



TOR VERGATA UNIVERSITY

***POTENTIAL ROLE OF IL-10-TREATED DENDRITIC
CELLS IN THE CONTROL OF THE IMMUNE
RESPONSE TO ALLERGENS***

Valentina Pacciani

DOCTORAL DISSERTATION

Faculty opponent
Prof. Maria Grazia Roncarolo
Vita-Salute San Raffaele University
HSR-TIGET, Milano

Supervisor
Dr. Federica Angelini
Tor Vergata University
Rome

Department of Public Health and Cellular Biology,

Tor Vergata University, Rome, Italy

2003-2006

TABLE OF CONTENTS

ABSTRACT	<i>pg 6</i>
LIST OF PAPERS	<i>pg 8</i>
ABBREVIATIONS	<i>pg 9</i>
INTRODUCTION	<i>pg 10</i>
Allergic Disease	<i>pg 10</i>
<i>Epidemiology of Asthma</i>	<i>pg 10</i>
<i>Immunopathogenesis of Allergic Disease</i>	<i>pg 12</i>
<i>Allergic Inflammation</i>	<i>pg 12</i>
<i>Immunological Mechanisms in Asthma</i>	<i>pg 16</i>
<i>Role of Dendritic Cells in Immune Regulation and Allergic</i>	
<i>Immune Response</i>	<i>pg 21</i>
<i>Airway DC</i>	<i>pg 21</i>
<i>Antigen Uptake and Lymphnode Migration of Airway DC</i>	
<i>pg 22</i>	
<i>Sensitization to Inhaled Allergens and Th2 Polarization</i>	
<i>by Airway DC</i>	<i>pg 24</i>
Immunological Tolerance	<i>pg 30</i>
<i>Central Tolerance</i>	<i>pg 30</i>

<i>Peripheral Tolerance</i>	<i>pg 33</i>
<i>T regulatory Cells</i>	<i>pg 36</i>
<i>Natural Occuring T regulatory Cells</i>	<i>pg 36</i>
<i>Adaptive T reg</i>	<i>pg 40</i>
<i>CD4+CD25+ T Regulatory Cells</i>	<i>pg 40</i>
<i>Type-1 Regulatory Cells (Tr1)</i>	<i>pg 41</i>
<i>Mechanisms of Tolerance to Allergens</i>	<i>pg 46</i>
<i>Role of CD4+CD25+ in allergy</i>	<i>pg 48</i>
<i>Role of Tr1 in allergy</i>	<i>pg 50</i>
<i>The Interplay between IL-10 Dendritic Cells, and T regulatory Cells in Allergy</i>	<i>pg 52</i>
<i>IL-10</i>	<i>pg 52</i>
<i>Immunoregulation of Dendritic Cells by IL-10</i>	<i>pg 56</i>
<i>Induction of T Regulatory Cells by Dendritic Cells</i>	<i>pg 57</i>
<i>Control of Dendritic Cell Function by T regulatory Cells</i>	<i>pg 59</i>
<i>AIM OF THE STUDY</i>	<i>pg 61</i>
<i>MATERIALS AND METHODS</i>	<i>pg 62</i>
<i>RESULTS</i>	<i>pg 68</i>
<i>DISCUSSION</i>	<i>pg 97</i>

CONCLUDING REMARKS

pg 103

REFERENCES

pg 104

Ringraziamenti

pg 135

PAPER

pg 137

ABSTRACT

Several lines of evidence indicate that a defect in immunoregulatory mechanisms is involved in the pathogenesis of allergic asthma.

The aim of this study was to determine whether IL-10-treated dendritic cells (DC) are able to modulate allergen-specific T cell responses in children affected by allergic asthma.

Forty-one children, aged between 4-14 years, allergic to House Dust Mite (HDM), and 10 healthy age-matched children were recruited. DC were differentiated from peripheral blood CD14⁺ precursors and cultured with GM-CSF and IL-4 for 5 days. Der p2 (a major HDM allergen) was added alone or in combination with IL-10 for 48 hours to obtain Dp2-DC and IL10 Dp2-DC, respectively. Alternatively, DC were differentiated in the presence of IL-10 and pulsed with Der p2 during the 2 last days of culture (Dp2-DC10).

The ability of the resulting DC to stimulate allergen-specific autologous T cells and to promote allergen-specific T cell anergy was analyzed.

Dp2-DC induced allergen-specific T cell proliferation in 32 out of 41 patients but not in healthy controls. In 25 out of 26 allergic patients both IL10 Dp2-DC and Dp2 DC10 induced a significantly lower allergen-specific T cell proliferation. The analysis of DC phenotype showed that IL-10 treatment during the last 2 days of culture down-regulated the expression of CD86 on Dp2-DC. However, no correlation between the reduction of CD86 expression and of T cell proliferation was observed. Dp2-DC promoted a selective activation of autologous CD4⁺ T cells (CD25 and intracellular CTLA-4 up-regulation), which remained unchanged after IL-10 treatment. Moreover, Dp2-DC stimulation induced a Th2 cytokine profile characterized by an increase of IL-5, IL-13 and IL-4 production and IL-5/IFN- γ ratio. In the same patients, the co-culture with both IL10 Dp2-DC and Dp2 DC10 caused a marked reduction of IL-5 and IL-13 production by T cells, with a parallel decrease of IL-5/IFN- γ ratio. Moreover, in 8 children we observed an increase of IL-10 production with IL10 Dp2-DC stimulation.

T cell lines generated with Dp2-DC10, compared to those generated with Dp2-DC, were hyporesponsive to reactivation with Der p2 in 4 out of 5 patients tested, both in terms of proliferation and cytokine production: IL-5, IL-13 and IL-5/IFN- γ ratio.

Our data show that IL-10 reduced the stimulatory capacity of DC through a mechanism independent from the downregulation of costimulatory signals. IL-10 treatment of DC promoted a suppression of allergen-specific Th2 cytokine production without a skewing to a Th1 profile, but with a parallel increase of IL-10 production by T cells. Moreover, Dp2-DC10 were able to promote T cell anergy associated with a reduction in Th2 cytokine production. These results represent an important step forward to the prospective clinical application of Dp2-DC10 to modulate allergen-specific T cell responses *in vivo*.

Key Words: Allergy, Th2, Dendritic cells, IL-10, Regulatory T cells

LIST OF PAPERS

- I. Pacciani V, Gregori S, Chini L, Chianca M, Corrente S, Rossi P, Roncarolo MG and Angelini F. Potential role of IL-10 treated dendritic cells in controlling the immune response to allergens. Manuscript in preparation.
- II. Angelini F, Piccinini S, Pacciani V, Di Cesare S, Rapini N, Del Duca E., Paone FM, Rossi P, Manca Bitti ML. Increase of $\alpha 4\beta 7$ integrin on T lymphocytes in children affected by Type 1 diabetes. 2007. Submitted.
- III. Gregori S, Pacciani V, Tomasoni D, Scirpoli M, Battaglia M, Magnani C, Hauben E, Roncarolo MG. Differentiation of type 1 T regulatory cells through the IL-10-dependent ILT4/HLA-G pathway. 2007. Submitted.
- IV. Angelini F, Pacciani V, Di Pede A, Polito A, Riccardi C, Rossi P and Chini L. Role of dendritic cells in the mechanism of sublingual immunotherapy in children affected by allergic asthma. 2007. Submitted.
- V. Angelini F, Del Duca E, Piccinini S, Pacciani V, Rossi P and Manca Bitti ML. Altered phenotype and function of dendritic cells in children with type 1 diabetes. Clin Exp Immunol 2005; 142: 341-346.

ABBREVIATIONS

ACD	Apoptotic cell death
AHR	Airway hyper reactivity
AICD	Activation-induced Cell Death
APC:	Antigen presenting cells
BAL	bronchoalveolar lavage
DC:	Dendritic cell
Der p2	Major group 2 allergen from <i>Dermatophagoides Pteronyssinus</i>
GVHD	Graft versus host disease
HDM	House dust mites
IBD	Inflammatory bowel disease
IDO	2,3 Indoleamine deoxygenase
LPS	Lyopolisaccarides
mDC	Myeloid dendritic cells
MHC:	Major histocompatibility complex
mTEC:	medullar thymic epithelial cell
NKT	Natural killer T cells
nTreg	Natural occurring T regulatory cells
PBMC	Peripheral blood mononuclear cells
pDC	Plasmacytoid dendritic cells
SIT	Specific immunotherapy
SLIT	Sublingual immunotherapy
TCR:	T cell receptor
TD1	Type 1 diabetes
Th1	T helper 1
Th2	T helper 2
Tr1	Type 1 regulatory cells
Treg	T regulatory cells

INTRODUCTION

ALLERGIC DISEASE

Allergy can be defined as the clinically evident reaction to ubiquitous allergens. Immunological sensitization to common environmental allergens, such as house dust mites, grass and tree pollens and cat dander, can result in diseases such as allergic rhinitis and conjunctivitis, asthma and atopic dermatitis, and in the most extreme cases, in anaphylaxis and death (**Kay AB et al., 2001; Holgate S et al., 2003**).

Clinical symptoms vary, partly depending on how the allergen is introduced into the body. Aeroallergens are frequently associated with asthma, causing characteristic tightness in the chest, wheezing and shortness of breath. Most individuals with allergic asthma also suffer from allergic rhinitis, and the two conditions have a common immunopathology.

Epidemiology of Asthma

Allergic rhinitis, asthma and atopic eczema are among the commonest causes of chronic ill health care costs. In Sweden, for example, the number of children with allergic rhinitis, asthma or eczema roughly doubled over a 12-year period (**Aberg N et al., 1995**) and in the United States the annual cost of treating asthma is about \$6 billion (**Smith DH et al., 1997**).

The prevalence of allergy varies between countries, as reported in the ISAAC study, (**ISAAC 1998**), with one study of young adults showing that the prevalence of sensitization to at least one allergen varies from 16% in Albacete, Spain, to 46% in Christchurch, New Zealand (**Burney P et al., 1997**). The prevalence is also generally much higher in the developed, rather than the developing, world. The recent increase in the prevalence of allergy and diseases associated with allergy in the developed world has major health and economic consequences.

Asthma prevalence in childhood, reaches in Germany 20% and in Australia 29% (**Sears MR et al., 1997**). In the USA asthma affects approximately 8-10% of the population and is the leading cause of hospitalization among children less than 15 years of age (**Elias JA et al., 2003**). Eighty percent of childhood asthma is reported to be IgE mediated.

There is a considerably lower prevalence of pediatric allergy in developing countries and there are also substantial differences between rural and urban areas. According to Von Ehrenstein, in a study performed in rural Bavaria, the prevalence of a doctor's diagnosis of asthma (3.4 vs. 6.4%) and current wheeze (5.6 vs. 8.1%) was significantly lower in farmers' children, whereas no such difference was observed for eczema. The effects were stronger in children of farmers with full-time activity as compared with their peers with only part-time farming parents, suggesting a dose-response relationship (**Von Ehrenstein OS et al., 2000**).

This and other observations support the so-called hygiene hypothesis. According to this theory bacterial and viral infections in early life might direct the immune system towards a prevalent type 1 helper T (Th1) cell response which produces interleukin (IL)-2 and interferon- γ (IFN- γ), thus preventing the development of allergic diseases which are characterized by a Th2 cell response with prevalent by IL-4, IL-5 and IL-13 production. Nevertheless, this theory is not easily reconciled with the increased prevalence of allergic asthma among poor blacks in the USA associated with sensitization to cockroaches and house-dust mite, and with some evidence about the increased prevalence of Th1 mediated diseases complicate the picture (**Yazdanbakhsh M et al., 2002**).

Immunopathogenesis of Allergic Diseases

Allergic Inflammation

All of us come across many allergens in our life, but a person without atopy mounts a low grade immunological response mainly characterized by production of allergen specific IgG1 and IgG4 antibodies, and by a modest cell proliferation and production of IFN- γ by Th1 cells. By contrast, when susceptible or atopic individuals are initially exposed or sensitized to allergens, this induces an exaggerated allergen-specific response which is characterized by CD4⁺ T cells producing a T helper 2 (Th2) profile of cytokines (IL-4, IL-5, IL-9 and IL-13 rather than IFN- γ and IL-2) and the presence of allergen-specific IgE (Tab. 1).

Table 1. Components of Immune Response to Allergens in Healthy and Allergic Individuals.

Specific antibody response in serum

Healthy: -No response

Detectable IgG1, IgG4 and IgA

High amounts of IgG4, relatively low amounts of IgG1 and detectable IgA and IgE

Allergic: -Relatively high amounts of IgE, together with low or high amounts of IgG1, IgG4 and IgA

T-cell response in allergen-specific cells and in PBMC

Healthy: -No response

Th0 response in PBMC and specific T-cell clones with low frequency

Tr1, particularly IL-10-dominating response with relatively high frequency

Allergic: Th2 response with varying quantities of IL-4, IL-5 and IL-13, in the presence of detectable IL-10 and IFN- γ

Clinical outcome

Healthy: -Healthy

Skin-prick test and IgE positivity; clinical disease cannot be induced

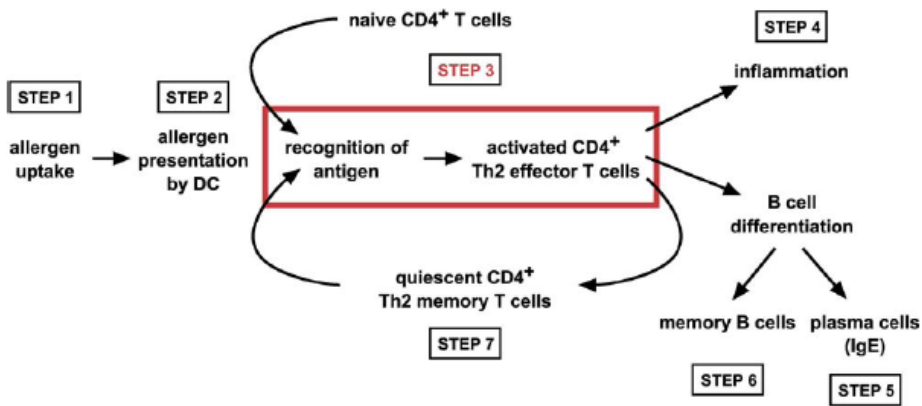
Skin-prick test and IgE positivity and healthy in normal circumstances; clinical disease can be induced by provocation tests and it is dose-dependent

Allergic: -Intermittent or persistent allergy in various clinical forms

In utero, T cells of the fetus are primed by common environmental allergens that cross the placenta. As a result, the immune response of virtually all newborn infants is dominated by Th2 cells (**Prescott S et al., 1998**). It has been proposed that during subsequent development the normal (i.e., nonatopic) infant's immune system shifts in favor of a Th1-mediated response to inhaled allergens (a process termed "immune deviation") (**Holt PG et al., 1999**), whereas in the potentially atopic infant there is a further increase in Th2 cells that were primed in utero. Microbes are probably the chief stimuli of protective Th1-mediated immunity. Macrophages that engulf microbes secrete IL-12, which induces Th1 cells and natural killer cells to produce IFN- γ , thereby shifting the immune system into an "allergy-protective" Th1-mediated response. Other factors may also influence whether Th1 or Th2 cells dominate the response, including the amount of allergen, the duration of exposure to the allergen, and the avidity of allergen-specific interactions between T cells and APC (**Constant SL et al., 1997; Rogers PR et al., 1999**).

Although the picture is more complex, the immunopathological hallmark of allergic disease is the infiltration of affected tissues by Th2 cells.

In particular, the allergen is taken up by phagocytic cells located in peripheral lung tissue and subsequently transported by DC to draining lymph nodes (step 1) where is presented to T cells (step 2). Naïve T cells recognizing the allergen for the first time become activated and undergo a differentiation into Th2 effector T cells (step 3). The fully activated Th2 effector T cells initiate and sustain the local inflammatory process through secretion of soluble mediators (step 4). Concomitantly, the Th2 effector T cells instruct allergen specific B cells to differentiate to antibody-secreting plasma cells (step 5) and memory B cells (step 6). Differentiated allergen-specific T cells develop into long-lived memory T cells (step 7). Memory T cells are quiescent in the absence of antigen, but on allergen re-exposure, they quickly become activated Th2 effector T cells again (step 3) (**Ngoc LP et al., 2005**) (Fig. 1).



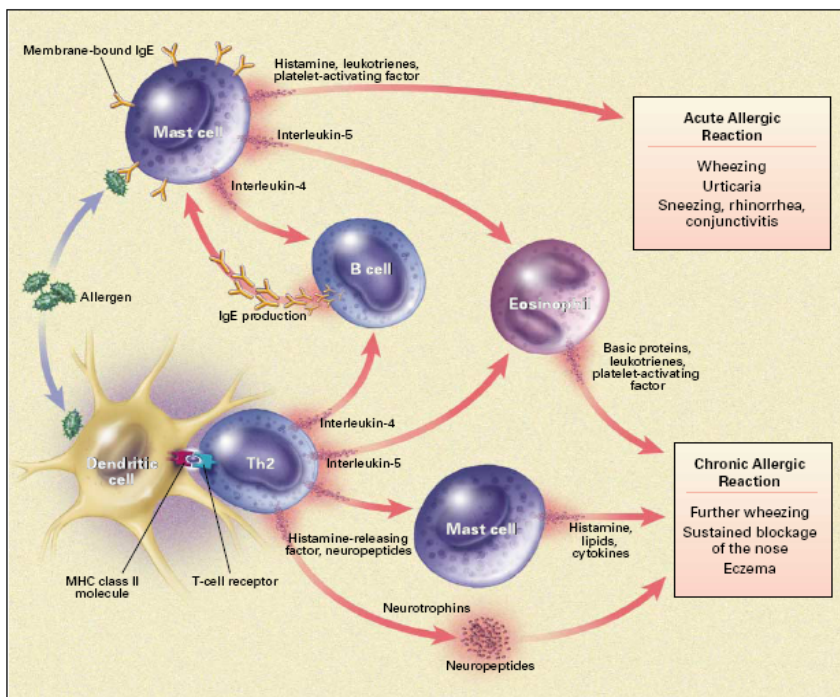
from Kroczek R et al. Allergy Clin Immunol 2005

Fig. 1. Schematic representation of the development and function of Th2 cells in airway disease

The cascade of events leading to the allergic inflammation is initiated by the process of tethering, activation, and adhesion to the endothelium followed by extravasation of inflammatory cells. This requires specific glycoprotein adhesion molecules, such as integrins and selectins, on both leukocytes and on endothelial cells, which are upregulated and show increased binding affinity in response to various inflammatory stimuli. Once the inflammatory cells have infiltrated into the tissue, they respond to chemotactic gradients established by chemoattractant cytokines and chemokines, which emanate from sites of injury or infection. More than 50 different chemokines are now recognized to be involved in the recruitment of inflammatory cells via the activation of more than 20 different surface receptors (**Rossi D et al., 2000**). These molecules play a central role in defining the nature of the inflammatory infiltrate in allergy (**Kay AB 2001**).

Exposure to an allergen following allergic sensitization leads to crosslinking of allergen specific IgE bound to the surface of mast cells and basophils, degranulation of these cells and release of histamine, preformed granule-associated mediators,

membrane-derived lipids, cytokines, and chemokines that cause the symptoms associated with early or acute allergic reactions, including wheezing and conjunctivitis (Kay AB 2001). The release of mediators and leukotrienes causes increases in vascular permeability, smooth-muscle contraction and mucus secretion. Late-phase allergic responses are characterized by the additional recruitment and activation of eosinophils and Th2 cells at the site of allergen challenge (Fig. 2).



from Kay AB et al N Engl J Med 2001

Fig. 2. Immune Mechanisms of Allergy

Immunological Mechanisms in Asthma

Over the decades, the prominent involvement of eosinophils, macrophages, mast cells, and lymphocytes in the inflammatory response in the airways of patients with asthma and the efficacy of steroids in the majority of patients with asthma became more clear and then led to the present-day concept that asthma is a chronic inflammatory disorder of the airways and that T cells are pivotal initiators and regulators of this response. Structural alterations including airway wall thickening, fibrosis in the lamina reticularis and adventitia of the airway, mucus metaplasia, myocyte hypertrophy and hyperplasia, and neovascularization are all readily appreciated in the asthmatic airway. This led to the hypothesis that the inflammatory response in the asthmatic airway causes these remodeling events, and to the belief that these events contribute to disease pathogenesis (**Elias JA et al., 2003**).

Immediate hypersensitivity is the basis of acute allergic reactions. It is caused by molecules released by mast cells when an allergen interacts with membrane bound IgE. The complex of allergen, IgE on the surface of the mast cell triggers a release of preformed histamine and other mediators prostaglandins. These mast-cell mediators have a critical role in anaphylaxis, rhinoconjunctivitis, and urticaria. The role of histamine in chronic asthma and eczema is probably minimal, however, as shown by the relative ineffectiveness of histamine antagonists in controlling these conditions.

Mast cells produce the three cysteinyl leukotrienes C₄, D₄, and E₄, which cause the contraction of smooth muscles, vasodilatation, increased vascular permeability, and the hypersecretion of mucus when they bind to specific receptors (**Drazen JM et al., 1999**).

Eosinophils, macrophages, and monocytes are also major sources of cysteinyl leukotrienes. Mast cells also contain tryptase, a four-chain neutral protease that activates the protease-activated receptors on endothelial and epithelial cells. The activation of these receptors initiates a cascade of events, including the up-regulation of adhesion molecules that selectively attract eosinophils and basophils (**Holgate ST 1999**).

In the cutaneous late-phase reaction, eosinophils and neutrophils accumulate, and then CD4⁺ T cells and basophils infiltrate the site **(Ying S et al., 1999)**. Late-phase asthmatic **(Robinson DS et al., 1993)** and nasal **(Durham SR et al., 1992)** reactions have a similar pattern of cellular infiltration, although basophils are not prominent in the lower airways **(Macfarlane AJ et al., 2000)**.

Th2-type cytokines such as interleukin-4, 5, 9, and 13 influence a wide range of events associated with chronic allergic inflammation.

IL-4 has a role in the initial derivation of the allergen-specific Th2-lineage cells, and IL-4 and IL-13 induce IgE class switching.

IL-5 is a lineage-specific eosinophil differentiation and activator factor. It can be detected in the serum of mice with eosinophilia, and antibody to IL-5 blocks the development of parasite-induced eosinophilia. Although eosinophilia is often associated with high levels of IgE antibody, IL-5 appears not be involved in this response, where IL-4 appears to be main controlling factor **(Sanderson CJ 1990)**.

IL-4 and IL-9 promote the development of mast cells; IL-9 and IL-13 help promote airway hyperresponsiveness; and IL-4, IL-9, and IL-13 promote the overproduction of mucus **(Kay AB 2001; Robinson DS 2000; Romagnani S 1994; Lloyd CM et al., 2001)**.

In the last years the important role of IL-13 in asthma has been elucidated. Originally discovered as an IL-4 like molecules, it is nowadays clear that these cytokines differ in their effector properties, IL-4 playing a more prominent role in the initiation and IL-13 in the effector phase of Th2 allergic inflammation **(Zhou Y at al 2001)**. Several studies using overexpression-transgenic animal models have provided impressive insights in the mechanism of IL-13 induced inflammation, through the chemokine receptor CCR2, and tissue fibrosis. They demonstrated, for instance, that the fibrotic response in asthma results from the ability of IL-13 to stimulate the production and activation of the fibrogenic cytokine TGF- β **(Lee CG et al., 2001)**.

Moreover, the observation that other cytokines such as IL-9, mediate their effect in the lung through the induction of IL-13, suggests that IL-13 might be a final common pathway for Th2-mediated inflammatory response (Tab.2).

EVENT	Th2-TYPE CYTOKINES INVOLVED	OTHER FACTORS INVOLVED
Production of IgE	Interleukin-4, interleukin-9, and interleukin-13	Interferon- γ , interleukin-12, and interleukin-18
Development and accumulation of eosinophils and basophils	Interleukin-4, interleukin-5, interleukin-9, and interleukin-13	Interleukin-3, granulocyte-macrophage colony-stimulating factor, eotaxin-1, eotaxin-2, eotaxin-3, RANTES, monocyte chemoattractant protein 3, monocyte chemoattractant protein 4, and vascular-cell adhesion molecule 1
Development of mast cells	Interleukin-4, interleukin-9, and interleukin-13	Interleukin-3 and stem-cell factor
Airway hyperresponsiveness	Interleukin-9 and interleukin-13	Interleukin-11 and growth factors involved in remodeling
Overproduction of mucus	Interleukin-4, interleukin-9, and interleukin-13	Histamine, leukotriene C ₄ , leukotriene D ₄ , substance P, and calcitonin-receptor-related peptide

Tab.2. The role of Cytokines produced by Th2 Cells in Chronic Allergic Inflammation

Other players involved in pathogenesis of asthma have been recently studied. Among these proinflammatory factors, the main are thymic stromal lymphopoietin (TSLP), IL-25, IL-21, tumor necrosis factor α (TNF- α) and natural killer T cells (NKT cells).

TSLP, which is highly expressed in Hassall's corpuscles in the thymic medulla, appears to be important in the periphery, where it enhances the capacity of DC to induce the development of Th2 cells (**Ito T et al., 2005**). In particular, overexpression of TSLP in the lungs of mice results in the development of severe allergic airway inflammation (**Zhou B et al., 2005; Al-Shami A et al., 2005**). TSLP is, in fact, expressed at high levels in the lungs of patients who have asthma (**Ying S et al., 2005**), which suggests that expression of TSLP by lung epithelial cells can indeed cause airway DC to enhance the development of Th2-driven inflammation.

IL-25 (also known as IL-17E) is produced by Th2 cells and mast cells and was found to induce the production of large quantities of Th2 cytokines in models of both infectious and pulmonary allergic disease (**Fort MM et al., 2001**). IL-25 might also enhance Th2 responses by actively inhibiting IFN- γ and IL-17 production, suggesting

that IL-25-producing cells might have a regulatory function, limiting pathologic (Th1-biased) inflammation at mucosal sites. Because IL-25 enhances Th2 cytokine production, however, it might also enhance the development of allergic inflammatory responses at mucosal sites by inducing eosinophilia, airway hyperreactivity and increased mucus production (**Hurst SD et al., 2002**).

IL-21 is a newly described T cell-produced cytokine related to IL-2, IL-4, and IL-15 that is capable of regulating T, NK, and, especially, Bcells (**Parrish-Novak J et al., 2000; Kasaian MT et al., 2002**). The role of IL-21 in Th differentiation is still somewhat controversial. In the mouse IL-21 is preferentially expressed by Th2 lymphocytes and inhibits IFN- γ production from developing Th1 cells (**Wurstel AR et al., 2002**). Paradoxically, though, exposure of human primary T and NK cells to IL-21 has also been reported to up-regulate IFN- γ , *T-bet*, and IL-12R β 2 (**Strengell M et al., 2002**).

TNF- α is a cytokine produced by mast cells and T cells, but its role in asthma has been controversial. However, a recent clinical study, in which patients who had refractory asthma were treated with the soluble TNF- α receptor ‘etanercept’, suggests that the TNF- α axis is upregulated and is proinflammatory in asthma (**Berry MA et al., 2006**). Whether the role of TNF- α is as important in mild-to-moderate asthma as it is in refractory asthma remains to be seen.

Another pro-allergic pro-asthmatic factor that is part of the innate immune system is the invariant T-cell receptor (TCR) natural killer T (iNKT) cell compartment. Some years ago, iNKT cells were shown to be required for the development of allergen-induced airway hyperreactivity (AHR) in mouse models of asthma (**Akbari O et al., 2003**). More recently, the activation of iNKT cells has been shown to be sufficient to induce AHR (**Meyer EH et al., 2006**) and it has been shown that iNKT cell-driven AHR can occur in the complete absence of adaptive immunity. These results suggest that glycolipids from respiratory pathogens might activate iNKT cells and directly cause wheezing and AHR.

Although the studies of allergen-induced AHR in mice strongly suggested that iNKT cells might be important in human asthma, direct assessment of the role of iNKT cells

in human asthma was necessary to establish this possibility. Frequency and distribution of iNKT cells in the lungs and in the circulating blood of patients with moderate-to-severe persistent asthma was assessed (**Akbari O et al., 2006**). Surprisingly, 60% of the pulmonary CD4⁺CD3⁺ cells in the lungs of these patients who had asthma were iNKT cells. These studies strongly suggest that iNKT cells play a prominent pathogenic role in human asthma. The presence of a large numbers of iNKT cells in the lungs of patients with asthma is surprising, and suggests that these cells might have been mistakenly identified in the past as conventional CD4⁺ Th2 cells. Most of the iNKT cells in the lungs of patients with asthma expressed CD4 and produced IL-4 and IL-13, but not IFN- γ , suggesting that a Th2-like subset of iNKT cells was recruited or expanded in the lungs of patients with asthma (**Akbari O et al., 2006**). In this and in another study by Ikegami et al, iNKT cells were not increased in the peripheral blood of patients with asthma, nor did circulating iNKT cells show any change in functionality (**Ikegami Y et al., 2004**); this indicates that the immunology of asthma must be studied not by the examination of peripheral blood alone but rather by the evaluation of cells from within the lung. The specific mechanisms by which the Th2-like subset of iNKT cells enters or expands in the lungs, and whether the number of iNKT cells in the lungs correlates with disease severity, are not yet clear.

Nevertheless, a very recent study showed low number of iNKT in airway biopsy, bronchoalveolar lavage (BAL), and sputum of allergic patients with no significant differences in the percentage of iNKT in atopic subjects compared to healthy controls (**Pandurangan V et al., 2007**).

Therefore, it seems clear that the precise role of this potent group of immunoregulatory cells in airway inflammation remain to be understood.

Role of Dendritic Cells in Immune Regulation and in Allergic Immune Response

Antigen-presenting cells are critical in initiating and controlling allergic inflammation and DC are particularly important in asthma.

DC are professional APC specialized for the initiation of T cell immunity **(Banchereau J et al., 1998; Cella M et al., 1997)**. Depending on their maturational state and their location, DC perform different functions within the immune system. DC normally reside in nonlymphoid tissues, such as the skin or the bronchial mucosa, in an immature form, where they are specialized for antigen capture. Activation of DC and subsequent migration from nonlymphoid tissues to regional lymph nodes have been shown to be early steps during inflammatory processes and critical events in the generation of cell-mediated immune responses against various pathogens. After antigen uptake, inflammatory stimuli are necessary to switch DC to a T cell stimulatory mode. This process has been called “maturation” and is associated with changes in the phenotype and function of DC, including upregulation of costimulatory molecules and adhesion molecules, expression of chemokine receptors, with concomitant down-regulation of tissue homing receptors for RANTES (CCL5), eotaxin (CCL11), and MIP3a (CCL20) **(Power CA et al., 1997; Stumbles PA et al., 1998; Beaulieu S et al., 2002)** and upregulation of lymph node homing chemokine receptors, which direct the migration of DC to the T-cell zones of draining lymph nodes where DC interact with recirculating T cells and initiate T cell immunity **(Cella M et al., 1997; De Smedt TB et al., 1996; Romani N et al., 1989)**.

Airway DC

Over the last 10 years, it has become increasingly clear that airway DC are crucial to the process of allergic Th2 sensitization, particularly through the use of mouse models of asthma. Transgenic and inducible knock out models have been developed to specifically study the role of DC in the pulmonary allergic response. From these

studies, a model has emerged in which airway DC are not only crucial for regulating the process of sensitization to inhaled antigens leading to allergy, but also for controlling established allergic inflammation. In addition to chemokines, other mechanisms are likely to regulate DC migration, including integrin and cadherin expression and extracellular matrix degrading enzymes.

Antigen Uptake and Lymphnode Migration of Airway DC

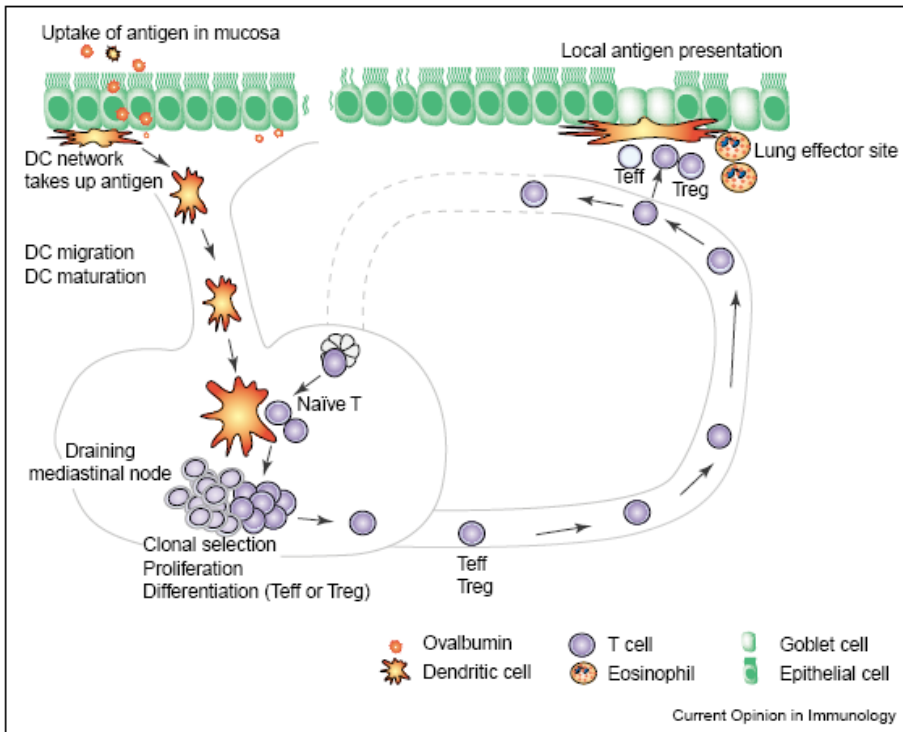
DC form a network in the upper layers of the epithelium and lamina propria of the airways. Here DC are said to be in an immature state, specialized for internalizing foreign antigens but not yet able to activate naïve T cells. With antigen uptake in the presence of a danger signal, DC undergo a maturation, whereby they lose their capacity to take up antigen and acquire a phenotype of professional APC expressing all the costimulatory molecules and chemokines to attract and stimulate naïve T cells (Fig. 3).

A molecular basis for DC activation has been provided with the discovery of pattern-recognition receptors (PRR). PRR recognize conserved microbial structures, termed pathogen-associated molecular patterns (PAMP), and signalling via PRR leads to DC activation, defined by upregulation of MHC class II and co-stimulatory molecules. The significance of this finding for induction of pulmonary immunity is underscored by the fact that lipopolysaccharide (LPS) is necessary for Th2 sensitization in mouse models of asthma (**Eisenbarth SC et al., 2002; Dabbagh K et al., 2002**).

DC transport the antigen from the mucosa to the draining lymph nodes of the lung, after degradation of the antigen in short immunogenic peptides and loading the peptide on major histocompatibility complex II (MHCII) molecules. DC migrate to the T cell-rich area of draining lymph nodes where naïve T lymphocytes continuously pass by (**Vermaelen KY et al., 2001; Lambrecht BN et al., 2000**).

In the lymph node mature DC form an immunologic synapse with T cells in which the MHC peptide interacts with the T-cell receptor, costimulatory molecules interact with T cell-expressed coreceptors, and cytokines are released to polarize the T-cell response. The process of migration to the lymph node is driven by chemokine signals

acting on the CCR7 receptor (Marsland BJ et al., 2005). The recognition of danger induces the surface expression of CCR7 on peripheral DC, but the responsiveness of CCR7 to CCL19 and CCL21 and the consequent lymph node migration of DC is controlled by lipid mediators, such as the leukotrienes and prostaglandins. Like skin DC, lung DC used the CCR8 receptor for the chemokine CCL-1 (also known as I-309 in human subjects and TCA-3 in mice) in concert with CCR7 for emigration of DC from the skin and lung, although the pathways governing DC migration from different tissues partially differ in molecular regulation. (Jakubzick C et al., 2006).



from Kuipers H et al. *Curr Opin Immunol* 2004

Fig. 3. The function of DC in allergic airway disease

Sensitization to Inhaled Allergen and Th2 Polarization by Airway DC

It was therefore long enigmatic how sensitization to natural allergens occurs. One requisite for priming to occur is that DC need to be activated by a sense of danger. This danger signal could be found in the allergen itself or in some accompanying microbial contaminant. Most clinically important allergens, such as the major Der p1 allergen from house dust mite (HDM), are proteolytic enzymes that can directly activate DC or epithelial cells (**Hammad H et al., 2001**). The biological function of the HDM allergen Der p2 is still unknown, although the possibility of a protease activity has been recently ruled out, since it had no effect on human alveolar cell lines (**Kauffman HF et al. 2006**). Other allergens, such as the experimental allergen OVA, do not have any intrinsic activating properties. For these antigens, contaminating molecules or environmental exposures (respiratory viruses and air pollution) might pull the trigger on DC activation (**Dahl ME et al., 2004**).

Based on studies on the functional interaction between mucosal T cells and DC, it is clear that effector Th2 responses *in vivo* in the lung continuously depend on antigen-presenting DC. One possible explanation would be that effector T cells *in vivo* remain dependent on costimulation.

Pulmonary DC upregulate the expression of CD40, CD80, CD86, ICOS-L, programmed death ligand (PD-L) 1, and PD-L2 during eosinophilic airway inflammation, particularly on contact with Th2 cells. (**De Heer HJ et al., 2004; Van Rijt LS et al., 2005; Van Rijt LS et al., 2004**). Costimulatory molecules might be involved in activation of effector T cells in the tissues.

Numerous models of asthma have demonstrated that blocking the interaction of costimulatory molecules of the B7 superfamily (CD80, CD86, ICOS-L) or tumour necrosis factor (TNF)-R family (OX40L) can reduce features of asthma (**Coyle AJ et al., 2000; Deurloo DT et al., 2003; Harris N et al., 1997**). However, challenge with DC derived from the BM of CD80/86 double knockout mice in sensitized mice induced similarly strong airway inflammation similar to the one observed after challenge with wild-type DC. It is likely that other costimulatory molecules besides CD80/CD86 are involved in activating T cells in secondary immune responses. Several

molecules on the surface of the DC, like ICOSL, OX40L, 4-1BBL, CD40 and ICAM-1, have been reported to have costimulatory capacity, and may be responsible for the induced reaction in the absence of CD80/CD86 **(Watts TH et al., 1999)**. Strikingly, it was also observed an increase in the B7 family members PDL-1 and PDL-2, ligands of the inhibitory PD-1 receptor, on DC within eosinophilic inflammation **(Van Rijt LS et al., 2005)**. PD-1 is generally seen as an inhibitory signal but recent data suggest that PDL-1 might also provide a costimulatory signal to T cells **(Shin T et al., 2003)**. Another costimulatory pathway able to compensate for the lack of CD80/86/B7RP-1 costimulation on DC would be OX40L. OX40 (CD134), a member of the TNFR family, is a major regulator of anti-apoptotic proteins such as Bcl-xL and Bcl-2, and strongly promotes the survival of antigen-activated primary CD4 T cells. In addition, OX40 is preferentially expressed by memory Th2 cells. Blocking of OX40–OX40L interaction impaired all features of asthma induced by adoptive transfer of OVA-specific Th2 cells **(Salek-Ardakani S et al., 2003)**. The requirement of CD40 and CD40L interaction has been investigated in several murine models for asthma with different protocols. It appeared that sensitized CD40^{-/-} and CD40L^{-/-} mice develop, respectively, less eosinophilia and airway hyper-reactivity in response to an allergen challenge when compared with wild-types **(Lei XF et al., 1998; Mehlhop PD et al., 2000)**. However, an inevitable disadvantage of using transgenic mice to study secondary immune responses is also that during sensitization, CD40-CD40L interaction is absent. Therefore, it is possible that the final consequence of asthma development in mice is determined by the sensitization phase because mice need to be sensitized before they can develop the effector phase. In another model where they could circumvent sensitization by using the OVA transgene in CD40-deficient mice to obtain sufficient OVA-specific T cells without sensitization, the results were completely opposite. Mice without CD40 developed more eosinophilia and a higher AHR compared with CD40^{+/+} OVA animals **(Takahashi H et al., 2003)**. These results suggest that during sensitization, CD40/ CD40L can act as a pro-inflammatory signal while having a protective role during the effector phase. The role of CD40/CD40L interaction between DC and T cells during a secondary immune

response in allergic airway inflammation still has to be further elucidated.

Besides their capacity to provide costimulation, an alternative characteristic that makes DC so important during secondary immune responses could be that lung DC are essential for the recruitment of Th2 cells by producing Th2-selective chemokines and cytokines.

In allergen-challenged mice, mDC might also be a prominent source of the chemokines CCL17 and CCL22, which are involved in attracting CCR4⁺ Th2 cells to the airways (**Kohl J et al., 2006**). The proallergic cytokine TSLP induces the production of large amounts of CCL17 by mDC, thus contributing to the recruitment of a large number of Th2 cells to the airways (**Zhou B et al., 2005**). In human subjects the HDM allergen Der p1 induces the production of CCL17 and CCL22 in monocyte-derived DC derived from asthmatic subjects with HDM allergy (**Hammad H et al., 2003**).

Mature DC produce two important polarizing cytokines: IL-12 recognized as the most powerful Th1 inducing cytokine and IL-10, a regulatory cytokine which has been shown to influence indirectly T cell polarization through its capacity to downregulate IL-12 production by DC (**Smedt T et al., 1997; Trinchieri G 2003; Smits HH et al., 2004**). It has been shown that retroviral overexpression of IL-12 in myeloid DC is sufficient to turn these cells into strong Th1 inducers, even in the Th2-prone milieu of the lung (**Kuipers H et al., 2004**). However, IL-12 is not necessary for Th1 development by DC, as LPS-stimulated IL-12p40^{-/-} DC still induce Th1 development in the lung (**Kuipers H et al., 2003**).

In contrast to the signals governing Th1 development, the mechanisms for DC-driven Th2 development have remained somewhat enigmatic. According to one theory, Th2 development occurs as a default in the absence of polarizing IL-12. Alternatively, some regard Th2 development as an instructive event requiring specific cytokines (such as IL-4 and IL-13) or cell surface molecules on DC. Although the prototypic Th2 cytokine IL-4 is important for Th2 development *in vivo*, it is not produced by DC directly, but can be induced in other cells by DC contact. Early sources of IL-4 (and IL-13) might be naïve T cells, eosinophils or CD1d-restricted NKT cells, reacting to antigens presented by CD1d on airway DC (**Voehringer D et al., 2004**). Recent work

from Amsen identified that the cell surface Notch ligand families delta and jagged influence Th1 and Th2 differentiation, respectively. Th2 differentiation induced by APC is abrogated in T cells lacking the Notch effector RBPJkappa. Notch directs Th2 differentiation by inducing GATA3, a transcription factor important for Th2 development and by directly regulating IL4 gene transcription through RBPJkappa sites in a 3' enhancer (**Amsen D et al., 2004**). The expression patterns of these ligands on DC correlate with the ability of known Th1- or Th2-inducing stimuli, such as cholera toxin, prostaglandin E2 or LPS to induce T cell differentiation (**Kapsenberg ML 2003**). Ectopic expression of jagged1 and delta1 skewed naïve CD4⁺ T cells towards Th2 and Th1, respectively.

Other cytokines are important during Th1 or Th2 development. IL-6 is a cytokine produced by DC and other cell types and has recently discovered roles in the abrogation of tolerance and in Th2 induction in several non-allergic models of disease. In addition, the production of IL-6 by pulmonary DC may favor Th2 differentiation by inhibiting Th1 responses (**Dodge IL et al., 2003**). IL-27 and IL-23 are produced by macrophages and DC. IL-27 and IL-23 can function as a proinflammatory cytokine because they synergize with IL-12 to induce IFN- γ production from NK cells and to promote Th1 responses. IL-27 also has anti-inflammatory properties. Addition of recombinant IL-27 to naïve T cells in culture under Th2-polarizing conditions results in decreased expression of *GATA-3*. Concurrent with the decrease in *GATA-3* was a decrease in IL-4 production. The decrease in Th2 cytokines caused by IL-27 is a result of inhibition of Th2 cell development. These results suggest that IL-27 might serve a dual role in T-cell development and the immune response by stimulating production of Th1 responses while inhibiting production of Th2 inflammatory responses (**Villarino AV et al., 2004**).

Others important players are involved in Th polarization. Among the transcription factors, *T-bet* and *Gata-3* are the most important involved in Th1 and Th2 development, respectively. The transcription factor *T-bet* is necessary to induce helper T cells to differentiate into Th1 cells and to produce IFN- γ . For these reasons, *T-bet* is thought to be central to the feedback loops that regulate Th1 and Th2 cells, and in this

way it could be important in asthma. Without any allergic sensitization of the animals, the bronchi in the *T-bet*^{-/-} mice were infiltrated with eosinophils and lymphocytes and showed signs of the airway remodelling typical of allergic asthma. Moreover, these animals had AHR, and their BAL contained increased amounts of cytokines produced by the Th2 cells. These spontaneous changes in the *T-bet*^{-/-} knockout mice were similar to those found in bronchi of wild-type mice that had been sensitized with a foreign protein and then challenged with an aerosol containing the allergen. These findings constitute strong evidence of the modulating role of IFN- γ in asthma and provide support for the hypothesis that an imbalance between Th1 and Th2 cells contributes to asthma (Finotto S et al., 2002). Furthermore, the transcription factor *T-bet* was recently found to be expressed in DC in addition to T cells. *T-bet* is required for optimal production of IFN- γ by DC under certain conditions. *T-bet*^{-/-} DC were less potent in inducing Th1 responses and produced less proinflammatory cytokines. These observations suggest that *T-bet* could regulate type 1 and 2 immunity by influencing genetic programs in both adaptive and innate immunity (Wang J et al., 2006).

Gata-3 is present at low levels in naïve CD4⁺ T cells. Its expression strongly increases during Th2 differentiation and decreases during Th1 differentiation. Expression of *Gata-3* using retroviral vectors or in transgenic mice, up-regulates expression of all Th2 cytokine genes, even when CD4⁺ cells are stimulated under Th1-polarizing conditions (Ouyang W et al., 1998; Zheng W et al., 1997; Ferber IA et al., 1999; Lee HJ et al., 2000). In addition, *Gata-3* strongly inhibits the production of IFN- γ and down-regulates IL-12R β 2 expression in an IL-4-independent manner T (Ouyang W et al., 1998, Ferber IA et al., 1999). *Gata-3* mRNA is increased in the airways of atopic asthmatic patients compared with those of normal control subjects (Nakamura Y et al., 1999). Moreover, transgenic T cell expression of a dominant-negative *Gata-3* allele attenuates allergic inflammation in a murine model of asthma, with decreased eosinophilia, mucus production, and IgE levels associated with decreased IL-4, IL-5, and IL-13 production (Zhang DH et al., 1999).

Finally, it has been shown that exposure of DC to agents such as epithelial cell-derived TSLP (Soumelis V et al., 2002) and PGE2 (Kalinski P et al., 1997), mast

cell-derived histamine (**Caron G et al., 2001**), and b2 agonists (**Panina-Bordignon P et al., 1997**), polarize the maturation of myeloid DC into Th2-promoting DC. Mice that conditionally overexpress thymic stromal lymphopoietin (TSLP) in the lungs mount vigorous Th2 responses in the airways in a process driven by DC (**Zhou B et al., 2005; Al-Shami A et al., 2005**). TSLP is increased in the airways of asthmatic patients (**Ying S et al., 2005**) and it can activate myeloid DC to prime naïve CD4⁺ T cells to differentiate into proinflammatory Th2 cells (**Watanabe N et al 2005**). The Th2 skewing effect induced by TSLP-activated DC was found to be dependent on OX40 ligand, a costimulatory molecule shown to play a critical role in the development of allergic lung inflammation (**Ito T et al 2005**).

IMMUNOLOGICAL TOLERANCE

The ability of the immune system to discriminate between self and non-self results in immunological tolerance, which can be defined as lack of responsiveness towards certain molecules, and can be acquired by central and peripheral mechanisms. CENTRAL TOLERANCE occurs during the ontogeny of T cells and leads to the elimination of self-reactive T cells by clonal deletion in the thymus. PERIPHERAL TOLERANCE takes place throughout life, and is usually designed to control responses towards foreign antigens, which are not harmful or antigens recognized with low affinity. Several not exclusive mechanisms are operational in peripheral lymphoid organs.

Central Tolerance

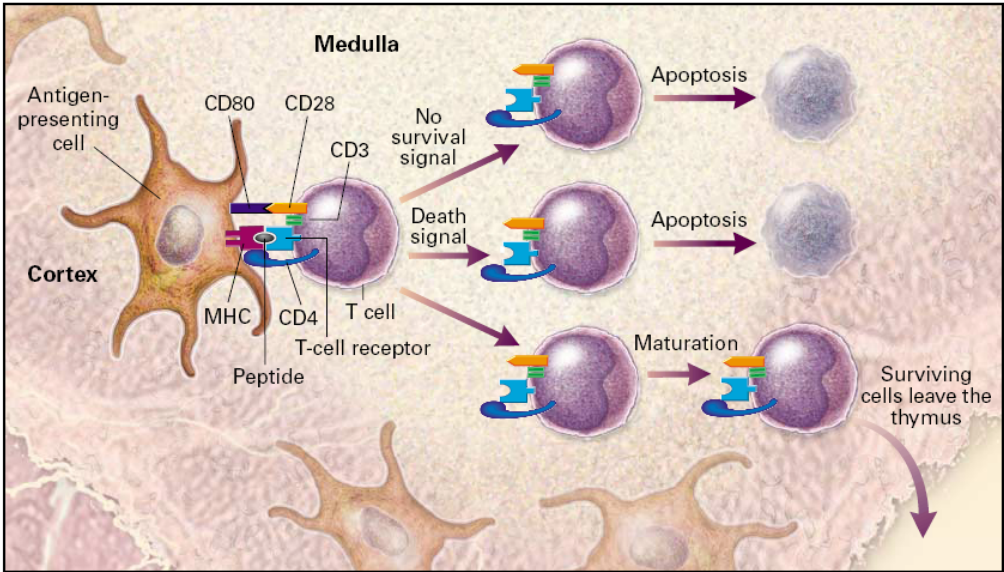
The thymus is the unique lymphoid organ responsible for the generation of the peripheral T lymphocytes repertoire with the ability to eliminate foreign pathogens while being tolerant to self-antigens. The process of differentiation of thymocytes into mature T cells is a dynamic process with a series of selection steps that allows only fully competent T cells to be exported to the periphery. During the development in the thymus only a very small percentage of the thymocytes generated are able to survive and eventually reach the periphery (**Shortman K 1992; Tough DF et al., 1994**).

The thymic cellular environment allows the generation of the peripheral T-cell repertoire through three main mechanisms: a) intrathymic random recombination of gene segments coding for the variable parts of the T-cell antigen receptor (TCR); b) clonal deletion of T cells bearing a TCR that binds with high avidity/affinity MHC-self peptides; c) generation of self-antigen specific regulatory T cells (Treg).

Hemopoietic progenitor T cells enter the thymus by the cortico-medullary boundary and migrate to subcapsular cortex where they undergo intense proliferation. Their differentiation depends on positive or negative selection, and is mediated by

transient synapses of immature thymocytes with thymic stromal cells. Thymic selection tunes the T cell repertoire through the testing of the signaling thresholds of newly rearranged molecules for peptides presented within the thymus. This process depends on several parameters: concentration and density of thymic MHC/self antigens complexes, as well as the affinity of randomly recombined for those complexes **(Sprent J, Webb SR 1995; Sebzda E et al., 1994)**. Positive selection of immature thymocytes is the result of low avidity/MHC-self peptide interactions. Thymocytes with TCR that binds to MHC-self peptide complex with high affinity are deleted during negative selection whereas those with TCR that does not bind to MHC fail positive selection and will die by default (neglect) **(Ashton-Rickardt PG et al., 1994; Jameson SC et al., 1995; Jameson SC et al., 1994; Surh CD et al., 1994)**.

Recent studies on autoimmune diseases that result from single gene mutations like autoimmune polyendocrinopathy syndrome type 1 (APS1) have provided new insights into how self-tolerance is maintained. APS1 is a syndrome characterized by mucocutaneous candidiasis, hypoparathyroidism and Addison Disease and results from homozygous mutations of the autoimmune regulator gene Aire, encoding a protein with structural and functional features suggestive of a transcription factor. Aire has recently been identified as an important mediator of central tolerance. The highest levels of Aire expression are detected in the thymus **(Anderson MS et al., 2002; Gotter J et al., 2004)** with its highest levels within the thymic medullary epithelial cells (mTEC), followed by thymic dendritic cells **(Heino M et al., 2000)**. Importantly, Aire is undetectable in the organs targeted by the autoimmune disease in APS1 patients. Studies on Aire-deficient mice have demonstrated that Aire drives the expression of many self-proteins in medullary mTEC **(Anderson MS et al., 2002)**. These organ-specific proteins are presented on the surface of mTEC by MHC molecules to developing T cells and thymocytes that recognize these proteins undergo negative selection. In the absence of Aire, there is a defect in the negative selection of organ specific T cells **(Liston A et al., 2003)**. Recently, it has been shown that also lymph node stromal cells can present endogenously expressed peripheral-tissue antigens and are therefore functionally akin to mTEC **(Lee JW et a., 2007)**. (Fig. 4).

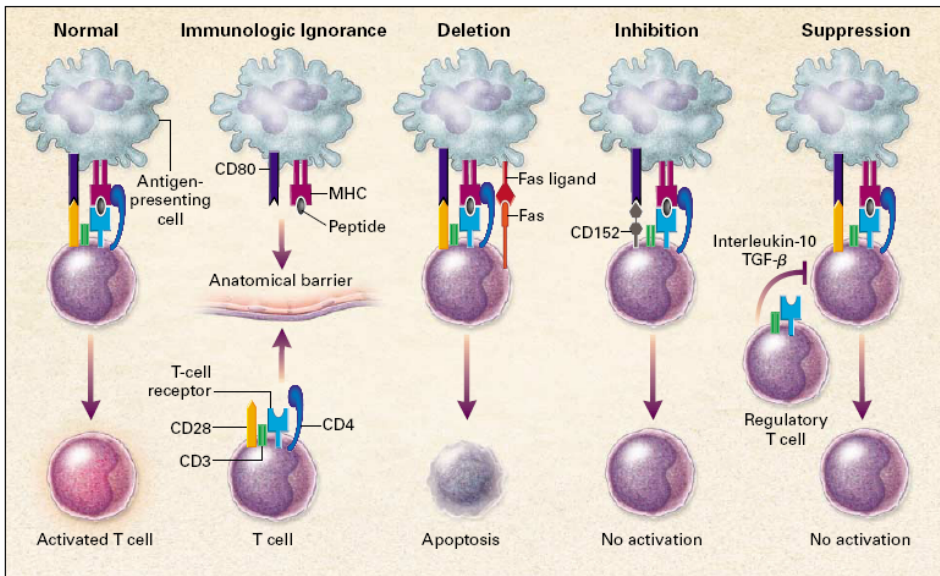


from Makai IR et al N Engl J Med 2001

Fig 4. Central mechanisms of the induction of Tolerance

Peripheral Tolerance

Thymic deletion is not sufficient for preventing the escape of self-reactive T cells in the periphery. Indeed, self-reactive T cells exist in normal healthy individuals (Pette M et al., 1990; Lohmann T et al., 1996; Semana G et al., 1999), suggesting that to control self-reactive T cells in order to maintain self-tolerance additional mechanisms occur in the periphery. Peripheral tolerance is operational during the entire lifespan and controls immune responses to self-antigens that are not expressed in the thymus, and to foreign antigens that are encountered in peripheral tissues. Mechanisms of peripheral T-cell tolerance include immunological ignorance, clonal deletion (Munn DH et al., 1996; Varadhachary AS, Salgame P. 1998), anergy (Sharpe AH. 1995), and active suppression mediated by regulatory cells (Chen L. 1998) (Fig. 5).



from Makai IR et al N Engl J Med 2001

Fig 5. Peripheral mechanisms of the induction of Tolerance

Several mechanisms can cause immunological ignorance: the antigen concentration that may be below the threshold required to induce the activation or deletion of T cells (**Akkraraju S et al., 1997; Ferber I et al., 1999**), antigens that may be physically separated from T cells (e.g., by the blood-brain barrier) (**Barker CF et al., 1977**), or antigens presented by MHC molecules in the absence of costimulation and therefore cannot induce T cell responses (**Janeway CA Jr. 1992**).

Anergy is a process that occurs when a T cell encounters its proper MHC-peptide complex under particular conditions and results in the induction of a hyporesponsive state, which affect IL-2 production and proliferation upon re-stimulation (**Lamb JR et al., 1983**). Several mechanisms are responsible for the induction of anergy including presentation of peptide antigens by a non-professional APC, which lack co-stimulatory molecules, such as CD80 and CD86. Moreover, during the course of inflammatory processes cell types other than professional APC, such as endothelial or epithelial cells, may express MHC class II alleles and are able to present peptides to T cells in the absence of co-stimulatory signals (**Powell JD. 2006; Asnagli H et al., 2001**). Alternatively, T cell anergy can occur when peptide recognition is not followed by CD80-CD86/CD28 interaction, but with the CD80-CD86/cytotoxic T lymphocyte associated antigen (CTLA)-4 (CD152), which delivers inhibitory signals (**Powell JD. 2006; Asnagli H et al., 2001**).

Deletion is an alternative mechanism of peripheral tolerance by which mature T cells undergo apoptosis when they encounter the specific antigen at high concentrations and/or are heavily activated. This process is known as activation-induced cell death (AICD), and it is mediated by the surface expression of Fas (CD95). The interaction between Fas e FasL on the proliferating T cells activates the cascade of caspase enzymes that results in the induction of apoptosis (**Fas SC et al., 2006**).

Regulatory T (Treg) cells are crucial players in the induction of peripheral tolerance to both self and not harmful foreign antigens. Cells with regulatory function exist within all major T and NK cell subsets, although most attention has been focused on Treg cells comprise in the CD4⁺ lymphocyte subset (Tab 3). The two most relevant classes of Treg described within the CD4⁺ subset are CD4⁺CD25⁺ Treg (**Shevach EM.**

2002; Wood KJ et al., 2003) and regulatory T type 1 (Tr1) cells (Roncarolo MG et al., 2000; Battaglia M et al., 2006). These two Treg subsets differ in a number of important biological features, including their specific cytokine secretion profile, cellular markers, ability to differentiate in response to antigen specific stimuli, and dependency on cytokines vs. cell–cell contact mechanisms for mediating suppressive activity (Wood KJ et al., 2003; Levings MK et al., 2005).

Subset	Phenotype	Origin	Mode of action
Naturally occurring T _{reg} cells	CD4 ⁺ CD25 ⁺ FOXP3 ⁺	Thymus and possibly periphery	Cell–cell contact and/or secretion of short-range membrane-bound or soluble TGF-β; secretion of IL-10 <i>in vivo</i>
T _R 1 cells*	CD4 ⁺ , secrete IL-10, and T _H 1 and T _H 2 cytokines (IFN-γ and IL-5, respectively)	Periphery	Secretion of IL-10, and possibly other mechanisms
IL-10-secreting regulatory T cells*	CD4 ⁺ , secrete IL-10 but not T _H 1 or T _H 2 cytokines	Periphery	Secretion of IL-10, and possibly cell–cell contact
T _H 3 cells	CD4 ⁺ , secrete TGF-β	Periphery	Secretion of TGF-β
T _H 1 cells	CD4 ⁺ , secrete IFN-γ	Periphery	Secretion of IFN-γ, which inhibits T _H 2 responses; and in some cases, secretion of IL-10, which regulates T _H 1 responses by inhibiting macrophage and DC production of IL-12
T _H 2 cells	CD4 ⁺ , secrete IL-4, IL-5 and IL-13	Periphery	Secretion of IL-4, which inhibits T _H 1 responses; secretion of IL-10, which inhibits T _H 1 responses by inhibiting macrophage and DC production of IL-12

Tab 3. Regulatory T-cell population

Regulatory T Cells

Natural Occurring T Regulatory Cells

Naturally occurring CD4⁺CD25⁺ Treg (nTreg) cells represents a minor population of CD4⁺ T cells (~10%), which emerge from the thymus. Thymic development of natural Treg appears to require a high avidity interaction between their and self-peptide/MHC ligands, and they expand in the periphery by recognizing the selecting self-peptide/MHC ligands (**Picca CC et al., 2006**). nTreg are characterized by the constitutively expression of CD25 (IL-2 receptor) molecule, as well as CTLA-4, glucocorticoid-induced tumor necrosis factor receptor (GITR), OX40 (CD134), L-selectin (CD62L) and the transcription factor forkhead box protein 3 (Foxp3) (**Sakaguchi S 2005**). Although the expression of CD25 has been used to specifically identify nTreg in naïve mice, the value of CD25 as a marker of human nTreg is limited because CD25 is highly expressed (as are CTLA-4 and GITR) on activated CD4⁺ T cells. Based on the level of expression of CD25, freshly isolated human T cells can be split into suppressive (CD25^{high}) and non-suppressive (CD25^{low}) cells . However, analysis at the clonal level revealed that even the small fraction of CD25^{high} cells is not an homogeneous population of suppressor cells . In addition, within the freshly isolated CD25^{high} subset, the HLA-DR positive cells can mediate early contact-dependent suppression that is associated with high Foxp3 expression, whereas HLA-DR negative CD25^{high} T cells promote early IL-10 and IL-4 production and induce late Foxp3-associated contact-dependent suppression . In contrast to CD25, the expression of Foxp3 is highly restricted to a subset of $\alpha\beta$ TCR T cells. Studies on mice suggest that Foxp3, which binds to DNA, localizes to the nucleus, acts as transcriptional repressor (**Shubert L et al., 2001**) and functions as a nTreg lineage specification factor (**Fontenot JD et al., 2005; Fontenot JD et al., 2003; Hori S et al., 2003**). It has been shown that Foxp3 expression correlates with suppressor activity (**Fontenot JD et al., 2005**), primarily in mice and less clearly in humans. It has been demonstrated that ectopic expression of Foxp3 can endow murine T cells with regulatory capacities *in*

vitro and *in vivo* (Ramsdell F. 2003). However, in contrast to results described in murine cells, in humans it is still controversial whether Foxp3 overexpression in naïve CD4⁺ T cells is sufficient to confer a regulatory function (Yagi H et al., 2004; Allan SE et al., 2005). Of note is that in human cells, Foxp3 is also expressed by activated non-suppressive CD4⁺CD25⁻ T cells (Allan SE et al., 2007; Wang J et al., 2007) suggesting that in addition to this transcription factor other components may be required for optimal suppressor activity (Schofield L et al., 1999).

Genetic mutation of Foxp3 gene, and the resulting deficiency or dysfunction of nTreg, is the primary cause of IPEX (immunodysregulation, polyendocrinopathy, enteropathy, X-linked syndrome), a monogenic human disease characterized by an allergic phenotype with dermatitis and hyper IgE and an autoimmune phenotype with enteropathy, type I diabetes, thyroiditis, haemolytic anemia and thrombocytopenia, and results from mutations of Foxp3 (Chatila TA et al, 2000).

nTreg cells do not produce IL-2 and are anergic *in vitro*. This hypo-responsiveness can be reversed by potent stimulation *via* TCR and high concentrations of IL-2. Importantly, IL-2 is not required for thymic development of nTreg cells, but it plays a central role in their peripheral homeostasis and function. Indeed, the presence of IL-2 is required for nTreg cells to exert their suppressive function.

Several mechanisms of nTreg-mediated suppression have been proposed including a direct T cell–T cell interaction, perforin and/or granzyme A-dependent killing, inhibitory cytokine-mediated suppression, modification of the DC function, and IL-2 consumption (Von Boehmer H 2005; Kim JM et al., 2006). *In vitro* studies suggest a role for cell–cell contact rather than secretion of inhibitory cytokines (Thornton AM et al., 1998). Whereas some groups have linked high levels of membrane-bound TGF- β to nTreg function *in vitro* (Nakamura K et al., 2001; Nakamura K, et al., 2004), others have shown that nTreg can suppress in the absence of TGF- β (Piccirillo CA et al., 2002). A recent report, which suggests that TGF- β is important for maintaining expression of Foxp3 and thus the suppressive function of nTreg, but it is not directly involved in their mechanism of action, may reconcile these discrepancies (Marie JC et al., 2005). However, *in vivo*, at least part of the

suppressive function of nTreg can be ascribed to inhibitory cytokines such as IL-10 and TGF- β , but the source of TGF- β that is needed for suppression may vary in different situations (**Fahlen L et al., 2005; Chen ML et al., 2005; Huang X et al., 2005**). Inhibitory receptors such as CTLA-4 have also been implicated as a direct or indirect effector mechanism of Treg that is associated with TGF- β , but this association remains controversial (**Sullivan TJ et al., 2001; Chen W et al., 1998; Shevach EM et al., 2002**). Finally, cytolytic activity has been invoked as possible mechanism of suppression. Human CD4⁺CD25⁺Foxp3-expressing T cells can be activated by a combination of antibodies to CD3 and CD28 to express Granzyme A (GZ-A) and kill activated CD4⁺ and CD8⁺ T cells by a perforin-dependent mechanism in a mechanism that does not involve Fas-Fas L interaction (**Grossmann WJ et al., 2004**). However, a recent study in mouse have been demonstrated that Granzyme B (GZ-B), but not perforin, is one of the key mechanism through which nTreg induce cell contact-mediated suppression. Indeed, mice GZ-B^{-/-} showed reduced suppression ability of nTreg (**Gondek DC et al., 2005**). nTreg may exert their inhibitory function by indirectly affecting APC or other cell types such as NK cells. Recently, it has been shown by two-photon microscopy that the presence of nTreg alters the migration behavior of autoreactive diabetogenic T cells *in vivo* (**Tang Q, et al., 2006**). This study suggests that nTreg affect the formation of immunological synapses of effector cells with their APC *in vivo*, either directly or indirectly. nTreg may compete for self-antigen and thus efficiently outcompete pathogenic T cells. Indeed, despite the fact that nTreg possess a diverse repertoire, their specificity seems to be predominantly directed to self-antigen (**Hsieh CS et al., 2004; Cozzo C et al., 2003**). Therefore, more than one mechanism of suppression mediated by nTreg cells is operational *in vivo*.

The regulatory function of nTreg cells has been demonstrated *in vivo* in several pre-clinical models of autoimmune pathology including autoimmune diabetes in non-obese diabetic (NOD) mice , experimental autoimmune encephalomyelitis (EAE) , colitis , and arthritis . In addition, nTreg cells have been demonstrated to exert regulatory function in pre-clinical mouse models of allogenic BM transplantation

(BMT). Depletion of either recipient or donor nTreg cells induced markedly accelerated graft-versus host disease (GvHD) lethality, whereas addition of freshly isolated nTreg cells to the donor graft not only delayed GvHD lethality, but also favoured the allogeneic hematopoietic engraftment. Importantly, it has been shown that following BMT, inhibition of GvHD by nTreg cells does not abolish graft-versus-tumor and graft-versus-leukaemia activity. Furthermore, depletion of nTreg provokes effective tumor immunity to autologous tumor cells in otherwise non responding animals, enhances immune responses to invading or cohabiting microbes (**Shimizu J et al., 1999; Onizuka S et al., 1999; Jones E et al., 2003**). Elevated numbers of CD4⁺CD25⁺ T cells have been found in various human cancers, and these cells have been demonstrated to mediate suppressive function and to be present at tumor sites, envisaging nTreg depletion as an interesting strategy for promoting tumor rejection. The relevance of immune suppressive function of Foxp3⁺CD4⁺CD25⁺ Treg cells has been studied also in human allergies, and impaired skin infiltration of Foxp3⁺CD4⁺CD25⁺ T cells was observed in acute atopic dermatitis lesions (**Verhagen J et al., 2006**). Therefore, nTreg not only play a central role in maintaining tolerance towards self-antigens but also suppress a variety of physiological and pathological immune responses to non-self antigens (**Sakaguchi S et al., 2006**). A growing number of infections have been shown to be also associated with nTreg development (**Belkaid Y, Rouse BT. 2005**). nTreg in this scenario may temper immune responses and thus diminish tissue damage in the host; however, this activity may come at the cost of prevention of pathogen clearance, thus leading to increased persistence or transmission of the microbe.

Adaptive T Regulatory Cells

CD4⁺CD25⁺ Treg

It has been generally accepted that a population of Treg cells phenotypically and functionally similar to nTreg can be generated in the periphery from naïve precursors, perhaps under particular conditions (**Walker MR et al., 2003; Walker MR et al.,**

2005). *De novo* generation of human CD4⁺CD25⁺ T cells can be obtained using high doses of anti-CD3 or anti-CD3 together with anti-CD28 . Alternatively, Treg specific for particular peptide-MHC complexes can be generated *in vitro* by culturing CD4⁺CD25⁻ T cells with allogeneic DC pulsed with peptide. The resulting peptide-specific Treg cells can be isolated by using HLA class II tetramers (**Walker MR et al., 2005**). Peripheral development of CD4⁺CD25⁺ Treg appears to be a stochastic process, with a certain fraction of activated cells remaining CD25^{bright}, beginning to express Foxp3, and acquiring suppressive capacity. Alternatively, CD25⁺ Treg cells expressing Foxp3 and presenting suppressive functions can be generated by priming both human and mouse T cell in the presence of TGF- β . Indeed, polyclonal stimulation CD4⁺CD25⁻ T cells, but not CD4⁺CD25⁺ T cells, in the presence of exogenous TGF- β promotes the expression of Foxp3 (**Fantini MC et al., 2004; Fantini MC et al., 2006**). The resulting Foxp3 expressing T cells are anergic and suppress T cells responses *in vitro* , and *in vivo* . Moreover, it has been shown that activation of CD4⁺CD25⁻ T cells with allogeneic APC in the presence of exogenous TGF- β results in the generation of Treg cells phenotypically and functionally similar to nTreg cells . In this system the ability of TGF- β to promote Treg cells is strickly dependent on IL-2 . Importantly, TGF- β -induced Treg cells have the ability to educate other CD4⁺CD25⁻ cells to become Treg cells , suggesting that IL-2 and TGF- β stimulation triggers a cytokine-dependent self-perpetuating loop to sustain specific Treg cell activity and expansion .

Similarly, it has been reported that Treg cells can be induced *in vivo*. Thorstenson et al., (**Thorstenson KM et al., 2001**) used the DO11.10 transgenic adoptive transfer system to show that CD4⁺CD25⁺ T cells may also arise in the course of peripheral tolerance induction. CD25⁺ DO11.10 cells could be induced in hosts that had been adoptively transferred with DO11.10 cells and immunized with OVA peptide intravenously or orally. In the studies, the absolute number of CD25⁺ cells that was generated following adoptive transfer and immunization, and the relative percentage of CD25⁺ DO11.10 cells, inversely correlated with the amount of antigen present and the strength of immunogenic stimulus. Although Foxp3 staining was not feasible at the time of these studies, the CD25⁺ cells were shown to be defective in IL-2 production

and to mediate suppressive function *in vitro*. These studies suggested that CD25⁺ suppressor cells could be generated from mature peripheral CD4⁺ cells. Additionally, two studies have demonstrated that delivery of antigen coupled to the DC-specific anti-DEC205 monoclonal antibody led to the appearance of CD25⁺ Treg, in the periphery (**Kretschmer K et al., 2005; Mahnke K et al., 2003**). Similar to previously described results, the highest percentage of Treg was seen with low doses of antigen in the absence of inflammatory stimuli. These Treg had undergone fewer cell divisions than CD25⁻ antigen-specific cells. However, immunization with a high dose of anti-DEC205HA and anti-CD40 (the latter to activate DC) led to the presence of approximately fivefold more Foxp3⁺ cells at earlier time points and twofold fewer at later points than with a low dose of anti-DEC205HA alone, and this population seemed to undergo as many divisions as did CD25⁻ cells.

Type-1 Regulatory T cells

In 1997, Groux et al. described antigen-specific Treg cells characterized by the ability to secrete high levels of IL-10 and suppress T cell responses *in vitro* and *in vivo* via IL-10 and TGF- β . This subset was defined as Tr1 cells based on the fact that it was the first Treg cell population characterized at the clonal level. Tr1 cells can be generated *in vitro* and *in vivo* upon priming of naïve T cells with antigen in the presence of IL-10 and are characterized by their unique pattern of cytokine production, which is distinct from that of classical Th1 and Th2 cells. Tr1 cells, upon TCR-mediated activation, produce high levels of IL-10 and TGF- β , and IL-5, but low IL-2 and IFN- γ and no IL-4. Tr1 cells display low proliferative capacity, but can expand in the presence of IL-2 and IL-15, independently from their activation *via* the TCR (**Bacchetta R et al., 2002**). Activated Tr1 cells express the IL-2 receptor (IL-2R) α chain, and high levels of the IL-15R α chain, together with both the IL-2/15R β and IL-2R γ chains. Upon activation Tr1 cells also express activation markers such as CD40L, CD69, CD28, CTLA-4 and HLA-DR (**Bacchetta R et al., 2002**). In contrast to CD25⁺ Treg cells, Tr1 cells do not constitutively express Foxp3, but upon activation can up-regulated this transcription

factor to levels similar to those observed in activated CD4⁺CD25⁻ T cells (**Levings MG et al., 2005; Vieira PL et al., 2004**).

Human Tr1 cells in the resting phase express both Th1-associated (CXCR3 and CCR5) and Th2-associated (CCR3, CCR4, and CCR8) chemokine receptors (**Sebastiani S et al., 2001**). Interestingly, CCR8 is expressed at higher levels compared with Th2 cells, and upon activation, human Tr1 cells migrate preferentially in response to I-309, a ligand for CCR8 (**Sebastiani S et al., 2001**). CCR8 seems also to be relevant *in vivo*, because it has been recently demonstrated that in a model of helminth infection, its expression is strongly associated with IL-10-producing CD4⁺ T cells, which resemble Tr1 cells (**Freeman CM et al., 2005**). In contrast, CD4⁺ T cells from normal donors with a Tr1 cell phenotype have been found to express CCR9, suggesting that these cells home to the gut (**Papadakis KA et al., 2003**). Although significant efforts have been made to identify molecules expressed by Tr1 cells to discriminate them from the nTreg cells and from effector T cells, no specific marker for Tr1 cells has been reported so far.

Tr1 cells are anergic and proliferate poorly upon polyclonal-mediated or antigen-specific activation (**Groux H et al., 1997; Bacchetta R et al., 1994**), and these cells do not expand significantly under standard culture conditions. The autocrine production of IL-10 by Tr1 cells contributes to their low proliferative capacity, since addition of anti-IL-10 monoclonal antibody partially restores this response (**Groux H et al., 1997; Bacchetta R et al., 1994**).

Tr1 cells regulate immune responses through the secretion of the immunosuppressive cytokines IL-10 and TGF- β , and they suppress both naïve and memory T-cell responses *in vivo* and *in vitro* (**Groux H et al., 1997; Barrat FJ et al., 2002; Bacchetta R et al 1994; Levings MK et al., 2001; Veldman C et al., 2004**). Nevertheless, a mechanism implicating direct cell-cell contact with the target cells has also been postulated (**Hawrylowicz CM et al., 2005**). Although antigen-specific Tr1 cells need to be activated via the TCR in order to exert their suppressive function, once activated, Tr1 cells can mediate bystander suppressive activity against other antigens. This bystander suppression is likely mediated by the local release of IL-10 and TGF- β

(**Groux H. 2003**), which act on both APC and T cells. IL-10 downregulates expression of costimulatory molecules and pro-inflammatory cytokine production by APC and directly inhibits IL-2 and tumor necrosis factor- α (TNF- α) production by CD4⁺ T cells (**Pestka S et al., 2004**). Similarly, TGF- β downregulates the function of APC (**Strobl H et al., 1999**) and inhibits proliferation and cytokine production by T cells (**Cerwenka A et al., 1999**). The suppressive effects of Tr1 cells are reversed by addition of anti-IL10 and anti-TGF- β neutralizing monoclonal antibodies (**Levings MK et al., 2005; Groux H et al., 1997; Bacchetta R et al., 1994; Levings MK et al., 2005; Cavani A et al., 2000**), but additional mechanisms may also contribute.

Tr1 are involved in many T cell-mediated diseases. The first evidence that human Tr1 cells are involved in maintaining peripheral tolerance *in vivo* came from studies in severe combined immunodeficient (SCID) patients successfully transplanted with HLA-mismatched allogeneic stem cells. Despite the HLA disparity, these patients did not develop GvHD in the absence of immunosuppressive therapy. Interestingly, high levels of IL-10 were detected in the plasma of these patients, and a significant proportion of donor-derived T cells, which were specific for the host HLA antigens and produced high levels of IL-10, could be isolated *in vitro* (**Bacchetta R et al., 1994**). More recently, high frequencies of donor T cells producing IL-10 in response to recipient alloantigens have been found to correlate with the absence of acute GvHD after allogeneic HSC transplantation in cancer patients, while low frequencies were strongly associated with severe GvHD (**Weston LE et al., 2006**). Finally, spontaneous development of tolerance to kidney or liver allograft in transplanted patients is associated with the presence of CD4⁺ T cells that suppress naïve T-cell responses via production of IL-10 or TGF- β (**Van Buskirk AM et al., 2000**). Together these data indicate that Tr1 cells can regulate immune responses and induce tolerance *in vivo* in bone marrow and solid organ transplantation.

There are many evidences displaying that Tr1 cells regulate also responses to self-antigens and autoimmunity. Kitani and colleagues isolated self-major histocompatibility complex (MHC)-reactive Tr1 cell clones from peripheral blood of healthy individuals (**Kitani A et al., 2000**). These Tr1 cells inhibit proliferation of

primary CD4⁺ T cells and tetanus toxoid-specific T-cell clones via IL-10 and TGF-β. Similarly, T cells from non-diabetic individuals carrying HLA class II molecules associated with type 1 diabetes show an IL-10 response to islet peptides, while T cells from diabetic subjects produce predominantly IFN-γ (Arif S et al., 2004). These findings clearly demonstrate that self-antigen-specific Tr1 cells are present in humans and that they may play a role in maintaining self-tolerance. It is possible that skewing of self-reactive T cells toward a Treg or Th1 phenotype defines the balance between homeostasis and autoimmunity, respectively, and that unbalance in favor of Th1 cells results in pathology.

Tr1 cells specific for infectious agents or tumor antigens may interfere with the host's immune response and thus be detrimental. Presumably, it is advantageous for pathogens to evolve strategies to enhance the differentiation of Tr1 cells, which would then limit the protective immune response and allow long-term infection of the host (McGuirk P et al., 2002). Hemagglutinin from *B. pertussis* inhibits IL-12 and enhances IL-10 production from DC in the lung and bronchial lymph nodes (Gray CP et al., 2002). In chronic helminthes infections, where patients have relatively little sign of dermatitis despite the presence of small worms in the skin, antigen-specific Tr1 cells can be isolated. These cells were able to inhibit proliferation of effector T cell clones (Satoguina J et al., 2002). Several studies report the existence of Tr1 cells specific for tumor antigens. It has been shown that myeloma cells prime DC towards a state that favors the generation of T cells with a Tr1 rather than an effector phenotype (Fiore F et al., 2005). Exposure to cyclooxygenase-2-overexpressing glioma induces mature DC to overexpress IL-10 and decreased IL-12p70 production. These DC induce a Tr1 cell response, which is characterized by robust secretion of IL-10 and TGF-β with negligible IL-4 secretion by CD4⁺ T cells, and an inhibitory effect on responder T cells (Akasaki Y et al., 2004). In addition, Hodgkin lymphoma infiltrating CD4⁺ lymphocytes (HLIL), unlike their PBMC counterpart, have been found to be anergic, and suppress cell proliferation *in vitro*. Furthermore, HLIL contain large populations of both IL-10-secreting Tr1 and CD4⁺CD25⁺ Treg cells. Thus, HLIL are highly enriched

in Treg cells, which create a profoundly immunosuppressive environment that provides an ineffective immune clearance of cancer cells (**Marshall NA et al., 2004**).

Mechanism of Tolerance to Allergens

Because most allergens are immunologically inert proteins, the usual outcome of their inhalation is tolerance, and inflammation does not develop on chronic exposure (**De Heer et al., 2004; Ostroukhova M et al., 2004**). Several mechanisms have been proposed to describe this phenomenon. The most probable explanation is that partially activated DC migrate to the lymphoid organs and induce an abortive proliferative response of T cells leading to death (**Gett AV et al., 2003**). It is presently unclear whether tolerance induction is mediated by DC at different state of maturation, or by specialized DC subsets. Steinman has suggested that peripheral tolerance induction is induced by immature DC (**Steinman RM et al., 2003**). Bone marrow myeloid DC kept in an immature state by peroxisome proliferator activating receptor (PPAR) agonist treatment and subsequently injected into the trachea, they suppressed development of airway inflammation in an IL-10-dependent manner, whereas injection of mature DC induced Th2 priming (**Lambrecht BN et al., 2000; Hammad H et al., 2004**). However, Umetsu et al. demonstrated that tolerance in the lung is depended upon CD86 and ICOS-L, suggesting that some degree of DC maturity is necessary for tolerance induction, and is accompanied by vigorous naïve T cell division (**Akbari O et al., 2002; Tsitoura DC et al 1999**).

In Humans, myeloid DC (mDC) and plasmacytoid DC (pDC) were initially called DC1 and DC2, referring to their capacity to preferentially induce the differentiation of naïve T cells into Th1 and Th2 effector cells, respectively. The finding that asthmatic patients have more pDC in peripheral blood compared to healthy individuals (**Matsuda H et al., 2002**) could be related to the Th2-dominant phenotype in asthma. However, several studies described a plasticity of the two DC subsets in directing T cell responses (**Cella M et al., 2000; Langenkamp A et al., 2000**). These studies suggest that not only their lineage but also the cytokine microenvironment, and/or inflammatory mediators can influence the outcome of the immune response.

Another level of complexity arose when it was been demonstrated that respiratory tolerance might be control by a subset of pDC (**De Heer HJ et al., 2004; Oriss TB et al., 2005**). The precise mechanisms by which pDC promote tolerance are unknown, but in the absence of pDC, mDC become more immunogenic and induce the secretion of effector cytokines. pDC express high-level of programmed death ligand 1 which can deliver a negative signal to both T cells and mDC (**De Heer HJ et al., 2004; Kohl J et al., 2006**). Additionally, pDC produce the tryptophan-metabolizing enzyme indoleamine 2,3-dioxygenase (IDO), which exerts a strong inhibitory activity on T-cell proliferation (**Fallarino F et al., 2004**) and prevents inflammatory airway disease (**Hayashi Tet al., 2004**). Interestingly, IDO expression has been recently demonstrated also in pulmonary CD11c⁺ cells (**Swanson KA et al., 2004**), suggesting that not only pDC but also mDC may control inflammation in the lung. Finally, the tolerogenic properties of pDC can be related to their immature phenotype, since it has been demonstrated that immature pDC can induce Treg (**De Heer HJ et al., 2004**).

Based on the mutually inhibitory effects of Th1 and Th2 cytokines, it has been proposed that skewing towards Th1 response may protect against the development of allergic disease. Indeed, there is a considerable body of evidence from studies of allergic patients receiving immunotherapy, to support a protective effect for IFN- γ (**Durham SR et al., 1998**). Studies *in vitro* (**Manetti R et al., 1993; Parronchi P et al., 1992, Manetti R et al., 1999; Parronchi P et al., 1999; Brugnolo F et al., 2003; Fili L et al., 2006**) and *in vivo* (**Tournoy KG et al., 2002; Smart JM et al., 2002; Shahid SK et al., 2002; Romagnani S 2004; Turcanu V et al., 2003; Hamid QA et al., 1997**) support the hypothesis that allergen-specific Th2 responses are subjected to the negative control by Th1-polarizing cytokines, such as IFNs, and IL-12. Conversely, other studies have suggested that Th1 cells may contribute to Th2 priming or to inflammatory responses in the lung (**Bocek P et al., 2004; Dahl ME et al., 2004; Kuipers H et al., 2004; Heaton T et al., 2005**). Therefore although in murine models a clear-cut role of Th2 cells in promoting asthma has been demonstrated, data in humans indicate that both Th1 and Th2 cells contributes to allergic inflammatory

processes. Renewed interest in Treg cells within the last few years has led to the general consensus that Treg play a pivotal role in controlling allergic responses.

Role of CD4⁺CD25⁺ in allergy

The function of nTreg cells has been investigated in mouse models of allergic airway disease. *In vitro* studies showed the capacity of *ex vivo*-derived nTreg cells to inhibit both the differentiation of mouse T cells towards a Th2 phenotype (**Xu D et al., 2003; Stassen M et al., 2004**) and the production of cytokines by established Th2 cells, but the latter occurred only after the pre-activation of nTreg cells *in vitro* (**Stassen M et al., 2004**). The *in vivo* data, however, seem to be more complex. CD25⁺ depletion failed to show any effect on inflammation or AHR, although, modulated the Th2 response (**Jaffar Z et al., 2004**). By contrast, using recombination-activating gene 2 (Rag2)^{-/-} ovalbumin (OVA)-specific TCR transgenic mice the capacity of unfractionated T cells and CD4⁺CD25⁺-depleted T cells to control antigen-induced airway eosinophilia was compared. Results demonstrated that depletion of CD4⁺CD25⁺ T cells upregulated Th2-cell-mediated allergic inflammation (**Suto A et al., 2001**). Similarly, in mouse model resistant to allergen-induced AHR (C3H) it has been shown that depletion of CD4⁺CD25⁺ Treg cells promoted AHR, eosinophilia, and IgE, with a concomitant increase of Th2 cytokine production (**Lewkowich IP et al., 2005**). Moreover, *in vivo* transfer of nTreg reduced AHR, recruitment of eosinophils, and Th2 cytokine expression in the lung after allergen challenge. This suppression was dependent on IL-10, but IL-10 production by the Treg cells themselves is not required for suppression (**Kearley J et al., 2005**). In a different system, was demonstrated a role for membrane-bound TGF- β -expressing CD4⁺ T cells in the induction of airway tolerance elicited with low doses of inhaled antigen (**Ostroukhova, M et al., 2004**).

Although it is generally accepted that Treg cells in mouse model of allergic inflammation are crucial to control airways inflammation, the antigen-specificity of Treg cells remains controversial. Indeed, using the DO11.10 mouse model of allergic inflammation it has been demonstrated that suppression mediated by Treg cells is not

Ag-specific (Li J et al., 2006). Conversely, Der p1 peptide administration in DO11.10 mice have been shown to promote peripheral tolerance through the induction of Ag-specific Treg which can exert, once activated by Der p1, a bystander suppression (Zuleger CL et al., 2005).

Several studies have examined the role of nTreg in modulating allergic disease in humans. Some reports have suggested a defect in the number and/or function of allergen-specific Treg cells in atopic individuals. (Ling EM et al., 2004; Akdis CA et al., 1998; Karlsson MR et al., 2004; Verhagen J 2006). CD4⁺CD25⁺ T cells from patients with hay fever suppressed allergen-induced proliferation of autologous CD25⁻ T cells and their IL-5 production at lower extent than in non-atopic donors. Treg cells were Foxp3⁺, but their suppressive activity was not related to CTLA-4, IL-10, or TGF- β (Ling EM et al., 2004). The loss in inhibitory activity of CD25⁺ T cells from allergic donors was most pronounced during symptomatic periods, when pollen counts were highest. Similarly, CD25⁺ T cells from both allergic and non-allergic individuals potently suppressed T-cell proliferation and Th2 cytokine production in response to birch allergen outside of the pollen season; however, during the birch pollen season, CD25⁺ T cells from allergic patients but not from non-allergic controls were defective in downregulating birch-pollen induced IL-13 and IL-5 production (Grindebacke H et al., 2004). In healthy non-allergic subjects, the proliferative response of T cells to cows' milk antigen is essentially absent, however, depletion of CD25⁺ T cells resulted in significant T-cell proliferation, suggesting that CD25⁺ T cells normally suppress the responses to dietary antigens (Taams LS et al., 2002, Sletten GB et al., 2007). Similarly, children who had outgrown cows' milk sensitivity had higher frequencies of circulating Treg cells and decreased *in vitro* proliferative responses to bovinem β -lactoglobulin (a milk protein) in PBMC compared with children who maintained clinically active milk sensitivity. Nevertheless, the reduced proliferative response to milk (β -lactoglobulin) in children who had outgrown milk-induced gastrointestinal symptoms could be reversed by removal of the CD25⁺ cells from the cultured PBMC, but this increase in T-cell proliferation did not occur in allergic individuals. These results indicate that in cows' milk-induced colitis, CD25⁺ inhibitory Treg become

evident after cows' milk challenge in allergic children who become tolerant to cows' milk. The suppressive activity of these Treg cells was not related to the release of soluble factors, but was due to a cell contact-dependent phenomenon (**Karlsson MR et al., 2004**). These latter findings are apparently at variance with the results of another study performed in children suffering from peanut allergy. Children who outgrew their peanut allergy exhibited increased numbers of allergen-specific interferon (IFN)- γ -producing and reduced levels of IL-4-producing T cells in comparison with those who remained peanut-sensitive, thus becoming nearly similar to peanut non-allergic children. This finding suggests that the lack of peanut allergy or the recovery from this form of hypersensitivity is due to an immune deviation in the specific response from Th2 to Th1 rather than to the enhancement of immune suppression (**Turcanu V et al., 2003**).

Role of Tr1 in allergy

Many studies in mouse model had shown the importance of Tr1 cells in controlling allergy and asthma. Intranasal treatment with OVA induced T cell tolerance by inhibiting T cell proliferation, IgE and IgG1 production. In this system tolerance to OVA was associated with the generation of Tr1 cells in bronchial lymph nodes (**Barbey C et al., 2004**). Akbari et al., showed that in the bronchial lymph nodes of mice exposed to respiratory allergen mature pulmonary DC induced the development of Tr1 cells, *via* the inducible ICOS-ICOS-ligand pathway (**Akbari O et al 2002**). In addition, it has been described the *in vivo* development of a population of Treg cells during a Th1 polarized response, which were distinct from Treg cells. These antigen-specific Treg cells produced both IL-10 and IFN- γ , expressed the transcription factors Foxp3 and *T-bet*, and potently inhibited the development of AHR (**Stock P et al., 2004**). The suppressive activity of *Mycobacterium vaccae* on airway eosinophilia was found to be clearly related to the induction of allergen-specific Treg cells able to produce IL-10 and TGF- β (**Zuany-Amorim C et al., 2002**). Although, in a subsequent study *M. vaccae* administration during allergen sensitization or challenge suppressed

asthmatic features without inducing an increase in either IFN- γ or IL-10 in the lung lavage fluid (**Smit JJ et al., 2003**). Tr1 cells are also important in downregulating immune responses toward allergens such as nickel (**Kinter AL et al., 2004**), insect venom (**Durez P et al., 1993**), and cat allergens (**Bergmann, M. & Sautner, T. 2002**). Cavani et al. has been isolated Nickel-specific Tr1 clones from skin and peripheral blood of atopic patients affected by ACD to Nickel (Ni). They demonstrated that Ni-specific Tr1 cells inhibit the APC functions of monocytes (antigen-presenting capacity) and DC (maturation, antigen-presenting capacity and IL-12 relapse), in a IL-10-dependent manner, and directly suppress Ni-specific Th1 responses (**Cavani A et al., 2000**).

The defect in allergen-specific Tr1 cells in human subjects has been reported by Akdis and colleagues. The authors demonstrated an increased frequency of allergen-specific Tr1 cells with a concomitant decreased allergen-specific Th2 cells in non-allergic compared to allergic subjects. These results clearly indicate that the balance between allergen-specific Tr1 and Th2 cells is a key determinant in the development of allergic responses in humans (**Akdis M et al., 2004**).

The Interplay between IL-10, Dendritic Cells and Regulatory T cells in Allergy

IL-10

IL-10, an anti-inflammatory cytokine, has originally been described as cytokine-synthesis-inhibiting factor (CSIF) with regard to its effects exerted on IFN- γ production of Th1 cells. It is predominantly produced by T lymphocytes, monocytes, and macrophages. IL-10 exerts suppressive effects on a wide range of different populations of lymphocytes and it inhibits the antigen presenting capacity of different types of APC, including DC, Langerhan's cells, and macrophages. Moreover, IL-10 inhibits the production of pro-inflammatory cytokines and IL-12 (a cytokine important for the development of Th1 cells) (Moore KW et al., 2001), chemokine secretion (Jinquan T et al., 1993; Kasama T et al., 1994), and the expression of MHC class II and costimulatory molecules (Moore KW et al., 2001; Fiorentino DF et al., 1991). IL-10 inhibits the maturation of DC from monocyte precursors (Buelens C et al., 1997), modifies chemokine receptor expression (Sozzani S et al., 1998; Takayama T et al., 2001), increases β 2-integrin ligand (e.g., ICAM-1) expression (Willems F et al., 1994). IL-10 has also been shown to promote the development of B1 cells (Ishida H et al., 1992), to enhance the survival of B cells which correlated with increased expression of the anti-apoptotic protein bcl-2, T cells, and tumor cells (Levy Y et al., 1994; Cohen SB et al., 1997), and, paradoxically, to induce apoptosis in chronic B cell leukemia cells (Fluckiger AC et al., 1994). IL-10 promotes the activity of NK cells (Cai G et al 1999) and has a stimulatory effect on CD8⁺ T cells by enhancing their cytotoxic capacity and proliferation (Moore KW et al., 2001; Asadullah K et al., 2003; Groux H et al., 1998; Schwarz MA et al., 1994).

The principle function of IL-10, however, is to limit the magnitude of an immune response, as mice lacking IL-10 exhibit spontaneous enterocolitis (Kuhn R et al., 1993). More relevant to peripheral immunity, mice lacking IL-10 by gene deletion have increased Th1 responses (Yang X et al., 1999) and, consequentially, enhanced

clearance of bacterial (Murray PJ et al., 1999; Sewnath ME et al., 2001), fungal (Vazquez-Torres A et al., 1999), or toxoplasmic (Gazzinelli RT et al., 1996) infection. These mice also show exaggerated asthmatic (Tournoy KG et al., 2000) and allergic responses (Berg DJ et al., 1995; Grunig G et al., 1997).

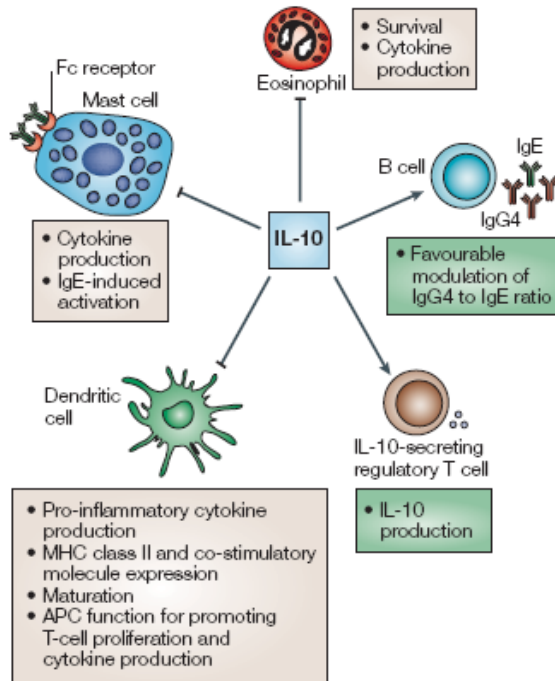
IL-10 modulates many cells and effector functions that are associated with allergic and it is thought to have a role in naturally regulating immune homeostasis in the lungs (Akbari O et al., 2001). Consistent with these findings, there is an inverse association between IL-10 levels and the severity of allergic and asthmatic disease (Lim S et al., 1998). IL-10 is expressed at lower levels in the nasal mucosa of HDM patients with severe allergic rhinitis compared to patients with mild symptoms (Muller B., 2007). In asthmatic patients, the wheal size from skin prick with allergen is negatively associated with IL-10. Moreover, bronchial hyper-responsiveness (BHR) in non-atopics was associated with elevated allergen-specific and polyclonal IL-10 production (Heaton T et al., 2005). Atopic subjects have intrinsic inability to up-regulate IL-10 production (Gentile DA et al., 2004). Studies with BAL fluid from asthmatic patients have revealed lower IL-10 levels than in healthy controls, and T cells from children suffering from asthma have been shown to produce less IL-10 mRNA than T cells from healthy children (Borish L et al., 1996; Koning H et al., 1997).

A suppressive role for IL-10 has also been demonstrated in both allergen-specific immunotherapy (SIT) and normal responses to allergens (Blaser K et al., 2004). Increased expression of allergen-induced *in vitro* IL-10 mRNA in PBMC of allergic rhinitis patients has been demonstrated after specific venom immunotherapy (Savolainen J et al., 2004). During allergen- SIT, intracellular IL-10 significantly increased in the antigen-specific T cell population and activated CD4⁺ T lymphocytes during the early phase of the therapy. Afterwards, intracytoplasmic IL-10 was also increased in monocytes and B cells. These results indicate that IL-10 secreted by T cells represents a crucial step for the consequent induction T-cell anergy and the modulation of APC that are required for the maintenance of tolerance (Akdis CA et

al., 1998). The proliferative and cytokine responses could be reconstituted by *ex vivo* neutralization of endogenous IL-10, supporting the pivotal role of IL-10 is actively in promoting tolerance after allergen-SIT (**Akdis CA et al., 1999**). Moreover, in *Dermatophagoides-pteronyssinus*-sensitive patients, the allergen-specific sublingual immunotherapy (SLIT) increased IL-10 and IFN- γ production upon allergen stimulation of PBMC before the clinical improvement (**Cosmi L et al., 2006**).

In animal models IL-10 has been shown to be effective in inhibiting allergic inflammation. IL-10 administration before allergen treatment induced antigen-specific T-cell unresponsiveness and promote peripheral T-cell tolerance (**Enk AH et al., 1994**). Moreover, intranasal administration of constructs carrying the transgenes for murine IL-10 (**Stampfli MR et al., 1999**) and adoptive transfer of IL-10-transfected T cells (**Oh JW et al., 2002**) or OVA-specific cells engineered to produce IL-10 (**Hansen G et al 2000**), have both been shown effective in blocking allergic airway inflammation.

Therapies shown to be beneficial for the treatment of allergy and asthma, such as glucocorticoids, have been shown to increase IL-10 secretion by T cells (**Corral LG et al., 1999**). Furthermore, the clinical efficacy of glucocorticoids seems to be linked to the ability of T cells to produce IL-10 (**Sawicka E et al 2003**). It has been demonstrated that allergen-specific Tr1 cells induced *in vitro* with vitamin D3 and dexamethasone potently inhibit allergen-induced Th2 cytokine production in an IL-10-dependent manner (**Barrat FJ et al., 2002**). Furthermore, administration of vitamin D3 to glucocorticoid-resistant patients enhanced T-cell responsiveness to dexamethasone with an increase in IL-10 production (**Xystrakis E et al., 2006**) (Fig. 6).



from Hawrylowicz CM et al. Nat Rev Immunol 2005

Fig 6. Functions of IL-10 that are relevant in allergy and asthma

Immunoregulation of Dendritic Cells by IL-10

IL-10 has been shown to modulate APC, including DC. When human or murine DC exposed to IL-10 display reduced surface expression of MHC class I and class II molecules and costimulatory molecules of the B7 family (**Jonuleit H, Schmitt E 2003; Schuler G, Steinman RM 1985; Bonifaz L et al., 2002; Mahnke K et al., 2003; Mahnke K et al., 2002**). In addition, the release of pro-inflammatory cytokines by DC, i.e. IL-1 β , IL-6, TNF- α and most markedly IL-12, is abolished after IL-10 treatment (**Chang CH et al., 1995; Koch F et al., 1996**). However, these effects have been shown when immature DC were exposed to IL-10 (**Steinbrink K et al., 1997; Misra N et al., 2004**).

IL-10-transduced murine bone marrow-derived mDC showed reduced expression of MHC class II and costimulatory molecules and thereby an impaired ability to promote T cell proliferation (**Takayama T et al., 2001; Takayama T et al., 1998; Lu L et al., 1999**). Moreover, IL-10-transduced DC showed lower levels of CCR7 and simultaneously increased CCR5 levels, which seemed responsible for an impaired, *in vivo* homing of DC from peripheral tissues to secondary lymphoid organs (**Takayama T et al., 2001**). Studies have demonstrated, however, that the tolerogenic effect of IL-10-treated DC is not simply due to decreased cytokine production or costimulatory molecule expression. Indeed, IL-10 modulates DC maturation enabling DC to induce T cells with regulatory properties (**Steinbrink K et al., 1997; Sato K et al., 2002; Steinbrink K et al., 2002**). Freshly isolated Langerhans cells treated with IL-10 inhibit Th1 cells proliferation (**Enk AH et al., 1993**).

IL-10 not only modulate already differentiated DC, but also promote the differentiation of tolerogenic DC. Wakkach et al. (**Wakkach A et al., 2003**) showed that addition of IL-10 during *in vitro* differentiation of bone-marrow derived DC resulted in a population of CD45RB^{high} tolerogenic DC. These CD11c^{low}CD45RB^{high} DC are present in the spleen and lymph nodes of normal mice and are significantly enriched in the spleen of IL-10 transgenic mice. These natural or *in vitro*-derived DC display plasmacytoid morphology, an immature-like phenotype, and secrete high levels of IL-

10 after activation. Moreover, OVA peptide-pulsed CD11c^{low}CD45RB^{high} DC specifically induce differentiation of Tr1 cells *in vitro* and *in vivo*.

The therapeutic use of these IL-10-modulated DC is under investigation since injection of *in vitro* generated IL-10-modified DC have been shown to be able to prevent EAE (Okura Y et al., 2003) and GvHD (Muller G et al., 2002; Sato K et al., 2003).

Induction of Regulatory T Cells by Dendritic Cells

Several evidences indicate that Treg cells can be induced by both DC in an immature or semi-mature stage. Presentation of antigens by immature DC in the absence of danger signals results in tolerance (Steinman et al., 2000 e Steinman et al., 2002). Repetitive stimulation of naïve cord blood CD4⁺ T cells with allogenic immature DC results in the differentiation of IL-10-producing Treg (Jonuleit H et al., 2000) that suppress T-cell responses via a cell-contact dependent mechanism. Furthermore, it has been reported that repeated stimulation of naïve peripheral blood CD4⁺ T cells with allogenic iDC induces the differentiation of human Tr1 cells *in vitro* (Levings MK, et al., 2005). In this system, after three rounds of stimulation with iDC, T cells become anergic and acquire regulatory function. These T 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. They also suppress T-cell responses via an IL-10- and TGF- β -dependent mechanism. Similarly, semi-mature DC originated from the exposure of immature DC to TNF- α in the absence of danger signals acquire some characteristic of mature DC but, due to their inability to produce polarizing signals, they induce the differentiation of Tr1 cells (Menges M et al., 2002).

Not only immature DC but also specialized subsets of tolerogenic DC may prime Treg cells (Battaglia M et al., 2006; Lanzavecchia A et al., 2001; Banchereau J et al., 1998; Penna G et al., 2005; Mahnke K et al., 2005; Battaglia M et al., 2004). Biological agents, such as IL-10 (Steinbrink K et al., 1997; Steinbrink K et al., 2002; Gregori S et al., 2007 submitted), TGF- β (Sato K et al., 2003), IFN- α (Carbonneil C et al., 2004) induce tolerogenic DC inhibiting the antigen-presenting

capacity and promoting the differentiation of Treg *in vitro* (Woltman AM et al., 2003; Adorini L et al 2004; Matyszak MK et al., 2000). During the differentiation from peripheral progenitors, DC exposed to IL-10 for the last 2 days of culture show a strongly reduced capacity to stimulate a CD4⁺ T cell response in an allogeneic MLR in a dose-dependent manner (Steinbrink K et al., 1997). These anergic T cells were able to suppress activation and function of T cells in an antigen-specific manner. (Steinbrink K et al., 1999; Steinbrink K et al., 2002). We recently demonstrated that IL-10 induces the differentiation of a unique subset of myeloid DC (DC-10) which are CD14⁺CD11c⁺CD11b⁺CD83⁺HLA-DR⁺CD71⁺CD1a⁻, display a myeloid mature phenotype (CD80⁺CD86⁺), express ILT2, ILT3 and ILT4, secrete high levels of IL-10 but low IL-12, and display low stimulatory capacity. Importantly, DC-10 are potent inducer of Tr1 cells in a allogeneic system (Gregori S et al., 2007 manuscript submitted).

Several immunosuppressive agents including glucocorticoids, mycophenolate mofetil, vitamin D3, and sirolimus have been shown to promote DC with tolerogenic phenotype and function (Penna G et al., 2005). These agents impair DC maturation and inhibit up-regulation of costimulatory molecules, secretion of proinflammatory cytokines, in particular IL-12, and allostimulatory capacity.

Certain pathogens or allergens, such as *Bordetella pertussis* (McGuirk P et al., 2002), *Lactobacillus reuteri*, *L. casei* (Smits HH, et al., 2005), and ferruginol (Takei M et al., 2005), evade immunity by priming DC to become tolerogenic DC, which induce Tr1 cells.

Not only mDC but also other subsets of DC, including pDC, can induce the differentiation of Treg. pDC polarize T cells towards IL-10 production and regulatory activity *in vitro* (Gilliet M et al., 2002; Kuwana M et al., 2001; Kuwana M 2002). In addition, repetitive stimulation of naïve CD4⁺ T cells with murine CD8 α ⁺ pDC isolated from mesenteric lymph nodes results in the generation of a population of Tr1-like cells with regulatory properties (Bilsborough J et al., 2003).

Control of lung Dendritic Cell Function by regulatory T cells

It is clear that DC control effector T cell responses at sites of inflammation. An obvious question, therefore, is whether Treg cells also exert their action by regulating the function of DC within sites of inflammation, by producing IL-10, TGF- β or through cell–cell contact. Induction of CD86 on DC by inflammation might lead to the local expansion of Treg cells and, after a few days of inflammation, Treg cell numbers might outnumber pathogenic effector T cells (**Yamazaki S et al., 2003**). Within sites of chronic inflammation, such as colonic inflammation induced by transfer of CD45RB⁺ cells into severe combined immunodeficiency syndrome (SCID) mice, Treg cells interact locally with DC and pathogenic effector T cells to suppress effector T cell activation by downregulating OX40L (**Mottet C et al., 2003**). This might also occur in asthma, where OX40L is critical for effector Th2 cells (**Salek-Ardakani S et al., 2003**). The chemokines produced by DC in response to allergen recognition not only attract pathogenic Th2 cells, but also CCR4- and CCR8-expressing Treg cells (**Hammad H et al., 2003; Iellem A et al., 2001**).

How Treg downregulate inflammation is a matter of debate. In one scenario, Treg might keep DC in an immature state, unable to induce Th immunity (**Serra P et al., 2003**). In a very interesting study, mice that were deficient in Runx3, a critical downstream mediator of TGF- β signalling, had lung DC that were in a spontaneously activated and mature state, leading to Th2 mediated lung inflammation to environmental antigens (**Fainaru O et al., 2004**). It was also shown that murine natural Treg cells have the capacity to induce IDO activity and tryptophan catabolism in myeloid DC, thus leading to T cell suppression (**Fallarino F et al., 2003**). High level IDO expression, such as induced by treatment with CpG motifs has been shown to suppress eosinophilic airway inflammation (**Hayashi T et al., 2004**).

If Treg cell suppression of allergic immune responses is mediated at the level of the DC, it will be important to find out if DC from atopic individuals have a reduced capacity to interact with or stimulate Treg function or alternatively are resistant to Treg regulation. Inflammatory cytokines produced by DC (e.g. IL-6) might render effector T

cells irresponsive to Treg suppression (**Pasare C, Medzhitov R 2003**). Alternatively, decreased Treg activity in atopic individuals might contribute to chronic inflammation (**Ling EM et al., 2004**).

During generation of an efficient immune response, lung DC have to overcome suppression by Treg, and the dominant way in they seem to do this is by producing the cytokine IL-6, which counteracts the suppression by naturally occurring CD4⁺CD25⁺ Treg (**Doganci A et al., 2005**). Several articles now support the concept that Treg alter airway DC function. In mice resistant to HDM-induced asthma and AHR (C3Hmice), Treg depletion with the CD25-depleting antibody similarly led to increased numbers of pulmonary mDC with increased expression of MHC class II, CD80, and CD86 and an increased capacity to stimulate T-cell proliferation and Th2 cytokine production. In normally susceptible A/J mice Treg did not suppress inflammation and AHR. These data suggest therefore that resistance to allergen- driven-AHR is mediated in part by CD4⁺CD25⁺ Treg suppression of DC activation and that the absence of this regulatory pathway contributes to susceptibility (**Lewkowich IP et al., 2005**).

In human subjects with allergy, there is a reduction in the number and possibly function of Treg, (**Kuipers H, Lambrecht BN 2004**) but it is unclear at present whether this would also lead to altered function of DC in these patients.

AIM OF THE STUDY

Several studies have indicated that an alteration of immune regulatory mechanisms is involved in the development of allergic diseases.

In particular, an impaired number or function of T regulatory subsets as well as a defective production of IL-10 and TGF- β by T cells have been recently reported in individuals affected by allergic diseases.

DC treated with IL-10 were found to be potent inhibitors of the immune response. This effect is mediated by the induction of Tr1 and the production of high amount of IL-10. Tr1 generated in this way are able to inhibit both Th1 and Th2 responses.

The aim of this thesis is to determine whether IL-10-treated DC are able to induce Tr1 cells in children allergic to HDM. These conditioned DC could be used as cellular therapy for allergy or for other diseases characterized by inappropriate immune response, such as autoimmune diseases.

To this end, monocyte-derived DC were obtained from allergic patients and healthy controls, pulsed with Der p2 (a major HDM allergen) in presence of IL-10 (using 2 different experimental strategies) and further used to stimulate autologous T cells.

The specific aims of the study can be summarized in the following way:

1. To characterize Der p2-specific T cell response in HDM patients in terms of cell proliferation, T cell phenotype and cytokine production.
2. To determine whether IL-10-treated DC are able to modulate allergen-specific T cell responses in HDM patients.
3. To induce Der p2-specific T cells with regulatory capacities by using DC treated with IL-10.

MATERIALS AND METHODS

PATIENTS

Inclusion criteria.

The study included 41 children (24 males and 17 females) between 4 and 14 years, sensitized to House Dust Mites (HDM) and affected by intermittent to mild persistent asthma diagnosed according to the GINA guidelines (**GINA, 2006**).

All patients had clinical symptoms after allergen exposure, and elevated IgE and/or positive skin prick tests to HDM: *Dermatophagoides Pteronissimus* (*Dt Pt*), and *Farinae* (*Dt F*). None of the children was affected by a concomitant autoimmune diseases or infection and at the time of the enrollment and none of them had been taking either local or oral antibiotics, corticosteroids, or immunosuppressive drugs since at least 2 months. The demographic and clinical characteristic of the patients are reported in Tab 4.

Ten age-matched healthy controls (5 male and 5 female) aged between 3 and 14 years without a family history of allergic symptoms, and negative for skin prick test and serum specific IgE were used as controls.

Table 4. Demographic and clinical description of study population.

	PATIENTS (n=41)
Sex (females/males)	17/24
Age (yrs)	*8.5±3.3
Range	3-14.4
Duration of asthma (yrs)	3.6±2.7
Family history of asthma and/or allergy (%)	27/41 (65.85)
Other symptoms of allergy (%)	21/41 (51.2)
Respiratory infections (%)	5/41 (18.5)
Total IgE	144.5±165.1
<i>Dt. Pt</i> specific IgE	15.3±31
<i>Dt. F</i> specific IgE	10.5±17.8
Occasional use of symptom relievers (%)	34/41 (82.9)

*Data are expressed as means ±SD, unless differently stated.

Serum IgE quantization.

IgE specific for inhalant and food allergens were tested with Pharmacia RAST, according to the manufacturer's instructions. Serum samples were blind-coded prior to the quantization of total and specific IgE and titers were expressed as IU/ml.

Skin prick tests.

The Skin Prick Test was performed on the volar forearm. We evaluated skin test reactivity to cow milk, egg, *Dermatophagoides Pteronissimus* and *Farinae*, grass, birch, *Alternaria Alternata*, *Aspergillus Fumigatus* and *Clodosporium*, *Olea* tree, cat and dog dander. As positive and negative controls, histamine (10 mg/ml) and 0.9% sodium chloride in 50% glycerol, respectively, were included. Test was considered positive when the size of the wheals had a diameter ≥ 3 mm than any measurable diameter of the negative control test.

Peripheral blood cells

Peripheral blood monocyte cells (PBMC) were obtained from allergic patients and healthy controls according to the ethical commitment and were purified by gradient centrifugation over Ficoll-Hypaque (Amersham Biosciences, Uppsala, Sweden). Blood samples range between 5 and 15ml.

Dendritic cells differentiation.

Dendritic cells (DC) were differentiated from peripheral blood monocytes (CD14⁺). CD14⁺ cells were isolated from PBMC by positive selection, using anti-CD14-conjugated magnetic Microbeads (MACS; Miltenyi Biotec, Belgish Gladbach, Germany) according to the manufacturer's instructions. CD14⁺ population routinely resulted in >95% purity as assessed by flow cytometry. The negative fraction (CD14⁻) was collected and cryopreserved for later uses. DC were differentiated from CD14⁺ cells cultured with rhGM-CSF (100 ng/ml, R&D Systems Minneapolis, MN, USA) and rhIL-4 (10 ng/ml, R&D Systems) for 5 days in RPMI 1640 containing 5% pooled AB human serum (BioWhittaker, Walkersville, MD, USA), 100 U/ml penicillin/streptomycin (Bristol-Myers Squibb, New York, USA), 2mM L-glutamine

(GIBCO BRL Gaithersburg, MD, USA) (DC medium). 5 days of culture, extractive purified Der p2 (1 µg/ml, Lofarma, Milano, Italy), was added for additional 2 days (Dp2-DC). The optimal concentration of Der p2 was defined on the basis of pilot experiments. In some experiments after 5 days of culture, DC were treated with Der p2 (1 µg/ml) and activated with lipopolysaccharide from E. Coli (LPS) (1 µg/ml, Sigma Chemicals, St Louis, MO, USA) for additional 2 days (LPS Dp2-DC). In parallel, DC were differentiated from CD14⁺ cells in the presence of GM-CSF and IL-4 for 5 days and left un-stimulated (iDC) or matured with LPS (1 µg/ml) for additional 2 days (LPS-DC).

IL-10-treated Dp2-DC were obtained by differentiating CD14⁺ cells in the presence of GM-CSF and IL-4 for 5 days, and cultured with Der p2 (1 µg/ml) and rhIL-10 (40 ng/ml, Endogen, Woburn, MA, USA) for additional 2 days (see IL10-DC, protocol n.1). Dp2-DC10 were differentiated from CD14⁺ cells cultured with GM-CSF, IL-4, and rhIL-10 (10 ng/ml, BD Biosciences, San Jose CA, USA) for 5 day. After 5 day of culture, DC were treated with Der p2 (1 µg/ml) for additional two days (see DC10, protocol n.2).

The purity and maturation state of DC was routinely checked by flow cytometric analysis to determine expression of CD14, CD1a, CD80, CD83, CD86, and HLA DR.

T cell differentiation

Protocol n.1: CD14⁻ cells (freshly isolated T cells) were thawed and cultured with autologous iDC, Dp2-DC, IL-10 Dp2-DC at 10:1 T cells/DC ratio in RPMI 1640 containing 10% fetal calf serum (FCS) (BioWhittaker), 100 U/ml penicillin/streptomycin (Bristol-Myers Squibb), 2mM L-glutamine (GIBCO BRL). After 6 days of culture, T cell lines were collected, washed, and cultured with 50 U/ml of rhIL-2 (Sigma Chemicals, St Louis, MO, USA) for additional 14 days. Subsequently, T cell lines were collected, washed, and repeatedly stimulated with original type of autologous DC freshly differentiated from cryopreserved CD14⁺ cells. At any time points, a portion of T cell cells was analyzed for T cell phenotype and proliferation (see protocol n.1).

Protocol n.2. CD14⁻ cells (freshly isolated T cells) were thawed and cultured with autologous iDC, Dp2-DC, Dp2-DC10 at 10:1 T cells/DC ratio in X-vivo 15 medium (BioWhittaker), supplemented with 5% pooled AB human serum (BioWhittaker) and 100 U/mL penicillin/ streptomycin (Bristol-Myers Squibb). After 7 days of culture, rhIL-2 (20 U/mL, Chiron, Amsterdam, Holland) was added, and cells were expanded for additional 7 days. 14 days after initiation of the culture, T cell lines were collected, washed, and tested for their ability to respond Der p2 (see protocol n.2).

Flow cytometric analyses.

DC were stained with monoclonal antibodies against CD14, CD1a, CD80, CD83, CD86, and HLA-DR as well as with isotype controls, FITC-or PE-conjugated (all from Pharmingen, San Diego, CA, USA or BD Biosciences, San Jose CA, USA).

T cell were stained with monoclonal antibodies against CD3, CD4, CD8, CD25, CD69, and CTLA-4, as well as isotype controls, FITC-or PE- CyCr-, Cy5- and APC-conjugated (from Pharmingen, or BD Biosciences). For intracellular expression of CTLA-4, after surface staining, Cytoperm/Cytofix kit (BD-Pharmingen) was used, according to manufacturer's instructions. After washing twice, cells were incubated with anti-CTLA-4 for 20 min, washed and analyzed by flow cytometry. Cells were analyzed by a multiparametric flow cytometry with FACSCalibur (Beckton Dickinson, San Jose, CA, USA), interfaced with Cellquest software (Beckton Dickinson).

Der p2-specific T cell proliferative response

To analyze Der p2-specific T-cell proliferation, autologous CD14⁻ cells (5×10^4 /well) were thawed and cultured either with iDC, Dp2-DC, IL10-Dp2-DC or Dp2-DC10 (5×10^3 /well) (at 10:1, T cells/DC ratio) in 96-well round-bottom microplates in triplicate in a final volume of 200 μ l of medium. Alternatively T cells were stimulated with PHA (5 μ g/ml, Sigma, St Louis MO, USA). After 5 days of culture, T-cell proliferation was assessed by overnight [³H]Thymidine incorporation (1 μ Ci/well, Amersham International, Amersham U.K.). In parallel after 5 days of culture, supernatants were collected for cytokine detection.

To test the anergy, T cell lines (5×10^4 /well) were cultured alone or with autologous monocytes (5×10^4 /well) (at 1:1 T cells/monocytes ratio) in absence or presence of Der p2 (1 μ g/ml) in 96-well round-bottom microplates, in a final volume of 200 μ l of medium. After 3 days of culture, T-cell proliferation was assessed by overnight incorporation [3 H]Thymidine (1 μ Ci/well ,Amersham International, Amersham U.K.). In parallel after 2 days of culture, supernatants were collected for cytokine detection.

Cytokine production assay.

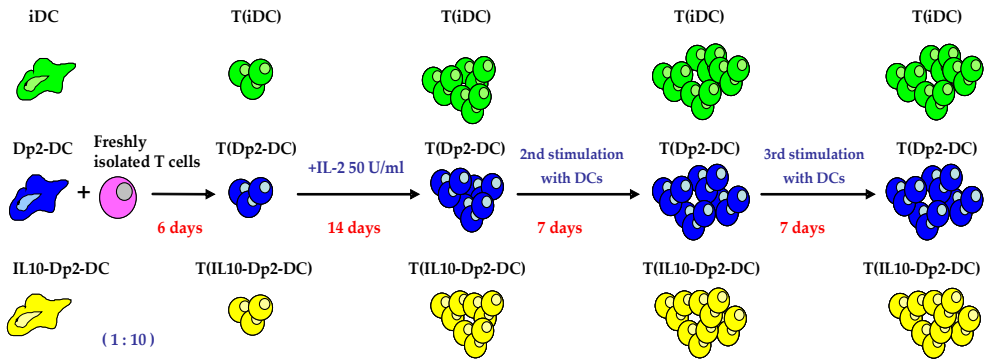
To detect IL-4, IL-5, IL-10, IL-13, TNF- α , IFN- γ concentrations in supernatants of DC-T cell co-cultures, was used the Th1/Th2 cytometric bead array-kit (CBA) (BD Biosciences, San Jose, USA) or, alternatively, the Bioplex Protein Array system (BioRad, Hercules CA, USA), according to the manufacturer's instruction.

The detection limit was 20 pg/ml for ELISA and CBA and 2 pg/ml for Bioplex.

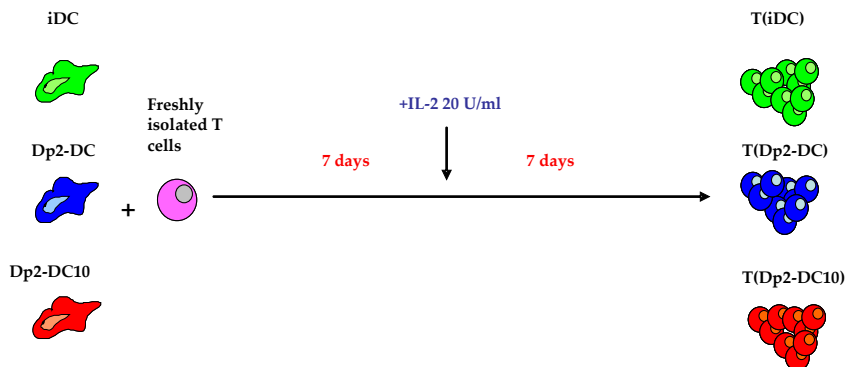
Statistical analysis

Student's t test was used to compare the differences of cell proliferation and cytokine production between patients and controls and between different cell culture conditions. The relationship between CD86 expression and T cell proliferation reductions was assessed by Person's correlation test. The SPSS 13.0 analysis software was employed. P value <0.05 was considered statistically significant.

Protocol n.1



Protocol n.2



RESULTS

1. CHARACTERIZATION OF Der p2 SPECIFIC RESPONSE IN ALLERGIC PATIENTS

1a. Selection of the Culture Conditions to promote Der p2-specific T Cell Response in Allergic Patients.

To set up conditions to promote Derp2-specific T-cell response in patients allergic to HDM, we performed co-culture experiments using DC differentiated from CD14⁺ peripheral blood monocytes with GM-CSF and IL-4 for 5 days, and pulsed for additional 2 days with different concentrations of Der p2 (range from 0.1 to 10 $\mu\text{g/ml}$) and autologous T cells. We observed a dose-response curve with the maximal proliferation at the concentration of 1 $\mu\text{g/ml}$ of Derp 2 which was used in all subsequent experiments (Fig.7).

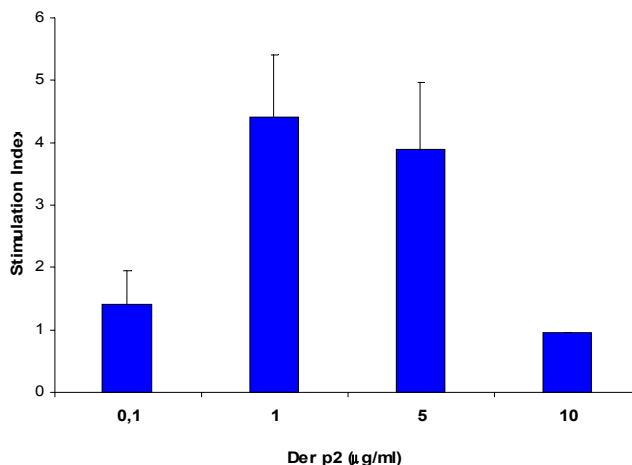


Fig. 7. Dose-response curve to Der p2. T cells from allergic patients were co-cultured with autologous DC differentiated from peripheral blood CD14⁺ cells cultured with GM-CSF and IL-4 for 5 days, and pulsed with the indicated concentrations of Der p2 for additional 2 days. Proliferative responses were evaluated after 5 days of culture by thymidine incorporation for an additional 16 hrs. Results are expressed as ratio between Der p2-specific T-cell response and unstimulated T-cell response (Stimulation Index). The average \pm SD of 7 independent experiments is presented.

It is well known that mature DC are better antigen presenting cells than immature DC (**Banchereau J et al., 1998**). Moreover, it has been recently reported that LPS can promote allergic inflammation (**Hollingsworth JW et al., 2006**). To identify the antigen specific stimulator to be used in our study, we differentiated DC from CD14⁺ peripheral monocytes of allergic patients with GM-CSF and IL-4 for 5 days, and pulsed with Der p2 (1 µg/ml) for additional 2 days in the absence (Dp2-DC) or in the presence of LPS (LPS Dp2-DC). As control, we used DC obtained by culturing CD14⁺ peripheral monocytes with GM-CSF and IL-4 for 5 days, and left unstimulated (iDC) or activated with LPS (LPS DC) for additional 2 days. Autologous T cells stimulated with LPS Dp2-DC displayed a proliferative response comparable to that observed with un-pulsed LPS-DC. Conversely, stimulation of T cells with autologous Dp2-DC resulted in a Der p2-specific T-cell response (Fig. 8). These results indicate that Dp2-DC are good Der p2-specific T cell stimulators, and therefore were used in all subsequent experiments.

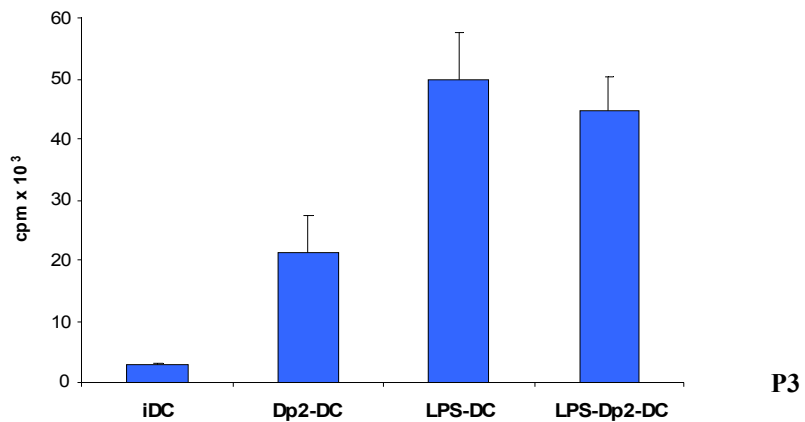


Fig. 8. Dp2-DC are good antigen specific stimulator. T cells from allergic patients were co-cultured with autologous Dp2-DC or Dp2-DC maturated with LPS (1 µg/ml) (LPS Dp2-DC). In parallel, T cells were stimulated with un-pulsed DC left inactivated (iDC) or activated with LPS (1µg/ml) (LPS DC). Proliferative responses were evaluated after 5 days of culture by thymidine incorporation for an additional 16 hrs. Results from one representative patient out of three patients analyzed are shown.

We then analyzed the phenotype of Dp2-DC generated from CD14⁺ peripheral monocytes of 8 allergic patients. We observed an increase in the expression of CD86 in terms of percentage and Mean Fluorescence Intensity (MFI) ($42.7 \pm 14.2\%$, MFI 35.5 ± 17.9 and $33,3 \pm 13,7\%$; MFI 27.4 ± 13.4 in Dp2-DC and iDC, respectively, $n=8$ $p=NS$) and no differences in the expression of CD80 ($47,3 \pm 19,9\%$; MFI $18,9 \pm 7,5$ and $43,7 \pm 18,7\%$; MFI 17.3 ± 7.1 in Dp2-DC and iDC, respectively $n=8$) (Fig. 9). Together these results indicate that pulsing of DC with Der p2 promotes a partial maturation of DC

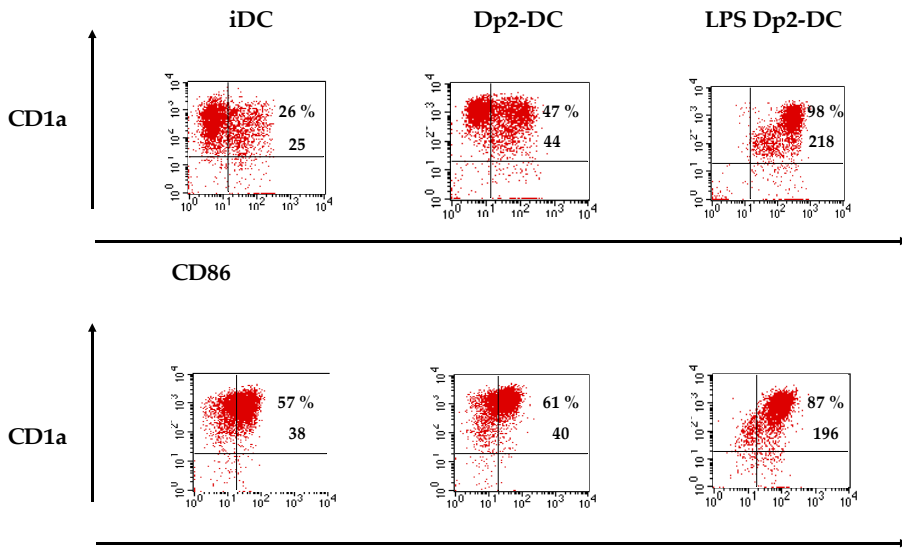


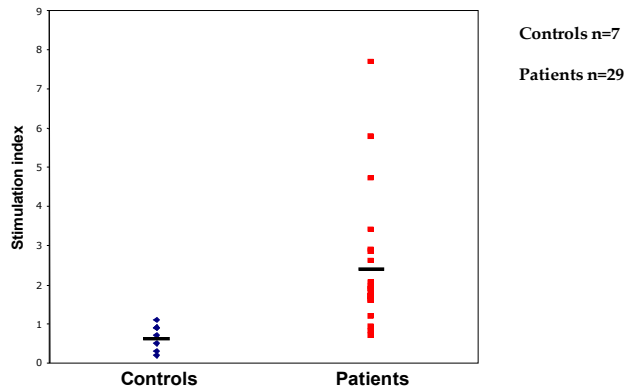
Fig. 9

Fig. 9. Dp2-DC phenotype. Monocyte-derived DC were differentiated from CD14⁺ cells cultured with IL-4 and GM-CSF for 5 days, and pulsed (Dp2-DC) or not (iDC) for additional 2 days with Der p2 (1 μg/ml). Expression level of CD86 and CD80 were evaluated by FACS analysis. A representative patient out of 8 patients analyzed is presented.

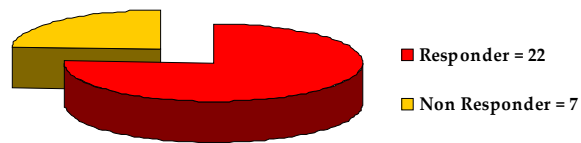
1b. Dp2-DC induce Derp2-specific Th2 Response.

To induce Derp2-specific T-cell response in HDM patients, we stimulated T cells obtained from PBMC after CD14⁺ depletion with autologous Dp2-DC. As controls we used T cells from 7 aged-matched healthy children. Responder was defined any patient displaying a stimulation index (SI) \geq of the mean SI+2SD obtained by stimulating T cells from healthy controls with autologous Dp2-DC. In our study, the cut off is set at 1.3 (Fig. 10a). Among the allergic children analyzed, Der p2-specific T-cell proliferation was observed in 22 out of 29 patients. Therefore, a Der p2 response was observed in 75.8% of the children allergic to HDM studied (Fig. 10b) consistent with previous reports (**Taketomi EA et al., 2006; Tsai JJ et al., 2000**).

To characterize the Der p2-specific T-cell response elicited by Dp2-DC, we analyzed the phenotype and the cytokine production profile of T cells from responder patients. Analyses of the T cell phenotype revealed that stimulation with autologous Dp2-DC promoted the up-regulation of activation markers CD25 (inducible α -chain IL-2 Receptor) and intracellular CTLA-4. The mean of T cells expressing CD25 and CTLA-4 after stimulation with Dp2-DC [T(Dp2-DC)] was 41.1 \pm 11.1% and 25.3 \pm 9.9%, respectively, compared to T cells stimulated with iDC [T(iDC)] (24.4 \pm 7.2% and 18.9 \pm 8.6%, respectively). (Fig. 11). These effects were observed in CD4⁺ but not CD8⁺ T cells (data not shown).

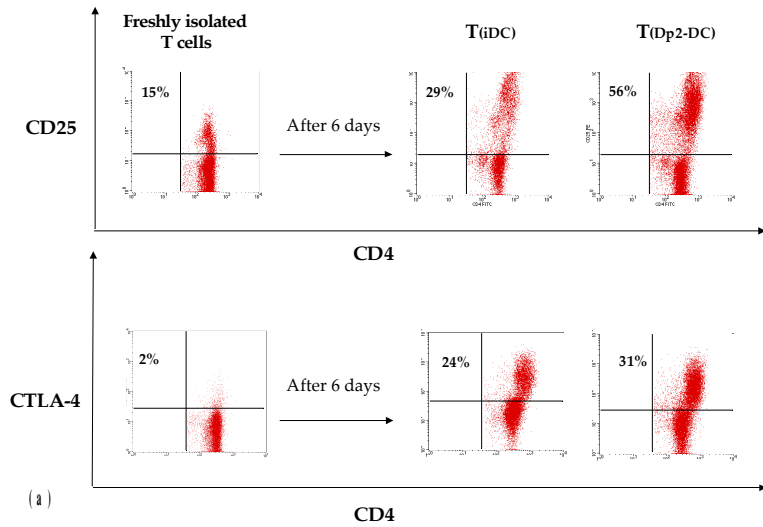


(a)



(b)

Fig. 10.Dp2-DC induce Der p2-specific T cell proliferation in allergic patients. a) T cells from HDM patients and healthy controls were co-cultured with autologous Dp2-DC. Proliferative responses were evaluated after 5 days of culture by thymidine incorporation for an additional 16 hrs. Results are expressed as Stimulation Index (SI) and the horizontal bars represent the means. b) Prevalence of Der p2-responders and non responders among the total population of allergic children analyzed.



P16

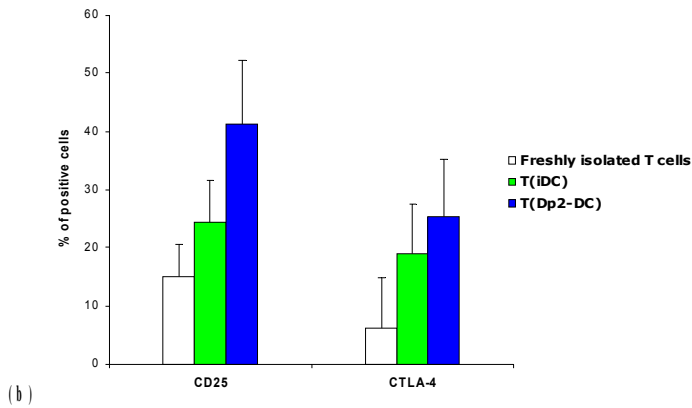


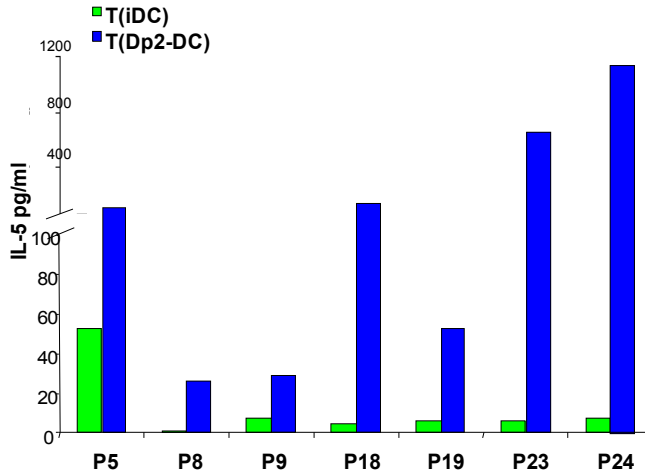
Fig. 11 .T cells stimulated with Dp2-DC display an activated phenotype. Freshly isolated T cells from allergic patients were co-cultured with autologous immature DC (iDC) or Der p2-pulsed DC (Dp2-DC).

a) The expression of CD25 and intracellular CTLA-4 CD4⁺ freshly isolated T cells and CD4⁺ T cells after 6 days of culture was evaluated by flow cytometry. A representative patient out of 6 patients analyzed is shown. b) The average \pm SD of CD25⁺ and intracellular CTLA-4⁺ CD4⁺ T cells detected in 6 patients is presented.

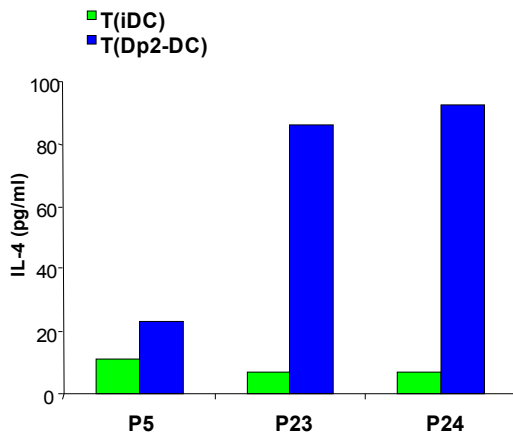
We then analyzed the cytokine production profile of T cells after 6 days of co-culture with Dp2-DC. Detectable amounts of IL-5 were observed in 7 out of 13 patients (53.8%), with a mean of 336.2 ± 181.2 pg/ml (Fig. 12a). In addition, among these 7 patients, 3 patients (42.8%) showed also detectable levels of IL-4 after Der p2 stimulation, with a mean of 67.4 ± 27.3 pg/ml (Fig. 12b). These data indicate that Der p2-specific T-cell response is Th2 like. This result was confirmed by the increase of IL-5/IFN- γ ratio after Der p2-stimulation (Fig. 12c).

In conclusion, we demonstrated that stimulation of T cells from allergic children to HDM with autologous Dp2-DC promotes the induction of Der p2-specific Th2 cell response.

(a)



(b)



(c)

<i>Patients</i>	IL-5/IFN-γ	
	T(iDC)	T(Dp2-DC)
P5	0,06	0,25
P8	0,00	0,04
P9	0,04	0,07
P18	0,04	5,03
P19	0,05	0,10
P23	0,01	2,11
P24	0,07	9,50

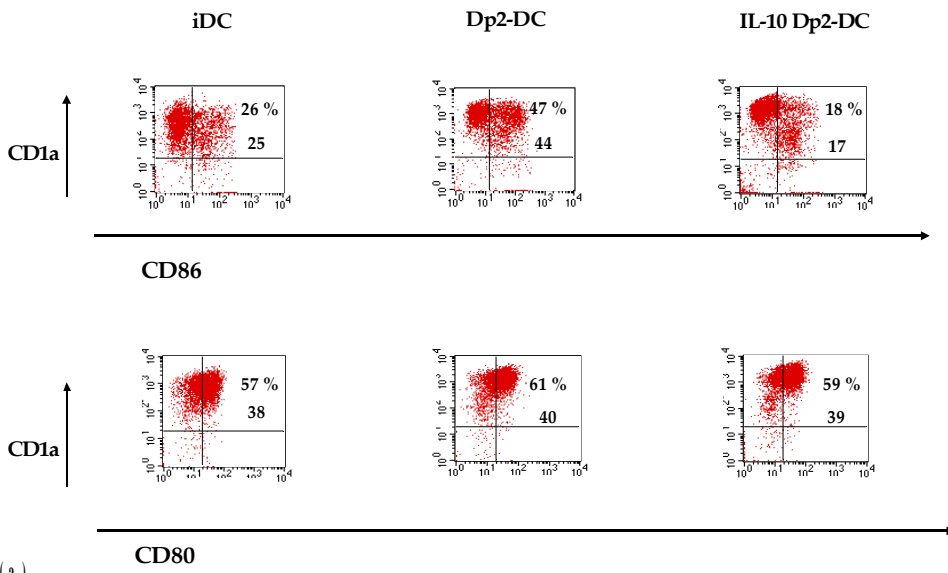
Fig. 12. Dp2-specific T cells are Th2. T cells from allergic patients were co-cultured with autologous iDC or Dp2-DC. Culture supernatants were collected after 6 days of culture and IL-5 (a) and IL-4 (b) were determined by cytometric bead array-kit (CBA). c) The IL-5/IFN- γ ratio was calculated by dividing the amount (express in pg/ml) of the IL-5 by the amount of IFN- γ (also expressed in pg/ml).

2. MODULATION OF Der p2-SPECIFIC T-CELL RESPONSE BY IL-10 TREATED-DC

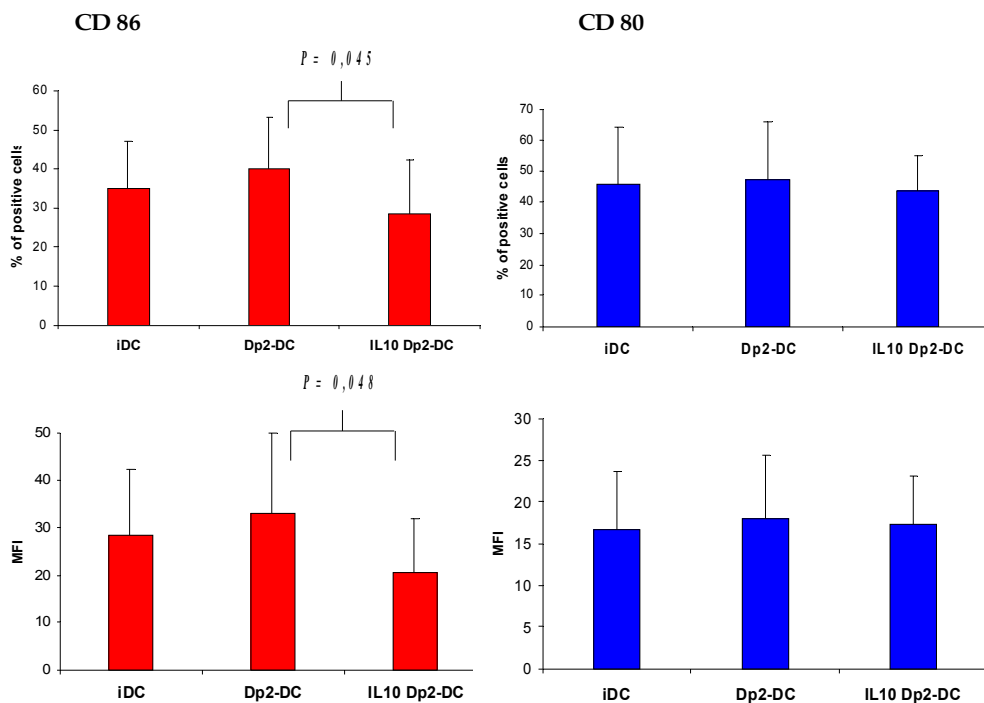
2a. IL-10 modulates Der p2-pulsed DC

IL-10 is an immunomodulatory cytokine that has key role in controlling inflammatory processes, tolerance induction and maintenance of immunological homeostasis. It exerts suppressive effects on different populations of lymphocytes, it downmodulates cytokine production by T cells and monocytes/macrophages, and proliferation of antigen-specific CD4⁺ T effector cells, by inhibiting the antigen presenting capacity of APC (**Moore KW et al., 2001**). Moreover, IL-10 induces antigen-specific T-cell anergy (**Groux H et al., 1996**) and, importantly, induces Tr1 cells both *in vitro* (**Groux H et al., 1997**) and *in vivo* (**Battaglia M et al., 2006**). Furthermore, IL-10 plays an important role in controlling allergic inflammation. IL-10 inhibits the proliferative T-cell response in PBMC to various allergens (**Akdis CA et al., 2000**). A suppressive role for IL-10 has also been demonstrated in both normal responses to allergens and in allergic individuals after SIT (**Blaser K et al., 2004 Muller UR et al., 1998; Akdis CA et al., 1998 Pierkes M et al., 1999**). Moreover, these studies indicate that IL-10-secreting Treg cells are involved in the suppression of allergic Th2 cell responses in humans.

These observations strongly suggested us to investigate the ability of IL-10 to modulate Der p2-specific responses through its effect on DC. Then, we first analyzed the role of IL-10 in modulating Dp2-DC. DC were differentiated from peripheral CD14⁺ monocytes of allergic patients with GM-CSF and IL-4 for 5 days, and then pulsed with Der p2 (1µg/ml) for additional two days in the absence (Dp2-DC) or in the presence of exogenous IL-10 (40ng/ml) (IL10 Dp2-DC). Phenotypic analysis of the resulting DC demonstrated that IL-10 treatment significantly downregulated the CD86 expression, both in terms of cell percentage and MFI in IL-10 Dp2-DC (28.6±13.5%; MFI 32.9±17.2) compared to that observed in Dp2-DC (42.7±14.2%; MFI 28.4±13.8, n=12 *p*=0.045). Conversely, the CD80 expression was slightly affected by the IL-10 treatment (43.8±11.2%; MFI 17.3±5.8 vs. 47.3±18.8%; MFI 16.6±7.1 in IL10 Dp2-DC and Dp2-DC respectively, n=12 *p*=NS). (Fig. 13).



(a)



(b)

Fig. 13. IL-10 modulates Dp2-DC phenotype and cytokines production. Monocyte-derived DC from allergic patients were differentiated from CD14+ cells cultured with IL-4 and GM-CSF for 5 days and then pulsed with Der p2 (1 µg/ml) in the absence (Dp2-DC) or in the presence of exogenous IL-10 (40ng/ml)(IL10 Dp2-DC) for additional 2 days. As control, unpulsed DC (iDC) were used.

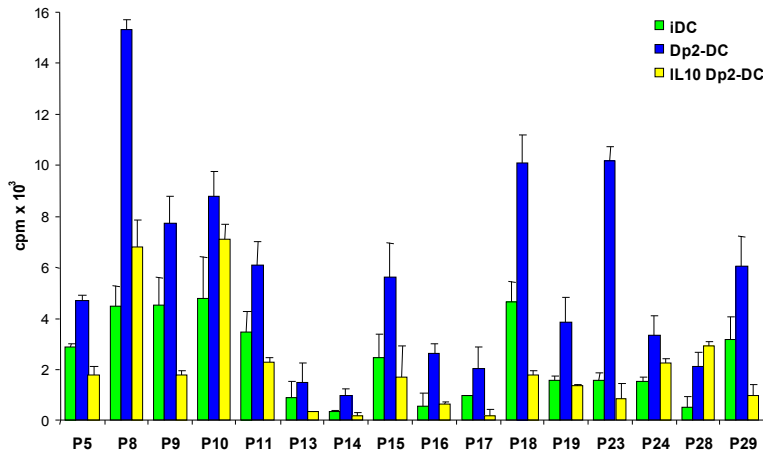
a) Expression level of CD86 and CD80 were evaluated by FACS analysis. A representative patient out of 12 patients analyzed is shown. b) The average of positive cells percentage±SD (upper panel) and the average MFI±SD (lower panel) of 12 patients are presented. $p < 0.05$ when IL10 Dp2-DC were compared to Dp2-DC.

2b. IL-10-treated Dp2-DC inhibit Der p2-specific T-Cell Response

We next determine the ability of IL-10-treated Dp2-DC to modulate Der p2-specific T-cell response. T cells from 16 allergic patients were stimulated with both Dp2-DC and IL10 Dp2-DC. In 15 out of 16 allergic patients, IL-10-treated Dp2-DC induced a significantly lower Der p2-specific T-cell proliferation compared to Dp2-DC

(SI: 0.9 ± 0.5 vs. 2.3 ± 0.8 in T cells stimulated with IL-10 Dp2-DC and with Dp2-DC respectively, $n=15$ $p=0.00007$) with an average inhibition of $69.2 \pm 20.3\%$ (Fig. 14).

(a)



(b)

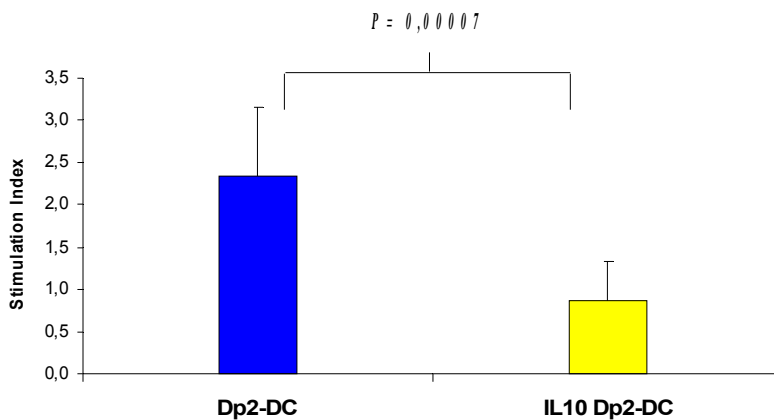


Fig. 14. IL-10-treated Dp2-DC inhibit Der p2-specific T-cell proliferation. Monocyte-derived DC were differentiated from CD14⁺ cells in IL-4 and GM-CSF for 5 days and then pulsed (Dp2-DC) or not (iDC) with Der p2 (1 μ g/ml) for additional 2 days. Alternatively, DC were pulsed with Der p2 (1 μ g/ml) in the presence of IL-10 (40ng/ml) for additional 2 days (IL10 Dp2-DC). T cells from allergic patients were co-cultured with autologous iDC, Dp2-DC, or IL10 Dp2-DC. Proliferative responses were evaluated after 5 days of culture by thymidine incorporation for an additional 16 hrs.

a) Mean \pm SD of triplicates of individual patient are presented. b) The average proliferation \pm SD of 15 patients is presented. Results are expressed in SI. $p < 0.05$ when T cells were stimulated with IL10 Dp2-DC compared to T cells stimulated with Dp2-DC.

IL10 Dp2-DC were unable to inhibit Derp2-specific T-cell proliferation in only one patient (P28). Interestingly, this result was consistent with the lack of downregulation of CD86 expression on DC mediated by IL-10 treatment (Fig. 15a, 15b). Based on this observation, we investigated whether a correlation between reduction of T-cell proliferation induced by IL10 Dp2-DC and downregulation of CD86 expression on IL-10 Dp2-DC was present in the cohort of allergic children analyzed. No correlation between the two variables was observed (Fig. 15c), indicating that IL-10 does not decrease the stimulatory capacity of Dp2-DC through modulation of costimulatory molecules.

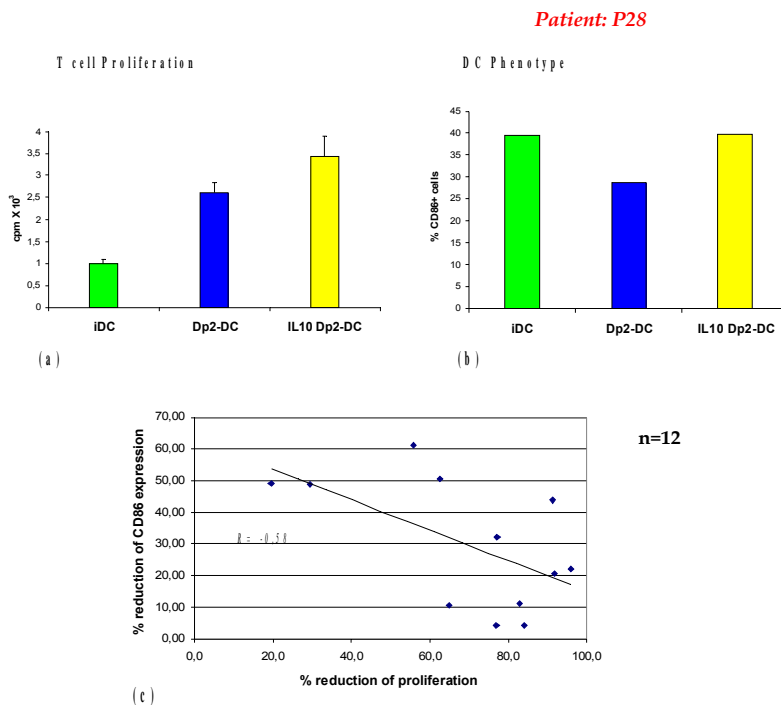


Fig. 15. In patient P28 IL-10-treated Dp2-DC did not inhibit Der p2-specific T-cell proliferation. Monocyte-derived DC were differentiated from CD14+ cells cultured with IL-4 and GM-CSF for 5 days, and then pulsed (Dp2-DC) or not (iDC) with Der p2 (1 µg/ml) for additional 2 days. Alternatively, DC were pulsed with Der p2 (1 µg/ml) in the presence of IL-10 (40ng/ml) for additional 2 days (IL10 Dp2-DC). T cells from P28 were co-cultured with autologous iDC, Dp2-DC, or IL10 Dp2-DC. a) Proliferative responses were evaluated after 5 days of culture by thymidine incorporation for an additional 16 hrs. Mean±SD of triplicates are presented. b) Percentage of CD86+ DC was evaluated by FACS analysis. In c), the percent reduction in proliferation of T cells stimulated with IL-10 treated Dp2-DC of 12 patients and the respective percent reduction of CD86 expression on DC surface were plotted.

We next determined whether the inhibition of T-cell proliferation depends on the prevention of T-cell activation. Analysis of the expression of CD25 and of intracellular CTLA-4 on T cell stimulated with IL10 Dp2-DC was performed. Results in Figure 16 showed that the levels of CD25 and of intracellular CTLA-4 expression in CD4⁺ T cells remained unchanged (Fig. 16), indicating that IL-10 Dp2-DC did not prevent T-cell activation.

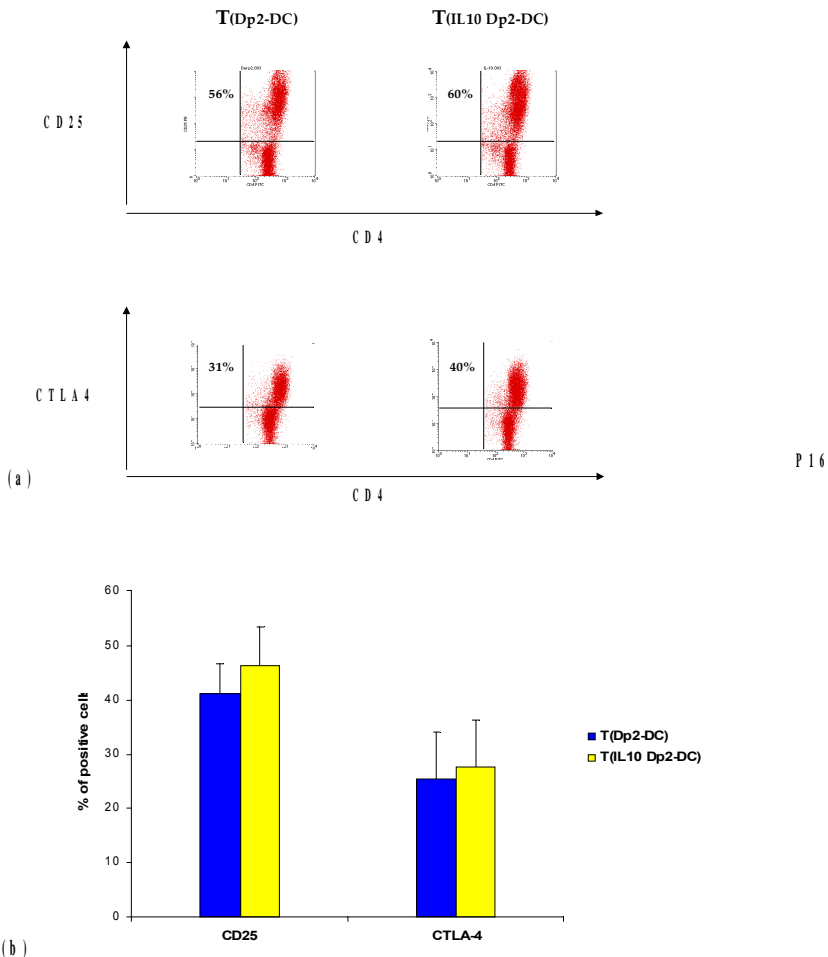
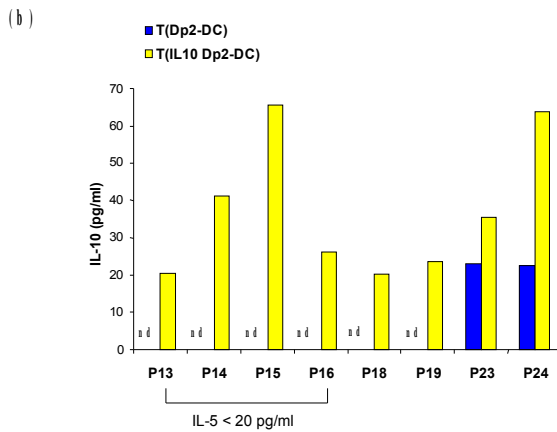
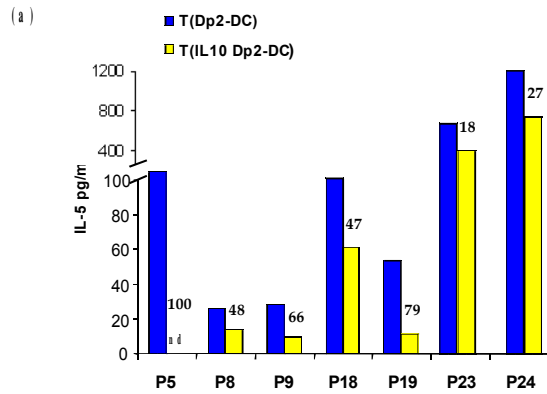


Fig. 16. IL10 Dp2-DC do not modify T cell activation. Freshly isolated T cells from allergic patients were co-cultured with autologous Dp2-DC and IL10-treated Dp2-DC.

a) The expression of CD25⁺CD4⁺ and intracellular CTLA-4⁺CD4⁺ T cells after 6 days of culture was evaluated by flow cytometry. A representative patient out of 6 patients analyzed is shown. b) The average±SD percentages of CD25⁺CD4⁺ and intracellular CTLA-4⁺ CD4⁺ T cells detected in 6 patients are presented.

We then investigated the effect of IL-10 Dp2-DC stimulation on T cell cytokine production. We previously demonstrated that 7 patients displayed a Th2 cytokine profile after Der p2 stimulation using Dp2-DC (Fig. 12). In the same patients, we observed that stimulation of T cells with IL-10-treated Dp2-DC resulted in a significant reduction in IL-5 production (222.1 ± 144.4 pg/ml vs. 336.2 ± 181.2 pg/ml in T cells stimulated with IL10 Dp2-DC and Dp2-DC respectively, $n=7$ $p=0.029$) with an average inhibition of $55.1 \pm 28.8\%$. This data was confirmed by the reduction of IL-5/IFN- γ ratio (1.1 ± 0.4 vs. 2.5 ± 1.4 in T cells activated with IL10 Dp2-DC and Dp2-DC respectively, $n=7$). Importantly, although the IL-5/IFN- γ ratio was reduced, the IFN- γ production by T cells stimulated with IL-10 treated Dp2-DC was not increased (243.5 ± 93.5 pg/ml vs. 416 ± 118.4 pg/ml by T cells primed with IL10 Dp2-DC and Dp2-DC respectively, $n=7$), indicating that the IL-5/IFN- γ ratio reduction was due to the inhibition of IL-5 secretion. These results indicate that IL-10 treated Dp2-DC promote a preferential suppression of Th2 cytokines without skewing to a Th1 profile. Interestingly, an increase of IL-10 production by T cells stimulated with IL-10-treated Dp2-DC was observed in 8 patients (37.0 ± 7.0 pg/ml vs. 5.7 ± 4.0 pg/ml, $n=8$ $p=0.001$). Of note, in 4 of these patients, a reduction of IL-5 production was also observed (Fig. 17), suggesting a putative role of IL-10 Dp2-DC in promoting IL-10 production by T cells.

Taken together, these results indicate that IL-10-treated DC modulate allergen-specific Th2 responses and might promote the induction of IL-10-producing T cells.



(c)

Patients	IL-5/IFN- γ		
	T(iDC)	T(Dp2-DC)	T(IL10 Dp2-DC)
P5	0,06	0,25	0,00
P8	0,00	0,04	0,02
P9	0,04	0,07	0,04
P18	0,04	5,03	0,51
P19	0,05	0,10	0,09
P23	0,01	2,11	1,90
P24	0,07	9,50	4,84

Fig. 17. IL-10 treated Dp2-DC modulate the Th2 cytokine profile. T cells from allergic patients were co-cultured with autologous Dp2-DC and IL10 Dp2-DC. Culture supernatants were collected after 6 days of culture and IL-5 a) and IL-10 b) were determined by cytometric bead array-kit (CBA). c) The IL-5/IFN- γ ratio was calculated by dividing the amount (express in pg/ml) of the IL-5 by the amount of IFN- γ (also expressed in pg/ml).

3. ROLE OF IL-10 ON DENDRITIC CELLS: EFFECT ON DIFFERENTIATION AND EFFECTOR FUNCTION.

The tolerogenic effect of IL-10-modulated DC has been previously demonstrated. Indeed, DC matured in presence of IL-10 induced the differentiation of anergic CD4⁺ and CD8⁺ T cells (**Steinbrink K et al., 1999**), with the ability to suppress T cell responses via cell-cell contact mechanism (**Steinbrink K et al., 2002**). The ability of IL-10-treated Dp2-DC to modulate allergic-specific T-cell responses prompted us to determine whether they would be able to generate anergic allergen-specific T cells with suppressor activity. To this end, we designed a protocol to differentiate antigen-specific T-cell lines with regulatory activity through repetitive Der p2-specific stimulation with IL-10-treated Dp2-DC. We aimed to compare T cell lines generated with Dp2-DC [T (Dp2-DC)] with T cell lines generated with IL-10-treated Dp2-DC [T(IL10 Dp2-DC)] obtained upon 3 rounds of stimulation with freshly differentiated Dp2-DC (Fig. 18). Unfortunately, we collected a single blood sample from each of our patients and we were not able to complete our experimental plan. Indeed, the differentiation of freshly DC was impaired due to the low number of CD14⁺ cells routinely obtained from our pediatric patients.

Therefore, we sought for an alternative protocol which would provide us a sufficient number of DC and T cells to be analyzed. We therefore set up a new protocol based on the use of DC differentiated in the presence of IL-10 (DC10) (**Gregori S et al., 2007 submitted**). DC10 are potent inducers of Tr1 cells, since a single round of stimulation is sufficient to promote Tr1 differentiation (**Gregori S et al., 2007 submitted**). Thus, this protocol has the advantage to shorten the experimental procedure utilizing fewer cells (Fig. 19).

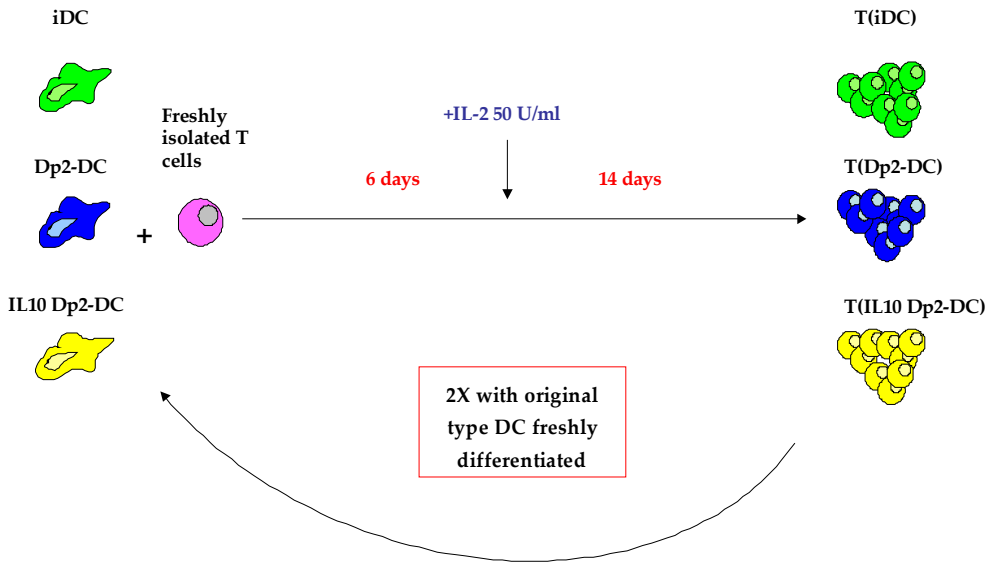


Fig. 18. Protocol n.1 (see methods)

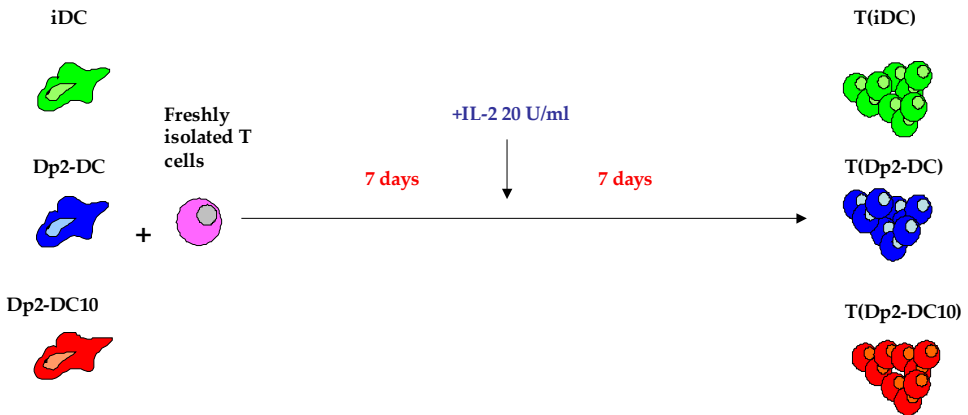


Fig. 19. Protocol n.2 (see methods)

To validate the possibility to use DC10 to differentiate Der p2-specific T cell lines with regulatory function, we first compared phenotype and effector function of DC differentiated in the presence of IL-10 (DC10) and DC treated with IL-10 during the last 2 days of differentiation (IL10-DC) (Fig. 20).

DC Differentiation

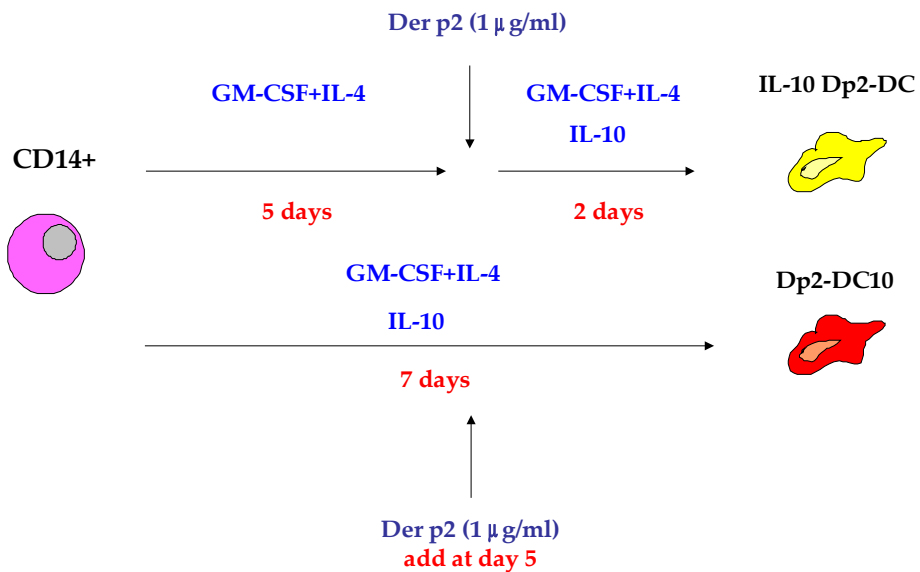


Fig. 20. DC Differentiation. DC were differentiated from CD14+ cells culturing with GM-CSF and IL-4 for 5 days. Der p2 (1µg/ml) was added in combination with IL-10 (40ng/ml) for 48 hours to obtain IL10 Dp2-DC (upper panel). Alternatively, DC were differentiated from CD14+ cells culturing with GM-CSF and IL-4 for 5 days in the presence of IL-10 (10ng/ml) and pulsed with Der p2 (1µg/ml) for additional 48 hours to obtain Dp2-DC10 (lower panel).

3a. Comparison of phenotype and allogenic stimulatory capacity of DC10 and IL10-DC

DC10 were differentiated from CD14⁺ cells cultured with GM-CSF and IL-4 in the presence of exogenous IL-10 for 7 days. Alternatively, IL-10 DC were differentiated from CD14⁺ cells cultured with GM-CSF and IL-4 for 5 days, and then treated with IL-10 for additional 2 days. As control, we used iDC and LPS DC. The resulting DC were compared both in terms of phenotype and allogenic stimulatory capacity. As previously demonstrated (**Gregori S et al., 2007 submitted**) DC10 were CD14⁺, CD1a⁻ and expressed HLA-DR, CD80, CD83, and CD86, whereas IL10 DC were CD14⁻, CD1a⁺ and expressed low levels of HLA-DR, CD80, CD83, and CD86 (Fig. 21). This result indicates that addition of exogenous IL-10 at the beginning of the DC differentiation or during the last two days of culture resulted in the generation of DC with different phenotype.

DC10 and IL-10 DC were then tested for their ability to promote allogenic T cell response. Results in Fig. 22 show that both DC10 and IL-10 DC displayed lower stimulatory capacity compared to iDC. Therefore, despite the different expression of costimulatory molecules between DC10 and IL-10 DC, both cells were comparable in inhibiting allogenic T cell response.

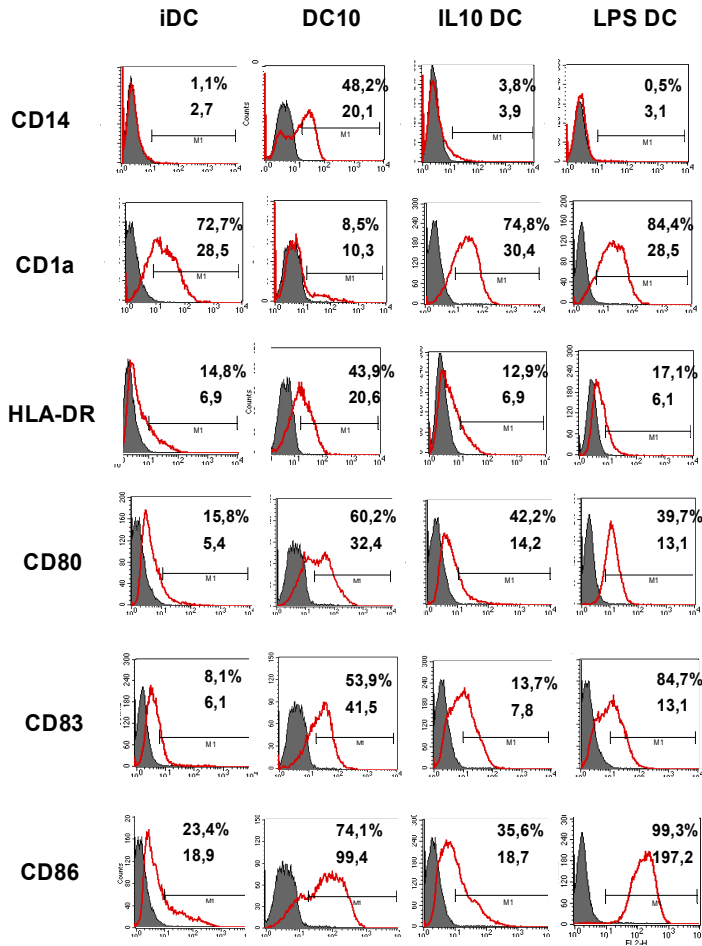


Fig. 21. DC10 and IL10 DC phenotype. Monocyte-derived DC were differentiated from CD14⁺ cells cultured with IL-4, GM-CSF, and IL-10 for 7 days (10ng/ml every 2 days) (DC10) or with GM-CSF and IL-4 for 5 days and then IL-10 (40ng/ml) was added for additional 2 days. As control, unpulsed DC (iDC) and mature DC (LPS DC) were used. Expression level of CD14, CD1a, HLA DR, CD80, CD83, and CD86 were evaluated by FACS analysis. The cell percentage and the MFI set according to the isotype-matched control are shown. A representative donor out of 2 donors analyzed is presented.

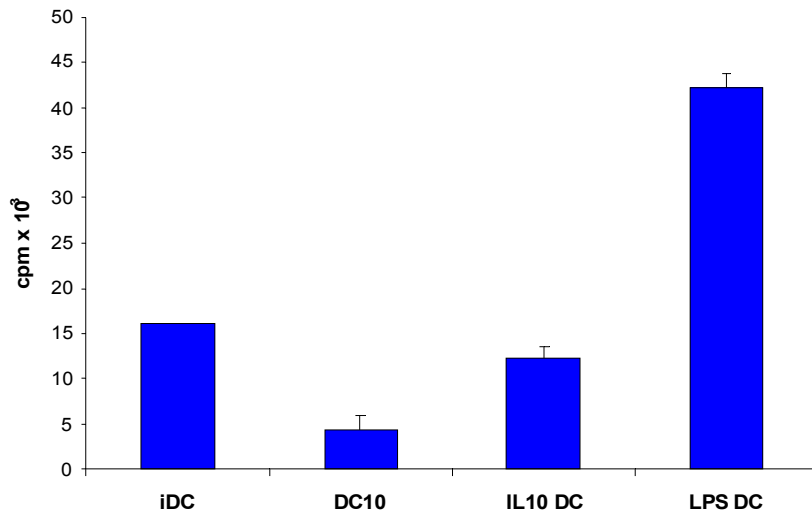


Fig. 22. DC10 and IL10-DC display a low stimulatory capacity. Naïve CD4⁺ T cells were cultured with allogenic iDC, DC10, IL10-DC and LPS-DC. After 4 days of culture, proliferative responses were evaluated by addition of thymidine for an additional 16 hrs. Mean of triplicates \pm SD are presented. A representative donor out of 2 independent donors tested is presented.

4. MODULATION OF T CELL RESPONSE BY DC DIFFERENTIATED IN PRESENCE OF IL-10

4a. Dp2-DC10 inhibit Der p2-specific T-cell responses

We determined the ability of Dp2-DC10 to inhibit Der p2-specific T-cell response in 12 allergic patients. In 10 out of 12 patients, who responded to Der p2, Dp2-DC10 induced a significantly lower allergen-specific T-cell proliferation (SI: 0.3 ± 0.2 vs. 4.0 ± 1.7 in T cells stimulated with Dp2-DC10 and Dp2-DC respectively, $n=10$ $p=0.00011$) (Fig. 23). This result indicates that Dp2-DC10 are comparable to IL10 Dp2-DC in inhibiting Der p2-specific T-cell proliferation.

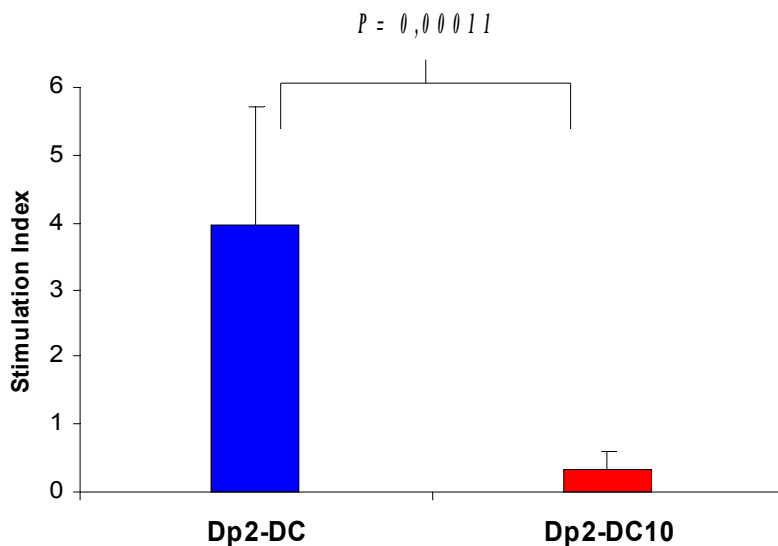
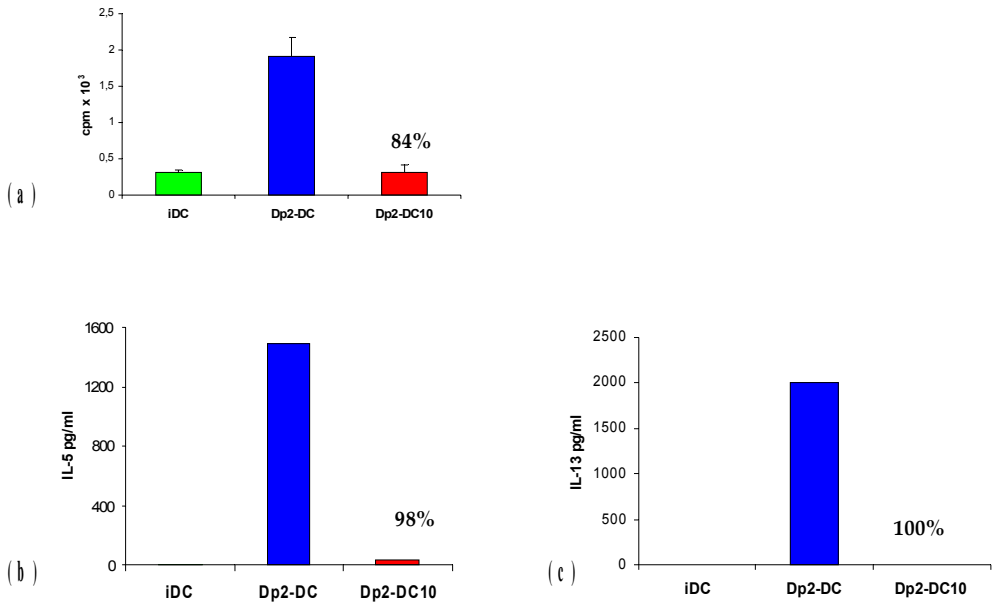


Fig. 23. Dp2-DC10 inhibit Der p2-specific T-cell proliferation. Monocyte-derived DC were differentiated from CD14+ cells and cultured with IL-4 and GM-CSF in presence of IL-10 for 5 days, and then pulsed with Der p2 (1 μ g/ml) (Dp2-DC) for additional 2 days. T cells from allergic patients were co-cultured with autologous Dp2-DC or Dp2-DC10. Proliferative responses were evaluated after 5 days of culture by thymidine incorporation for an additional 16 hrs. The average proliferation \pm SD of 10 patients is presented. Results are expressed in SI. $P < 0.05$ when T cells were stimulated with Dp2-DC10 compared to T cells stimulated with Dp2-DC.

Analyses of the cytokine production in 10 responder allergic patients revealed in 8 out of 10 (80%) detectable amount of Der p2-specific IL-5 (342.5 ± 174.1 pg/ml vs. 5.1 ± 1.1 pg/ml in T cell stimulated with Dp2-DC and with iDC, respectively, $n=8$, $p=0.037$). Interestingly, in 7 out of 10 patients (70%) Der p2-specific IL-5 was strongly reduced when T cells were cultured in the presence of Dp2-DC10 (60.4 ± 32.5 pg/ml). Moreover, the ratio IL-5/IFN- γ was markedly reduced (0.7 ± 0.2 vs. 87.9 ± 53.1 in T cell stimulated with Dp2-DC10 and with Dp2-DC, respectively, $n=8$). This observation was supported by the data on IL-13 production which was found substantially defective in the presence of Dp2-DC10 (15.7 ± 10.2 pg/ml vs. 369.8 ± 255.8 pg/ml in Dp2-DC10 and Dp2-DC, respectively, $n=8$). Although the average production of IL-5 and IL-13 was markedly reduced (68% and 80%, respectively), the decrease did not reach statistical significance due to the high variability of IL-5 and IL-13 secretion among patients (IL-5 ranged between 12 and 1488 pg/ml, IL-13 ranged between 8 and 2000 pg/ml) (Fig. 24).



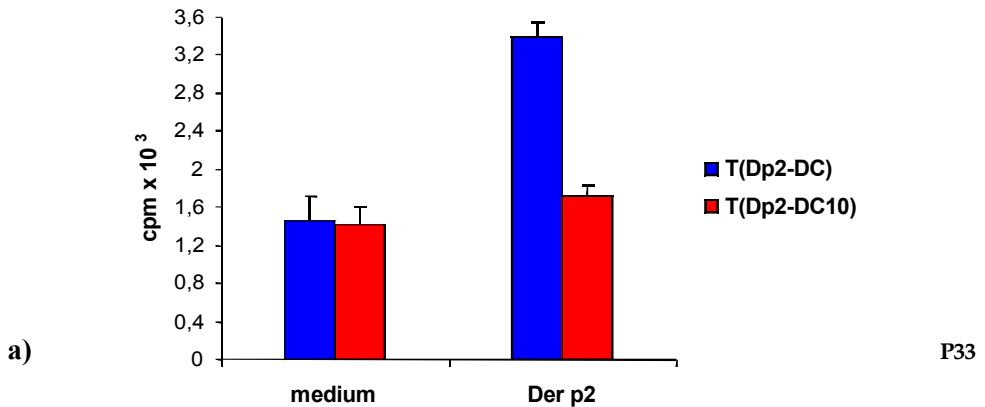
P36

Fig. 24. Dp2-DC10 inhibit Ag-specific Th2 cell response. Monocyte-derived DC were differentiated from CD14⁺ cells and cultured with IL-4 and GM-CSF in presence of IL-10 for 5 days, and then pulsed with Der p2 (1 μ g/ml) (Dp2-DC) for additional 2 days. T cells from allergic patients were co-cultured with autologous Dp2-DC or Dp2-DC10.

a) Proliferative responses were evaluated after 5 days of culture by thymidine incorporation for an additional 16 hrs. Mean of triplicates \pm SD are presented. Culture supernatants were collected after 5 days of culture and IL-5 (b) and IL-13 (c) were determined by Bioplex Protein Array system. A representative patient out of 10 patients analyzed is shown.

4b. Dp2-DC10 induce anergic Tcells

To test the ability of Dp2-DC10 to promote Der p2-specific T cell anergy in allergic patients, we used a standardized protocol (Gregori S et al., 2007 *submitted*) (see Material and Methods) in which T cells were stimulated for 14 days with iDC, Dp2-DC, and Dp2-DC10. After stimulation, T cell lines were tested for their ability to proliferate in response to Der p2. Interestingly, T cells generated with Dp2-DC10 were hypo-responsive to reactivation with Der p2 in 4 out of 5 patients (80%) tested. An average reduction of $60.8 \pm 15.9\%$ in Ag-induced proliferation of T cell lines generated with Dp2-DC10 compared to T cell lines generated with Dp2-DC was observed ($p=0.05$). (Fig. 25).



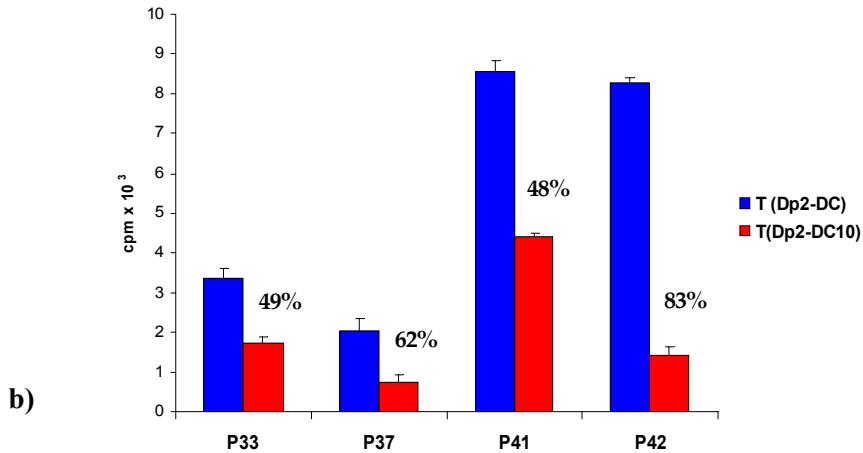


Fig. 25. Dp2-DC10 induce anergic T cells. Monocyte-derived DC were differentiated from CD14⁺ cells and cultured with IL-4 and GM-CSF in presence of IL-10 for 5 days pulsed with Der p2 (1 µg/ml) (Dp2-DC) for additional 2 days. T cells from allergic patients were co-cultured with autologous Dp2-DC or Dp2-DC10 for 14 days. T cells were collected, and cultured with autologous CD14⁺ in the absence (medium) or in the presence of Der p2 (1 µg/ml). Proliferative responses were evaluated after 2 days of culture by thymidine incorporation for an additional 16 hrs. Mean of triplicates±SD are presented.

a) A representative patient out of 4 patients analyzed is presented. Control proliferation after co-culture of T(Dp2-DC) and T(Dp2-DC10) cell lines with unstimulated CD14⁺ are also shown. b) Proliferative response of 4 patients in which anergy was induced is shown.

T cell lines generated with Dp2-DC10 showed a lower production of IL-5 (48.3±317.8 pg/ml) compared to T cell lines generated with Dp2-DC (297.1±182.3 pg/ml). In addition, inhibition of IL-13 (24.2±9.7 vs. 351.1±212.6 pg/ml in T(Dp2-DC10) and T(Dp2-DC), respectively, n=4), and reduction of IL-5/IFN- γ ratio (1.9±1.3 vs. 7.5±3.5 in T(Dp2-DC10) and T(Dp2-DC), respectively, n=4) were observed (Fig 26 a-c).

a)

IL-5	T(Dp2-DC) pg/ml	T(Dp2-DC10) pg/ml
P33	44	50
P37	120	72
P41	748	67,2
P42	276	4

b)

IL-13	T(Dp2-DC) pg/ml	T(Dp2-DC10) pg/ml
P33	62,8	26,4
P37	100	35,2
P41	864	35,2
P42	377,6	0

c)

IL5/IFN- γ	T(Dp2-DC)	T(Dp2-DC10)
P33	4,8	4,5
P37	3,4	0,8
P41	14,4	0,6
P42	0	0

Fig. 26. Dp2-DC10 induce anergic T cells. Monocyte-derived DC were differentiated from CD14+ cells and cultured with IL-4 and GM-CSF in presence of IL-10 for 5 days and then pulsed with Der p2 (1 μ g/ml) (Dp2-DC) for additional 2 days. T cells from allergic patients were co-cultured with autologous Dp2-DC or Dp2-DC10 for 14 days. T cells were collected, and cultured with autologous CD14+ in presence of Der p2 (1 μ g/ml). Culture supernatants were collected after 2 days of culture and IL-5 (a) and IL-13 (b) were determined by Bioplex Protein Array system. c) The IL-5/IFN- γ ratio was calculated by dividing the amount (express in pg/ml) of the IL-5 by the amount of IFN- γ (also expressed in pg/ml).

These results indicate that Dp2-DC10 are able to promote antigen-specific T cell anergy associated with a reduction in the Th2 cytokine production.

In parallel we tested the ability of Dp2- DC10 in promoting allergen-specific T cells in 3 healthy aged-matched controls. In none of them we observed any response to Der p2, and induction of anergic T cells (data not shown).

DISCUSSION

In the present study, we characterized the immune response to *Der p2*, the most important allergens from *Dt.Pt.*, in patients allergic to HDM. We demonstrated that DC pulsed with *Der p2* (Dp2-DC) induced *Der p2*-specific Th2 response in HDM-allergic patients, but not in healthy controls. We then investigated the ability of Dp2-DC treated or differentiated in the presence of exogenous IL-10, IL-10 Dp2-DC and Dp2-DC10, respectively, to modulate the immune response to *Der p2*. We demonstrated that both IL-10 Dp2-DC and Dp2-DC10 inhibit *Der p2*-specific T cell proliferation and Th2 cytokine production. Interestingly, we showed that Dp2-DC differentiated in presence of IL-10 are able to promote T-cell anergy associated with a reduction in Th2 cytokine production. In conclusion, we demonstrate that Dp2-DC are useful tools to promote *Der p2*-specific Th2 response in HDM-allergic patients. Moreover, our data strongly support the conclusion that IL-10 enables DC to modulate allergen-specific Th2 response, and drives the differentiation of tolerogenic DC able to promote the induction anergic T cells, which may represent the precursors of regulatory T cells.

We showed that autologous DC pulsed with the allergen *Der p2* efficiently stimulated *Der p2*-specific Th2 cell response. The use of allergen-pulsed-DC to stimulate Th2 cell response in a autologous system has been previously demonstrated. Autologous *Der p1*-pulsed DC have been shown to be able to stimulate T cells from allergic patients and to preferentially increase IL-4 production without modifying IFN- γ production. Conversely, autologous *Der p1*-pulsed DC favoured a type 1 response in healthy donors (**Hammad H et al., 2001**). Our results are in line with previous reports in which stimulation of total PBMC from HDM allergic patients and healthy controls with *Der p2* promoted T cell proliferation and Th2 cytokine induction (**O'Brien RM et al., 1992**; **Bullens DM et al., 2005**). Here we show, for the first time, that *Der p2*-specific T cell response can be elicited by autologous *Der p2*-DC in HDM allergic patients.

We detected a specific proliferative response to Der p2 in HDM patients but not healthy controls. Within the atopic cohort, 75.8% of subjects responded to Der p2, consistent previous reports in which 80-90% of mite-sensitive subjects have IgE antibodies to *Der p1* and *2* allergens (**Taketomi EA et al., 2006; Tsai JJ et al., 2000**). The high frequency of Der p2 reactive patients is probably due to the presence in Der p2 protein of a immunodominant T cell epitope that behaves as a universal epitope recognized by most human MHC class II molecules (**Wu B et al., 2002**).

Dp2-DC promoted Der p2-specific T cell proliferation, and, importantly, in the majority of allergic patients induced a Th2 cytokine profile. In our study we were not able to detect cytokine production after 48 hours of T cell activation (data not shown), in contrast to previous reports in which a pick of Th2 cytokine production was detected after 48-72 hours of T cell-DC co-culture (**Hammad H et al., 2001; Bullens DM et al., 2005; Bullens DM et al., 2004; Noma T et al., 2004**). In our culture system a substantial amount of IL-5 and IL-13 was detected after 5 days of T cell stimulation with autologous Der p2-pulsed DC. Nevertheless, in some patients IL-5 and IL-4 were undetectable. The lack of IL-5 and IL-4 production in some patients, already reported by others (**Nurse B et al., 2000**), could be due to the low frequency of Th2 clones in peripheral blood. It has been indeed reported that a high number of IL-5- and IL-4-producing T cells are accumulated and infiltrate the inflamed bronchial mucosa (**Borgonovo B et al., 1997**). Similarly to previous reports (**Wurtzen PA et al., 1998; Looney RJ et al., 1994; Nurse B et al., 2000**), we were unable to detect IL-4 in the majority of our patients. The discrepancy between IL-5 (detected in 16 out of 23 patients) and IL-4 production (detected in 3 out of 23 patients) could be explained by the fact that pick of IL-4 production occurs before that of IL-5 and rapidly declines, as indicated in time course analyses on primary cultures (**Kimura M et al., 2000; Van Bever HP et al., 1998; Jung T et al., 1996**). Alternatively, the phase of disease, acute vs. chronic, might justify a differential involvement of IL-4 and IL-5, respectively, in the allergic inflammatory response. Likewise, although IL-13 is involved in acute phase of disease, it differs in its effector properties from IL-4. IL-4 plays a prominent role in the initiation of the Th2 response whereas IL-13 in the effector phase of the

response (**Zhou Y et al., 2001**). Moreover, IL-4 plays a pivotal role as growth factor for B and Th2 cells, which might result in the exhaustion of IL-4 in the majority of the cell cultures from allergic patients. Some patients and healthy controls showed a substantial IFN- γ production after Der p2 stimulation (data not shown). This effect has been previously reported (**Bullens DM et al., 2005, Akdis CA et al., 2004**) and an increase of Th1 response has been recently demonstrated in chronic allergic inflammation (**Heaton T et al., 2005**).

We demonstrated that Der p2 induced a partial maturation of DC in the absence of other maturation stimuli. Although it is well established that the addition of LPS or CD40 cross-linked promotes DC maturation and optimizes the antigen presentation capacity of the resulting DC (**Banchereau J et al., 1998**), we showed that addition of LPS to Der p2-pulsed DC did not improve their Der p2-specific presenting capacity. Conversely, Der p2 induced a partial maturation of DC and unable them to promote a Der p2-specific response.

Der p2 induced a selective upregulation of CD86 on Dp2-DC. It has been suggested that the type of costimulatory molecules expressed by DC is essential for determining Th differentiation: CD86 seems to be more important than CD80 in the promoting a Th2 response (**Kuchroo KV et al., 1995**). Indeed, in mice exposed to ovalbumin aerosols, treatment with anti-CD86 but not with anti-CD80 mAbs significantly inhibits allergic reactions such as the production of ovalbumin-specific IgE and IgG1 antibodies, and airway eosinophilia and hyperresponsiveness (**Haczku A et al., 1999**). In atopic dermatitis, CD86 is predominantly expressed on Langerhans cells and seems to play an important role in the pathogenesis of the disease since *Dt.Pt.*-specific T cell proliferation is completely inhibited by the addition of anti-CD86 mAbs (**Ohki O et al., 1997**). Moreover, previous evidence demonstrates that B7 -CD28 costimulation is required for the production of Th2 cytokines such as IL-5 and IL-13 by allergen-specific T cells from asthmatic patients. Interestingly, this effect seems to be dependent on CD86 in the periphery and on CD80 in the inflamed tissues (**Jaffar Z et al., 1999**).

The immunosuppressive properties of IL-10 have been well documented (**reviewed in Moore KW et al., 2001**). The inhibitory influence of IL-10 on antigen

presenting function of DC may be due to several phenotypic and functional alteration, including downregulation of MHC-class II and costimulatory molecules, and inhibition of variety of pro-inflammatory cytokines (**Chang CH et al., 1995; Koch F et al 1996; De Smedt T et al., 1997; Sato K et al., 1999; Willems F et al., 1994; Fiorentino DF et al., 1991; De Waal Malefyt R et al., 1991**). In our study we investigated the ability of IL-10 to modulate monocyte-derived-DC from HDM allergic patients using 2 different protocols. DC were either treated with IL-10 during the last 2 days of differentiation (IL-10 DC), or DC were differentiated in the presence of IL-10 (DC10). Despite their different phenotype, both DC lines were able to modulate T cell response. IL-10-modulated Dp2-DC induce a significantly lower allergen-specific T cell proliferation in all responder patients associated with a marked reduction of both IL-5 and IL-13 production, and a parallel decrease of IL-5/IFN- γ ratio. These results are in line with several studies in which the pivotal role of IL-10 (**Cottrez F et al., 2000; Zuany-Amorim C et al., 2002; Akdis CA et al., 2004; Akbari O et al., 2001; Xystrakis E et al., 2006**) and of IL-10-treated DC (**Bellinghausen I et al., 2001; Bellinghausen I et al., 2006**) in inhibiting Th2 response has been shown. Of note, recently Koya demonstrated that IL-10-treated DC are potent suppressors of the development of AHR, inflammation, and Th2 cytokine production in mice (**Koya T et al., 2007**). Similarly to the DC10, tolerogenic DC used in this study were obtained by culturing bone marrow cells with GM-CSF and IL-10. Interestingly, in our patients, the reduction of IL-5 and IL-13 after IL-10 treatment of DC, occurred without a skewing toward a Th1 cytokine profile. This hold an important clinical implication since it suggests that modification of DC function by IL-10 might attenuate allergic response without increasing the risk of Th1-mediated diseases. Therapeutic strategies for controlling Th2-immune response based on the upregulation of Th1-immunity are not desirable, not only for the potential induction of autoimmunity, but also for the possible risk of exacerbation of allergic immune response. Increasing evidence indicates the role of IFN- γ and CD8⁺IFN- γ ⁺ cells in the pathogenesis of atopy (**Heaton T et al., 2005**).

In agreement with previous reports (**Steinbrink et al., 1997; Steinbrink et al., 2002**), we showed that IL-10 treatment promoted a significant downregulation of CD86 expression on the resulting DC. Nevertheless, we did not observe a correlation between CD86 downregulation and the reduction of allergen-specific T cell proliferation, suggesting that the poor stimulatory capacity of IL 10- treated Dp2-DC is not due to inhibition of costimulation. Moreover, although Dp2-DC10 displayed a mature phenotype, since they expressed CD80 and CD86 at levels similar to those observed in mature DC, they elicited a low T cell proliferation. Taken together these data indicate that the poor stimulatory capacity of IL 10-treated Dp2-DC and of Dp2-DC10 does not reside in the lack of costimulation. It is indeed generally approved that the capacity of DC to inhibit T cell proliferation, to promote T cell anergy and Tr1 cells differentiation is not associated with reduced expression of costimulatory molecules (**Gray CP et al., 2002; Mc Guirk P et al., 2002**). Recent studies support the hypothesis that other mechanisms including inhibitory costimulatory molecules ICOS-L (**Akbari O et al., 2002**), or tolerogenic molecules ILT-3 and ILT-4 (**Manavalan JS et al., 2003**) are involved in the generation of anergic T cells with regulatory activity by tolerogenic DC. Importantly, IL-10 has been reported to up-regulate ILT-3 and ILT-4 expression on DC (**Velten FW et al., 2004; Manavalan JS et al., 2003**) Furthermore, we recently identified a unique population of DC (DC-10), which is present *in vivo* and can be generated *in vitro* in the presence of IL-10, produces high levels of IL-10 and low IL-12, and is characterized by the expression of CD14, CD11c, CD11b, CD83, CD71 and HLA-DR, but not CD1a. DC-10 have a stable phenotype, display low stimulatory capacity, and are powerful Tr1 cell inducers. DC-10 express high levels of tolerogenic molecules ILT-2, ILT-3, ILT-4, and HLA-G. DC-10 induce Tr1 cells *via* the IL-10-dependent ILT-4/HLA-G pathway (**Gregori S et al., 2007 submitted**). It is probable that an up-regulation of tolerogenic markers, including ILT-3 and ILT-4, occurred also on DC10 generated from our patients rendering them capable of promoting allergen-specific anergic T cells containing already differentiated or precursors of allergen-specific Tr1 cells.

In vitro T-cell priming by immature DC, as well as by DC rendered tolerogenic with biological or pharmacological agents, can drive the differentiation of regulatory T cells (**Roncarolo MG et al., 2006; Levings MK et al., 2005; Chang CC et al., 2002; Penna G et al., 2005; Jonuleit H et al., 2000**). Immature DC differentiated *in vitro* and treated with exogenous IL-10 display reduced allo-stimulatory capacity and were shown to induce anergic Ag-specific T cells (**Zheng Z et al., 2004**). Furthermore, DC matured in the presence of exogenous IL-10 induce Ag-specific anergic T cells (**Steinbrink K et al., 1997**), which suppress T-cell proliferation (**Steinbrink K et al., 2002**). It has been shown that Tr1 differentiation can be obtained by stimulation of human peripheral blood naïve CD4⁺ T cells with allogeneic immature DC only after repetitive stimulation (**Levings MK et al., 2005; Jonuleit H et al., 2000**). In our study we were not able to promote T cell anergy using IL-10- treated Dp2-DC, due to the low number of cells obtained from our allergic children which did not allow us to perform the necessary rounds of stimulation. Importantly, we demonstrated that single stimulation of autologous T cells with Dp2-DC10 promoted anergic T cells that secreted lower amounts of Th2 cytokines. Interestingly, TNF- α production by anergic T cells was preserved (data not shown), indicating that innate immunity was not affected, but the tolerogenic effect of DC10 was directed mainly to Th2 effector cells. These results are in line with our recent data demonstrating that DC-10 drive Tr1 differentiation after a single round of stimulation in allogeneic system (**Gregori S et al., 2007 submitted**). We can therefore speculate that Der p2-specific anergic T cells induced by DC10 are precursor or contain already differentiated Tr1 cells, although the suppressor capacity of these cells is currently under investigation together with their antigen specificity.

A previous report demonstrated the presence of Der p1-specific Tr1 and Th2 cells in healthy donors as well as in atopic subjects. Nevertheless, in allergic patients was observed a lower Der p1-specific Tr1/Th2 ratio compared to healthy controls, indicating that allergen-specific Tr1 cells are present in allergic patients but in lower frequency compared to Th2 cells (**Akdis CA et al., 2004**). Thus, we can hypothesise that an imbalance of Tr1/Th2 ratio is present in our HDM-allergic children. In this

light, our work represents an important step forward to restoring the balance between Tr1 and Th2, which is altered in allergic disease. Dp2-DC10 represent a good tool to re-establish tolerance and the defective Tr1 compartment of the immune system in allergic patients.

CONCLUDING REMARKS

In the present study we demonstrated that allergen-specific anergic T cells, which may exert regulatory activity, can be generated *in vitro* using DC differentiated in the presence of exogenous IL-10 in allergic patients. This finding represent a step forward for the prospective clinical use of autologous DC, rendered tolerogenic with IL-10, as vectors for the immunotherapy of allergic disease, to restore tolerance.

Immunization with *ex vivo* generated DC has been proven feasible in other conditions and would be particularly attractive in allergy since a disease modifying treatment is not currently available. Moreover, the advent of component resolved diagnosis of allergy and the increasing availability of recombinant allergens and synthetic peptides makes of DC based immunotherapy a realistic strategy to cure allergy in the future.

REFERENCES

- Aberg N, Hesselmar B, Aberg B, Eriksson B. Increase of asthma, allergic rhinitis and eczema in Swedish schoolchildren between 1979 and 1991. *Clin Exp Allergy* 1995; 25:815-9.
- Adorini L, Interleukin-12, a key cytokine in Th1-mediated autoimmune diseases, *Cell. Mol. Life Sci* 1999;55:1610–1625.
- Adorini L, Penna G, Giarratana N, Roncari A, Amuchastegui S, Daniel KC, Uskokovic M. Dendritic cells as key targets for immunomodulation by Vitamin D receptor ligands. *J Steroid Biochem Mol Biol.* 2004;89(90):437–441.
- Akasaki Y, Liu G, Chung NH, Ehtesham M, Black KL, Yu JS. Induction of a CD4+ T regulatory type 1 response by cyclooxygenase- 2-overexpressing glioma. *J Immunol* 2004;173:4352–4359.
- Akbari O, DeKruyff RH, Umetsu DT. Pulmonary dendritic cells producing IL-10 mediate tolerance induced by respiratory exposure to antigen. *Nat Immunol.* 2001;2(8):725-31.
- Akbari O, Faul JL, Hoyte EG, Berry GJ, Wahlstrom J, Kronenberg M, DeKruyff RH, Umetsu DT. CD4+ invariant T-cellreceptor + natural killer T cells in bronchial asthma. *N Engl J Med* 2006; 354:1117-1129.
- Akbari O, Freeman GJ, Meyer EH, Greenfield EA, Chang TT, Sharpe AH, Berry G, DeKruyff RH, Umetsu DT. Antigen-specific regulatory T cells develop via the ICOS-ICOS-ligand pathway and inhibit allergen-induced airway hyperreactivity. *Nat Med* 2002; 8:1024-1032.
- Akbari O, Stock P, Meyer E, Kronenberg M, Sidobre S, Nakayama T, Taniguchi M, Grusby MJ, DeKruyff RH, Umetsu DT: Essential role of NKT cells producing IL-4 and IL-13 in the development of allergen-induced airway hyperreactivity. *Nat Med* 2003; 9:582-588.
- 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(6):603-9. Review
- Akdis CA, Blesken T, Akdis M, Wuthrich B, Blaser K. Role of interleukin 10 in specific immunotherapy. *J Clin Invest.* 1998;102(1):98-106.
- Akdis CA, Joss A, Akdis M, Faith A, Blaser K. A molecular basis for T cell suppression by IL-10: CD28-associated IL-10 receptor inhibits CD28 tyrosine phosphorylation and phosphatidylinositol 3-kinase binding. *FASEB J* 2000; 14:1666-1668.
- Akdis M, Verhagen J, Taylor A, Karamloo F, Karagiannidis C, Cramer R, Thunberg S, Deniz G, Valenta R, Fiebig H, Kegel C, Disch R, Schmidt-Weber CB, Blaser K, Akdis CA. 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(11):1567-75.

- Akkaraju S, Ho WY, Leong D, Canaan K, Davis MM, Goodnow CC. A range of CD4 T cell tolerance: partial inactivation to organ-specific antigen allows nondestructive thyroiditis or insulinitis. *Immunity*. 1997;7(2):255-71
- Allan SE, Crome SQ, Crellin NK, Passerini L, Steiner TS, Bacchetta R, Roncarolo MG, Levings MK. Activation-induced FOXP3 in human T effector cells does not suppress proliferation or cytokine production. *Int Immunol*. 2007 Apr;19(4):345-54.
- Allan SE, Passerini L, Bacchetta R, Crellin N, Dai M, Orban PC, Ziegler SF, Roncarolo MG, Levings MK. The role of 2 FOXP3 isoforms in the generation of human CD4+ Tregs. *J Clin Invest*. 2005;115(11):3276-84.
- Al-Shami A, Spolski R, Kelly J, Keane-Myers A, Leonard W. A role for TSLP in the development of inflammation in an asthma model. *J Exp Med* 2005; 202:829-839.
- Amsen D, Blander JM, Lee GR, Tanigaki K, Honjo T, Flavell RA. Instruction of distinct CD4 T helper cell fates by different notch ligands on antigen-presenting cells. *Cell* 2004; 117:515-526.
- Anderson MS, Venzani ES, Klein L, Chen Z, Berzins SP, Turley SJ, von Boehmer H, Bronson R, Dierich A, Benoist C, Mathis D. Projection of an immunological self shadow within the thymus by the aire protein. *Science* 2002; 298(5597):1395-401.
- Arif S, Tree TI, Astill TP, Tremble JM, Bishop AJ, Dayan CM, Roep BO, Peakman M. Autoreactive T cell responses show proinflammatory polarization in diabetes but a regulatory phenotype in health. *J Clin Invest* 2004;113:451–463.
- Asadullah K, Sterry W, Volk HD. Interleukin-10 therapy--review of a new approach. *Pharmacol Rev*. 2003 Jun;55(2):241-69. Review
- Ashton-Rickardt PG, Tonegawa S. A differential-avidity model for T-cell selection. *Immunol Today* 1994; 15(8):362-6.
- Asnagli H, Murphy KM. Stability and commitment in T helper cell development. *Curr Opin Immunol*. 2001;13(2):242-7. Review
- Bacchetta R, Bigler M, Touraine JL, Parkman R, Tovo PA, Abrams J, de Waal Malefyt R, de Vries JE, Roncarolo MG. High levels of interleukin 10 production in vivo are associated with tolerance in SCID patients transplanted with HLA mismatched hematopoietic stem cells. *J Exp Med* 1994; 179:493-502.
- Bacchetta R, Bigler M, Touraine JL, Parkman R, Tovo PA, Abrams J, de Waal Malefyt R, de Vries JE, Roncarolo MG. High levels of interleukin 10 production in vivo are associated with tolerance in SCID patients transplanted with HLA mismatched hematopoietic stem cells. *J Exp Med* 1994;179:493–502.
- 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.

- Baecher-Allan C, Brown JA, Freeman GJ, Hafler DA. CD4⁺CD25^{high} regulatory cells in human peripheral blood. *J Immunol.* 2001;167(3):1245-53
- Baecher-Allan C, Hafler DA. Human regulatory T cells and their role in autoimmune disease. *Immunol Rev.* 2006 Aug;212:203-16. Review.
- Banchereau J, Steinman RM. Dendritic cells and the control of immunity. *Nature* 1998;392:245–252.
- Barbey C, Donatelli-Dufour N, Batard P, Corradin G, Spertini F. Intranasal treatment with ovalbumin but not the major T cell epitope ovalbumin 323-339 generates interleukin-10 secreting T cells and results in the induction of allergen systemic tolerance. *Clin Exp Allergy.* 2004;34(4):654-62.
- Barker CF, Billingham RE. Immunologically privileged sites. *Adv Immunol.* 1977; 25:1-54. Review.
- Barrat FJ, Cua DJ, Boonstra A, Richards DF, Crain C, Savelkoul HF, de Waal-Malefyt R, Coffman RL, Hawrylowicz CM, O'Garra A. 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.
- Battaglia M, Gianfrani C, Gregori S, Roncarolo MG. IL-10-producing T regulatory type 1 cells and oral tolerance. *Ann NY Acad Sci* 2004;1029:142–153.
- Battaglia M, Gregori S, Bacchetta R, Roncarolo MG. Tr1 cells: from discovery to their clinical application. *Semin Immunol* 2006;18: 120–127.
- Battaglia M, Stabilini A, Migliavacca B, Horejs-Hoeck J, Kaupper T, Roncarolo MG. Rapamycin and interleukin- 10 treatment induces T regulatory type 1 cells that mediate antigen-specific transplantation tolerance. *Diabetes* 2006;55: 40–49
- Beaulieu S, Robbiani DF, Du X, Rodrigues E, Ignatius R, Wei Y, Ponath P, Young JW, Pope M, Steinman RM, Mojsov S. Expression of a functional eotaxin (CC chemokine ligand 11) receptor CCR3 by human dendritic cells. *J Immunol.* 2002;169(6):2925-36.
- Belkaid Y, Rouse BT. Natural regulatory T cells in infectious disease. *Nat Immunol* 2005;6:353–360.
- Bellinghausen I, Brand U, Steinbrink K, Enk AH, Knop J, Saloga J. Inhibition of human allergic T-cell responses by IL-10-treated dendritic cells: differences from hydrocortisone-treated dendritic cells. *J Allergy Clin Immunol.* 2001;108(2):242-9
- Bellinghausen I, König B, Bottcher I, Knop J, Saloga J. Inhibition of human allergic Th2 immune responses by induced regulatory T cells requires the combination of IL-10-treated dendritic cells and TGF- β for their induction. *Clin Exp Allergy* 2006; 36(12):1546-1555.
- Berg DJ, Leach MW, Kuhn R, Rajewsky K, Muller W, Davidson NJ, Rennick D. Interleukin 10 but not interleukin 4 is a natural suppressant of cutaneous inflammatory responses. *J. Exp Med* 1995;182:99–108

- Bergmann, M. & Sautner, T. Immunomodulatory effects of vasoactive catecholamines. *Wien. Klin. Wochenschr* 2002;114,752–761.
- Berry MA, Hargadon B, Shelley M, Parker D, Shaw DE, Green RH, Bradding P, Brightling CE, Wardlaw AJ, Pavord ID. Evidence of a role of tumor necrosis factor alpha in refractory asthma. *N Engl. J Med* 2006; 354:697-708.
- Billsborough J, George TC, Norment A, Viney JL. Mucosal CD8alpha+ DC, with a plasmacytoid phenotype, induce differentiation and support function of T cells with regulatory properties. *Immunology* 2003;108:481–492.
- Blaser K, Akdis CA. Interleukin-10, T regulatory cells and specific allergy treatment. *Clin Exp Allergy* 2004;34(3):328-31.
- Bocek P Jr, Foucras G, Paul WE. Interferon gamma enhances both in vitro and in vivo priming of CD4+ T cells for IL-4 production. *J Exp Med.* 2004;199(12):1619-30.
- Bonifaz L, Bonnyay D, Mahnke K, Rivera M, Nussenzweig MC, Steinman RM. Efficient targeting of protein antigen to the dendritic cell receptor DEC-205 in the steady state leads to antigen presentation on major histocompatibility complex class I products and peripheral CD8(+) T cell tolerance. *J Exp Med* 2002;196:1627–38.
- Borgonovo B, Casorati G, Frittoli E, Gaffi D, Crimi E, Burastero SE. Recruitment of circulating allergen-specific T lymphocytes to the lung on allergen challenge in asthma. *J Allergy Clin Immunol.* 1997;100(5):669-78.
- Borish L, Aarons A, Rumblyrt J, Cvietusa P, Negri J, Wenzel S. Interleukin-10 regulation in normal subjects and patients with asthma. *J Allergy Clin Immunol.* 1996; 97(6):1288-96.
- Brugnolo F, Sampognaro S, Fintoni T, Cosmi L, Annunziato F, Manuelli C, Campi P, Maggi E, Romagnani S, Parronchi P. The novel synthetic immune response modifier R-848 (Resiquimod) has a powerful effect on the in vitro switching of human allergen-specific CD4+ Th2 lymphocytes into IFN- γ producing cells. *J Allergy Clin Immunol* 2003;111:380–388.
- Buelens C, Verhasselt V, De Groote D, Thielemans K, Goldman M, Willems F. Human dendritic cell responses to lipopolysaccharide and CD40 ligation are differentially regulated by interleukin-10. *Eur J Immunol.* 1997;27(8):1848-52.
- Bullens DM, De Swert A, Dilissen E, Kasran A, Kroczeck RA, Cadot P, Casaer P, Ceuppens JL. House dust mite-specific T cells in healthy non-atopic children. *Clin Exp Allergy.* 2005;35(12):1535-41
- Bullens DM, Van Den Keybus C, Dilissen E, Kasran A, Ceuppens JL. Allergen-specific T cells from birch-pollen-allergic patients and healthy controls differ in T helper 2 cytokine and in interleukin-10 production. *Clin Exp Allergy.* 2004;34(6):879-87
- Burney P, Malmberg E, Chinn S, Jarvis D, Luczynska C, Lai E. The distribution of total and specific serum IgE in the European Community Respiratory Health Survey. *J. Allergy Clin. Immunol* 1997;99, 314–322.

- Cai G, Kastelein RA, Hunter CA. IL-10 enhances NK cell proliferation, cytotoxicity and production of IFN- γ when combined with IL-18. *Eur. J. Immunol* 1999; 29:2658–65
- Carbonneil C, Saidi H, Donkova-Petrini V, Weiss L. Dendritic cells generated in the presence of interferon-alpha stimulate allogeneic CD4⁺ T-cell proliferation: modulation by autocrine IL-10, enhanced T-cell apoptosis and T regulatory type 1 cells. *Int Immunol* 2004;16:1037–1052.
- Caron G, Delneste Y, Roelandts E, Duez C, Bonneoy JY, Pestel J, Jeannin P. Histamine polarizes human dendritic cells into Th2 cell-promoting effector dendritic cells. *J Immunol* 2001; 167(7):3682-3686.
- Cavani A, Nasorri F, Prezzi C, Sebastiani S, Albanesi C, Girolomoni G. Human CD4⁺ T lymphocytes with remarkable regulatory functions on dendritic cells and nickelspecific Th1 immune responses. *J Invest Dermatol* 2000;114:295–302.
- Cella M, Engering A, Pinet V, Pieters J, Lanzavecchia A. Inflammatory stimuli induce accumulation of MHC class II complexes on dendritic cells. *Nature* 1997 ;388(6644):782-7.
- Cella M, Facchetti F, Lanzavecchia A, Colonna M. Plasmacytoid dendritic cells activated by influenza virus and CD40L drive a potent TH1 polarization. *Nat Immunol* 2000; 1:305–10.
- Cerwenka A, Swain SL. TGF-beta1: immunosuppressant and viability factor for T lymphocytes. *Microbes Infect* 1999;1: 1291–1296.
- Chang CC, Ciubotariu R, Manavalan JS, Yuan J, Colovai AI, Piazza F, Lederman S, Colonna M, Cortesini R, Dalla-Favera R, Suciufoca N. Tolerization of dendritic cells by T(S) cells: the crucial role of inhibitory receptors ILT3 and ILT4. *Nat Immunol* 2002;3:237–343.
- Chang CH, Furue M, Tamaki K. B7-1 expression of Langerhans cells is up-regulated by proinflammatory cytokines, and is downregulated by interferon-gamma or by interleukin-10. *Eur J Immunol* 1995;25:394–8.
- Chatila TA, Blaeser F, Ho N, Lederman HM, Voulgaropoulos C, Helms C, Bowcock AM. JM2, encoding a fork head-related protein, is mutated in X-linked autoimmunity-allergic dysregulation syndrome. *J Clin Invest.* 2000;106(12):R75-81
- Chen L. Immunological ignorance of silent antigens as an explanation of tumor evasion. *Immunol Today* 1998; 19(1):27-30.
- Chen ML, et al. Regulatory T cells suppress tumor-specific CD8 T cell cytotoxicity through TGF-beta signals in vivo. *Proc Natl Acad Sci USA* 2005;102:419–424.
- Chen W, Jin W, Wahl SM. Engagement of cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) induces transforming growth factor beta (TGF-beta) production by murine CD4(+) T cells. *J Exp Med* 1998;188:1849–1857.
- Cohen SB, Crawley JB, Kahan MC, Feldmann M, Foxwell BM. Interleukin-10 rescues T cells from apoptotic cell death: association with an upregulation of Bcl-2. *Immunology* 1997; 92: 1–5

- Constant SL, Bottomly K. Induction of Th1 and Th2 CD4⁺ T cell responses: the alternative approaches. *Annu Rev Immunol* 1997;15:297-322.
- Corral, LG, Haslett PA, Muller GW, Chen R, Wong LM, Ocampo CJ, Patterson RT, Stirling DI, Kaplan G. Differential cytokine modulation and T cell activation by two distinct classes of thalidomide analogues that are potent inhibitors of TNF- α . *J. Immunol* 1999; 163, 380–386.
- Cosmi L, Santarasci V, Angeli R, Liotta F, Maggi L, Frosali F, Rossi O, Falagiani P, Riva G, Romagnani S, Annunziato F, Maggi E. Sublingual immunotherapy with Dermatophagoides monomeric allergoid down-regulates allergen-specific immunoglobulin E and increases both interferon-gamma- and interleukin-10-production. *Clin Exp Allergy* 2006;36(3):261-72.
- Cottrez F, Hurst SD, Coffman RL, Groux H. T regulatory cells 1 inhibit a Th2-specific response in vivo. *J Immunol.* 2000;165(9):4848-53.
- Coyle AJ, Lehar S, Lloyd C, Tian J, Delaney T, Manning S, Nguyen T, Burwell T, Schneider H, Gonzalo JA, Gosselin M, Owen LR, Rudd CE, Gutierrez-Ramos JC. The CD28-related molecule ICOS is required for effective T cell-dependent immune responses. *Immunity* 2000; 13:95–105.
- Cozzo C, Larkin J, 3rd, Caton AJ. Cutting edge: self-peptides drive the peripheral expansion of CD4⁺CD25⁺ regulatory T cells. *J Immunol* 2003;171:5678–5682
- Dabbagh K, Dahl ME, Stepick-Biek P, Lewis DB. Toll-like receptor 4 is required for optimal development of Th2 immune responses: role of dendritic cells. *J Immunol* 2002; 168:4524-4530.
- Dahl ME, Dabbagh K, Liggitt D, Kim S, Lewis DB. Viral-induced T helper type 1 responses enhance allergic disease by effects on lung dendritic cells. *Nat Immunol* 2004;5:337-43.
- D'Cruz LM, L. Development and function of agonist-induced CD25⁺Foxp3⁺ regulatory T cells in the absence of interleukin 2 signaling. *Nat Immunol* 2005;6(11):1152-
- De Heer HJ, , Hammad H, Soullie T, Hijdra D, Vos N, Willart MA, Hoogsteden HC, Lambrecht BN. Essential role of lung plasmacytoid dendritic cells in preventing asthmatic reactions to harmless inhaled antigen. *J Exp Med* 2004;200: 89-98.
- De Smedt T, Pajak B, Muraille E, Lespagnard L, Heinen E, De Baetselier P, Urbain J, Leo O, Moser M. Regulation of dendritic cell numbers and maturation by lipopolysaccharide in vivo. *J Exp Med.* 1996;184(4):1413-24.
- De Smedt T, Van Mechelen M, De Becker G, Urbain J, Leo O, Moser M. Effect of interleukin-10 on dendritic cell maturation and function. *Eur J Immunol.* 1997;27(5):1229-3.
- De Waal Malefyt R, Abrams J, Bennett B, Figdor CG, de Vries JE. Interleukin 10(IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes. *J Exp Med.* 1991;174(5):1209-20
- De Wit D, Amraoui Z, Vincart B, Michel O, Michils A, Van Overvelt L, Willems F, Goldman M. Helper T-cell responses elicited by Der p 1-pulsed

dendritic cells and recombinant IL-12 in atopic and healthy subjects. *J Allergy Clin Immunol.* 2000;105(2 Pt 1):346-52

- Deurloo DT, van Berkel MA, van Esch BC et al. Hofhuis F, Nijkamp FP, Oosterwegel MA, van Oosterhout AJ. CD28/CTLA4 double deficient mice demonstrate crucial role for B7 co-stimulation in the induction of allergic lower airways disease. *Clin Exp Allergy* 2003; 33:1297–304.
- Dieckmann D, Plottner H, Berchtold S, Berger T, Schuler G. Ex vivo isolation and characterization of CD4(+)CD25(+) T cells with regulatory properties from human blood. *J Exp Med.* 2001;193(11):1303-10.
- Dodge IL, Carr MW, Cernadas M, Brenner MB: IL-6 production by pulmonary dendritic cells impedes Th1 immune responses. *J Immunol* 2003, 170:4457-4464
- Doganci A, Sauer K, Karwot R, Finotto S. Pathological role of IL-6 in the experimental allergic bronchial asthma in mice. *Clin Rev Allergy Immunol* 2005;28:257-70.
- Drazen JM, Israel E, O’Byrne PM. Treatment of asthma with drugs modifying the leukotriene pathway. *N Engl J Med* 1999; 340:197-206. Dreher, D., Kok, M., Obregon, C., Kiama, S. G., Gehr, P., & Nicod, L. P. Salmonella virulence factor SipB induces activation and release of IL-18 in human dendritic cells. *J Leukoc Biol* 2002; 72, 743– 751.
- Durez, P, Abramowicz D, Gerard C, Van Mechelen M, Amraoui Z, Dubois C, Leo O, Velu T, Goldman M. In vivo induction of interleukin 10 by anti-CD3 monoclonal antibody or bacterial lipopolysaccharide: differential modulation by cyclosporin A. *J. Exp. Med* 1993;177, 551–555.
- Durham SR, Till SJ. Immunologic changes associated with allergen immunotherapy. *J Allergy Clin Immunol.* 1998;102(2):157-64. Review
- Durham SR, Ying S, Varney VA, Jacobson MR, Sudderick RM, Mackay IS, Kay AB, Hamid QA. Cytokine messenger RNA expression for IL-3, IL-4, IL-5 and granulocyte/macrophage-colony-stimulating factor in the nasal mucosa after local allergen provocation: relationship to tissue eosinophilia. *J Immunol* 1992;148:2390-4.
- Edinger M, Hoffmann P, Ermann J, Drago K, Fathman CG, Strober S, et al. CD4+CD25+ regulatory T cells preserve graft-versus-tumor activity while inhibiting graft-versus-host disease after bone marrow transplantation. *Nat Med.* 2003 Sep;9(9):1144-50.
- Eisenbarth SC, Piggott DA, Huleatt JW, Visintin I, Herrick CA, Bottomly K. Lipopolysaccharide-enhanced, toll-like receptor 4-dependent T helper cell type 2 responses to inhaled antigen. *J Exp Med* 2002; 196:1645-1651.
- Elias JA, Lee CG, Zheng T, Ma B, Homer RJ, Zhu Z. New insights into the pathogenesis of asthma. *J Clin Invest.* 2003;111(3):291-7.
- Enk AH, Angeloni VL, Udey MC, Katz SI. Inhibition of Langerhans cell antigen-presenting function by IL-10. A role for IL-10 in induction of tolerance. *J Immunol* 1993;151:2390–8.

- Enk AH, Saloga J, Becker D, B1P6madzadeh M, Knop J. Induction of hapten-specific tolerance by interleukin 10 in vivo. *J Exp Med* 1994;179(4):1397-402.
- Ermann J, Hoffmann P, Edinger M, Dutt S, Blankenberg FG, Higgins JP, et al. Only the CD62L+ subpopulation of CD4+CD25+ regulatory T cells protects from lethal acute GvHD. *Blood*. 2005 Mar 1;105(5):2220-6.
- Fahlen L, Read S, Gorelik L, Hurst SD, Coffman RL, Flavell RA, Powrie F. T cells that cannot respond to TGF-beta escape control by CD4+CD25+ regulatory T cells. *J Exp Med* 2005;201:737-746.
- Fainaru O, Woolf E, Lotem J, Yarmus M, Brenner O, Goldenberg D, Negreanu V, Bernstein Y, Levanon D, Jung S. Runx3 regulates mouse TGF-beta-mediated dendritic cell function and its absence results in airway inflammation. *EMBO J* 2004; 23:969-979.
- Fallarino F, Asselin-Paturel C, Vacca C, Bianchi R, Gizzi S, Fioretti MC. Murine plasmacytoid dendritic cells initiate the immunosuppressive pathway of tryptophan catabolism in response to CD200 receptor engagement. *J Immunol* 2004;173:3748-54.
- Fallarino F, Grohmann U, Hwang KW, Orabona C, Vacca C, Bianchi R, Belladonna ML, Fioretti MC, Alegre ML, Puccetti P. Modulation of tryptophan catabolism by regulatory T cells. *Nat Immunol* 2003; 4:1206-1212.
- Fantini MC, Becker C, Monteleone G, Pallone F, Galle PR, Neurath MF. 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(9):5149-53
- Fantini MC, Becker C, Tubbe I, Nikolaev A, Lehr HA, Galle P, et al. Transforming growth factor beta induced FoxP3+ regulatory T cells suppress Th1 mediated experimental colitis. *Gut*. 2006;55(5):671-80
- Fas SC, Fritzsching B, Suri-Payer E, Krammer PH. Death receptor signaling and its function in the immune system. *Curr Dir Autoimmun*. 2006;9:1-17. Review
- Ferber IA, Lee HJ, Zonin F, et al.:GATA-3 significantly downregulates IFN-gamma production from developing Th1 cells in addition to inducing IL-4 and IL-5 levels. *Clin Immunol* 1999; 91(2):134-144.
- Fili L, Ferri S, Guarna F, Sampognaro S, Manuelli C, Liotta F, Cosmi L, Matucci A, Vultaggio A, Annunziato F, Maggi E, Guarna A, Romagnani S, Parronchi P.Redirection of allergen-specific TH2 responses by a modified adenine through Toll-like receptor 7 interaction and IL-12/IFN release. *J Allergy Clin Immunol*. 2006;118(2):511-7.
- Finotto S, Neurath MF, Glickman JN, Qin S, Lehr HA, Green FH, Ackerman K, Haley K, Galle PR, Szabo SJ, Drazen JM, De Sanctis GT, Glimcher LH. Development of spontaneous airway changes consistent with human asthma in mice lacking T-bet. *Science* 2002;295:336-8.
- Fiore F, Nuschak B, Peola S, Mariani S, Muraro M, Foglietta M. 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(4):1155–63.

- Fiorentino DF, Zlotnik A, Vieira P, Mosmann TR, Howard M, Moore KW, O'Garra A. IL-10 acts on the antigen-presenting cell to inhibit cytokine production by Th1 cells. *J Immunol*. 1991;146(10):3444-51
- Fluckiger AC, Durand I, Banchereau J. Interleukin 10 induces apoptotic cell death of B chronic lymphocytic leukemia cells. *J. Exp. Med* 1994;179:91– 99
- Fontenot JD, Gavin MA, Rudensky AY. Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. *Nat Immunol* 2003;4:330–336.
- Fontenot JD, Rasmussen JP, Gavin MA, Rudensky AY. A function for interleukin 2 in Foxp3-expressing regulatory T cells. *Nat Immunol*. 2005;6(11):1142-51
- Fontenot JD, Rasmussen JP, Williams LM, Dooley JL, Farr AG, Rudensky AY. Regulatory T cell lineage specification by the forkhead transcription factor foxp3. *Immunity* 2005;22:329–341.
- Fort MM, Cheung J, Yen D, Li J, Zurawski SM, Lo S, Menon S, Clifford T, Hunte B, Lesley R. IL-25 induces IL-4, IL-5, and IL-13 and Th2-associated pathologies in vivo. *Immunity* 2001; 15:985-995.
- Freeman CM, Chiu BC, Stolberg VR, Hu J, Zeibecoglou K, Lukacs NW, Lira SA, Kunkel SL, Chensue SW. CCR8 is expressed by antigen-elicited, IL-10-producing CD4+CD25+ T cells, which regulate Th2-mediated granuloma formation in mice. *J Immunol* 2005;174:1962–1970
- Furtado GC, Olivares-Villagomez D, Curotto de Lafaille MA, Wensky AK, Latkowski JA, Lafaille JJ. Regulatory T cells in spontaneous autoimmune encephalomyelitis. *Immunol Rev*. 2001;182:122-34
- Gazzinelli RT, Wysocka M, Hieny S, Schar-ton-Kersten T, Cheever A. In the absence of endogenous IL-10, mice acutely infected with toxoplasma gondii succumb to a lethal immune response dependent on CD4⁺ T cells and accompanied by overproduction of IL-12, IFN- γ and TNF- α . *J. Immunol* 1996; 157:798–805
- Gentile DA, Schreiber R, Howe-Adams J, Trecki J, Patel A, Angelini B, Skoner DP. Diminished dendritic cell interleukin 10 production in atopic children *Ann Allergy Asthma Immunol*. 2004;92(5):538-44
- Gett AV, Sallusto F, Lanzavecchia A, Geginat J. T cell fitness determined by signal strength. *Nat Immunol* 2003; 4:355-360.
- Gilliet M, Liu YJ. Generation of human CD8 T regulatory cells by CD40 ligand-activated plasmacytoid dendritic cells. *J Exp Med* 2002;195:695–704.
- Global Initiative for Asthma (GINA). WHO/NHLBI workshop report. Lung and Blood Institute, National Institutes of Health. 2006 Revision. <http://www.ginasthma.com/>
- Gotter J, Brors B, Hergen-hahn M, Kyewski B. Medullary epithelial cells of the human thymus express a highly diverse selection of tissue-specific genes colocalized in chromosomal clusters. *J Exp Med* 2004; 199(2):155-66.

- 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.
- Gregori S, Pacciani V, Tomasoni D, Scirpoli M, Battaglia M, Magnani C, Hauben E, Roncarolo MG. Differentiation of type 1 T regulatory cells through the IL-10-dependent ILT4/HLA-G pathway. 2007. Submitted
- Grindebacke H, Wing K, Andersson AC, Suri-Payer E, Rak S, Rudin A. Defective suppression of Th2 cytokines by CD4CD25 regulatory T cells in birch allergics during birch pollen season. *Clin Exp Allergy* 2004;34:1364–1372.
- Grossman WJ, Verbesky JW, Barchet W, Colonna M, Atkinson JP, Ley TJ. Human T regulatory cells can use the perforin pathway to cause autologous target cell death. *Immunity* 2004; 21:589-601.
- Gondek DC, Lu LF, Quezada SA, Sakaguchi S, Noelle RJ. Cutting edge: contact-mediated suppression by CD4+CD25+ regulatory cells involves a granzyme B-dependent, perforin-independent mechanism. *J Immunol.* 2005;174(4):1783-6.
- 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.
- Groux H, Bigler M, de Vries JE, Roncarolo MG. Inhibitory and stimulatory effects of IL-10 on human CD8+ T cells. *J Immunol* 1998;160:3188–3193.
- Groux H, Bigler M, de Vries JE, Roncarolo MG. Interleukin-10 induces a long-term antigen-specific anergic state in human CD4+ T cells. *J Exp Med* 1996;184:19–29.
- Groux H. Type 1 T-regulatory cells: their role in the control of immune responses. *Transplantation* 2003;75:8S-12S.
- 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;185:1089–99
- Haczku A, Takeda K, Redai I, Hamelmann E, Cieslewicz G, Joetham A, Loader J, Lee JJ, Irvin C, Gelfand EW. Anti-CD86 (B7.2) treatment abolishes allergic airway hyperresponsiveness in mice. *Am J Respir Crit Care Med* 1999;159:1638-1643.
- Hamid QA, Shotman E, Jacobson MR, Walker SM, Durham SR. Increases in IL-12 messenger RNA+ cells accompany inhibition of allergen-induced late skin responses after successful grass pollen immunotherapy. *J Allergy Clin Immunol* 1997; 99:254–60.
- Hammad H, Charbonnier AS, Duez C, Jacquet A, Stewart GA, Tonnel AB, Pestel J. Th2 polarization by Der p 1--pulsed monocyte-derived dendritic cells is due to the allergic status of the donors. *Blood.* 2001;98(4):1135-41
- Hammad H, de Heer HJ, Souillie T, Angeli V, Trottein F, Hoogsteden HC, Lambrecht BN. Activation of peroxisome proliferator-activated receptor

pathway in dendritic cells inhibits development of eosinophilic airway inflammation in a mouse model of asthma. *Am J Pathol* 2004;164:263-271.

- Hammad H, Smits HH, Ratajczak C, Nithianathan A, Wierenga EA, Stewart GA, Jacquet A, Tonnel AB, Pestel J. Monocytederived dendritic cells exposed to Der p 1 allergen enhance the recruitment of Th2 cells: major involvement of the chemokines TARC/CCL17 and MDC/CCL22. *Eur Cytokine Netw* 2003;14:219-228.
- Hanash AM, Levy RB. Donor CD4+CD25+ T cells promote engraftment and tolerance following MHC-mismatched hematopoietic cell transplantation. *Blood*. 2005 Feb 15;105(4):1828-36.
- Hansen G, McIntire JJ, Yeung VP, Berry G, Thorbecke GJ, Chen L, DeKruyff RH, Umetsu DT. CD4+ Th cells engineered to produce latent TGF- β 1 reverse allergen-induced airway hyperreactivity and inflammation. *J Clin Invest* 2000;11:89–96.
- Harris N, Campbell C, Le Gros G, Ronchese F. Blockade of CD28/B7 co-stimulation by mCTLA4-H γ 1 inhibits antigeninduced lung eosinophilia but not Th2 cell development or recruitment in the lung. *Eur J Immunol* 1997; 27:155–61.
- Hawrylowicz CM, O'Garra A. Potential role of interleukin-10-secreting regulatory T cells in allergy and asthma. *Nat Rev Immunol* 2005;5(4):271-83. Review
- Hayashi T, Beck L, Rossetto C, Gong X, Takikawa O, Takabayashi K, Broide DH, Carson DA, Raz E. Inhibition of experimental asthma by indoleamine 2,3-dioxygenase. *J Clin Invest* 2004;114:270-279.
- Heaton T, Rowe J, Turner S, Aalberse RC, de Klerk N, Suriyaarachchi D, Serralha M, Holt BJ, Hollams E, Yerkovich S, Holt K, Sly PD, Goldblatt J, Le Souef P, Holt PG. An immunoepidemiological approach to asthma: identification of in-vitro T-cell response patterns associated with different wheezing phenotypes in children. *Lancet*. 2005 Jan;365(9454):142-9
- Heino M, Peterson P, Sillanpaa N, Guerin S, Wu L, Anderson G, Scott HS, Antonarakis SE, Kudoh J, Shimizu N, Jenkinson EJ, Naquet P, Krohn KJ. RNA and protein expression of the murine autoimmune regulator gene (Aire) in normal, RelB-deficient and in NOD mouse. *Eur J Immunol* 2000; 30(7):1884-93.
- Holgate ST. The epidemic of allergy and asthma. *Nature* 1999;402: Suppl:B2-B4.
- Holgate, S. T. & Broide, D. New targets for allergic rhinitis: a disease of civilization. *Nature Rev. Drug Discov* 2003; 2: 902–914.
- Hollingsworth JW, Whitehead GS, Lin KL, Nakano H, Gunn MD, Schwartz DA, Cook DN. TLR4 signaling attenuates ongoing allergic inflammation. *J Immunol*. 2006;176(10):5856-62
- Holt PG, Macaubas C, Stumbles PA, Sly PD. The role of allergy in the development of asthma. *Nature* 1999;402:Suppl:B12-B17.

- Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. *Science* 2003;299:1057–1061
- Horwitz DA, Zheng SG, Gray JD, Wang JH, Ohtsuka K, Yamagiwa S. Regulatory T cells generated ex vivo as an approach for the therapy of autoimmune disease. *Semin Immunol.* 2004 Apr;16(2):135-43.
- Horwitz DA, Zheng SG, Gray JD. The role of the combination of IL-2 and TGF-beta or IL-10 in the generation and function of CD4+ CD25+ and CD8+ regulatory T cell subsets. *J Leukoc Biol.* 2003 Oct;74(4):471-8.
- Hsieh C, Liang Y, Tyznik A, Self S, Liggitt D, Rudensky A. Recognition of the peripheral self by naturally arising CD25+ CD4+ T cell receptors. *Immunity* 2004;21:267–277.
- Huang X, Zhu J, Yang Y. Protection against autoimmunity in nonlymphopenic hosts by CD4+ CD25+ regulatory T cells is antigenspecific and requires IL-10 and TGF-beta. *J Immunol* 2005;175:4283–4291
- Hurst SD, Muchamuel T, Gorman DM, Gilbert JM, Clifford T, Kwan S, Menon S, Seymour B, Jackson C, Kung TT et al.: New IL-17 family members promote Th1 or Th2 responses in the lung: in vivo function of the novel cytokine IL-25. *J Immunol* 2002, 169:443-453.
- Iellem A, Mariani M, Lang R, Recalde H, Panina-Bordignon P, Sinigaglia F, D'Ambrosio D: Unique chemotactic response profile and specific expression of chemokine receptors CCR4 and CCR8 by CD4(+)CD25(+) regulatory T cells. *J Exp Med* 2001, 194:847-853.
- Ikegami Y, Yokoyama A, Haruta Y, Hiyama K, Kohno N: Circulating natural killer T cells in patients with asthma. *J Asthma* 2004; 41:877-882.
- Ishida H, Hastings R, Kearney J, Howard M. Continuous anti-interleukin 10 antibody administration depletes mice of Ly-1 B cells but not conventional B cells. *J. Exp. Med* 1992;175:1213–20
- Ito T, Wang YH, Duramad O, Hori T, Delespesse GJ, Watanabe N, Qin FX, Yao Z, Cao W, Liu YJ: TSLP-activated dendritic cells induce an inflammatory T helper type 2 cell response through OX40 ligand. *J Exp Med* 2005; 202:1213-1223.
- Jaffar Z, Roberts K, Pandit A, Linsley P, Djukanovic R, Holgate S. B7 costimulation is required for IL-5 and IL-13 secretion by bronchial biopsy tissue of atopic asthmatic subjects in response to allergen stimulation. *Am J Respir Cell Mol Biol* 1999;20:153-162
- Jaffar Z, Sivakuru T, Roberts K: CD4+CD25+ T Cells Regulate Airway Eosinophilic Inflammation by Modulating the Th2 Cell Phenotype. *J Immunol* 2004;172:3842-3849.
- Jakubzick C, Tacke F, Llodra J, van Rooijen N, Randolph GJ. Modulation of dendritic cell trafficking to and from the airways. *J Immunol* 2006;176:3578-84.
- Jameson SC, Hogquist KA, Bevan MJ. Positive selection of thymocytes. *Annu Rev Immunol* 1995;13:93-126.

- Jameson SC, Hogquist KA, Bevan MJ. Specificity and flexibility in thymic selection. *Nature* 1994; 369(6483):750-2.
- Janeway CA Jr. The immune system evolved to discriminate infectious nonself from noninfectious self. *Immunol Today* 1992;13(1):11-6. Review.
- Jinquan T, Larsen CG, Gesser B, Matsushima K, Thestrup-Pedersen K. Human IL-10 is a chemoattractant for CD8+T lymphocytes and an inhibitor of IL-8-induced CD4+ T lymphocyte migration. *J. Immunol* 1993;151:4545–51
- Jones E, Dahm-Vicker M, Golgher D, Gallimore A. CD25+ regulatory T cells and tumor immunity. *Immunol Lett* 2003;85:141–143.
- Jonuleit H, Schmitt E, Schuler G, Knop J, Enk AH. Induction of interleukin 10-producing, nonproliferating CD4(+) T cells with regulatory properties by repetitive stimulation with allogeneic immature human dendritic cells. *J Exp Med* 2000;192: 1213–1222.
- Jonuleit H, Schmitt E, Stassen M, Tuettenberg A, Knop J, Enk AH. Identification and functional characterization of human CD4(+)CD25(+) T cells with regulatory properties isolated from peripheral blood. *J Exp Med*. 2001;193(11):1285-94
- Jonuleit H, Schmitt E. The regulatory T cell family: Distinct subsets and their interrelations. *J Immunol* 2003;171:6323– 7.
- Jung T, Wijdenes J, Neumann C, de Vries JE, Yssel H. Interleukin-13 is produced by activated human CD45RA+ and CD45RO+ T cells: modulation by interleukin-4 and interleukin-12. *Eur J Immunol* 1996;26(3):571-7
- Kalinski P, Hilkens CM, Snijders A, Snijdewint FG, Kapsenberg ML. IL-12-deficient dendritic cells, generated in the presence of prostaglandin E2, promote type 2 cytokine production in maturing human naive T helper cells. *J Immunol* 1997;159(1):28-35.
- Kapsenberg ML. Dendritic-cell control of pathogen-driven T-cell polarization. *Nat Rev Immunol* 2003;3:984-993.
- Karlsson MR, Rugtveit J, Brandtzaeg P. Allergen-responsive CD+CD25+ regulatory T cells in children who have outgrown cow's milk allergy. *J Exp Med* 2004;199:1679–1688
- Kasaian MT, Whitters MJ, Carter LL et al. IL-21 limits NK cell responses and promotes antigen-specific T cell activation: A mediator of the transition from innate to adaptive immunity. *Immunity* 2002; 16(4):559–569.
- Kasama T, Strieter RM, Lukacs NW, Burdick MD, Kunkel SL. Regulation of neutrophil-derived chemokine expression by IL-10. *J. Immunol* 1994;152:3559–69
- Kauffman HF, Tamm M, Timmerman JA, Borger P. House dust mite major allergens Der p 1 and Der p 5 activate human airway-derived epithelial cells by protease-dependent and protease-independent mechanisms. *Clin Mol Allergy* 2006;4:5.
- Kay, A. B. Allergy and allergic diseases. First of two parts. *N. Engl. J. Med* 2001;344, 30–37.

- Kearley J, Barker JE, Robinson DS, Lloyd CM. Resolution of airway inflammation and hyperreactivity after in vivo transfer of CD4+CD25+ regulatory T cells is interleukin 10 dependent. *J Exp Med*. 2005;202(11):1539-47.
- Kim JM, Rudensky A. The role of the transcription factor Foxp3 in the development of regulatory T cells. *Immunol Rev*. 2006;212:86-98. Review
- Kimura M, Tsuruta S, Yoshida T IL-4 production by PBMCs on stimulation with mite allergen is correlated with the level of serum IgE antibody against mite in children with bronchial asthma. *J Allergy Clin Immunol* 2000;105(2 Pt 1):327-32.
- Kinter AL, Hennessey M, Bell A, Kern S, Lin Y, Daucher M, Planta M, McGlaughlin M, Jackson R, Ziegler SF, Fauci AS. CD25+CD4+ regulatory T cells from the peripheral blood of asymptomatic HIV-infected individuals regulate CD4+ and CD8+ HIV-specific T cell immune responses in vitro and are associated with favorable clinical markers of disease status. *J. Exp. Med* 2004; 200, 331–343.
- Kitani A, Chua K, Nakamura K, Strober W. Activated Self-MHC-Reactive T Cells Have the Cytokine Phenotype of Th3/T Regulatory Cell 1 T Cells. *J Immunol* 2000;165:691–702.
- Koch F, Stanzl U, Jennewein P, Janke K, Heufler C, Kampgen E. High level IL-12 production by murine dendritic cells: upregulation via MHC class II and CD40 molecules and downregulation by IL-4 and IL-10. *J Exp Med* 1996;184:741–6.
- Kohl J, Baelder R, Lewkowich IP, Pandey MK, Hawlisch H, Wang L. A regulatory role for the C5a anaphylatoxin on type 2 immunity in asthma. *J Clin Invest* 2006;116:783-96
- Koning H, Neijens HJ, Baert MR, Oranje AP, Savelkoul HF. T cells subsets and cytokines in allergic and non-allergic children. II. Analysis and IL-5 and IL-10 mRNA expression and protein production. *Cytokine*. 1997;9(6):427-36
- Koya T, Matsuda H, Takeda K, Matsubara S, Miyahara N, Balhorn A, Dakhama A, Gelfand EW. IL-10-treated dendritic cells decrease airway hyperresponsiveness and airway inflammation in mice. *J Allergy Clin Immunol* 2007; Epub ahead of print
- Kretschmer K, Apostolou I, Hawiger D, Khazaie K, Nussenzweig MC, von Boehmer H. Inducing and expanding regulatory T cell populations by foreign antigen. *Nat Immunol* 2005;6:1219–1227.
- Kuchroo VK, Das MP, Brown JA, Ranger AM, Zamvil SS, Sobel RA, Weiner HL, Nabavi N, Glimcher LH. B7–1 and B7–2 costimulatory molecules activate differentially the Th1/Th2 developmental pathways: application to autoimmune disease therapy. *Cell* 1995;80:707-718.
- Kuhn R, Lohler J, Rennick D, Rajewsky K, Muller W. Interleukin- 10-deficient mice develop chronic enterocolitis. *Cell* 1993;75:263–74
- Kuipers H, Heirman C, Hijdra D, Muskens F, Willart M, Van Meirvenne S, et al. Dendritic cells retrovirally overexpressing IL-12 induce strong Th1

responses to inhaled antigen in the lung but fail to revert established Th2 sensitization. *J Leukoc Biol* 2004;76:1028-38.

- Kuipers H, Hijdra D, De Vries VC, Hammad H, Prins JB, Coyle AJ. Lipopolysaccharide-induced suppression of airway Th2 Responses does not require IL-12 production by dendritic cells. *J Immunol* 2003; 171:3645-54.
- Kuipers H, Lambrecht BN. The interplay of dendritic cells, Th2 cells and regulatory T cells in asthma. *Curr Opin Immunol* 2004;16:702-8.
- Kuwana M, Kaburaki J, Wright TM, Kawakami Y, Ikeda Y. Induction of antigenspecific human CD4(+) T cell anergy by peripheral blood DC2 precursors. *Eur J Immunol* 2001;31:2547-2457.
- Kuwana M. Induction of anergic and regulatory T cells by plasmacytoid dendritic cells and other dendritic cell subsets. *Hum Immunol* 2002;63:1156-1163.
- Lamb JR, Skidmore BJ, Green N, Chiller JM, Feldmann M. Induction of tolerance in influenza virus-immune T lymphocyte clones with synthetic peptides of influenza hemagglutinin. *J Exp Med.* 1983;157(5):1434-47.
- Lambrecht BN, De Veerman M, Coyle AJ, Gutierrez-Ramos JC, Thielemans K, Pauwels RA. Myeloid dendritic cells induce Th2 responses to inhaled antigen, leading to eosinophilic airway inflammation. *J Clin Invest* 2000; 106:551-9.
- Lambrecht BN, Pauwels RA, Fazekas De St Groth B. Induction of rapid T cell activation, division, and recirculation by intratracheal injection of dendritic cells in a TCR transgenic model. *J Immunol* 2000;164:2937-2946.
- Langenkamp A, Messi M, Lanzavecchia A, Sallusto F. Kinetics of dendritic cell activation: impact on priming of TH1, TH2 and nonpolarized T cells. *Nat Immunol* 2000;1:311-6.
- Lanzavecchia A, Sallusto F. Regulation of T cell immunity by dendritic cells. *Cell* 2001;106:263-266.
- Lee CG, Homer RJ, Zhu Z, Lanone S, Wang X, Koteliansky V, Shipley JM, Gotwals P, Noble P, Chen Q, Senior RM, Elias JA. Interleukin-13 induces tissue fibrosis by selectively stimulating and activating transforming growth factor beta(1). *J Exp Med.* 2001;194(6):809-21.
- Lee HJ, Takemoto N, Kurata H, et al. GATA-3 induces T helper cell type 2 (Th2) cytokine expression and chromatin remodeling in committed Th1 cells. *J Exp Med* 2000; 192(1):105-115
- Lee JW, Epardaud M, Sun J, Becker JE, Cheng CA, Yonekura A, Heath JK & Turley SJ. Peripheral antigen display by lymph node stroma promotes T cell tolerance to intestinal self. *Nat Immunol* 2007; 8(2): 181-190.
- Lei XF, Ohkawara Y, Stampfli MR, Mastruzzo C, Marr RA, Snider D, Xing Z, Jordana M. Disruption of antigen induced inflammatory responses in CD40 ligand knockout mice. *J Clin Invest* 1998; 101:1342-53.
- 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.

- 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.
- Levings MK, Sangregorio R, Galbiati F, Squadrone S, deWaal Malefyt R, Roncarolo MG. IFN-alpha and IL-10 induce the differentiation of human type 1 T regulatory cells. *J Immunol* 2001;166:5530–5539.
- Levings MK, Sangregorio R, Sartirana C, Moschin AL, Battaglia M, Orban PC, et al. Human CD25+CD4+ T suppressor cell clones produce transforming growth factor beta, but not interleukin 10, and are distinct from type 1 T regulatory cells. *J Exp Med.* 2002;196(10):1335-46
- Levy Y, Brouet JC. Interleukin-10 prevents spontaneous death of germinal center B cells by induction of the bcl-2 protein. *J. Clin. Invest* 1994;93:424–28
- Lewkowich IP, Herman NS, Schleifer KW, Dance MP, Chen BL, Dienger KM, Sproles AA, Shah JS, Kohl J, Belkaid Y, Wills-Karp M. CD4+CD25+ T cells protect against experimentally induced asthma and alter pulmonary dendritic cell phenotype and function. *J Exp Med.* 2005;202(11):1549-61.
- Lewkowich IP, Rempel JD, HayGlass KT. Prevention of allergen-specific, Th2-biased immune responses in vivo: role of increased IL-12 and IL-18 responsiveness. *J Immunol.* 2005;175(8):4956-62.
- Li J, Bracht M, Shang X, Radewonuk J, Emmell E, Griswold DE, Li L. Ex vivo activated OVA specific and non-specific CD4+CD25+ regulatory T cells exhibit comparable suppression to OVA mediated T cell responses. *Cell Immunol.* 2006;241(2):75-84
- Lim S, Crawley E, Woo P, Barnes PJ. Haplotype associated with low interleukin-10 production in patients with severe asthma *Lancet.* 1998;352(9122):113
- Ling EM, Smith T, Nguyen XD, Pridgeon C, Dallman M, Arbery J, Carr VA, Robinson DS. Relation of CD4+CD25+ regulatory T-cell suppression of allergen-driven T-cell activation to atopic status and expression of allergic disease. *Lancet* 2004; 363:608-615
- Liston A, Lesage S, Wilson J, Peltonen L, Goodnow CC. Aire regulates negative selection of organ-specific T cells. *Nat Immunol* 2003; 4(4):350-4.
- Liu H, Hu B, Xu D, Liew FY. CD4+CD25+ regulatory T cells cure murine colitis: the role of IL-10, TGF-beta, and CTLA4. *J Immunol.* 2003;171(10):5012-7
- Lloyd, C. M., Gonzalo, J. A., Coyle, A. J. & Gutierrez-Ramos, J. C. Mouse models of allergic airway disease. *Adv. Immunol* 2001;77, 263–295.
- Lohmann T, Leslie RD, Londei M. T cell clones to epitopes of glutamic acid decarboxylase 65 raised from normal subjects and patients with insulin-dependent diabetes. *J Autoimmun* 1996;9(3):385-9.
- Looney RJ, Pudiak D, Rosenfeld SI. Cytokine production by mite-specific T cells from donors with mild atopic disease. *J Allergy Clin Immunol.* 1994;93(2):476-83

- Lu L, Werneck MBF, Cantor H. The immunoregulatory effects of Qa-1. *Immunol Rev* 2006;212:51–59.
- Lu, L., Lee, W. C., Takayama, T., Qian, S., Gambotto, A., Robbins, P. D., Thomson, A. W. Genetic engineering of dendritic cells to express immunosuppressive molecules (viral IL-10, TGF-beta, and CTLA4Ig). *J. Leukoc. Biol* 1999;66, 293–296.
- Macfarlane AJ, Kon OM, Smith SJ, et al. Basophils, eosinophils, and mast cells in atopic and nonatopic asthma and in late-phase allergic reactions in the lung and skin. *J Allergy Clin Immunol* 2000;105:99-107.
- Mahnke K, Enk AH. Dendritic cells: key cells for the induction of regulatory T cells? *Curr Top Microbiol Immunol* 2005;293:133–150.
- Mahnke K, Knop J, Enk AH. Induction of tolerogenic DCs: ‘you are what you eat’. *Trends Immunol* 2003;24:646–51.
- Mahnke K, Qian Y, Knop J, Enk AH. Induction of CD4+/CD25+ regulatory T cells by targeting of antigens to immature dendritic cells. *Blood* 2003;101:4862–4869.
- Mahnke K, Schmitt E, Bonifaz L, Enk AH, Jonuleit H. Immature, but not inactive: the tolerogenic function of immature dendritic cells. *Immunol Cell Biol* 2002;80:477–83.
- Manavalan JS, Rossi PC, Vlad G, Piazza F, Yarilina A, Cortesini R, Mancini D, Suci-Foca N. High expression of ILT3 and ILT4 is a general feature of tolerogenic dendritic cells. *Transpl Immunol* 2003;11:245–258.
- Manetti R, Annunziato F, Tomasevic L, Gianni V, Parronchi P, Romagnani S. Polyinosinic acid:polycytidylic acid promotes T helper type 1-specific immune responses by stimulating macrophage production of interferon alpha and interleukin-12. *Eur J Immunol* 1999;25:2656–2660
- Manetti R, Parronchi P, Giudizi MG, Piccinni MP, Maggi E, Trinchieri G. Natural killer cell stimulatory factor (interleukin-12) induces T helper type 1 (Th1)-specific immune responses and inhibits the development of IL-4-producing Th cells. *J Exp Med* 1993;177:1199–1204
- Marie JC, Letterio JJ, Gavin M, Rudensky AY. TGF-beta1 maintains suppressor function and Foxp3 expression in CD4+CD25+ regulatory T cells. *J Exp Med*. 2005;201(7):1061.
- Marshall NA, Christie LE, Munro LR, Culligan DJ, Johnston PW, Barker RN. Immunosuppressive regulatory T cells are abundant in the reactive lymphocytes of Hodgkin lymphoma. *Blood* 2004;103(5):1755–62.
- Marsland BJ, Battig P, Bauer M, Ruedl C, Lassing U, Beerli RR. CCL19 and CCL21 induce a potent proinflammatory differentiation program in licensed dendritic cells. *Immunity* 2005;22:493-505
- Matsuda H, Suda T, Hashizume H, Yokomura K, Asada K, Suzuki K, Chida K, Nakamura H. Alteration of balance between myeloid dendritic cells and plasmacytoid dendritic cells in peripheral blood of patients with asthma. *Am J Respir Crit Care Med* 2002; 166:1050–4.

- 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.
- McGuirk P, McCann C, Mills KH. Pathogenspecific 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.
- Mehlhop PD, van de Rijn M, Brewer JP, Kisselgof AB, Geha RS, Oettgen HC, Martin TR. CD40L, but not CD40, is required for allergen-induced bronchial hyperresponsiveness in mice. *Am J Respir Cell Mol Biol* 2000; 23: 646–51.
- Menges M, Rossner S, Voigtlander C, Schindler H, Kukutsch NA, Bogdan C, Erb K, Schuler G, Lutz MB. Repetitive injections of dendritic cells matured with tumor necrosis factor alpha induce antigen-specific protection of mice from autoimmunity. *J Exp Med* 2002;195:15–21
- Meyer EH, Goya S, Akbari O, Berry GJ, Savage PB, Kronenberg M, Nakayama T, DeKruyff RH, Umetsu DT. Glycolipid activation of invariant T cell receptor+ NK T cells is sufficient to induce airway hyperreactivity independent of conventional CD4+ T cells. *Proc Natl Acad Sci USA* 2006;103:2782-2787.
- Misra N, Bayry J, Lacroix-Desmazes S, Kazatchkine MD, Kaveri SV. Cutting Edge: Human CD4+CD25+ T cells restrain the maturation and antigen-presenting function of dendritic cells. *J Immunol* 2004;172:4676–80.
- 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. Review
- Morgan ME, Flierman R, van Duivenvoorde LM, Witteveen HJ, van Ewijk W, van Laar JM, et al. Effective treatment of collagen-induced arthritis by adoptive transfer of CD25+ regulatory T cells. *Arthritis Rheum.* 2005; 52(7):2212-21.
- Mottet C, Uhlig HH, Powrie F. Cutting edge: cure of colitis by CD4+CD25+ regulatory T cells. *J Immunol* 2003;170:3939-3943.
- Muller B. Interleukin-10 in Nasal Mucosa Protects Against Allergic Symptoms. *J Allergy Clin Immunology* 2007; 19 (2): 525.
- Muller G, Muller A, Tuting T, Steinbrink K, Saloga J, Szalma C. Interleukin-10-treated dendritic cells modulate immune responses of naive and sensitized T cells in vivo. *J Invest Dermatol* 2002;119:836–41.
- Muller UR, Akdis CA, Fricker M, Akdis M, Bettens F, Blesken T, Blaser K. Successful immunotherapy with T cell epitope peptides of bee venom phospholipase A2 induces specific T cell anergy in bee sting allergic patients. *J Allergy Clinimmunol* 1998;101:747–54.
- Munn DH, Pressey J, Beall AC, Hudes R, Alderson MR. Selective activation-induced apoptosis of peripheral T cells imposed by macrophages. A potential mechanism of antigen-specific peripheral lymphocyte deletion. *J Immunol* 1996; 156(2):523-32.

- Murray PJ, Young RA. Increased antimycobacterial immunity in interleukin-10-deficient mice. *Infect. Immun* 1999;67:3087–95
- Nakamura K, Kitani A, Fuss I, Pedersen A, Harada N, Nawata H, Strober W. TGF-beta 1 plays an important role in the mechanism of CD4+CD25+ regulatory T cell activity in both humans and mice. *J Immunol* 2004;172:834–842.
- Nakamura K, Kitani A, Strober W. Cell contact-dependent immunosuppression by CD4(+)CD25(+) regulatory T cells is mediated by cell surface-bound transforming growth factor beta. *J Exp Med* 2001;194:629–644.
- Nakamura Y, Ghaffar O, Olivenstein R, Taha RA, Soussi-Gounni A, Zhang DH, et al.: Gene expression of the GATA-3 transcription factor is increased in atopic asthma. *J Allergy Clin Immunol* 1999; 103(2,Pt 1):215–222.
- Ng WF, Duggan PJ, Ponchel F, Matarese G, Lombardi G, Edwards AD, et al. Human CD4(+)CD25(+) cells: a naturally occurring population of regulatory T cells. *Blood*. 2001;98(9):2736-44
- Ngoc LP, Gold DR, Tzianabos AO, Weiss ST, Celedon JC. Cytokines, allergy and asthma. *Curr Opin Allergy Clin Immunol* 2005; 5: 161-6
- Noma T, Sugawara Y, Ogawa N, Saeki T, Yamaguchi K, Kawano Y. Dermatophagoides-induced interleukin-10 production by peripheral blood lymphocytes from patients with asthma in remission. *Pediatr Allergy Immunol* 2004;15(5):459-68
- Nurse B, Puterman AS, Haus M, Berman D, Weinberg EG, Potter PC. PBMCs from both atopic asthmatic and nonatopic children show a TH2 cytokine response to house dust mite allergen. *J Allergy Clin Immunol*. 2000; 106 (1 Pt):84-91
- O'Brien RM, Thomas WR, Wootton AM. T cell responses to the purified major allergens from the house dust mite *Dermatophagoides pteronyssinus*. *J Allergy Clin Immunol*. 1992;89(5):1021-31
- Oh JW, Seroogy CM, Meyer EH, Akbari O, Berry G, Fathman CG, Dekruyff RH, Umetsu DT. CD4 T-helper cells engineered to produce IL-10 prevent allergen-induced airway hyperreactivity and inflammation. *J Allergy Clin Immunol*. 2002;110(3):460-8.
- Ohki O, Yokozeki H, Katayama I, Nishioka K. Functional CD86 (B7.2/B70) is predominantly expressed on Langerhans cells in atopic dermatitis. *Br J Dermatol*. 1997;136:838-845.
- Okura Y, Jee Y, Matsumoto Y. Acquired thymic tolerance to autoimmune encephalomyelitis is associated with activation of peripheral IL-10-producing macrophages/dendritic cells. *Int Immunol*. 2003;15(3):437-46
- Onizuka S, Tawara I, Shimizu J, Sakaguchi S, Fujita T, Nakayama E. Tumor rejection by in vivo administration of anti-CD25 (interleukin-2 receptor alpha) monoclonal antibody. *Cancer Res* 1999;59:3128–3133.
- Oriss TB, Ostroukhova M, Seguin-Devaux C, Dixon-McCarthy B, Stolz DB, Watkins SC. Dynamics of dendritic cell phenotype and interactions with CD41 T cells in airway inflammation and tolerance. *J Immunol* 2005;174:854-63.

- Ostroukhova M, Seguin-Devaux C, Oriss TB, Dixon-McCarthy B, Yang L, Ameredes BT. Tolerance induced by inhaled antigen involves CD4(1) T cells expressing membrane-bound TGF-beta and FOXP3. *J Clin Invest* 2004;114:28-38.
- Ouyang W, Ranganath SH, Weindel K, et al. Inhibition of Th1 development mediated by GATA-3 through an IL-4-independent mechanism. *Immunity* 1998; 9(5):745–755.
- Pandurangan V, Seumois G, Pickard C, Powell RM, Angco G, Sammut D, Gadola SD, Friedmann PS and Djukanović R. Invariant Natural Killer T Cells in Asthma and Chronic Obstructive Pulmonary Disease. *N Engl Med* 2007; 356: 1410-1422.
- Panina-Bordignon, P., Mazzeo, D., Lucia, P. D., D’Ambrosio, D., Lang, R., Fabbri, L., Self, C., & Sinigaglia, F. Beta2-agonists prevent Th1 development by selective inhibition of interleukin 12. *J Clin Invest.* 1997; 100, 1513– 1519.
- Papadakis KA, Landers C, Prehn J, Kouroumalis EA, Moreno ST, Gutierrez-Ramos JC, Hodge MR, Targan SR. CC chemokine receptor 9 expression defines a subset of peripheral blood lymphocytes with mucosal T cell phenotype and Th1 or T-regulatory 1 cytokine profile. *J Immunol* 2003;171:159–165.
- Parrish-Novak J, Dillon SR, Nelson A, et al.: Interleukin 21 and its receptor are involved in NK cell expansion and regulation of lymphocyte function. *Nature* 2000; 408(6808):57–63.
- Parronchi P, Brugnolo F, Annunziato F, Manuelli C, Sampognaro S, Mavilia C et al. Phosphorothioate oligodeoxynucleotides promote the in vitro development of human allergen-specific CD4+ T cells into Th1 effectors. *J Immunol* 1999;163:5946–5953.
- Parronchi P, De Carli M, Manetti R, Simonelli C, Sampognaro S, Piccinni MP et al. IL-4 and IFN(s) (alpha and gamma) exhibit opposite regulatory effects on the development of cytolytic potential by TH1 or TH2 human T cell clones. *J Immunol* 1992;149:2977–2982.
- Pasare C, Medzhitov R. Toll pathway-dependent blockade of CD4+CD25+ T cell-mediated suppression by dendritic cells. *Science* 2003;299:1033-1036.
- Penna G, Giarratana N, Amuchastegui S, Mariani R, Daniel KC, Adorini L. Manipulating dendritic cells to induce regulatory T cells. *Microbes Infect* 2005;7:1033–1039.
- Pestka S, Krause CD, Sarkar D, Walter MR, Shi Y, Fisher PB. Interleukin-10 and related cytokines and receptors. *Annu Rev Immunol* 2004;22:929–979.
- Pette M, Fujita K, Kitze B, Whitaker JN, Albert E, Kappos L, Wekerle H: Myelin basic protein-specific T lymphocyte lines from MS patients and healthy individuals. *Neurology* 1990; 40(11):1770-6.
- Picca CC, Larkin J, Boesteanu A, Lerman MA, Rankin AL, Caton AJ. Role of TCR specificity in CD4+ CD25+ regulatory T-cell selection. *Immunol Rev.* 2006;212:74-85. Review

- Piccirillo CA, Letterio JJ, Thornton AM, McHugh RS, Mamura M, Mizuhara H, Shevach EM. CD4(+)CD25(+) regulatory T cells can mediate suppressor function in the absence of transforming growth factor beta1 production and responsiveness. *J Exp Med* 2002;196:237–246.
- Pierkes M, Bellinghausen I, Hultsch T, Metz G, Knop J, Saloga J. Decreased release of histamine and sulfidoleukotrienes by human peripheral blood leukocytes after wasp venom immunotherapy is partially due to induction of IL-10 and IFN-gamma production of T cells. *J Allergy Clin Immunol* 1999; 103:326–32.
- Powell JD. The induction and maintenance of T cell anergy. *Clin Immunol.* 2006;120(3):239-46.
- Power, C. A., Church, D. J., Meyer, A., Alouani, S., Proudfoot, A. E., ClarkLewis, I., Sozzani, S., Mantovani, A., & Wells, T. N. Cloning and characterization of a specific receptor for the novel CC chemokine MIP-3alpha from lung dendritic cells. *J Exp Med.* 1997; 186, 825–835.
- Powrie F. Immune regulation in the intestine: a balancing act between effector and regulatory T cell responses. *Ann N Y Acad Sci.* 2004 Dec;1029:132-41
- Prescott S, Macaubas C, Holt BJ, Smallacombe TB, Loh R, Sly PD, Holt PG. Transplacental priming of the human immune system to environmental allergens: universal skewing of initial T cell responses toward the Th2 cytokine profile. *J Immunol* 1998; 160:4730-7.
- Ramsdell F. Foxp3 and natural regulatory T cells: key to a cell lineage? *Immunity.* 2003;19(2):165-8. Review
- Reddy J, Illes Z, Zhang X, Encinas J, Pyrdol J, Nicholson L, et al. Myelin proteolipid protein-specific CD4+CD25+ regulatory cells mediate genetic resistance to experimental autoimmune encephalomyelitis. *Proc Natl Acad Sci U S A.* 2004 Oct 26;101(43):15434-9
- Robinson DS, Hamid Q, Bentley A, Ying S, Kay AB, Durham SR. Activation of CD4+ T cells, increased TH2-type cytokine mRNA expression, and eosinophil recruitment in bronchoalveolar lavage after allergen inhalation challenge in patients with atopic asthma. *J Allergy Clin Immunol* 1993;92(3):397-403.
- Robinson, DS TH2 cytokines in allergic disease. *Br. Med. Bull* 2000;56, 956–968.
- Rogers PR, Croft M. Peptide dose, affinity, and time of differentiation can contribute to the Th1/Th2 cytokine balance. *J Immunol* 1999;163: 1205-13.
- Romagnani S. Immunologic influences on allergy and the TH1/TH2 balance. *J Allergy Clin Immunol* 2004; 113:395– 400.
- Romagnani, S. Regulation of the development of type 2 T-helper cells in allergy. *Curr. Opin. Immunol* 1994;6, 838–846.
- Romani N, Koide S, Crowley M, Witmer-Pack M, Livingstone AM, Fathman CG, Inaba K, Steinman RM. Presentation of exogenous protein antigens by dendritic cells to T cell clones. Intact protein is presented best by immature, epidermal Langerhans cells. *J Exp Med.* 1989;169(3):1169-78.

- Roncarolo MG, Gregori S, Battaglia M, Bacchetta R, Fleischhauer K, Levings MK. Interleukin-10-secreting type 1 regulatory T cells in rodents and humans. *Immunol Rev* 2006;212:28-50. Review.
- Roncarolo MG, Levings MK. The role of different subsets of T regulatory cells in controlling autoimmunity. *Curr Opin Immunol* 2000;12:676-683.
- Rossi, D. & Zlotnik, A. The biology of chemokines and their receptors. *Annu. Rev. Immunol.* 2000;18, 217-242.
- Sakaguchi S, Ono M, Setoguchi R, Yagi H, Hori S, Fehervari Z, Shimizu J, Takahashi T, Nomura T. Foxp3⁺ CD25⁺ CD4⁺ natural regulatory T cells in dominant self-tolerance and autoimmune disease. *Immunol Rev.* 2006;212:8-27. Review
- Sakaguchi S. Naturally arising Foxp3-expressing CD25⁺CD4⁺ regulatory T cells in immunological tolerance to self and nonself. *Nat Immunol* 2005;6:345-352.
- Salek-Ardakani S, Song J, Halteman BS et al. OX40 (CD134) controls memory T helper 2 cells that drive lung inflammation. *J Exp Med* 2003; 198:315-24.
- Salek-Ardakani S, Song J, Halteman BS, Jember AG, Akiba H, Yagita H, Croft M. OX40 (CD134) controls memory T helper 2 cells that drive lung inflammation. *J Exp Med* 2003; 198:315-324.
- Salomon B, Lenschow DJ, Rhee L, Ashourian N, Singh B, Sharpe A, et al. B7/CD28 costimulation is essential for the homeostasis of the CD4⁺CD25⁺ immunoregulatory T cells that control autoimmune diabetes. *Immunity.* 2000;12(4):431-40
- Sanderson CJ. The biological role of interleukin 5. *Int J Cell Cloning.* 1990; 8 Suppl 1:147-53; discussion 153-4. Review
- Sato K, Nagayama H, Tadokoro K, Juji T, Takahashi TA. Extracellular signal-regulated kinase, stress-activated protein kinase/c-Jun N-terminal kinase, and p38mapk are involved in IL-10-mediated selective repression of TNF-alpha-induced activation and maturation of human peripheral blood monocyte-derived dendritic cells. *J Immunol.* 1999;162(7):3865-72.
- Sato K, Yamashita N, Baba M, Matsuyama T. Modified myeloid dendritic cells act as regulatory dendritic cells to induce anergic and regulatory T cells. *Blood* 2003;101:3581-3589.
- Sato K, Yamashita N, Baba M, Matsuyama T. Regulatory dendritic cells protect mice from murine acute graft-versus-host disease and leukemia relapse. *Immunity.* 2003;18(3):367-79
- Sato K, Yamashita N, Matsuyama T. Human peripheral blood monocyte-derived interleukin-10-induced semi-mature dendritic cells induce anergic CD4(+) and CD8(+) T cells via presentation of the internalized soluble antigen and cross-presentation of the phagocytosed necrotic cellular fragments. *Cell Immunol* 2002;215:186-94.
- Satoguina J, Mempel M, Larbi J, Badusche M, Loliger C, Adjei O, et al. Antigen-specific T regulatory-1 cells are associated with immunosuppression

in a chronic helminth infection (onchocerciasis). *Microbes Infect* 2002;4(13):1230–91

- Savolainen J, Laaksonen K, Rantio-Lehtimäki A, Terho EO. Increased expression of allergen-induced in vitro interleukin-10 and interleukin-18 mRNA in peripheral blood mononuclear cells of allergic rhinitis patients after specific immunotherapy. *Clin Exp Allergy*. 2004;34(3):413-9.
- Sawicka E, Zuany-Amorim C, Manlius C, Trifilieff A, Brinkmann V, Kemeny DM, Walker C. Inhibition of TH1- and TH2-mediated airway inflammation by the sphingosine 1 phosphate receptor agonist FTY720. *J. Immunol* 2003;171, 6206–6214.
- Schofield L, McConville MJ, Hansen D, Campbell AS, Fraser-Reid B, Grusby MJ, Tachado SD. CD1d-restricted immunoglobulin G formation to GPI-anchored antigens mediated by NKT cells. *Science* 1999;283:225–229.
- Schubert L, Jeffery E, Zhang Y, Ramsdell F, Ziegler S. Scurfin (FOXP3) acts as a repressor of transcription and regulates T cell activation. *J Biol Chem* 2001;276:37672–37679
- Schuler G, Steinman RM. Murine epidermal Langerhans cells mature into potent immunostimulatory dendritic cells in vitro. *J Exp Med* 1985;161:526–46.
- Schwarz MA, Hamilton LD, Tardelli L, Narula SK, Sullivan LM. Stimulation of cytolytic activity by interleukin-10. *J Immunother Emphasis Tumor Immunol* 1994;16:95–104.
- Sears MR. Epidemiology of childhood asthma. *Lancet*. 1997;350(9083):1015-20.
- Sebastiani S, Allavena P, Albanesi C, Nasorri F, Bianchi G, Traidl C, Sozzani S, Girolomoni G, Cavani A. Chemokine receptor expression and function in CD4+ T lymphocytes with regulatory activity. *J Immunol* 2001;166:996–1002.
- Sebzda E, Wallace VA, Mayer J, Yeung RS, Mak TW, Ohashi PS. Positive and negative thymocyte selection induced by different concentrations of a single peptide. *Science* 1994; 263(5153):1615-8.
- Semana G, Gausling R, Jackson RA, Hafler DA. T cell autoreactivity to proinsulin epitopes in diabetic patients and healthy subjects. *J Autoimmun* 1999; 12(4):259-67.
- Serra P, Amrani A, Yamanouchi J, Han B, Thiessen S, Utsugi T, Verdaguer J, Santamaria P. CD40 ligation releases immature dendritic cells from the control of regulatory CD4+CD25+ T cells. *Immunity* 2003;19:877-889.
- Setoguchi R, Hori S, Takahashi T, Sakaguchi S. Homeostatic maintenance of natural Foxp3+ CD25+ CD4+ regulatory T cells by interleukin (IL)-2 and induction of autoimmune disease by IL-2 neutralization. *J Exp Med*. 2005;201(5):723-35.
- Sewnath ME, Olszyna DP, Birjmohun R, ten Kate FJ, Gouma DJ, van Der PT. IL-10-deficient mice demonstrate multiple organ failure and increased mortality during *Escherichia coli* peritonitis despite an accelerated bacterial clearance. *J. Immunol* 2001;166:6323–31

- Sharpe AH. Analysis of lymphocyte costimulation in vivo using transgenic and 'knockout' mice. *Curr Opin Immunol* 1995; 7(3):389-95.
- Shevach EM. CD4⁺ CD25⁺ suppressor T cells: more questions than answers. *Nat Rev Immunol* 2002;2:389–400.
- Shimizu J, Yamazaki S, Sakaguchi S. Induction of tumor immunity by removing CD25⁺CD4⁺ T cells: a common basis between tumor immunity and autoimmunity. *J Immunol* 1999;163:5211–5218.
- Shin T, Kennedy G, Gorski K, Tsuchiya H, Koseki H, Azuma M, Yagita H, Chen L, Powell J, Pardoll D, Housseau F. Cooperative B7-1/2 (CD80/ CD86) and B7-DC costimulation of CD41 T cells independent of the PD-1 receptor. *J Exp Med* 2003; 198:31–8.
- Shortman K. Cellular aspects of early T-cell development. *Curr Opin Immunol* 1992; 4(2):40-6.
- Sletten GB, Halvorsen R, Egaas E, Halstensen TS. Memory T Cell Proliferation in Cow's Milk Allergy after CD25⁺ Regulatory T Cell Removal Suggests a Role for Casein-Specific Cellular Immunity in IgE-Mediated but Not in Non-IgE-Mediated Cow's Milk Allergy. *Int Arch Allergy Immunol*. 2007;142(3):190-8.
- Smedt T, Van Mechelen M, De Becker G, Urbain J, Leo O, Moser M. Effect of interleukin-10 on dendritic cell maturation and function. *Eur. J. Immunol*. 1997; 27:1229-1235.
- Smit JJ, Van Loveren H, Hoekstra MO, Schijf MA, Folkerts G, Nijkamp FP. Mycobacterium vaccae administration during allergen sensitization or challenge suppresses asthmatic features. *Clin Exp Allergy*. 2003;33(8):1083-9
- Smith DH, Malone DC, Lawson KA, Okamoto LJ, Battista C, Saunders WB. A national estimate of the economic costs of asthma. *Am J Respir Crit Care Med* 1997;156:787-93.
- Smits HH, Engering A, van der Kleij D, de Jong EC, Schipper K, van Capel TM, Zaat BA, Yazdanbakhsh M, Wierenga EA, van Kooyk Y, Kapsenberg ML. Selective probiotic bacteria induce IL-10-producing regulatory T cells in vitro by modulating dendritic cell function through dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin. *J Allergy Clin Immunol* 2005;115: 1260–1267.
- Smits HH, van Beelen AJ, Hessle C, Westland R, de Jong E, Soeteman E, Wold A, Wierenga EA, Kapsenberg ML. Commensal Gram-negative bacteria prime human dendritic cells for enhanced IL-23 and IL-27 expression and enhanced Th1 development. *Eur J Immunol* 2004;34:1371-1380.
- Soumelis V, Reche PA, Kanzler H, Yuan W, Edward G, Homey B, Gilliet M, Ho S, Antonenko S, Lauerma A et al.: Human epithelial cells trigger dendritic cell mediated allergic inflammation by producing TSLP. *Nat Immunol* 2002; 3:673-680
- Sozzani S, Ghezzi S, Iannolo G, Luini W, Borsatti A, et al. Interleukin 10 increases CCR5 expression and HIV infection in human monocytes. *J. Exp. Med*. 1998;187:439–44

- Sprent J, Webb SR: Intrathymic and extrathymic clonal deletion of T cells. *Curr Opin Immunol* 1995; 7(2):196-205.
- Stampfli MR, Cwiartka M, Gajewska BU, Alvarez D, Ritz SA, Inman MD, Xing Z, Jordana M. Interleukin-10 gene transfer to the airway regulates allergic mucosal sensitization in mice. *Am J Respir Cell Mol Biol*. 1999;21(5):586-96.
- Stassen M, Jonuleit H, Muller C, Klein M, Richter C, Bopp T, Schmitt S, Schmitt E. Differential regulatory capacity of CD25+ T regulatory cells and preactivated CD25+ T regulatory cells on development, functional activation, and proliferation of TH2 cells. *J Immunol* 2004;173, 267–274.
- Steinbrink K, Graulich E, Kubsch S, Knop J, Enk AH. CD4(+) and CD8(+) anergic T cells induced by interleukin-10-treated human dendritic cells display antigen-specific suppressor activity. *Blood* 2002;99:2468–76.
- Steinbrink K, Jonuleit H, Muller G, Schuler G, Knop J, Enk AH. Interleukin-10-treated human dendritic cells induce a melanomaantigen- specific anergy in CD8(+) T cells resulting in a failure to lyse tumor cells. *Blood* 1999;93:1634–1642.
- Steinbrink K, Wolf M, Jonuleit H, Knop J, Enk AH. Induction of tolerance by IL-10- treated dendritic cells. *J Immunol* 1997;159:4772–4780.
- Steinman RM, Hawiger D, Nussenzweig MC. Tolerogenic dendritic cells. *Annu Rev Immunol* 2003; 21:685-711.
- Steinman RM, Pope M. Exploiting dendritic cells to improve vaccine efficacy. *J Clin Invest*. 2002 ;109(12):1519-26. Review.
- Steinman RM, Turley S, Mellman I, Inaba K. The induction of tolerance by dendritic cells that have captured apoptotic cells. *J Exp Med*. 2000; 191(3):411-417.
- Stock P, Akbari O, Berry G, Freeman GJ, Dekruyff RH, Umetsu DT. Induction of T helper type 1-like regulatory cells that express Foxp3 and protect against airway hyper-reactivity. *Nat Immunol*. 2004;5(11):1149-56.
- Strengell M, Sareneva T, Foster D, Julkunen I, Matikainen S. IL-21 up-regulates the expression of genes associated with innate immunity and Th1 response. *J Immunol* 2002; 169(7):3600–3605.
- Strobl H, Knapp W. TGF-beta1 regulation of dendritic cells. *Microbes Infect* 1999;1: 1283–1290.
- Stumbles PA, Thomas JA, Pimm CL, Lee PT, Venaille TJ, Proksch S, Holt PG. Resting respiratory tract dendritic cells preferentially stimulate T helper cell type 2 (Th2) responses and require obligatory cytokine signals for induction of Th1 immunity. *J Exp Med* 1998;188:2019-2031
- Su L, Creusot RJ, Gallo EM, Chan SM, Utz PJ, Fathman CG et al. Murine CD4+CD25+ regulatory T cells fail to undergo chromatin remodeling across the proximal promoter region of the IL-2 gene. *J Immunol*. 2004; 173(8):4994-5001.
- Sullivan TJ, Letterio JJ, van Elsas A, Mamura M, van Amelsfort J, Sharpe S, Metzler B, Chambers CA, Allison JP. Lack of a role for transforming growth

factor-beta in cytotoxic T lymphocyte antigen-4-mediated inhibition of T cell activation. *Proc Natl Acad Sci USA* 2001;98:2587–2592.

- Surh CD, Sprent J. T-cell apoptosis detected in situ during positive and negative selection in the thymus. *Nature* 1994; 372(6501):100-3.
- Suto A, Nakajima H, Kagami SI, Suzuki K, Saito Y, Iwamoto I. Role of CD4+ CD25+ regulatory T cells in T helper 2 cell-mediated allergic inflammation in the airways. *Am. J. Respir. Crit. Care Med* 2001;164, 680–687.
- Swanson KA, Zheng Y, Heidler KM, Zhang ZD, Webb TJ, Wilkes DS. Flt3-ligand, IL-4, GM-CSF, and adherence-mediated isolation of murine lung dendritic cells: assessment of isolation technique on phenotype and function. *J Immunol* 2004;173:4875-81.
- Taams LS, Vukmanovic-Stejić M, Smith J, Dunne PJ, Fletcher JM, Plunkett FJ, Ebeling SB, Lombardi G, Rustin MH, Bijlsma JW, Lafeber FP, Salmon M, Akbar AN. Antigen-specific T cell suppression by human CD4+CD25+ regulatory T cells. *Eur J Immunol* 2002;32:1621–1630.
- Takahashi H, Ebihara S, Ebihara S, Kanda A, Kamanaka M, Sato T, Habu S, Kikutani H, Sasaki H. Increased susceptibility to airway responses in CD40-deficient mice. *Clin Exp Immunol* 2003; 133:22–9.
- Takayama, T., Morelli, A. E., Onai, N., Hirao, M., Matsushima, K., Tahara, H., Thomson, A. W. Mammalian and viral IL-10 enhance C–C chemokine receptor 5 but down-regulate C–C chemokine receptor 7 expression by myeloid dendritic cells: impact on chemotactic responses and in vivo homing ability. *J. Immunol* 2001;166, 7136–7143
- Takayama, T., Nishioka, Y., Lu, L., Lotze, M. T., Tahara, H., Thomson, A. W. Retroviral delivery of viral interleukin-10 into myeloid dendritic cells markedly inhibits their allostimulatory activity and promotes the induction of T-cell hyporesponsiveness. *Transplantation* 1998;66, 1567–1574.
- Takayama, T., Tahara, H., Thomson, A. W. Differential effects of myeloid dendritic cells retrovirally transduced to express mammalian or viral interleukin-10 on cytotoxic T lymphocyte and natural killer cell functions and resistance to tumor growth. *Transplantation* 2001;71, 1334– 1340.
- Takei M, Umeyama A, Arihara S. Epicubenol and Ferruginol induce DC from human monocytes and differentiate IL-10-producing regulatory T cells in vitro. *Biochem Biophys Res Commun* 2005;337:730–738.
- Taketomi EA, Silva DA, Sopelete MC, Gervasio AM, Alves R, Sung SJ. Differential IgE reactivity to Der p 1 and Der p 2 allergens of *Dermatophagoides pteronyssinus* in mite-sensitized patients. *J Investig Allergol Clin Immunol*. 2006;16(2):104-9.
- Tang Q, Bluestone JA. Visualizing regulatory T cell control of autoimmune responses in nonobese diabetic mice. *Nat Immunol* 2006;7:83–92.
- Tang Q, Henriksen KJ, Bi M, Finger EB, Szot G, Ye J, et al. In vitro-expanded antigen-specific regulatory T cells suppress autoimmune diabetes. *J Exp Med*. 2004;199(11):1455-65

- Taylor PA, Lees CJ, Blazar BR. The infusion of ex vivo activated and expanded CD4(+)CD25(+) immune regulatory cells inhibits graft-versus-host disease lethality. *Blood*. 2002;99(10):3493-9.
- The International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee. Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema: ISAAC. *Lancet* 1998;351, 1225–1232.
- Thornton AM, Shevach EM. CD4+CD25+ immunoregulatory T cells suppress polyclonal T cell activation in vitro by inhibiting interleukin 2 production. *J Exp Med* 1998;188:287–296.
- Thorstenson KM, Khoruts A. Generation of anergic and potentially immunoregulatory CD25+CD4 T cells in vivo after induction of peripheral tolerance with intravenous or oral antigen. *J Immunol* 2001;167:188–195.
- Tough DF, Sprent J: Turnover of naive and memory-phenotype T cells. *J Exp Med* 1994; 179(4):1127-35.
- Tournoy KG, Kips JC, Pauwels RA. Endogenous interleukin-10 suppresses allergen-induced airway inflammation and nonspecific airway responsiveness. *Clin. Exp. Allergy* 2000;30:775– 83
- Tournoy KG, Kips JC, Pauwels RA. Is Th1 the solution for Th2 in asthma? *Clin Exp Allergy* 2002; 31:17–29.
- Trinchieri G. Interleukin-12 and the regulation of innate resistance and adaptive immunity. *Nat Rev Immunol* 2003; 3:133-146.
- Tsai JJ, Shen HD, Chua KY. Purification of group 2 Dermatophagoides pteronyssinus allergen and prevalence of its specific IgE in asthmatics. *Int Arch Allergy Immunol*. 2000;121(3):205-10
- Tsitoura DC, Dekruyff RH, Lamb JR, Umetsu DT. Intranasal exposure to protein antigen induces immunological tolerance mediated by functionally disabled CD4+ T cells. *J Immunol* 1999;163:2592-2600.
- Turcanu V, Maleki SJ, Lack G. Characterization of lymphocyte responses to peanuts in normal children, peanut allergic children, and allergic children who acquired tolerance to peanuts. *J Clin Invest* 2003;111:1065–1072.
- Van Bever HP, Vereecke IF, Bridts CH, De Clerck LS, Stevens WJ. Comparison between the in vitro cytokine production of mononuclear cells of young asthmatics with and without immunotherapy (IT) *Clin Exp Allergy*. 1998;28(8):943-9.
- Van Buskirk AM, et al. Human allograft acceptance is associated with immune regulation. *J Clin Invest* 2000;106:145–155.
- Van Rijt LS, Jung S, Kleinjan A, Vos N, Willart M, Duez C, et al. In vivo depletion of lung CD11c+ dendritic cells during allergen challenge abrogates the characteristic features of asthma. *J Exp Med* 2005;201: 981-91.
- Van Rijt LS, Vos N, Willart M, Kleinjan A, Coyle AJ, Hoogsteden HC, et al. Essential role of dendritic cell CD80/CD86 costimulation in the induction, but not reactivation, of TH2 effector responses in a mouse model of asthma. *J Allergy Clin Immunol* 2004;114:166-73.

- Van Scott MR, Justice JP, Bradfield JF, Enright E, Sigounas A, Sur S. IL-10 reduces Th2 cytokine production and eosinophilia but augments airway reactivity in allergic mice. *Am J Physiol Lung Cell Mol Physiol.* 2000;278(4):L667-74
- Varadhachary AS, Salgame P. CD95 mediated T cell apoptosis and its relevance to immune deviation. *Oncogene* 1998; 17(25):3271-6.
- Vazquez-Torres A, Jones-Carson J, Wagner RD, Warner T, Balish E. Early resistance of interleukin-10 knockout mice to acute systemic candidiasis. *Infect. Immun* 1999; 67:670–74
- Veldman C, Hohne A, Dieckmann D, Schuler G, Hertl M. Type I regulatory T cells specific for desmoglein 3 are more frequently detected in healthy individuals than in patients with pemphigus vulgaris. *J Immunol* 2004;172:6468–6475.
- Velten FW, Duperrier K, Bohlender J, Metharom P, Goerdts S. A gene signature of inhibitory MHC receptors identifies a BDCA3(+) subset of IL-10-induced dendritic cells with reduced allostimulatory capacity in vitro. *Eur J Immunol.* 2004;34(10):2800-11
- Verhagen J, Akdis M, Traidl-Hoffmann C, Schmid-Grendelmeier P, Hijnen D, Knol EF, Behrendt H, Blaser K, Akdis CA. Absence of T-regulatory cell expression and function in atopic dermatitis skin. *J Allergy Clin Immunol.* 2006;117(1):176-83.
- Vermaelen KY, Carro-Muino I, Lambrecht BN, Pauwels RA. Specific migratory dendritic cells rapidly transport antigen from the airways to the thoracic lymph nodes. *J Exp Med* 2001;193:51–60.
- Vieira PL, Christensen JR, Minaee S, O'Neill EJ, Barrat FJ, Boonstra A, Barthlott T, Stockinger B, Wraith DC, O'Garra A. 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.
- Villarino AV, Huang E, Hunter CA. Understanding the pro- and anti-inflammatory properties of IL-27. *J Immunol* 2004;173:715-20.
- Voehringer D, Shinkai K, Locksley RM. Type 2 immunity reflects orchestrated recruitment of cells committed to IL-4 production. *Immunity* 2004;20:267-277.
- Von Boehmer H. Mechanisms of suppression by suppressor T cells. *Nat Immunol.* 2005; 6(4):338-44. Review.
- Von Ehrenstein OS, Von Mutius E, Illi S, Baumann L, Bohm O, von Kries R. Reduced risk of hay fever and asthma among children of farmers. *Clin Exp Allergy.* 2000;(2):187-93.
- Wakkach A, Fournier N, Brun V, Breitmayer JP, Cottrez F, Groux H. Characterization of dendritic cells that induce tolerance and T regulatory 1 cell differentiation in vivo. *Immunity* 2003;18:605–17.

- Walker MR, Carson BD, Nepom GT, Ziegler SF, Buckner JH. De novo generation of antigen-specific CD4+CD25+ regulatory T cells from human CD4+CD25- cells. *Proc Natl Acad Sci USA* 2005;102:4103–4108.
- Walker MR, Kaspirowicz DJ, Gersuk VH, Benard A, Van Landeghen M, Buckner JH, Ziegler SF. Induction of FoxP3 and acquisition of T regulatory activity by stimulated human CD4+CD25- T cells. *J Clin Invest* 2003;112:1437–1443.
- Wang J, Fathman JW, Lugo-Villarino G, Scimone L, von Andrian U, Dorfman DM, et al. Transcription factor T-bet regulates inflammatory arthritis through its function in dendritic cells. *J Clin Invest* 2006;116: 414-21.
- Wang J, Ioan-Facsinay A, van der Voort EI, Huizinga TW, Toes RE. Transient expression of FOXP3 in human activated nonregulatory CD4+ T cells. *Eur J Immunol.* 2007;37(1):129-38
- Watanabe N, Hanabuchi S, Marloie-Provost MA, Antonenko S, Liu YJ, Soumelis V. Human TSLP promotes CD40 ligand-induced IL-12 production by myeloid dendritic cells but maintains their Th2 priming potential. *Blood* 2005;105:4749-51.
- Watts TH, DeBenedette MA. T cell co-stimulatory molecules other than CD28. *Curr Opin Immunol* 1999; 11:286–93.
- Weston LE, Geczy AF, Briscoe H. Production of IL-10 by alloreactive sibling donor cells and its influence on the development of acute GVHD. *Bone Marrow Transplant* 2006;37:207–212.
- Willems F, Marchant A, Delville JP, Gerard C, Delvaux A, et al. Interleukin-10 inhibits B7 and intercellular adhesion molecule-1 expression on human monocytes. *Eur. J. Immunol.* 1994;24:1007–9
- Woltman AM, van Kooten C. Functional modulation of dendritic cells to suppress adaptive immune responses. *J Leukoc Biol* 2003;73:428–441.
- Wood KJ, Sakaguchi S. Regulatory T cells in transplantation tolerance. *Nat Rev Immunol* 2003;3:199–210.
- Wu B, Elst LV, Carlier V, Jacquemin MG, Saint-Remy JM. The Dermatophagoides pteronyssinus group 2 allergen contains a universally immunogenic T cell epitope. *J Immunol.* 2002;169(5):2430-5.
- Wurster AL, Rodgers VL, Satoskar AR, et al.: Interleukin 21 is a T helper (Th) cell 2 cytokine that specifically inhibits the differentiation of naive Th cells into interferon gamma-producing Th1 cells. *J Exp Med* 2002; 196(7):969–977.
- Wurtzen PA, van Neerven RJ, Arned J, Ipsen H, Sparholt SH. Dissection of the grass allergen-specific immune response in patients with allergies and control subjects: T-cell proliferation in patients does not correlate with specific serum IgE and skin reactivity. *J Allergy Clin Immunol.* 1998;101(2 Pt 1):241-9
- Xu, D. et al. CD4+CD25+ regulatory T cells suppress differentiation and functions of TH1 and TH2 cells, Leishmania major infection, and colitis in mice. *J. Immunol.* 2003;170, 394–399
- Xystrakis E, Kusumakar S, Boswell S, Peek E, Urry Z, Richards DF, Adikibi T, Pridgeon C, Dallman M, Loke TK, Robinson DS, Barrat FJ, O'Garra A,

- Lavender P, Lee TH, Corrigan C, Hawrylowicz CM Reversing the defective induction of IL-10-secreting regulatory T cells in glucocorticoid-resistant asthma patients. *J Clin Invest*. 2006;116(1):146-55.
- Yagi H, Nomura T, Nakamura K, Yamazaki S, Kitawaki T, Hori S, Maeda M, Onodera M, Uchiyama T, Fujii S, Sakaguchi S. Crucial role of FOXP3 in the development and function of human CD25+CD4+ regulatory T cells. *Int Immunol*. 2004;16(11):1643-56.
 - Yamagiwa S, Gray JD, Hashimoto S, Horwitz DA. A role for TGF-beta in the generation and expansion of CD4+CD25+ regulatory T cells from human peripheral blood. *J Immunol* 2001;166:7282-7289.
 - Yamazaki S, Iyoda T, Tarbell K, Olson K, Velinzon K, Inaba K, Steinman RM. Direct expansion of functional CD25+ CD4+ regulatory T cells by antigen-processing dendritic cells. *J Exp Med* 2003;198:235-247.
 - Yang X, Gartner J, Zhu LH, Wang SH, Brunham RC. IL-10 gene knockout mice show enhanced Th1-like protective immunity and absent granuloma formation following *Chlamydia trachomatis* lung infection. *J. Immunol*. 1999;162:1010-17
 - Yazdanbakhsh M, Kreamsner PG, van Ree R. Allergy, parasites, and the hygiene hypothesis. *Science*. 2002;296(5567):490-4.
 - Ying S, O'Connor B, Ratoff J, Meng Q, Mallett K, Cousins D, Robinson D, Zhang G, Zhao J, Lee TH. Thymic stromal lymphopoietin expression is increased in asthmatic airways and correlates with expression of Th2-attracting chemokines and disease severity. *J Immunol* 2005;174:8183-8190.
 - Ying S, Robinson DS, Meng Q, Barata LT, McEuen AR, Buckley MG, Walls AF, Askenase PW, Kay AB.. C-C chemokines in allergen-induced late-phase cutaneous responses in atopic subjects: association of eotaxin with early 6-hour eosinophils, and of eotaxin-2 and monocyte chemoattractant protein-4 with the later 24-hour tissue eosinophilia, and relationship to basophils and other C-C chemokines (monocyte chemoattractant protein-3 and RANTES). *J Immunol* 1999;163:3976-84.
 - Zhang DH, Yang L, Cohn L, et al.: Inhibition of allergic inflammation in a murine model of asthma by expression of a dominant-negative mutant of GATA-3. *Immunity* 1999; 11(4):473-482
 - Zheng SG, Wang JH, Gray JD, Soucier H, Horwitz DA. Natural and induced CD4+CD25+ cells educate CD4+CD25- cells to develop suppressive activity: the role of IL-2, TGF-beta, and IL-10. *J Immunol*. 2004 May 1;172(9):5213-21.
 - Zheng SG, Wang JH, Koss MN, Quismorio F, Jr., Gray JD, Horwitz DA. CD4+ and CD8+ regulatory T cells generated ex vivo with IL-2 and TGF-beta suppress a stimulatory graft-versus-host disease with a lupus-like syndrome. *J Immunol*. 2004 Feb 1;172(3):1531-9.
 - Zheng Z, Narita M, Takahashi M, Liu A, Furukawa T, Toba K, Aizawa Y. Induction of T cell anergy by the treatment with IL-10-treated dendritic cells. *Comp Immunol Microbiol Infect Dis*. 2004;27(2):93-103

- Zheng W, Flavell RA: The transcription factor GATA-3 is necessary and sufficient for Th2 cytokine gene expression in CD4 T cells. *Cell* 1997; 89(4):587–596.
- Zhou B, Comeau M, De Smedt T, Liggitt H, Dahl M, Lewis D, Gyarmati D, Aye T, Campbell D, Ziegler S. Thymic stromal lymphopoietin as a key initiator of allergic airway inflammation in mice. *Nat Immunol* 2005; 6:1047-1053.
- Zhou Y, McLane M, Levitt RC. Th2 cytokines and asthma. Interleukin-9 as a therapeutic target for asthma. *Respir Res* 2001;2(2):80-4.
- 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(6):625-9
- Zuleger CL, Gao X, Burger MS, Chu Q, Payne LG, Chen D. Peptide induces CD4(+)CD25+ and IL-10+ T cells and protection in airway allergy models. *Vaccine* 2005;23(24):3181-6

Ringraziamenti

Se sono riuscita a realizzare questo lavoro lo devo soprattutto all'aiuto di tante persone che ho incontrato in questi ultimi 3 anni.

Innanzitutto vorrei ringraziare il Prof Paolo Rossi per avermi dato l'opportunità ed i mezzi per diventare un PhD.

Un enorme grazie va alla Prof.ssa Maria Grazia Roncarolo per avermi dato l'opportunità di frequentare e studiare nel suo laboratorio. Grazie per i gli enormi insegnamenti che mi ha dato, e per aver avuto la possibilità di confrontarmi giornalmente con il mondo della ricerca ai più alti livelli, mettendomi perennemente alla prova, permettendomi così di crescere professionalmente.

Alla Dott Federica Angelici e alla Dott. Silvia Gregori che mi hanno guidato in questo percorso, va il mio ringraziamento più grande. A loro devo tutto quello che sono diventata oggi. Grazie alla loro esperienza e professionalità, ai loro suggerimenti, hanno permesso la realizzazione di questo lavoro. Grazie anche per il loro aiuto non solo intellettuale, ma soprattutto umano.

Grazie alla Dott Loredana Chini, alle infermiere ed i medici, in particolare Stefania, del Centro Interdisciplinare di Pediatria Specialistica del PTV, che hanno permesso il reclutamento dei piccoli pazienti. Grazie anche ai piccoli pazienti e ai loro genitori!

Grazie a Luisa, Silvia, Lola, Franceschina; Betta, Simona, Elio, Zok e tutte le persone con cui ho avuto il piacere di lavorare a Roma. Grazie per il loro aiuto e per la loro amicizia.

Un grazie al mio fedele e amico Simone, con il quale ho condiviso non solo il duro cammino di "studente" fino ad oggi, ma le soddisfazioni, le delusioni, i viaggi, le paure e le ansie, le risate...e l'Amicizia che ci lega ormai da 10 anni!!!!

Grazie a Manuela, Rosi, Daniela, Chiara, Laura, Nuria, Andrea, Giorgia e tutte le persone che ho incontrato nel laboratorio Tiget di Milano. Grazie per aver condiviso con me le loro competenze professionali e soprattutto per avermi fatto sentire "a casa".

E grazie a Lella e alla sua infinita pazienza e disponibilità.

Vorrei ringraziare i miei genitori, senza i quali, ancora una volta, tutto questo non sarebbe stato possibile, e mia sorella che giorno dopo giorno mi stupisce sempre più, dimostrandomi il suo affetto e il suo sostegno. Un ringraziamento particolare va a mia madre, il mio punto di riferimento, alla quale dedico questa tesi. Perché senza il suo sostegno, i suoi insegnamenti, la sua forza, il suo amore incondizionato non sarei mai riuscita ad affrontare questi ultimi 3 anni e a superare uno dei momenti più difficili della mia vita.

Grazie infine a tutte le persone che mi sono state vicine in questi anni. Un grazie particolare a Fabio, non solo per avermi aiutato per questa tesi, ma soprattutto per essermi stato vicino in tanti momenti difficili, per avermi insegnato a “guardare oltre” e ad affacciarmi al domani con un sorriso....e grazie per aver avuto la pazienza di attendere....