



The biodiversity hypothesis and immunotolerance in allergy

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Abstract

Background The human immune system is capable of detecting a multitude of substances and organisms in the environment and responding to these in a variety of ways. This includes immune responses to pathogens, on the one hand, and immunotolerance in the form of immunoregulatory responses, resulting in either the termination of immune responses or in tolerance to harmless and endogenous substances, on the other. The development of immunotolerance is an active process that is essentially characterized by interaction with microbiota and environmental components and is primarily mediated by regulatory T cells.

Methods This article provides an overview of selected scientific articles and is addressed also to non-specialists. It is based on a literature search in PubMed, specialist databases, and guidelines.

Results According to the diversity hypothesis, exposure in early childhood to broad biodiversity is now considered to reduce the risk of developing allergic diseases.

Conclusion Therefore, tolerance induction emerges in allergology not only as a potential concept for prevention, but also as a treatment approach in atopic diseases.

Keywords Immunotolerance · Treg · Biodiversity · Microbiome · Cutaneous sensitization

Abbreviations

DC	Dendritic cells
IgE	Immunoglobulin E
IL	Interleukin
iTreg	“Induced” regulatory T cells
MHC	Major histocompatibility complex
nTreg	“Natural” regulatory T cells
SCORAD	Score of Atopic Dermatitis
Th cells	T helper cell subtypes
Treg	Regulatory T cells

The immune system

The human immune system has undergone constant evolution in response to environmental pressure. This is particularly true of boundary organs such as the skin and gut, where the immune system is represented by sentinel cells, predominantly dendritic cells (DC). DC belong to the innate or natural immune system and, as immature cells, achieve permanent control over the boundary areas by forming a vast network with their cytoplasmic processes that reach to the surface, absorbing pathogens invading from the environment and picking up danger signals. The detection of danger signals takes place via pattern recognition receptors and triggers activation programs in the DC in the course of which the cells mature: they alter their expression pattern, migrate to the respective draining lymph nodes, process the absorbed foreign structures, and present antigens derived from these structures to major histocompatibility complexes (MHC) [1, 2]. Once in the lymph nodes, the now matured DC translate the information detected in the boundary areas into biological signals for the acquired or adaptive immune system. Translation takes place via costimu-

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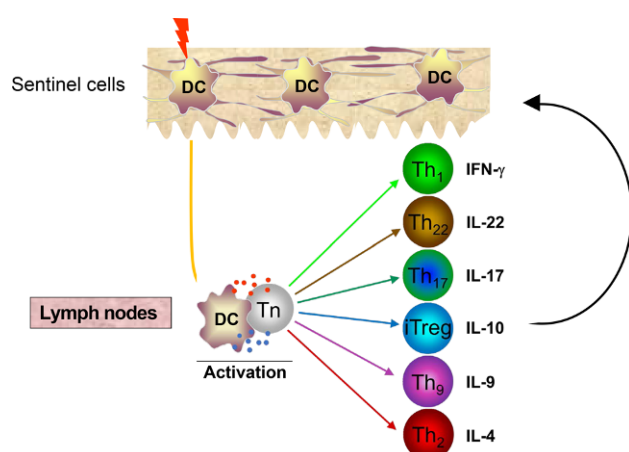


Fig. 1 The immune response to environmental signals. Dendritic cells detect signals from the environment and translate these into biological information following migration into the draining lymph nodes. This results in the activation of naive T cells, which, using characteristic cytokines, are able to polarize into different subtypes depending on the quality of the signals. DC dendritic cell, IFN interferon, IL interleukin, Th T-helper cell, Tn naive T cells, iTreg “induced” Tregs

latory molecules on the surface of the DC, messengers secreted in the micromilieu of the lymph node, as well as via antigens presented in the MHC context, and is aimed primarily at T-lymphocytes with an appropriate T-cell receptor [3]. As part of this process, the sum of all signals determines not only whether a suitable T cell bound by the T-cell receptor is activated—it also defines the polarized subtype into which the T cell develops and which “homing” receptors it will express in order to migrate back to the surface organ initiating this process. Thus, DC have an important adapter function between innate and adaptive immunity: they are capable of activating naive T cells (Tn) and determining the quality of the immune response induced depending on the information received from the environment [4]. The differently polarized effector T-cell subtypes formed in this way are characterized by the production of typical key cytokines and are involved in the defense against a variety of pathogens (Fig. 1). For example, viruses, intracellular bacteria, and parasites induce Th1 cells characterized by dominant interferon- γ production, while extracellular bacteria and fungi induce Th17 cells that produce interleukin-17A, among others, and helminths induce Th2 cells with characteristic interleukin 4 (IL-4) production. Other hitherto less well characterized Th subtypes include Th9 and Th22 cells. While Th9 cells, with their key cytokine IL-9, play a role in the defense against parasites [5, 6], Th22 cells are involved in immune responses to bacterial infections as well as in tissue remodeling with their IL-22 production [7]. However, the defense functions of these T-cell subgroups also at odds with their pathological effector functions [8]: Th1 and Th17 cells are able to mediate autoimmune diseases such as psoriasis and multiple sclerosis, while

Th2 cells form the basis for atopic diseases such as asthma, neurodermatitis, allergic rhinitis, and food allergies [9, 10]. Both cases involve misdirected immune responses—responses mounted against endogenous components in the first case and against harmless, exogenous components in the second. This points to a central challenge facing the immune system: first, to generate effective yet terminated immune responses to pathogens and, second, to recognize and tolerate endogenous components and harmless substances as such [11]. In order to achieve these tasks, the immune system has mechanisms not only to induce immunity, but also to induce tolerance.

Tolerance development

The induction of immunological tolerance consists of complex processes involving a variety of cells. Thus, potentially autoreactive T cells in the thymus are eliminated during negative selection as part of central tolerance. However, there is also a specific T-cell subtype, the regulatory T cells (Treg), which, by involvement of the T-cell receptor, actively suppress the activation and function of other immune cells. Tregs are formed either during T-cell development as “natural” Tregs in the thymus (nTreg) or differentiate in the periphery from mature naive T cells to “induced” Tregs (iTreg) in the course of an immune response [12–15]. Tregs that are directed against allergens are referred to as allergen-specific iTregs. The repertoire of structures identified by T-cell receptors includes both auto- and foreign antigens, thereby contributing to self-tolerance and to the restriction or termination of immune responses. Maintaining a balanced ratio between inflammatory and regulatory signals guarantees that healthy individuals not only have self-tolerance, but also tolerate harmless components such as skin and gut bacteria, contact substances, and allergens [16]. This ratio is unbalanced in allergy sufferers. Comparative studies on T cells in allergic individuals and healthy test subjects were already undertaken over 10 years ago. Both allergy sufferers and healthy subjects were found to have disparately differentiated T-cell subtypes with the same antigen specificity and comparable quality [17]. However, there was a shift in the balance between subpopulations: while the Treg subtype with the anti-inflammatory messenger interleukin 10 (IL-10) specific for frequent allergens was primarily found in healthy individuals, IL-4-producing Th2 cells predominated for the same allergens in allergy sufferers.

This shows that the healthy immune system also actively recognizes allergens but, in contrast to the allergy sufferer, it translates these differently to the adaptive immune system and induces tolerance according to the harmless nature of allergens. It has been shown that, as part of this, IL-10 is a marker messenger for regulatory T cells and tolerance-type immune responses. It is now assumed that an

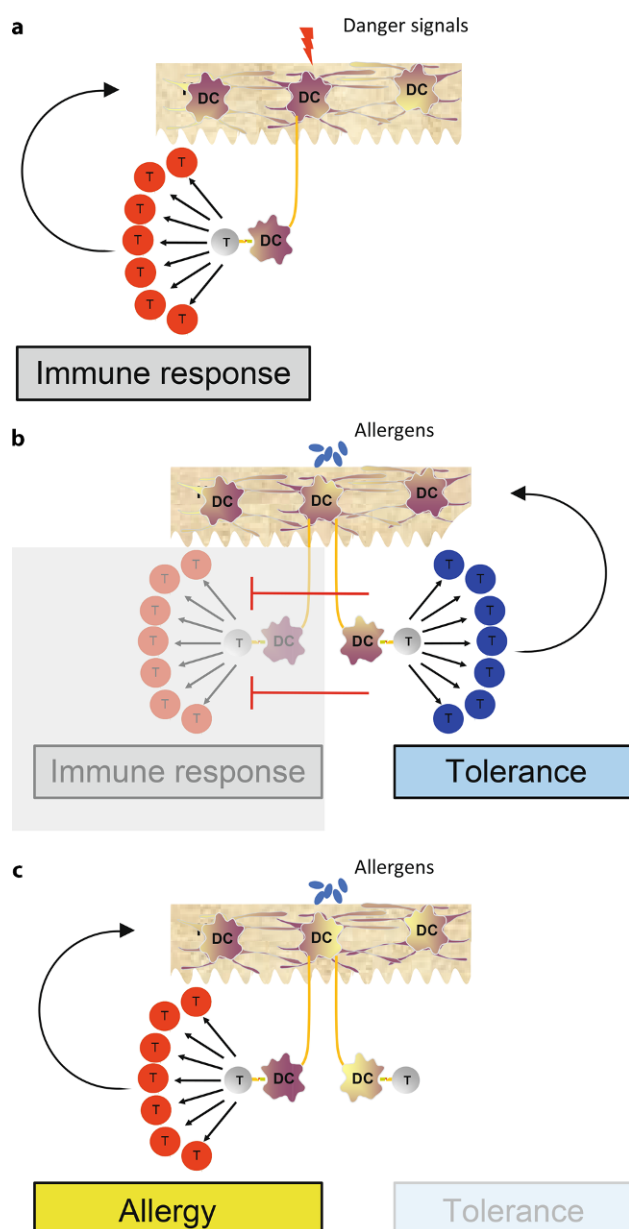


Fig. 2 A comparison of the immune response in healthy individuals and allergy sufferers. While the detection of danger signals triggers an immune response with effector T cells (a), harmless allergens induce regulatory T cells in healthy individuals; these T cells actively suppress effector cells of the same specificity, thereby maintaining immunological tolerance to harmless substances (b). In allergy sufferers, the tolerance system fails and Th2-polarized effector T cells over-respond to harmless allergens (c)

immunotolerance system that has expanded early enough and which acts in a pluripotent manner can effectively prevent the onset of allergic diseases, autoimmune diseases, and other chronic inflammatory disorders. Furthermore, this tolerance development is necessary to ensure self-tolerance to skin and intestinal pathogens as well as to contact substances ([18]; Fig. 2).

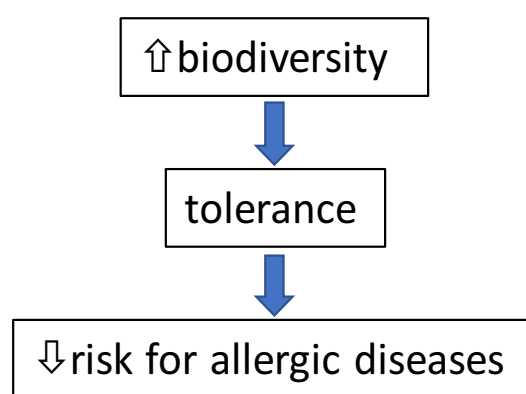


Fig. 3 Biodiversity and allergic diseases. Exposure to broad diversity in early childhood, e.g., while growing up on a farm, results in the development of a well-functioning tolerance system, thereby reducing the risk for developing allergic diseases. (From stefano—stock.adobe.com)

Efficient immune regulation as a means to prevent allergies

Numerous studies conducted over the last 25 years have shown that growing up on a farm (Fig. 3) and the resultant exposure to the dust and microbes found there can reduce the risk of asthma and prevent atopic sensitization [19]. David P. Strachan first formulated the so-called hygiene hypothesis in 1989: Strachan observed a correlation between the onset of allergic rhinitis and the number of older siblings. He suspected that infections caused by sibling contact in early childhood provided protection against allergic diseases and, therefore, that the declining average number of siblings and, at the same time, ever improving hygiene conditions were able to explain the rising prevalence of allergic/atopic diseases [20]. Other studies on the effect of the number of siblings [21–23], as well as studies comparing East and West Germany in the 1990s [24, 25], yielded similar results, thereby supporting the hypothesis that the main cause of the rise in allergic and atopic diseases was the reduced exposure to pathogens. It was originally assumed that improved hygiene conditions would result in fewer infections and thus fewer Th1 and, to

compensate for this, more Th2-dominated immune responses characteristic of allergic diseases. As the evidence improved, it became clear that early exposure to a wide range of microbial substances rather than contact with pathogenic microorganisms reduces the risk of developing allergic diseases [26, 27]. This led to the hygiene hypothesis being increasingly replaced by the biodiversity hypothesis. Tari Haahtela and Harri Alenius and their research group [28] were able to show that the composition of the skin microbiota is affected by the living environment: the microbial diversity of the environment and, in particular, the presence of gammaproteobacteria correlated with protection against allergic sensitization and increased IL-10 production. A positive correlation to IL-10 production was seen in particular for the abundance of the gammaproteobacteria genus *Acinetobacter* in healthy individuals, not, however, in allergy sufferers. These data show that exposure to a wide range of microbes reduces the risk of allergic disease. The extrapolation of this concept from aeroallergens to food allergens led to studies that considered early exposure and the resulting immunological exposure to food as the basis for allergy prevention.

Nutritional diversity in early childhood as a means of allergy prevention

Many allergy sufferers experience the “atopic march”: young children manifesting allergic disease, usually neurodermatitis, in infancy go on to acquire other related diseases, e.g., hay fever, allergic asthma, or food allergies, in the course of their lives [29, 30]. For example, studies have shown that the exposure of children with severe neurodermatitis to peanut proteins in house dust correlates significantly with increased sensitization (IgE) to these proteins [31, 32]. The resulting hypothesis of a food allergy acquired via the skin was later expanded to include wheat components in wheat-dependent exercise-induced anaphylaxis and red meat in alpha-Gal syndrome [33]. Cutaneously mediated sensitization occurs in the former via exposure to hydrolyzed wheat proteins in cosmetic products [34], and via tick bites on the skin in the latter [35]. These findings reinforced the concept that cutaneous exposure to noncutaneous proteins, such as food proteins, induces sensitization and the production of specific IgE antibodies and subsequently promotes type I allergies to these food proteins. The default pathway of the gastrointestinal immune system, on the other hand, is to translate the food proteins found in the gut and their signals into immunological tolerance [36]. Therefore, in order to counteract sensitization via the skin, the gastrointestinal immune system needs to be exposed early on and adequately to these foods, before an allergy can be established via the skin. This theory was investigated in a large-scale study: infants aged 4–11 months with severe dermatitis were randomly assigned to two groups—one

that would consume peanuts (exposure group) and one that would avoid peanuts in line with conventional recommendations (avoidance group). Infants that were not sensitized to peanut (negative skin prick test) or that exhibited a mild skin prick test reaction (1–4 mm) and a negative provocation test were included. All infants underwent oral provocation testing at baseline and at the age of 5 years. Of the infants that originally tested negative in skin prick tests, 13.9% of the avoidance group developed a food allergy to peanut protein compared with only 0.4% of the exposure group. Of the infants that initially tested slightly positive to skin prick testing and negative to provocation testing, 34% of the avoidance group and 0% in the exposure group exhibited a food allergy [37]. According to this, early oral exposure to peanut protein in children at high risk of allergy resulted in immunological tolerance induction, and significantly fewer children developed peanut allergies than did unexposed children in the comparison group. The follow-up study investigated whether this oral immunological tolerance persists long-term. Children in both groups (avoidance and exposure) were instructed to avoid peanut protein consumption for 1 year, after which they underwent repeat provocation testing. Among the originally skin prick test-negative children, peanut allergy occurred in 15.2% of the avoidance group and 1.9% of the exposure group. In the children that were skin prick test-positive at baseline, the number of allergy sufferers rose in the avoidance group to 37.8% and to 3.6% in the exposure group [38]. This demonstrates the potency of natural tolerance mechanisms when induced in the right way and at the right time. However, achieving immunological tolerance depends on a multitude of factors and is significantly more challenging to accomplish than simply through short-term exposure. Thus, the success of the peanut study could not be repeated with early exposure to six different foods (peanut, boiled egg, cow's milk, sesame, fish, and wheat) [39]. A total of 1303 infants exclusively breastfed for a 3-month period were randomized either to exposure to one of the six foods mentioned above (exposure group) or to continued exclusive breastfeeding for a further 3 months (control group). Upon comparison of food allergies at the ages of 1–3 years, this study also appeared to be successful: 7.3% of the control group (524 children) developed a food allergy, whereas the percentage of allergy sufferers in the exposure group was significantly lower at only 2.4% (208 children). However, these data were evaluated according to the “per protocol analysis” principle, meaning that only those participants that adhered strictly to the study protocol were considered in the evaluation. If one applies the intention-to-treat principle of evaluation, which takes all evaluable data into account, the number of allergy sufferers rises in both groups. However, the percentage of children with food allergy changed significantly to 5.6% (567 children) only in the exposure group, and was

thus no longer significantly different to the percentage of children with food allergy in the control group (7.1% of 595 children).

Immunotolerance as a treatment goal

Allergen-specific immunotherapy has long been an effective approach to treating immediate-type reactions that are already established. Although the mechanisms that achieve this effect are manifold, the induction of IL-10-producing regulatory T cells has also been described as correcting the imbalance between allergen-specific T-cell subtypes in the allergy sufferer [40]. While immunotherapy was initially established empirically, it became increasingly possible to better describe the mechanisms on the various levels of the immune system. Above all, however, thanks to the introduction of the Therapy Allergen Ordinance (“Therapieallergene-Verordnung”), evidence-based proof of efficacy of the therapy allergens available for immunotherapy is now standard.

Interestingly, there is a considerable discrepancy in the evidence on prevention within the biodiversity hypothesis in terms of type I sensitization (IgE) and the manifestation and development of atopic dermatitis (neurodermatitis) [19]. The pathogenesis of atopic dermatitis is based on a complex genetic predisposition that is reflected primarily in a reduced skin barrier and a susceptibility to develop Th2 immunity [10]. Both promote the proliferation of specific bacteria on the skin, explaining why dysbiosis, i.e., a breakdown in the skin microflora, is considered an early and key event in the development of cutaneous dermatitis [10]. Longitudinal studies have shown that the composition of the skin's bacterial ecosystem is relatively stable in healthy individuals despite external factors. Although there are intraindividual differences depending on the local composition of the skin, the diversity of the microorganisms involved and their relative composition is overall preserved as an individual signature over a long period of time [41]. In atopic dermatitis, this stability in the bacterial ecosystem breaks down when lesions occur, and microbial diversity diminishes in favor of staphylococcal dominance, in particular *Staphylococcus aureus* [42]. The effect of Th2 immunity and its characteristic cytokines further promotes dysbiosis: first, antimicrobial peptides that normally represent up-regulated, natural, antimicrobial substances are suppressed [43, 44]; second, one sees reduced production of, e.g., the structural protein of the skin, filaggrin, and thus a weakening of the skin barrier [45], as well as suppression of the antibacterial immune response in the direction of Th17 [46]. This results in chronic inflammation triggered by substances produced by the staphylococci and supported by characteristic Th2 cytokines such as IL-4. A reversal in the immune response ensues: whereas the inflammatory response abates in the absence of staphylococcal components recognized as danger sig-

nals, their presence in combination with IL-4 results in strong down-regulation of the IL-10 that suppresses the inflammatory response, and thus to chronification [47]. Indeed, using a biologic agent, a monoclonal antibody that blocks the alpha-chain of the IL-4 receptor, can neutralize the effect of IL-4 (and also interleukin-13). An antibody of this kind has already been approved as a targeted therapy and showed significant dose-dependent improvement in patients with moderate to severe atopic dermatitis [48–50].

Given the crucial role of micro-organisms in immunity and tolerance, the question arises as to whether the prevention or treatment of inflammatory diseases can be developed based on influencing skin bacterial diversity. This has already been achieved for the gut microbiome in patients with chronic recurrent *Clostridium difficile* infections: while the standard treatment (antibiotic therapy with vancomycin) resulted in resolution in only 30% of patients, the additional infusion of a solution of healthy feces achieved treatment success after the second infusion in 90% of patients [51]. Only scant data are available as yet on the modulation of the cutaneous microbiota as a treatment or prevention approach. The study to yield positive results in this regard was published as early as 2008 by our group: patients with mild atopic dermatitis received either a cream containing lysates from the nonpathogenic bacterium *Vitreoscilla filiformis* or a placebo cream. A significant difference in the Score of Atopic Dermatitis (SCORAD) was seen as early as 15 days, reaching a significant intergroup difference between the placebo group and the verum group at 29 days [52]. Subsequent mechanistic analyses showed that the recognition of *V. filiformis* components by dendritic cells resulted in increased production of the suppressor IL-10 and induced regulatory T cells, which in turn attenuated inflammatory responses. Thus, in the human model of atopic dermatitis, exposure to the nonpathogenic bacterium *V. filiformis* significantly reduced dermatitis severity [18].

Conclusion

Today, biodiversity is seen as essential to ensuring the formation of a well-functioning immune system that actively develops tolerance. Studies to translate these findings into actual preventive measures are currently underway or in design. How and whether biodiversity can treat already established atopic diseases or allergies has only been demonstrated by way of example. This evidence, however, is promising in that completely novel strategies could make treatments with few side effects possible using immunomodulation. Therefore, further investigations need to define whether and how the stability of tolerance can be maintained and its specificity improved.

Conflict of interest S. Kaesler, Y. Skabytska, T. Volz and T. Biedermann declare that they have no competing interests.

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