Review

IL-10-Producing Type 1 Regulatory T Cells and Allergy

Kui Wu[1], Yutian Bi[1], Kun Sun[2] and Changzheng Wang[2, 3]

As an important subset of regulatory T (Treg) cells, IL-10-producing type 1 regulatory T cells (Tr1), have some different features to thymic-derived naturally occurring $CD4^+CD25^+Foxp3^+$ Treg cells(nTreg cells). Similar to nTreg cells, Tr1 also play important roles in the control of allergic inflammation in several ways. There is a fine balance between Tr1 and Th2 responses in healthy subjects. Skewing of allergic-specific effctor T cells to a Tr1 phenotype appears to be a critical event in successful allergen-specific immunotherapy and glucocorticoids and β_2-agonists treatment. Tr1 suppress Th2 cells and effector cells of allergic inflammation, such as eosinophils, mast cells, basophils, through producing IL-10, and perhaps TGF-β. Understanding of Tr1 may be helpful in developing new strategies for treatment of allergic diseases. *Cellular & Molecular Immunology*. 2007;4(4):269-275.

Key Words: allergic disease, immune dysregulation, interleukin 10, regulatory T cell, type 1 regulatory T cell

Introduction

Regulatory T (Treg) cells play crucial roles in the induction of peripheral tolerance to self and foreign antigens. Increasing evidence suggests these cells play important roles in the immunological dysregulation underlying autoimmune disease, chronic inflammatory diseases, and cancer, as well as allergic diseases. There are two most relevant classes of Treg cells within $CD4^+$ T cells, type 1 regulatory T cells (Tr1), and $CD4^+CD25^+$Treg cells (1). These two subsets of Treg cells differ in a number of important biological features, including their specific cytokine production, surface markers, ability to differentiate in antigen-specific responses, and patterns to exert their functions, i.e., through secretion of cytokines or cell-cell contact mechanisms.

Allergic asthma is characterized by airway hyper-responsiveness and chronic mucosal inflammation mediated by $CD4^+$ Th2 lymphocytes. Increasing evidence has suggested impaired regulatory T cell activity can cause autoimmune diseases and allergy (2). The roles of these two Treg cell subsets in allergy have been commented before. Nonetheless, the precise molecular mechanisms involved in the differentiation and function of these cells remain to be fully elucidated, especially Tr1. In this review, we will mainly focus on biological features and the possible roles of IL-10-producing Tr1 cells in the pathogenesis of allergy, as well as the potential use of Tr1 cells in current and future therapy for allergic diseases.

Features of Tr1

Biological features of Tr1

The term "Tr1 cell" refers to the $CD4^+$ T cell populations that producing IL-10 with regulatory activity. These cells are characterized by a distinct cytokine production profile, which distinguishes them from Th1 and Th2 cells (3). Upon activation, Tr1 cells produce high levels of IL-10, TGF-β, IL-5, low amounts of IFN-γ and IL-2, but no IL-4. Depending on the experimental systems used for the induction of Tr1 cells, their cytokine production profile can vary with regards to TGF-β, IFN-γ, and/or IL-5 production, but their levels of IL-10 production are all high, and IL-4 production is undetectable. IL-10 secreted by Tr1 cells is detectable after 4 h, and reaches top concentration 12-24 h after activation.

Activated Tr1 cells express IL-2 receptor α chain (IL-2Rα) and high levels of the IL-15Rα chain, together with both the IL-2/IL-15Rβ and IL-2/IL-15Rγ chains (4). After TCR-mediated activation, expression of activation markers, including CD40L, CD69, CD28, cytotoxic T-lymphocyte antigen-4 (CTLA-4), and human leukocyte antigen-DR (HLA-DR), is upregulated in Tr1 cells. Human rest Tr1 cells express CXCR3, CCR5, CCR3, CCR4 and CCR8 chemokine receptors, and CXCR3 and CCR5 associated with Th1 cells, and CCR3, CCR5 and CCR8 associated with Th2 cells (5).

[1]Department of Respiratory Disease, Daping Hospital, Third Military Medical University, Chongqing 400042, China;

[2]Institute of Respiratory Disease, Xinqiao Hospital, Third Military Medical University, Chongqing 400037, China;

[3]Corresponding to: Dr. Changzheng Wang, Institute of Respiratory Disease, Xinqiao Hospital, Third Military Medical University, Chongqing, 400037, China. Tel: +86-23-6877-4603, E-mail: czwang@netease.com

Received Jun 18, 2007. Accepted Aug 8, 2007.

Some experts have tried to identify specific markers expressed by Tr1 cells to discriminate them from naturally occurring $CD4^+CD25^+$ Treg cells. Naturally occurring $CD4^+CD25^+$ Treg cells constitutively express Foxp3, which not only controls their differentiation, but has been regarded as a specific marker for these Treg cells (6). Several groups have shown that Tr1 cells do not constitutively express Foxp3, but after activation, Foxp3 can be upregulated to levels similar to activated $CD4^+CD25^-$T cells (7, 8).

Induction of IL-10-producing Tr1 cells

It has been shown naturally occurring $CD4^+CD25^+$ Treg cells are selected in thymus and thus have predefined antigen specificity, and migrate to peripheral blood in mature state. But there are also some studies demonstrating that $CD4^+CD25^+$ Treg cells can develop in the peripheral (9). $CD4^+CD25^+$ Treg cells can also be induced by some agents in the presence of high levels of IL-2, and these cells have comparative regulatory activity with naturally occurring $CD4^+CD25^+$ Treg cells (10).

On the contrary, Tr1 cells are inducible cells, and they arise from naïve precursors in the presence of IL-10. Tr1 cells can be induced *in vitro* and *in vivo* from naïve T cells by immunosuppressive drugs (8), soluble protein, and peptide antigens (11). It has also been reported that Tr1 cells can be induced from fully differentiated Th1 and Th2 cells under chronic stimulation, and these cells only secrete IL-10 without any other cytokines (12). IL-15 supports Tr1 cell proliferation, even in the absence of TCR activation, and in combination with IL-2, significantly enhanced the expansion of Tr1 cell clones *in vitro* (4). Long-term culture in IL-15 does not alter the phenotype or function of Tr1 cell clones, but it does enhance production of IFN-γ.

Dendritic cells (DCs) are the most potent professional antigen-presenting cells now we know, and they classically initiate antigen-specific immune response. This process involves the terminal maturation of DCs. In contrast, when DCs remain immature, they induce tolerance *via* deletion of antigen-specific effector T cells and/or differentiation of Treg cells (1, 13). It has been shown repeated stimulation of naïve peripheral blood $CD4^+$ T cells with allogeneic immature DCs induces human Tr1 cells *in vitro*, and these cells secrete high levels of IL-10 and TGF-β, significant amounts of IFN-γ and IL-5, low IL-2, and no IL-4, and suppress T cell responses in an IL-10- and TGF-β-dependent mechanism (7).

In addition to immature DCs, specific tolerogenic DCs can prime Tr1 cells too. Tolerogenic DCs can be induced by biological or by pharmacological agents. These agents include immunomodulatory cytokines, such as IL-10, TGF-β; certain pathogens or allergens, such as *Lactobacillus reuteri*, *L. case* (14); tumor antigens, such as myeloma cells (15); and endogenous proteins, such as heavy chain ferritin (16), or be engineered to express tolerogenic molecules, such as IL-10, TGF-β, CTLA-4 (17), or Serrate (a ligand for Notch proteins) (18). Finally, DCs treated with immunosuppressive drugs, such as vitamin D3 and/or dexamethasone, which also modulate DC maturation, prime T cells to become anergic and suppressive (19, 20).

Mechanisms of suppression

$CD4^+CD25^+$ Treg cells function in a cell-cell contact manner. Although it is not clear whether IL-10 and/or TGF-β play roles in $CD4^+CD25^+$ Treg cells mediated suppression *in vivo*, studies have established that IL-10 has no roles in the function of human $CD4^+CD25^+$ Treg cells *in vitro* (21). In contrast, Tr1 cells function through the secretion of the immunosuppressive cytokines IL-10 and TGF-β both *in vivo* and *in vitro* (1). Although Tr1 cells are activated in antigen-specific manner, once activated, Tr1 cells can mediate bystander suppressive activity against other antigens, and this bystander suppression is mediated by the local release of IL-10 (22). TGF-β may also attribute the function of Tr1 cells, as there are still some studies showing that Tr1 cells mainly function through IL-10 (2, 22). The suppressive effects of Tr1 cells are partially or fully blocked by addition of anti-IL-10 and anti-TGF-β neutralizing monoclonal antibodies (7, 23). But contact-dependent signals, such as programmed death-1 (PD-1), glucocorticoid-induced TNF receptor (GITR), membrane TGF-β (mTGF-β), and cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), appear to be important in some situations (24). The precise mechanisms still need to be fully investigated.

Role of Tr1 in allergy

IL-10-producing Tr1 cells in pathogenesis of allergy

Allergic diseases, such as allergic asthma, rhinitis, and atopic dermatitis are characterized by chronic inflammatory disorders caused by Th2-type immune responses against common "innocuous" environmental antigens (allergens) in susceptible individuals. Th2 cells produce IL-4, IL-5, IL-9, and IL-13 and mediate several regulatory and effector functions in the production of allergen-specific IgE by B cells, development and recruitment of eosinophils, production of mucus, contraction of smooth muscles, and airway hyperresponsiveness (AHR). Th1 cells might also efficiently contribute to the chronicity and effector phase in allergic diseases (25) or dampen allergic inflammation (26).

Characteristic of Tr1 responses in allergy

Several studies have demonstrated that a peripheral T-cell repertoire to allergens exists that recognizes the same T-cell epitopes as allergic patients (24). Specific Tr1 cells represent the dominant subset against allergens in healthy individuals, on the contrary, there was high frequency of allergen-specific Th2 cells in allergic individuals. *In vitro* generated Tr1 cells by antigen stimulation in the presence of IL-10, could prevent Th2 sensitization and IgE production if adoptively transferred prior to sensitization (27). Tr1 cells function through producing immunosuppressive cytokines, including IL-10 and TGF-β and they produce high levels of IL-10 rather than TGF-β (28). The IL-10 levels in bronchoalveolar lavage fluid of asthma patients were lower than those in healthy controls, and T cells from asthma children produce

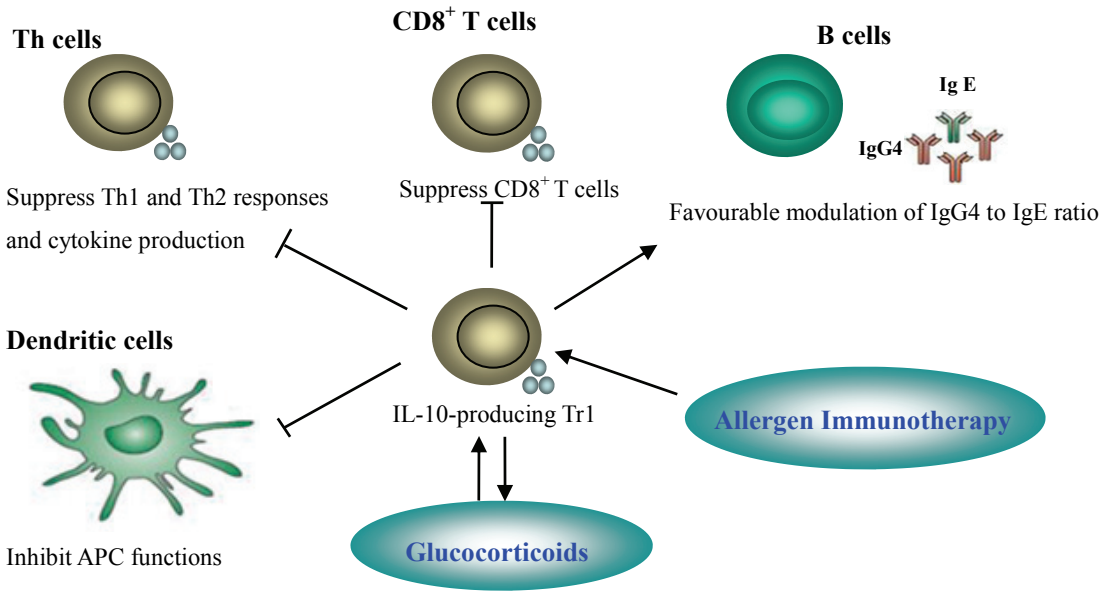

Figure 1. Glucocorticoids and allergen immunotherapy function through induction of IL-10 producing Tr1 cells. Tr1 cells secreted IL-10 and TGF-β, and these cytokines could suppress Th2 dominat allergic responses.

less IL-10 mRNA than those from healthy children. As observed in a large cohort of allergic and non-allergic children, increased IL-4, IL-5 and IL-13 were associated with allergy, and IL-10 was associated with negative allergy skin tests (29). Bullens and co-workers (30) showed that *ex vivo* specific stimulation by recombinant allergens, T cells from allergic display the expected Th2 pattern, on the contrary, T cells from healthy controls produce IL-10 and IFN-γ, and express ICOS. IL-10 is a potent antiinflammatory and immunosuppressive cytokine that mediates its major immunosuppressive function by inhibiting APC function and cytokine production by macrophages and dendritic cells, leading to profound inhibition of Th1 cell-mediated immunity (31). IL-10 also regulates effector responses associated with established allergic and asthmatic disease, including inhibition of cytokine production by Th2 cells as well as mast cells and eosinophil function and modulation of IgG4:IgE ratios (12, 31). IL-10 also reduces proinflammatory cytokine release from mast cells, downregulates eosinophil function and activity, and suppresses IL-5 production by human resting Th0 and Th2 cells. There appears to be an inverse correlation between IL-10 levels and the incidence and/or severity of asthmatic and allergic disease (24, 29). Negoro (32) investigated the single nucleotide polymorphism (SNP) in Japanese allergic children, and found the genotype with lower IL-10 production is associated with higher IgE levels in the serum. Grunig and colleagues (33) reported that *in vitro* restimulated lung cells from sensitized IL-$10^{-/-}$ mice produce exaggerated amounts of IL-4, IL-5 and IFN-γ; and *in vivo*, repeated allergen inhalation in IL-$10^{-/-}$ outbred mice resulted in a 50% - 60% mortality rate, while mortality was rare in similarly treated WT mice, and exhibited exaggerated airway inflammation and heightened levels of IL-5 and IFN-γ in BAL fluids and heightened eosinophilic airway inflammation as compared with wild type mice. In animal models, transfer of the IL-10 gene directly to the lung (34) or adoptive transfer of IL-10 transfected $CD4^+$ T cells (39) blocked allergic airway inflammation. Together these findings indicate an association between increased IL-10 production and decreased allergic reactions. In mice, IL-10 administration before allergen treatment induced antigen specific T cell unresponsiveness and blocked the generation of allergic inflammation (36).

Tr1 cells suppress allergic Ig responses

$CD4^+CD25^+Foxp3^+$ Treg cells can directly suppress B cell Ig response and Ig class switch recombination (CSR) (37). In contrast to $CD4^+CD25^+$ Treg cells, Tr1 cells also cross-talk to B cells through secreting IL-10 and TGF-β, which leads to regulation of antibody production. IL-10 counterregulates antigen-specific IgE and IgG4 antibody synthesis. IL-10 suppresses both total and allergen-specific IgE, and simultaneously increases IgG4 production. IL-10 has two major effects on B cells. IL-10 decreases ε transcript expression and IgE production when added to PBMCs during the first 3 days of culture. However, IL-10 induces further upregulation of IgE production when added to already committed B cells and enhances γ4 transcript expression and IgG4 production induced by IL-4 (38). Thus IL-10 not only generates tolerance in T cells, it also regulates specific isotype formation and skews the specific response from an IgE- to an IgG4-dominated phenotype. In healthy individuals antibody response to Der p 1 is characterized by specific IgA and IgG4 levels, small amounts of IgG1, and almost undetectable IgE antibodies in serum (39). Although further studies are required, this might account for the role of IgA

and TGF-β, as well as IgG4 and IL-10, in mucosal immune responses to allergens in healthy individuals. These findings suggest a regulatory function in addition to the well-known suppressor function of Tr1 cells.

Role of Tr1 cells in immunotherapy

Allergen specific immunotherapy (SIT) has been used for about 100 years in treatment of allergic diseases, such as asthma and rhinitis. Although the precise mechanisms of SIT still need to be investigated, evidence has shown successful allergen-SIT is associated with an increase in production of IgG antibodies, primarily the IgG4 subtype, along with the generation of IgE-modulating $CD8^+$ T cells and decrease of mast cells and eosinophils, as well as a decrease in the release of mediators. The induction of peripheral T-cell tolerance plays a crucial role in allergen-SIT and is initiated by the autocrine action of IL-10 and TGF-β, which are increasingly produced by antigen-specific Tr1 cells (40). Reactivation of tolerized T cells can result in the distinct production of either Th1 or Th2 cytokine profiles depending on the cytokines present in the tissue microenvironment, and can therefore direct allergen SIT towards either successful or unsuccessful treatment.

Tr1 cells in SIT induced T cell responses

IL-10-producing Treg cells can be induced by repeated intranasal administration of an antigenic peptide of myelin basic protein (MBP) and that these regulatory cells inhibit the proliferation of naïve MBP specific T cells both *in vitro* and *in vivo* (28). During allergen SIT, IL-10 levels increased significantly at day 7, and reached a maximum at day 28 together with fully established peripheral tolerance, and the proliferative and cytokine responses could be reconstituted by *ex vivo* neutralization of endogenous IL-10, indicating that IL-10 is actively involved in the development of anergy in specific T cells (41). Phospholipase A is the major allergen of bee venom, in both venom SIT and phospholipase A-petide SIT (PLA-PIT), IL-10 production increased and reached maximal levels after 4 weeks, and antigen- and peptide-induced proliferative responses and Th1 and Th2 cytokine production decreased, at this time, specific anergy has been fully established. $IL\text{-}10^+$ T cells significantly increased after 7 days, and $IL\text{-}10^+$ monocytes and B cells were also increased after 4 weeks of allergen SIT (42). And the same features of peripheral tolerance were found in the T cells of healthy beekeepers that had previously been stung by large numbers of bees. These naturally anergized individuals show increased numbers of IL-10-producing $CD4^+CD25^+$ T cells and monocytes similar to allergic patients after bee venom SIT. Neutralization of endogenous IL-10 in PBMC cultures from these individuals fully reconstituted the proliferative T-cell response and cytokine production. In a randomized conventional HDM immunotherapy study (43), all SIT-treated allergic rhinitis patients showed reduced symptom scores and late-phase cutaneous responses to HDM compared with baseline levels, and $CD4^+IL\text{-}10^+$ T cells increased simultaneously, and IL-10 staining co-localized with $CD4^+$ $CD25^+$T cells. In humans (44), IL-10-producing cells were also recently increased in cutaneous biopsies of patients undergoing bee venom immunotherapy. Other studies also reported similar results (39, 45, 46).

Tr1 cells in SIT induced Ig responses

Hyperimmunogulobin E characterized the Ig responses of allergic diseases. In the early phase of SIT, serum levels of both IgG and IgE specificly increased. The increase in antigen-specific IgG4 levels was more pronounced, and the ratio of specific IgE to IgG4 decreased by 10 to 100 fold. A similar change in specific isotype ratio was observed in SIT of various allergies. IL-10 counterregulates antigen-specific IgE and IgG4 antibody synthesis (42). In healthy individuals antibody response to Der p1 is characterized by specific IgA and IgG4 levels, small amounts of IgG1, and almost undetectable IgE antibodies in serum. House dust mite SIT did not significantly change specific IgE levels after 70 days of treatment; however, significant increases in specific IgA, IgG1, and IgG4 levels were observed (39). The increase of specific IgA and IgG4 levels in serum coincides with increased TGF-β and IL-10 levels, respectively. Although further studies are required, this might account for the role of IgA and TGF-β, as well as IgG4 and IL-10, in mucosal immune responses to allergens in healthy individuals (42). Akdis (41) also showed that increased IL-10 suppresses IgE and enhances IgG4 synthesis, resulting in a decreased antigen-specific IgE: IgG4 ratio, as observed normally in patients after SIT with whole allergen or antigenic T cell peptides. These findings suggest a regulatory function in addition to the well-known suppressor function of Treg cells.

Tr1 in other types of SIT

Allergen-derived T cell epitope peptides have been used in clinical trial of immunotherapy, because they are safer than native allergens, as they do not cross-link IgE. Tarzi et al. reported after receiving PLA2 peptides, volunteers with allergy to bee venom showed a decrease in the magnitude of the late-phase cutaneous reaction to bee venom, the proliferation of venom-stimulated PBMCs decreased and the production of IL-13 and IFN-γ reduced, otherwise production of IL-10 increased (47). In house dust mite extract sensitized mice model, Der p1 peptide containing a major T cell epitope protected sensitized mice from airway inflammation and eosinophilia, and $CD25^+CD4^+$ and IL-10 producing cell populations increased (48).

Immunostimulatory DNA-based vaccines can prevent airway inflammation mediated by Th2 cells in experimental models when administered before or at the time of allergen exposure. Jarman et al. have shown in mice that sensitized and challenged with Der p1, treatment with pDNA vaccines reduced the infiltration of inflammatory cells, goblet cell hyperplasia and mucus production, and attenuated subepithelial fibrosis, together with the increasing of IL-10 (49).

Sublingual immunotherapy (SLIT) has been used in Europe for decades, and has been proved to be safer and more convenient than conventional SIT. It had been supposed that IL-10 may also attribute the mechanism of SLIT. In

children with allergy rhinitis undergoing pollen SLIT with high dose and low dose, the expression of IL-10 mRNA was increased at 2 years of therapy, concomitant with the decreased expression of IL-5 mRNA (50).

Role of Tr1 cells in chemotherapy of allergy

Glucocorticoids are the first-line antiinflammatory treatment for asthma. Their multiple inhibitory properties, including the inhibition of Th2 cytokine synthesis, are likely to contribute to clinical efficacy. Glucocorticoids also enhance IL-10 production *in vitro* by human $CD4^+$ and $CD8^+$ T cells (50). It has been shown that glucocorticoid treatment induces the synthesis of IL-10 by airway cells in asthmatic patients, and glucocorticoids also can enhance the production of IL-10 by polyclonally stimulated T cells, and that these IL-10-producing cells inhibited IFN-γ production by human $CD4^+$ T cells in an IL-10-dependent manner (51).

A proportion of asthmatic patients fail to benefit from oral glucocorticoid therapy and are thus denoted as having glucocorticoid-resistant (SR, derived from "steroid resistant") or insensitive asthma. SR is associated with *in vitro* and *in vivo* alterations in cellular responses to exogenous glucocorticoids. Hawrylowicz et al. have demonstrated that $CD4^+$ T cells from SR asthma patients fail to induce IL-10 synthesis following *in vitro* stimulation in the presence of dexamethasone as compared with their glucocorticoid-sensitive counterparts (SS, derived from "steroid sensitive") (52), suggesting a link between induction of IL-10 synthesis and clinical efficacy of glucocorticoids. Combination of dexamethasone and calcitriol, the active form of vitamin D3, induced high numbers of IL-10-producing T cells that made negligible amounts of Th1 and Th2 cytokines, and adoptive transfer of these IL-10-secreting Treg cells inhibited the development of murine experimental allergic encephalomyelitis *in vivo* (53); and they inhibited the proliferation of naïve T cells *in vitro* (8). More recently, Xystrakis and colleagues (54) reported that dexamethasone does not enhance secretion of IL-10 by SR $CD4^+$ T cells and addition of vitamin D3 with dexamethasone to cultures of SR $CD4^+$ T cells enhanced IL-10 synthesis to levels observed in cells from SS patients cultured with dexamethasone alone. Furthermore, pretreatment with IL-10 fully restored IL-10 synthesis in these cells in response to dexamethasone. And administration of vitamin D3 to healthy individuals and SR asthmatic patients enhanced subsequent responsiveness to dexamethasone for induction of IL-10 *in vitro*. This strongly suggests that vitamin D3 could potentially increase the therapeutic response to glucocorticoids in SR patients.

Conclusion

Peripheral T-cell tolerance is a key immunologic mechanism in healthy immune response to allergens. The roles of Treg cells, immunosuppressive cytokines, or both as a mechanism by which allergen SIT and healthy immune response to allergens is mediated have been studied. Treg cells contribute to the control of allergen-specific immune responses in 5 major ways (55): (1) suppression of antigen-presenting cells that support the generation of effector Th2 and Th1 cells; (2) suppression of Th2 and Th1 cells; (3) regulatory function on B cells by suppression of allergen-specific IgE and induction of IgG4, IgA, or both; (4) suppression of mast cells, basophils, and eosinophils; and (5) interaction with resident tissue cells and remodeling. In addition to the treatment of established allergy, it is essential to consider prophylactic approaches before initial sensitization has taken place. Preventive and therapeutic vaccines that induce Treg responses can be developed. Allergen-specific Treg cells might in turn dampen both the Th1 and Th2 cells and cytokines, ensuring a well-balanced immune response. However, it has to be considered that Treg cells might not always be responsible for beneficial effects because several studies have shown that they could be responsible for the chronicity of infections and tumor tolerance. Treg cell populations have been proved possible but difficult to grow, expand, and clone *in vitro*. A crucial area for future studies is the identification of drugs, cytokines, or costimulatory molecules that induce *in vivo* growth while preserving the suppressor function of Treg cells.

References

1. Battaglia M, Gregori S, Bacchetta R, Roncarolo MG. Tr1 cells: from discovery to their clinical application. Semin Immunol. 2006;18:120-127.
2. Romagnani S. Regulatory T cells: which role in the pathogenesis and treatment of allergic disorders? Allergy. 2006;61:3-14.
3. Groux H, O'Garra A, Bigler M, et al. A $CD4^+$ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. Nature. 1997;389:737-742.
4. Bacchetta R, Sartirana C, Levings MK, Bordignon C, Narula S, Roncarolo MG. Growth and expansion of human T regulatory type 1 cells are independent from TCR activation but require exogenous cytokines. Eur J Immunol. 2002;32:2237-2245.
5. Sebastiani S. Chemokine recepotr expression and function in $CD4^+$ T lymphocytes with regulatory activity. J Immunol. 2001;166:996-1002.
6. Fontenot JD, Rudensky AY. A well adapted regulatory contrivance: regulatory T cell development and the forkhead family transcription factor Foxp3. Nat Immunol. 2005;6: 331-337.
7. Levings MK, Gregori S, Tresoldi E, Cazzaniga S, Bonini C, Roncarolo MG. Differentiation of Tr1 cells by immature dendritic cells requires IL-10 but not $CD25^+CD4^+$ Tr cells. Blood. 2005;105:1162-1169.
8. Vieira PL, Christensen J R, O'Garra A, 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. 2004;172:5986-5993.
9. Walker MR, Carson BD, Nepom GT, Ziegler SF, Buckner JH. *De novo* generation of antigenspecific $CD4^+CD25^+$ regulatory T cells from human $CD4^+$CD25 cells. Proc Natl Acad Sci U S A. 2005;102:4103-4108.
10. Godfrey WR. Cord blood $CD4^+CD25^+$ derived T regulatory cell lines express FoxP3 protein and manifest potent suppressor function. Blood. 2005;105:750-758.
11. Grundstrom S, Cederbom L, Sundstedt A, Scheipers P, Ivars F.

Superantigen-induced regulatory T cells display different suppressive functions in the presence or absence of natural $CD4^+CD25^+$ regulatory T cells *in vivo*. J Immunol. 2003;170: 5008-5017.
12. Hawrylowicz CM, O'Ogarra A. Potential role of interleukin 10 secreting regulatory T cells in allergy and asthma. Nat Rev Immunol. 2005;5:271-283.
13. Smits HH, de Jong EC, Wierenga EA, Kapsenberg ML. Different faces of regulatory DCs in homeostasis and immunity. Trends Immunol. 2005;26:123-129.
14. Smits HH, Engering A, van der Kleij D, et al. Selective probiotic bacteria induce IL-10-producing regulatory T cells *in vitro* by modulating dendritic cell function through dendritic cellspecific intercellular adhesion molecule 3-grabbing nonintegrin. J Allergy Clin Immunol. 2005;115:1260-1267.
15. Fiore F, Nuschak B, Peola S, et al. Exposure to myeloma cell lysates affects the immune competence of dendritic cells and favors the induction of Tr1 like regulatory T cells. Eur J Immunol. 2005;35:1155-1163.
16. 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.
17. Lu L, Lee WC, Takayama T, et al. Genetic engineering of dendritic cells to express immunosuppressive molecules (viral IL10, TGF-β, and CTLA4-Ig). J Leukoc Biol. 1999;66:293-296.
18. Hackstein H, Morelli AE, Thomson AW. Designer dendritic cells for tolerance induction: guided not misguided missiles. Trends Immunol. 2001;22:437-442.
19. Penna G, Adorini L. 1α,25-dihydroxyvitamin D3 inhibits differentiation, maturation, activation, and survival of dendritic cells leading to impaired alloreactive T cell activation. J Immunol. 2000;164:2405-2411.
20. Matyszak MK, Citterio S, Rescigno M, Ricciardi-Castagnoli P. Differential effects of corticosteroids during different stages of dendritic cell maturation. Eur J Immunol. 2000;30:1233-1242.
21. Dieckmann D, Bruett CH, Ploettner H, Lutz MB, Schuler G. Human $CD4^+CD25^+$ regulatory, contact-dependent T cells induce interleukin 10-producing, contact-independent type 1-like regulatory T cells. J Exp Med. 2002;196:247-253.
22. Groux H. Type 1 Tregulatory cells: their role in the control of immune responses. Transplantation. 2003;75:8s-12s.
23. Levings MK, Roncarolo MG. Phenotypic and functional differences between human $CD4^+CD25^+$ and type 1 regulatory T cells. Curr Top Microbiol Immunol. 2005;293:303-326.
24. 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.
25. Trautmann A, Schmid-Grendelmeier P, Kruger K, et al. T cells and eosinophils cooperate in the induction of bronchial epithelial apoptosis in asthma. J Allergy Clin Immunol. 2002; 109:329-337.
26. Finotto S, Neurath MF, Glickman JN, et al. Development of spontaneous airway changes consistent with human asthma in mice lacking T-bet. Science. 2002;295:336-338.
27. Cottrez F, Hurst SD, Coffman RL, Groux H. T regulatory cells 1 inhibit a Th2-specific response *in vivo*. J Immunol. 2000;165: 4848-4853.
28. Sundstedt A, O'Neill EJ, Nicolson KS, Wraith DC. Role for IL-10 in suppression mediated by peptideinduced regulatory T cells *in vivo*. J Immunol. 2003;170:1240-1248.
29. 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. 2005;365:142-149.
30. Bullens DM, De Swerdt A, Dilissen E, et al. House dust mite-specific T cells in healthy non-atopic children. Clin Exp Allergy. 2005;35:1535-1541.
31. Moore KW, de Waal Malefyt R, Coffman RL, O'Garra A. Interleukin-10 and the interleukin-10 receptor. Annu Rev Immunol. 2001;19:683-765.
32. Negoro T, Orihara K, Irahara T, et al. Influence of SNPs in cytokine-related genes on the severity of food allergy and atopic eczema in children. Pediatr Allergy Immunol. 2006;17:583-590.
33. Grunig G, Corry DB, Leach MW, Seymour BW, Kurup VP, Rennick DM. Interleukin-10 is a natural suppressor of cytokine production and inflammation in a murine model of allergic bronchopulmonary aspergillosis. J Exp Med.1997;185:1089-1099.
34. Stampfli MR, Cwiartka M, Gajewska BU, et al. Interleukin-10 gene transfer to the airway regulates allergic mucosal sensitization in mice. Am J Respir Cell Mol Biol. 1999;21:585-596.
35. Oh JW, Seroogy CM, Meyer EH, et al. CD4 T-helper cells engineered to produce IL-10 prevent allergen-induced airway hyperreactivity and inflammation. J Allergy Clin Immunol. 2002;110:460-468.
36. Enk AH, Saloga J, Becker D, Mohamadzadeh M, Knop J. Induction of hapten-specific tolerance by interleukin 10 *in vivo*. J Exp Med. 1994;179:1397-1402.
37. Lim HW, Hillsamer P, Banham AH, Kim CH. Direct suppression of B cells by $CD4^+CD25^+$ regulatory T cells. J Immunol. 2005;175:4180-4183.
38. Jeannin P, Lecoanet S, Delneste Y, Gauchat JF, Bonnefoy JY. IgE versus IgG4 production can be differentially regulated by IL-10. J Immunol. 1998;160:3555-3561.
39. Jutel M, Akdis M, Budak F, et al. IL-10 and TGF-β cooperate in regulatory T cell response to mucosal allergens in normal immunity and specific immunotherapy. Eur J Immunol. 2003;33:1205-1214.
40. Taylor A, Verhagen J, Blaser K, Akdis M, Akdis CA. Mechanisms of immune suppression by interleukin-10 and transforming growth factor-β: the role of T regulatory cells. Immunology. 2006;117:433-442.
41. 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.
42. Akdis CA, Blesken T, Akdis M, Wuthrich B, Blaser K. Role of interleukin 10 in specific immunotherapy. J Clin Invest. 1998; 102:98-106.
43. Gardner LM, Thien FC, Douglass JA, Rolland JM, O'Hehir RE. 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. 2006;34:1209-1219.
44. Nasser SM, Ying S, Meng Q, Kay AB, Ewan PW. Interleukin-10 levels increase in cutaneous biopsies of patients undergoing wasp venom immunotherapy. Eur J Immunol. 2001; 31:3704-3713.
45. Zemann B, Schwaerzler C, Griot-Wenk M, et al. Oral administration of specific antigens to allergy-prone infant dogs induces IL-10 and TGF-β expression and prevents allergy in adult life. J Allergy Clin Immunol. 2003;111:1069-1075.
46. Francis JN, Till SJ, Durham SR. Induction of IL-10^+CD4^+ $CD25^+$ T cells by grass pollen immunotherapy. J Allergy Clin Immunol. 2003;111:1255-1261.
47. Tarzi M, Klunker S, Texier C, et al. Induction of interleukin-10 and suppressor of cytokine signalling-3 gene expression

following peptide immunotherapy. Clin Exp Allergy. 2006;36: 465-474.
48. Zuleger CL, Gao X, Burger MS, Chu Q, Payne LG, Chen D. Peptide induces $CD4^+CD25^+$ and $IL\text{-}10^+$ T cells and protection in airway allergy models. Vaccine. 2005;23:3181-3186.
49. Jarman ER, Lamb JR. Reversal of established $CD4^+$ type 2 T helper-mediated allergic airway inflammation and eosinophilia by therapeutic treatment with DNA vaccines limits progression towards chronic inflammation and remodeling. Immunology. 2004;112:631.
50. Savolaineu J, Jacobsen L, Valovirta E. Sublingual immunotherapy in children modulates allergen-induced *in vitro* expression of cytokine mRNA in PBMC. Allergy. 2006;61: 1184-1190.
51. Richards DF, Fernandez M, Caulfield J, Hawrylowicz CM. Glucocorticoids drive human $CD8^+$ T cell differentiation towards a phenotype with high IL-10 and reduced IL-4, IL-5 and IL-13 production. Eur J Immunol. 2000;30:2344-2354.
52. Hawrylowicz C, Richards D, Loke TK, Corrigan C, Lee T. A defect in corticosteroid-induced IL-10 production in T lymphocytes from corticosteroid-resistant asthmatic patients. J Allergy Clin Immunol. 2002;109:369-370.
53. 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. 2002;195:603-616.
54. Xystrakis E, Kusumakar S, Boswell S, et al. Reversing the defective induction of IL-10-secreting regulatory T cells in glucocorticoid-resistant asthma patients. J Clin Invest. 2006;116: 146-155.
55. Akdis M, Blaser K, Akdis CA. T regulatory cells in allergy: novel concepts in the pathogenesis, prevention, and treatment of allergic diseases. J Allergy Clin Immunol. 2005;116:961-968.