

New insights into the mechanisms of allergen-specific immunotherapy

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Purpose of review

Specific immunotherapy represents the only curative treatment of a specific allergy, and is therefore of great interest in terms of immunological mechanisms and therapeutic developments.

Recent findings

Allergen-specific regulatory T cells are induced after the initiation of specific immunotherapy, and are assumed to suppress effector T cells directly mediating allergic inflammation. Therefore regulatory T cells may play a key role in the re-induction of allergen tolerance. Multiple pathways in many systems are described to promote or enhance regulatory T cells. This review summarizes the current view on immunological mechanisms leading to and coming from regulatory T cells.

Summary

The highlighted mechanisms may not only contribute to improved specific immunotherapy, but also give insight into a clinically relevant therapy targeting regulatory T cells. The approach of addressing endogenous regulatory mechanisms specifically controlling inflammation instead of targeting inflammation itself is relevant for future pharmacological developments.

Keywords

anergy, FOXP3, suppression, tolerance, T_{reg}

Introduction

Allergic diseases result from an unbalanced response of the specific immune system, generating allergen-specific IgE antibodies, which mediate various clinical symptoms, such as immediate type hypersensitivity. The generation of allergen-specific IgE by B cells depends on the generation of IL-4-producing T helper (Th) type 2 cells. The existence of allergen-specific Th2 cells, however, is not sufficient for allergy pathogenesis, because these cells are also found in healthy individuals. In contrast, allergen-specific regulatory T cells (T_{regs}) occur at a higher frequency than their effector counterparts in healthy individuals [1•], and are capable of suppressing the proliferation and cytokine expression of Th1 and Th2 cells, and act on antigen-presenting cells (APC) [2•]. T_{regs} are defined on the basis of their function, in contrast to Th1 or Th2 cells, which are characterized by their gene products. The exact definition of the T_{reg} phenotype is therefore often difficult. T_{regs} include CD25⁺ T_{regs} expressing the FOXP3 transcription factor, which is considered a key factor necessary for the induction of T_{regs}, similarly to the role of T-bet and GATA-3 in the commitment to Th1 and Th2 cells, respectively. The ectotrophic expression of FOXP3 is sufficient to induce a regulatory T-cell phenotype [3], which is assumed to generate an anergic phenotype by the control of activatory transcription factors nuclear factor of activated T cells and nuclear factor kappa B [4,5]. In addition to the CD25⁺ T_{regs} the IL-10-expressing (Tr1) cells and the transforming growth factor beta (TGF-β)-expressing T_{regs} (Th3) were described [6,7]. These T-cell phenotypes are overlapping and the suppressive action may be similar.

Different vaccination strategies alter the balance between effector and regulatory T-cell populations, which regulate peripheral tolerance. The specific immunotherapy is a high allergen dose vaccination strategy, which reprogrammes the peripheral tolerance against allergens. It is currently the only curative treatment of specific allergy. Whereas in classic specific immunotherapy the allergen is administered subcutaneously, sublingual immunotherapy (SLIT) takes advantage of mucosal lymphoid systems, which appear to facilitate tolerance induction. Different clinically relevant vaccination strategies were evaluated and have been reviewed [8–11]. It was a great breakthrough in understanding the immunological background of immunotherapy, by demonstrating

Curr Opin Allergy Clin Immunol 5:525–530. © 2005 Lippincott Williams & Wilkins.

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Sponsorship: This work was supported by the Swiss National Foundation grant nos. 3100-065436 and 3100A0-100164, the Hartmann Müller Foundation Zürich, Bonizzi-Theler Foundation, the Ernst Goehner Foundation Zug and the Saurer Foundation Davos.

Current Opinion in Allergy and Clinical Immunology 2005, 5:525–530

Abbreviations

APC	antigen-presenting cell
DC	dendritic cell
GITR	glucocorticoid-induced TNFR-related protein
SLIT	sublingual immunotherapy
TGF-β	transforming growth factor beta
Th	T helper
T _{reg}	regulatory T cell

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1528-4050

peripheral tolerance induction and the development of an anergic state in activated, specific T cells. In recent work it appeared that specific immunotherapy generates IL-10 or TGF- β -secreting regulatory T cells, which are capable of specifically suppressing an IgE-related immune response and to trigger IgG4 and IgA antibody production by B cells [12,13]. A disadvantage of specific immunotherapy is the long-term duration of therapy along with the uncertainty as to whether tolerance will be achieved. The motivation to improve therapy, to facilitate tolerance induction and to find a predictive, diagnostic marker of successful specific immunotherapy is driving research on the cellular mechanisms of specific immunotherapy. Recent advances in the understanding of T_{regs} and the new concepts on these mechanisms are discussed in the current review.

The therapeutic effects of specific immunotherapy may act on different phases, which reflect our current knowledge of the cellular and molecular mechanisms of vaccination. Therefore, the current review is structured along the lines of events, as they may appear during specific immunotherapy.

Early immunological events in specific immunotherapy

A recent study [14] revealed the fate of the lymphocytes after allergen injection by the transfer of radioactively labeled autologous leukocytes and revealed that inflammatory activity at the site of injection (aqueous depot extract) started within the first hour and subsequently increased in a time-dependent manner. Interestingly, SLIT did not induce axillary or intestinal, inflammatory radiolabeled spots, even though patients had swallowed the allergen extract. It is evident that specific immunotherapy and SLIT are different in their lymphoid pathways and that the inflammation and recruitment of distinct leukocytes starts immediately. The engagement of the innate immune system, along with specific antibodies is important in this early phase, in which allergen is encountered, taken up and modulation of the specific immune defence is facilitated. Noticeably, allergen exposure during specific immunotherapy does not trigger IgE-mediated positive feedback regulation, which would lead to more IL-4 secretion and more IgE synthesis. In contrast, high allergen doses during specific immunotherapy promote the engagement of the low-affinity Fc ϵ RII (CD23), which is known as a negative feedback regulator of IgE-dependent responses [15]. The administered allergen in specific immunotherapy will not only be bound by specific IgE, but also by other immunoglobulin classes. IgG complexes with allergen are important in modulating the immune response and preventing IgE-mediated reactions. The existence of three types of Fc γ receptors is known. Fc γ RI and Fc γ RIII can mediate pro-inflammatory functions such as phagocytosis,

antibody-dependent cytotoxicity and the release of inflammatory mediators. Primarily, cells of the myeloid lineage express them [16]. On the other hand, Fc γ RII transmits inhibitory signals into the cells and is expressed on both myeloid and lymphoid cells. Mast cells from Fc γ RII knockout mice, in contrast with their wild-type counterparts, are highly sensitive to IgG-triggered degranulation [17], and show enhanced passive cutaneous anaphylaxis. These IgG subclasses are important switches for driving the immune response towards allergen tolerance. In this context, APCs are important in providing signals for the activation and differentiation of T cells. Activated dendritic cells (DCs) are potent T-cell stimulators and express both inhibitory and activating Fc γ Rs. It was suggested that IgG subclasses are critical for the development of the tolerogenic phenotype of APCs [18[•]]. Fc γ RIIB knockouts failed to develop nasal or oral tolerance [19^{••}]. Although these recent findings indicate interesting targets for therapeutic intervention, it should be recognized that Fc γ R-mediated signals support but are not necessarily required for the induction of allergen-specific tolerance. Specific immunotherapy with T-cell epitope-representing peptides does induce allergen tolerance [20] and T_{regs} [21], thus without any intermediate immunoglobulin binding.

An alternative immunoglobulin-independent strategy that interferes with APC functions involves the receptors for pathogen-associated molecular patterns, such as the Toll-like receptors (TLRs). It allows APCs to examine their environment for potentially dangerous pathogens and to carry this information to antigen-specific T cells, which convert this information into appropriate effector responses. Therefore, the activation of pathogen-associated molecular patterns could contribute to clinical outcome-specific immunotherapy, and thus represent an important target for vaccine adjuvants. However, the relationship between these receptors, DC maturation and T_{reg} development, immune suppression and the turnover of T_{regs} is not yet well understood. A recent study demonstrated that the suppressive function of T_{regs} critically depends on immature DCs [22[•]], and is readily reversed by the maturation of DCs that are induced by granulocyte-macrophage colony-stimulating factor and do not require TLR activation. In contrast, the reversal of anergy by T_{regs} was reported to depend on TLR activation of DCs. It involves the potentiation of T_{reg} responsiveness to IL-2 by the cooperative effects of IL-1 and IL-6, which are both produced by TLR-activated, mature DCs [22[•]]. Agonists for TLR-1 and TLR-4 were shown to decrease the allergic inflammation in experimental animal models, when given during sensitization [23]. The effect on T_{reg} expansion, however, was not analysed in that study [23]. Taken together, TLR-specific adjuvants may promote T_{reg} expansion, but do not provide decision signals preferentially to drive T_{reg}

differentiation over Th1/Th2 cells. Future studies may allow the design of adjuvants that facilitate T_{reg} induction without interfering with preexisting regulatory networks, controlling pathogens and autoantigens that require T-cell activation or anergy.

Induction and maintenance of specific tolerance

Specific immunotherapy results in immunological allergen unresponsiveness, which may result in decreased or absent allergen-specific immediate hypersensitivity skin-prick test responses, and reduced allergen-specific rhinitis or asthma symptoms, and in the case of insect venoms induce resistance to anaphylactic reactions. The immunological differences become apparent starting on days 3–7 [12,24]. A key finding in the mechanisms of specific immunotherapy against bee venom allergy induced IL-10-dependent T-cell unresponsiveness (anergy) *in vitro*, 3–7 days after the initiation of specific immunotherapy and is fully developed after 28 days [12,24,25]. In addition, it was shown that IL-10-producing $CD25^{+} T_{regs}$ increase after the initiation of specific immunotherapy [26]. In the case of insect ultra-rush vaccination, $CD25^{+}$ T cells reach maximal numbers in the periphery on day 15. The increase, however, starts as early as one day after therapy initiation [27]. There is evidence that FOXP3 expression increases between days 1 and 7 after the initiation of specific immunotherapy, which is accompanied by IL-10 and TGF- β expression in the same patients (G. Hofbauer, unpublished data). It is of great interest to understand the mechanisms of T_{reg} induction in specific immunotherapy, because it may allow the therapeutic enhancement or facilitation of the induction of peripheral tolerance and thus the endogenous capacity of the organism to control allergen reactivity. Current knowledge from the early events of specific immunotherapy is not yet sufficient to link allergen control with the induction of T_{regs} . However, the potential of tolerogenic APCs is evident as highlighted above. The antigen [28] and the absence of Th1 or Th2 driving conditions [29**] is essential for the induction of T_{regs} *in vitro*. It can thus be hypothesized that the generation of T_{regs} represents a default pathway that requires T-cell receptor activation as for any other T-cell differentiation, but the absence of decision signals for effector T cells [30*]. This allows several observations to be integrated: T_{regs} from cord blood, which are characterized by an uncommitted phenotype, also show a higher capacity for suppressive T_{reg} function than T_{regs} from adult donors [31]. TGF- β has been shown to promote FOXP3 expression [32–34], but is also known to inhibit the Th1 driving transcription factor T-BET [35] and the Th2-driving transcription factor GATA-3 [36,37]. We have demonstrated that glucocorticoids induce FOXP3 expression both *in vivo* and *in vitro* [29**]. This study also shows that IL-10 promotes FOXP3 expression *in vitro*, but only if IL-4 and IL-12

were neutralized. Both cytokines represent ‘decision’ signals for T-cell differentiation, and induce the transcription factors GATA-3 or T-BET, respectively. The induction of FOXP3 by glucocorticoids was independent of IL-4/IL-12-neutralizing antibodies. However, it is known that glucocorticoids inhibit GATA-3 and T-BET expression, thus replacing neutralizing conditions or TGF- β for controlling the decision signals in the process of FOXP3 induction. The induction of T_{regs} by the engagement of T-cell receptor and inhibitory receptors such as CD46 [38], CD200R [39], CTLA-4 [40] was demonstrated, and other inhibitory receptors may also have the capacity to regulate decision signals of T-cell differentiation negatively [41].

Mechanism of suppression

The induction of specific immune suppression is a key step in the re-induction of peripheral tolerance and thus in specific immunotherapy. Several mechanisms have been reported to be involved in this process and represent potential targets for supportive vaccination strategies in specific immunotherapy. Generally, T_{reg} -mediated suppression appeared to be antigen specific, which raised the question of how secreted cytokines could be involved in this process. However, recent findings showed that cytokines [42] and cytokine receptors [43**,44] can become part of the immunological synapse, which is formed between the APCs and the T cells. Therefore, soluble cytokines can be shuttled in an antigen-specific manner to a neighboring cell and the neighboring cell can specifically receive signals. The suppression mediated by T_{regs} renders the target cell into a non-responsive, non-apoptotic state (anergy). T-cell anergy can also be induced *in vitro*, when pure antigen-specific T cells are incubated with peptides in the absence of APCs [28,45,46]. Under these conditions, T cells possibly present the antigens themselves, but fail to costimulate each other, and therefore receive only one T-cell activation signal. For successful T-cell stimulation, two signals are necessary, whereas one signal alone results in T-cell anergy, which can be resolved by the addition of IL-2 [47] and IL-15 [48]. Therefore, T-cell anergy can be transient and anergized T cells are potentially available for later immune responses. Although the function of anergic cells as ‘inactive memory’ is speculative, it is interesting to note that peptide-induced T-cell anergy does not shut down the entire effector programme of T cells [28]. Increased IL-10 production [49], IL-5 and IL-13 as well as the upregulation of IL-2R were reported (CD25; [28]). As anergized cells have similar phenotypes to T_{regs} and target cells suppressed by T_{regs} it appears possible that the mechanisms of suppression are related to the two-signal paradigm. T_{regs} require IL-2 to mediate effective suppression [50,51], as anergic cells require IL-2 to become re-activated. An unbalanced activation of T cells by either signal one or two may cause suppression

as was shown for costimulatory receptors such as CD28 [52,53], OX40 [54], PD-1 [1^{••},55] and 4C8 [56]. CTLA-4 is constitutively found on T_{regs}, but its role in suppression is controversial as discussed [57–59]. The costimulatory receptors are capable of promoting phosphatidylinositol 3,4,5-trisphosphate phosphatase activity, which is a potent inhibitor of cell cycle progression [60].

Interference with signal 1 may underlie tolerance induction by anti-CD4 monoclonal antibody or the MHCII-binding receptor LAG-3 (CD223). Antibodies to CD223 inhibit suppression induced by T_{regs} both *in vitro* and *in vivo*. Natural CD25⁺ T_{regs} express CD223 upon activation, which is significantly enhanced in the presence of effector cells, whereas CD25⁺ T_{regs} from CD223 knock-out mice exhibit reduced regulatory activity [61^{••}].

The suppressive cytokines such as IL-10 or TGF- β may interfere with the downstream signal of signal 1 or 2, by recruiting phosphatases to the signaling complexes [62,63], leaving only partial activation and thus programmes T-cell anergy.

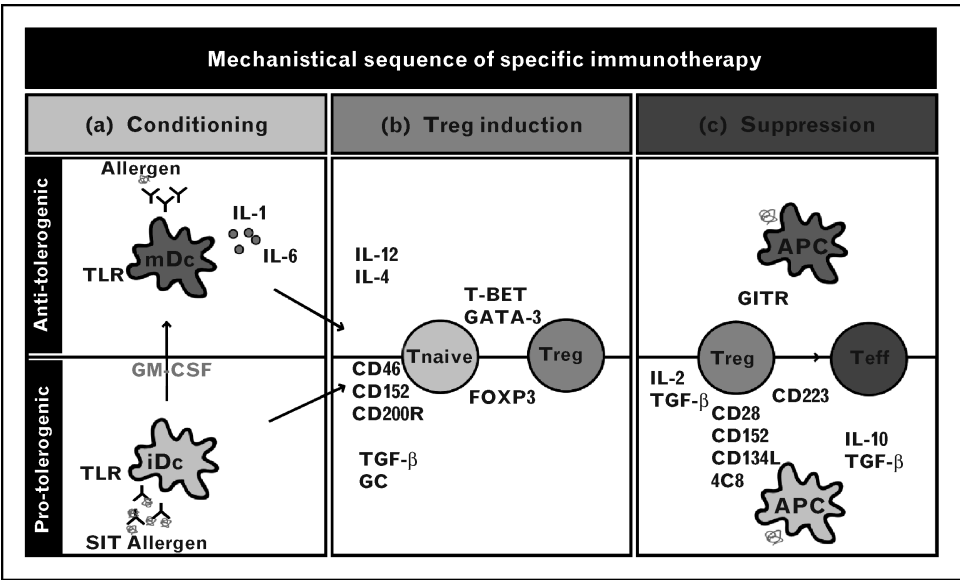
Interestingly, genes involved in cytotoxicity (perforin, granzyme), were reported to be involved in T_{reg} activity [64,65], suggesting that T_{regs} or at least certain subsets of them may also induce cell death in target cells. In most experimental systems, however, cell death could be excluded as a mechanism underlying T_{reg}-mediated suppression.

Maintenance of peripheral tolerance

The treatment success of specific immunotherapy is determined by stable tolerance against allergens. The current knowledge on the stability and turnover of T_{regs} may help to maximize the efficacy of the maintenance vaccination of specific immunotherapy. The glucocorticoid-induced TNFR-related protein (GITR), which is preferentially expressed on the surface of T_{reg} cells, provides a signal that abrogates T_{reg} suppression. A soluble form of GITR ligand induces GITR-dependent nuclear factor kappa B activation and blocks in-vitro suppression mediated by both resting and pre-activated polyclonal and antigen-specific T_{regs} [66]. The turnover or the suppressive capacity is not a constitutive parameter of T_{regs}, and TGF- β was reported to play a role in the expansion of T_{regs} *in vivo*. TGF- β may thus play a role in both T_{reg} induction and the maintenance of suppression. Similar to activated effector T cells, T_{regs} express the TGF- β RIII, endoglin, which may not only increase the affinity of T_{regs} to TGF- β , but also allows T cells to proliferate in response to low amounts of TGF- β [67^{••}]. Moreover, TGF- β is a switch and promotion factor for B cells towards IgA isotype and thereby controls peripheral tolerance, particularly in food allergies [68]. Similar to TGF- β , IL-10 was reported to promote IgG4 and shifted IgG4/IgA levels towards normal conditions and the ratio of specific IgE to IgG4 or IgA changes approximately 100–1000-fold [6]. Unfortunately, recent studies could not establish birch pollen-specific IgG4 [69] nor IgA as a marker for successful specific immunotherapy [70]. Therefore, markers of T-cell responses, specifically for

Figure 1. Current concepts of regulatory T-cell induction underlying specific immunotherapy.

Molecules are attributed to three phases, a conditioning phase (a) preceding allergen-induced regulatory T-cell (T_{reg}) induction (b), and the phase of re-challenge and T_{reg}-mediated suppression (c). The upper fields are factors counteracting, the lower fields factors that support T_{reg} induction. APC, Antigen-presenting cell; GC, glucocorticoids; iDC, immature dendritic cell; GITR, glucocorticoid-induced TNFR-related protein; mDC, mature dendritic cell; SIT, specific immunotherapy; TGF- β , transforming growth factor beta; TLR, Toll-like receptor.



T_{reg} induction and maintenance, may allow the direct monitoring of clinical success, which would greatly improve the treatment of allergies by specific immunotherapy.

Conclusion

The mechanisms of specific immunotherapy are characterized by an early induction of T_{regs} , which are capable of controlling allergen-specific effector T cells. This process is supported by immature DCs (Fig. 1a). As the effector T cells, particularly Th2 cells are necessary for IgE differentiation, it appears that the induction of sufficient numbers of T_{regs} is crucial for the success of specific immunotherapy. Peripheral tolerance to allergen in the normal immune response of healthy individuals to allergen exposure and in specific immunotherapy is mainly the result of sufficient T_{reg} numbers and not of the suppressive activity of T_{regs} . It is hypothesized that T_{regs} evolve when decision signals for Th1 or Th2 are absent (Fig. 1b), as is the case in specific immunotherapy. Therefore, therapeutic development may aim to control the microenvironment, including the maturation of DCs or T_{reg} induction before allergen exposure. The mechanism of suppression could not be defined by single receptors, but appears to involve an array of receptors (Fig. 1c) and can be categorized into two groups, those that are related to antigen-induced signaling and those to costimulatory signals. Research in the area of specific immunotherapy will take advantage of T_{reg} -relevant genes, to identify early markers of clinical success and to develop T_{reg} -focused vaccination strategies.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 577–578).

- 1 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* 2004; 199:1567–1575.
This work demonstrates for the first time that allergy in humans results from insufficient production of T_{reg} cells, which are specifically directed against allergen and are involved in peripheral tolerance induction and the generation of a normal, IgE or IgA driven healthy immune response to allergen.
- 2 Misra N, Bayry J, Lacroix-Desmazes S, *et al.* Cutting edge: human CD4+CD25+ T cells restrain the maturation and antigen-presenting function of dendritic cells. *J Immunol* 2004; 172:4676–4680.
This paper outlines the function of T_{regs} exceeding the T–T interaction. The effect of CD25+ T_{regs} on DCs is shown and demonstrates the involvement of IL-10 and TGF- β in this process.
- 3 Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. *Science* 2003; 299:1057–1061.
- 4 Schubert LA, Jeffery E, Zhang Y, *et al.* Scurfin (FOXP3) acts as a repressor of transcription and regulates T cell activation. *J Biol Chem* 2001; 276:37672–37679.
- 5 Bettelli E, Dastrange M, Oukka M. Foxp3 interacts with nuclear factor of activated T cells and NF-kappa B to repress cytokine gene expression and effector functions of T helper cells. *Proc Natl Acad Sci U S A* 2005; 102:5138–5143.
- 6 Jutel M, Akdis M, Budak F, *et al.* IL-10 and TGF-beta cooperate in the regulatory T cell response to mucosal allergens in normal immunity and specific immunotherapy. *Eur J Immunol* 2003; 33:1205–1214.
- 7 Weiner HL. Induction and mechanism of action of transforming growth factor-beta-secreting Th3 regulatory cells. *Immunol Rev* 2001; 182:207–214.
- 8 Baena-Cagnani CE, Passalacqua G, Baena-Cagnani RC, *et al.* Sublingual immunotherapy in pediatric patients: beyond clinical efficacy. *Curr Opin Allergy Clin Immunol* 2005; 5:173–177.
- 9 Wilson DR, Lima MT, Durham SR. Sublingual immunotherapy for allergic rhinitis: systematic review and meta-analysis. *Allergy* 2005; 60:4–12.
- 10 Bousquet J. Sublingual immunotherapy: from proven prevention to putative rapid relief of allergic symptoms. *Allergy* 2005; 60:1–3.
- 11 Wheeler AW, Woroniecki SR. Allergy vaccines – new approaches to an old concept. *Expert Opin Biol Ther* 2004; 4:1473–1481.
- 12 Akdis CA, Blaser K. IL-10-induced anergy in peripheral T cell and reactivation by microenvironmental cytokines: two key steps in specific immunotherapy. *FASEB J* 1999; 13:603–609.
- 13 Bellinghausen I, Knop J, Saloga J. The role of interleukin 10 in the regulation of allergic immune responses. *Int Arch Allergy Immunol* 2001; 126:97–101.
- 14 Pereira C, Botelho F, Tavares B, *et al.* Kinetics and dynamic evaluation of specific immunotherapy. *Allerg Immunol (Paris)* 2004; 36:375–386.
- 15 Payet-Jamroz M, Helm SL, Wu J, *et al.* Suppression of IgE responses in CD23-transgenic animals is due to expression of CD23 on nonlymphoid cells. *J Immunol* 2001; 166:4863–4869.
- 16 Ravetch JV, Bolland S. IgG Fc receptors. *Annu Rev Immunol* 2001; 19:275–290.
- 17 Takai T, Ono M, Hikida M, *et al.* Augmented humoral and anaphylactic responses in Fc gamma RII-deficient mice. *Nature* 1996; 379:346–349.
- 18 van Helvoort JM, Samsom JN, Chantry D, *et al.* Preferential expression of IgG2b in nose draining cervical lymph nodes and its putative role in mucosal tolerance induction. *Allergy* 2004; 59:1211–1218.
An important study together with that of Samsom *et al.* [19**], suggesting a differential role of the immunoglobulin subclasses on the mechanisms of mucosal tolerance. This study shows the lacking effect of relocated lymph nodes on the subclass profile of B cells, showing that the drainage of tissues and not the structure of the lymph nodes determines the immunoglobulin subclass.
- 19 Samsom JN, van Berkel LA, van Helvoort JM, *et al.* Fc gamma RIIb regulates nasal and oral tolerance: a role for dendritic cells. *J Immunol* 2005; 174:5279–5287.
Very interesting data demonstrating that signaling via Fc gamma RIIb on DCs, initiated by local IgG in the mucosa-draining lymph nodes, downregulates DC activation induced by nasally applied antigen. Results define conditions of antigen presentation that lead to T_{reg} induction and tolerance. Similar mechanisms are likely to act upon specific immunotherapy.
- 20 Muller U, Akdis CA, Fricker M, *et al.* Successful immunotherapy with T-cell epitope peptides of bee venom phospholipase A2 induces specific T-cell anergy in patients allergic to bee venom. *J Allergy Clin Immunol* 1998; 101:747–754.
- 21 Alexander C, Ying S, Kay AB, Larche M. Fel d 1-derived T cell peptide therapy induces recruitment of CD4+ CD25+; CD4+ interferon-gamma+ T helper type 1 cells to sites of allergen-induced late-phase skin reactions in cat-allergic subjects. *Clin Exp Allergy* 2005; 35:52–58.
- 22 Kubo T, Hatton RD, Oliver J, *et al.* Regulatory T cell suppression and anergy are differentially regulated by proinflammatory cytokines produced by TLR-activated dendritic cells. *J Immunol* 2004; 173:7249–7258.
Data presented in this paper are unexpected and give a new view on the potential contribution of DCs in the induction of T_{regs} . It is suggested that proinflammatory cytokines produced by TLR-activated, mature DCs are required for the reversal of T_{reg} anergy, but are not required to overcome T_{reg} suppression.
- 23 Velasco G, Campo M, Manrique OJ, *et al.* Toll-like receptor 4 or 2 agonists decrease allergic inflammation. *Am J Respir Cell Mol Biol* 2005; 32:218–224.
- 24 Akdis CA, Blesken T, Akdis M, *et al.* Role of interleukin 10 in specific immunotherapy. *J Clin Invest* 1998; 102:98–106.
- 25 Bellinghausen I, Metz G, Enk AH, *et al.* Insect venom immunotherapy induces interleukin-10 production and a Th2-to-Th1 shift, and changes surface marker expression in venom-allergic subjects. *Eur J Immunol* 1997; 27:1131–1139.
- 26 Gardner LM, Thien FC, Douglass JA, *et al.* Induction of T 'regulatory' cells by standardized house dust mite immunotherapy: an increase in CD4+ CD25+ interleukin-10+ T cells expressing peripheral tissue trafficking markers. *Clin Exp Allergy* 2004; 34:1209–1219.
- 27 Mamessier E, Dupuy P, Birnbaum J, *et al.* Early effect of specific wasp venom immunotherapy of T lymphocyte activation. In: European Academy of Allergy and Clinical Immunology Davos meeting. Davos, Switzerland, 2005 [Abstract no. 66].
- 28 Faith A, Akdis CA, Akdis M, *et al.* Defective TCR stimulation in anergized type 2 T helper cells correlates with abrogated p56(lck) and ZAP-70 tyrosine kinase activities. *J Immunol* 1997; 159:53–60.

- 29 Karagiannidis C, Akdis M, Holopainen P, *et al.* Glucocorticoids upregulate •• FOXP3 expression and regulatory T cells in asthma. *J Allergy Clin Immunol* 2004; 114:1425–1433.
- The data presented show that glucocorticoids transiently increase FOXP3 along with IL-10 upon inhalative or systemic treatment of asthma. This observation is confirmed by in-vitro data showing the generation of T_{reg}s upon stimulation of naive T cells in the presence of glucocorticoids. It is shown that FOXP3 is most effectively induced when IL-4 and IL-12 are neutralized.
- 30 Schmidt-Weber CB, Blaser K. Regulation and role of transforming growth factor-beta in immune tolerance induction and inflammation. *Curr Opin Immunol* 2004; 16:709–716.
- This review summarizes important features of T_{reg}s and focuses particularly on the role of TGF- β and its contribution to immune regulation.
- 31 Godfrey WR, Spoden DJ, Ge YG, *et al.* Cord blood CD4(+)CD25(+) derived T regulatory cell lines express FoxP3 protein and manifest potent suppressor function. *Blood* 2005; 105:750–758.
- 32 Chen W, Jin W, Hardegen N, *et al.* 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.
- 33 Fantini MC, Becker C, Monteleone G, *et al.* Cutting edge: TGF-beta induces a regulatory phenotype in CD4+CD25- T cells through Foxp3 induction and down-regulation of Smad7. *J Immunol* 2004; 172:5149–5153.
- 34 Marie JC, Letterio JJ, Gavin M, Rudensky AY. TGF- β 1 maintains suppressor function and Foxp3 expression in CD4+CD25+ regulatory T cells. *J Exp Med* 2005; 201:1061–1067.
- 35 Gorelik L, Constant S, Flavell RA. Mechanism of transforming growth factor beta-induced inhibition of T helper type 1 differentiation. *J Exp Med* 2002; 195:1499–1505.
- 36 Gorelik L, Fields PE, Flavell RA. Cutting edge: TGF-beta inhibits Th type 2 development through inhibition of GATA-3 expression. *J Immunol* 2000; 165:4773–4777.
- 37 Heath VL, Murphy EE, Crain C, *et al.* TGF-beta1 down-regulates Th2 development and results in decreased IL-4-induced STAT6 activation and GATA-3 expression. *Eur J Immunol* 2000; 30:2639–2649.
- 38 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* 2003; 421:388–392.
- 39 Gorczynski RM, Lee L, Boudakov I. Augmented induction of CD4+CD25+ Treg using monoclonal antibodies to CD200R. *Transplantation* 2005; 79:488–491.
- 40 Vasu C, Prabhakar BS, Holterman MJ. Targeted CTLA-4 engagement induces CD4+CD25+CTLA-4 high T regulatory cells with target (allo)antigen specificity. *J Immunol* 2004; 173:2866–2876.
- 41 Sinclair NR. Why so many coinhibitory receptors? *Scand J Immunol* 1999; 50:10–13.
- 42 Reichert P, Reinhardt RL, Ingulli E, Jenkins MK. Cutting edge: in vivo identification of TCR redistribution and polarized IL-2 production by naive CD4 T cells. *J Immunol* 2001; 166:4278–4281.
- 43 Maldonado RA, Irvine DJ, Schreiber R, Glimcher LH. A role for the immunological synapse in lineage commitment of CD4 lymphocytes. *Nature* 2004; 431:527–532.
- This publication is very important, because it not only shows that cytokine receptors are part of the immunological synapse, it also shows that the recruitment of receptors is regulated and part of the T-cell phenotype.
- 44 Vamosi G, Bodnar A, Vereb G, *et al.* IL-2 and IL-15 receptor alpha-subunits are coexpressed in a supramolecular receptor cluster in lipid rafts of T cells. *Proc Natl Acad Sci U S A* 2004; 101:11082–11087.
- 45 Faith A, Akdis CA, Akdis M, *et al.* An altered peptide ligand specifically inhibits Th2 cytokine synthesis by abrogating TCR signaling. *J Immunol* 1999; 162:1836–1842.
- 46 Fasler S, Aversa G, Terr A, *et al.* Peptide-induced anergy in allergen-specific human Th2 cells results in lack of cytokine production and B cell help for IgE synthesis. Reversal by IL-2, not by IL-4 or IL-13. *J Immunol* 1995; 155:4199–4206.
- 47 Jenkins MK, Chen CA, Jung G, *et al.* Inhibition of antigen-specific proliferation of type 1 murine T cell clones after stimulation with immobilized anti-CD3 monoclonal antibody. *J Immunol* 1990; 144:16–22.
- 48 Akdis CA, Akdis M, Blesken T, *et al.* Epitope-specific T cell tolerance to phospholipase A2 in bee venom immunotherapy and recovery by IL-2 and IL-15 *in vitro*. *J Clin Invest* 1996; 98:1676–1683.
- 49 Chou YK, Robey I, Woody CN, *et al.* Induction of T cell anergy by high concentrations of immunodominant native peptide is accompanied by IL-10 production and a block in JNK activity. *Cell Immunol* 1998; 188:125–136.
- 50 de la Rosa M, Rutz S, Dorninger H, Scheffold A. Interleukin-2 is essential for CD4+CD25+ regulatory T cell function. *Eur J Immunol* 2004; 34:2480–2488.
- 51 Malek TR. The main function of IL-2 is to promote the development of T regulatory cells. *J Leukoc Biol* 2003; 74:961–965.
- 52 Bour-Jordan H, Salomon BL, Thompson HL, *et al.* Costimulation controls diabetes by altering the balance of pathogenic and regulatory T cells. *J Clin Invest* 2004; 114:979–987.
- 53 Lin CH, Hunig T. Efficient expansion of regulatory T cells *in vitro* and *in vivo* with a CD28 superagonist. *Eur J Immunol* 2003; 33:626–638.
- 54 Takeda I, Ine S, Killeen N, *et al.* Distinct roles for the OX40-OX40 ligand interaction in regulatory and nonregulatory T cells. *J Immunol* 2004; 172:3580–3589.
- 55 Gray CP, Arosio P, Hersey P. Heavy chain ferritin activates regulatory T cells by induction of changes in dendritic cells. *Blood* 2002; 99:3326–3334.
- 56 Masuyama J, Kaga S, Kano S, Minota S. A novel costimulation pathway via the 4C8 antigen for the induction of CD4+ regulatory T cells. *J Immunol* 2002; 169:3710–3716.
- 57 Read S, Malmstrom V, Powrie F. Cytotoxic T lymphocyte-associated antigen 4 plays an essential role in the function of CD25(+)CD4(+) regulatory cells that control intestinal inflammation. *J Exp Med* 2000; 192:295–302.
- 58 Tang Q, Boden EK, Henriksen KJ, *et al.* Distinct roles of CTLA-4 and TGF-beta in CD4+CD25+ regulatory T cell function. *Eur J Immunol* 2004; 34:2996–3005.
- 59 Kataoka H, Takahashi S, Takase K, *et al.* CD25(+)CD4(+) regulatory T cells exert in vitro suppressive activity independent of CTLA-4. *Int Immunol* 2005; 17:421–427.
- 60 Schmidt-Weber CB, Wohlfahrt JG, Akdis C, Blaser K. The phosphatidylinositol phosphatase PTEN is under control of costimulation and regulates proliferation in human T cells. *Eur J Immunol* 2002; 32:1196–1204.
- 61 Huang CT, Workman CJ, Flies D, *et al.* Role of LAG-3 in regulatory T cells. •• *Immunity* 2004; 21:503–513.
- Important and convincing data showing that MHC-pathways ('signal 1') contributes to T_{reg}-mediated suppression. It is shown that anti-LAG-3 (CD223) as well as genetically induced CD223 defects affect T_{reg}-mediated suppression.
- 62 Joss A, Akdis M, Faith A, *et al.* IL-10 directly acts on T cells by specifically altering the CD28 co-stimulation pathway. *Eur J Immunol* 2000; 30:1683–1690.
- 63 Akdis CA, Joss A, Akdis M, *et al.* 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* 2000; 14:1666–1668.
- 64 Grossman WJ, Verbsky JW, Barchet W, *et al.* Human T regulatory cells can use the perforin pathway to cause autologous target cell death. *Immunity* 2004; 21:589–601.
- 65 Gondek DC, Lu LF, Quezada SA, *et al.* Cutting edge: contact-mediated suppression by CD4+CD25+ regulatory cells involves a granzyme B-dependent, perforin-independent mechanism. *J Immunol* 2005; 174:1783–1786.
- 66 Ji HB, Liao G, Faubion WA, *et al.* Cutting edge: the natural ligand for glucocorticoid-induced TNF receptor-related protein abrogates regulatory T cell suppression. *J Immunol* 2004; 172:5823–5827.
- 67 Schmidt-Weber CB, Letarte M, Kunzmann S, *et al.* The ancillary TGF-beta •• receptor endoglin modulates the TGF-beta response of T cells. *Int Immunol* 2005; 17:921–930.
- The study shows for the first time that endoglin (CD105), an ancillary TGF- β receptor is expressed by T cells, particularly by memory, but also some naive T cells. Its surface expression is dependent on T-cell activation, allowing the T cell to increase TGF- β affinity when encountering the specific antigen.
- 68 Frossard CP, Tropia L, Hauser C, Eigenmann PA. Lymphocytes in Peyer patches regulate clinical tolerance in a murine model of food allergy. *J Allergy Clin Immunol* 2004; 113:958–964.
- 69 Bodtger U, Ejrnaes AM, Hummelshoj L, *et al.* Is immunotherapy-induced birch-pollen-specific IgG4 a marker for decreased allergen-specific sensitivity? *Int Arch Allergy Immunol* 2005; 136:340–346.
- 70 Taudorf E, Moller C, Russell MW. Secretory IgA response in oral immunotherapy. Investigation in birch pollinosis. *Allergy* 1994; 49:760–765.