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Review

Murine models for mucosal tolerance in allergy

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ABSTRACT

Immunity is established by a fine balance to discriminate between self and non-self. In addition, mucosal surfaces have the unique ability to establish and maintain a state of tolerance also against non-self constituents such as those represented by the large numbers of commensals populating mucosal surfaces and food-derived or airborne antigens. Recent years have seen a dramatic expansion in our understanding of the basic mechanisms and the involved cellular and molecular players orchestrating mucosal tolerance. As a direct outgrowth, promising prophylactic and therapeutic models for mucosal tolerance induction against usually innocuous antigens (derived from food and aeroallergen sources) have been developed. A major theme in the past years was the introduction of improved formulations and novel adjuvants into such allergy vaccines. This review article describes basic mechanisms of mucosal tolerance induction and contrasts the peculiarities but also the interdependence of the gut and respiratory tract associated lymphoid tissues in that context. Particular emphasis is put on delineating the current prophylactic and therapeutic strategies to study and improve mucosal tolerance induction in allergy.

1. Introduction

Induction of immunological tolerance is one of the key mechanisms to ensure that immunity is directed exclusively against pathogens but not against innocuous ingested antigens in food or inhaled antigens thereby guaranteeing immunological homeostasis. In this review, we focus on established and novel approaches to induce tolerance with a special focus on the use of mucosal tolerance induction as prophylactic and therapeutic interventions in allergic diseases.

2. General principles of mucosal tolerance

The mucosal surfaces lining the airways, gut, and urogenital tract are constantly exposed to a multitude of environmental antigens and microbes [1]. Immune-surveillance at mucosal surfaces includes the action of immunological mechanisms that overlap with those that help maintain tolerance to self [2,3]. In healthy individuals, inhalation and/or ingestion of innocuous environmental antigens but also beneficial commensal-host interactions in the gut, the respiratory tract and other mucosal surfaces result in immunological tolerance and maintenance of mucosal homeostasis [4]. This is an active process during which induction of regulatory T cells in the periphery (pTreg), but also other suppressive cells, cytokines and regulatory antigen presenting cells (APCs) are the key factors, which are required to avoid pathological responses in the airways and the gut [5–7]. An imbalance in tolerance control mechanisms may result in the development of different forms of allergies, culminating in the generation of asthma or severe anaphylactic reactions, but also other inflammatory diseases with a disease-associated bias in T helper cell responses [2,8]. However, especially the immune pathways that trigger the initial induction of tolerance but also those which lead to a possible later break in tolerance are still largely unknown [9–11].

3. Basic mechanisms of the induction of mucosal tolerance

The phenomenon of tolerance to mucosally delivered allergens has first been described by *Dakin* who reported about tolerance induction as a strategy to avoid allergic reactions. He observed that Native Americans prevented contact hypersensitivity reactions to urushiole, found *e.g.* in plants of the genus *Toxicodendron*, by eating poisonous ivy leaves [12,13]. In 1911, *Wells* demonstrated that continuous feeding of inert protein antigens could lead to a state of antigen-specific

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unresponsiveness [14,15]. Later on, *Bienenstock* and co-workers introduced the concept of a common mucosal immune system in which immunization within one mucosal compartment could confer protection at distant mucosal sites through the movement of cells and humoral factors (antibodies) [16,17]. Mucosal tolerance induction has since been exploited and shown to be dependent on factors such as the antigen itself, the dose of the antigen (high versus low), the route of application, the sensitization schemes and the host factors (*e.g.* genetic, epigenetic, demographic and immunological factors, as well as the local microbiome) [18,19]. These interventions, whether *via* the nasal, sublingual, or oral route aim at the induction of specific pTreg cells or blocking antibodies that migrate/diffuse and control established systemic and local allergic responses also at distant mucosal sites such as the lung [20]. Models of oral and nasal allergen application using T cell receptor (TCR) transgenic or RAG-deficient mice have greatly helped to demonstrate that mucosal tolerance is impaired when the conversion of peripheral T cells into pTregs was impaired [21–23]. In that context, exact dosing and immunization schedules for mucosally administered antigens were shown to play a pivotal role. In mice, two forms of tolerance, *i.e.* high-dose and low-dose, can be discriminated. Administration of a single high dose of antigen (> 20 mg) results in lymphocyte deletion *via* CD95 (FAS)-dependent caspase activation and apoptosis or lymphocyte anergy as a result of TCR ligation in the absence of adequate co-stimulation or by CLTA-4 mediated feed-back regulation [19,24–28]. In contrast, low dose tolerance is induced by repeated exposure to lower doses of antigen (100 ng–1 mg), and is dependent on pTregs and active suppression [19,29–31]. Importantly, these two forms of tolerance induction (anergy and active suppression) are not mutually exclusive but may occur simultaneously. Tolerance induction depends also on the formulation of the antigen. While soluble antigens favor systemic tolerance induction, particulate antigens are generally more immunogenic and may prime immune responses [27,32,33].

The variation of application regimen, but also modifications in the formulation of mucosally, especially of orally, applied antigens provide possibilities to favor desired immunomodulation/-deviation. In this respect antigens prone to enzymatic degradation in the upper gastrointestinal tract can be protected by differential coating and/or containment strategies (*e.g.* enteric coating) and might thus have a better chance to reach the more distal mucosal surfaces in comparison to unmodified forms [34]. An important distinction based on the induced effector mechanisms should be made between long-lasting tolerance induction and short-lived down-regulation of effector cell function [35]. The goal of clinically induced tolerance is to reach a state of unresponsiveness that involves an immunologic deviation away from pro-allergic Th2 immunity that persists regardless of further allergen exposure [35,36].

4. How the microbiome shapes mucosal tolerance

It is generally believed that mucosal tolerance to environmental allergens requires microbial colonization early in life [37]. More than 10^{14} microorganisms of more than 500 different species make the intestine the major source of commensal microbes. The local microbiota of the airways and the gut have profound and long-term effects on the hosts' mucosal immunity [38–40]. The number of bacteria largely vary from the proximal to the distal end of the gastrointestinal tract, ranging from 10^2 to 10^3 per milliliter in the stomach to up to 10^{12} bacteria per gram (dry weight) of colonic contents [41,42]. Commensals also have the capacity to actively suppress inflammatory pathways in epithelial cells by *e.g.* blocking NF-κB activity [43,44], resulting in the release of transforming growth factor beta (TGF-β), retinoic acid (RA) and thymic stromal lymphopoietin (TSLP) from the epithelium that instruct the differentiation of tolerogenic dendritic cells (DCs) [45,46] (Fig. 1). Additionally, retinoid-related orphan receptor γt (RORγt) positive innate lymphoid cells (ILCs) can selectively suppress pathological $CD4^+$ T cell responses to commensal bacteria through MHCII engagement, limiting pathological immune cell responses to commensals and ensuring intestinal homeostasis [47]. It is hence conceivable that an intact response to the commensal bacteria and its components is essential for mucosal tolerance and that commensal dysbiosis is associated with biased type 2 immunity and susceptibility to allergic diseases [37,38,48,49]. The importance of the microbiome for intact immune tolerance has been shown in germ-free (GF) as well as antibiotic-treated mice. Mice lacking a normal microbiome show increased allergic responses in models of both allergic airway disease and food allergy [38,50–53]. Likewise, establishment of oral tolerance depends on the presence of the gut microbiota [54,55]. There is, however, also data showing that tolerance, particularly nasal tolerance, can be established in the absence of commensal bacteria [56,57].

5. Mechanisms underlying mucosal tolerance induction

5.1. Oral tolerance: function of the gut associated-lymphoid tissue (GALT)

IL-10 is the master regulator of intestinal mucosal homeostasis [58], which has been shown by the development of spontaneous enterocolitis upon its genetic deletion in mice [59]. However, no such pathology becomes evident in IL-10 knockout (KO) mice under GF conditions [60], indicating that under physiological conditions, the microbiome shapes mucosal immunity against potential pathogens in the intestines in an IL-10 dependent manner. At mucosal surfaces, critical factors influencing immunity and tolerance are determined by i) antigen size and ii) particularity, which dictate the mode of antigen uptake. The inductive sites, the so called Peyer's patches (PP), are constituted by lymphoid follicles that are covered by epithelium densely populated with microfold cells (M cells) (Fig. 1). Microfold cells represent $Siglec\text{-}F^+$ enterocytes [61], which are able to engulf particulate material and shuttle it to the lamina propria (LP), the mucosal effector site in the gut [62]. In contrast, soluble molecules are taken up by different modalities depending on the molecule size. Small molecules are able to diffuse freely from the gut lumen *via* tight junctions and reach the LP through a process called paracellular permeability [63]. Larger molecules reach the LP by active transcellular transport. In addition, exosomal-based pathways operated by MHC class II^+ enterocytes and transporting cargo within vesicles to the basolateral site of enterocytes seem to be operative as well [64,65]. Additionally, DCs as well as $CX3CR1^+$ macrophages sample soluble antigens by virtue of their cellular processes, which extend from the LP through the epithelium into the gut lumen [18,66].

Using *ex vivo* confocal imaging of intestinal lymphatics and sampling of intestinal draining lymph it has been shown that (soluble and particulate) antigenic material becomes transported within $CD103^+$ LP-derived DCs from the LP *via* afferent lymphatic vessels to the mesenteric lymph nodes (mLN) [67]. Before they migrate towards the mLN, DCs are located in special regions of the GALT where they function as sentinels and are forming an interconnected mesh within the LP of the intestine. Once arrived in the mLN, $CD103^+$ DCs are believed to play a critical role in the initiation of oral tolerance. Under steady-state conditions, more than three quarters of the $CD11c^+MHCII^+$ DCs found in the intestinal lymph are constituted by $CD103^+$ cDCs [68]. This is consistent with the observation that antigen-specific proliferation of $CD4^+$ and $CD8^+$ T cells can be induced by $CD103^+$ DCs, which had been isolated from mLN shortly after oral challenge [69,70]. Migration of $CD103^+$ LP DCs is a highly ordered, CCR7-dependent process. In fact, CCR7 deficiency is incompatible with the induction of oral tolerance [71,72], establishing the importance of constitutive antigen transport from the LP to the mLN to maintain mucosal integrity. The continuous flow of DCs towards the mLN might be a major mechanism how tolerance to food-derived antigens and the commensal flora [73] is initiated and maintained [74]. Epithelial cells heavily imprint the gut-resident $CD103^+$ DC by their production of instructive factors such as TSLP, TGF-β1 and RA [75,76]. Accordingly, the imprinted phenotype of