**Experimental food allergy models to study the role of innate
immune cells as initiators of allergen specific Th2 immune
responses**

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Abstract

Although our knowledge of the pathophysiology of food allergies has significantly improved over the last years, a more comprehensive understanding of basic immune mechanisms driving disease pathogenesis is important to develop new intervention strategies. The recent development of animal model systems recapitulating features of clinical food allergy provide an essential tool to study the immunology of IgE-mediated food allergies. While immunological effector responses have been well documented, how food allergic immune responses are initiated is not well defined. In this short review, we discuss the use of experimental mouse models both to study the role of innate immune cell populations in promoting disease and to test new treatment regimens that may prevent the onset of IgE-mediated food allergies.

Introduction

Food allergy is an adverse type-2-immune cell driven allergic response that occurs reproducibly on exposure to a given food. As the public health and economic impact of food allergies continues to grow, there is an urgent need to develop new intervention strategies to prevent and treat this disease. Despite their often criticized limitation to accurately mimic human pathophysiology and to predict treatment efficacy, experimental animal models have significantly contributed to a better understanding of the immunology of food allergy. The purpose of this review is to summarize the latest developments in the field of innate immune cells as initiators of food allergic responses. Furthermore, we will discuss potential new therapeutic modalities targeting innate immune cell populations which have emerged from experimental food allergy models and hold promise for future clinical studies.

Immunology of food allergy

Food allergies are characterized as adverse immune reactions to food proteins that affect up to 6% of children and 3-4% of adults [1]. Despite food allergies represent a growing clinical problem, disease etiology remains largely unknown. While genetic predisposition is a significant risk factor for the development of food allergies, the large increase in food allergies over the last two decades suggests that genetic predisposition alone cannot account for the observed phenomenon. Emerging evidence suggests that changes in lifestyle (e.g., diet, increased vaccination rate, antibiotics, changes in microbiome) modify innate and adaptive immunity which results in susceptibility to allergic sensitization to foods (reviewed in [2]).

Allergic responses to foods encompass a range of disorders from IgE-mediated food allergies to delayed cell-mediated reactions (also referred to as non-IgE food allergies) affecting the gastrointestinal tract, airways or skin. Throughout this review, we will focus on IgE-mediated food allergies. Food allergies are characterized by uncontrolled type-2 mediated immune responses that occur reproducibly on exposure to a given food. Both arms of the host's immune system, the innate- and the adaptive immune system contribute to disease pathogenesis. Cells of the innate immune system (mast cells, granulocytes, mononuclear phagocytes, innate lymphoid cells) are located at the interface between the external environment and the internal adaptive immune system. In contrast to the innate immune system, adaptive immune cells are able to generate allergen-specific receptor molecules and immunological memory. Thus, in response to allergen re-exposure, cells of the adaptive immune system are able to mount a memory immune response against the same allergen.

A key feature of the allergic cascade is the polarization of allergen specific T helper (Th)₂ cells. Th₂ cells represent important sources of pro-allergic cytokines and regulate B cell class switching to IgE through production of IL-4, recruit eosinophils through IL-5 or mast cells through IL-4/IL-9 signaling resulting in tissue eosinophilia and mast cell hyperplasia. B cell derived allergen-specific IgE is dispersed systemically and binds to its high affinity receptor FcεRI on tissue resident mast cells and circulating basophils resulting in allergen sensitization. Upon allergen re-exposure, IgE cross-linking initiates degranulation of mast cells and basophils which release a number of pro-allergic factors including pre-formed- or newly generated granule mediators, chemokines or cytokines causing smooth muscle contraction, vascular permeabilization and further recruitment of immune cells to sites of inflammation [3].

To better understand the immunological processes underlying the pathogenesis of food allergy, it is important to understand how cells of the innate immune system regulate adaptive immune responses to food allergens. IL-4 plays a critical role in the polarization of Th2 cells by regulating STAT6-mediated expression of GATA3, the master regulator of Th2 differentiation. Given the importance of IL-4 on the polarization of Th2 cells, IgE synthesis and mucosal mast cell expansion in the development of experimental IgE-mediated food allergy, identifying the initial source(s) of IL-4 is key for a better understanding on how food allergen-specific Th2 immune responses are initiated. As naïve T cells are poor producers of IL-4 and IL-4 is important for optimal Th2 polarization in most experimental settings, this raises the chicken-and-egg question of the cellular origin of IL-4. Recent studies using IL-4 reporter mice in models of helminth infection or allergic inflammation have highlighted numerous IL-4 competent innate immune cells that actively contribute to optimal Th2 polarization [4]. Emerging literature further suggests that epithelial cells play a fundamental role in the recruitment of IL-4 competent innate immune cells to sites of epithelial stress through secretion of IL-25, IL-33 and thymic stromal lymphopoietin (TSLP) [5],[6].

Here, we address how the use of different sensitization protocols in experimental murine food allergy models can work in synergy with human studies to investigate the role of innate immune cell populations as initiators of food allergic responses. Further, we discuss potential new treatment protocols that may interfere with the recruitment and activation of innate immune cell populations in the context of food allergy.

Experimental models of food allergy

Epicutaneous sensitization protocols

Studies in humans and mice have demonstrated that epicutaneous food allergen sensitization represents a significant risk factor for the development of food allergy, likely by bypassing the induction of oral tolerance [7]. Epicutaneous food allergen sensitization models often rely on physical impairment of the skin barrier induced by physical damage, chemical-induced damage or genetic manipulation resulting in local tissue inflammation. Physical damage of the skin epithelium can be induced by repeated tape-stripping of skin allowing for food allergen sensitization on a compromised skin barrier causing food allergy upon oral or systemic allergen challenge [8]. Chemicals used to promote epicutaneous food allergen sensitization include topical treatment with trinitrobenzene sulfonic acid (TNBS) [9], sodium dodecyl sulfate (SDS) [10] or calcipotriol (MC903), a vitamin D analogue that is widely used to induce atopic dermatitis (AD)-like skin lesions in mice. Because AD is a risk factor for the onset of food allergy and asthma in humans, recent studies made use of the MC903-mediated food allergen sensitization protocol to induce food allergy or allergic airway inflammation in mice [11],[12]. Alongside these methods many genetic approaches have been made to develop models with skin barrier defects, such as mice with defects in skin matrix proteins, e.g. filaggrin. Importantly, mutations in the filaggrin gene have been strongly associated with the pathogenesis of AD and food allergy in humans and mice [13]. Other genetic models allowing for epicutaneous food allergen sensitization include skin-specific over-expression of TSLP in keratinocytes resulting in gastrointestinal food allergy or allergic lung inflammation upon re-exposure of food allergens via the gastrointestinal tract or the airways, respectively [14]. Together, epicutaneous food allergen sensitization protocols represent valuable tools to study immune cell functions at distinct physical sites; the skin as site of allergen sensitization and the gastrointestinal tract or the airways as site of allergen challenge.

Recruitment of innate immune cell populations to sites of epicutaneous food allergen sensitization

Disruption of skin barrier function induced by mechanical, chemical or genetic manipulation is often required for optimal epicutaneous food allergen sensitization. In response to stress, skin epithelial cells secrete a number of cytokines, including IL-25, IL-33 and TSLP all of which have been implicated in promoting Th2 cytokine responses *in vivo* through attraction or stimulation of innate immune cells [15]. Signaling between skin epithelial cells and innate immune cells via TSLP and IL-33 has been

implicated in the pathogenesis of AD in humans and mice [16]. Recent studies highlighted that epicutaneous sensitization to food allergens on a developing AD-like skin lesion resulted in rapid infiltration of TSLP-elicited basophils that were both necessary and sufficient for the development of allergic responses to food in the gastrointestinal tract. Antibody-mediated depletion or genetic manipulation of TSLP-elicited basophils led to a significant reduction in Th2 polarization and allergen-specific IgE synthesis [12]. As basophils are potent producers of IL-4 in response to FcεRI cross-linking or TSLP signaling, basophil-mediated Th2 polarization and associated development of experimental IgE-mediated food allergy may be regulated through basophil intrinsic IL-4 production [17]. Further studies revealed that basophils and group 2 innate lymphoid cells (ILC2) accumulate in close proximity to each other in AD lesional skin of humans and mice. In these settings, basophil-derived IL-4 was shown to promote proliferation of ILC2s promoting AD-like skin inflammation [18]. Despite their disease promoting role in AD, whether skin ILC2s contribute to food allergen sensitization remains to be determined.

While basophils are known to act as Th2-inducing antigen presenting cells (APCs) and are required for Th2 polarization *in vitro* and *in vivo* [19], TSLP-primed dendritic cells (DCs) play a critical role in the differentiation of Th2 cells [20]. Given their strategic resident location in the skin, immature DCs may be important for internalization of food allergens and the presentation of processed food allergens to naïve T cells. Recent studies by Leyva-Castillo and colleagues demonstrated that optimal Th2 polarization is dependent on an orchestrated immune cascade in a model of AD. TSLP-activated DCs, through OX40L signaling, prime naïve CD4 T cells to produce IL-3 resulting in basophil recruitment and Th2 differentiation [21]. In addition to the above described cross-talk between basophils-DCs and T cells, basophils influence localized eosinophil recruitment, another IL-4 competent innate cell population, in a model of IgE-dependent eosinophilic skin inflammation [22]. Furthermore, targeting basophil responses in food-induced allergic inflammation resulted in a significant reduction of eosinophils to sites of allergen sensitization and challenge [23]. As eosinophils are capable of producing IL-4 and IL-13, it is likely that eosinophils contribute not only to local tissue inflammation, but also to Th2 polarization. Further studies are necessary to determine a potential role for eosinophils in epicutaneous food allergen sensitization. Innate immune cell pathways contributing to Th2 polarization in response to epicutaneous food allergen sensitization are illustrated in Figure 1.

Innate immune cells as initiators of food allergic responses in oral sensitization protocols

In contrast to allergen sensitization via epicutaneous routes, ingested food antigens are subject to denaturation and degradation in the digestive tract resulting in either immunological ignorance or induction of oral tolerance. Failure to induce tolerance to food proteins can result in the development of celiac disease or food allergies. The cellular and molecular events involved in the breakdown of oral tolerance, food allergen sensitization and the development of food allergies are incompletely understood (reviewed in [15]). Studies in mice demonstrated that oral administration of potent mucosal adjuvants e.g., cholera toxin (CT) or staphylococcus aureus enterotoxin B (SEB) together with food antigens is sufficient to overcome oral tolerance, promote food allergen sensitization and the development of IgE-mediated food allergy. Co-administration of allergens together with CT induces the production of antigen-specific IgE promoting anaphylactic responses in response to intra-gastric food allergen challenge of sensitized mice [24]. Mechanisms underlying food allergen sensitization in this model system rely on the up-regulation of co-stimulatory molecules OX40L [25] and TIM-4 [26] on intestinal DCs resulting in enhanced migration of DCs from the lamina propria to mesenteric lymph nodes where matured DCs present captured food antigens to naïve T cells to induce Th2 polarization [27]. Importantly, experimental manipulation of these Th2 polarizing co-stimulatory molecules have been shown to reduce Th2-associated food allergic responses in mice, suggesting their importance in food allergen sensitization [28]. A recent study using CT-mediated allergen sensitization to peanut highlighted that IL-33, but not TSLP or IL-25 promotes up-regulation of OX40L on DCs. IL-33, which is predominantly produced by epithelial cells, is known to increase mucosal permeability and promote Th2 skewing by attracting IL-4 competent innate immune cells [28],[29]. Among these, ILC2s - although poor producers of IL-4 - are major targets of IL-33 in the gastrointestinal tract. Despite their pathological role in models of allergic inflammation, Th2 priming under the control of OX40L-OX40 interactions in the CT food allergy model was independent of ILC2s [31]. These data suggest that other IL-4 competent cells such as tissue resident mast cells or eosinophils may be involved in OX40L-mediated polarization of Th2 cells. Studies by Chu et al. using intra-gastric immunization to the common food allergen peanut with the classical oral Th2-inducing adjuvant CT demonstrated that indigenous enteric eosinophils control OX40L expression on CD103⁺ DCs by means of secretion of the eosinophil-specific granule protein eosinophil peroxidase (EPO). In this model, eosinophil deficient mice were protected from Th2-mediated food allergy and anaphylaxis while Th2 polarization was restored by transfer of IL-4 sufficient or deficient eosinophils into eosinophil deficient hosts [32].

Other mouse models relying on oral allergen sensitization make use of enterotoxin B from *Staphylococcus aureus* (SEB) [33]. *Staphylococcus aureus* is a common organism colonising the airways, and therefore, exposure to *S. aureus* derived super-antigens may represent a physiologically relevant factor for allergic sensitisation to food proteins. SEB co-administered with food proteins applied via the oral route resulted in Th2-mediated IgE-dependent food allergy upon oral re-exposure of sensitized animals [34]. Similar to the CT model, SEB treatment resulted in the up-regulation of TIM-4 on intestinal DCs, and blockade of TIM-4 inhibited food-induced allergic responses [35]. Together, while DCs and indigenous enteric eosinophils can contribute to mucosal food allergen sensitization in the CT food allergy model, the role of indigenous enteric eosinophils in allergen sensitization in the SEB model remains to be determined (summarized in Figure 2). In contrast, ILC2 responses may not contribute to IgE-mediated food allergy in animals sensitized via the oral route [31], but have recently been shown to be critical mediators of IgE-mediated food allergy in mice systemically sensitized with alum before oral allergen feeding [36]. Thus, depending on the route of food allergen sensitization, different innate immune cells are required across various experimental systems to generate food allergen specific adaptive immune responses.

Genetic food allergy models to study innate immune cell functions

In addition to epicutaneous, oral or systemic food allergen sensitization protocols, recently established models of food allergy include genetic manipulation of key type-2 cytokines or their corresponding receptors. Enteral exposure to food allergens in mice harboring activating mutations of the IL-4 receptor α -chain (IL4raF709) induced strong allergen-specific IgE responses and intestinal mastocytosis resulting in systemic anaphylactic responses upon allergen challenge [36],[37]. Other models use transgenic overexpression of IL-9 predisposing mice for oral food allergen sensitization and the development of IgE-mediated intestinal anaphylaxis. The induction of food allergic responses in this model system required intestinal mast cells promoting increased intestinal permeability. Furthermore, overexpression of IL-9 in the intestine promoted local allergen-specific Th2 responses upon intra-gastric allergen feeding [39]. Another recently established genetic model of food allergy relies on the overexpression of IL-25 in the small intestinal epithelium (iL25Tg). Repeated oral sensitization of iL25Tg mice with the model food allergen ovalbumin was sufficient to promote symptomatic features of IgE-mediated food allergy including diarrhea, hypothermia, intestinal

mastocytosis and increased serum allergen-specific IgE levels. In this model system, IL-25 responsive ILC2 promoted susceptibility to experimental food allergy [36]. Collectively, genetic food allergy models represent valuable tools to assess multifactorial functions of innate immune cell populations in the pathogenesis IgE-mediated food allergic responses.

Potential new therapeutic targets arising from studies in experimental food allergy models

Currently, there is no cure for food allergies and available strategies to prevent or block food allergic responses include strict allergen avoidance or injection of epinephrine in emergency situations. Therefore, developing novel or improved therapeutic strategies is an active area of food allergy research. The recent use of pre-clinical experimental food allergy models led to the discovery of several innate immune cell pathways that promote the pathogenesis of food allergy. As a result prevention and treatment strategies have been successfully tested in animal food allergy models with promising results in clinical trials, including allergen-nonspecific- and allergen-specific therapeutic approaches (reviewed in [2]). For example, TSLP – a cytokine that is predominantly produced by innate immune cells – represents a promising new therapeutic target for preventing the onset of food allergies [40]. Given the importance of TSLP for AD pathogenesis in animal models [11] and AD representing a significant risk factor for the development of food allergies [41], targeting TSLP-TSLP-receptor interactions in AD patients may limit food allergen sensitization on impaired barrier skin and thus, prevent the progression to food allergy later in life. Importantly, blocking TSLP signaling in asthmatic patients attenuated allergen-induced asthmatic responses, highlighting its potential clinical value for the treatment of allergic inflammatory disorders [40]. Other epithelial-derived cytokines including IL-25 or IL-33 may represent potential new therapeutic targets for prevention or treatment of food allergies. In addition to interrupting the secretion of epithelial derived type-2 cytokines, targeting IL-4 signaling may represent a promising new therapeutic approach in the treatment of food allergies given its importance in Th2 polarization and the pathogenesis of IgE-mediated experimental food allergy. Recent clinical trials using dupilumab, an IL-4 receptor alpha blocking antibody revealed significant efficacy and safety for the treatment of AD [42]. Targeting IL-4 receptor signaling may not only show efficacy in patients with moderate to severe AD but may also limit food allergen sensitization on a compromised skin barrier.

While the suppression of immune responses is a common therapeutic strategy applied to various inflammatory disorders including allergic inflammation, there is rarely a benefit without potential harm. All biological targets discussed above actively interact with cellular and molecular innate immune cell functions that are important to maintain tissue homeostasis or promote tissue repair in the healthy host. A future challenge will be to determine the optimal therapeutic strategy (e.g. dosage, single or combinatorial treatment protocols) for the individual patient.

Conclusions

Experimental mouse models of food allergy significantly contributed to a better understanding of disease pathogenesis, validation of existing therapeutics and development of new treatment strategies. The use of these model systems highlighted a central role for various innate immune cell populations including basophils, mast cells, eosinophils, ILC2, or dendritic cells as initiators and amplifiers of pathologic allergen specific Th2 responses. While targeting the above discussed innate pathways of allergic inflammation show promising results in preventing or ameliorating disease in animal models, ongoing and future clinical trials will have to demonstrate the efficacy of such intervention strategies in food allergic patients. Together, despite experimental food allergy models do not completely mimic human pathophysiology, they have repeatedly demonstrated their utility in translational discoveries. A future challenge using animal models of food allergy will be the establishment of validated and predictive preclinical models to translate findings from bench to bedside [43].

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Figure Legends

Figure 1

Contribution of innate immune cells to Th2 polarization in epicutaneous food allergen sensitization protocols. Skin epithelial cells secrete TSLP and IL-33 following skin barrier impairment resulting in the recruitment and/or activation of innate immune cell populations. In response to TSLP, basophils are recruited rapidly from the bone marrow to sites of epithelial damage or stress. Basophil-derived IL-4 production promotes the accumulation of ILC2 to the skin that release significant amounts of IL-5 and IL-13 further attracting other immune cells such as eosinophils. Allergen uptake by DCs induces their maturation and migration to skin draining lymph nodes, where they present processed allergen epitopes to cognate T cells. Interaction of basophils with DCs induces the expression of the Th2 priming co-stimulatory molecules OX40L and TIM-4 on DCs, likely in an IL-4 dependent manner. In the presence of IL-4 or IL-13 derived from basophils, eosinophils, ILC2s or tissue resident mast cells, naïve T cells differentiate into effector Th2 cells. Th2 cells promote IgE isotype switching in B cells resulting in the secretion of allergen-specific IgE. Allergen-induced cross-linking of IgE on FcεRI expressed on basophils and mast cells induces cell degranulation and the release of pro-inflammatory mediators further amplifying the allergic cascade.

Figure 2

Contribution of innate immune cells to Th2 polarization in oral food allergen exposure protocols. Cholera toxin (CT) or enterotoxin B from *Staphylococcus aureus* (SEB) are potent mucosal adjuvants and their detoxified derivatives are important for the development of mucosal vaccines. In animal models, oral administration of CT or SEB together with food proteins induces OX40L- and IL-4 -dependent Th2 priming in the small intestine. Activation of indigenous enteric eosinophils leads to degranulation of eosinophil peroxidase (EPO) that promotes the activation of CD103+ DCs and their mobilization to mesenteric lymph nodes where they present allergen epitopes to naïve T cells resulting in Th2 differentiation. Epithelial-derived IL-33 promotes up-regulation of Th2 priming co-stimulatory receptors on CD103+ DCs likely through recruitment or activation of IL-4 competent tissue resident innate immune cells such as eosinophils or mast cells. In response to systemic allergen sensitization, IL-33 has been demonstrated to activate mucosal mast cells resulting in secretion of prodigious

523 amounts of IL-9 and pro-inflammatory mediators upon oral allergen challenge, a scenario that is also
524 likely in oral food allergen sensitization protocols.

Mucosal adjuvants: - CT
- SEB

food allergens