

Maria A. Curotto de Lafaille · Juan J. Lafaille

The role of regulatory T cells in allergy

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Abstract Atopic diseases are characterized by Th2 and IgE responses to common environmental and food antigens. In vivo, IgE production depends on interactions between allergen-specific B lymphocytes and Th2 lymphocytes. IgE levels are extremely low in normal individuals, suggesting that IgE production is under strong regulation. One of the reasons behind the lack of atopy in healthy individuals is the activity of regulatory T cells, which prevent naïve T helper cell precursors from acquiring a differentiated Th2 phenotype. In addition to naturally occurring regulatory T cells, atopy can be prevented by allergen-specific tolerant/regulatory cells induced through mucosal stimulation, and by mechanisms that directly suppress *I ϵ* sterile transcript production on activated B lymphocytes. This article reviews the recent progress on thymic-derived as well as peripherally induced regulatory T cells as they relate to atopy. The latter discussion also includes regulatory T cells that arise through immunotherapy.

Keywords Immunoglobulin E · Class switching · Atopy · CD4⁺CD25⁺ T cells · Th2 differentiation

Overview

Atopic diseases are characterized by the production of IgE antibodies to common non-infectious environmental or food proteins. The production of IgE antibodies is highly dependent on cognate T/B cell interactions involving the co-stimulatory molecules CD40/CD40 ligand (L) and on the cytokine IL-4 [63]. Atopy is also associated with, and depends on, the presence of allergen-specific Th2 lymphocytes.

Several factors may contribute to the development of atopic responses:

M. A. Curotto de Lafaille (✉) · J. J. Lafaille (✉)

Program of Molecular Pathogenesis, Skirball Institute of Biomolecular Medicine
and Department of Pathology,

New York University School of Medicine, 540 First Avenue, New York, NY 10016, USA

e-mail: curotto@saturn.med.nyu.edu · Tel.: +1-212-263-1469 · Fax: +1-212-263-5711

e-mail: lafaille@saturn.med.nyu.edu · Tel.: +1-212-263-1469/1489 · Fax: +1-212-263-5711

1. A genetic predisposition to biased Th2 responses by, for example, higher IL-4/IL-13 production or more efficient IL-4/IL-13 receptor signaling, or a higher propensity of the B cells to switch to IgE. While the former topic has been reviewed in detail elsewhere [19, 99, 111], we discuss aspects of the latter in the next section;
2. Genetic deficiencies in immunoregulatory mechanisms, including increased inflammatory response of epithelial cells, increased activity of dendritic cells, or deficiencies in regulatory T cells. We focus on reviewing the literature on regulatory T cells in Sections 2–5;
3. Environmental factors, such as infections, that may provide adjuvant activity simultaneous to allergen exposure. This is briefly discussed in Section 6;
4. Molecular properties of allergens that may generate inflammatory/co-stimulatory signals promoting stronger immune responses. This topic is discussed in Section 7.

1. Switching to IgE and its control in B lymphocytes

Naïve B cells express the constant region of IgM and IgD; switching to other constant regions (generating IgG, IgE or IgA) is brought about by B cell activation (Fig. 1). Of all secreted immunoglobulins, IgE is found at the lowest concentration in normal individuals, suggesting that the production of IgE is strongly controlled. B cell stimulation with anti-CD40 + IL-4, soluble CD40L + IL-4, LPS + IL-4, or cognate interaction with Th2 cells, leads to IgG1 and IgE class switching in vitro. However, even under these favorable conditions, the percentage of cells switching to IgE is low compared to the percentage of cells switching to IgG1. It has been shown that switching to IgE requires larger number of cell divisions than class switching to IgG1 [41]. This observation indicates that it may take several replication cycles to either relieve transcriptional suppression at the ϵ locus, or build up specific activator factors required for class switching to IgE. It is therefore possi-

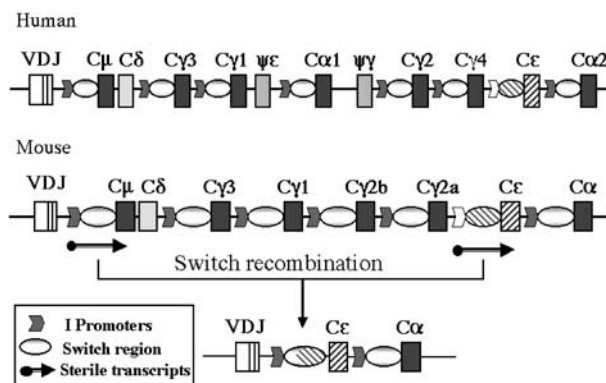


Fig. 1 Schematic organization of the human and mouse Ig constant region loci in B lymphocytes after VDJ rearrangement. The constant region genes (C μ , C δ , C ϵ or C α), the switch regions, and the intergenic sterile transcript switch region promoters (I promoters) are shown. ψ identifies a pseudogene. Class switching to IgE is preceded by the production of I ϵ sterile transcripts, followed by recombination between two switch regions and deletion of the intervening segments. Extensive review on class switching mechanisms can be found elsewhere [27, 49, 57]

ble that activated B cells may undergo apoptosis or differentiate into memory or plasma cells before switching to IgE can be accomplished.

Class switching to IgE is preceded by the production of germline transcripts driven by the IL-4-responsive I ϵ promoter (Fig. 1). Replacement of the I ϵ promoter and exon with an enhancer/promoter active in all B cells led to constitutive expression of the C ϵ germline transcript and IL-4-independent switching to IgE [112], and down-regulation of I ϵ transcription caused a reduction in IgE switching [113]. Activation of the I ϵ promoter requires binding of phosphorylated STAT-6, NF- κ B, BSAP, C/EBP, PU.1 and E2A transcription factors [63, 88]. Switching ultimately occurs through recombination between S regions, repetitive sequences located between the I promoter and the first exon encoding the constant region (Fig. 1). Switching, thus, entails DNA recombination and is often abbreviated as CSR (class switch recombination, reviewed in [27, 49, 57]).

In naïve human B cells, transcription through the I ϵ and I γ promoters appears to be actively suppressed by a transcriptional repressor complex containing the homeodomain proteins HoxC4 and Oct-1 together with the Ku70/Ku86 heterodimer. This complex binds to the switch regulatory-ATTT sequences (SRE) of human S ϵ and S γ regions, but not to human S α regions, which lack the SRE elements. CD40 engagement on B cells results in the dissociation of the complex from the SRE elements and activation of CSR [77].

Germline transcription of IgE and IgG1 in activated B cells can be inhibited by the combination of the cytokines IL-12 and IL-18 [115], IFN- γ [78, 113], transforming growth factor (TGF)- β , bacterial CpG-rich sequences [56], or expression of the proto oncogene Bcl-6 [40].

The effect of IL-12 + IL-18 on anti-CD40 + IL-4-stimulated B cells depends on the induction of IFN- γ production by B cells [101, 115]. The mechanism of IFN- γ inhibition of ϵ germline transcription was studied using a reporter gene under the control of the IL-4 responsive element of the I ϵ promoter. Inhibition was dependent on STAT-1 and SOCS-1, and resulted in the loss of STAT-6 activation over time [101]. On the other hand, bacterial CpG, which binds Toll-like receptor 9 (TLR9) on B lymphocytes, can inhibit germline ϵ and γ 1 germline transcripts by a STAT-1-, IFN- γ -independent mechanism. Bacterial CpG induces the expression of the transcription factor T-bet on B lymphocytes, which has been shown to be required for germline transcription and CSR to IgG2a [56, 67]. Interestingly, CpG-containing oligonucleotides not only prevent IgE switching in naïve B cells, but also prevent bronchial hyperresponsiveness in already sensitized animals [50].

The proto-oncogene Bcl-6 is a transcriptional repressor expressed by germinal center B cells. Mice deficient in Bcl-6 develop an inflammatory disease characterized by Th2 differentiated cells and hyper IgE and IgG1 production [114]. At the B cell level, it has been shown that Bcl-6 represses I ϵ transcription by competing with STAT-6 for binding to the I ϵ promoter [40].

The inhibitory effects of IL-21 and the transcription factor Id2 on B lymphocytes are interesting in that they affect ϵ but not γ 1 germline transcription. IL-21 is a cytokine of the IL-2/IL-15 family, which signals using the common γ chain (CD132). IL-21 receptor-deficient mice display a reduced antigen-specific IgG1 response and an enhanced IgE response, suggesting that IL-21 down-regulates IgE switching [64]. Indeed, addition of IL-21 to LPS + IL-4 B cell cultures led to increased IgG1 and decreased IgE switching [89]. The mechanism by which IL-21 inhibits ϵ germline transcription remains unknown; however, it does not involve inhibition of STAT-6 or NF- κ B activation [89]. The selective down-regulation of IgE switching by IL-21 points to a possible therapeutic use of this cy-

tokine, although *in vivo* administration of IL-21 reduced IgE production less dramatically than the *in vitro* data would have predicted [89]. In addition, it is unknown whether IL-21 could regulate IgE production by switched memory B cells, a key aspect for the treatment of atopy.

Id2 is a transcription factor of the Id family that is involved in the negative regulation of E2A proteins. Id2-deficient mice have increased IgE levels in serum, and Id2-deficient B cells preferentially switch to IgE *in vitro*. Id2 is induced by TGF- β in B lymphocytes, and suppresses germline I ϵ transcription by interacting with E2A and Pax5 and inhibiting their DNA binding to the I ϵ promoter [88].

2. Suppression of Th2 responses and IgE class switching by CD4⁺ regulatory T cells

Work performed mostly during the 1970s amply documented that antigen-specific IgE responses could be down-regulated by the transfer of T cells [62, 93, 94].

Using a mouse model of hyper IgE response, we demonstrated that transfer of polyclonal CD4⁺ regulatory T cells (referred to as Treg cells or Tregs) prevented the development of antigen-specific Th2 cells, the formation of germinal centers, and class switching to IgE. The suppression in this model did not affect the initial proliferation and IL-2 production of antigen-specific T cells, but blocked the transition from this early activation to the differentiated T helper state. Regulatory T cells present in the CD4⁺CD25⁺ and CD4⁺CD25⁻ populations were equally efficient in protecting mice against hyper IgE [24]. In this hyper IgE system, the transfer of regulatory T cells after the IgE response was established did not substantially decrease IgE levels. This model illustrates one of the roles of regulatory T cells, i.e. to impair the surge of fully differentiated T helper cells during normal immune responses. While these responses generate IgG antibodies, they do not produce T helper cells with high inflammatory potential and do not result in high production of IgE antibodies. In addition, this model shows that this type of regulation is very important in preventing Th2/IgE responses, but is much less effective in eradicating an established response.

One of the most dramatic effects of regulatory T cell deficiency is the IPEX syndrome (X-linked immune dysregulation, polyendocrinopathy, enteropathy, and X-linked inheritance syndrome) also known as XLAAD (X-linked autoimmunity-allergic dysregulation syndrome). Patients with IPEX syndrome have a profound immune dysregulation that causes aggressive autoimmune and atopic disease. Signs of atopy include eczema, eosinophilia and high IgE [33]. IPEX is a monogenic disease replicated by the natural mouse mutant strain Scurfy, and it is caused by a mutation in the transcription repressor gene *Foxp3* [13, 15, 110]. *Foxp3* is expressed in thymus-derived CD4⁺CD25⁺ regulatory T cells and has been shown to establish an anergic/regulatory cellular program when over-expressed in T cells [29, 44, 47].

A variety of protocols have been developed to generate regulatory T cells *in vitro* from naïve cells (reviewed in [23, 55, 107]). Notably, incomplete stimulation (low co-stimulation), or stimulation in the presence of the cytokines TGF- β or IL-10 or other anti-inflammatory compounds, leads to T cells with regulatory potential. Both TGF- β and IL-10 have the capacity to stimulate their own production by the target cells. Regulatory T cells producing IL-10 have been called Tr1 cells [37], while T cells producing TGF- β have been called Th3 cells [17]. Ovalbumin-specific Tr1 cells were shown to suppress IgE but not

IgG1 or IgG2 production when injected into BALB/c mice before immunization with ovalbumin. The suppression of IgE production was abolished by treatment with anti-IL-10 receptor antibodies [20].

Antigen-specific regulatory T cells can be induced *in vivo*. For instance, CD4⁺ Tr1-like T cells were induced by treatment of mice with a heat-killed *Mycobacterium vaccae* suspension at the time of immunization with ovalbumin, which was used as a model allergen. The regulatory T cells were CD45RB^{low}, secreted IL-10 and suppressed eosinophilia and bronchial hyperresponsiveness (BHR). The suppressor activity of *M. vaccae*-induced Treg cells *in vivo* and *in vitro* was dependent both on IL-10 and TGF- β [117].

Ovalbumin-specific CD4⁺CD25⁺ Treg cells developed in ovalbumin-specific DO11.10 TCR transgenic x RAG-deficient mice crossed with mice expressing ovalbumin under the surfactant protein C promoter, but not in DO11.10 TCR transgenic x RAG-deficient mice in which antigen was not expressed [38]. In this experimental system (in which the generation of Treg cells occurred through pathways akin to the ones reported by Jordan et al. [45] and Apostolou et al. [5]) Treg cells were able to control IL-4 and IgE production, and the inflammatory infiltrate in the lungs was diminished in the presence of Tregs; however, antigen-specific CD4⁺CD25⁺ Treg cells did not affect bronchial hyperreactivity [38].

CD4⁺ CD45RC^{low} self-reactive regulatory T cells are induced in the Lewis rats by chronic injection of HgCl₂. These cells have the capacity to prevent Th2-dependent HgCl₂-induced autoimmunity in (LEW x BN) F1 hybrids, and EAE in LEW rats. These regulatory cells produce IL-2, IFN- γ , TGF- β and IL-10 upon stimulation, and their protective role is abolished by injection of anti-TGF- β antibodies [66].

Tolerant cells with regulatory activity and with characteristics of Tr1 or Th3 cells can be induced *in vivo* by respiratory or oral immunization, respectively. Most environmental allergens contact the body through the mucosal surfaces of the respiratory and digestive tract. A large body of experimental data from animal models using antigens such as ovalbumin has shown that immunization through the respiratory or oral routes in the absence of adjuvant generally leads to immune tolerance [2, 43, 62, 75]. Tolerance is achieved by deletion of antigen-specific cells, and by the induction of anergic/regulatory T cells. Mucosal delivery of antigen induces Th2-type cytokine production in antigen-specific T cells, with IL-4 and IL-10 being produced after respiratory exposure, and IL-4 and TGF- β after oral exposure. Repeated stimulation of T cells with antigen-pulsed dendritic cells from the airways or the digestive tract shifts the cytokine balance towards the suppressor cytokines IL-10 and TGF- β , respectively, and confers tolerogenic/suppressive properties to the T cells [2].

From the point of view of their generation, regulatory T cells can be divided into two groups, those that display regulatory properties before they leave the thymus and those that acquire regulatory properties as a consequence of peripheral induction [11]. The relationship between the two groups is, at the present time, not clear. However, it has been shown that naïve T cells that do not normally develop into Treg cells (such as those of the DO11.10 RAG-deficient mice), can be induced to do so by *in vitro* or *in vivo* tolerogenic protocols (reviewed in [23, 73]). The different modes of Treg generation and the putative properties of these two regulatory cell types are schematically depicted in Fig. 2.

Given the inhibitory effect of IFN- γ , IL-12 and IL-18 in B cell class switching to IgE (see above), as well as the known antagonistic effect of Th1 cytokines in Th2 cell differentiation and proliferation, it was interesting to determine whether fully differentiated Th1 cells could be beneficial in allergy. Predisposition to Th1 responses is associated with re-

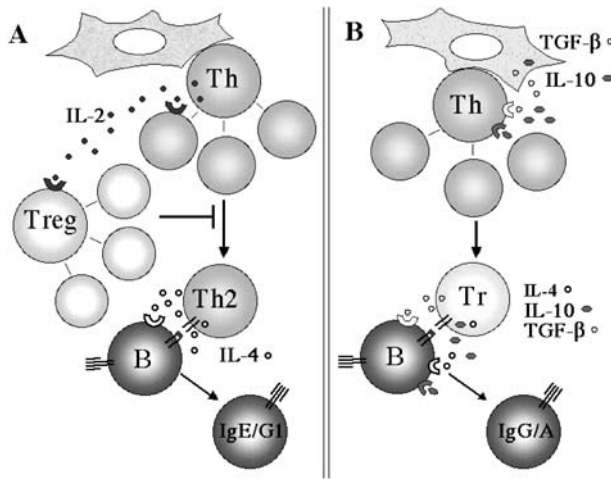


Fig. 2 Two ways of regulating IgE production. **A** Impairment of Th2 differentiation by Treg cells. Naïve, allergen-specific Th precursor cells respond to allergen presented on dendritic cells, proliferate and secrete IL-2. IL-2 stimulates Treg cells [32], which in turn inhibit the differentiation of Th cells to Th2 and B lymphocyte class switching to IgE. **B** Induction of “tolerant” allergen-specific T cells by stimulation through mucosa. Allergen-specific Th precursor cells stimulated by IL-10/TGF- β -producing dendritic cells differentiate into IL-4/IL-10/TGF- β -producing regulatory T cells (*Tr*), which provide help to allergen-specific B lymphocytes to switch to non-pathogenic IgG and IgA isotypes

duced incidence of allergy and asthma [70, 95, 96, 108]. Administration of IFN- γ , IL-12, or IL-12 + IL-18 was shown to inhibit airways responses to allergens, although IL-18 alone can contribute to Th2 and IgE responses [18, 34, 39, 42, 109]. However, adoptive transfer of allergen-specific Th1 cells did not suppress but rather contributed to Th2-mediated airway inflammation in mouse models of asthma [39, 71, 72]. It thus appears that the treatment of established Th2 responses with Th1 cells or cytokines will entail considerable risk of worsening inflammation. Both Th1 and Th2 cells are pro-inflammatory T helper cell types, although each Th type promotes a different kind of inflammation. It is becoming increasingly apparent that regulatory T cells are different from both Th types and, in fact, effectively control the development of both Th types.

3. Regulatory T cells in human atopy

Regulatory T cells protect individuals against autoimmune diseases, but also make more difficult the development of highly differentiated Th1 and Th2 cells during immune responses [23, 73]. Thus, one of the mechanisms leading to atopy could involve a failure in regulatory T cell function. This failure could be temporary, local, or permanent (genetically determined) and systemic.

Human CD4⁺CD25⁺ regulatory T cells from peripheral blood have been characterized in several laboratories, and it has been shown that they display similar properties to the rodent CD4⁺CD25⁺ anergic cell population (reviewed in [23, 73]). In brief, human CD4⁺CD25⁺ cells from normal individuals have an anergic phenotype, being deficient in IL-2 production and proliferation in response to polyclonal activators, such as phytohe-

magglutinin, anti-CD3 antibodies or allogeneic cells. These anergic responses were reverted by IL-2 and/or IL-15 addition. Human CD25⁺ cells also exert contact-dependent suppressor activity on proliferation and IL-2 production by CD4⁺CD25⁻ cells. Human CD4⁺CD25⁺ cells could confer suppressive activity to CD4⁺CD25⁻ T cells in a process referred to as infectious tolerance. Suppressor cells induced by infectious tolerance exerted suppression in a cell contact independent, cytokine (IL-10 or TGF- β)-dependent manner (reviewed in [23]).

Recent experiments indicate that the human CD4⁺CD25⁺ population contains antigen-specific regulatory T cells. Purified CD4⁺CD25⁺ cells suppressed the proliferation of CD4⁺CD25⁻ stimulated with food (cow's milk antigen), self (hHSP60) and vaccine antigens (tetanus toxoid). Human CD4⁺CD25⁺ cells displayed a diverse repertoire and had short telomers, thus representing cells that have divided repeatedly. The authors proposed that these cells arise after repeated peripheral stimulation [92].

Some recent studies have started to address the role of regulatory T cells in human atopic diseases. Since regulatory T cells can prevent Th2 differentiation and class switching to IgE to immunizing antigens, it is tempting to speculate that the development of atopy may involve a failure in regulatory T cell function.

Mutations that seriously compromise regulatory T cell function, such as the IPEX mutation, produce a dramatic dysregulation of immune responses, causing severe autoimmunity and allergy. In fact, scurfy mice (see section above) die at 3–4 weeks of age. Considering the clinical symptoms of atopic individuals, it wouldn't be expected that atopy entailed a complete failure in Treg function. Instead, partial, temporary, antigen-specific, or tissue-specific deficiencies in Treg cells would be expected. Because of the obvious limitations of human studies, analysis of Treg cells in atopic patients have utilized peripheral blood lymphocytes, and have analyzed CD4⁺CD25⁺ regulatory T cell numbers and function *ex vivo* and *in vitro*. Although these are important initial contributions, one must bear in mind that peripheral blood may not reflect the status of regulatory T cells in draining lymph nodes or in the affected tissues. In addition, although the CD4⁺CD25⁺ T cell population of rodents and humans is mostly composed of regulatory T cells [76, 81], we and others have shown that the CD4⁺CD25⁻ population also contains regulatory T cells (reviewed in [23]).

Lara-Marquez et al. [54] studied the percentage of CD25⁺CD4⁺ T cells in acute atopic asthmatics, stable atopic asthmatics, atopic non-asthmatics and healthy non-atopics. They found an increased percentage of CD45RO⁺CD4⁺CD25⁺ cells in PBMCs of acute atopic asthmatics and stable atopic asthmatics compared to normal non-atopic patients. Atopic non-asthmatics did not differ significantly from normal patients. Tiemessen et al. [98] tested the suppressor activity of purified CD4⁺CD25⁺ cells from patients with IgE-mediated cow's milk allergy. They described that CD4⁺CD25⁺ cells from the allergic patients were able to suppress the proliferation of autologous CD4⁺CD25⁻ cells stimulated with cow's milk antigen, and thus, that they were not functionally impaired.

Bellinghausen et al. [8] studied the allergen-specific proliferative response, cytokine production and suppressor activity of CD4⁺CD25⁺ peripheral blood T cells from patients with allergic rhino-conjunctivitis to birch pollen and grass pollen, and from non-atopic healthy donors. The authors divided the atopic patients into two groups according to their high or low production of IL-4 and IL-10 in culture. They found that CD4⁺CD25⁺ cells from the low cytokine producer allergic patients (the majority of allergics) behaved as anergic and suppressors. CD4⁺CD25⁺ cells from the high cytokine producer patients prolifer-

ated and secreted cytokines *in vitro*, and displayed greatly reduced suppressor activity. Interestingly, there was no correlation between low suppressor activity and any clinical or laboratory parameter tested.

We compared the distribution of CD25⁺ cells in CD45RA⁺ and CD45RO⁺ CD4⁺ T cells in atopic asthmatic patients, atopic dermatitis patients with asthma, and normal non-atopic donors. We found no differences in the percentage of CD45RO⁺ CD4⁺CD25⁺ cells between the groups. Because CD25 is a marker of recent activation, part of the CD25⁺ cells could be recently activated cells rather than regulatory T cells. To address this issue, we purified the CD4⁺CD25⁺ cells from the patient samples and determined the expression levels of the transcription factor Foxp3, a marker of suppressor function in CD4 T cells but not of recently activated CD25⁺ effector T cells. We observed no differences in Foxp3 mRNA expression between atopic and non-atopic individuals (unpublished results).

These limited analyses of CD25⁺ Treg cells in atopic patients have not yet provided an explanation for the failure of immunoregulatory mechanisms in these patients. The results described above indicate that atopic patients are not devoid of the CD4⁺CD25⁺ cell population and that, in large proportion of atopic patients, this population is functional, and is comprised of Foxp3-expressing anergic cells. However, peripheral blood CD4⁺CD25⁺ T cells from some of the atopic patients showed decreased *in vitro* suppressor activity with little or no decrease in numbers. It is possible that in these individuals the Treg activity was neutralized, temporarily or permanently. For instance, the suppressor activity of CD4⁺CD25⁺ cells *in vitro* can be neutralized by IL-2, high co-stimulation, or engagement of the glucocorticoid-induced TNF receptor-related family gene (GITR, also known as TNFRSF18) [58, 82].

4. CD8⁺, $\gamma\delta$ and NK1.1 T cells in the regulation of Th2 and IgE responses

CD8⁺ T cells, $\gamma\delta$ T cells and NK T cells are potentially important regulators of immune responses because of their capacity to develop effector functions such as cytokine secretion and cytotoxic activity.

Kemeny's group described the regulation of IgE by antigen-specific CD8 T cells that arise during immunization in rats and mice [103]. Regulation of IgE by CD8⁺ T cells required priming and expansion of antigen-specific CD8 T cells, and appeared to induce immune deviation (IFN- γ production). The cytokine profile of the CD8 T cells was not important for regulation, since both Tc1 and Tc2 cells could protect upon transfer into naïve mice. Protection depended on IL-12/IFN- γ production by the recipient mice. The authors proposed a model whereby antigen-specific CD8 T cells interact with dendritic cells, inducing the production of IL-12, which in turn induces IFN- γ secretion by CD4 T cells. Antigen-specific CD8⁺ cells can thus affect the Th2/IgE response by limiting its magnitude and duration [97, 103].

Some subsets of $\gamma\delta$ T cells are expanded in the bronchoalveolar lavage of asthmatic patients [52, 84], although the total number of $\gamma\delta$ T cells is often decreased in peripheral blood of asthmatics [16, 51]. The role of $\gamma\delta$ T cells in Th2 responses and asthma has been investigated in several animal models with mixed results. These cells are found in lymphoid organs and in mucosal tissues, including the airways, as intraepithelial lymphocytes. The mucosal localization suggests a potential effect on the primary responses and/or the inflammatory responses to airborne allergens. In an asthma model in BALB/c mice, genet-

ic deficiency of $\gamma\delta$ T cells resulted in lower IgE, IgG1, and IL-5 levels, and reduced lung eosinophilia [116]. Svensson et al. [90] described reduced IgE production and airway eosinophilia in $\gamma\delta$ T cell-deficient mice, but no differences in IL-4 production between $\gamma\delta$ -deficient mice and wild-type mice. However, other studies found no differences between wild-type and $\gamma\delta$ -deficient mice in Th2/IgE responses [106]. In models of airway tolerization that compared the response of wild-type and $\gamma\delta$ -deficient mice, $\gamma\delta$ T cells were found to be required for tolerization at low antigenic dose only [31], or not to be required [79]. On the other hand, $\gamma\delta$ T cells isolated from rats tolerized to antigen by inhalation were able to suppress the IgE response of recipient rats [59]. $\gamma\delta$ T cells were also found to down-regulate $\alpha\beta$ T cell-mediated contact sensitivity reactions to haptens [91].

NK T cells are a subset of T lymphocytes that express NK cell lineage receptors, are selected by the MHC class I molecule CD1, and, in mice, express conserved V α 14-J α 281 T cell receptor genes [7, 9, 26, 102]. Because of their capacity to quickly produce high amounts of IL-4 (and IFN- γ) upon activation *in vivo*, they were thought to be involved in the induction of Th2 cells. However, mice genetically deficient NK T cells were able to develop Th2 and IgE responses to a variety of stimuli [60, 83]. Recent studies in a mouse model of asthma established that NK T cells, through secretion of IL-4 and IL-13, contribute to airway inflammation [3]. Thus, NK T cells may not be required for Th2 development but may influence inflammatory tissue responses. Moreover, NK T cells, through their production of IFN- γ , were responsible for the down-regulation of antigen-specific IgE production in mice that received α -galactosylceramide, an activating ligand of CD1d-restricted NK T cells. The inhibition appeared to be IgE specific [21, 61].

5. Immune responses to allergens in normal individuals and in atopics treated by specific immunotherapy: a role for regulatory T cells?

We discussed above some of the extensive experimental data showing the induction of tolerance after mucosal immunization in animal models. Comparative analysis of immune responses to allergens in normal exposed individuals and in atopics that underwent successful immunotherapy, indicates that in both cases atopy may be avoided if IL-10/TGF- β -producing allergen-specific tolerant/regulatory T cells are present.

In non-atopic individuals, chronic or frequent allergen exposure often induces IgG antibody responses with predominance of IgG4, whereas atopic exposed individuals produce both IgG4 and IgE antibodies [1, 14]. Successful immunotherapy correlated with specific increases in IgG4 and IgA antibodies, and with the production of IL-10 and TGF- β by CD4⁺ T cells. In addition to its effect in suppressing proliferation and cytokine production, IL-10 was also shown to stimulate IgG4 production and inhibit IgE production *in vitro* [4]. Since IgG4 production is IL-4 dependent, this type of response was called a “modified Th2 response” by Platts-Mills [69]. Thus, the combination of IL-4 and IL-10 may favor IgG4 over IgE production.

Jutel et al. [46] demonstrated that after immunotherapy to house dust mite or birch pollen antigens in atopic patients and in normal exposed individuals, allergen-specific *in vitro* suppressor activity could be detected in the CD4⁺CD25⁺ population of PBMC. *In vitro* suppression was abrogated by neutralization of IL-10 and TGF- β in the cultures. Francis et al. [30] described increased IL-10 production by CD4⁺CD25⁺ cells from allergen-stimulated cultures of cells from patients that underwent pollen immunotherapy. IL-10 was not

detected in stimulated cells from untreated allergic patients or non-allergic individuals. Thus, successful immunotherapy may lead to the induction of regulatory T cell activity.

6. Viral respiratory infections and asthma

Infections during childhood can influence the development of atopy and asthma. Based upon epidemiological studies that correlated a larger family size with lower risk of developing allergies and asthma, Strachan [87] hypothesized that a higher exposure to infections during childhood protects against atopic asthma. This theory, known as the “hygiene hypothesis”, has been further developed to explain the increased incidence of allergies and asthma observed in recent years, associating this fact to a cleaner environment during childhood. However, certain respiratory viral infections in young children are believed to increase the chances of atopy and asthma later in life, and common respiratory infections are often triggers of severe pulmonary problems in patients with established asthma [35]. Infection with respiratory syncytial virus (RSV) is common in children during the first 2 years of life. RSV infects the epithelial cells of the respiratory tract, causing mild symptoms in most children. A percentage of these children develop more severe wheezing and broncheolitis, and often require hospitalization. Longitudinal studies have shown that severe RSV infection is associated with an increased frequency of wheezing and asthma in later childhood [22, 35].

In animal models of asthma, RSV infection concomitant with ovalbumin airway challenge in sensitized mice leads to increased airway hyperresponsiveness (AHR) and Th2-mediated inflammation, despite the fact that RSV infection induces IFN- γ production by respiratory epithelial cells [6, 65]. RSV infection increased recruitment of ovalbumin-specific resting Th2 cells to the lung even in the absence of ovalbumin airway challenge [85].

In another example of enhancement of asthma-type reactions by paramyxoviral infections, Walter et al. [104] described chronic inflammation and AHR in mice after a single infection with Sendai virus, and long after the viral infection was cleared.

It is therefore clear that the epidemiological human data and the experimental animal data provide a model in which inflammatory changes affecting primarily the respiratory epithelial cells can have an enhancement effect on allergen responses in the airways.

7. Do allergens possess intrinsic immunomodulatory activities?

Allergens are mostly non-infectious ubiquitous substances present in the food or carried in the air [100]. Most common allergens, such as pollen, dust mites and food allergens, contact the body through the respiratory and digestive mucosa. In normal individuals, exposure to non-infectious antigens through mucosal tissues frequently elicits tolerance. However, environmental factors (such as infections and pollutants [74, 86]) that affect the inflammatory/activation state of mucosal tissues may favor allergic responses. Mucosal tolerance to allergens could be overcome by the ability of some allergens to generate innate immune system signals (danger), analogous to those delivered by microorganisms, that result in increased co-stimulation and the triggering of full-blown immune responses.

One of the most dramatic examples of allergy induction through respiratory exposure is the fungus *Aspergillus fumigatus*. *A. fumigatus* is an opportunistic fungal pathogen in humans that can induce strong hypersensitivity reactions [36]. Sensitization of mice through

the airways with *A. fumigatus* extracts that did not contain live organisms led to strong Th2, IgE responses, and airway inflammation and hyperresponsiveness [53]. The allergic response induced by *A. fumigatus* in the airways did not require systemic priming or the use of adjuvants. Other allergens, such as an extract from pollen of *Ambrosia artemisiifolia* (ragweed) and an extract from *Aspergillus oryzae*, but not chicken ovalbumin, were able to induce Type-2 allergic airway inflammation after respiratory exposure [48].

What are the properties of allergens not shared by ovalbumin? As allergen molecules began to be cloned and sequenced, it came to the attention of investigators that many of the dominant allergens displayed protease activity. Moreover, airways sensitization correlated with protease activity in the immunizing extracts. Addition of protease activity to ovalbumin resulted in Type-2 inflammation and production of IgE/IgG1 antibodies to ovalbumin [48]. Protease activity has been shown in other major allergens, such as Der p1 and Fed d1. Der p1, the major allergen of the house dust mite *Dermathofagoides pteronyssinus*, is a serine protease [80]. Further evidence of potential role of proteases in asthma is the increased risk of asthma for factory workers exposed to laundry detergent proteases [28, 68]. Proteases can also contribute to allergic reactions outside the airways, for instance protease-containing contact lens cleaning solutions induced optic angioedema and conjunctivitis accompanied by increased protease-specific IgE serum titers and positive skin prick test [10].

At this moment the mechanisms whereby protease activity influences Th2-type immune responses are unclear. Der p1 can cleave human CD25, CD23 [80], α -1 proteinase inhibitor and elastase inhibitors [12], disrupt tight junctions of epithelial cells [105], and promote Th2 responses and IgE production in vitro [80]. Both CD25 and CD23 cleavage could explain a decrease in down-regulatory mechanisms during allergen exposure, while the disruption of epithelial tight junctions could lead to increased allergen uptake. Proteolytic cleavage of components of the complement cascade may also affect Th2 responses [25, 48].

In summary, protease activity or other inflammation-promoting activities of allergens may be one of the factors (together with other environmental and genetic factors) that facilitate the development of atopy.

8. Concluding remarks

Th2/IgE responses appear to be under the control of regulatory T cells in normal individuals, and it follows that this regulation may be defective in atopic patients. However, the extent of regulatory T cell dysfunction in patients is not clear. Better markers for Treg function are needed, as well as better evaluation of patients' immune responses in secondary lymphoid organs and peripheral tissues.

The extensive research on regulatory T cell markers continues at a fast pace, and it is therefore likely that new molecules and pathways will be identified that will be unique to regulatory T cells and useful to determine the degree of Treg dysfunction in atopic individuals.

Another important aspect that remains unknown is the therapeutic potential of Treg-stimulating treatments. Part of the problem is that in many of the models in which Tregs are effective, their suppressive role is evident relatively early in the process. It is therefore important to determine whether some type of Treg cell will be effective in down-regulat-

ing Th2/IgE responses if the intervention begins at a time point at which large numbers of allergen-specific memory cells are present in the system.

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