

# Mucosal Tolerance and Immunity: Regulating the Development of Allergic Disease and Asthma

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## Key Words

Dendritic cells · Costimulatory molecules · Asthma ·  
IL-10 · Tolerance

## Abstract

Allergic diseases and asthma are characterized by eosinophilic inflammation induced by Th2 lymphocytes. However, the immunological events and the molecular and cellular mechanisms that protect against and regulate these pathological immune responses are poorly understood. In this review, we discuss the role of immunological tolerance, regulatory T cells, and dendritic cells (DCs) in these protective processes. In addition, we discuss factors like the maturation state of DCs, source and effect of IL-10 production as well as specific combinations of costimulatory molecules in the antigen-presenting cell: T cell synapse, which determine whether tolerance or immunity develops in the respiratory mucosa in response to inhaled allergen.

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## Introduction

The mucosa of the lung and intestine is the major epithelial surface at which the body interfaces with the envi-

ronment, and the major site where the body normally encounters foreign antigen. The mucosal epithelium, which in an adult individual covers an area of about 400 m<sup>2</sup> [1], is continuously exposed to a vast quantity of antigens in the air that we breathe, in food that we eat, and from pulmonary and intestinal microbes, toxins and parasites. The immune system must cope with these constant challenges, discriminate between pathogenic and innocuous antigens and develop appropriate immune responses. Under normal circumstances, soluble nonreplicating proteins delivered to the intact mucosal surfaces do not provoke strong immune reactions, but instead induce a state of antigen-specific hyporesponsiveness, although under other circumstances, such proteins may induce Th2-biased allergic inflammatory responses and asthma. The state of tolerance against innocuous antigens, which prevents the development of allergic disease and asthma, develops through several immunological mechanisms. In particular clonal deletion, anergy and active suppression, mediated by regulatory cells secreting interleukin 10 (IL-10), have been implicated in the downregulation of immune responses at other sites and in the lung [2–4]. The immunological events, cellular mechanisms and antigen-presenting cell (APC) types involved in regulating immune responses and inducing the T cell hyporesponsiveness to antigens inhaled into the lung are only recently becoming understood.

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## Allergic Disease and Asthma

Asthma is a complex trait that is characterized by inflammation in the lungs consisting of Th2 lymphocytes, eosinophils, and basophils. This Th2-biased inflammatory response is associated with increased mucus production, mucosal edema, reversible airway obstruction, airway hyperresponsiveness (AHR) and remodeling of the airways. The inflammatory response in asthma is strongly, although not exclusively, associated with Th2-biased sensitization to indoor and outdoor allergens [5, 6], and with elevated serum IgE levels [7, 8]. Moreover, sensitization to environmental allergens is a major risk factor for the development of asthma and persistent wheezing. Although the pathogenic mechanisms of asthma and atopy are now understood to be largely related to Th2-biased inflammation, the mechanisms that protect against these diseases are much less well understood.

## Antigen Uptake and Presentation

Both Th2-biased immune responses as well as immune responses that protect against allergic disease and asthma depend on activation of antigen-specific T cells by APCs. Respiratory antigens that have evaded the mechanical mechanisms that normally prevent entry into the lungs (cough reflex, mucus/ciliary action, phagocytic clearance by alveolar macrophages) are taken up by pulmonary dendritic cells (DCs), which are present as a network permeating the respiratory epithelium. The antigens entering into the lungs are thus continuously sampled by DCs, which migrate from the lungs to the bronchial lymph nodes where they present antigen to and activate the innate and adaptive components of the immune system. DCs, which are the most potent immunostimulatory APCs and uniquely associated with the induction of active immune responses [9], process antigen into immunogenic peptides, and present them in the context of MHC molecules to naïve T cells in the lymph nodes draining the lung [10–12].

## DCs and Peripheral Tolerance

DCs have the capacity to secrete a large number of cytokines and to express different costimulatory molecules, thereby strongly influencing the qualitative and quantitative form of the subsequent immune response. For example, subsets of DCs (DC1) that produce IL-12

and express high levels of costimulatory molecules, such as B7.1 and B7.2, induce Th1 responses, associated with the production of IFN- $\gamma$  and IL-2 [13]. In contrast, other DC subsets (DC2) and possibly those expressing low levels of costimulatory molecules, particularly those in the pulmonary parenchyma during repeated exposure to low levels of antigen, induce Th2 responses, associated with the production of IL-4, IL-5 and IL-13 [14–17]. Predisposition towards the development of Th2 responses may be genetically determined, and occur more frequently in atopic individuals. In other situations, DCs induce T cell unresponsiveness, anergy or apoptosis. Thus, in the thymus, DCs mediate negative selection of T cells [18–20], and induce tolerance to autoantigens, such as myelin basic protein, thereby limiting the development of experimental autoimmune encephalomyelitis [21]. Similarly, in the intestinal tract and in peripheral lymphoid tissues, DCs have the capacity to present antigens in a tolerogenic manner [22–25]. This type of peripheral tolerance might be mediated by a specialized subset of DCs in secondary lymphoid tissue that constitutively expresses and presents peripheral antigens for the purpose of perpetual elimination of autoreactive T cells. Thus, DCs may phagocytose disintegrating cells or apoptotic cells, process and present these antigens, and tolerize antigen-reactive T cells [26], or may induce T cell tolerance by direct induction of programmed cell death [27]. Precisely how and when a DC promotes tolerance versus active immunity is still unclear, but may be related to the maturational state of the DC, and to the expression of different costimulatory molecules.

## Mature versus Immature DCs

One possible mechanism proposed to explain how DCs can both induce primary immune responses and also inhibit inflammatory processes is that tolerance is mediated by immature or semi-mature DCs expressing low levels of T cell receptor ligands and costimulatory molecules, whereas immunity is generated by mature DCs expressing high levels of costimulatory molecules. According to this hypothesis, antigen presentation in the absence of costimulatory signals by immature DCs in secondary lymphoid tissue, a scenario in conflict with some experimental data [28], could lead to incomplete T cell activation, anergy, and possible T cell deletion. This idea is consistent with the concept that tissue DCs migrate on a continuous basis to secondary lymphoid tissue [29], and the fact that migration of DCs in the absence of matura-

tional stimuli could allow immature or semi-mature DCs to present tissue antigens to T cells in a substimulatory context. Thus DCs in lymph nodes express gut- and skin-restricted antigens [30, 31], indicating that migratory DCs sample antigens in peripheral tissues and transport these antigens to the draining lymph nodes under homeostatic conditions. In addition, Hawiger et al. [32] showed that presentation of antigen by DCs in the absence of inflammation or infection leads to T cell tolerance. Soluble antigen was targeted to the MHC class II pathway of DCs in situ by noninflammatory measures using an antibody specific for a specialized DC endocytosis receptor, DEC205. Under these conditions, antigen presentation by immature DCs prompted a short proliferative burst of cognate CD4 T cells followed by deletion of antigen-specific T cells. The lack of a response to subsequent peptide immunization indicated that the recipients had been rendered tolerant. In agreement with a previous report that linked peripheral tolerance to immature bone-marrow-derived APCs [33], the study by Hawiger et al. [32] established that immature DCs induce peripheral tolerance by eliminating autoreactive T cells.

The idea that immature DCs induce peripheral tolerance, however, although supported by some experimental evidence, contradicts other data. For example, several autoimmune syndromes result from immaturity and failure of DC function resulting in the failure of peripheral tolerance induction. This concept is advanced by the fact that DCs obtained from the peripheral blood of patients with autoimmune diabetes, systemic lupus erythematosus, Grave's disease and multiple sclerosis exhibited a relatively immature phenotype, and expressed lower levels of costimulatory molecules and APC function [34–37]. These studies suggest that mature DC function is in fact required for responses to environmental factors associated with protection against autoimmune disease, and that mature DC function aids in the development of peripheral tolerance and in the suppression of autoreactive T cells.

#### Tolerance and Protective Immunity in Allergic Diseases

The conflicting nature of mature versus immature DC function in the development of tolerance may be due to the fact that tolerance can occur by several mechanisms. Whereas immature DCs may be important in inducing deletional tolerance or anergy, for example, in central tolerance, mature DCs expressing high levels of costimula-

tory molecules may be required for induction of regulatory cells [15] and for induction of peripheral tolerance mediated by suppression, particularly in response to exogenous environmental allergens.

In allergic disease and asthma, T cell tolerance against allergens, induced by respiratory exposure to allergen, protects against the development of allergic disease and asthma, and appears to be mediated at least in part by the development of regulatory T (Tr) cells [38]. T cell tolerance, induced by immunization with allergen (e.g., allergen immunotherapy or allergy shots) may also protect against the development of asthma. Allergen immunotherapy has been shown to reduce the quantity of IL-4 produced by allergen-specific memory CD4<sup>+</sup> T cells in allergic individuals to levels observed with T cells from nonallergic subjects, or to levels induced with nonallergic antigens such as tetanus toxoid [39]. It is also possible that allergen immunotherapy may induce immune deviation from Th2 to Th1 type responses, for example by the development of IFN- $\gamma$  producing Th1 CD4 cells, CD8 T cells or  $\gamma\delta$  T cells, which has been thought to protect against allergy and asthma. However, although IFN- $\gamma$  inhibits the production of Th2 cytokines and although overproduction of IFN- $\gamma$  in patients with multiple sclerosis protects against the development of atopy [40, 41], Th1 cells are normally not found in the lungs of nonatopic, nonasthmatic individuals. Moreover, the production of IFN- $\gamma$  contributes to the pathogenesis of human asthma [42]. In addition, adoptive transfer of allergen-specific Th1 cells fails to counterbalance Th2 cell-induced AHR. Instead, allergen-specific Th1 cells caused severe airway inflammation [43, 44]. Thus, while it is clear that T cells secreting Th2 cytokines, particularly in combination with eosinophils, play a key role in the pathogenesis of airway inflammation and asthma [45, 46], pure Th1 responses do not form the major immunological mechanism that protects against asthma and allergy. However, it is possible that production of IFN- $\gamma$  may in some cases, perhaps in combination with other cytokines, contribute to protection against the development of asthma.

In contrast to Th1 responses, allergen-specific CD4 T cell tolerance, induced by respiratory exposure to allergen, can protect against the development of Th2-biased responses and allergen-induced AHR [16, 44, 47]. In humans, the degree of allergen exposure determines whether tolerance develops, with high levels of exposure inducing greater tolerance [48]. The mechanism by which respiratory antigens induce T cell tolerance includes T cell clonal deletion, anergy as well as active suppression mediated by regulatory cells secreting IL-10 or TGF- $\beta$ , as is thought to

occur in oral tolerance [2–4]. The development of respiratory tolerance is initiated by uptake of antigen in the lungs by immature DCs [49]. Within 24 h after respiratory exposure to antigen such as ovalbumin, the pulmonary DCs migrate to the draining bronchial lymph nodes, where they mature, transiently produce IL-10, and express high levels of B7.1 and B7.2 costimulatory molecules [15]. Even in the absence of inflammation, DCs migrate from the lungs to the bronchial lymph nodes. In the lymph nodes, DCs induce an initial phase of allergen-specific T cell activation, proliferation and expansion, followed by depletion of these T cells from the lymphoid organs, though a stable population of these cells survive but remain refractory (functionally disabled) to antigenic rechallenge [16]. The establishment of a tolerant state in allergen-specific T cells, however, prevents the development of airway inflammation and AHR.

The inhibition of AHR by T cell tolerance is in part mediated by allergen-specific CD4 T cells exerting potent regulatory/suppressor cell activity. These antigen-specific CD4 Tr cells produce IL-10, and can be generated by activating CD4 T cells with IL-10 producing DCs from bronchial lymph nodes of tolerized mice [38]. These DCs express high levels of costimulatory molecules including inducible costimulatory molecule (ICOS) ligand (ICOSL), and possess a low capacity for phagocytosis, all of which are characteristics of mature DCs. The production of IL-10 by these DCs most likely plays a major role in the generation of Tr cells because monoclonal antibodies (mAbs) against IL-10 blocked this process. Moreover, the *in vivo* and *in vitro* inhibitory capacity of the Tr effector cells was abrogated by neutralization of IL-10 with an anti-IL-10 mAb, or by interrupting ICOS-ICOSL interactions with an anti-ICOSL mAb. In addition, interference with the ICOS-ICOSL signaling pathway not only abrogated the inhibitory capacity of Tr effector cells, but also blocked the development of IL-10 production and the induction of T cell tolerance following intranasal exposure to ovalbumin [38]. Thus Tr cells play an important role in mediating respiratory tolerance and protection against asthma via pathways that involve IL-10 and ICOS-ICOSL interactions. Moreover, these pathways play a critical role in initiating CD4 T cell unresponsiveness following respiratory exposure to antigen [38].

Other types of cells may also be involved in regulating Th2-driven allergic inflammatory responses. For example, Tr1 cells have been generated by other investigators by addition of large quantities of exogenous IL-10 during the stimulation of antigen-specific T cells, or with immunosuppressive drugs (corticosteroids plus vitamin D) [50,

51]. In addition, Th3 cells secreting TGF- $\beta$ 1 can inhibit the proinflammatory function of both Th2 and Th1 cells in experimental models [3, 4, 52–54]. Furthermore, CD4+CD25+ cells, which regulate a variety of other responses and inflammatory conditions [55, 56], may also regulate Th2 responses. However, it is not yet clear whether any of these T cells with regulatory activity are involved in the regulation of asthma, although Th3 cells may be involved in control of inflammation in the gastrointestinal mucosa.

### Costimulation in Asthma and Allergy

In several systems, immunological tolerance is induced by immature DCs expressing low levels of costimulatory molecules or generated by inhibitory costimulatory molecules. For example, some systems utilize immature DCs derived after extensive propagation *in vitro* in the presence of GM-CSF, which express limited costimulatory molecules, but which may not fully reflect immune mechanisms in the lung [57]. In other systems, activation with DCs expressing low levels of costimulatory molecules induces immunological tolerance manifested by anergy or deletion of antigen-specific T cells [30, 58]. Still other systems demonstrate the importance of inhibitory costimulatory pathways for T cells, such as CTLA-4 and PD-1. In these systems, treatment with a neutralizing anti-CTLA-4 mAb increased IgE production, eosinophilic inflammation and AHR, increased Th2 cytokine production, but decreased TGF- $\beta$  production in mice. Another member of the CD28 family is the PD-1 (programmed death) receptor, which interacts with the B7 family members PD-L1 and PD-L2 [59]. PD-1 expression is upregulated in T, B and myeloid cells upon activation. Binding of PD-1 to both PD-L1 and PD-L2 has been associated mainly with inhibition of T cell function, in both CD4 and CD8 T cells although some studies reported T cell stimulation, especially production of IL-10. Nevertheless, studies with PD-1-deficient mice exhibited characteristics of autoimmunity, suggesting its involvement in the regulation of T and B cell tolerance. Involvement of PD-1 in allergic responses remains to be investigated. Finally, the latest identified member of the B7 family is B7-H3 [60]. B7-H3 expression is induced on DCs and monocytes by proinflammatory cytokines. The receptor for B7-H3 is still unknown, and is distinct from CD28, CTLA-4, ICOS and PD-1. B7-H3 costimulation induces proliferation in both CD4 and CD8 T cells, induces cytotoxic activity and selectively increases expression of IFN- $\gamma$  [61]. Whether any of these

mechanisms play a role in the respiratory tract is as yet unclear.

### OX40 Costimulation in Asthma

Another costimulatory molecule that may greatly affect the development of tolerance versus immunity is the tumor necrosis factor (TNF)/TNF receptor (TNFR) family member, OX40 and OX40L, which are expressed on T cells and APCs, respectively. CD40-activated but not freshly isolated DCs express the TNF family member OX40L in mice [62] and humans [63], and mRNA for OX40L and OX40 is upregulated in lymph nodes during CD4 T cell priming [64]. In human tonsils, OX40 protein is most strongly expressed on CD4 T cell blasts in the T zone [65], which is compatible with activated CD4 T cells receiving OX40 signals when they are primed by DCs [66]. This suggests a sequential model of naïve CD4 T cell activation, with OX40 expression being dependent on an initial CD28 signal. Subsequent activation of DCs through CD40 by CD4 T cell CD40L in turn upregulates DC expression of OX40L. OX40 and CD28 signals are highly synergistic for CD4 T cell proliferation and survival [67]. Moreover, Bansal-Pakala et al. [68] suggest that the OX40 signal, by enhancing T cell proliferation and survival, actually prevents the development of tolerance.

OX40 was shown also to play a critical role in the development of allergic inflammation in a murine model of asthma. Using mice deficient in OX40, several investigators showed that recruitment of large numbers of eosinophils into the lungs, induction of IgE, and production of high levels of Th2 cytokines both in serum and bronchoalveolar lavage were dependent on the presence of OX40 [69, 70]. In addition, goblet cell hyperplasia, mucus production, and AHR were suppressed in the absence of OX40, suggesting that this molecule is required in development of the asthmatic reaction. These studies and others [64, 71, 72] suggest that OX40 plays an important role in development of Th2 responses and in asthma.

### Tolerance and Costimulation in the Respiratory Tract

In the lung, activation of T cells with costimulatory signaling including ICOSL and B7.2 on DCs, preferentially costimulates IL-10 production [73, 74] and leads to the development of antigen-specific Tr cells. ICOS-ICOSL signaling in the presence of IL-10 appears to be critically

important in the development of such Tr cells in the lung, which potentially inhibited the proliferation of ovalbumin-specific T cells, and inhibited the development of AHR and airway inflammation when transferred to allergen-sensitized recipients [38].

The role of IL-10 in limiting airway inflammation and AHR has been controversial. IL-10 has been considered to be an essential Th2 cytokine [75], particularly because (1) IL-10 inhibits Th1 cytokine production by inhibiting IL-12 synthesis [76], and (2) IL-10<sup>-/-</sup> mice develop poor Th2 responses and resist the development of AHR [77]. However, IL-10 may have several roles in asthma, not only by playing a critical function in initiating the development of Th2-polarized responses, but also by performing an important regulatory role late during immune responses, especially by downmodulating Th2-driven inflammation [77–80]. In addition to the previously described finding that administration of IL-10 inhibits the expression of AHR, our group has proven that IL-10-producing Tr cells prevent the development of AHR even in allergen-sensitized mice. This suggests that Tr cells normally develop during respiratory exposure to allergen and protect against allergic asthma [38].

### ICOS and ICOSL in Tolerance

Since blockade of ICOS-ICOSL interactions blocked the development of respiratory tolerance and the development of Tr cells, we believe that ICOS expression on T cells and ICOSL expression on DCs are important costimulatory molecules in tolerance. In contrast to CD28, ICOS is not expressed constitutively on naïve T cells but is induced rapidly on T cells after TCR engagement [81–86], suggesting that ICOS provides co-stimulatory signals to activated T cells. ICOS expression on T cells is enhanced by both TCR and CD28 signals [83–86], and is markedly reduced in the absence of B7.1 and B7.2 [85, 87], indicating that some of the functions ascribed to CD28 might be due in part to ICOS signaling. ICOS expression is upregulated on both Th1 and Th2 cells during the initial phase of differentiation, but levels remain high on Th2 cells and decrease on Th1 cells [83, 85]. In contrast to B7.1 and B7.2, ICOSL is expressed not only on hematopoietic cells but also on other cell types, such as resting B cells, some macrophages and dendritic cells [82, 87–89], as well as on a small subset of CD3<sup>+</sup> T cells [90].

During the initial activation of naïve T cells, the effects of ICOS on T cell proliferation and IL-2 production are modest in comparison to those of CD28. For example,

blocking ICOS-ICOSL interaction with an ICOS-Ig fusion protein does not inhibit the proliferation of naïve DO.11.10 TCR transgenic T cells in response to peptide [85]. Rather, signaling through ICOS appears to be more important for regulating cytokine production by recently activated and effector T cells [81]. Stimulatory anti-ICOS mAbs or a bivalent ICOSL-Ig fusion protein can enhance production of IL-4, IL-5, IL-10, IFN- $\gamma$ , TNF- $\alpha$  and GM-CSF [81, 85]. Blockade of ICOS during initial stimulation of naïve T cells enhances Th1 differentiation, presumably due to lack of opposing Th2 cytokine or regulatory cytokine (IL-10) production. When restimulated, differentiated ICOS-/- T cells produce IFN- $\gamma$  but fail to produce IL-4 [73, 91, 92]. The defect in Th2 cytokine production is not due to an obligatory role for ICOS for Th2 cell differentiation, but results rather from a crucial role for ICOS in regulating IL-4 and IL-13 production by effector cells [93].

The ICOS-ICOSL pathway is important in the activation and function of effector Th2 cells specifically in inducing Th2 cytokine production [73, 81, 87]. ICOS-ICOSL activation appears to promote IL-10 production, along with production of IL-4, IL-5 and GM-CSF [84, 94] but little IL-2. Mice deficient in ICOS exhibit major reductions in the production of IgE [73], Th2 cytokines and in the development of AHR, indicating an important role for ICOS in the development of allergic respiratory inflammatory responses [95]. ICOS-/- mice are susceptible to induction of inflammatory lung disease by airway challenge with ovalbumin in primed mice, but produce less IL-4 and IL-13 [91]. ICOS blockade reduces lung inflammation and AHR after adoptive transfer of highly polarized Th2 cells to naive mice [83]. Blockade of ICOS 21 days after priming significantly reduces lung inflammation after airway antigen challenge [95], whereas blockade of the B7-CD28 pathway after priming does not inhibit Th2 effector cytokine production. This is consistent with the idea that Th2 effector cell function is very much dependent on the ICOS-ICOSL pathway, and that Th2 effector cell development is only partly dependent on this pathway.

Studies of experimental autoimmune encephalomyelitis (EAE), a Th1 disease mediated by myelin-specific CD4 T cells, suggest that ICOS may also have a significant effect on the development of regulatory T cells. ICOS-/- mice showed greatly enhanced susceptibility to EAE [91], although blockade of the ICOS-ICOSL pathway with ICOS-Ig inhibited the ability of myelin-specific TCR transgenic T cells to induce EAE [60, 96]. These conflicting results may be explained by the fact that the ICOS-

ICOSL pathway may be involved at several stages of T cell development. ICOS is expressed early after activation of naïve T cells, providing a signal distinct from CD28 for the activation and viability of T cells, and may also be involved in the function of IL-10 producing regulatory T cells. Costimulation through ICOS is more effective than costimulation through CD28 at enhancing IL-10 production [81]. Furthermore, the IL-10-IL-12 regulatory loop is crucial in regulating EAE [97], because IL10-/- but not IL4-/- mice develop exacerbated EAE [98]. Therefore, Th1 diseases may be downmodulated by IL-10-producing T cells, which depend on ICOS-ICOSL stimulation for development and function.

ICOS-dependent IL-10-producing T cells also regulate Th2-mediated diseases, as we demonstrated that ICOS costimulates the induction of Tr cells that inhibit the function of antigen-specific T cells and the development of AHR [38]. This role of ICOS was unexpected, although in retrospect those findings are not surprising, because ICOS costimulation induces large quantities of IL-10 production [73, 83] by T cells, and since ICOS-/- mice develop enhanced EAE [91, 99].

The idea that ICOS-ICOSL interactions costimulate the development of both Th2-driven inflammation and Tr-cell-mediated tolerance suggests that these distinct processes are in fact related. Both Th2 cells and Tr cells are associated with respiratory mucosal responses, require costimulation through CD28 and ICOS for activation, and both cell types produce IL-4 and IL-10 (although in relatively different quantities). Th2 cells produce primarily IL-4, IL-13 and IL-10, whereas Tr cells produce primarily IL-10 and low levels of IL-4 [50], but not IL-13 [38]. Moreover, the functions of Tr and Th2 cells in our mouse model of asthma were clearly distinct, because Tr cells, but not Th2 cells, blocked the development of AHR. Our studies demonstrated that pulmonary DCs from mice exposed to intranasal ovalbumin induced T cell production of both IL-4 and IL-10 initially, but with subsequent stimulation synthesis of IL-4 decreased, while production of IL-10 was maintained. The specific signals that preferentially induce development of Tr cells rather than Th2 cells are not entirely clear, but may involve IL-10 production by DCs. Thus, in the presence of IL-10-producing DCs (or in the presence of exogenously derived IL-10), Tr cells develop [50], whereas in the absence of IL-10, e.g., when antigen is presented by DCs from IL-10-/- mice or from mice with allergic asthma [15], Tr cells fail to develop. Furthermore, we suggest that the development of Th2 cells and of allergic diseases may represent an aberration of Tr cell development, possibly due to inadequate pro-

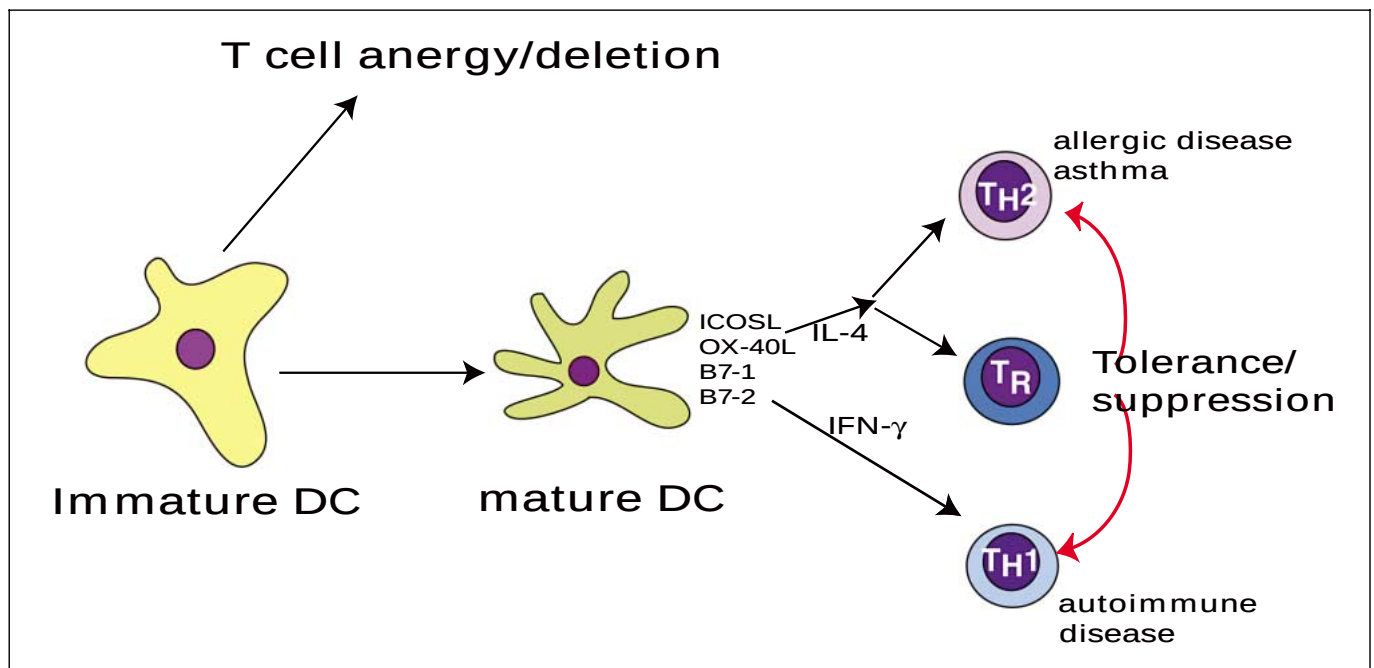


Fig. 1. Tolerance to inhaled antigen involves several immune mechanisms, including anergy, deletion and the development of Tr cells. The state of the DC and the costimulatory molecules expressed by the DC in the lung and bronchial lymph node determine the fate of the responding T cell. Antigen presentation of inhaled antigen by mature DCs, which express low levels of costimulatory molecules, induces antigen-specific T cell anergy or deletion. Antigen presentation by mature DCs, expressing high levels of costimulatory molecules, induces the differentiation and maturation of CD4 T cells. The

production of IL-12 by DCs enhances the differentiation of Th1 cells, whereas expression of ICOSL enhances the development of Th2 and Tr cells. Both Th2 and Tr cells initially produce IL-4, suggesting that they arise together, but Tr cells stop producing IL-4 but continue to produce IL-10 during the differentiation process. The development of Tr cells is enhanced by DC production of IL-10. Tr cells have a regulatory/suppressive function, and are capable of inhibiting the function of both Th1 and Th2 effector cells.

duction of IL-10 [100]. Thus, limited IL-10 and enhanced IL-4 and IL-13 production in allergic asthma may result in a failure to develop allergen-specific Tr cells and thus promotes development of Th2 cells. The balance between Tr and Th2 cells in allergic inflammatory diseases might be even more crucial than a putative counteraction between Th1 and Th2 cells. Further study of the signals that differentially affect Th2 cell and Tr cell development is required to fully understand the development of tolerance and its relationship to the pathogenesis of allergic asthma.

#### Tolerance and Commensal Bacteria

Over the past 20 years, the prevalence in industrialized countries of inflammatory diseases such as asthma and allergies, as well as autoimmune diseases, has doubled. Because environmental factors induce these diseases in

genetically predisposed individuals, and since the genetic composition of the population has not changed substantially over the past twenty years, it is assumed that changes in the environment, perhaps improved hygiene and fewer childhood infections, are responsible for this increase in prevalence. Infectious diseases, including tuberculosis, hepatitis A and bacterial infections are much less common today than twenty years ago. In addition, the commensal bacterial flora in the gastrointestinal and respiratory tracts, which may activate the innate immune system to protect against the development of airway inflammation, is altered.

We believe that the natural environment in the past, prior to industrialization, maintained the respiratory and gastrointestinal mucosal systems in a state that favored the development of T cell tolerance to nonreplicating antigens encountered at mucosal surfaces [101]. The establishment and maintenance of such tolerance require the presence of commensal bacteria in the gastrointestinal

and upper respiratory tracts, since mucosal tolerance cannot be induced in germfree animals [102, 103]. Commensal bacteria may enhance production of IL-10 through mechanisms involving the innate immune system, and receptors such as nucleotide-binding oligomerization domain 2 (*NOD2*). *NOD2* is a member of the NOD family of proteins involved in the regulation of programmed cell death and host defense against pathogens [104]. *NOD2* expression is highly restricted to monocytes, and when activated by LPS from certain bacteria, it induces nuclear translocation of NF- $\kappa$ B in a manner that appears to down-modulate inflammation. Alterations in the *NOD2* function due to polymorphisms in the *NOD2* gene are associated with Crohn's disease, an inflammatory bowel disease [105, 106]. The anti-inflammatory effects induced in the gastrointestinal tract by *NOD2* may be related to the enteric 'immunosuppressive' effects observed with non-virulent *Salmonella* strains, which block NF- $\kappa$ B activation by inhibiting I $\kappa$ B degradation [107]. Infection with high levels of helminths may also favor the development of tolerance to nonreplicating environmental antigens by enhancing IL-10 production [108, 109]. Therefore, we suggest that some microorganisms may diminish inflammation at mucosal surfaces, and that changes in the environment in westernized societies limit exposure to these types of organisms and thereby diminish the normal tolerance-inducing state of mucosal surfaces. This would result in enhanced mucosal inflammation. These changes prevent the development of allergen-specific Tr cells in some individuals. Instead, Th2 cells develop (an aberrant form of Tr cells), which in turn induce allergic inflammatory responses. The responsible environmental changes may include frequent use of antibiotics, or changes in diet [110], which alter the normal gastrointestinal flora, resulting in reduced IL-10 and TGF- $\beta$  production by epithelial cells, DCs and B cells in the mucosa, causing increased development of Th2 cells and allergy.

Whereas nonreplicating antigens such as allergens as well as commensal bacteria normally induce tolerance, replicating, tissue invasive pathogens generate inflammatory responses that eliminate these organisms. In these instances, specific activating TLR are stimulated by pathogen-associated molecular patterns that activate the innate immune system and the acquired immune system. These inflammatory responses induced by infection prevent the development of tolerance, and can promote the development of Th2-biased allergen sensitization [111].

## Conclusions

The development of productive immune responses versus tolerance in the lungs is regulated by subsets of DCs expressing specific profiles of cytokines and costimulatory molecules (fig. 1). Immature DCs, expressing limited quantities of costimulatory molecules and not producing IL-12, induce T cell anergy and deletion. In contrast, mature DCs, expressing high levels of costimulatory molecules induce Th1, Th2 or Tr cells, depending on the expression of specific costimulatory molecules and cytokines. Tr cells producing IL-10 are capable of actively suppressing respiratory inflammation and asthma induced by Th2 cells. The specific response in the respiratory tract therefore reflects the relative development of Th1, Th2 and Tr effector cells.

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