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Review

The role of commensal bacteria in the regulation of sensitization to food allergens

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ABSTRACT

The prevalence of life-threatening anaphylactic responses to food is rising at an alarming rate. The emerging role of the gut microbiota in regulating food allergen sensitization may help explain this trend. The mechanisms by which commensal bacteria influence sensitization to dietary antigens are only beginning to be explored. We have found that a population of mucosa-associated commensal anaerobes prevents food allergen sensitization by promoting an IL-22-dependent barrier protective immune response that limits the access of food allergens to the systemic circulation. This early response is followed by an adaptive immune response mediated in part by an expansion of Foxp3$^+$ Tregs that fortifies the tolerogenic milieu needed to maintain non-responsiveness to food. Bacterial metabolites, such as short-chain fatty acids, may contribute to the process through their ability to promote Foxp3$^+$ Treg differentiation. This work suggests that environmentally induced alterations of the gut microbiota offset the regulatory signals conferred by protective bacterial species to promote aberrant responses to food. Our research presents exciting new possibilities for preventing and treating food allergies based on interventions that modulate the composition of the gut microbiota.

1. Introduction

Differentiating innocuous environmental antigens from serious threats is a particular challenge in the intestinal mucosa, which is constantly exposed to antigens derived from both food and the commensal microbiota. Oral tolerance typically refers to the process by which the immune system limits the response to dietary antigens. Experimentally, administration of antigen by the oral route induces mucosal and systemic non-responsiveness to subsequent peripheral challenge [1]. Rodent models have provided insight into the physiological processes required to maintain tolerance to food and suggest that sensitization to dietary allergens increases when these processes fail.

Since nutrients are absorbed in the small intestine studies on the mechanisms regulating oral tolerance have focused primarily on antigen-specific immune responses in the gut associated lymphoid tissues (GALT) that drain this site. Emerging evidence, however, points to a central role for commensal bacteria in preventing food allergen sensitization. Dietary antigens share the intestinal lumen with trillions of astoundingly diverse bacteria comprising approximately one thousand different species [2]. These bacteria colonize the gut at increasing densities from stomach to rectum. In the small intestine, bacterial load is kept low due to the high motility of intestinal contents and the bactericidal activity of bile salts, with densities of 10^4–10^5 bacteria per milliliter of effluent in the proximal small intestine and 10^8 bacteria per milliliter of effluent in the ileum [3]. Bacterial load is by far greatest in the colon, where densities can reach 10^{11} organisms per gram of luminal content [3]. The high microbial content of the ileum and colon exerts a formidable influence on the framework of the mucosal immune system in the gut, not only to promote local homeostatic interactions, but also to modulate immune responses to antigens at peripheral sites beyond the mucosa. We have found that particular populations of bacteria are required to prevent sensitization to dietary antigens; when protective bacteria-derived signals are lacking, tolerance to dietary antigen is not induced. Our data support the idea that an environmentally induced alteration of the commensal microbiota is driving the rapidly increasing prevalence of allergic responses to food in Western societies [4].

In this review, we will first discuss what is currently known about the regulation of tolerance to dietary antigen before discussing how commensal bacteria influence this process.

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2. Mechanisms of orally induced non-responsiveness

The mechanisms governing non-responsiveness to dietary antigens remain poorly understood; the ways in which dietary antigens cross the intestinal epithelial barrier, are presented to the immune system, and elicit a response are still being defined. It is clear, however, that the maintenance of tolerance involves both cellular and humoral processes that are induced in the intestinal lamina propria (LP) and the mesenteric lymph node (MLN). In order to be recognized by the immune system dietary antigen must first cross the epithelium of the small intestine. Antigen can be transcytosed by specialized epithelial cells called microfold (M) cells, which reside in the follicle-associated epithelium of Peyer's patches (PP) [5]. M cells lack the brush border glycocalyx present on other enterocytes and instead contain large vesicles in their cytoplasm that enable transepithelial transport of luminal antigens [6,7]. The basolateral surface of M cells is in close contact with the underlying GALT where antigen presenting cells (APCs) can take up and process these luminal antigens for presentation to naïve T cells [8].

M cells were initially thought to be the major route by which dietary antigens cross the epithelial barrier. More recent evidence, however, suggests that other types of epithelial cells may assist in this process. Goblet cells, best known for their ability to produce the thick mucus glycocalyx overlaying the epithelium, may play a role in antigen transport. Fluorescent imaging techniques indicate that goblet cell-associated passages (GAPs) allow labeled, orally delivered antigen to cross from the lumen into the LP [9]. This process is constitutively active in the small intestine, suggesting that GAPs may be an important, underappreciated route for antigen uptake. In addition to active transport through enterocytes, antigen can pass between epithelial cells. Intestinal epithelial cells (IECs) are joined by tight junctions, adherens junctions, and desmosomes that together form a "molecular gasket" to seal the paracellular space [10]. Under homeostatic conditions, solutes, proteins, and certain microbial components can infiltrate this complex and pass between cells. Paracellular transport is regulated closely by the cytokine milieu, which can dramatically alter the expression of tight junction proteins under conditions of inflammation [10]. Some evidence also suggests that extensions from a subset of dendritic cells (DC) in the small intestine reach through epithelial tight junctions to sample luminal antigens [11–13].

Once antigen has passed from the intestinal lumen into the GALT it is taken up by APCs for presentation to naïve T cells. Several functionally distinct subsets of APCs in the GALT can be defined by their surface marker expression. Two major populations have been implicated in oral tolerance: a DC subset identified by the expression of CD11c and CD103 and a macrophage subset defined by the expression of CD11b and CX_3CR1 [1]. $CD11c^+$ $CD103^+$ DCs express high levels of MHC class II and the homing receptor CCR7 that enables migration to the MLN [14,15]. $CD11c^+$ $CD103^+$ $CCR7^+$ DCs are the major cell population responsible for picking up and processing antigens crossing the epithelial barrier [16,17]. Once antigen has been processed, these DCs traffic through the lymph to the MLN where they encounter and stimulate naïve $CD4^+$ T cells. The fate of an antigen-specific $CD4^+$ T cell following stimulation depends upon the cytokine milieu in which the encounter occurs. Under homeostatic conditions, high concentrations of TGF-β and retinoic acid (RA) in the MLN promote a tolerogenic environment that favors the differentiation of regulatory T cells (Tregs) [18,19]. The TGF-β required for this process is produced both by $CD103^+$ DCs themselves as well as by IECs [20,21]. Interestingly, IEC-derived TGF-β has been shown to be important for promoting TGF-β production by $CD103^+$ DCs and enhancing their ability to drive Treg differentiation [22]. RA is derived from dietary vitamin A and is metabolized primarily by $CD11c^+CD103^+$ DCs, which express high levels of retinaldehyde dehydrogenase (RALDH) and aldehyde dehydrogenase (ALDH) enzymes [18,19]. RA enhances the TGF-β dependent upregulation of Foxp3, the transcription factor that controls Treg differentiation [18]. In the absence of dietary vitamin A, the frequency of Tregs in the MLN and intestinal LP is significantly reduced [23,24].

Of the two major APC subsets in the GALT, the role of CX_3CR1^+ macrophages has been controversial. Using an in vitro transwell system, Rescigno et al first visualized the ability of a subset of DCs to extend their dendrites between epithelial tight junctions [12]. This finding was corroborated using confocal microscopy, which found the dendrites to extend primarily in the villi of the terminal ileum. The DC subset involved was later characterized as $CD11c^+$ $CD11b^+$ CX_3CR1^+ DCs that derived from myeloid precursors [11]. However, some question remained as to the role of these cells in oral tolerance, as they were shown to be poor APCs for the stimulation of T cell proliferation in vitro [25]. Moreover, CX_3CR1^+ DCs do not migrate under homeostatic conditions and have been observed in the MLN only after infection with an intestinal pathogen or following antibiotic treatment [26]. Given these characteristics, CX_3CR1^+ cells are thought to be more representative of a macrophage rather than a DC subset. Yet, their importance in establishing oral tolerance is clear; in the absence of CX_3CR1 expression, the uptake of fed antigen and the expansion of cognate T cells is reduced, resulting in increased delayed-type hypersensitivity (DTH) reactions in response to antigen challenge [27]. Recent work suggests that CX_3CR1^+ macrophages are the first to acquire luminal antigen, which they then pass via cell-to-cell contact and gap junctions to $CD103^+$ DCs that migrate and interact with naïve T cells [27]. Other work suggests that the major function of these cells is to produce IL-10, which supports the proliferation and expansion of antigen-specific $Foxp3^+$ Tregs in the LP [28].

After dietary antigen-specific T cells recognize their cognate antigen and differentiate into $Foxp3^+$ Tregs, they upregulate the homing molecules CCR9 and $\alpha_4\beta_7$ that direct migration back to the small intestinal LP [28,29]. Once there, $Foxp3^+$ Tregs expand and suppress aberrant responses to dietary antigens through the production of IL-10, TGF-β, and IL-35 [30]. In the absence of induced Tregs, the cytokine milieu of the MLN is highly Th2-skewed with increases in $CD4^+$ T cells producing IL-4, IL-13 and IL-5 [31]. Food allergen sensitization occurs when naïve $CD4^+$ T cells differentiate into Th2 cells in the presence of IL-4 [32]. Th2 cells then help to promote an allergic response by inducing B cell class-switching to the IgE isotype. IgE subsequently binds to its high affinity Fc receptor, FcεRI, which is expressed predominantly on mast cells. Re-exposure to dietary antigen crosslinks bound IgE, inducing mast cell degranulation and the release of mediators that precipitate an allergic, and potentially anaphylactic, reaction [33].

The cytokine environment may not be the only factor regulating tolerance to food. Recent work suggests that mucus is more than just a physical barrier between IECs and the intestinal lumen and actively promotes tolerance by repressing the expression of inflammatory cytokines by DCs [34]. In the presence of the mucin protein MUC2, DCs produce more IL-10 and express higher levels of RALDH and ALDH enzymes. Mice deficient in mucus production ($Muc2^{-/-}$ mice) have a decreased proportion of $Foxp3^+$ Tregs in the LP and exhibit increased DTH responses after antigen feeding. This effect of MUC2 is mediated by its binding to a galectin-3-dectin-1-FcγRIIB complex on DCs, activating β-catenin signaling to induce a tolerogenic phenotype that contributes to mucosal homeostasis [34].

Antibody secreting B cells also contribute to the induction of oral tolerance. IgA is the most abundantly produced immunoglobulin isotype and can be found in two forms: a monomeric form in

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