-1 and DC-SIGN. PRRs recognize pathogen-associated molecular patterns, which are repeating structures common to categories of microorganisms (gram-negative bacteria, gram-positive bacteria, fungi, and viruses). PRRs can be on the cell surface (eg, TLR2 and TLR4) or within the cell (eg, TLR7 and TLR9, as well as NOD1 and NOD2, which recognize fragments of bacterial cell-wall proteoglycans). PRRs are found on a wide range of cell types, including DCs, and can promote phagocytosis of microorganisms, promote chemotaxis to sites of infections, and induce the release of effector molecules, such as chemokines and cytokines. CLRs specifically bind to carbohydrates, such as glucans, which are prominent constituents of the cell walls of fungi, plants, and mycobacteria; high-mannose structures, which are expressed by some viruses, fungi, and bacteria; and fucose structures, which are found on the surface of helminths and some bacteria.

SUBSETS OF T_H CELLS: After activation by DCs, naive $CD4^+$ T cells differentiate into various subsets of T_H cells that can be divided into categories based on their cytokine secretion. T_H1 cells secrete IFN-γ; T_H2 cells secrete IL-4, IL-5, and IL-13; T_H17 cells secrete IL-17; and regulatory T (Treg) cells produce IL-10, TGF-β, or both. This specialized cytokine secretion is associated with a specialization of function, such that T_H1 cells promote clearance of intracellular pathogens, T_H2 cells provide B-cell help and promote humoral immunity, T_H17 cells enhance neutrophil responses and promote clearance of extracellular bacteria, and Treg cells suppress the other T-cell subsets to prevent excess or damaging immune responses.

induce sensitization and allergic symptoms in susceptible subjects. Approximately 400 food allergens have currently been identified, representing 71 protein families, which is only 0.6% of the more than 12,000 protein families known to date.[4] The most allergenic foods are cow's milk, hen's egg, peanut, tree nuts, fish, shellfish, wheat, soy, and sesame, and these are the cause of the large majority of food allergy cases in the United States.[3] The fact that food allergy is only triggered by such a small group of proteins suggests that these proteins share uncommon characteristics that add to their allergenicity. One explanation might be that these foods act as adjuvants and stimulate DCs to enhance allergen-specific T_H2 differentiation.[9]

Our laboratory observed that the major peanut allergen and glycoprotein Ara h 1 stimulates human monocyte-derived dendritic cells (MoDCs) to induce T_H2 differentiation in naive T cells. Ara h 1 was further shown to bind to the C-type lectin receptor (CLR) dendritic cell–specific intercellular adhesion molecule 3–grabbing nonintegrin (DC-SIGN). Deglycosylated Ara h 1 did not have a T_H2-skewing effect, indicating that allergen-bound carbohydrate structures act as a T_H2-skewing adjuvant.[27] Correspondingly, other studies have reported that glycation of proteins enhances their uptake by DCs and their T-cell immunogenicity,[28] as well as their ability to induce T_H2 differentiation.[29,30] Incubation of DCs with Lewis-x trisaccharides suppressed production of the T_H1-inducing cytokine IL-12,[30] which provides an explanation for the T_H2 skewing induced by these glycans. Recently, we observed that a previously undescribed nonglycosylated peanut protein upregulates the expression of retinaldehyde dehydrogenase 2 in human myeloid dendritic cells (mDCs). RA produced by these DCs acts on naive T cells to induce the gut-homing $\alpha_4\beta_7$ integrin and production of IL-5.[31]

Intrinsic T_H2-skewing properties have also been shown in respiratory allergens, such as house dust mite (HDM). Glycans in HDM extract induce T_H2 differentiation through binding to the CLR dectin-2 on DCs and subsequent induction of cysteinyl leukotriene production.[32] The mannose receptor, another CLR, binds to and mediates internalization of a variety of allergens, such as Der p 1 and Der p 2 (HDM) and Ara h 1. Mannose receptor also mediated Der p 1–induced T_H2 skewing by human MoDCs through upregulation of indoleamine 2,3-dioxygenase activity.[33] Der p 2 has further been shown to share structural and functional homology with MD-2, the LPS-binding component of the Toll-like receptor (TLR) 4 signaling complex. Because of this homology, Der p 2 in the presence of a very low concentration of LPS signals directly through TLR4, which plays an essential role in the induction of sensitization to Der p 2 and experimental allergic asthma.[34]

Interestingly, activation of the CLR specific intercellular adhesion molecule 3–grabbing nonintegrin-related 1 (SIGNR1, the murine homologue of DC-SIGN) on lamina propria (LP) DCs was shown to have a tolerogenic rather than T_H2-skewing effect. BSA coupled with mannoside selectively targeted SIGNR1-expressing LP DCs and induced production of IL-10, which promoted the generation of $CD4^+$ type 1 Treg cells.[35] Isoflavones are anti-inflammatory molecules that occur naturally in soybean. In a murine model of peanut allergy, oral exposure to these isoflavones reduced allergic symptoms upon challenge with peanut. Moreover, isoflavones inhibited cholera toxin (CT)–induced DC maturation in the MLNs and in human MoDCs and decreased production of T_H2 cytokines in cocultures of CT-primed DCs and $CD4^+$ naive T cells. The immunosuppressive capacity of isoflavones might account for the fact that soy is less allergenic than peanut, whereas proteins from both species share extensive amino acid sequence homology.[36]

DCs IN INDUCTION OF T_H2 DIFFERENTIATION AND FOOD ALLERGY

The mechanisms involved in the differentiation of T_H2 cells have long been more elusive than the factors driving T_H1 responses but have received much attention in recent years.[37] T_H2-type immunity has presumably evolved in response to extracellular multicellular parasites, such as helminths. However, as stated above, T_H2 cells also play a central role in the pathophysiology of asthma and other allergic inflammatory diseases. DCs have been shown to play an important, probably essential role in the induction of primary T_H2 responses to helminths and allergens.[38,39] A substantial body of research has been performed regarding the phenotype and function of DCs in murine models of food allergy. The importance of DCs in establishing a food-induced allergic response has been shown by means of adoptive transfer of DCs from mice with cow's milk allergy into naive recipients. The recipients developed cow's milk–specific IgE and IgG responses in the absence of previous immunization.[40] Interestingly, the same group reported that DCs from mice with cow's milk allergy were more resistant to apoptosis induced by cow's milk–specific $CD4^+$ T cells than DCs from control mice. T cell–mediated apoptosis of DCs took place only in the presence of the specific antigen, was dependent on MHC class II, and was partially dependent on CD95–CD95 ligand.[41] It was further observed in a different murine allergy model that purified apoptosis-resistant DCs from allergic mice prompted T_H2 differentiation in cocultured naive T cells and induced allergen-specific IgE and T_H2 responses upon adoptive transfer into naive syngeneic recipients.[42] These findings suggest that apoptosis resistance is a feature of DCs that contributes to the generation, maintenance, or both of IgE-mediated allergy. Nevertheless, enhanced T_H2 skewing might be unique to apoptosis-resistant DCs from allergic mice because studies in other murine models have shown that apoptosis-resistant DCs can enhance proinflammatory, T_H1-driven immune responses as well.[43,44]

A combination of antigen with a T_H2-skewing adjuvant, such as CT, is generally used to establish food allergy in mice through the oral route of exposure. Oral sensitization to peanut extract plus CT was observed to induce a shift in DC subsets toward more $CD11c^+CD11b^+$ DCs and less $CD11c^+CD103^+$ DCs in the mucosa but not in the MLNs.[45] These effects were mainly induced by CT because responses to CT alone and CT plus peanut extract were comparable. In a different study oral exposure to CT instead increased $CD11c^+CD103^+$ DCs in the MLNs. Moreover, oral CT induced DC maturation and upregulation of OX40 ligand (OX40L) in MLN DCs. OX40L was shown to mediate the CT-induced T_H2 differentiation.[46] In a murine model investigating sensitization to the cow's milk allergen α-lactalbumin through various physiologic routes, it was observed that a potent route of sensitization was through the skin. It was further shown that CT induced an efflux of antigen-bearing langerin-negative dermal DCs to the lymph nodes, which generated a proallergic T_H2 response.[47] In sum, these data indicate that CT enhances sensitization by inducing migration of T_H2-skewing DC subsets to sites of naive T-cell priming and upregulation of OX40L on these DCs. It is currently unknown whether food allergens share characteristics with CT in regard to DC activation.

T-cell immunoglobulin and mucin domain-containing protein (TIM) 4 is expressed by DCs in lymphoid organs, and its ligand, TIM-1, is expressed by T cells. These molecules have been found to be critical regulators of T_H2 differentiation. In a murine model of food allergy using the antigen ovalbumin and staphylococcal enterotoxin B as an adjuvant, upregulation of TIM-4 and other costimulatory molecules was found in intestinal mucosal DCs. Blockade of TIM-4 or its ligand, TIM-1, dampened T_H2 differentiation and intestinal allergy.[48] Corresponding results were obtained in a different study, which used peanut as the antigen and CT as the adjuvant. CT induced upregulation of TIM-4 in DCs, which was essential for the induction of peanut-specific T_H2 responses and intestinal allergy and dependent on TLR4 expression in DCs.[49] Evidence for an important role of TIM-4 in the induction of allergic responses has also been found in human subjects. Treatment of patients with HDM allergy with allergen-specific immunotherapy (SIT) using the major allergen Der p 1 decreased expression of TIM-4 on DCs and TIM-1 on T cells. The expression of TIM-4 on DCs mildly correlated with the clinical symptom score. Also in this study the TIM-4/TIM-1 ligation was shown to enhance T_H2 responses.[50] The lectin galectin-9 is a ligand of TIM-3 and suppresses T_H1 and T_H17 differentiation. Expression of galectin-9 was found to be enhanced in IECs of patients with food allergy. Stimulation of murine DCs with galectin-9 through TIM-3 induced expression of TIM-4. Moreover, blocking of galectin-9 reduced specific IgE and T_H2 responses in established allergy in mice and decreased expression of TIM-1 in LP $CD4^+$ T cells and TIM-4 in LP DCs. These data indicate that galectin-9–induced expression of TIM-4 in DCs plays a role in sustaining allergic responses.[51]

In contrast to these positive regulators of T_H2 differentiation and allergen sensitization, production of the T_H1-inducing cytokine IL-12 by DCs can strongly inhibit oral sensitization to peanut, even when administered with CT as adjuvant. In comparison with normal mice, those with a defect in IL-12 production by DCs produced higher specific IgE responses and more severe allergic symptoms after sensitization. Neutralization of IL-12 by specific antibody also increased susceptibility to food allergy.[52] More evidence for an inhibitory effect of IL-12 on allergic responses comes from a study in which murine DCs pulsed with peanut extract were cocultured with T cells isolated from peanut-sensitized mice in the presence or absence of heat-killed *Escherichia coli* (HKE). Addition of HKE resulted in a decrease in T_H2 cytokine production and an increase in levels of the T_H1 cytokine IFN-γ. These effects were mediated by HKE-induced TLR activation, and antibodies neutralizing IL-12 could partially abrogate the effects of HKE.[53]

Whereas there is a relative abundance of data derived from murine models of allergy, very little is known about the phenotype and function of DCs in allergy in human subjects. Comparing DC responses between allergic and tolerant subjects might provide insight into whether and how abnormal DC functioning leads to allergy, and examples of DC abnormalities have been found. In patients with atopic dermatitis, of whom the majority were sensitized to aeroallergens, TLR-induced production of IL-12 and TNF in mDCs, as well as release of IFN-α in plasmacytoid dendritic cells (pDCs), was lower than in nonallergic control subjects.[54] In a different study pDCs from patients with inhalant allergies produced less IFN-α in response to TLR9 stimulation than pDCs from nonallergic subjects.[55] Together, these data indicate that DCs of allergic subjects are less responsive to proinflammatory, T_H1-inducing stimuli. In a follow-up study by the latter group, it was shown that SIT restored TLR9 responsiveness in pDCs from treated patients.[56] A different group observed lower expression of the costimulatory molecules CD86 in pDCs and CD40 in mDCs from patients with Hymenoptera venom allergy compared with nonallergic control subjects. Interestingly, TLR9 expression in pDCs was also lower in allergic subjects and increased after SIT.[57] In the only study we found comparing DC responses between subjects with food allergy and those with food tolerance, cow's milk–stimulated but not resting pDCs and mDCs from peripheral blood of patients with cow's milk allergy produced greater levels of TNF, IL-6, GM-CSF, and IL-10 than DCs of control subjects.[58] Neither TLR expression nor direct DC responsiveness to TLR ligands was compared. The authors point out that the mechanism of the milk-induced response remains to be defined because although the subjects were uniquely sensitized with milk-specific IgE compared with control subjects, stimulation of DCs through FcεRI with anti-IgE was insufficient to reproduce the effect of allergen stimulation.

INTERACTION OF DCs WITH OTHER INNATE IMMUNE CELLS

Direct interaction of allergens with DCs through pattern-recognition receptors (PRRs), such as CLRs, has been shown to activate DCs to induce T_H2 skewing. In addition, indirect activation of DCs through other cell types or cooperation of DCs with other immune cells can give rise to T_H2 differentiation. Epithelial cells are located at the sites of allergen entry into the body and interact closely with DCs *in situ*. It was observed that human bronchial epithelial cells and various other cell types produce the cytokine thymic stromal lymphopoietin (TSLP), which stimulates DCs to produce the T_H2 cell–attracting chemokines CCL17 and CCL22. Moreover, TSLP-activated DCs prime naive T_H cells to produce IL-4, IL-5, and IL-13.[59] Induction of T_H2 cells by TSLP-stimulated human DCs depends on OX40L specifically induced by TSLP.[60] As such, the natural T_H2 adjuvant TSLP in human subjects appears to act through the same mechanism as the experimental adjuvant CT in murine models of (food) allergy.[46] Human IECs have also been shown to express TSLP, which confers T_H2-inducing ability on gut DCs. Interestingly, human gut-associated DCs are biased toward inducing T_H2 responses, even in the presence of bacterial stimulation.[61] A recent study showed that TSLP promotes systemic basophilia, resulting in increased production of T_H2 cytokines and IgE. Wild-type mice infected with the helminth *Trichuris muris* exhibited increased basophils in the periphery, whereas $Tslpr^{-/-}$ mice did not. Critically, TSLP-elicited basophils isolated from $Tslpr^{+/+}$ mice and adoptively transferred into $Tslpr^{-/-}$ mice infected with *T muris* were sufficient to restore protective T_H2 cytokine responses.[62] It was further shown that IL-3–activated human basophils also express TSLP receptor, indicating that basophils might be involved in TSLP-induced T_H2 immunity in human subjects.

The role of basophils has recently been suggested to extend beyond the effector phase of the allergic response. In addition to their FcεRI-mediated degranulation upon allergen encounter after sensitization, basophils can also act as APCs in the induction of T_H2 responses. This has been shown to occur in response to the protease allergen papain,[63] the experimental allergen ovalbumin,[64] and helminths.[65] In contrast, induction of T_H2 responses to HDM allergen was shown to be dependent on inflammatory

DCs. However, in this model exposure to inhaled HDM led to recruitment of IL-4–competent basophils, and depletion of basophils partially reduced T_H2 immunity.[39] Hence basophils might play an important role in induction of T_H2 differentiation by creating a T_H2-skewing cytokine microenvironment, in which DCs present antigen to naive T cells. It is currently unknown whether human basophils can act independently as APCs to induce T_H2 responses to (food) allergens. Nevertheless, it is well possible that human basophils enhance T_H2 differentiation and existing allergic responses through production of T_H2 cytokines.

Another cell type that may contribute to a T_H2-skewing environment is the invariant natural killer T (iNKT) cell. iNKT cells recognize lipid antigens presented via the nonpolymorphic CD1d molecule on DCs and other APCs, and exhibit characteristics that place them at the border between innate and adaptive immunity. An interesting recent study showed that sphingolipid isolated from cow's milk induces iNKT cell proliferation and T_H2-type cytokine secretion. iNKT cells from children with cow's milk allergy produced more IL-4 and IL-13 in response to cow's milk sphingolipid than those from nonallergic children.[66] Thus, cow's milk sphingolipid-specific iNKT cells could give rise to a proallergic microenvironment which facilitates the induction of cow's milk protein-specific T_H2 cells and sensitization to cow's milk.

Epithelial cells are important players in sensitization to allergens, as has recently been elegantly shown for HDM. HDM is naturally contaminated with LPS, which activates lung epithelial cells through TLR4 and induces these cells to produce the innate proallergic cytokines TSLP, GM-CSF, IL-25, and IL-33. Production of TSLP and GM-CSF results in the recruitment and activation of DCs, which initiate the HDM-specific T_H2 response. Interestingly, TLR4 expression on lung epithelial cells, but not on DCs, is necessary and sufficient for DC activation and T_H2 priming.[67] The cytokines IL-25 and IL-33 have been shown to act on natural helper cells, nuocytes, and innate T_H2 cells, which are innate immune cells initially discovered in mice. These cells play a role in the induction of T_H2 responses and early protection against helminths and produce IL-5 and IL-13 upon stimulation with IL-25 or IL-33.[68] A recent study described the equivalent of these cells in human subjects and named them type 2 innate lymphoid cells. These cells are present in the lung and gut and are enriched in nasal polyps of patients with chronic rhinosinusitis, indicating that type 2 innate lymphoid cells might play a role in allergic inflammation.[69] Moreover, IL-33 appears to be important in allergy because it induces production of IL-5 and IL-13 in mast cells and IL-4, IL-5, IL-6, and IL-13 in basophils. Expression of IL-33 has been shown to be increased in epithelial cells from patients with asthma.[70]

ROLE OF DCs IN IMMUNOTHERAPY FOR FOOD ALLERGY

SIT is effective for the treatment of inhalant allergies, such as allergic rhinitis and allergic asthma, as well as for Hymenoptera venom allergy. The 2 most common modes of treatment of these allergies are subcutaneous allergen immunotherapy and sublingual allergen immunotherapy (SLIT).[71] Food allergy has been difficult to treat with SIT because of serious and sometimes life-threatening side effects, especially with subcutaneous allergen immunotherapy. Nevertheless, in recent years, success has been achieved by treating patients with food allergy with oral allergen immunotherapy (OIT) or SLIT, resulting in desensitization and in some cases tolerance to the opposing food.[72] Numerous studies have shown that allergen-specific Treg cells play a key role in the mechanism of SIT.[73] Although very little is known about the role of DCs in SIT, DCs might well be important in the induction of Treg cells over the course of treatment. For instance, DCs in oral epithelium are mainly comprised of oral mucosal Langerhans cells, which have been proposed to specialize in maintaining tolerance in an area exposed to a large variety of antigens.[74] It has been observed that oral mucosal Langerhans cells derived from patients with grass pollen allergy, as well as nonatopic donors, rapidly take up the timothy grass allergen Phl p 5, which attenuates their maturation and increases production of TGF-β and IL-10.[75] These features contribute to their capacity to induce allergen-specific Treg cells. Moreover, an interesting recent study showed that pDCs and $FoxP3^+$ Treg cells constitute important DC and T-cell compartments in human tonsils. Tonsil pDCs were observed to have the ability to generate functional $FoxP3^+$ Treg cells from naive T cells, and tonsil $FoxP3^+$ Treg cells suppressed tonsil T-cell responses to common food and aeroallergens.[76] Thus oral mucosal Langerhans cells and tonsil pDCs are important in oral tolerance and might be involved in the mechanisms of SLIT and OIT.

Alternatively, therapies can be developed that specifically target DCs in patients with food allergy. A recent study described the design of a DNA plasmid fusion protein, which can be used to target DNA coding for a specific allergen to FcεRI on DCs.[77] The FcεRI-bound molecule will be internalized by DCs, leading to translation of the DNA, production of allergen, and presentation of allergen-derived peptides to T cells. The DC-specific promoter fascin was incorporated into the gene expression plasmid to avoid allergen expression in other FcεRI-expressing cells, such as basophils and mast cells. An important advantage of this approach over conventional SIT is that the allergen will be contained within DCs. Hence activation of basophils and mast cells will be reduced and side effects will be minimized. A comparable method was shown in an older study to reduce allergic symptoms in a murine model of peanut allergy.[78] In addition to the use of allergen-encoding DNA, it might be possible to target DCs with allergenic proteins fused to antibodies against internalizing receptors on DCs and hereby modulate the allergen-specific T-cell response. It has been shown recently that targeting of antigens to human DCs through the CLR DC-asialoglycoprotein receptor, but not through dectin-1 or DC-SIGN, favors the generation of antigen-specific suppressive $CD4^+$ T cells that produce IL-10.[79]

Over the years, various mucosal adjuvants have been identified that might enhance the immunogenicity and tolerogenicity of coadministered allergens in immunotherapy suitable for treatment of food allergy, such as SLIT.[80] Most of these studies were performed with murine models for allergy. Ligands of TLR2 and TLR4 were shown to enhance production of IL-10 and IL-12 in DCs and to be effective in inducing Treg cells and T_H1 responses through the sublingual route. Administration of these ligands together with allergen enhanced tolerance induction.[81,82] Synthetic drugs, such as glucocorticoids, plus vitamin D3 are powerful inducers of IL-10 production by DCs and $CD4^+$ T cells and inhibit DC maturation.[83] Furthermore, certain probiotic bacterial strains induce high production of IL-10 and IL-12 by mucosal DCs and enhanced efficacy of SLIT in a murine asthma model.[84] These adjuvants, as well as vectors targeting allergen to DCs, have only sparsely been tested in human subjects but might enhance the efficacy of SLIT and perhaps OIT in human food allergy.[80]

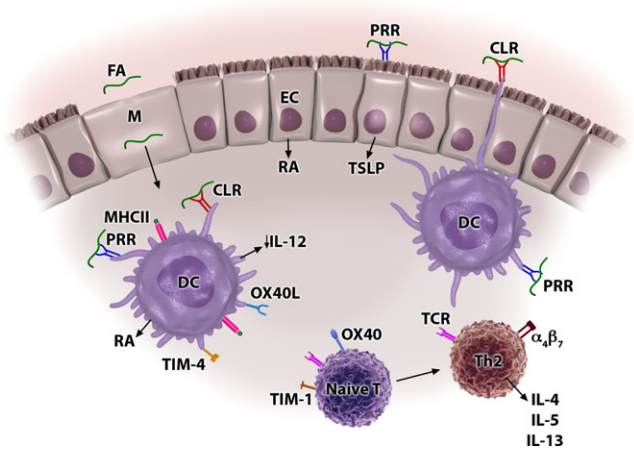


FIG 1. Proposed mechanisms by which gut-associated DCs can contribute to sensitization to food proteins. The epithelial cell *(EC)* layer forms the barrier between gut lumen and the gut-associated lymphoid tissue *(GALT)*. DCs, as well as naive T and B cells, reside in the lymphoid follicle. Food allergen *(FA)* may either be transported from the gut lumen into the lymphoid follicle by specialized microfold cells *(M cells)* where it will encounter DCs in Peyer patches or MLNs, or sampled directly from the gut lumen by DCs. In both cases, FA-derived peptides will be presented by DCs to GALT-resident naive T cells. Sensing of food allergen by DCs through CLRs or other PRRs might lead to a decrease in TLR-induced IL-12 production, as well as upregulation of OX40L, TIM-4, or both. Epithelial cells may also be involved in direct sensing of food allergen through PRRs and this might result in the release of TSLP, which induces OX40L expression in DCs. OX40L and TIM-4 on DCs bind, respectively, to OX40 and TIM-1 on naive T cells. Together, these factors might contribute to the induction of food allergen–specific T_H2 responses by DCs. In addition, epithelial cells and gut-associated DCs express retinaldehyde dehydrogenase and thus produce RA, which upregulates the gut-homing integrin $\alpha_4\beta_7$ in T cells. *TCR,* T-cell receptor.

Given the pivotal role of DCs in regulating the balance between immunity and tolerance, much effort has been put into the generation of tolerogenic DCs in recent years. *In vitro*–generated tolerogenic DCs could potentially be used to induce antigen-specific tolerance in a wide variety of applications, such as autoimmunity, allergy and other hypersensitivity, gene and cell therapy, and transplantation.[85] Tolerogenic DCs can be induced *in vitro* by anti-inflammatory biological agents, such as IL-10, TGF-β, or vitamin D3; by immunosuppressive drugs, such as dexamethasone or rapamycin; and by genetic modification.[85,86] In murine models for autoimmune disorders, treatment with tolerogenic DCs has been successful in reducing disease severity.[86,87] Thus far, tolerogenic DCs have not been applied in models of allergy.

CONCLUSION

Our understanding of the role of DCs in food allergy in human subjects is very limited. Most of our knowledge of the mechanism behind sensitization to food allergens originates from murine models. These studies have shown that expression of OX40L and TIM-4 in DCs, as well as decreased IL-12 production, is associated with T_H2 skewing (Fig 1). Interaction of DCs with other cell types involved in innate immunity, such as epithelial cells, basophils, and iNKT cells, strongly influences the resulting T_H cell response. Epithelial cells produce TSLP, which programs DCs to induce T_H2 skewing, whereas basophils and iNKT cells might enhance the T_H2-inducing microenvironment by producing IL-4 and IL-13. We are beginning to understand the functional and biochemical properties of food allergens that favor induction of T_H2 responses, and enhance the T_H2-skewing ability of DCs. By comparing food allergens with helminth-derived products and other known T_H2 adjuvants, further insight might be gained into the factors that drive T_H2 differentiation and the receptors on DCs and other innate immune cells that are involved in sensing food allergens. Studies in human subjects have indicated that DCs from allergic patients are less mature and less responsive to T_H1-inducing stimuli. These characteristics might increase susceptibility to the T_H2-skewing adjuvant effects of food allergens. DCs appear to play an important role in the induction of allergen-specific Treg cells, which is a key event in SIT. The efficacy of treatments for food allergy, such as OIT or SLIT, might be enhanced by the use of adjuvants or vectors designed to enhance the tolerogenic properties of DC. Further characterization of differences in DC responses between patients with food allergy and nonallergic subjects is necessary to expand our knowledge of human DC function in food allergy and to determine whether observations in murine models can be extrapolated to human subjects. Whereas studying peripheral blood DCs will be informative, investigating oral and gut-associated DCs might be the best strategy to gain insight into the DC responses associated with sensitization and tolerance to food allergens.

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