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Mechanisms of tolerance and allergic sensitization in the airways and the lungs

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Abstract

The respiratory mucosa is constantly exposed to non-infectious substances that have the potential of triggering inflammation. While many particles are excluded, soluble molecules can reach the epithelium surface, where they can be uptaken by dendritic cells and stimulate an adaptive immune response. Most mucosal responses result in tolerance to subsequent antigen encounters, which is mediated by Foxp3⁺ regulatory Tcells. Genetic and environmental factors, added to the ability of certain allergens to induce innate responses, can predispose to allergic sensitization. In this review we discuss recent advances in the understanding of the mechanisms of tolerance and allergic sensitization to airborne allergens.

Introduction

In normal conditions, inhalation of non-infectious protein antigens induces a state of tolerance, which is essential to maintain mucosal homeostasis under constant exposure to environmental antigens. Studies of respiratory tolerance in rodents have provided valuable information on the mechanisms that prevent allergic sensitization through the airways. Resident lung cells set the stage for a non-inflammatory steady state. Airway macrophage interactions with epithelial cells involving integrins and TGF- β are necessary to prevent inflammation in the lung. Antigen-presentation by migratory lung DC to CD4⁺ cells in draining LN and induction of Foxp3⁺ iTreg cells are essential steps in the establishment of tolerance. Allergic sensitization occurs by a failure in the mechanisms of tolerance, leading to development of inflammatory airway diseases such as rhinitis and asthma. Allergies are caused by Th2 lymphocytes that secrete IL-4, IL-5 and IL-13, and by IgE antibodies. In this review we will focus on recent data on airways immune tolerance and sensitization.

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Induction of tolerance

Antigens entering through the respiratory mucosa normally induce tolerance

Exposure to environmental allergens does not elicit clinical symptoms in non-allergic individuals. Human studies of cord blood and neonatal responses to allergens indicate that this is not due to absence of immune response, but rather to development of a protective response. Experiments of inhalation of allergens in rodents by the Patrick Holt group in the 1980s and 1990s clearly established that inhaled antigens induce a state of dominant tolerance that could be transferred by T cells. These days, the nomenclature dominant tolerance implies the activity of regulatory T cells. Similarly to oral tolerance, tolerance to antigens entering through the respiratory tract is antigen-specific and systemic [1].

Adaptive (or inducible) Regulatory T cells

It has been shown that several strategies leading to tolerance induction do so by inducing regulatory cells. Although not all regulatory T cells express Foxp3 [2,3], absence of Foxp3 compromises immune tolerance leading to severe autoimmunity both in animal models [4] and human patients [5]. Foxp3⁺ regulatory T cells are generated either in the thymus (nTreg cells) or induced in the periphery (iTreg cells) [6]

The study of peripheral Treg cell conversion was greatly facilitated with TCR-transgenic RAG deficient mice, which are unable to produce a functional TCR from their endogenous genes and, as a consequence, lack nTreg cells [6]. Therefore, it becomes possible to examine the conversion of such T cells into Foxp3⁺ Treg cells, in the absence of a possible contamination due to the expansion of pre-existing nTreg cells.

It was shown that addition of TGF- β to *in vitro* cultures of OVA-specific T cells from DO11.10 mice led to induction of Foxp3⁺ Treg cells [7]. Furthermore, the use of low concentration of OVA peptide for *in vitro* activation of Foxp3⁻ T cells also resulted in induction of Foxp3⁺ T cells [8]. Interestingly, B cells were shown to be more efficient than DCs in driving Treg conversion, presumably by their inability to provide full co-stimulatory signals [8].

There are now many examples of *in vivo* generation of iTreg cells from naïve T cells from TCR transgenic RAG-deficient mice. Oral and nasal exposure to OVA was shown to lead to the conversion of TCR-transgenic RAG-deficient T cells into Foxp3⁺ iTreg cells, a process that was dependent on TGF- β [9]. Furthermore, mucosal tolerance (both oral and nasal) was completely abolished if Foxp3⁺ iTreg generation was prevented [10].

Exposure of T cells to a low dose of persistent antigen also resulted in Foxp3⁺ iTreg induction [11], as did the administration of antigen covalently coupled with anti DEC205 antibody [12]. It was also possible to document the *in vivo* induction of iTreg cells in TCR-transgenic RAG^{-/-} mice specific to a given male following transplantation of male skin grafts onto female TCR-transgenic mice treated with tolerogenic anti-CD4 MAb [13]. Immature DCs or DCs treated with vitD3 (a reagent that prevents subsequent DC maturation) when adoptively transferred into male-specific TCR transgenic female mice equally lead to peripheral induction of Foxp3⁺ Treg cells and dominant tolerance to male skin grafts [14]. The peripheral induction of Foxp3⁺ iTreg cells, both *in vitro* and *in vivo*, requires TGF- β as it is abrogated in the presence of neutralizing anti-TGF- β MAb [9,13,14].

Using TCR transgenic T cells specific to a moth cytochrome c (MCC) peptide, it was possible to show that induction of peripheral Foxp3 expression was a consequence of activation by a low dose of a strong agonist, while weak agonists only induce a transient population of Foxp3 expressing cells [15].

Spontaneous conversion of non-regulatory T cells into Foxp3⁺ Tregs was also shown following adoptive transfer experiments of CD4⁺CD25⁻ T cells into congenic mice [16,17]. However, in animals that are not TCR-transgenic RAG^{-/-} it is always difficult to exclude a contribution of Treg expansion by Foxp3⁺CD25⁻ cells.

The anatomical location where Treg cells operate *in vivo* is important. Treg cells able to prevent transplant rejection can be found infiltrating the tolerated transplant [18]. Remarkably, Treg cells accumulate preferentially within the tolerated graft, e.g. transplanted skin, but not within the host-origin skin. It will be important therefore to clarify whether a local immune privileged-like site may be created through the action of Treg cells. Such Treg-induced immune privileged sites may be important for the generation of more iTreg cells ("infectious" tolerance)[19].

In mice displaying normal T cell repertoire, tolerance to inhaled OVA was demonstrated to depend on regulatory T cells expressing TGF- β on their surface [20]. For the reasons discussed above, it was not possible to determine whether these TGF- β ⁺ cells were derived from nTreg cells or de novo-induced iTreg cells. It was however possible to establish that TGF- β ⁺ Treg cells expressed Delta-like and Jagged Notch ligands and were thus able to activate the Notch1 expressed on T cells. Reversal of Notch1 activation with γ -secretase inhibitor blocked the suppression brought about by TGF- β ⁺ Treg cells [21]. Thus, notch signaling is involved in Treg-mediated suppression of allergic inflammation. However, as will be discussed below, Notch signaling delivered by Jagged-2⁺ DC is involved in Th2 cell induction. Naturally, the presence of modifying factors on the Tregs versus DC must be critical in determining the suppressed versus the Th2 fate, respectively.

A different population of regulatory T cells, named Tr1 cells, have been described [22]. These cells do not express Foxp3, are peripherally induced by antigenic stimulation in an IL-10 rich environment, and are characterized by IL-10 production. It was recently shown that triggering of the aryl hydrocarbon receptor (AhR) can promote the differentiation of human Tr1 cells, while AhR activation in presence of TGF- β induces Foxp3⁺ Treg cells [23]. Furthermore, a synergy between c-Maf and AhR signaling was identified for the induction of Tr1 cells [24].

IL-10-producing Treg cells with potent suppressive ability upon transfer were generated in the lung-draining bronchial lymph nodes by exposure to antigen delivered intranasally, in a process that required ICOS-ICOSL interactions [25].

The hygiene hypothesis and iTreg cells

Epidemiological observations revealed that the prevalence of allergic sensitization was lower in children growing up in a farm environment than in children from urban dwellings. Other factors that were found to be associated with protection from allergic sensitization in childhood were having older siblings and being exposed to domestic animals [26].

The hygiene hypothesis explained these observations by linking the exposure to a richer microbial environment to protection from allergic sensitization. Initially, it was thought that these protective factors lead to development of Th1 response, thus that protection involved a mechanism of immune deviation. The fact that autoimmune diseases, many of which are mediated by Th1 or Th17-biased responses, have also become more prevalent in recent decades, argues against immune-deviation as the main mechanism driven by microbial environment products.

A more general defect in the maturation of the regulatory T cell compartment is likely. Foxp3⁺ Treg cells are dependent on activated effector cells as their main source of IL-2,

which is required for Treg cell homeostasis and function. A low T effector activity during early childhood may result in an underdeveloped Treg cell compartment. Moreover, microbial antigens have an effect on the number and maturation of mucosal DC. These, in turn, are essential for Foxp3⁺ iTreg cell induction. A recent report has shown that gut microbiota can induce local Treg development [27]. The human commensal *B. fragilis* colonization of the mouse gut, or one of its immunomodulatory molecules (polysaccharide A) can induce Foxp3 expression by T cells, endowing them with IL-10-producing ability. Of note, oral exposure to a potent NKT cell agonist – α -galactosylceramide – was shown to lead to induction of Foxp3 expression by NKT cells in the gut. This process was TGF- β -dependent as no Foxp3 induction occurs in mice with NKT cells devoid of a functional TGF- β receptor [28]. All these data are consistent with the observation that germ-free mice are impaired in their ability to establish long-term oral tolerance unless exposed to bacterial products [29]. As mentioned, mucosal tolerance is dependent on Foxp3⁺ iTreg cells [10].

Dendritic cell, macrophages, and other cells inducing tolerance or preventing tissue damage caused by respiratory antigens

Tolerogenic DC [25], alveolar macrophages [30] and other presenting cell types have been implicated in the induction of tolerance in the lungs and airways.

The place of induction of the Foxp3⁺ cells required for immune tolerance in the respiratory tract [10] is not known. However, it is known that it is dependent on lung dendritic cells migrating to the draining bronchial lymph nodes, a process that is dependent on CCR7 [31]. CD103⁺ gut DC were shown to be important in the generation of iTreg cells [32,33]; these cells migrate to the gut-draining mesenteric lymph nodes. Although both CD103⁺ and CD103⁻ lung DC migrated to the draining lymph nodes [31], the migration requirement to establish mucosal tolerance in the lung resembles the requirement for oral tolerance, which requires intact mesenteric lymph nodes.

In the lung-draining lymph nodes, a comparison of dendritic cell populations between mice subjected to tolerance versus inflammation conditions show the predominance of cells with markers of plasmacytoid DC in the tolerant mice; in contrast, CD11c⁺CD11b⁺, MHC class II^{high} conventional DC predominated in the inflammatory condition [34].

Recently, a population of myeloid-derived suppressor cells induced by high dose LPS was identified. These cells could suppress lung dendritic cells' ability to reactivate Th2 cells locally. This observation may link the known beneficial effect of LPS on the incidence of allergic disease with the cell type capable of suppressing the Th2 response [35].

In the lungs, a delicate balance must exist in relation to the generation of active TGF- β . As noted above, all studies indicate that TGF- β is required for Foxp3⁺ iTreg cell development, and that iTreg cell development is essential to establish mucosal tolerance. On the flip side, excess TGF- β is associated with lung fibrosis. In the lungs, TGF- β is locally activated by cells that express the integrin α v β 6. As expected, mice lacking α v β 6 develop exaggerated inflammation, because TGF- β activation is impaired; however, these mice are protected from pulmonary fibrosis [36].

Tissues have other means to prevent damage. A key enzyme involved in tissue protection is HO-1 [37]. HO-1 catalyses heme degradation leading to the local production of equimolar amounts of carbon monoxide (CO), biliverdin, and free-iron, which induces the expression of heavy chain ferritin, an iron-binding protein. All three metabolites resulting from heme degradation by HO-1 may have an immune protective effect [38].

Furthermore, the local immune response seems to be under tight control of local availability of specific essential amino acids [39]. The tryptophan-catabolizing enzyme indoleamine 2,3-deoxygenase (IDO) was proposed to be a key component of fetal tolerance [40]. IDO was also implicated in the maintenance of tolerance following co-stimulation blockade by CTLA4Ig, leading to Treg cell induction [41]. These observations were recently generalized with the finding that several other enzymes that catabolize essential amino acids are over-expressed under tolerogenic conditions [39].

Allergic sensitization

Allergic sensitization: IL-4 and IL-13

IL-4 and IL-13 are the most important cytokines for the induction of allergic asthma. IL-4 can directly induce differentiation of Th2 cells that produce an array of inflammatory cytokines, including IL-4 itself, IL-5 and IL-13. Although differentiation of Th2 cells can be induced in the absence of IL-4, IL-4 greatly amplifies the response. IL-4 is the main cytokine involved in IgE production, and through Th2 amplification, in eosinophilic inflammation. IL-13 is mostly responsible for the effects on lung tissue, such as the bronchial hyperresponsiveness, overproduction of mucus by epithelial cells, thickening of smooth muscle and subepithelial fibrosis (reviewed in [42]).

Polymorphisms in IL-4, IL-13, or their receptors, that result in higher production of the cytokines, or increased signaling through STAT6, are associated with increase risk of asthma [43]. Whether a genetic predisposition toward higher production of Th2 cytokines or increased Th2 cytokine signaling can by itself override induction of tolerance to innocuous antigens is not clear. Most likely other genetic and environmental factors, as well as the ability of particular allergens to stimulate innate immunity, are required to break tolerance [44]. For example, mice of the BALB/c strain are genetically predisposed to mount Th2 responses [45], yet they can become tolerant if they are administered chicken ovalbumin (OVA) through the oral or respiratory routes. Th2 cytokines are not induced during this tolerization process [10], and this explains why these mice become tolerant even though they are prone to Th2 responses. However, co-administration of low doses of LPS [46], or protease allergens [47] through the airways, would induce allergic sensitization to OVA.

Early respiratory infections in children have been associated with later development of asthma [48]. Enhanced allergic sensitization after respiratory virus infections can also be observed in experimental systems. Kim and collaborators found that after the clearance of respiratory virus infection in mice, a population of NKT-stimulated IL-13-producing macrophages persisted in the lungs and facilitated allergic sensitization [49].

The interplay between IL-4 and TGF- β is highly relevant. As indicated above, naïve cells can be induced to differentiate into Foxp3⁺ iTreg cells by TCR stimulation in the presence of TGF- β . Interestingly, Foxp3 expression on differentiated Th2 cells silences the IL-4 locus [50]. On the other hand, IL-4 inhibits TGF- β -induced iTreg differentiation *in vitro*, promoting instead the production of IL-9 and IL-10 by T cells [50,51]. Thus, if IL-4 is present *in vivo* during the initial response to a mucosal antigen, the induction of iTregs could be suppressed and Th2 differentiation can occur.

Upstream of IL-4 and IL-13: Notch, TSLP, IL25 and IL-33

Stimulation of naïve T cells in the presence of IL-4 induces STAT6-dependent IL-4R signaling and differentiation of Th2 cells. However, signals other than IL-4 can initiate Th2 differentiation [52]. For example, DC incubated with parasite extracts, cholera toxin or PGE2 can induce Th2 responses independently of IL-4 [53,54]. Under these Th2-inducing conditions the Notch ligands Jagged1 or Jagged2 are expressed in APC, and there is strong

evidence demonstrating that Jagged expression in DC promotes Th2 differentiation, and that Notch expression by T cells is required for Th2 differentiation [55,56].

In recent year it has become apparent the cytokines TSLP, IL-33 and IL-25 (IL-17E), all of which can be produced by epithelial cells, can initiate Th2 responses in the airways and probably contribute to the amplification of allergic responses [44,57-61]. TSLP is an epithelial cell-derived cytokine that can strongly activate myeloid DC. TSLP activated-DC promote differentiation of Th2 cells, expansion of memory Th2 cells and enhancement of the production of IL-5 and IL-13 through OX40/OX40 ligand interactions [60]. TSLP-DC also augments expression of the IL-25R (IL-17RB) in Th2 memory cells but not other cells.

IL-33 is a member of the IL-1 family that was identified as the ligand for ST2 (IL-1RL1), a receptor stably expressed in Th2 cells. IL-33 is expressed by non-hematopoietic tissue cells such as fibroblasts, epithelial and endothelial cells, localizes to the cell nucleus, can be released by necrosis but is inactivated by apoptosis [62,63]. IL-33 is thus consider an “alarmin”. It was recently demonstrated that, in contrast to IL-1 and IL18, IL-33 is biologically active independently of caspase-1 cleavage [64]. The regulation of IL-33 release and the multiple effects of IL-33 are not yet fully understood. IL-33 is a potent inducer of Th2 responses but has also been associated with non-atopic diseases [61].

IL-25 was initially identified as a cytokine produced by differentiated Th2 cells but not naïve cells. In untreated wild type mice, low levels of IL-25 transcripts were detected in the gastrointestinal tract and the uterus but not in lymphoid organs. Subsequently, it was found that in addition to Th2 cells, IL-25 could also be produced by IgE-stimulated mast cells, alveolar macrophages and epithelial cells stimulated by allergens [57].

Infusion of IL-25 into mice stimulated the production of IL-4, IL-5, IL-13 and IgE, and induced allergic-type pathology in the lung. Tissue pathology was dependent on IL-13 but not IL-4 production, and could be induced in the absence of T and B lymphocytes [65]. In this study, the IL-25-responsive, IL-13 producing population was identified as MHC II^{hi} CD11c^{lo} and lineage negative. Interestingly, IL-25-induced hyperresponsiveness to the broncho-constrictor methacholine in mice deficient for typical Th2 cytokines IL-4, IL-5, IL-9 and IL-13 [66].

Following the initial observations of Fort and collaborators on the IL-13 producing IL-25-responding cells, several groups have further characterized nonB-nonT cell populations considered to be of the “innate immune system” that respond to IL-25 and IL-33 with production of IL-13 and other Th2 cytokines, and that may be involved in initiating allergic responses. Lineage negative cells in gut-associated lymphoid tissue were found to be Sca-1⁺c-Kit⁺, to produce Th2 cytokines in response to IL25 and IL-33 and to expand after helminth infection or cytokine treatment [67–70]. While these cells were shown to be important for the immune response resulting in clearance of helminth parasites, their role in sensitization to aero-allergens allergens is not known. After allergic sensitization, or in response to intranasal administration of IL-25, a population of IL-17RB⁺iNKT was detected that could induce airway hyper responsiveness upon adoptive transfer [71].

Conclusions

The last years have seen a tremendous increase in the understanding of the immune responses to allergens. Advances in the regulatory T cell field lead to the discovery of peripheral induced Tregs and their importance in tolerance and homeostasis at mucosal tissues. A considerable amount of work has unveiled innate immune mechanisms and cells linked to the induction of Th2 cytokines. There has also been progress in understanding how changes in the environment and life style have lead in recent decades to an increase in

allergic diseases, as well as the nature of gene-environment interactions in promoting allergy. The next challenge is to integrate the knowledge of these diverse fields to be able to make predictions as to which individuals have the highest likelihood of develop allergies, and to develop strategies to blunt the increased incidence of allergic inflammation. From the population point of view the challenge ahead is to device strategies that promote tolerance and avoid sensitization at early age.

References and recommended reading

*of special interest

**of outstanding interest

1. Holt PG, McMenamin C. Defence against allergic sensitization in the healthy lung: the role of inhalation tolerance. *Clin Exp Allergy*. 1989; 19:255–262. [PubMed: 2660967]
2. Levings MK, Roncarolo MG. Phenotypic and functional differences between human CD4+CD25+ and type 1 regulatory T cells. *Curr Top Microbiol Immunol*. 2005; 293:303–326. [PubMed: 15981486]
3. Thomson CW, Lee BP, Zhang L. Double-negative regulatory T cells: non-conventional regulators. *Immunol Res*. 2006; 35:163–178. [PubMed: 17003518]
4. Kim JM, Rasmussen JP, Rudensky AY. Regulatory T cells prevent catastrophic autoimmunity throughout the lifespan of mice. *Nat Immunol*. 2007; 8:191–197. [PubMed: 17136045]
5. Chatila TA, Blaeser F, Ho N, Lederman HM, Voulgaropoulos C, Helms C, Bowcock AM. JM2, encoding a fork head-related protein, is mutated in X-linked autoimmunity-allergic dysregulation syndrome. *J Clin Invest*. 2000; 106:R75–81. [PubMed: 11120765]
6. Curotto de Lafaille MA, Lafaille JJ. Natural and adaptive foxp3+ regulatory T cells: more of the same or a division of labor? *Immunity*. 2009; 30:626–635. [PubMed: 19464985]
7. Chen W, Jin W, Hardegen N, Lei KJ, Li L, Marinos N, McGrady G, Wahl SM. Conversion of peripheral CD4+CD25- naive T cells to CD4+CD25+ regulatory T cells by TGF-beta induction of transcription factor Foxp3. *J Exp Med*. 2003; 198:1875–1886. [PubMed: 14676299]
8. Kim JM, Rudensky A. The role of the transcription factor Foxp3 in the development of regulatory T cells. *Immunol Rev*. 2006; 212:86–98. [PubMed: 16903908]
9. Mucida D, Kutchukhidze N, Erazo A, Russo M, Lafaille JJ, Curotto de Lafaille MA. Oral tolerance in the absence of naturally occurring Tregs. *J Clin Invest*. 2005; 115:1923–1933. [PubMed: 15937545]
10. Curotto de Lafaille MA, Kutchukhidze N, Shen S, Ding Y, Yee H, Lafaille JJ. Adaptive Foxp3+ regulatory T cell-dependent and -independent control of allergic inflammation. *Immunity*. 2008; 29:114–126. [PubMed: 18617425]
11. Apostolou I, von Boehmer H. In vivo instruction of suppressor commitment in naive T cells. *J Exp Med*. 2004; 199:1401–1408. [PubMed: 15148338]
12. Kretschmer K, Apostolou I, Hawiger D, Khazaie K, Nussenzweig MC, von Boehmer H. Inducing and expanding regulatory T cell populations by foreign antigen. *Nat Immunol*. 2005; 6:1219–1227. [PubMed: 16244650]
13. Cobbold SP, Castejon R, Adams E, Zelenika D, Graca L, Humm S, Waldmann H. Induction of foxP3+ regulatory T cells in the periphery of T cell receptor transgenic mice tolerized to transplants. *J Immunol*. 2004; 172:6003–6010. [PubMed: 15128783]
14. Yates SF, Paterson AM, Nolan KF, Cobbold SP, Saunders NJ, Waldmann H, Fairchild PJ. Induction of regulatory T cells and dominant tolerance by dendritic cells incapable of full activation. *J Immunol*. 2007; 179:967–976. [PubMed: 17617588]
15. Gottschalk RA, Corse E, Allison JP. TCR ligand density and affinity determine peripheral induction of Foxp3 in vivo. *J Exp Med*. 2010; 207:1701–1711. [PubMed: 20660617]
16. Curotto de Lafaille MA, Lino AC, Kutchukhidze N, Lafaille JJ. CD25- T cells generate CD25+Foxp3+ regulatory T cells by peripheral expansion. *J Immunol*. 2004; 173:7259–7268. [PubMed: 15585848]

17. Liang S, Alard P, Zhao Y, Parnell S, Clark SL, Kosiewicz MM. Conversion of CD4⁺ CD25⁻ cells into CD4⁺ CD25⁺ regulatory T cells in vivo requires B7 costimulation, but not the thymus. *J Exp Med*. 2005; 201:127–137. [PubMed: 15630140]
18. Graca L, Cobbold SP, Waldmann H. Identification of regulatory T cells in tolerated allografts. *J Exp Med*. 2002; 195:1641–1646. [PubMed: 12070291]
19. Qin S, Cobbold SP, Pope H, Elliott J, Kioussis D, Davies J, Waldmann H. "Infectious" transplantation tolerance. *Science*. 1993; 259:974–977. [PubMed: 8094901]
20. Ostroukhova M, Seguin-Devaux C, Oriss TB, Dixon-McCarthy B, Yang L, Ameredes BT, Corcoran TE, Ray A. Tolerance induced by inhaled antigen involves CD4⁺ T cells expressing membrane-bound TGF-beta and FOXP3. *J Clin Invest*. 2004; 114:28–38. [PubMed: 15232609]
21. Ostroukhova M, Qi Z, Oriss TB, Dixon-McCarthy B, Ray P, Ray A. Treg-mediated immunosuppression involves activation of the Notch-HES1 axis by membrane-bound TGF-beta. *J Clin Invest*. 2006; 116:996–1004. [PubMed: 16543950]
22. Roncarolo MG, Gregori S, Battaglia M, Bacchetta R, Fleischhauer K, Levings MK. Interleukin-10-secreting type 1 regulatory T cells in rodents and humans. *Immunol Rev*. 2006; 212:28–50. [PubMed: 16903904]
- 23*. Gandhi R, Kumar D, Burns EJ, Nadeau M, Dake B, Laroni A, Kozoriz D, Weiner HL, Quintana FJ. Activation of the aryl hydrocarbon receptor induces human type 1 regulatory T cell-like and Foxp3⁺ regulatory T cells. *Nat Immunol*. 2010
- 24*. Apetoh L, Quintana FJ, Pot C, Joller N, Xiao S, Kumar D, Burns EJ, Sherr DH, Weiner HL, Kuchroo VK. The aryl hydrocarbon receptor interacts with c-Maf to promote the differentiation of type 1 regulatory T cells induced by IL-27. *Nat Immunol*. 2010 This paper, together with 23, show that AHR activation can lead to induction of Tr1 cells.
25. Akbari O, Freeman GJ, Meyer EH, Greenfield EA, Chang TT, Sharpe AH, Berry G, DeKruyff RH, Umetsu DT. Antigen-specific regulatory T cells develop via the ICOS-ICOS-ligand pathway and inhibit allergen-induced airway hyperreactivity. *Nat Med*. 2002; 8:1024–1032. [PubMed: 12145647]
26. Strachan DP. Hay fever, hygiene, and household size. *BMJ*. 1989; 299:1259–1260. [PubMed: 2513902]
- 27*. Round JL, Mazmanian SK. Inducible Foxp3⁺ regulatory T-cell development by a commensal bacterium of the intestinal microbiota. *Proc Natl Acad Sci U S A*. 2010; 107:12204–12209. This paper shows that gut microbiota can impact local Treg cell induction. [PubMed: 20566854]
28. Monteiro M, Almeida CF, Caridade M, Ribot JC, Duarte J, Agua-Doce A, Wollenberg I, Silva-Santos B, Graca L. Identification of regulatory Foxp3⁺ invariant NKT cells induced by TGF-beta. *J Immunol*. 2010; 185:2157–2163. [PubMed: 20639482]
29. Wannemuehler MJ, Kiyono H, Babb JL, Michalek SM, McGhee JR. Lipopolysaccharide (LPS) regulation of the immune response. LPS converts germfree mice to sensitivity to oral tolerance induction. *J Immunol*. 1982; 129:959–965. [PubMed: 6980928]
30. Thepen T, Kraal G, Holt PG. The role of alveolar macrophages in regulation of lung inflammation. *Ann N Y Acad Sci*. 1994; 725:200–206. [PubMed: 8030991]
31. Hintzen G, Ohl L, del Rio ML, Rodriguez-Barbosa JJ, Pabst O, Kocks JR, Kregge J, Hardtke S, Forster R. Induction of tolerance to innocuous inhaled antigen relies on a CCR7-dependent dendritic cell-mediated antigen transport to the bronchial lymph node. *J Immunol*. 2006; 177:7346–7354. [PubMed: 17082654]
32. Sun CM, Hall JA, Blank RB, Bouladoux N, Oukka M, Mora JR, Belkaid Y. Small intestine lamina propria dendritic cells promote de novo generation of Foxp3⁺ T reg cells via retinoic acid. *J Exp Med*. 2007; 204:1775–1785. [PubMed: 17620362]
33. Coombes JL, Siddiqui KR, Arancibia-Carcamo CV, Hall J, Sun CM, Belkaid Y, Powrie F. A functionally specialized population of mucosal CD103⁺ DCs induces Foxp3⁺ regulatory T cells via a TGF-beta and retinoic acid-dependent mechanism. *J Exp Med*. 2007; 204:1757–1764. [PubMed: 17620361]
34. Oriss TB, Ostroukhova M, Seguin-Devaux C, Dixon-McCarthy B, Stolz DB, Watkins SC, Pillemer B, Ray P, Ray A. Dynamics of dendritic cell phenotype and interactions with CD4⁺ T cells in airway inflammation and tolerance. *J Immunol*. 2005; 174:854–863. [PubMed: 15634907]

35. Arora M, Poe SL, Oriss TB, Krishnamoorthy N, Yarlagadda M, Wenzel SE, Billiar TR, Ray A, Ray P. TLR4/MyD88-induced CD11b(+)Gr-1(int)F4/80(+) non-migratory myeloid cells suppress Th2 effector function in the lung. *Mucosal Immunol*.
36. Munger JS, Huang X, Kawakatsu H, Griffiths MJ, Dalton SL, Wu J, Pittet JF, Kaminski N, Garat C, Matthay MA, et al. The integrin α v β 6 binds and activates latent TGF β 1: a mechanism for regulating pulmonary inflammation and fibrosis. *Cell*. 1999; 96:319–328. [PubMed: 10025398]
37. Yamashita K, Ollinger R, McDaid J, Sakahama H, Wang H, Tyagi S, Csizmadia E, Smith NR, Soares MP, Bach FH. Heme oxygenase-1 is essential for and promotes tolerance to transplanted organs. *FASEB J*. 2006; 20:776–778. [PubMed: 16473885]
38. Gozzelino R, Jeney V, Soares MP. Mechanisms of cell protection by heme oxygenase-1. *Annu Rev Pharmacol Toxicol*. 2010; 50:323–354. [PubMed: 20055707]
39. Cobbold SP, Adams E, Farquhar CA, Nolan KF, Howie D, Lui KO, Fairchild PJ, Mellor AL, Ron D, Waldmann H. Infectious tolerance via the consumption of essential amino acids and mTOR signaling. *Proc Natl Acad Sci U S A*. 2009; 106:12055–12060. [PubMed: 19567830]
40. Munn DH, Zhou M, Attwood JT, Bondarev I, Conway SJ, Marshall B, Brown C, Mellor AL. Prevention of allogeneic fetal rejection by tryptophan catabolism. *Science*. 1998; 281:1191–1193. [PubMed: 9712583]
41. Fallarino F, Grohmann U, You S, McGrath BC, Cavener DR, Vacca C, Orabona C, Bianchi R, Belladonna ML, Volpi C, et al. The combined effects of tryptophan starvation and tryptophan catabolites down-regulate T cell receptor zeta-chain and induce a regulatory phenotype in naive T cells. *J Immunol*. 2006; 176:6752–6761. [PubMed: 16709834]
- 42*. Finkelman FD, Hogan SP, Hershey GK, Rothenberg ME, Wills-Karp M. Importance of cytokines in murine allergic airway disease and human asthma. *J Immunol*. 2010; 184:1663–1674. [PubMed: 20130218]
- 43*. Vercelli D. Discovering susceptibility genes for asthma and allergy. *Nat Rev Immunol*. 2008; 8:169–182. [PubMed: 18301422]
- 44*. Paul WE, Zhu J. How are T(H)2-type immune responses initiated and amplified? *Nat Rev Immunol*. 2010; 10:225–235. Together with 42 and 43, these are three clear and comprehensive reviews. [PubMed: 20336151]
45. Bix M, Wang ZE, Thiel B, Schork NJ, Locksley RM. Genetic regulation of commitment to interleukin 4 production by a CD4(+) T cell-intrinsic mechanism. *J Exp Med*. 1998; 188:2289–2299. [PubMed: 9858515]
46. Eisenbarth SC, Piggott DA, Huleatt JW, Visintin I, Herrick CA, Bottomly K. Lipopolysaccharide-enhanced, toll-like receptor 4-dependent T helper cell type 2 responses to inhaled antigen. *J Exp Med*. 2002; 196:1645–1651. [PubMed: 12486107]
47. Yu HS, Angkasekwinai P, Chang SH, Chung Y, Dong C. Protease allergens induce the expression of IL-25 via Erk and p38 MAPK pathway. *J Korean Med Sci*. 2010; 25:829–834. [PubMed: 20514301]
48. Bartlett NW, McLean GR, Chang YS, Johnston SL. Genetics and epidemiology: asthma and infection. *Curr Opin Allergy Clin Immunol*. 2009; 9:395–400. [PubMed: 19644362]
49. Kim EY, Battaile JT, Patel AC, You Y, Agapov E, Grayson MH, Benoit LA, Byers DE, Alevy Y, Tucker J, et al. Persistent activation of an innate immune response translates respiratory viral infection into chronic lung disease. *Nat Med*. 2008; 14:633–640. [PubMed: 18488036]
50. Kwon HK, So JS, Lee CG, Sahoo A, Yi HJ, Park JN, Lim SY, Hwang KC, Jun CD, Chun JS, et al. Foxp3 induces IL-4 gene silencing by affecting nuclear translocation of NF κ B and chromatin structure. *Mol Immunol*. 2008; 45:3205–3212. [PubMed: 18395799]
51. Dardalhon V, Awasthi A, Kwon H, Galileos G, Gao W, Sobel RA, Mitsdoerffer M, Strom TB, Elyaman W, Ho IC, et al. IL-4 inhibits TGF β -induced Foxp3+ T cells and, together with TGF β , generates IL-9+ IL-10+ Foxp3(–) effector T cells. *Nat Immunol*. 2008; 9:1347–1355. [PubMed: 18997793]
52. Schmitz J, Thiel A, Kuhn R, Rajewsky K, Muller W, Assenmacher M, Radbruch A. Induction of interleukin 4 (IL-4) expression in T helper (Th) cells is not dependent on IL-4 from non-Th cells. *J Exp Med*. 1994; 179:1349–1353. [PubMed: 8145047]

53. Jankovic D, Kullberg MC, Noben-Trauth N, Caspar P, Paul WE, Sher A. Single cell analysis reveals that IL-4 receptor/Stat6 signaling is not required for the in vivo or in vitro development of CD4⁺ lymphocytes with a Th2 cytokine profile. *J Immunol.* 2000; 164:3047–3055. [PubMed: 10706693]
54. Gagliardi MC, Sallusto F, Marinaro M, Langenkamp A, Lanzavecchia A, De Magistris MT. Cholera toxin induces maturation of human dendritic cells and licenses them for Th2 priming. *Eur J Immunol.* 2000; 30:2394–2403. [PubMed: 10940931]
55. Krishnamoorthy N, Oriss TB, Paglia M, Fei M, Yarlagadda M, Vanhaesebroeck B, Ray A, Ray P. Activation of c-Kit in dendritic cells regulates T helper cell differentiation and allergic asthma. *Nat Med.* 2008; 14:565–573. [PubMed: 18454155]
56. Amsen D, Antov A, Flavell RA. The different faces of Notch in T-helper-cell differentiation. *Nat Rev Immunol.* 2009; 9:116–124. [PubMed: 19165228]
57. Saenz SA, Taylor BC, Artis D. Welcome to the neighborhood: epithelial cell-derived cytokines license innate and adaptive immune responses at mucosal sites. *Immunol Rev.* 2008; 226:172–190. [PubMed: 19161424]
58. Liu YJ. Thymic stromal lymphopoietin and OX40 ligand pathway in the initiation of dendritic cell-mediated allergic inflammation. *J Allergy Clin Immunol.* 2007; 120:238–244. quiz 245–236. [PubMed: 17666213]
59. He R, Geha RS. Thymic stromal lymphopoietin. *Ann N Y Acad Sci.* 2010; 1183:13–24. [PubMed: 20146705]
60. Liu YJ. TSLP in epithelial cell and dendritic cell cross talk. *Adv Immunol.* 2009; 101:1–25. [PubMed: 19231591]
61. Liew FY, Pitman NI, McInnes IB. Disease-associated functions of IL-33: the new kid in the IL-1 family. *Nat Rev Immunol.* 2010; 10:103–110. [PubMed: 20081870]
62. Cayrol C, Girard JP. The IL-1-like cytokine IL-33 is inactivated after maturation by caspase-1. *Proc Natl Acad Sci U S A.* 2009; 106:9021–9026. [PubMed: 19439663]
63. Luthi AU, Cullen SP, McNeela EA, Duriez PJ, Afonina IS, Sheridan C, Brumatti G, Taylor RC, Kersse K, Vandenabeele P, et al. Suppression of interleukin-33 bioactivity through proteolysis by apoptotic caspases. *Immunity.* 2009; 31:84–98. [PubMed: 19559631]
64. Talabot-Ayer D, Lamacchia C, Gabay C, Palmer G. Interleukin-33 is biologically active independently of caspase-1 cleavage. *J Biol Chem.* 2009; 284:19420–19426. [PubMed: 19465481]
65. Fort MM, Cheung J, Yen D, Li J, Zurawski SM, Lo S, Menon S, Clifford T, Hunte B, Lesley R, et al. IL-25 induces IL-4, IL-5, and IL-13 and Th2-associated pathologies in vivo. *Immunity.* 2001; 15:985–995. [PubMed: 11754819]
66. Ballantyne SJ, Barlow JL, Jolin HE, Nath P, Williams AS, Chung KF, Sturton G, Wong SH, McKenzie AN. Blocking IL-25 prevents airway hyperresponsiveness in allergic asthma. *J Allergy Clin Immunol.* 2007; 120:1324–1331. [PubMed: 17889290]
67. Neill DR, Wong SH, Bellosi A, Flynn RJ, Daly M, Langford TK, Bucks C, Kane CM, Fallon PG, Pannell R, et al. Nuocytes represent a new innate effector leukocyte that mediates type-2 immunity. *Nature.* 464:1367–1370. [PubMed: 20200518]
68. Price AE, Liang HE, Sullivan BM, Reinhardt RL, Eislely CJ, Erle DJ, Locksley RM. Systemically dispersed innate IL-13-expressing cells in type 2 immunity. *Proc Natl Acad Sci U S A.* 107:11489–11494. [PubMed: 20534524]
69. Saenz SA, Siracusa MC, Perrigoue JG, Spencer SP, Urban JF Jr, Tocker JE, Budelsky AL, Kleinschek MA, Kastelein RA, Kambayashi T, et al. IL25 elicits a multipotent progenitor cell population that promotes T(H)2 cytokine responses. *Nature.* 464:1362–1366. [PubMed: 20200520]
70. Moro K, Yamada T, Tanabe M, Takeuchi T, Ikawa T, Kawamoto H, Furusawa J, Ohtani M, Fujii H, Koyasu S. Innate production of T(H)2 cytokines by adipose tissue-associated c-Kit(+)Sca-1(+) lymphoid cells. *Nature.* 463:540–544. [PubMed: 20023630]
71. Stock P, Lombardi V, Kohlrantz V, Akbari O. Induction of airway hyperreactivity by IL-25 is dependent on a subset of invariant NKT cells expressing IL-17RB. *J Immunol.* 2009; 182:5116–5122. [PubMed: 19342692]