

For reprint orders, please contact:
reprints@future-drugs.com



Regulatory CD4⁺ T cells in allergic asthma

Vincent A Carlier, Luc P VanderElst, Marc G Jacquemin and Jean-Marie R Saint-Remy[†]

Active suppression by regulatory T cells (T_{regs}) appears to play a key role in the downregulation of T-cell responses to foreign antigens. Several subtypes of T_{regs} have been described but their mechanisms of action remain unclear. Recent data demonstrate that the suppressive capacity of natural T_{regs} could be associated with cytotoxicity due to the release of granzymes, which are capable of apoptosis induction in target effector T lymphocytes and in antigen-presenting cells, such as dendritic cells. The mechanism of such nonspecific T_{regs} is discussed. Peptide immunotherapy is thought to induce regulatory cells capable of suppressing autoimmune and allergic diseases. We have recently optimized a vaccination strategy by which cytotoxic antigen-specific adaptive T_{regs} can be elicited towards allergens involved in allergic asthma. Such a strategy could be of value in the treatment of allergic asthma.

Expert Rev. Clin. Immunol. 2(5), 737–744 (2006)

Allergic bronchial asthma

The prevalence of allergic asthma has almost doubled in the last 25 years and has now reached epidemic proportions. It is defined as an inflammation affecting the bronchi, airway hyperreactivity (AHR) and obstruction of the bronchial tree after exposure to specific or non-specific triggering factors. Airway inflammation may result in remodeling due to the interplay of cells with soluble factors in the respiratory tract. Effector cells, such as T lymphocytes, eosinophils, mast cells and basophils, are activated by aeroallergen exposure. This is accompanied by organ-selective homing of inflammatory cells under the influence of a complex network of chemokines in the lungs [1]. Endowed with a prolonged survival, these cells interact with sub-epithelial tissues and become reactivated within the organ [2], leading to a full spectrum of effector mechanisms, including eosinophil activation, IgE synthesis, mucus hyperproduction and remodeling [3,4].

T-helper type 2-based etiology?

Mucosal surfaces, such as the bronchial epithelium, are continuously exposed to environmental antigens. This must be handled in a way that does not harm the host. This is

achieved by a fully reactive mucosal immune system inducing a state of tolerance towards inhaled antigens, while, at the same time, maintaining the capacity to develop strong protective responses towards environmental pathogens, such as viruses and bacteria.

The production of T-helper (Th)2-type cytokines (i.e., interleukin [IL]-4, -5, -13) is increased in broncho-alveolar fluids, in cells and in airway biopsies taken from asthma sufferers [5]. This is associated with a decrease in the production of the main cytokines responsible for Th1 differentiation of CD4⁺ T cells, that is, interferon (IFN)- γ and IL-12. Also, GATA binding protein 3, a Th2-type transcription factor, is expressed in CD4⁺ T cells from asthmatic airways [6], which points towards CD4⁺ Th2 lymphocytes being the primary cause of pulmonary inflammation, AHR, airway eosinophilia and mucus hypersecretion. This allergy paradigm has been validated with the widely used ovalbumin (OVA)-induced asthma model in the mouse, in which OVA aerosols induce AHR, eosinophilia, inflammation and specific IgE antibodies. Furthermore, in an IL-4 knock-out model [7], AHR to OVA challenge was impaired considerably, while in an IL-5

CONTENTS

Allergic bronchial asthma

CD4⁺CD25⁺ regulatory T cells

Adaptive T_{regs}

Allergen-specific immunotherapy: a niche for T_{regs}

Expert commentary

Five-year view

Key issues

References

Affiliations

[†] Author for correspondence
University of Leuven, Center for
Molecular and Vascular Biology,
Campus Gasthuisberg, O&N,
Herestraat 49,
3000 Leuven, Belgium
Tel.: +32 1633 0561
Fax: +32 1633 0560
jeanmarie.saint-remy@med.
kuleuven.be

KEYWORDS:
asthma, cytotoxicity,
FAS-L, granzymes,
inflammation, regulation

knock-out model, hyper responsiveness and eosinophilia were fully prevented [8], which confirmed the participation of Th2-derived cytokines in the pathology of allergic asthma.

The latter cannot, however, be explained solely by a Th2 paradigm [9]. Treatment with anti-IL-5 monoclonal antibodies suppressed eosinophilia but was ineffective in decreasing bronchial hyperreactivity in humans [10]. Other inflammatory components incriminate the Th1 cytokine IFN- γ . This was demonstrated, *inter alia*, by blood evaluation showing that asthma severity was clearly associated with an increased production of IFN- γ by CD8⁺ cells [11]. As well as the Th1-cell counterbalancing effect on Th2 cell development [12] and eosinophilia [13], IFN- γ -producing cells can, in fact, strongly exacerbate allergic asthma, at least in the mouse. In a mouse model of asthma, migration of adoptively transferred Th1 lymphocytes to the lungs was detrimental, with increased inflammation and pathological alterations [14].

Altogether, a simple Th1–Th2 paradigm appears difficult to reconcile with observed data, both in humans and mice.

CD4⁺CD25⁺ regulatory T cells

An alternative view on the pathogenesis of asthma is that it is not the inflammatory infiltrate that leads to asthma but a reduced capacity to prevent and/or suppress such inflammation. Regulatory T cells (T_{regs}) are well designed for such a purpose. They exhibit immunosuppressive properties on both Th2 and Th1 cells [15], thereby inhibiting the production of IL-4, -5 and -9 cytokines, which are important for the differentiation of mast cells, basophils, homing of Th2 cells and mucus hyperproduction.

These observations suggest, but do not yet prove, that most of the aspects of allergic asthma could be the consequences of an inefficient regulatory process, rather than uncontrolled accumulation of inflammatory cells. If this is the case, T_{regs} will be vital to the future of allergic asthma therapy.

The T_{regs} that are most fully described all carry the phenotype CD4⁺CD25⁺ cells. These are generated in the thymus, represent approximately 5% of all CD4⁺ T lymphocytes and can suppress various inflammatory processes, including inflammatory bowel diseases and autoimmune diabetes in mouse models [16,17]. Depletion of these cells elicits diverse inflammatory pathologies. Their development is associated with FOXP3 [18], a transcription factor responsible for the silencing of some cytokine gene promoters. Mutations in the FOXP3 gene result in the absence of natural T_{regs} and are the underlying cause of the immune dysregulation, polyendocrinopathy, enteropathy X-linked (IPEX) syndrome (which leads to atopic dermatitis), eosinophilia, increased serum IgE levels and lethal infections.

CD4⁺CD25⁺ regulatory cells can produce IL-10 and transforming growth factor (TGF)- β and are commonly activated by dendritic cells presenting self-peptides. Interestingly, these two immunosuppressive cytokines are produced at low level and, in mouse models, neutralizing antibodies to the respective cell-surface receptors cannot overcome the suppression, indicating

that other mechanisms are involved [19–21]. *In vitro*, these natural T_{regs} suppress adaptive T-cell responses in a cell-contact dependent manner. They express cytotoxic T-lymphocyte-associated protein (CTLA)-4 that can ligate B7 molecules at the surface of dendritic cells, resulting in the induction of the enzyme indoleamine 2,3-dioxygenase (IDO) in human dendritic cells, leading to tryptophan depletion, autoreactive T-cell starvation and death [22]; a process that is enhanced by IL-10 [23].

Whether or not such a mechanism is involved in the control of asthma symptoms is investigated by an experiment carried out in an OVA-induced model of asthma. Coadministration of Toll-like receptor-9 ligands (e.g., immunostimulatory sequence oligodeoxynucleotides [ISS-ODN]) and high-dose lipopolysaccharide induced IDO activity in the lungs that correlated with reduced AHR to nonspecific challenge and with reduced eosinophil accumulation in bronchoalveolar lavage (BAL) fluid [24]. The level of IDO activity achieved in nonimmune cells generated a hostile and cytotoxic microenvironment for infiltrating Th1 and -2 cells.

Is there a functional deficit in the regulatory cell population of allergic individuals?

A murine model of asthma first provided evidence that a lower frequency of CD4⁺CD25⁺ T_{regs} could result in increased hyperreactivity that could be reversed by blocking the proinflammatory cytokine IL-6 [25].

In humans, a recent study showed that CD4⁺CD25⁺ T_{regs} have a limited capacity to suppress polyclonal T-lymphocyte proliferation in patients with seasonal allergy, compared with nonallergic controls [26].

These results are compatible with those of Akdis and colleagues, who demonstrated that allergen specific T cells from allergic subjects produced mainly IL-4 and that those derived from nonallergics produced mainly IL-10 [15]. This is also consistent with observations made regarding allergic and nonallergic children, in whom IL-10 was associated with negative skin tests [27]. It is, therefore, a tenable hypothesis that allergic inflammatory responses are due to a CD4⁺CD25⁺ T_{reg} deficiency. However, the therapeutic potential of natural T_{regs} is limited by their low numbers and the risk of uncontrolled pan-immunosuppression. Adaptive T_{regs} could possibly circumvent these difficulties.

Adaptive T_{regs}

Adaptive T_{regs} are formed in the periphery and several subsets of such cells have been derived using various stimulation methods. Th3 cells produce high levels of TGF- β and variable amounts of IL-4 and IL-10. They are found in mesenteric lymph nodes after tolerance induction by oral antigen administration [28] and can suppress autoimmune diseases, such as encephalomyelitis.

Type 1 T regulatory cells (T_R1) produce high concentrations of IL-10 and, to a lesser extent, TGF- β and do not express FOXP3. They can be derived *in vitro* from naive human CD4 cells after exposure to IL-10, IFN- α or IL-4 and -10 [29].

Chronic activation of either human or murine CD4⁺ T cells with IL-10 induces populations of cells with low proliferating capacities and high production of IL-10 [30]. Kemper and colleagues were able to generate human T_R1 regulatory lymphocytes through dual activation with antibodies recognizing the CD3 and CD46 molecules [31]. The T_R1 subset of suppressor cells is certainly diverse in terms of induction mechanisms and the relationships between Th3 and T_R1 remain to be defined.

Whether adaptive antigen-specific T_{regs} can be used to keep allergic inflammation under control is a matter of debate. Thus, Stock and colleagues described the *in vitro* induction of a population of regulatory CD4⁺ T cells sharing some characteristics with T_R1 cells, such as production of IL-10 (in this particular situation they also produced IFN- γ and expressed FOXP3) that was able to protect against AHR in an OVA-induced asthma model [32]. Oral or intravenous administration of antigen has resulted in the production of adaptive antigen-specific CD4⁺CD25⁺ T_{regs} [33] but also in the development of strongly suppressive Th3 cells [34]. Th2-driven inflammation in mice can be inhibited by subcutaneous administration of peptide [35,36], leading to the development of antigen-specific T_{reg} cells expressing FOXP3 and, presumably, deriving from nonregulatory cells.

Allergen-specific immunotherapy: a niche for T_{regs}

Allergen immunotherapy consists of subcutaneous administration of increasing doses of allergens and is effective in allergic rhinitis and, to some extent, in asthma [37,38]. It is associated with decreased Th2 responses and the development of antigen-induced IL-10-secreting T_{regs} [39,40]. Some adjuvants are more appropriate for inducing allergen-specific T_{regs}, for instance, mice treated with mycobacteria are protected against lung inflammation by T_{reg} cells producing IL-10 and IFN- γ [41]. In humans, however, the benefits of allergen immunotherapy are overshadowed by the risk of harmful side effects, such as anaphylaxis. For this reason, alternative strategies, which would reduce the risk of side effects but increase the generation of T_{regs}, have to be defined.

Allergen-derived peptides containing T-cell epitopes and selected for their lack of IgE binding capacity have been used. Clinical trials have demonstrated increased production of IL-10 concomitant with decreased Th-1 and -2 cytokines [42,43]. Differences in CD4⁺ T-cell populations were analyzed before and after treatment with peptides and revealed that antigen-specific proliferation is decreased after peptide immunotherapy. The observation that isolated human CD4⁺ T cells inhibited the proliferation of bystander cells in coculture experiments suggested that allergen peptide immunotherapy was able to induce regulatory CD4⁺ T cells [44]. Bystander suppression represents an important feature of induced regulatory cells, by which an epitope-specific T_{reg} can suppress the response of effector cells specific for another protein, provided that the presentation of the two antigenic determinants is made by the same antigen-presenting cell (APC) [45].

Nonspecific mechanisms of suppression

Progress towards a therapeutic strategy by which antigen-specific T_{regs} would keep symptoms of asthma under control requires a better understanding of the mechanisms exerting suppression. It is fair to state that, at this point in time, the specifics of such mechanisms remain controversial, not to mention that significant differences exist between mice and humans.

Contact-dependent mechanisms

Cell-contact dependency was demonstrated when the suppressive activity of natural CD4⁺CD25⁺ T_{regs} was abrogated in Transwell experiments, where regulatory cells were separated by a membrane from target CD4⁺CD25⁻ cells [46,47]. A caveat with this methodology is that molecules acting in a paracrine manner can lose their effect, owing to the increased distance separating both cell types. Tryptophan is an essential amino acid for effector CD4⁺ lymphocytes and its deprivation can be initiated from IDO induction. Ligation of the costimulatory molecule B7 on the target cell by membrane-bound CTLA-4 induces IDO synthesis, a phenomenon observed with murine CD4⁺CD25⁺ cells [48]. Anti-CTLA-4 antibodies were demonstrated to inhibit the regulation by mouse T_{reg} cells [49] but conflicting results were obtained by different groups concerning experiments with T lymphocytes from human peripheral blood and, so far, no clear CTLA-4 mediated suppression could be assigned with human cells [50].

Secretion of IL-10 and TGF- β

The effects of IL-10 appear to be restricted to costimulation pathways, as it only inhibits tyrosine phosphorylation of CD28, not of zeta-chain associated protein kinase (ZAP)-70, which is induced by CD3 ligation [51,52]. IL-10 receptor binding activates phosphorylation of tyrosine kinase (TYK) 2 and Janus kinase (JAK) 1 tyrosine kinases and also phosphorylation of signal transducer and activator of transcription (STAT)-1 α and STAT-3 [53], leading to the inhibition of tyrosine phosphorylation of the cytoplasmic tail of CD28, preventing its binding to Phosphatidylinositol (PI) 3-kinase and thereby signal processing. In mice, IL-10 can increase the expression of suppressor of cytokine signaling molecules [54], decrease the expression of costimulatory molecules on APC and inhibit full maturation of dendritic cells [55].

TGF- β is a complex molecule, not only in terms of target T cells but also in terms of activities. Apart from its well known pathogenesis in human asthma, including injured airway remodeling [56] and neutrophil and lymphocyte recruitment [57], TGF- β is mainly described as an inhibitor of inflammation, capable of acting on all cell types by activation of Smad pathways.

TGF- β is important for the expansion of CD4⁺CD25⁺ cells, owing to their suppressive capacity and the conversion of mouse naive CD4⁺CD25⁻ cells to CD4⁺CD25⁺ cells expressing FOXP3 [58,59]. TGF- β is also important in the regulation of apoptosis, although it provides signals for both cell survival and apoptosis. For example, it inhibits apoptosis induced by

FAS/FASL in activated T cells [60], as well as in other systems using osteoblasts, microglia and smooth muscle cells [61]. However, TGF- β induces apoptosis in B-cell lymphoma by increasing I κ B- α transcription and inhibition of nuclear factor (NF)- κ B, known to be an anti-apoptotic transcription factor [62]. In addition, TGF- β has the capacity to activate p38 mitogen-activated protein kinase, which triggers apoptotic pathways through caspase-8 or -9 activation [63]. In human epithelial cells, apoptosis and necrosis can be induced after increased production of reactive oxygen species and simultaneous decrease in antioxidant enzymes by TGF- β treatment [64].

These pro- or anti-apoptotic effects vary from one cell-type to another and the extracellular matrix plays a major role through its interaction with TGF- β signaling.

However, no definitive data have been generated that demonstrate that TGF- β could induce apoptosis in immune cells. TGF- β affects CD4⁺ lymphocyte differentiation by inhibiting GATA-3 or T-BET expression [65,66] but it also regulates T-cell proliferation, mainly by inhibiting IL-2 transcription [67] and possibly by upregulation of cyclin-dependent kinase inhibitors and downregulation of c-myc.

T_{regs} & granule exocytosis

An intriguing finding in some T_{regs} is that they are equipped with granules containing granzymes (GZs), a group of serine proteases, that cleave and activate procaspases in the target cell, resulting in DNA fragmentation and depolarization of mitochondrial membranes, which leads to apoptosis. This suggests that granule exocytosis could be involved in the regulation of various immune responses.

The first clue that this could be the case was obtained from T_R1 cells. Cobbold and colleagues compared gene expression in an induced T_R1-like regulatory clone with that of resting CD4⁺CD25⁺ splenocytes in order to identify specific clusters of genes that were up- or down-regulated [68]. They identified shared genes that are increased on both cell

types, such as *GITR*, *OX40* and *CD103*, but, remarkably, they found a strong overexpression of *GZ-A* transcript in T_R1-like cells.

Grossman and colleagues examined expression patterns at the single-cell level using GZ-specific antibodies and an intracellular flow cytometry technique [69]. They clearly demonstrated that human CD4⁺ T-cell activation, either with anti-CD3/CD28 or CD3/CD46 antibodies, resulted in increased expression of GZ-B in up to 90% of cells. They further examined naive and memory CD4⁺ lymphocytes. CD3/CD46 stimulation induced the largest proportion of GZ-B in naive and memory cells, while CD3/CD28 only induced GZ-B-positive cells in the memory population. As mentioned previously, CD3/CD46 stimulation of peripheral CD4 cells results in phenotypic differentiation towards T_R1 cells, producing high levels of IL-10. These adaptive T_R1 cells were tested for their ability to kill allogenic tumor target cells. Killing was non-specific (target cells were devoid of MHC class I and II molecules), FAS–FASL-independent but it was perforin-dependent. Grossman and colleagues also demonstrated that induced T_R1 cells were able to kill auto-logous activated CD4⁺, CD8⁺ cells, CD14⁺ monocytes and immature dendritic cells [70].

Most interestingly, natural CD4⁺CD25⁺ cells expressed GZ-A but not GZ-B after CD3/CD46 stimulation. They were also able to kill the same targets as the T_R1 cells in a perforin-dependent cytotoxicity [70].

The use of a GZ-B-deficient mouse strain indicated the importance of GZ proteases in the suppressive ability of natural CD4⁺CD25⁺ T_{reg}. Gondek and colleagues demonstrated that natural T_{regs} from GZ-B^{-/-} mice have a 50% decrease in their capacity to inhibit the proliferation of CD4⁺CD25⁺ effector T cells [71]. However, strikingly and in contrast with previous results with human cells, suppression was independent of perforin, as perforin-deficient mice were still able to suppress proliferation. Most importantly, the contact-dependent suppression resulted in effector-cell apoptosis but also in their decreased proliferative capacity. The decreased, but not

Table 1. Major characteristics of CD4⁺ regulatory cells.

Cell type	Origin	Classical suppressive mechanisms	Cytotoxicity: mechanisms	Cytotoxicity: target cells	Ref.
Natural CD4 ⁺ CD25 ⁺	Thymus	Cell contact, CTLA-4, TGF- β , IL-10	GZ-A, GZ-B, perforin*	Activated T cells, immature DC, CD14 ⁺ monocytes	[72,73]
T _R 1-like	Periphery	IL-10 (+/- IFN- γ , IL-5), cell contact	GZ-B, perforin*	Activated T cells, immature DC, CD14 ⁺ monocytes	[71]
Th3	Periphery	TGF- β (+/- IL-10), cell contact	?	T cells	
Other induced T _{reg}	Periphery (FOXP3 ⁺)	IFN- γ , IL-10	?	T cells	[29]
	Periphery (FOXP3 ⁻)	IFN- γ , IL-10	GZ-B, FAS-L	Activated T cells, immature DC, B cells	[74]

*Perforin dependency is controversial: human and mouse models show opposed results.

DC: Dendritic cell; GZ: Granzyme; IFN: Interferon; IL: Interleukin; Th3: T-helper type 3; TGF: Transforming growth factor; T_R1: Type 1 regulatory T cell.

abrogated, suppressive capacity of these GZ-B-deficient T_{regs} showed, however, that suppression mechanisms are multiple, even within a single subclass of regulatory CD4⁺ lymphocyte.

The authors' group has defined a peptide immunization method that generates adaptive suppressor CD4⁺ T cells and demonstrated its benefit in bronchial asthma. T_{regs} with phenotypic characteristics shared with natural CD4⁺CD25⁺ T_{reg} (but negative for FOXP3 at resting state) have been isolated and demonstrated to lyse target cells (e.g., APCs) in a MHC class II and strictly contact-dependent manner [72]. Moreover, adoptive transfer of such T_{regs} completely and selectively abrogated bronchial inflammation, lung infiltrates and AHR in mice sensitized to a recombinant form of the Der p 2 aeroallergen. Such T_{regs} kill target cells by apoptosis induction through FAS–FASL interaction and secretion of GZ family members, notably GZ-A and -B.

Taken together, these data clearly implicate apoptosis in the regulatory capacity of different activated suppressor cells. Similar to the FOXP3 deficiency, a defect in GZs leads to different autoimmune pathologies [73], suggesting the important contribution of these molecules to the control of immune responses.

Thus, subtypes of regulatory CD4⁺ cells can act after activation, not only through classically described mechanisms but also by generating apoptotic signals. This can lead to a putatively more efficient immune regulation by killing T lymphocytes and APC (TABLE 1). Whether apoptosis induction by T_{regs} represents an important facet of such cells for *in vivo* suppressive activity remains to be demonstrated. However, the possibility of generating 'à la carte' antigen-specific cytotoxic T_{regs} represents a fascinating approach with potential for allergic and autoimmune diseases.

Expert commentary

Allergic bronchial asthma results from complex, intricate mechanisms linking innate and adaptive immunity. The mechanism leading to one of the main symptoms of asthma, airway hyper-reactivity, has not yet been precisely determined and could be independent of the development of lung inflammatory infiltrates. The latter, consisting of cells belonging to the adaptive arm of the immune response, is dominated by T cells, which are deemed to represent the driving force in attracting cells (including eosinophils and basophils) into the lung. Therefore, a logical approach to the treatment of asthma would be to prevent effector T-cell activation and/or migration into the lungs (FIGURE 1). Effector T cells (whatever the subset to which they belong), CD4⁺ Th1 and Th2, CD8⁺ and natural

killer T (NKT) cells, are activated after cognate interaction with a specific peptide presented into the context of MHC-II, I or the CD1d molecule, respectively. Preventing effector T-cell activation could, therefore, be achieved readily by elimination of the peptide-presenting cells, preferably in a strictly antigen-specific context. This is precisely what cytotoxic T_{regs} have to offer.

Such properties contrast with T_{regs} that are deemed to be activated upon recognition of a cognate peptide but that express their suppressive activity mainly through the secretion of suppressive cytokines such as IL-10 and TGF-β, which is, therefore, no longer specific.

Although many questions remain to be answered before embarking on clinical application (see below), this approach might fulfil an immunologist's dream: to switch off an unwanted immune response using a biological scalpel.

Five-year view

The field of regulatory cells has evolved very rapidly over the last 10 years and it is expected to continue to do so. Other subsets of T_{regs} will be discovered, either naturally present in the repertoire or elicited in the periphery, during the course

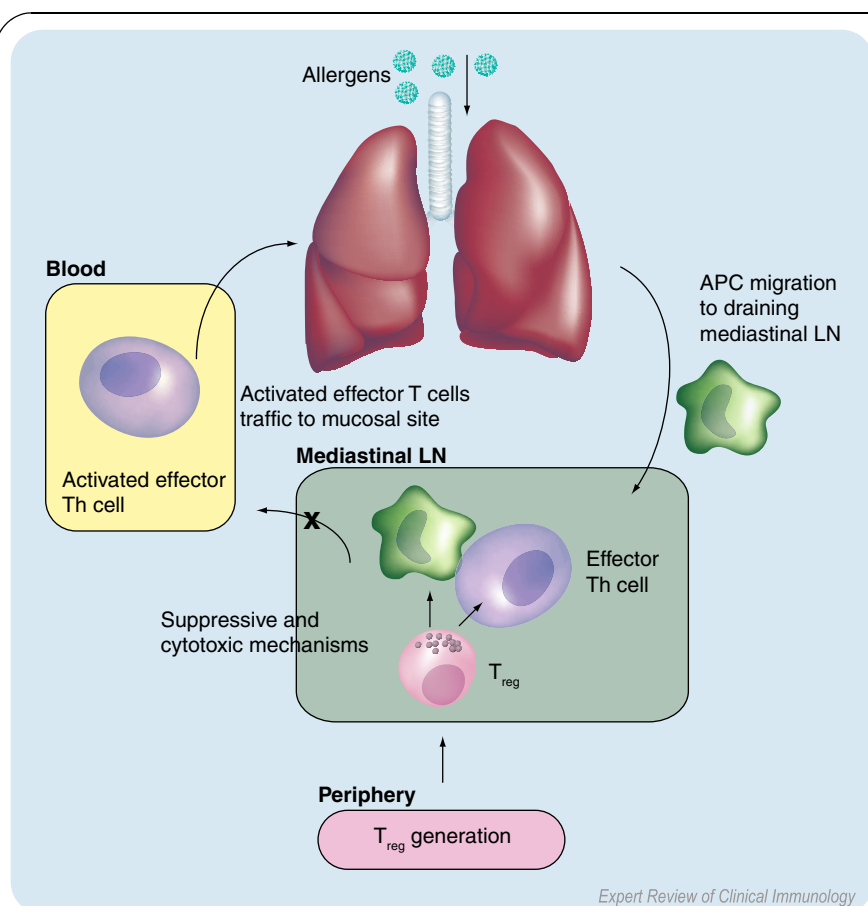


Figure 1. Regulatory CD4⁺ T cells generated after peptide immunization can migrate to draining mediastinal LN where they can kill APC and allergen-specific effector T cells, preventing their recirculation and the formation of cellular infiltrates promoters of inflammation.

APC: Antigen-presenting cell; Th: T-helper cells; T_{reg}: Regulatory T cell; LN: Lymph node.

of natural immunization or through vaccination. However, the data presently available are definitive enough to be able to envision a number of clinical applications, at least in allergic and auto-immune diseases. Peptides activating and expanding T_{regs} with cytolytic properties require full characterization, as well as the conditions under which they have to be

administered, including a choice for the right adjuvants. Their mechanism of action has to be investigated fully, with a particular emphasis on specificity. For *in vivo* applications, lifetime expectancy of T_{regs} and their capacity to migrate to tissues, such as the lung or lymph nodes, have to be understood and controlled.

Key issues

- Effector T cells are instrumental in the development of allergic asthma. Preventing T-cell activation and/or migration into the lungs could be a valuable therapeutic approach for asthma.
- Conventional immunotherapy or peptide administration without adjuvant elicit variable degrees of regulatory T cells (T_{regs}) expansion.
- A subset of T_{regs} elicited upon peptidic administration in adjuvant develops cytotoxic properties towards antigen-presenting cells after cognate interaction.
- Controlling the conditions under which such cytotoxic T_{regs} can be elicited *in vivo* could offer a therapeutic approach for asthma.
- The mechanism of action of such cytotoxic T cells should be deciphered at the molecular level, as well as the behavior of such cells *in vivo*, including lifetime expectancy and capacity to migrate to tissues.

References

Papers of special note have been highlighted as:

- of interest
- of considerable interest

- Gutierrez-Ramos JC, Lloyd C, Kapsenberg ML, Gonzalo JA, Coyle AJ. Non-redundant functional groups of chemokines operate in a coordinate manner during the inflammatory response in the lung. *Immunol. Rev.* 177, 31–42 (2000).
- Akdis M, Trautmann A, Klunker S *et al.* T helper (Th) 2 predominance in atopic diseases is due to preferential apoptosis of circulating memory/effector Th1 cells. *Faseb. J.* 17(9), 1026–1035 (2003).
- Akdis M, Simon HU, Weigl L *et al.* Skin homing (cutaneous lymphocyte-associated antigen-positive) CD8⁺ T cells respond to superantigen and contribute to eosinophilia and IgE production in atopic dermatitis. *J. Immunol.* 163(1), 466–475 (1999).
- Whittaker L, Niu N, Temann UA *et al.* Interleukin-13 mediates a fundamental pathway for airway epithelial mucus induced by CD4⁺ T cells and interleukin-9. *Am. J. Respir. Cell Mol. Biol.* 27(5), 593–602 (2002).
- Robinson DS, Hamid Q, Ying S *et al.* Predominant TH2-like bronchoalveolar T-lymphocyte population in atopic asthma. *N. Engl. J. Med.* 326(5), 298–304 (1992).
- Nakamura Y, Ghaffar O, Olivenstein R *et al.* Gene expression of the GATA-3 transcription factor is increased in atopic asthma. *J. Allergy Clin. Immunol.* 103(2 Pt 1), 215–222 (1999).
- Brusselle GG, Kips JC, Tavernier JH *et al.* Attenuation of allergic airway inflammation in IL-4 deficient mice. *Clin. Exp. Allergy* 24(1), 73–80 (1994).
- Foster PS, Hogan SP, Ramsay AJ, Matthaei KI, Young IG. Interleukin 5 deficiency abolishes eosinophilia, airways hyperreactivity, and lung damage in a mouse asthma model. *J. Exp. Med.* 183(1), 195–201 (1996).
- O'Byrne PM, Inman MD, Adelroth E. Reassessing the Th2 cytokine basis of asthma. *Trends Pharmacol. Sci.* 25(5), 244–248 (2004).
- **Underlines the challenges in therapies aiming at preventing asthma solely by acting on the T helper (Th)1–Th2 balance.**
- Leckie MJ, ten Brinke A, Khan J *et al.* Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyper-responsiveness, and the late asthmatic response. *Lancet* 356(9248), 2144–2148 (2000).
- Cho SH, Stanciu LA, Begishvili T *et al.* Peripheral blood CD4⁺ and CD8⁺ T cell type 1 and type 2 cytokine production in atopic asthmatic and normal subjects. *Clin. Exp. Allergy* 32(3), 427–433 (2002).
- Abbas AK, Murphy KM, Sher A. Functional diversity of helper T lymphocytes. *Nature* 383(6603), 787–793 (1996).
- Iwamoto I, Nakajima H, Endo H, Yoshida S. Interferon- γ regulates antigen-induced eosinophil recruitment into the mouse airways by inhibiting the infiltration of CD4⁺ T cells. *J. Exp. Med.* 177(2), 573–576 (1993).
- Hansen G, Berry G, DeKruyff RH, Umetsu DT. Allergen-specific Th1 cells fail to counterbalance Th2 cell-induced airway hyperreactivity but cause severe airway inflammation. *J. Clin. Invest.* 103(2), 175–183 (1999).
- Akdis M, Verhagen J, Taylor A *et al.* Immune responses in healthy and allergic individuals are characterized by a fine balance between allergen-specific T regulatory 1 and T helper 2 cells. *J. Exp. Med.* 199(11), 1567–1575 (2004).
- Fehervari Z, Sakaguchi S. Development and function of CD25⁺CD4⁺ regulatory T cells. *Curr. Opin. Immunol.* 16(2), 203–208 (2004).
- Salomon B, Lenschow DJ, Rhee L *et al.* B7/CD28 costimulation is essential for the homeostasis of the CD4⁺CD25⁺ immunoregulatory T cells that control autoimmune diabetes. *Immunity* 12(4), 431–440 (2000).
- Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. *Science* 299(5609), 1057–1061 (2003).
- **Key paper on FOXP3 function.**
- Barrat FJ, Cua DJ, Boonstra A *et al.* *In vitro* generation of interleukin 10-producing regulatory CD4(+) T cells is induced by immunosuppressive drugs and inhibited by T helper type 1 (Th1)- and Th2-inducing cytokines. *J. Exp. Med.* 195(5), 603–616 (2002).

- 20 Vieira PL, Christensen JR, Minaee S *et al.* IL-10-secreting regulatory T cells do not express Foxp3 but have comparable regulatory function to naturally occurring CD4⁺CD25⁺ regulatory T cells. *J. Immunol.* 172(10), 5986–5993 (2004).
- 21 Waldmann H, Graca L, Cobbold S *et al.* Regulatory T cells and organ transplantation. *Semin. Immunol.* 16(2), 119–126 (2004).
- 22 Munn DH, Sharma MD, Mellor AL. Ligation of B7–1/B7–2 by human CD4⁺ T cells triggers indoleamine 2,3-dioxygenase activity in dendritic cells. *J. Immunol.* 172(7), 4100–4110 (2004).
- 23 Grohmann U, Orabona C, Fallarino F *et al.* CTLA-4-Ig regulates tryptophan catabolism *in vivo*. *Nat. Immunol.* 3(11), 1097–1101 (2002).
- 24 Hayashi T, Beck L, Rossetto C *et al.* Inhibition of experimental asthma by indoleamine 2,3-dioxygenase. *J. Clin. Invest.* 114(2), 270–279 (2004).
- 25 Doganci A, Eigenbrod T, Krug N *et al.* The IL-6R α chain controls lung CD4⁺CD25⁺ T_{reg} development and function during allergic airway inflammation *in vivo*. *J. Clin. Invest.* 115(2), 313–325 (2005).
- 26 Ling EM, Smith T, Nguyen XD *et al.* Relation of CD4⁺CD25⁺ regulatory T cell suppression of allergen-driven T cell activation to atopic status and expression of allergic disease. *Lancet* 363(9409), 608–615 (2004).
- Investigates the link between disease and activity of CD4⁺CD25⁺ regulatory T cells (T_{regs}): the activity of T_{regs} from atopics was decreased, with the lowest suppressive capacities observed during the pollen season.
- 27 Heaton T, Rowe J, Turner S *et al.* An immunoepidemiological approach to asthma: identification of *in-vitro* T cell response patterns associated with different wheezing phenotypes in children. *Lancet* 365(9454), 142–149 (2005).
- 28 Chen Y, Kuchroo VK, Inobe J, Hafler DA, Weiner HL. Regulatory T cell clones induced by oral tolerance: suppression of autoimmune encephalomyelitis. *Science* 265(5176), 1237–1240 (1994).
- 29 Levings MK, Sangregorio R, Galbiati F *et al.* IFN- α and IL-10 induce the differentiation of human type 1 T regulatory cells. *J. Immunol.* 166(9), 5530–5539 (2001).
- 30 Groux H, O'Garra A, Bigler M *et al.* A CD4⁺ T cell subset inhibits antigen-specific T cell responses and prevents colitis. *Nature* 389(6652), 737–742 (1997).
- 31 Kemper C, Chan AC, Green JM *et al.* Activation of human CD4⁺ cells with CD3 and CD46 induces a T-regulatory cell 1 phenotype. *Nature* 421(6921), 388–392 (2003).
- 32 Stock P, Akbari O, Berry G *et al.* Induction of T helper type 1-like regulatory cells that express Foxp3 and protect against airway hyper-reactivity. *Nat. Immunol.* 5(11), 1149–1156 (2004).
- Describes the *in vivo* induction of T_{regs} from naive CD4⁺CD25⁺ T cells and sharing characteristics of both CD4⁺CD25⁺ T_{reg} and Th1 cells.
- 33 Thorstenson KM, Khoruts A. Generation of anergic and potentially immunoregulatory CD25⁺CD4⁺ T cells *in vivo* after induction of peripheral tolerance with intravenous or oral antigen. *J. Immunol.* 167(1), 188–195 (2001).
- 34 Dao Nguyen X, Robinson DS. Fluticasone propionate increases CD4CD25⁺ T regulatory cell suppression of allergen-stimulated CD4CD25⁺ T cells by an IL-10-dependent mechanism. *J. Allergy Clin. Immunol.* 114(2), 296–301 (2004).
- 35 Apostolou I, von Boehmer H. *In vivo* instruction of suppressor commitment in naive T cells. *J. Exp. Med.* 199(10), 1401–1408 (2004).
- 36 Bynoe MS, Evans JT, Viret C, Janeway CA Jr. Epicutaneous immunization with autoantigenic peptides induces T suppressor cells that prevent experimental allergic encephalomyelitis. *Immunity* 19(3), 317–328 (2003).
- 37 Norman PS. Immunotherapy: 1999–2004. *J. Allergy Clin. Immunol.* 113(6), 1013–1023 (2004).
- 38 Wilson DR, Lima MT, Durham SR. Sublingual immunotherapy for allergic rhinitis: systematic review and meta-analysis. *Allergy* 60(1), 4–12 (2005).
- 39 Nouri-Aria KT, Wachholz PA, Francis JN *et al.* Grass pollen immunotherapy induces mucosal and peripheral IL-10 responses and blocking IgG activity. *J. Immunol.* 172(5), 3252–3259 (2004).
- 40 Till SJ, Francis JN, Nouri-Aria K, Durham SR. Mechanisms of immunotherapy. *J. Allergy Clin. Immunol.* 113(6), 1025–1034 (2004).
- 41 Zuany-Amorim C, Sawicka E, Manlius C *et al.* Suppression of airway eosinophilia by killed *Mycobacterium vaccae*-induced allergen-specific regulatory T cells. *Nat. Med.* 8(6), 625–629 (2002).
- 42 Fellrath JM, Kettner A, Dufour N *et al.* Allergen-specific T cell tolerance induction with allergen-derived long synthetic peptides: results of a phase I trial. *J. Allergy Clin. Immunol.* 111(4), 854–861 (2003).
- 43 Oldfield WL, Larche M, Kay AB. Effect of T cell peptides derived from Fel d 1 on allergic reactions and cytokine production in patients sensitive to cats: a randomised controlled trial. *Lancet* 360(9326), 47–53 (2002).
- 44 Verhoef A, Alexander C, Kay AB, Larche M. T cell epitope immunotherapy induces a CD4⁺ T cell population with regulatory activity. *PLoS Med.* 2(3), 78 (2005).
- First demonstration that peptide immunotherapy in humans can generate a population of suppressive CD4⁺ cells that is different from the natural CD4⁺CD25⁺ regulatory cell lineage.
- 45 Alpan O, Bachelder E, Isil E, Arnheiter H, Matzinger P. 'Educated' dendritic cells act as messengers from memory to naive T helper cells. *Nat. Immunol.* 5(6), 615–622 (2004).
- 46 Martin B, Banz A, Bienvenu B *et al.* Suppression of CD4⁺ T lymphocyte effector functions by CD4⁺CD25⁺ cells *in vivo*. *J. Immunol.* 172(6), 3391–3398 (2004).
- 47 Takahashi T, Kuniyasu Y, Toda M *et al.* Immunologic self-tolerance maintained by CD25⁺CD4⁺ naturally anergic and suppressive T cells: induction of autoimmune disease by breaking their anergic/suppressive state. *Int. Immunol.* 10(12), 1969–1980 (1998).
- 48 Fallarino F, Grohmann U, Hwang KW *et al.* Modulation of tryptophan catabolism by regulatory T cells. *Nat. Immunol.* 4(12), 1206–1212 (2003).
- 49 Takahashi T, Tagami T, Yamazaki S *et al.* Immunologic self-tolerance maintained by CD25(+)CD4(+) regulatory T cells constitutively expressing cytotoxic T lymphocyte-associated antigen 4. *J. Exp. Med.* 192(2), 303–310 (2000).
- 50 Jonuleit H, Schmitt E, Stassen M *et al.* Identification and functional characterization of human CD4(+)CD25(+) T cells with regulatory properties isolated from peripheral blood. *J. Exp. Med.* 193(11), 1285–1294 (2001).
- 51 Akdis CA, Joss A, Akdis M, Faith A, Blaser K. A molecular basis for T cell suppression by IL-10: CD28-associated IL-10 receptor inhibits CD28 tyrosine phosphorylation and phosphatidylinositol 3-kinase binding. *Faseb. J.* 14(12), 1666–1668 (2000).

- 52 Joss A, Akdis M, Faith A, Blaser K, Akdis CA. IL-10 directly acts on T cells by specifically altering the CD28 co-stimulation pathway. *Eur. J. Immunol.* 30(6), 1683–1690 (2000).
- 53 Finbloom DS, Winestock KD. IL-10 induces the tyrosine phosphorylation of tyk2 and Jak1 and the differential assembly of STAT1 α and STAT3 complexes in human T cells and monocytes. *J. Immunol.* 155(3), 1079–1090 (1995).
- 54 Ding Y, Chen D, Tarcsfalvi A *et al.* Suppressor of cytokine signaling 1 inhibits IL-10-mediated immune responses. *J. Immunol.* 170(3), 1383–1391 (2003).
- 55 Steinbrink K, Wolfl M, Jonuleit H, Knop J, Enk AH. Induction of tolerance by IL-10-treated dendritic cells. *J. Immunol.* 159(10), 4772–4780 (1997).
- 56 Vignola AM, Chanez P, Chiappara G *et al.* Transforming growth factor- β expression in mucosal biopsies in asthma and chronic bronchitis. *Am. J. Respir. Crit. Care Med.* 156(2 Pt 1), 591–599 (1997).
- 57 Adams DH, Hathaway M, Shaw J *et al.* Transforming growth factor- β induces human T lymphocyte migration *in vitro*. *J. Immunol.* 147(2), 609–612 (1991).
- 58 Chen W, Jin W, Hardegen N *et al.* Conversion of peripheral CD4⁺CD25⁻ naive T cells to CD4⁺CD25⁺ regulatory T cells by TGF- β induction of transcription factor Foxp3. *J. Exp. Med.* 198(12), 1875–1886 (2003).
- 59 Huber S, Schramm C, Lehr HA *et al.* Cutting edge: TGF- β signaling is required for the *in vivo* expansion and immunosuppressive capacity of regulatory CD4⁺CD25⁺ T cells. *J. Immunol.* 173(11), 6526–6531 (2004).
- 60 Wahl SM, Orenstein JM, Chen W. TGF- β influences the life and death decisions of T lymphocytes. *Cytokine Growth Factor Rev.* 11(1–2), 71–79 (2000).
- 61 Pollman MJ, Naumovski L, Gibbons GH. Vascular cell apoptosis: cell type-specific modulation by transforming growth factor- β 1 in endothelial cells versus smooth muscle cells. *Circulation* 99(15), 2019–2026 (1999).
- 62 Arsura M, Wu M, Sonenshein GE. TGF β 1 inhibits NF- κ B/Rel activity inducing apoptosis of B cells: transcriptional activation of I κ B α . *Immunity* 5(1), 31–40 (1996).
- 63 Schrantz N, Bourgeade MF, Mouhamad S *et al.* p38-mediated regulation of an Fas-associated death domain protein-independent pathway leading to caspase-8 activation during TGF β -induced apoptosis in human Burkitt lymphoma B cells BL41. *Mol. Biol. Cell* 12(10), 3139–3151 (2001).
- 64 Thannickal VJ, Fanburg BL. Activation of an H2O2-generating NADH oxidase in human lung fibroblasts by transforming growth factor β 1. *J. Biol. Chem.* 270(51), 30334–30338 (1995).
- 65 Gorelik L, Constant S, Flavell RA. Mechanism of transforming growth factor β -induced inhibition of T helper type 1 differentiation. *J. Exp. Med.* 195(11), 1499–1505 (2002).
- 66 Gorelik L, Fields PE, Flavell RA. Cutting edge: TGF- β inhibits Th type 2 development through inhibition of GATA-3 expression. *J. Immunol.* 165(9), 4773–4777 (2000).
- 67 Thornton AM, Shevach EM. CD4⁺CD25⁺ immunoregulatory T cells suppress polyclonal T cell activation *in vitro* by inhibiting interleukin 2 production. *J. Exp. Med.* 188(2), 287–296 (1998).
- 68 Cobbold SP, Adams E, Graca L, Waldmann H. Serial analysis of gene expression provides new insights into regulatory T cells. *Semin. Immunol.* 15(4), 209–214 (2003).
- 69 Grossman WJ, Verbsky JW, Tollefsen BL *et al.* Differential expression of granzymes A and B in human cytotoxic lymphocyte subsets and T regulatory cells. *Blood* 104(9), 2840–2848 (2004).
- Introduces the single-cell analysis with granzyme-specific monoclonal antibodies applied on induced T_{regs}. The flow-based killing assay is also of interest.
- 70 Grossman WJ, Verbsky JW, Barchet W *et al.* Human T regulatory cells can use the perforin pathway to cause autologous target cell death. *Immunity* 21(4), 589–601 (2004).
- 71 Gondek DC, Lu LF, Quezada SA, Sakaguchi S, Noelle RJ. Cutting edge: contact-mediated suppression by CD4⁺CD25⁺ regulatory cells involves a granzyme B-dependent, perforin-independent mechanism. *J. Immunol.* 174(4), 1783–1786 (2005).
- 72 Janssens W, Carlier V, Wu B *et al.* CD4⁺CD25⁺ T cells lyse antigen-presenting B cells by Fas–Fas ligand interaction in an epitope-specific manner. *J. Immunol.* 171(9), 4604–4612 (2003).
- Presents the first evidence that allergen-specific adaptive T_{regs} exert a cytotoxicity on target cells.
- 73 Russell JH, Ley TJ. Lymphocyte-mediated cytotoxicity. *Annu. Rev. Immunol.* 20, 323–370 (2002).
- Excellent reference and overview of granule exocytosis and circulation.

Affiliations

- Vincent A Carlier, MSc
University of Leuven,
Center for Molecular and Vascular Biology,
Campus Gasthuisberg, O&N, Herestraat 49,
3000 Leuven, Belgium
Tel.: +32 1633 0562
Fax: +32 1633 0560
vincent.carlier@med.kuleuven.be
- Luc P VanderElst, MSc
University of Leuven,
Center for Molecular and Vascular Biology,
Campus Gasthuisberg, O&N, Herestraat 49,
3000 Leuven, Belgium
Tel.: +32 1633 0562
Fax: +32 1633 0560
luc.vanderelst@med.kuleuven.be
- Marc G Jacquemin, MD, PhD
University of Leuven,
Center for Molecular and Vascular Biology,
Campus Gasthuisberg, O&N, Herestraat 49,
3000 Leuven, Belgium
Tel.: +32 1634 5776
Fax: +32 1634 5990
marc.jacquemin@med.kuleuven.be
- Jean-Marie R Saint-Remy, MD, PhD
University of Leuven,
Center for Molecular and Vascular Biology,
Campus Gasthuisberg, O&N, Herestraat 49,
3000 Leuven, Belgium
Tel.: +32 1633 0561
Fax: +32 1633 0560
jeanmarie.saint-remy@med.kuleuven.be