The role of regulatory T cells in respiratory infections and allergy / asthma

Peter McGuirk[1], Sarah C Higgins and Kingston H.G. Mills*

Immune Regulation Research Group, School of Biochemistry and Immunology,
Trinity College, Dublin 2, Ireland

*Corresponding author: Prof Kingston Mills, School of Biochemistry and Immunology, Trinity College, Dublin 2, Ireland; Phone –353-1-8963573; Fax -353-1-6772400; email: kingston.mills@tcd.ie

[1]Current address: Opsona Therapeutics Ltd, Institute of Molecular Medicine, St James's Hospital, Dublin 8.

Abstract

The role of distinct CD4$^+$ T cell populations in regulating the nature and strength of immune responses is well documented, and in the past has principally focused on the cross regulation of Th1 and Th2 cells, which secrete IFN-γ and IL-4 respectively. However, the identification of T cells capable of suppressing responses mediated by both Th1 and Th2 cells, termed regulatory T (Treg) cells, has prompted a paradigm shift in our understanding of the regulation of immune responses to infection and environmental antigens. This review will focus on the role of Treg cells in the lungs following infection with respiratory pathogens and will discuss the targeting of Treg cells in the development of new therapies for immune-mediated respiratory diseases, such as allergy and asthma.

Key words: regulatory T cell, respiratory infection, suppression, allergy, asthma.

Introduction

The continual exposure to a large number of air-borne pathogens within the respiratory tract, presents the immune system with a relentless challenge. An appropriate immune response encompasses the ability to discriminate between self and non-self, and importantly to differentiate between harmful and innocuous antigens. In order to protect the host, mechanisms must be in place to prevent deleterious reactions, such as immune responses to self-antigens, leading to autoimmune diseases, immune response to food antigens in the intestine or exaggerated immune responses to benign environmental antigens. The existence of suppressor T cells and their role in the maintenance of immune homeostasis was first proposed in the early

1970s [1]. However, the field eventually floundered because these T cells could not be phenotyped and attempts to clone the putative suppressor T cells failed.

Following the re-discovery of suppressor T cells as regulatory T (Treg) cells in the mid 1990s, it is now fully accepted that distinct subtypes of $CD4^+$ T cells, discriminated on the basis of phenotype or cytokine secretion, play a major role in maintenance of self tolerance, largely by their ability to suppress responses mediated by effector T cells. Treg cells can be divided broadly into two distinct subsets, natural Treg (nTreg) and inducible or adaptive Treg (iTreg) cells.

$CD4^+CD25^+$ nTreg cells mature in the thymus and represent 5-10% of the peripheral $CD4^+$ T cell population and have been shown to exhibit potent immunosuppressive activity both *in vitro* and *in vivo* [2-4]. Since CD25 is expressed on all activated T cells, it is not an ideal marker for nTreg cells. Attempts to find alternative phenotypic markers lead to the discovery that these nTreg cells express the forkhead-winged helix transcription factor Foxp3. This transcription factor has been shown to be critical in the development and function of nTreg cells, as both mice and humans deficient in a functional Foxp3 protein develop autoimmune diseases [5,6]. It has also been demonstrated that $CD4^+CD25^-$ T cells can convert to $CD4^+CD25^+Foxp3^+$ T cells in the periphery under the influence of TGF-β and retinoic acid [7,8]. These peripherally converted Treg cells are induced during infection and cancer and also function to suppress effector T cells.

Recent studies have shown that subsets of $Foxp3^+$ T cells can be distinguished based on differential expression of certain cell surface markers. For example, CD103 ($\alpha_E\beta_7$ integrin) is expressed at high levels on $Foxp3^+$ T cells found in secondary lymphoid organs, whereas, the chemokine receptor CCR4, which is also present on Th1 cells, is expressed at high levels on

Foxp3^{+} T cells found in the skin but not in the thymus [9,10]. In addition, it is now apparent that Foxp3^{+} Treg cells also express transcription factors previously associated with particular effectors T cells, inducing the Th1 transcription factor, T-bet and the Th2 and Th17 related transcription factor, IRF4 [11,12]. It is possible that common transcription factors facilitates shared homing receptors, allowing the Foxp3^{+} Treg cells to home to the same site as effector cells, in order to prevent excessive inflammation by effector T cells. It has recently been reported that a subpopulation of Foxp3^{+} nTreg cells that express CD39, an ectonuclease that cleaves ATP to form adenosine, are the functionally important Treg subtype in suppressing IL-17-producing T cells (Th17) cells, that mediate autoimmune diseases [13].

A number of studies demonstrated that Fox3p^{+} Treg cells mediate suppression by inhibiting the induction of IL-2 mRNA in the responder Foxp3^{-} T cells [14,15]. However, it is now apparent that the mechanism of immune suppression by Foxp3^{+} Treg cells is more complex than simply inhibiting IL-2 production and is mediated through a combination of both cell-contact dependent and independent mechanisms. It has been suggested that secretion of molecules, such as galectin-1 and fibrinogen-like protein 2 (FGLP2) play a role in suppression mediated by Foxp3^{+} nTreg cells. Galectin-1 is preferentially expressed in Treg cells and is upregulated upon TCR activation. Blocking of galectin -1 has been shown to significantly reduce the inhibitory properties of both mouse and human Treg cells [16]. FGLP2, a member of the fibrinogen superfamily, has been shown to be expressed in significantly higher levels in CD4^{+}CD25^{+}Foxp3^{+} T cells when compared with CD4^{+}CD25^{-}Foxp3^{-} T cells. Furthermore, recombinant FGLP2 has been shown to inhibit T cell proliferation in response to anti-CD3 and anti-CD28 stimulation and Treg cells from FGLP2 knockout mice have been shown to be less

effective at suppressing alloantigen T cell responses than Treg cells from FGLP2 competent mice [17].

In addition to the secretion of inhibitory factors, Foxp3^{+} Treg cells have also been shown to mediate immune suppression via cell-cell contact dependent mechanisms. It has been demonstrated that interaction of CTLA-4 on Treg cells with CD80 and CD86 on antigen presenting cells (APC), such as dendritic cells (DC), blocks the ability of DC to promote the activation of effector T cells [18]. The finding that Treg cells from CTLA-4 knockout mice are defective in their ability to suppress effector T cell responses, together with the observation that CTLA-4 defective mice spontaneously develop systemic autoimmunity further demonstrates the vital role of CTLA-4 in Foxp3^{+} Treg cell mediated immune suppression [19]. Similarly, ligation of lymphocyte activation gene-3 (LAG-3) on Treg cells with MHC class II on APC results in an inhibitory signal that suppresses DC maturation and ability to stimulate effector T cell responses [20].

In contrast to naturally occurring CD4^{+}CD25^{+}Fox3p^{+} Treg cells, inducible or adaptive Treg cells are thought to acquire their suppressor activity through antigenic activation of CD4^{+}CD25^{-}Fox3p^{-} T cells in the periphery. The suppressor activity of these Treg cells is believed to be cell-cell contact independent and is mediated primarily through the actions of anti-inflammatory cytokines, including IL-10 and TGF-β. IL-10 secreting Treg cells, also known as Tr1 cells, were shown to efficient suppressors of antigen-specific immune responses in murine models of inflammatory bowel disease. T cells from OVA-TCR-transgenic mice repeatedly stimulated with OVA and IL-10 differentiated into Tr1 cells *in vitro* [21]. These Tr1 cells produced IL-10, low to moderate levels of IL-5 and TGF-β, but with little or no IL-2, IL-4 or IFN-γ. Naïve CD4^{+} T cells can be manipulated *in vitro* to generate homogenous population of

IL-10 producing Tr1 cells [22]. The ability of Tr1 cells to suppress proliferative responses has been demonstrated by co-culture of naïve CD4^{+} T cells and Tr1 cells in the presence of allogeneic APC [21]. Similarly, Tr1 clones specific for filamentous haemaggultinin (FHA) from *Bordetella pertussis* were shown to be capable of suppressing proliferation and cytokine production by a Th1 clone against an unrelated antigen, influenza virus haemagglution [23].

TGF-β has been shown to play a critical role in the induction of Treg cells both *in vitro* and *in vivo* [24,25]. Interestingly, TGF-β also play an important role in the induction of pro-inflammatory Th17 cells [26], although recent studies have suggested that TGF-β does not directly promote Th17 cell differentiation, but acts indirectly by inhibiting activating of Th1 and Th2 cell, the products of which can suppress development of Th17 cells [27]. It has been shown that nTreg cells can be converted to Th17 cells in response to IL-6 in conditions were TGF-β levels are limiting [28,29]. In contrast, T cells that have been induced to become Treg cells under the influence of TGF-β are resistant to IL-6-induced conversion to Th17 [30]. TGF-β has also been shown, in the presence of IL-4, to reprogramme established Th2 cells to lose their ability to produce Th2 cytokines and switch to IL-9 production; these cells have been called Th9 cells [31]. Using a skin graft model, Lu *et al*., showed that CD4^{+}CD25^{+} T cells but not CD4^{+}CD25^{-} cells are able to secrete a significant amount of IL-9, which in turn activated mast cells and induced graft tolerance [32].

Treg cells that exclusively secrete TGF-β, known as Th3 cells, have also been described. These Th3 cells were originally identified in mice after oral tolerance induction to myelin basic protein (MBP) for their ability to suppress MBP-specific encephalomyelitis (EAE) *in vivo* in a TGF-β-dependent manner, as suppression was abrogated by injection of anti-TGF-β antibody [33,34]. Th3 cells provide help for IgA production and suppress both Th1 and Th2 cells [35].

Furthermore, as TGF-β is broadly expressed and acts on multiple cell types, TGF-β-secreting Th3 cells probably have a major role in many aspects of immune regulation and homeostasis [36].

DC play a key role in the initiation and regulation of T cell responses and help to link the innate and adaptive immune systems. It has been suggested that plasmacytoid DC selectively promote the induction of Th1 cells, while myeloid DC favour the induction of Th2 cells. However, it is more likely that the activation status of the DC, rather than the lineage, determines its ability to selectively promote T cell subtypes [22,37]. Ligation of LAG-3 and CTLA-4 on Treg cells with their cognate receptors on DC has been shown to suppress DC maturation and as a consequence the activation of effector T cells responses. In addition, the interaction of CTLA-4 of Treg cells with CD80 or CD86 on DC has also been shown to promote the secretion of indoeamine 2, 3-dioxygenase (IDO), a potent suppressive molecule which induces the catabolism of tryptophan into pro-apoptotic metabolites [38].

Another key process in directing naïve T cells to differentiate into effector Th1 or Th2 cells is the induction of DC maturation through pathogen recognition receptors (PRRs). Many toll-like receptor (TLR) ligands, such as LPS, CpG motifs in bacterial DNA and viral dsRNA have been shown to induce IL-12 production and to activate DC that promote the differentiation of Th1 cells [39,40]. In contrast, products of helminth parasites, yeast hyphae and cholera toxin (CT) activate DC, which directs the induction of Th2 cells [40-42]. Since Tr1 and Th3 cells arise from naïve or resting CD4$^+$ T cells in the periphery, it was highly conceivable that DCs activated with an appropriate stimulus, such as certain pathogen-derived molecules, could selectively promote the induction of Treg cells. Indeed, certain pathogen-derived molecules, including FHA and adenylate cyclase toxin (CyaA) from *B. pertussis*, non-structural protein

(NS) 4 from hepatitis C virus and CT have been shown to promote the induction of IL-10 producing Tr1 cells, through their interaction with DCs or with other innate cells, such as macrophages. [43-45].

Induction of Treg cells by respiratory pathogens

Mycobacterium tuberculosis

It is estimated that one third of the world's population is infected with *Mycobacterium tuberculosis*, the bacterium that causes tuberculosis, a disease responsible for significant morbidity and mortality worldwide. [46].   The majority of individuals infected with *M. tuberculosis* develop a delayed-type hypersensitivity response 2-3 weeks after infection [47]. Intradermal injection with purified protein derivative (PPD) from *M. tuberculosis* is used to screen for *M. tuberculosis* infection. However, the absence of skin reactivity to PPD has been described in immunocompetent individuals with active *M. tuberculosis* infection [48]. T cell proliferation in response to PPD as well as non-specific mitogens has been shown to be significantly impaired in anergic patients and is associated with high levels of IL-10-producing T cells in the peripheral blood [48]. Studies in IL-10 transgenic mice demonstrate that increased susceptibility to reactivation tuberculosis and suppression of protective Th1 responses is strongly influenced by the expression of IL-10 in the chronic phase of infection [49]. A recent study, using a model of OVA-induced eosinophilic airway inflammation demonstrated that another strain of mycobacterium, *M. vaccae*, had suppressive activity on airway eosinophilia. [50]. Treatment of mice with killed *M. vaccae* suspension (SRP299) induced allergen-specific CD4$^+$CD45RB^{low} Treg cells, which conferred protection against airway epithelium damage. The regulatory activity of CD4$^+$ T cells from SRP299-treated mice was highly potent, as cell numbers

as low as 0.25×10^6 cells significantly suppressed allergen-induced eosinophilic inflammation. This study also demonstrated that SRP299-induced Treg cells reduced other pathophysiological readouts, such as bronchial hyperresponsiveness. In addition, there are now numerous studies demonstrating the presence of high numbers of Foxp3^{+} and TGF-β secreting Treg cells in the lungs of mice and humans infected with *M. tuberculosis,* [51-54] suggesting that the presence of Treg cells in the lungs facilities the latent and persistent nature of the infection. As such, Treg cells generated by certain mycobacterial products may provide a valuable therapeutic strategy for allergic diseases.

B. pertussis

Infection with a number of pathogens is associated with the production of high levels of IL-10 and/or TGF-β by innate cells. In certain cases, specific pathogen-derived immunoregulatory molecules have been identified that stimulate IL-10 from macrophages and/or DC. *B. pertussis*, the etiologic agent of whooping cough, causes a severe and protracted respiratory disease, often complicated by secondary infections that can have a lethal outcome in young children. Recovery from infection in humans and mice is associated with the development of *B. pertussis*-specific Th1 cells [55,56]. However, antigen-specific Th1 immune responses in the lungs of infected mice are severely suppressed during the acute phase of infection [57]. The *B. pertussis* virulence factors, FHA and CyaA, have been shown to inhibit IL-12 and enhance IL-10 production from macrophages and DC and selectively stimulate the induction of IL-10 secreting Tr1 cells from naïve T cells [43,58]. Furthermore, Tr1 clones specific for FHA have been generated at low frequency from the lungs of acutely infected mice, but could not be generated from the spleens of infected or convalescent mice or the lungs of naïve mice [43].

These findings suggest that certain pathogens have developed strategies to exploit Treg cells to subvert protective immune responses and thereby prolong their survival in the host.

Although the induction of Treg cells following infection in many cases promotes bacterial persistence, it is important to note that pathogen-specific Treg cells are not exclusively detrimental to the host, as Treg cells play a role in controlling infection-induced immunopathology. This has been illustrated in a study on infection of TLR-4 deficient mice with the respiratory pathogen *B. pertussis.* Innate and adaptive IL-10 is defective in response to *B. pertussis* in TLR-4 defective mice. During the acute phase of infection Th1 responses were shown to be enhanced and IL-10-producing T cells significantly reduced in TLR-4 deficient mice. Furthermore, this was associated with enhanced inflammatory cytokine production, cellular infiltration, and severe pathological changes in the lungs of the TLR-4 deficient mice [59]. These findings suggest that ligation of conserved pathogen molecules on specific pathogen recognition receptors promotes the induction of Treg cells that prevents immunopathology. Therefore, the induction of Treg cells may represent a protective strategy adopted by the host to limit collateral damage mediated by excessive pathogen-stimulated inflammatory responses.

Pneumocystis carinii

P. carinii (PC) is an opportunistic pulmonary pathogen which can causes life-threatening pneumonia in immunocompromised individuals, such as AIDS patients. While an indispensable role for CD4$^+$ T cells in bacterial clearance is well established [60], it has also been suggested that under certain circumstances CD4$^+$ T cell-mediated inflammatory responses contribute significantly to pathogenesis of PC-induced pneumonia [61]. A number of recent studies have suggested an important role for CD4$^+$CD25$^+$Foxp3$^+$ Treg cells in controlling excessive T-cell

mediated inflammatory responses during PC infection. Hori et al have shown that adoptive transfer of CD4$^+$CD25$^-$ T cells into PC infected RAG2$^{-/-}$ mice leads to a lethal pneumonia within 13 days of transfer. In contrast, transfer of CD4$^+$CD25$^+$ Treg cells did not induce pneumonia and prevented the development of disease induced by CD4$^+$CD25$^-$ T cells [62]. In addition, studies by McKinley et al have demonstrated significantly increased lung injury in PC infected SCID mice injected with CD4$^+$CD25$^-$ T cells but not with CD4$^+$CD25$^+$ Foxp3$^+$ T cells [63]. The finding that CD4$^+$CD25$^+$Foxp3$^+$ Treg cells inhibit both pulmonary inflammation and elimination of the pathogen mediated by CD4$^+$CD25$^-$ T cells illustrates a dual role for Treg cells in infection, inhibiting both deleterious and protective immune responses.

Implications for allergy and asthma

In atopic individuals, presentation of allergens to naïve T cells leads to the development of effector Th2-type cytokines – IL-4, IL-5, IL-9 and IL-13. Generation of allergen-specific IgE antibodies responsible for subsequent hypersensitivity reactions is the hallmark of this type of immune response. This is followed by infiltration of eosinophils, neutrophils, basophils, T cells and macrophages. The possibility that Treg cells inhibit allergic diseases has received growing support from both animal and human studies. It has recently been demonstrated that alveolar type II epithelial cells preferentially promote the induction of Treg cells [64]. In addition, when compared with healthy controls, asthma patients have decreased levels of TGF-β and Foxp3$^+$ Treg cells in their lungs [65]. Furthermore, allergen-specific CD4$^+$CD25$^+$ Treg cells co-expressing CCR4 were found to home to the lung and inhibit the proliferation of allergen-specific Th2 cells in a murine model of airway inflammation [66]. Another recent study has shown that vaccination with bovis bacille Calmette-Guerin (BCG) protected mice from

subsequent OVA-sensitization. BCG vaccination prevented airway eosinophilia and was associated with increased numbers of CD4$^+$CD25$^+$Foxp3$^+$ Treg cells, increased expression of CTLA-4 and increased levels of the anti-inflammatory cytokines IL-10 and TGF-β [67].

Existing therapies for allergic diseases, such as treatment with glucocorticoids and β-2-agonists may function to promote the numbers and function of Tr1-like cells [68,69]. Both inhaled and glucocorticoid treatment in moderate and severe asthma patients have been shown to increase the frequency of Foxp3$^+$ and IL-10$^+$ CD4$^+$ T cells in the peripheral blood [68]. In addition, a study found increased numbers of CD3$^+$CD25$^+$Foxp3$^+$ cells, some of which co-expressed IL-10, in the nasal mucosa of patients with hay fever after grass pollen immunotherapy [70]. IL-10 promotes IgG4 production, an antibody isotype generally considered to be protective in the context of allergic responses; IgG4 has been shown to inhibit IgE [71]. Furthermore, it has been demonstrated that treatment with dexamethasone and vitamin D3 enhances IL-10-secreting Treg cells, which inhibit allergen-specific Th2 cells [72]. It has recently been reported that IL-23 and Th17 enhance airway inflammation by promoting neutrophil recruitment but also by enhancing Th2 cell-mediated eosinophilic inflammation in a murine asthma model [73]. Therefore, effective immunomodulatory therapies against asthma may need to suppress Th17 as well as Th2 responses.

Studies examining the relationship between allergies and parasitic infections have demonstrated that children with chronic helminth infection have reduced allergic responses to the house dust mite [74]. Infection of mice with the gastrointestinal nematode Heligmosomoides polygyrus was found to indiuced Treg cells that suppress experimentally induced airway allergy to the house dust mite allergen Der p1 [75]. These studies support the hypothesis that the induction of Treg cells by respiratory or enteric pathogens may play an important role in the

protective effect of infections against the development of allergic disorders [76]. Furthermore, identification of immunomodulatory molecules, such as FHA from *B. pertussis* or products of helminth parasites [77] [78] that promote the development of Treg cells *in vivo*, may provide us with novel immunotherapeutic drugs for the prevention and treatment of autoimmune diseases and allergy (Fig 1). Support for this possibility stems from the demonstration that parenteral administration of FHA to mice significantly ameliorated disease in a T-cell mediated model of colitis. In this model, SCID mice injected with $CD4^+CD45RB^{high}$ T cells develop a severe inflammatory bowel disease with symptoms similar to that observed in human Crohn's disease. In contrast to PBS-treated mice, FHA-treated mice had significantly reduced weight loss, reduced colon shrinkage and lower levels of inflammatory cytokines [79].

Conclusions

Asthma, allergy and autoimmune diseases are still a major cause of morbidity and mortality in humans worldwide. For the most part, treatment of many of these disorders is heavily reliant on immunosuppressive drugs, which can lack specificity or have serious side effects. Approaches based on understanding the immunology of the disease and designing interventions that inhibit aberrant immune responses have considerable potential in the development of new therapeutics. As reviewed in this article, several respiratory pathogens have evolved strategies to prevent the host from eliminating them by inducing Treg cells that subvert protective immune responses. A novel immunological approach for the treatment of allergy may be to exploit the immunomodulatory function of these pathogens and their products. Furthermore, as Treg cells are thought to be more prevalent at mucosal surfaces, immunization with pathogen-derived immunomodulatory molecules via the nasal route may favour the induction of Treg cells capable

of suppressing allergen-specific Th2 cells at the local site of inflammation. However, while manipulation of the Treg cell to effector T cell ratio *in vivo* may have considerable potential for the prevention and treatment of allergic disorders, identification and purification of safe but effective specific immunomodulatory molecules from these pathogens is required before their therapeutic potential can be realised.

Fig 1. Role of Treg cells in the prevention of allergic disorders. Under normal homeostatic conditions aberrant inflammatory responses are suppressed by Foxp3$^+$ nTreg cells. However, in certain individuals where immunological tolerance is defective, when allergens enter the lung they bind to immature DC and promote the differentiation of Th2 cells, which can differentiate into IL-9-secreting Th9 cells. The production of IL-4, IL-5 and IL-9 by activated Th2 and Th9 cells promotes extravasation of eosinophils from the vasculature, leading to immune-mediated pathology and damage to the lung epithelium. However, administration of bacterial immunomodulators (IMs) capable of promoting the differentiation of iTreg cells may help to reduce these aberrant inflammatory responses by suppressing the induction and/or expansion of reactive Th2 and Th9 cells and thus facilitating the attenuation of allergen-mediated disorders.

References

1. Gershon RK, Kondo K: **Infectious immunological tolerance**. *Immunology* 1971, **21**:903-914.
2. Sakaguchi S: **Regulatory T cells: key controllers of immunologic self-tolerance**. *Cell* 2000, **101**:455-458.
3. Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M: **Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases**. *J Immunol* 1995, **155**:1151-1164.
4. Shevach EM: **CD4+ CD25+ suppressor T cells: more questions than answers**. *Nat Rev Immunol* 2002, **2**:389-400.
5. Kriegel MA, Lohmann T, Gabler C, Blank N, Kalden JR, Lorenz HM: **Defective suppressor function of human CD4+ CD25+ regulatory T cells in autoimmune polyglandular syndrome type II**. *J Exp Med* 2004, **199**:1285-1291. Epub 2004 Apr 1226.
6. Ramsdell F: **Foxp3 and natural regulatory T cells: key to a cell lineage?** *Immunity* 2003, **19**:165-168.
7. Chen W, Jin W, Hardegen N, Lei KJ, Li L, Marinos N, McGrady G, Wahl SM: **Conversion of peripheral CD4+CD25- naive T cells to CD4+CD25+ regulatory T cells by TGF-beta induction of transcription factor Foxp3**. *J Exp Med* 2003, **198**:1875-1886.
8. Mucida D, Pino-Lagos K, Kim G, Nowak E, Benson MJ, Kronenberg M, Noelle RJ, Cheroutre H: **Retinoic acid can directly promote TGF-beta-mediated Foxp3(+) Treg cell conversion of naive T cells**. *Immunity* 2009, **30**:471-472; author reply 472-473.
9. Lehmann J, Huehn J, de la Rosa M, Maszyna F, Kretschmer U, Krenn V, Brunner M, Scheffold A, Hamann A: **Expression of the integrin alpha Ebeta 7 identifies unique subsets of CD25+ as well as CD25- regulatory T cells**. *Proc Natl Acad Sci U S A* 2002, **99**:13031-13036.
10. Sather BD, Treuting P, Perdue N, Miazgowicz M, Fontenot JD, Rudensky AY, Campbell DJ: **Altering the distribution of Foxp3(+) regulatory T cells results in tissue-specific inflammatory disease**. *J Exp Med* 2007, **204**:1335-1347.
11. Koch MA, Tucker-Heard G, Perdue NR, Killebrew JR, Urdahl KB, Campbell DJ: **The transcription factor T-bet controls regulatory T cell homeostasis and function during type 1 inflammation**. *Nat Immunol* 2009, **10**:595-602.
12. Zheng Y, Chaudhry A, Kas A, deRoos P, Kim JM, Chu TT, Corcoran L, Treuting P, Klein U, Rudensky AY: **Regulatory T-cell suppressor program co-opts transcription factor IRF4 to control T(H)2 responses**. *Nature* 2009, **458**:351-356.
13. Fletcher JM, Lonergan R, Costelloe LKi, K. , Moran BT, O' Farrrelly C, Tubridy N, Mills KHG: **CD39+Foxp3+ regulatory T cells suppress pathogenic Th17 cells and are impaired in multiple sclerosis.** . *J Immunol* 2009, **in press**.
14. Takahashi T, Kuniyasu Y, Toda M, Sakaguchi N, Itoh M, Iwata M, Shimizu J, Sakaguchi S: **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* 1998, **10**:1969-1980.
15. Thornton AM, Shevach EM: **Suppressor effector function of CD4+CD25+ immunoregulatory T cells is antigen nonspecific**. *J Immunol* 2000, **164**:183-190.

16. Garin MI, Chu CC, Golshayan D, Cernuda-Morollon E, Wait R, Lechler RI: **Galectin-1: a key effector of regulation mediated by CD4+CD25+ T cells**. *Blood* 2007, **109**:2058-2065.
17. Shalev I, Liu H, Koscik C, Bartczak A, Javadi M, Wong KM, Maknojia A, He W, Liu MF, Diao J, et al.: **Targeted deletion of fgl2 leads to impaired regulatory T cell activity and development of autoimmune glomerulonephritis**. *J Immunol* 2008, **180**:249-260.
18. Onishi Y, Fehervari Z, Yamaguchi T, Sakaguchi S: **Foxp3+ natural regulatory T cells preferentially form aggregates on dendritic cells in vitro and actively inhibit their maturation**. *Proc Natl Acad Sci U S A* 2008, **105**:10113-10118.
19. Wing K, Onishi Y, Prieto-Martin P, Yamaguchi T, Miyara M, Fehervari Z, Nomura T, Sakaguchi S: **CTLA-4 control over Foxp3+ regulatory T cell function**. *Science* 2008, **322**:271-275.
20. Liang B, Workman C, Lee J, Chew C, Dale BM, Colonna L, Flores M, Li N, Schweighoffer E, Greenberg S, et al.: **Regulatory T cells inhibit dendritic cells by lymphocyte activation gene-3 engagement of MHC class II**. *J Immunol* 2008, **180**:5916-5926.
21. Groux H, O'Garra A, Bigler M, Rouleau M, Antonenko S, de Vries JE, Roncarolo MG: **A CD4+ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis**. *Nature* 1997, **389**:737-742.
22. Barrat FJ, Cua DJ, Boonstra A, Richards DF, Crain C, Savelkoul HF, de Waal-Malefyt R, Coffman RL, Hawrylowicz CM, O'Garra 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 1alpha,25-Dihydroxyvitamin d3 has a direct effect on naive CD4(+) T cells to enhance the development of Th2 cells**. *J Exp Med* 2002, **195**:603-616.
23. McGuirk P, Johnson PA, Ryan EJ, Mills KH: **Filamentous hemagglutinin and pertussis toxin from Bordetella pertussis modulate immune responses to unrelated antigens**. *J Infect Dis* 2000, **182**:1286-1289.
24. Chen C, Lee WH, Yun P, Snow P, Liu CP: **Induction of autoantigen-specific Th2 and Tr1 regulatory T cells and modulation of autoimmune diabetes**. *J Immunol* 2003, **171**:733-744.
25. Chen W, Wahl SM: **TGF-beta: the missing link in CD4+CD25+ regulatory T cell-mediated immunosuppression**. *Cytokine Growth Factor Rev* 2003, **14**:85-89.
26. Zhou L, Chong MM, Littman DR: **Plasticity of CD4+ T cell lineage differentiation**. *Immunity* 2009, **30**:646-655.

    ●● A key review describing the plasticity of T cell lineages

27. Das J, Ren G, Zhang L, Roberts AI, Zhao X, Bothwell AL, Van Kaer L, Shi Y, Das G: **Transforming growth factor {beta} is dispensable for the molecular orchestration of Th17 cell differentiation**. *J Exp Med* 2009.
28. Osorio F, LeibundGut-Landmann S, Lochner M, Lahl K, Sparwasser T, Eberl G, Reis e Sousa C: **DC activated via dectin-1 convert Treg into IL-17 producers**. *Eur J Immunol* 2008, **38**:3274-3281.

29. Yang XO, Nurieva R, Martinez GJ, Kang HS, Chung Y, Pappu BP, Shah B, Chang SH, Schluns KS, Watowich SS, et al.: **Molecular antagonism and plasticity of regulatory and inflammatory T cell programs**. *Immunity* 2008, **29**:44-56.

30. Zheng SG, Wang J, Horwitz DA: **Cutting edge: Foxp3+CD4+CD25+ regulatory T cells induced by IL-2 and TGF-beta are resistant to Th17 conversion by IL-6**. *J Immunol* 2008, **180**:7112-7116.

31. Veldhoen M, Uyttenhove C, van Snick J, Helmby H, Westendorf A, Buer J, Martin B, Wilhelm C, Stockinger B: **Transforming growth factor-beta 'reprograms' the differentiation of T helper 2 cells and promotes an interleukin 9-producing subset**. *Nat Immunol* 2008, **9**:1341-1346.

    ●● The identification of a new novel population of CD4+ T cell that secrete IL-9, termed Th9 cells, that may prove to be very important in the pathogenic if allergic disorders.

32. Lu LF, Lind EF, Gondek DC, Bennett KA, Gleeson MW, Pino-Lagos K, Scott ZA, Coyle AJ, Reed JL, Van Snick J, et al.: **Mast cells are essential intermediaries in regulatory T-cell tolerance**. *Nature* 2006, **442**:997-1002.

33. Chen Y, Kuchroo VK, Inobe J, Hafler DA, Weiner HL: **Regulatory T cell clones induced by oral tolerance: suppression of autoimmune encephalomyelitis**. *Science* 1994, **265**:1237-1240.

34. Fukaura H, Kent SC, Pietrusewicz MJ, Khoury SJ, Weiner HL, Hafler DA: **Induction of circulating myelin basic protein and proteolipid protein-specific transforming growth factor-beta1-secreting Th3 T cells by oral administration of myelin in multiple sclerosis patients**. *J Clin Invest* 1996, **98**:70-77.

35. Weiner HL: **Induction and mechanism of action of transforming growth factor-beta-secreting Th3 regulatory cells**. *Immunol Rev* 2001, **182**:207-214.

36. 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.

37. de Jong EC, Vieira PL, Kalinski P, Schuitemaker JH, Tanaka Y, Wierenga EA, Yazdanbakhsh M, Kapsenberg ML: **Microbial compounds selectively induce Th1 cell-promoting or Th2 cell-promoting dendritic cells in vitro with diverse th cell-polarizing signals**. *J Immunol* 2002, **168**:1704-1709.

38. Grohmann U, Orabona C, Fallarino F, Vacca C, Calcinaro F, Falorni A, Candeloro P, Belladonna ML, Bianchi R, Fioretti MC, et al.: **CTLA-4-Ig regulates tryptophan catabolism in vivo**. *Nat Immunol* 2002, **3**:1097-1101.

39. Hemmi H, Takeuchi O, Kawai T, Kaisho T, Sato S, Sanjo H, Matsumoto M, Hoshino K, Wagner H, Takeda K, et al.: **A Toll-like receptor recognizes bacterial DNA**. *Nature* 2000, **408**:740-745.

40. Moser M, Murphy KM: **Dendritic cell regulation of TH1-TH2 development**. *Nat Immunol* 2000, **1**:199-205.

41. Gagliardi MC, Sallusto F, Marinaro M, Langenkamp A, Lanzavecchia A, De Magistris MT: **Cholera toxin induces maturation of human dendritic cells and licences them for Th2 priming**. *Eur J Immunol* 2000, **30**:2394-2403.

42. d'Ostiani CF, Del Sero G, Bacci A, Montagnoli C, Spreca A, Mencacci A, Ricciardi-Castagnoli P, Romani L: **Dendritic cells discriminate between yeasts and hyphae of**

the fungus Candida albicans. Implications for initiation of T helper cell immunity in vitro and in vivo**. *J Exp Med* 2000, **191**:1661-1674.

43. McGuirk P, McCann C, Mills KH: **Pathogen-specific T Regulatory 1 Cells Induced in the Respiratory Tract by a Bacterial Molecule that Stimulates Interleukin 10 Production by Dendritic Cells: A Novel Strategy for Evasion of Protective T Helper Type 1 Responses by Bordetella pertussis**. *J Exp Med* 2002, **195**:221-231.

44. Ross PJ, Lavelle EC, Mills KHG, Boyd AP: **Adenylate cyclase toxin from Bordetella pertussis synergises with lipopolysaccharide to promote innate IL-10 production and enhance the induction of Th2 and regulatory T cells**. *Infect. Immun.* 2003:Submitted for publication.

45. Lavelle EC, McNeela E, Armstrong ME, Leavy O, Higgins SC, Mills KH: **Cholera toxin promotes the induction of regulatory T cells specific for bystander antigens by modulating dendritic cell activation**. *J Immunol* 2003, **171**:2384-2392.

46. Bloom BR, Small PM: **The evolving relation between humans and Mycobacterium tuberculosis**. *N Engl J Med* 1998, **338**:677-678.

47. Tsicopoulos A, Hamid Q, Varney V, Ying S, Moqbel R, Durham SR, Kay AB: **Preferential messenger RNA expression of Th1-type cells (IFN-gamma+, IL-2+) in classical delayed-type (tuberculin) hypersensitivity reactions in human skin**. *J Immunol* 1992, **148**:2058-2061.

48. Boussiotis VA, Tsai EY, Yunis EJ, Thim S, Delgado JC, Dascher CC, Berezovskaya A, Rousset D, Reynes JM, Goldfeld AE: **IL-10-producing T cells suppress immune responses in anergic tuberculosis patients**. *J Clin Invest* 2000, **105**:1317-1325.

49. Turner J, Gonzalez-Juarrero M, Ellis DL, Basaraba RJ, Kipnis A, Orme IM, Cooper AM: **In vivo IL-10 production reactivates chronic pulmonary tuberculosis in C57BL/6 mice**. *J Immunol* 2002, **169**:6343-6351.

50. Zuany-Amorim C, Sawicka E, Manlius C, Le Moine A, Brunet LR, Kemeny DM, Bowen G, Rook G, Walker C: **Suppression of airway eosinophilia by killed Mycobacterium vaccae-induced allergen-specific regulatory T-cells**. *Nat Med* 2002, **8**:625-629.

51. Chen X, Zhou B, Li M, Deng Q, Wu X, Le X, Wu C, Larmonier N, Zhang W, Zhang H, et al.: **CD4(+)CD25(+)FoxP3(+) regulatory T cells suppress Mycobacterium tuberculosis immunity in patients with active disease**. *Clin Immunol* 2007, **123**:50-59.

52. Mason CM, Porretta E, Zhang P, Nelson S: **CD4+ CD25+ transforming growth factor-beta-producing T cells are present in the lung in murine tuberculosis and may regulate the host inflammatory response**. *Clin Exp Immunol* 2007, **148**:537-545.

53. Qin XJ, Shi HZ, Liang QL, Huang LY, Yang HB: **CD4+CD25+ regulatory T lymphocytes in tuberculous pleural effusion**. *Chin Med J (Engl)* 2008, **121**:581-586.

54. Ribeiro-Rodrigues R, Resende Co T, Rojas R, Toossi Z, Dietze R, Boom WH, Maciel E, Hirsch CS: **A role for CD4+CD25+ T cells in regulation of the immune response during human tuberculosis**. *Clin Exp Immunol* 2006, **144**:25-34.

55. Mills KH, Barnard A, Watkins J, Redhead K: **Cell-mediated immunity to Bordetella pertussis: role of Th1 cells in bacterial clearance in a murine respiratory infection model**. *Infect Immun* 1993, **61**:399-410.

56. Ryan M, Murphy G, Gothefors L, Nilsson L, Storsaeter J, Mills KH: **Bordetella pertussis respiratory infection in children is associated with preferential activation of type 1 T helper cells**. *J Infect Dis* 1997, **175**:1246-1250.

57. McGuirk P, Mahon BP, Griffin F, Mills KH: **Compartmentalization of T cell responses following respiratory infection with Bordetella pertussis: hyporesponsiveness of lung T cells is associated with modulated expression of the co-stimulatory molecule CD28**. *Eur J Immunol* 1998, **28**:153-163.

58. Hickey FB, Brereton CF, Mills KH: **Adenylate cycalse toxin of Bordetella pertussis inhibits TLR-induced IRF-1 and IRF-8 activation and IL-12 production and enhances IL-10 through MAPK activation in dendritic cells**. *J Leukoc Biol* 2008, **84**:234-243.

59. Higgins SC, Lavelle EC, McCann C, Keogh B, McNeela E, Byrne P, O'Gorman B, Jarnicki A, McGuirk P, Mills KH: **Toll-like receptor 4-mediated innate IL-10 activates antigen-specific regulatory T cells and confers resistance to Bordetella pertussis by inhibiting inflammatory pathology**. *J Immunol* 2003, **171**:3119-3127.

60. Hanano R, Kaufmann SH: **Effect on parasite eradication of Pneumocystis carinii-specific antibodies produced in the presence or absence of CD4(+) alphabeta T lymphocytes**. *Eur J Immunol* 1999, **29**:2464-2475.

61. Roths JB, Sidman CL: **Both immunity and hyperresponsiveness to Pneumocystis carinii result from transfer of CD4+ but not CD8+ T cells into severe combined immunodeficiency mice**. *J Clin Invest* 1992, **90**:673-678.

62. Hori S, Carvalho TL, Demengeot J: **CD25+CD4+ regulatory T cells suppress CD4+ T cell-mediated pulmonary hyperinflammation driven by Pneumocystis carinii in immunodeficient mice**. *Eur J Immunol* 2002, **32**:1282-1291.

63. McKinley L, Logar AJ, McAllister F, Zheng M, Steele C, Kolls JK: **Regulatory T cells dampen pulmonary inflammation and lung injury in an animal model of pneumocystis pneumonia**. *J Immunol* 2006, **177**:6215-6226.

64. Gereke M, Jung S, Buer J, Bruder D: **Alveolar type II epithelial cells present antigen to CD4(+) T cells and induce Foxp3(+) regulatory T cells**. *Am J Respir Crit Care Med* 2009, **179**:344-355.

65. Luo ZX, Liu EM, Deng B, Li X, Chen KH, Wang LJ, Huang Y, Fu Z: [**Role of Foxp3 expression and CD4+CD25+ regulatory T cells on the pathogenesis of childhood asthma**]. *Zhonghua Er Ke Za Zhi* 2006, **44**:267-271.

66. Saito K, Torii M, Ma N, Tsuchiya T, Wang L, Hori T, Nagakubo D, Nitta N, Kanegasaki S, Hieshima K, et al.: **Differential regulatory function of resting and preactivated allergen-specific CD4+ CD25+ regulatory T cells in Th2-type airway inflammation**. *J Immunol* 2008, **181**:6889-6897.

67. Li Q, Shen HH: **Neonatal bacillus Calmette-Guerin vaccination inhibits de novo allergic inflammatory response in mice via alteration of CD4+CD25+ T-regulatory cells**. *Acta Pharmacol Sin* 2009, **30**:125-133.

68. Karagiannidis C, Akdis M, Holopainen P, Woolley NJ, Hense G, Ruckert B, Mantel PY, Menz G, Akdis CA, Blaser K, et al.: **Glucocorticoids upregulate FOXP3 expression and regulatory T cells in asthma**. *J Allergy Clin Immunol* 2004, **114**:1425-1433.

69. Peek EJ, Richards DF, Faith A, Lavender P, Lee TH, Corrigan CJ, Hawrylowicz CM: **Interleukin-10-secreting "regulatory" T cells induced by glucocorticoids and beta2-agonists**. *Am J Respir Cell Mol Biol* 2005, **33**:105-111.

70. Radulovic S, Jacobson MR, Durham SR, Nouri-Aria KT: **Grass pollen immunotherapy induces Foxp3-expressing CD4+ CD25+ cells in the nasal mucosa**. *J Allergy Clin Immunol* 2008, **121**:1467-1472.

● Demonstrates the potential of immunotherapeutic approaches for allergies based on Treg cell induction.

71. Till SJ, Francis JN, Nouri-Aria K, Durham SR: **Mechanisms of immunotherapy**. *J Allergy Clin Immunol* 2004, **113**:1025-1034; quiz 1035.
72. Xystrakis E, Kusumakar S, Boswell S, Peek E, Urry Z, Richards DF, Adikibi T, Pridgeon C, Dallman M, Loke TK, 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.
73. Wakashin H, Hirose K, Maezawa Y, Kagami S, Suto A, Watanabe N, Saito Y, Hatano M, Tokuhisa T, Iwakura Y, et al.: **IL-23 and Th17 cells enhance Th2-cell-mediated eosinophilic airway inflammation in mice**. *Am J Respir Crit Care Med* 2008, **178**:1023-1032.

●● Reports that Th17 cells may cooperate with Th2 cells to induce neutrophilic and eosinophilic airway inflammation.

74. Scrivener S, Yemaneberhan H, Zebenigus M, Tilahun D, Girma S, Ali S, McElroy P, Custovic A, Woodcock A, Pritchard D, et al.: **Independent effects of intestinal parasite infection and domestic allergen exposure on risk of wheeze in Ethiopia: a nested case-control study**. *Lancet* 2001, **358**:1493-1499.
75. Wilson MS, Taylor MD, Balic A, Finney CA, Lamb JR, Maizels RM: **Suppression of allergic airway inflammation by helminth-induced regulatory T cells**. *J Exp Med* 2005, **202**:1199-1212.
76. van der Kleij D, Yazdanbakhsh M: **Control of inflammatory diseases by pathogens: lipids and the immune system**. *Eur J Immunol* 2003, **33**:2953-2963.
77. Walsh KP, Brady MT, Finlay CM, Boon L, Mills KH: **Infection with a helminth parasite attenuates autoimmunity through TGF-beta-mediated suppression of Th17 and Th1 responses**. *J Immunol* 2009, **183**:1577-1586.
78. Stepek G, Auchie M, Tate R, Watson K, Russell DG, Devaney E, Harnett W: **Expression of the filarial nematode phosphorylcholine-containing glycoprotein, ES62, is stage specific**. *Parasitology* 2002, **125**:155-164.
79. Braat H, McGuirk P, Ten Kate FJ, Huibregtse I, Dunne PJ, Hommes DW, Van Deventer SJ, Mills KH: **Prevention of experimental colitis by parenteral administration of a pathogen-derived immunomodulatory molecule**. *Gut* 2007, **56**:351-357.