

EDITORIAL

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Dietary allergenic proteins and intestinal immunity: a shift from oral tolerance to sensitization

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The gastrointestinal (GI) tract is exposed daily to a myriad of environmental antigens including dietary proteins, airborne materials and commensal microbes. The GI tract must discriminate between invasive organisms and harmless antigens, decide whether to initiate an active immune response and prevent dissemination of infections or alternatively induce a local and systemic immunological tolerance response. Furthermore, the GI tract must simultaneously identify dietary proteins and food components (proteins, lipids, carbohydrates and micronutrients such as minerals and vitamins), promote digestion, modification and absorption of nutrients. The GI immune system plays a critical role in the homeostatic regulation and maintenance of these functions. To do this, the intestinal immune system possesses special features that promote the induction of tolerance including specialized cells such as distinctive subsets of antigen presenting cells such as dendritic cells (DCs) and B and T cells and specialized lymphoid structures that promote suppressive immune responses [gut-associated lymphoid tissue, Peyer's patches and mesenteric lymph nodes (MLN)] [1–3]. Dysfunction of the intestinal immune system often leads to the development of intestinal inflammatory diseases including coeliac disease, inflammatory bowel disease and food allergy.

In most individuals ingestion of soluble dietary antigens leads to the development of systemic immunologic hyporesponsiveness termed oral tolerance (Fig. 1) [2]. Dietary antigens are generally sampled by M cells within the follicle-associated epithelium of the PP's in the small intestine. They are then transferred to local DC's that can either present the MHC-II restricted antigens directly to CD4⁺ T cells within the PP, or alternatively, the food antigen loaded DC's traffick to the MLN via the afferent lymphatics and present to naïve MLN CD4⁺ T cells. The

intestinal immune system contains a number of conventional subpopulations of DC's including CD8 α ⁺CD11b⁺ (myeloid) and CD8 α ⁺CD11b[–] (lymphoid) DCs and an unusual subset identified as CD8 α [–]CD11b⁺ DCs [4]. The CD8 α ⁺CD11b[–] DCs generate suppressive cytokines IL-10 and TGF- β . Presentation of dietary antigens to naïve T cells in the presence of this milieu leads to the development of regulatory T cells which generate IL-10 and IFN- γ and/or T-helper (Th)3 cells which produce TGF- β . The regulatory T cells either remain within the MLN or migrate to the PP or lamina propria (LP) and release these suppressive cytokines; notably these suppressive cytokines also enhance local IgA production. In food allergic individuals there is an abrogation of oral tolerance leading to a hypersensitivity response to dietary antigens [5]. DC presentation of dietary allergenic antigens leads to the development of effector CD4⁺ Th2-type T cells. Upon stimulation the CD4⁺ effector cells release a cocktail of Th2 cytokines (IL-4, IL-5, IL-9 and IL-13) leading to the generation of antigen-specific IgE, maturation and activation of mast cells and eosinophils and priming of the intestine for an allergic inflammatory responses (Fig. 1) [5].

The molecular basis for the augmentation of the intestinal CD4⁺ Th2-type response is currently unclear. A study by Cardoso et al. [6] in the present issue of *Clinical and Experimental Allergy* employed a murine model of dietary allergenic protein-induced intestinal inflammation to study the effect of dietary allergenic antigens on the modulation of gut immune responses. They demonstrate that induction of peanut-induced intestinal inflammation was associated with a cellular infiltrate mainly comprised of eosinophils, mast cells, phagocytes, natural killer (NK) and plasma cells. Furthermore, they demonstrate a reduction in $\gamma\delta$ ⁺ and CD4⁺CD25⁺ Forkhead/winged helix boxed (FoxP)3⁺ cells in the PP's. Analysis of the cytokine profile revealed increased expression of the Th2-associated transcriptional factor GATA-3 and cytokines (IL-4 and IL-13), also TNF- α and reduced IFN- γ . The increased GATA-3 and Th2 cytokines (IL-4 and IL-13) and reduction in IFN- γ in peanut-

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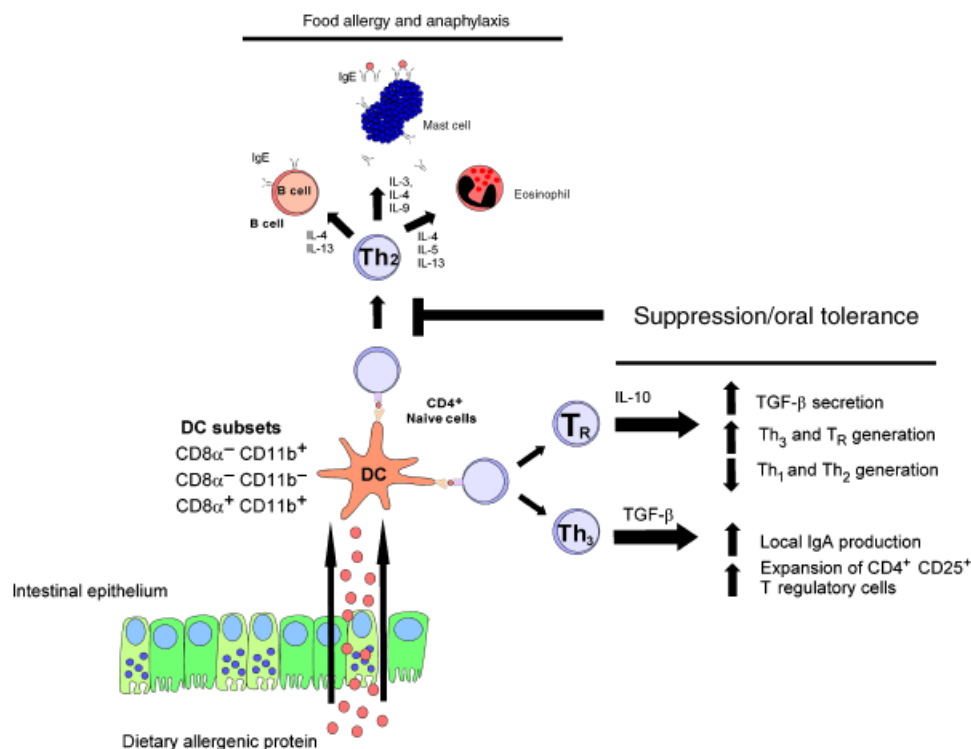


Fig. 1. Dietary allergenic proteins are taken up by dendritic cells (DCs) and are presented to naïve CD4⁺ T cells in the mesenteric lymph node or Peyer's patch. In non-allergic individuals these T cells differentiate into regulatory T (T_R) cells which produce IL-10 and/or T-helper (Th)₃ cells which produce TGF- β . These cytokines stimulate local IgA production, further expansion of CD4⁺CD25⁺ T regulatory cells and suppression of CD4⁺ effector (Th₁ and Th₂) cell development leading to oral tolerance. In contrast, in allergic individuals the naïve T cells differentiate into CD4⁺ Th₂ effector cells leading to elevated levels of Th₂ cytokines, IL-4, IL-5, IL-9 and IL-13. Elevated levels of these cytokines stimulate IgE production, mast cell and eosinophil recruitment and activation and onset of food allergy and anaphylaxis.

sensitized and challenged mice is consistent with the amplification of a CD4⁺ Th₂-type response leading to intestinal mastocytosis and eosinophilia and enhanced peanut-specific IgG1 and IgE. Previous investigations employing mouse models of food-induced intestinal inflammation have demonstrated a critical role for mast cells, Fc ϵ R1 and IgE in the pathogenesis of allergic GI response to oral allergens [7–9]. Interestingly the exacerbation of food-induced intestinal allergic inflammation was also associated with a reduction in intra-epithelial $\gamma\delta$ ⁺ T cells. Previous investigations have demonstrated that depletion of $\gamma\delta$ T cells *in vivo* abrogates oral tolerance and adoptive transfer of $\gamma\delta$ T cells isolated from fed mice can reconstitute oral tolerance suggesting a role for $\gamma\delta$ T cells in oral tolerance [10, 11]. The reduction of $\gamma\delta$ T cells in peanut-sensitized and challenged mice may explain the exacerbation of the intestinal allergic inflammation. However, the reduction in $\gamma\delta$ T cells was only observed in peanut-sensitized and challenged mice and not following peanut challenge alone. This suggests that cutaneous sensitization and amplification of Th₂ response by oral challenge promoted the reduction in $\gamma\delta$ ⁺ T cells.

Majority of the experimental models of intestinal allergic inflammation including the study by Cardoso and

colleagues require prior antigen sensitization in the presence of adjuvant to promote the induction of a Th₂ antibody and T cell response. To gain insight into the effect of dietary allergenic protein on intestinal immunity in naïve mice, Cardoso and colleagues also challenged non-sensitized (naïve) mice with oral peanut seeds. Importantly, the investigators demonstrated that consumption of peanuts in the absence of sensitization induced an altered intestinal immune response. Notably, the authors identified that oral consumption induced an increase in CD19⁺ cells and NK⁺ cells within the LP and a reduction in CD4⁺CD25⁺FoxP3⁺ cells in the PP's. Whether this reduction is due to transmigration of the FoxP3⁺ cells into LP of the intestine remains unclear, however, no increase in CD3⁺ LP cells was observed suggesting that dietary allergenic antigens may promote a reduction in FoxP3⁺ cells in the PP's. The reduction in FoxP3⁺ cells following consumption of dietary allergic proteins is interesting and may possibly provide an explanation for oral sensitization in humans. Patients with X-linked autoimmunity-allergic dysregulation syndrome which is caused by a FoxP3 mutation, often develop atopic dermatitis, elevated serum IgE and food allergy [12]. Furthermore, outgrowth of cow's milk allergy by children is

associated with the development of CD4⁺CD25⁺ T cells which possess regulatory function [13]. Experimental investigations have previously demonstrated an important role for CD4⁺CD25⁺ T cells in oral tolerance [14, 15].

Cardoso and colleagues show that the consumption of dietary allergenic proteins was associated with a significant increase in NK⁺ cells within the LP. The NK marker is predominantly expressed on NK cells and a subset of T cells known as NK T cells. NK T cells have been shown to rapidly produce high levels of IFN- γ and/or IL-4 following engagement of the T cell receptor and their involvement in CD4⁺ Th2-type allergic responses is unclear [16]. The rapid production of IL-4 is thought to lead to the initiation of CD4⁺ Th2 responses. Indeed an essential role for NK T cells in the production of IL-4 and IL-13 and in the development of allergic asthma has been demonstrated [17]. However, activation of NK T cells has also been shown to inhibit Th2 cell differentiation and IgE production [18]. Notably, NK T cells have also been shown to be critical for induction of oral tolerance. Employing an oral nickel tolerance model, investigators demonstrated a break in oral tolerance in *Jalpha18*^{-/-} mice which lack invariant NK T cells [19]. These mice fail to develop regulatory cells following nickel challenge and adoptive transfer of the NK T cells lead to the generation of regulatory T cells and induction of oral tolerance. However, oral tolerance can be induced in mice deficient in NK T cells suggesting that immunological tolerance does not require NK T cells, at least under some conditions [20]. It would be intriguing to examine non-sensitized mice 2–3 weeks following oral challenge with dietary allergenic proteins to determine whether the increase in NK cells is associated with the induction of a CD4⁺ Th2-type response.

The molecular basis of how dietary allergenic protein stimulates the induction of a CD4⁺ Th2-type immune response is a question of fundamental importance in the allergy field. Experimental investigations characterizing of dietary allergens has revealed that these foods consist of a complex mixture of proteins with varied molecular weight, structure and function. The most widespread group of plant proteins that contains allergens are cupin and prolamin superfamilies [21]. The cupin superfamily consists of proteins include allergenic seed storage proteins of the vicilin and legumin type present in soybeans, peanuts and tree nuts. These proteins consist of seed storage proteins, lipid transfer proteins and α -amylase and protease inhibitors. The peanuts consist of a number of allergens including Ara h1–3/4. The peanut allergen Ara h1 is a member of the vicillin protein family and is resistant to proteolytic digestion and denaturation. Ara h2, 6 and 7 are members of the 2S albumin family; Ara h2 is a weak trypsin inhibitor and has been shown to partially inhibit trypsin-mediated digestion of Ara h1. Ara h3/4 is a 11S legumin-like seed storage protein. The antiproteolytic

and protease inhibitory properties of these proteins are thought to contribute to oral sensitization and predisposition to intestinal allergic inflammation. However, as atopy is only restricted to ~30% of the population, simply ingestion of these allergens alone cannot possibly explain oral sensitization. A major component contributing to atopy is genetic susceptibility. Indeed, even in the case of respiratory allergy (e.g. asthma), over 100 contributory genes have been implicated to interact with environmental exposures [22]. Combination of these genetic susceptibility factors and environmental factors 'consumption of dietary protein allergens at the vulnerable stage of development' may create a favourable environment for the induction of oral sensitization and predisposition to intestinal allergic inflammation. Notably, there is evidence accumulating that atopy and intestinal allergic inflammation is linked with increased intestinal permeability. Food allergy patients also have increased intestinal permeability, which correlates with the severity of their clinical symptoms [23]. Furthermore, recent clinical studies have demonstrated an increase in food protein sensitivity in paediatric liver transplant patients treated with the immunosuppressive tacrolimus [24]. Notably, tacrolimus, a calcineurin inhibitor, has been shown to increase intestinal permeability by dysregulating intercellular junctional integrins [25]. The presence of genetic susceptibility factors involved in the regulation of intestinal permeability has not yet been identified.

The results of the study by Cardoso and colleagues in this issue of *Clinical and Experimental Allergy* demonstrate that dietary allergenic protein consumption induces an altered intestinal immune response resulting in a reduction in intestinal immune suppressor/tolerance pathways. It is possible but yet unproven that reduction in these tolerance pathways may create a favourable environment for the development of CD4⁺ Th2 response and oral antigen sensitization.

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