Eur Respir J 2007; 29: 1246–1255
DOI: 10.1183/09031936.00094306

MINI-SERIES "T-CELL CO-STIMULATORY MOLECULES"
Edited by M. Belvisi and K.F. Rabe
Number 2 in this Series

T-cell co-stimulatory molecules: their role in allergic immune reactions

T. Kallinich, K.C. Beier, U. Wahn, P. Stock and E. Hamelmann

ABSTRACT: The development of allergic diseases, such as allergic asthma, depends upon the initiation and maintenance of T-helper cell type-2-skewed allergen-specific immune reactions. Although it is clear that susceptibility to this process is under genetic and environmental control, the fine-tuning and regulation of the type-2 T-helper cell immune response is not yet fully understood. In this second article in the present series, current understanding regarding the involvement of T-cells and antigen-presenting cells is summarised, with emphasis on the interaction between these two types of immune regulatory cells by means of co-stimulatory molecules.

KEYWORDS: Allergic airway inflammation, asthma pathogenesis, co-stimulation, T-cells

SPECIFIC CD4+ T-CELL SUBSETS CONTRIBUTE TO ALLERGIC AIRWAY DISEASE DEVELOPMENT

CD4+ T-cells play a central role in the initiation and maintenance of the allergic airway reaction. By expression of major histocompatibility complex (MHC) class II molecules and allergen-specific T-cell receptors (TCRs) they link innate and adoptive immune responses. They induce the synthesis of allergen-specific immunoglobulin (Ig)E and recruit and activate effector cells, such as eosinophils, *via* the secretion of soluble factors. Their pivotal role in controlling the early and late asthmatic reaction was convincingly demonstrated in human patients, as well as in animal models of allergic airway inflammation. Activated T-cells were found in increased numbers in the bronchial tissues of asthmatic subjects [1, 2], and depletion of CD4+ T-cells in a mouse model of allergic airway disease impressively prevented both the development of airway hyperresponsiveness (AHR) and the infiltration of eosinophils into the airways [3].

T-cells were originally subdivided, according to their functional properties, into two polarised subsets [4]. Type-1 T-helper cells (Th) predominantly produce the cytokines tumour necrosis factor (TNF)-β, interferon-γ and interleukin (IL)-2 and stimulate a strong cell-mediated immune response, particularly against intracellular pathogens. In contrast, Th2 have the capacity to secret greater amounts of IL-4, -5, -9 and IL-13, and are therefore crucial for allergic immune reactions.

The first report on the specific cytokine profile of T-cells involved in the pathogenesis of human asthma was published in the early 1990s, demonstrating that T-cells in bronchoalveolar lavage fluid (BALF) from asthmatic patients predominantly produce the Th2-type cytokines granulocyte-macrophage colony-stimulating factor and IL-3, -4 and -5 [5, 6]. The functional importance of these cells for the development of allergic airway inflammation was further persuasively demonstrated by analyses in rodents. Mice lacking pivotal elements required for the induction of Th2 immune reactions, *e.g.* signal transducer and activator of transcription 6 [7, 8], failed to develop symptoms of allergic airway inflammation. Similarly, athymic mice with no functional peripheral T-cells also lack eosinophilic airway infiltration and increased AHR following allergen sensitisation, unless production of the Th2 cytokine IL-5 is restored by systemic administration [9]. Although the involvement of Th2 cytokines explains the biological basis of many features of the allergic reaction (*e.g.* recruitment

AFFILIATIONS
Dept of Paediatrics, Division of Pneumology and Immunology, Charité Medical University of Berlin, Berlin, Germany.

CORRESPONDENCE
E. Hamelmann
Dept of Paediatrics
Division of Pneumology and Immunology
Charité Medical University of Berlin
Campus Virchow-Klinikum
Augustenburger Platz 1
13353 Berlin
Germany
Fax: 49 30450566931
E-mail: eckard.hamelmann@charite.de

Received:
July 18 2006
Accepted after revision:
January 06 2007

STATEMENT OF INTEREST
None declared.

Previous articles in this series: No. 1: Beier KC, Kallinich T, Hamelmann E. Master switches of T-cell activation and differentiation. *Eur Respir J* 2007; 29: 804–812.

European Respiratory Journal
Print ISSN 0903-1936
Online ISSN 1399-3003

of eosinophils, activation of mast cells, airway remodelling and IgE production), it has been observed more recently that asthmatic individuals may also exhibit an increased Th1-like response to allergens [10, 11], especially in established disease [12–15]. Conversely, acute segmental allergen challenges lead to a reduction in Th1 cytokine-producing T-cell numbers [16]. In summary, the dogma of the allergic reaction being purely a Th2-driven disease, especially as an attempt to explain asthma pathology, is questioned; however, the important role of Th2 cytokines, such as IL-4, -5 and -13, has been demonstrated by a large body of evidence.

The important role of the various Th2 cytokines in the development of allergic airway inflammation was further demonstrated by analysis of the pathology of mice over-expressing certain Th2 cytokines, by use of cytokine-blocking agents or genetically deficient mice unable to produce specific cytokines (table 1). In conclusion, these data clearly underline the importance of the Th2 subset in the pathogenesis of allergic (airway) diseases.

Encouraged by these experimental *in vitro* and *in vivo* data, trials were performed with the aim of therapeutically targeting Th2 cytokines in human subjects with persistent asthma (Global Initiative for Asthma stage 2 or worse). Single intravenous application of a humanised monoclonal antibody (mAb) directed against IL-5 (SB-240563/mepolizumab) in patients with mild persistent asthma significantly reduced the numbers of eosinophils in the peripheral blood and sputum, but had no significant effect on the late asthmatic response, or on nonspecific AHR to histamine [37]. Later, in patients with mild/moderate asthma undergoing a prolonged protocol of repeated antibody treatment, it was shown that, although eosinophils were nearly wiped out in the periphery, signs of eosinophil activation and degranulation were still present in the lung tissues, probably accounting for the lack of efficacy [38]. In another trial, in patients with severe persistent asthma, with a different humanised mAb directed against IL-5, SCH55700, numbers of circulating eosinophils were also reduced, but, besides a small increase in baseline forced expiratory volume in one second (FEV_1), there was no effect on other clinical indices of disease activity [39]. In mild-to-moderate asthmatic subjects, application of a recombinant soluble IL-4 receptor by inhalation during dose-reduction and withdrawal of inhaled corticosteroids prevented a decline in FEV_1 or an increase in asthma symptoms [40, 41]. Since there was a lack of further evidence of efficacy, this strategy has since been discontinued, as have those with an anti-IL-5 approach.

TABLE 1 Involvement of type-2 T-helper cell cytokines in the pathogenesis of allergic airway inflammation (selected mouse studies)

	Cytokine-blocking agent	Cytokine overexpression	Cytokine deficiency
IL-4	[17, 18]		[19, 20]
IL-5	[21, 22]	[23–25]	[20, 26, 27]
IL-9	[28]	[29, 30]	[31]
IL-13	[32–34]	[35]	[36]

IL: interleukin.

These rather frustrating outcomes of trials in which the blockade of a single Th2 cytokine was attempted may be explained in various ways: 1) the applied compounds did not sufficiently deplete and antagonise eosinophils and IL-4, respectively; 2) eosinophils might not be totally required or even important in the clinical manifestation of asthma [42]; 3) other trigger factors, such as viral infections, exercise or airway pollution, might account for asthma pathology and/or exacerbation; and 4) depletion of a single cytokine is not sufficient, due to the high redundancy of effector function of a wide variety of cytokines [38]. It is, therefore, tempting to speculate that elimination of the entire Th2 function or the complete prevention of differentiation towards the Th2 type may overcome the problem of redundant cytokine function (discussed in the next article in the present series [43]). Also, different treatment protocols, including early and/or prolonged intervention and the use of antibody cocktails aimed at a variety of cytokines, might result in increased efficacy in asthma therapy.

Furthermore, there are new data showing that strategies targeting T-cells and/or T-cell products that are not linked with a Th1/Th2 bias may be effective in the treatment of asthma. Selective blockade of TNF-α using the TNF receptor-2 Fc fusion protein etanercept, as well as the application of methotrexate, to suppress TNF-α as well as antibody production, led to a reduction in asthma severity [44, 45]. In summary, these data are in line with a concept of impaired immunostasis being the main reason for asthma development, with unwanted immune reactions of allergen-specific CD4+ T-cells due to the lack of regulatory T-cell (Tr) functions inducing the allergic cascade, regardless of Th1 or Th2 bias [46].

ALLERGIC AIRWAY INFLAMMATION REGULATION BY SPECIFIC T-CELLS

The term regulatory T-cell (Tr) refers to cells that actively control or suppress the function of other cells, generally in an inhibitory fashion. Tr appear to control the development of autoimmune disease and transplant rejection, and may also play a critical role in controlling the expression of asthma and allergy. Their specific characterisation, as well as their mode of action, is still undergoing intense investigation [47]. However, different Tr subtypes with certain attributes and functions may be described.

Naturally occurring Tr are positively selected in the thymus by encountering self antigen. They constitute 5–10% of CD4+ T-cells in the periphery in both mice and humans. These cells constitutively express CD25 (IL-2 receptor), as well as the transcription factor forkhead box protein (Fox) P3 [48–50], which acts as a specific factor for the differentiation of the Tr lineage and closely correlates with the suppressor activity of the cells [51].

The precise function of FoxP3, which until recently was thought to be expressed only by natural CD25+ Tr, is not known, but absence of FoxP3 in humans results in immunodysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome [52, 53], also know as X-linked autoimmunity–allergic

dysregulation syndrome [54]. Patients with IPEX syndrome share many phenotypic features with scurfy mice, which have a natural mutation in the gene encoding FoxP3, and lack natural CD25+ Tr. Scurfy mice develop diarrhoea, malabsorption, autoimmune haemolytic anaemia, thrombocytopenia, leukocytosis, lymphadenopathy, hepatosplenomegaly, hypogonadism and dry skin, which are all corrected by adoptive transfer of natural CD25+ Tr into these mice [55]. In addition, forced expression of the FoxP3 gene, which encodes a transcription repressor, converts naive murine T-cells into Tr that phenotypically and functionally resemble natural CD25+ Tr.

Although the absence of *FOXP3* expression in patients with IPEX syndrome and scurfy mice eliminates development of natural CD25+ Tr, whether IPEX syndrome patients and scurfy mice also lack adaptive Tr that differentiate in the periphery from CD25- T-cells is not yet clear. However, since patients with IPEX syndrome develop eczema, food allergy, elevated IgE levels and peripheral eosinophilia, associated with elevated Th2 cytokine production [54], the present authors suggest that IPEX syndrome patients lack both natural CD25+ Tr and antigen-specific adaptive Tr, which may be particularly effective in regulating Th2 responses to allergens.

In mice, natural CD25+ Tr have been shown to limit allergen-induced airway inflammation but not the development of airway hyperreactivity [56]. In these experimental settings, pre-activation of natural Tr by nonspecific signals appears to be required in order to obtain strong inhibition of allergen-induced airway inflammation [57].

In children who have outgrown cow's milk sensitivity, an increased frequency of circulating natural Tr is associated with decreased *in vitro* proliferative responses to the specific allergen bovine β-lactoglobulin [58]. Natural CD25+ Tr from nonallergic, but not from allergic, donors suppress proliferation and IL-5 secretion by allergen-stimulated CD4+CD25- effector T-cells, indicating that natural Tr may also suppress allergic immune responses to inhaled allergens, *e.g.* cat allergen or grass-pollen [59]. Interestingly, in allergic donors, the inhibitory activity of CD25+ Tr on the production of Th2 cytokines was most suppressed when pollen counts were highest during the specific season. Accordingly, natural Tr from both allergic and nonallergic individuals suppressed T-cell proliferation and Th2 cytokine production in response to birch allergen most potently outside, but not during the birch pollen season [60].

Although it is clear that natural Tr are positively selected in the thymus after encounter with self antigens, it is likely that allergen-specific adaptive Tr develop in the periphery after encounters with exogenous allergens (*e.g.* from food or plants). Adaptive Tr do not have clearly defined markers that would make them easily identified or isolated; thus their further characterisation is difficult to accomplish.

Several studies have analysed the significance of co-stimulatory pathways in the generation and expansion of Tr. First, it was demonstrated that rat Tr respond less well than CD25- T-cells to conventional co-stimulation, but are readily expanded *in vitro* by superagonistic CD28-specific mAbs, which are potent mitogens for all T-cells without the need for TCR engagement [61]. *In vivo*, functional Tr are preferentially expanded over other T-cell subsets by CD28 stimulation. These data suggest that CD28-driven activation of Tr may be effective in the treatment of inflammatory and autoimmune diseases, but side-effects should be carefully taken into account [62].

In addition, co-stimulation of CD4+ T-cells with anti-CD52 mAb leads to the development of Tr that suppress the polyclonal responses of CD4+ T-cells [63]. These Tr had the potential to suppress graft-*versus*-host disease-like pathology in severe combined immunodeficiency syndrome mice injected with human peripheral blood mononuclear cells. It was suggested that anti-CD52-induced Tr, which can be expanded and which confer antigen specificity, have the potential to be a desirable tool in cellular immunotherapy.

T-CELLS SHOW SPECIFIC DIFFERENTIATION AND MIGRATION IN ALLERGIC AIRWAY INFLAMMATION

The differentiation of T-cell function depends upon the recognition of a specific peptide presented by antigen-presenting cells (APCs) in combination with additional co-stimulatory signals, as described in the first article in the present series [64]. In recent years, a variety of studies have broadened knowledge of the cellular interaction leading to T-cell differentiation in allergic airway inflammation.

The allergic immune response is initiated by pulmonary dendritic cells (DCs), derived from blood monocytes with low granulocyte-differentiation antigen (Gr)-1 and high CX_3C chemokine receptor 1 densities [65]. They are located underneath the basal membrane of the airway epithelium, where they encounter and incorporate allergens and migrate to the draining thoracic lymph nodes (fig. 1) [66]. As soon as the DCs carrying the allergen peptides arrive in the T-cell area of the local lymph node, they stimulate specific naive or memory T-cells by increasing expression of co-stimulatory and MHC class II molecules. Under conditions in which allergen exposure is accompanied by an inflammatory stimulus, differentiation towards functional effector T-cells, Tr or memory T-cells is induced [67]. Activated effector T-cells leave the lymph node and enter the pulmonary tissues, the site of further antigen deposition, where they are retained in a nonproliferative state, recognising antigens presented by local DCs [68]. They further enhance the recruitment and maturation of other immune cells involved in the allergic cascade, such as eosinophils and mast cells, *via* the secretion of cytokines and chemokines.

IMPACT OF INNATE IMMUNITY ON T-CELL DIFFERENTIATION AND DEVELOPMENT OF THE ASTHMA PHENOTYPE

The innate immune system links environmental factors with the specific responses of adaptive immunity. A family of evolutionarily highly conserved receptors, known as Toll-like receptors (TLRs), are expressed by APCs and recognise pathogen-associated molecular patterns. This interaction leads to secretion of different cytokines by the APCs, including IL-12, particularly promoting the development of Th1 [69]. The epidemiological observation that the modified exposure to microbial particles in westernised areas is associated with a reduced prevalence of atopic diseases, the so-called ''hygiene hypothesis'', may thus be explained by the reduced production of IL-12 shifting the immune responses to a predominantly Th2

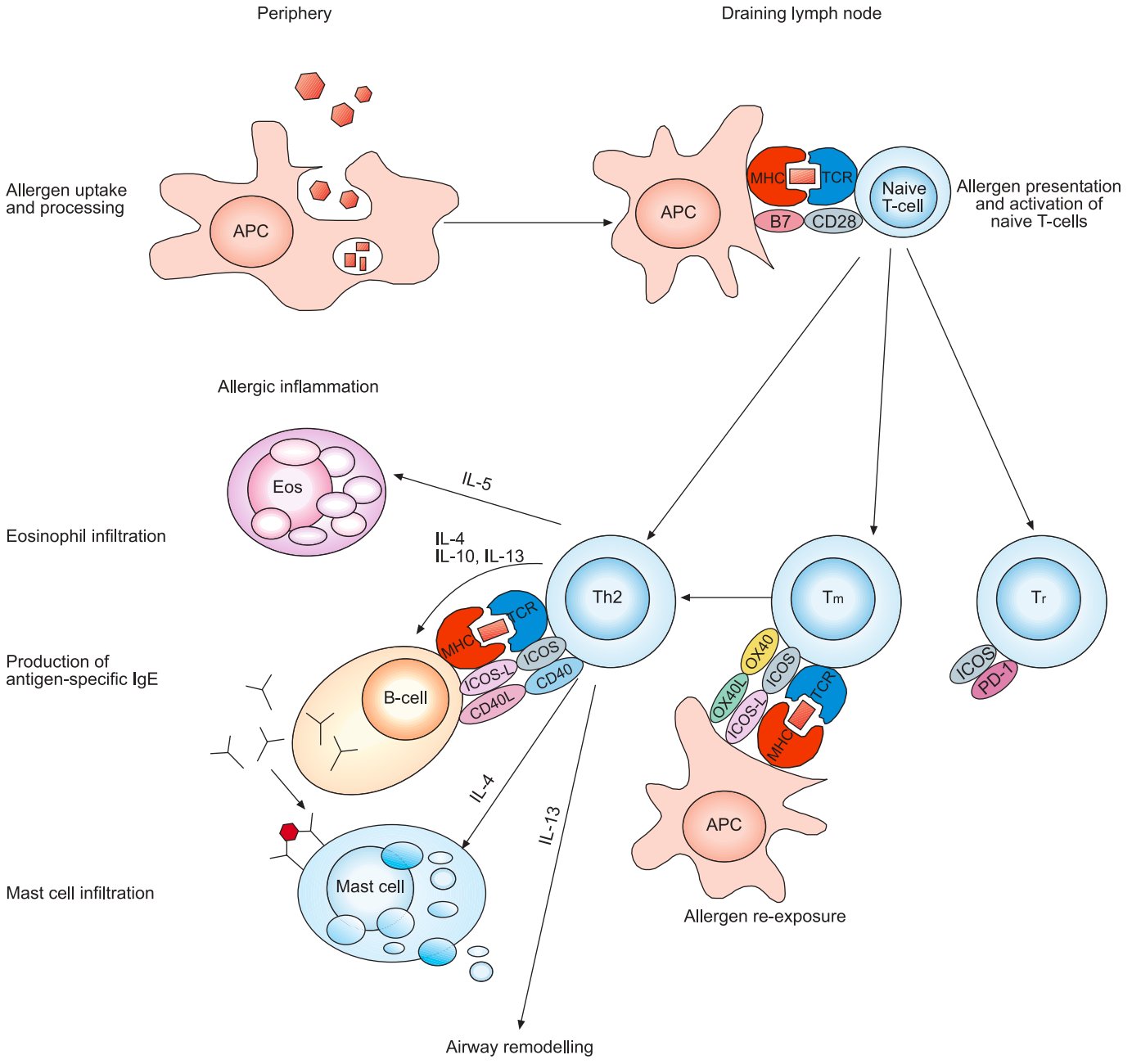

FIGURE 1. Cellular and molecular events in the development of allergic reaction. APC: antigen-presenting cell; MHC: major histocompatibility complex; TCR: T-cell receptor; IL: interleukin; Eos: eosinophil; Ig: immunoglobulin; Th: T-helper cell; ICOS: inducible co-stimulatory antigen; ICOS-L: ICOS ligand; CD40L: CD40 ligand; Tm: memory T-cell; OX40: CD134; OX40L: OX40 ligand; Tr: regulatory T-cell; PD: programmed cell death.

type. This hypothesis was supported by recent findings that genetic variations in the *TLR2*, *TLR4*, *TLR6* and *TLR10* genes were associated with atopy in children [70–74].

Similar to TLRs, CD14 is a pattern recognition receptor for certain microbial molecules and expressed by APCs. Associations of different polymorphisms within the *CD14* gene and cardinal features of atopic diseases, such as IgE levels [75], eczema [76], asthma severity [77] and bronchial hyperresponsiveness [78], were identified. In contrast, other studies did not replicate these associations [72, 79], possibly due to genetic variations in different study populations or differences in the kinetics of the microbial exposure [80].

CO-STIMULATORY SIGNALS REGULATE ALLERGIC AIRWAY INFLAMMATION

The development of allergen-induced airway inflammation and AHR is regulated by fine-tuning of several co-stimulatory factors. The first co-stimulatory signal for activation of naive T-cells is delivered by the interaction of CD28 with its ligands expressed on DCs, CD80 (B7.1) and CD86 (B7.2) (fig. 1) [81]. This interaction induces the expression of anti-apoptotic

TABLE 2 Role of co-stimulatory molecules in the development of allergen-induced immune and airway responses studied in various deficient germline mice

Deficiency	BALF eosinophils	AHR	IgE	Th2 cytokines	Th1 (IFN-γ)	[Ref.]
CD28	↓	ND	↓	↓	↔	[96]
	↓	ND	↓	↓	↓	[97]
CD80	↓	↓	↓	↓	↑	[95]
CD86	↓	↓	↓	↓	↑	[95]
CD80/CD86	↓	↓	↓	↓	↑	[95]
	↓	↓	↓	↓	↑	[98]
ICOS	ND	ND	↓	↓	↑	[99]
	↓	ND	↓	↓	ND	[100]
	ND	ND	↓	↓	↑	[101]
ICOS-L	ND	ND	ND	↓	↑	[102]
	↓	ND	↓	↓	↑	[103]
OX40	ND	ND	ND	↓	↓	[104]
	↓	↓	↓	↓	ND	[105]
OX40L	ND	ND	ND	↓	↓	[106]
	↓	↓	↓	↓	↓	[107]
	↓	↓	↓	↓	ND	[108]

BALF: bronchoalveolar lavage fluid; AHR: airway hyperresponsiveness; Ig: immunoglobulin; Th: T-helper cell; IFN: interferon; ICOS: inducible co-stimulatory antigen; ICOS-L: ICOS ligand; OX40: CD134; OX40L: OX40 ligand; ↓: decreased; ↑: increased; ↔: unchanged; ND: not determined.

molecules of the B-cell leukaemia/lymphoma gene product (Bcl) family, permitting the clonal expansion of the activated T-cell [82] and inducing the production of cytokines IL-2, -4 and -5. It is currently a matter of debate as to whether the differential ligation of CD28 with either CD80 or CD86, respectively, favours differentiation into Th1- or Th2-type T-cells [83, 84]. Certainly, the cytokine milieu at the time of antigen presentation is crucial to the type of T-cell differentiation; increased production of IL-12 by DCs favours the development of Th1-mediated cellular immune responses, whereas the preferential synthesis of IL-4 and -6 by DCs that are localised at mucosal surfaces (DC2 cells) may trigger polarisation towards the Th2 phenotype [85, 86].

The role of CD28 in the pathogenesis of human asthma was studied using T-cells obtained from BALF and bronchial biopsy specimens from asthmatic patients [87–90]. Interestingly, the proliferation of human BALF-derived T-cells from patients with atopic asthma after *in vitro* stimulation with allergen was inhibited by the addition of anti-CD86, but not of anti-CD80 mAb [90], underscoring the critical role of CD86 in allergen-induced T-cell (Th2) responses.

The activation of T-cells through CD28 is negatively regulated by the inducible molecule cytotoxic T-lymphocyte antigen (CTLA)-4. Accordingly, inhibition of CD28 co-stimulation by means of CTLA-4–Ig suppressed the production of IL-5, -13 and -16 and RANTES (regulated on activation, normal T-cell expressed and secreted) upon allergen-specific *in vitro* stimulation by T-cells isolated from bronchial biopsy specimens from asthmatic subjects [88, 89]. A more detailed analysis of these experiments revealed that allergen-mediated Th2-type cytokine production in bronchial biopsy specimens was dependent upon signalling *via* both CD80 and CD86 [87].

In human bronchial asthma, an association between certain single nucleotide polymorphisms (SNPs) within the *CTLA-4* gene and increased total serum IgE levels (+49A/G [91]), bronchial hyperresponsiveness (BHR) (-1147C/T and +49A/G [92]) and asthma severity (-318C/T [92]) was demonstrated. In two other cohorts, no association of the +49A/G and -318C/T polymorphisms and the development of the asthma phenotype was found [93, 94].

The critical role of CD28 signalling in the development of allergic airway diseases was further confirmed in mice deficient in this co-stimulatory pathway. Mice deficient in CD86 developed less airway inflammation in response to allergen sensitisation and airway challenges than their normal littermates [95]. In the same line, CD28-deficient or CD80/CD86 double knockout mice showed virtually no signs of allergic airway inflammation (*e.g.* production of IgE, eosinophilic airway inflammation and development of AHR) following allergen sensitisation and airway challenges (table 2) [95, 97]. Mice deficient in CTLA-4 developed a lethal lymphoproliferative disease, which reflects the importance of this molecule in the restriction of T-cell-mediated immune responses [109].

After initial T-cell activation, effector T-cell function seems to be less dependent on the engagement of CD28 [110, 111]. Other co-stimulatory molecules, which are only expressed upon T-cell activation, take over and seem to play the dominant role in the control of the ongoing immune reaction.

A prominent member of this second line of T-cell control is inducible co-stimulatory antigen (ICOS). Initial studies on its functional properties demonstrated that co-stimulation *via* ICOS mainly resulted in high expression of IL-10 and -4 and minor production of other Th1 and Th2 cytokines during the initial priming and effector T-cell responses [112–114]. It was further demonstrated that the production of certain cytokines correlated with the expression density of ICOS on the cell surface [115]. Intermediate ICOS expression was associated with high production of Th2 cytokines, whereas high levels of ICOS predominantly translated into high IL-10 production. In line with these findings, it was shown that mature pulmonary DCs in the bronchial lymph nodes of mice exposed to respiratory allergen induced the development of Tr, in a process that required T-cell co-stimulation *via* the ICOS-ICOS ligand (ICOS-L) pathway. The Tr produced IL-10 and showed potent inhibitory activity; when adoptively transferred into sensitised mice, Tr blocked the development of AHR. Both the development and the inhibitory function of regulatory cells were dependent upon the presence of IL-10 and on ICOS-ICOS-L interactions. These studies demonstrate that Tr and the ICOS-ICOS-L signalling pathway are critically involved in respiratory tolerance and downregulating pulmonary inflammation in asthma [116, 117].

Other authors have shown that adoptively transferred ICOS+ cells were capable of inducing airway inflammation upon

subsequent allergen airway challenges, highlighting the essential role of ICOS in the development of allergic airway inflammation [115].

One study showed an association of SNPs within the *ICOS* gene (-693A/A and -1413A/A) with increased total IgE and allergic sensitisation to airborne allergens in a Hutterite population. Furthermore, the -1413 SNP is located at a nuclear factor-κB-binding site and peripheral blood mononuclear cells homozygous for the A allele (-1413A/A) produce significantly greater amounts of the cytokines IL-4, IL-5 and IL-13 and TNF-α. This points towards a role of ICOS in allergic diseases in humans [118].

Mice lacking ICOS or ICOS-L expression are strongly impaired in T-cell IL-4 production, resulting in impaired production of antigen-specific class-switched antibodies, such as IgG1, IgG2a, IgA and, especially, IgE (table 2) [100, 101, 103]. Interestingly, ICOS-deficient mice still develop profound IL-5-mediated eosinophilic airway inflammation upon allergen sensitisation and subsequent airway challenges, indicating that ICOS may not primarily be involved in global Th2 differentiation, but is rather required for the expression of IL-4 in the effector phase [100, 119].

Another inducible co-stimulatory molecule involved in the pathogenesis of the allergic airway reaction is CD134 (OX40). This molecule is mainly expressed by activated Th2. OX40 is required for the cytoplasmic expression of the transcription factors Bcl-xL and Bcl-2, which deliver anti-apoptotic signals for pre-activated T-cells [120]. These signals are essential for the accumulation of T-cells during the primary immune response, as well as during the subsequent formation of a memory cell pool. In mice lacking OX40 signalling, strong impairment of allergic airway inflammation, with significantly reduced development of AHR, Th2 cytokine production and IgE synthesis, was observed (table 2) [105, 107].

Co-stimulation is also an effective means of downregulating T-cell functions. Signalling through the negative co-stimulatory molecule, programmed cell death (PD)-1, leads to arrest in cell cycle phase G_0–G_1, thus limiting an ongoing immune reaction [121]. The importance of PD-1-mediated T-cell suppression for peripheral tolerance was supported by the observation that PD-1-deficient mice developed autoimmune diseases, *e.g.* cardiomyopathy [122], autoimmune glomerulonephritis and arthritis [123].

In a murine model of airway inflammation, allergen sensitisation and airway challenge lead to increased expression of PD-1 ligand 1 (PD-L1) on pulmonary DCs, macrophages and B-cells isolated from lung tissues [124]. Despite this abundant expression of PD-L1, this molecule seems not to be involved in the control of allergic airway inflammation, since blockade by mAb did not alter the allergic phenotype [124]. In contrast, another PD-1 ligand, PD-L2, was expressed only in very low amounts by lymphocytes of unchallenged mice, with only moderate increases after allergen challenge. However, blockade of this specific ligand significantly inhibited the development of AHR, lung eosinophilia and production of Th2 cytokines [124]. This gives PD-L2 a so-far unique role in the negative regulation of allergen-induced airway responses.

SUMMARY

A complex network of enhancing and inhibitory co-stimulatory signals regulates T-cell differentiation and effector functions. Owing to their central role in allergen-mediated airway inflammation, T-cell co-stimulatory molecules constitute promising targets for novel therapeutic interventions. The state of the art regarding experimental data on the use of these signals in modulating allergen-induced T-cell responses, and the outlook for future approaches targeting co-stimulation for intervention in allergic airway diseases is discussed in more detail in the third and final part of the present series [43].

REFERENCES

1 Azzawi M, Bradley B, Jeffery PK, *et al.* Identification of activated T lymphocytes and eosinophils in bronchial biopsies in stable atopic asthma. *Am Rev Respir Dis* 1990; 142: 1407–1413.
2 Bradley BL, Azzawi M, Jacobson M, *et al.* Eosinophils, T-lymphocytes, mast cells, neutrophils, and macrophages in bronchial biopsy specimens from atopic subjects with asthma: comparison with biopsy specimens from atopic subjects without asthma and normal control subjects and relationship to bronchial hyperresponsiveness. *J Allergy Clin Immunol* 1991; 88: 661–674.
3 Gavett SH, Chen X, Finkelman F, Wills-Karp M. Depletion of murine CD4+ T lymphocytes prevents antigen-induced airway hyperreactivity and pulmonary eosinophilia. *Am J Respir Cell Mol Biol* 1994; 10: 587–593.
4 Mosmann TR, Coffman RL. TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. *Annu Rev Immunol* 1989; 7: 145–173.
5 Robinson DS, Hamid Q, Ying S, *et al.* Predominant TH2-like bronchoalveolar T-lymphocyte population in atopic asthma. *N Engl J Med* 1992; 326: 298–304.
6 Robinson D, 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: 313–324.
7 Kuperman D, Schofield B, Wills-Karp M, Grusby MJ. Signal transducer and activator of transcription factor 6 (Stat6)-deficient mice are protected from antigen-induced airway hyperresponsiveness and mucus production. *J Exp Med* 1998; 187: 939–948.
8 Tomkinson A, Kanehiro A, Rabinovitch N, Joetham A, Cieslewicz G, Gelfand EW. The failure of STAT6-deficient mice to develop airway eosinophilia and airway hyperresponsiveness is overcome by interleukin-5. *Am J Respir Crit Care Med* 1999; 160: 1283–1291.
9 Hamelmann E, Oshiba A, Schwarze J, *et al.* Allergen-specific IgE and IL-5 are essential for the development of airway hyperresponsiveness. *Am J Respir Cell Mol Biol* 1997; 16: 674–682.
10 Maxwell MJ, Muthu P, Pritty PE. Accidental colchicine overdose. A case report and literature review. *Emerg Med J* 2002; 19: 265–267.
11 Heaton T, Rowe J, Turner S, *et al.* An immunoepidemiological approach to asthma: identification of *in-vitro* T-cell

response patterns associated with different wheezing phenotypes in children. *Lancet* 2005; 365: 142–149.
12 Litonjua AA, Sparrow D, Guevarra L, O'Connor GT, Weiss ST, Tollerud DJ. Serum interferon-gamma is associated with longitudinal decline in lung function among asthmatic patients: the Normative Aging Study. *Ann Allergy Asthma Immunol* 2003; 90: 422–428.
13 Koh YI, Choi IS, Lee HC. Relationship between changes in interferon-γ production by peripheral blood T cells and changes in peak expiratory flow rate in patients with chronic stable asthma. *Clin Exp Allergy* 2002; 32: 1734–1738.
14 Krug N, Madden J, Redington AE, *et al.* T-cell cytokine profile evaluated at the single cell level in BAL and blood in allergic asthma. *Am J Respir Cell Mol Biol* 1996; 14: 319–326.
15 Smart JM, Kemp AS. Increased Th1 and Th2 allergen-induced cytokine responses in children with atopic disease. *Clin Exp Allergy* 2002; 32: 796–802.
16 Krug N, Erpenbeck VJ, Balke K, *et al.* Cytokine profile of bronchoalveolar lavage-derived $CD4^+$, $CD8^+$, and γδ T cells in people with asthma after segmental allergen challenge. *Am J Respir Cell Mol Biol* 2001; 25: 125–131.
17 Coyle AJ, Le Gros G, Bertrand C, *et al.* Interleukin-4 is required for the induction of lung Th2 mucosal immunity. *Am J Respir Cell Mol Biol* 1995; 13: 54–59.
18 Corry DB, Folkesson HG, Warnock ML, *et al.* Interleukin 4, but not interleukin 5 or eosinophils, is required in a murine model of acute airway hyperreactivity. *J Exp Med* 1996; 183: 109–117.
19 Brusselle GG, Kips JC, Tavernier JH, *et al.* Attenuation of allergic airway inflammation in IL-4 deficient mice. *Clin Exp Allergy* 1994; 24: 73–80.
20 Hamelmann E, Takeda K, Haczku A, *et al.* Interleukin (IL)-5 but not immunoglobulin E reconstitutes airway inflammation and airway hyperresponsiveness in IL-4-deficient mice. *Am J Respir Cell Mol Biol* 2000; 23: 327–334.
21 Hamelmann E, Oshiba A, Loader J, *et al.* Antiinterleukin-5 antibody prevents airway hyperresponsiveness in a murine model of airway sensitization. *Am J Respir Crit Care Med* 1997; 155: 819–825.
22 Hamelmann E, Takeda K, Schwarze J, Vella AT, Irvin CG, Gelfand EW. Development of eosinophilic airway inflammation and airway hyperresponsiveness requires interleukin-5 but not immunoglobulin E or B lymphocytes. *Am J Respir Cell Mol Biol* 1999; 21: 480–489.
23 Lee JJ, McGarry MP, Farmer SC, *et al.* Interleukin-5 expression in the lung epithelium of transgenic mice leads to pulmonary changes pathognomonic of asthma. *J Exp Med* 1997; 185: 2143–2156.
24 Lee NA, McGarry MP, Larson KA, Horton MA, Kristensen AB, Lee JJ. Expression of IL-5 in thymocytes/T cells leads to the development of a massive eosinophilia, extramedullary eosinophilopoiesis, and unique histopathologies. *J Immunol* 1997; 158: 1332–1344.
25 Lefort J, Bachelet CM, Leduc D, Vargaftig BB. Effect of antigen provocation of IL-5 transgenic mice on eosinophil mobilization and bronchial hyperresponsiveness. *J Allergy Clin Immunol* 1996; 97: 788–799.
26 Hogan SP, Koskinen A, Matthaei KI, Young IG, Foster PS. Interleukin-5-producing CD4+ T cells play a pivotal role in aeroallergen-induced eosinophilia, bronchial hyperreactivity, and lung damage in mice. *Am J Respir Crit Care Med* 1998; 157: 210–218.
27 Foster PS, Hogan SP, Ramsay AJ, Matthaei KI, Young IG. Interleukin 5 deficiency abolishes eosinophilia, airways hyperreactivity, and lung damage in a mouse asthma model. *J Exp Med* 1996; 183: 195–201.
28 Kung TT, Luo B, Crawley Y, *et al.* Effect of anti-mIL-9 antibody on the development of pulmonary inflammation and airway hyperresponsiveness in allergic mice. *Am J Respir Cell Mol Biol* 2001; 25: 600–605.
29 McLane MP, Haczku A, van de Rijn M, *et al.* Interleukin-9 promotes allergen-induced eosinophilic inflammation and airway hyperresponsiveness in transgenic mice. *Am J Respir Cell Mol Biol* 1998; 19: 713–720.
30 Temann UA, Geba GP, Rankin JA, Flavell RA. Expression of interleukin 9 in the lungs of transgenic mice causes airway inflammation, mast cell hyperplasia, and bronchial hyperresponsiveness. *J Exp Med* 1998; 188: 1307–1320.
31 Townsend JM, Fallon GP, Matthews JD, Smith P, Jolin EH, McKenzie NA. IL-9-deficient mice establish fundamental roles for IL-9 in pulmonary mastocytosis and goblet cell hyperplasia but not T cell development. *Immunity* 2000; 13: 573–583.
32 Wills-Karp M, Luyimbazi J, Xu X, *et al.* Interleukin-13: central mediator of allergic asthma. *Science* 1998; 282: 2258–2261.
33 Grunig G, Warnock M, Wakil AE, *et al.* Requirement for IL-13 independently of IL-4 in experimental asthma. *Science* 1998; 282: 2261–2263.
34 Taube C, Duez C, Cui ZH, *et al.* The role of IL-13 in established allergic airway disease. *J Immunol* 2002; 169: 6482–6489.
35 Zhu Z, Homer RJ, Wang Z, *et al.* Pulmonary expression of interleukin-13 causes inflammation, mucus hypersecretion, subepithelial fibrosis, physiologic abnormalities, and eotaxin production. *J Clin Invest* 1999; 103: 779–788.
36 Walter DM, McIntire JJ, Berry G, *et al.* Critical role for IL-13 in the development of allergen-induced airway hyperreactivity. *J Immunol* 2001; 167: 4668–4675.
37 Leckie MJ, ten Brinke A, Khan J, *et al.* Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyper-responsiveness, and the late asthmatic response. *Lancet* 2000; 356: 2144–2148.
38 Flood-Page PT, Menzies-Gow AN, Kay AB, Robinson DS. Eosinophil's role remains uncertain as anti-interleukin-5 only partially depletes numbers in asthmatic airway. *Am J Respir Crit Care Med* 2003; 167: 199–204.
39 Kips JC, O'Connor BJ, Langley SJ, *et al.* Effect of SCH55700, a humanized anti-human interleukin-5 antibody, in severe persistent asthma: a pilot study. *Am J Respir Crit Care Med* 2003; 167: 1655–1659.
40 Borish LC, Nelson HS, Corren J, *et al.* Efficacy of soluble IL-4 receptor for the treatment of adults with asthma. *J Allergy Clin Immunol* 2001; 107: 963–970.
41 Borish LC, Nelson HS, Lanz MJ, *et al.* Interleukin-4 receptor in moderate atopic asthma. A phase I/II randomized, placebo-controlled trial. *Am J Respir Crit Care Med* 1999; 160: 1816–1823.
42 Boushey HA, Fahy JV. Targeting cytokines in asthma therapy: round one. *Lancet* 2000; 356: 2114–2116.

43 Beier KC, Kallinich T, Hamelmann E. Novel targets for the treatment of allergic airway disease. *Eur Respir J* 2007 (in press).

44 Berry MA, Hargadon B, Shelley M, *et al.* Evidence of a role of tumor necrosis factor α in refractory asthma. *N Engl J Med* 2006; 354: 697–708.

45 Corrigan CJ, Shiner RJ, Shakur BH, Ind PW. Methotrexate therapy of oral corticosteroid-dependent asthmatics reduces serum immunoglobulins: correlation with clinical response to therapy. *Clin Exp Allergy* 2005; 35: 579–584.

46 Bach JF. The effect of infections on susceptibility to autoimmune and allergic diseases. *N Engl J Med* 2002; 347: 911–920.

47 Stock P, DeKruyff RH, Umetsu DT. Inhibition of the allergic response by regulatory T cells. *Curr Opin Allergy Clin Immunol* 2006; 6: 12–16.

48 Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. *Science* 2003; 299: 1057–1061.

49 Fontenot JD, Rudensky AY. A well adapted regulatory contrivance: regulatory T cell development and the forkhead family transcription factor Foxp3. *Nat Immunol* 2005; 6: 331–337.

50 Sakaguchi S. Naturally arising Foxp3-expressing CD25+CD4+ regulatory T cells in immunological tolerance to self and non-self. *Nat Immunol* 2005; 6: 345–352.

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.

52 Bennett CL, Christie J, Ramsdell F, *et al.* The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. *Nat Genet* 2001; 27: 20–21.

53 Wildin RS, Ramsdell F, Peake J, *et al.* X-linked neonatal diabetes mellitus, enteropathy and endocrinopathy syndrome is the human equivalent of mouse scurfy. *Nat Genet* 2001; 27: 18–20.

54 Chatila TA, Blaeser F, Ho N, *et al. JM2*, encoding a fork head-related protein, is mutated in X-linked autoimmunity–allergic disregulation syndrome. *J Clin Invest* 2000; 106: R75–R81.

55 Fontenot JD, Gavin MA, Rudensky AY. Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. *Nat Immunol* 2003; 4: 330–336.

56 Hadeiba H, Locksley RM. Lung CD25 CD4 regulatory T cells suppress type 2 immune responses but not bronchial hyperreactivity. *J Immunol* 2003; 170: 5502–5510.

57 Stassen M, Jonuleit H, Muller C, *et al.* 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.

58 Karlsson MR, Rugtveit J, Brandtzaeg P. Allergen-responsive CD4+CD25+ regulatory T cells in children who have outgrown cow's milk allergy. *J Exp Med* 2004; 199: 1679–1688.

59 Ling EM, Smith T, Nguyen XD, *et al.* Relation of CD4+CD25+ regulatory T-cell suppression of allergen-driven T-cell activation to atopic status and expression of allergic disease. *Lancet* 2004; 363: 608–615.

60 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.

61 Lin CH, Hunig T. Efficient expansion of regulatory T cells *in vitro* and *in vivo* with a CD28 superagonist. *Eur J Immunol* 2003; 33: 626–638.

62 Suntharalingam G, Perry MR, Ward S, *et al.* Cytokine storm in a phase 1 trial of the anti-CD28 monoclonal antibody TGN1412. *N Engl J Med* 2006; 355: 1018–1028.

63 Watanabe T, Masuyama J, Sohma Y, *et al.* CD52 is a novel costimulatory molecule for induction of CD4+ regulatory T cells. *Clin Immunol* 2006; 120: 247–259.

64 Beier KC, Kallinich T, Hamelmann E. Master switches of T-cell activation and differentiation. *Eur Respir J* 2007; 29: 804–812.

65 Geissmann F, Jung S, Littman DR. Blood monocytes consist of two principal subsets with distinct migratory properties. *Immunity* 2003; 19: 71–82.

66 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.

67 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.

68 Reinhardt RL, Bullard DC, Weaver CT, Jenkins MK. Preferential accumulation of antigen-specific effector CD4 T cells at an antigen injection site involves CD62E-dependent migration but not local proliferation. *J Exp Med* 2003; 197: 751–762.

69 Manetti R, Parronchi P, Giudizi MG, *et al.* Natural killer cell stimulatory factor (interleukin 12 [IL-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.

70 Eder W, Klimecki W, Yu L, *et al.* Toll-like receptor 2 as a major gene for asthma in children of European farmers. *J Allergy Clin Immunol* 2004; 113: 482–488.

71 Yang IA, Barton SJ, Rorke S, *et al.* Toll-like receptor 4 polymorphism and severity of atopy in asthmatics. *Genes Immun* 2004; 5: 41–45.

72 Fageras BM, Hmani-Aifa M, Lindstrom A, *et al.* A TLR4 polymorphism is associated with asthma and reduced lipopolysaccharide-induced interleukin-12 (p70) responses in Swedish children. *J Allergy Clin Immunol* 2004; 114: 561–567.

73 Tantisira K, Klimecki WT, Lazarus R, *et al.* Toll-like receptor 6 gene (*TLR6*): single-nucleotide polymorphism frequencies and preliminary association with the diagnosis of asthma. *Genes Immun* 2004; 5: 343–346.

74 Lazarus R, Raby BA, Lange C, *et al. TOLL*-like receptor 10 genetic variation is associated with asthma in two independent samples. *Am J Respir Crit Care Med* 2004; 170: 594–600.

75 Baldini M, Lohman IC, Halonen M, Erickson RP, Holt PG, Martinez FD. A polymorphism in the 5′ flanking region of the CD14 gene is associated with circulating soluble

CD14 levels and with total serum immunoglobulin E. *Am J Respir Cell Mol Biol* 1999; 20: 976–983.
76 Litonjua AA, Belanger K, Celedon JC, *et al.* Polymorphisms in the 5′ region of the CD14 gene are associated with eczema in young children. *J Allergy Clin Immunol* 2005; 115: 1056–1062.
77 Zambelli-Weiner A, Ehrlich E, Stockton ML, *et al.* Evaluation of the CD14/-260 polymorphism and house dust endotoxin exposure in the Barbados Asthma Genetics Study. *J Allergy Clin Immunol* 2005; 115: 1203–1209.
78 O'Donnell AR, Toelle BG, Marks GB, *et al.* Age-specific relationship between CD14 and atopy in a cohort assessed from age 8 to 25 years. *Am J Respir Crit Care Med* 2004; 169: 615–622.
79 Kedda MA, Lose F, Duffy D, Bell E, Thompson PJ, Upham J. The CD14 C-159T polymorphism is not associated with asthma or asthma severity in an Australian adult population. *Thorax* 2005; 60: 211–214.
80 Eder W, Klimecki W, Yu L, *et al.* Opposite effects of CD 14/-260 on serum IgE levels in children raised in different environments. *J Allergy Clin Immunol* 2005; 116: 601–607.
81 Vermaelen K, Pauwels R. Accelerated airway dendritic cell maturation, trafficking, and elimination in a mouse model of asthma. *Am J Respir Cell Mol Biol* 2003; 29: 405–409.
82 Harding FA, McArthur JG, Gross JA, Raulet DH, Allison JP. CD28-mediated signalling co-stimulates murine T cells and prevents induction of anergy in T-cell clones. *Nature* 1992; 356: 607–609.
83 Natesan M, Razi-Wolf Z, Reiser H. Costimulation of IL-4 production by murine B7-1 and B7-2 molecules. *J Immunol* 1996; 156: 2783–2791.
84 Kuchroo VK, Das MP, Brown JA, *et al.* B7-1 and B7-2 costimulatory molecules activate differentially the Th1/Th2 developmental pathways: application to autoimmune disease therapy. *Cell* 1995; 80: 707–718.
85 Stumbles PA, Thomas JA, Pimm CL, *et al.* 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.
86 Constant SL, Brogdon JL, Piggott DA, *et al.* Resident lung antigen-presenting cells have the capacity to promote Th2 T cell differentiation *in situ*. *J Clin Invest* 2002; 110: 1441–1448.
87 Jaffar ZH, Stanciu L, Pandit A, Lordan J, Holgate ST, Roberts K. Essential role for both CD80 and CD86 costimulation, but not CD40 interactions, in allergen-induced Th2 cytokine production from asthmatic bronchial tissue: role for αβ, but not γδ, T cells. *J Immunol* 1999; 163: 6283–6291.
88 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.
89 Hidi R, Riches V, Al Ali M, *et al.* Role of B7-CD28/CTLA-4 costimulation and NF-κB in allergen-induced T cell chemotaxis by IL-16 and RANTES. *J Immunol* 2000; 164: 412–418.
90 Larche M, Till SJ, Haselden BM, *et al.* Costimulation through CD86 is involved in airway antigen-presenting cell and T cell responses to allergen in atopic asthmatics. *J Immunol* 1998; 161: 6375–6382.
91 Howard TD, Postma DS, Hawkins GA, *et al.* Fine mapping of an IgE-controlling gene on chromosome 2q: analysis of CTLA4 and CD28. *J Allergy Clin Immunol* 2002; 110: 743–751.
92 Lee SY, Lee YH, Shin C, *et al.* Association of asthma severity and bronchial hyperresponsiveness with a polymorphism in the cytotoxic T-lymphocyte antigen-4 gene. *Chest* 2002; 122: 171–176.
93 Nakao F, Ihara K, Ahmed S, *et al.* Lack of association between CD28/CTLA-4 gene polymorphisms and atopic asthma in the Japanese population. *Exp Clin Immunogenet* 2000; 17: 179–184.
94 Heinzmann A, Plesnar C, Kuehr J, Forster J, Deichmann KA. Common polymorphisms in the CTLA-4 and CD28 genes at 2q33 are not associated with asthma or atopy. *Eur J Immunogenet* 2000; 27: 57–61.
95 Mark DA, Donovan CE, De Sanctis GT, *et al.* B7-1 (CD80) and B7-2 (CD86) have complementary roles in mediating allergic pulmonary inflammation and airway hyperresponsiveness. *Am J Respir Cell Mol Biol* 2000; 22: 265–271.
96 Mathur M, Herrmann K, Qin Y, *et al.* CD28 interactions with either CD80 or CD86 are sufficient to induce allergic airway inflammation in mice. *Am J Respir Cell Mol Biol* 1999; 21: 498–509.
97 Burr JS, Kimzey SL, Randolph DR, Green JM. CD28 and CTLA4 coordinately regulate airway inflammatory cell recruitment and T-helper cell differentiation after inhaled allergen. *Am J Respir Cell Mol Biol* 2001; 24: 563–568.
98 Deurloo DT, van Berkel MA, van Esch BC, *et al.* 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: 1297–1304.
99 McAdam AJ, Greenwald RJ, Levin MA, *et al.* ICOS is critical for CD40-mediated antibody class switching. *Nature* 2001; 409: 102–105.
100 Dong C, Juedes AE, Temann UA, *et al.* ICOS co-stimulatory receptor is essential for T-cell activation and function. *Nature* 2001; 409: 97–101.
101 Tafuri A, Shahinian A, Bladt F, *et al.* ICOS is essential for effective T-helper-cell responses. *Nature* 2001; 409: 105–109.
102 Wong SC, Oh E, Ng CH, Lam KP. Impaired germinal center formation and recall T-cell-dependent immune responses in mice lacking the costimulatory ligand B7-H2. *Blood* 2003; 102: 1381–1388.
103 Mak TW, Shahinian A, Yoshinaga SK, *et al.* Costimulation through the inducible costimulator ligand is essential for both T helper and B cell functions in T cell-dependent B cell responses. *Nat Immunol* 2003; 4: 765–772.
104 Gramaglia I, Jember A, Pippig SD, Weinberg AD, Killeen N, Croft M. The OX40 costimulatory receptor determines the development of CD4 memory by regulating primary clonal expansion. *J Immunol* 2000; 165: 3043–3050.
105 Jember AG, Zuberi R, Liu FT, Croft M. Development of allergic inflammation in a murine model of asthma is dependent on the costimulatory receptor OX40. *J Exp Med* 2001; 193: 387–392.

106 Chen AI, McAdam AJ, Buhlmann JE, *et al.* Ox40-ligand has a critical costimulatory role in dendritic cell:T cell interactions. *Immunity* 1999; 11: 689–698.
107 Arestides RS, He H, Westlake RM, *et al.* Costimulatory molecule OX40L is critical for both Th1 and Th2 responses in allergic inflammation. *Eur J Immunol* 2002; 32: 2874–2880.
108 Hoshino A, Tanaka Y, Akiba H, *et al.* Critical role for OX40 ligand in the development of pathogenic Th2 cells in a murine model of asthma. *Eur J Immunol* 2003; 33: 861–869.
109 Tivol EA, Borriello F, Schweitzer AN, Lynch WP, Bluestone JA, Sharpe AH. Loss of CTLA-4 leads to massive lymphoproliferation and fatal multiorgan tissue destruction, revealing a critical negative regulatory role of CTLA-4. *Immunity* 1995; 3: 541–547.
110 London CA, Lodge MP, Abbas AK. Functional responses and costimulator dependence of memory CD4+ T cells. *J Immunol* 2000; 164: 265–272.
111 Gause WC, Mitro V, Via C, Linsley P, Urban JF Jr, Greenwald RJ. Do effector and memory T helper cells also need B7 ligand costimulatory signals? *J Immunol* 1997; 159: 1055–1058.
112 Hutloff A, Dittrich AM, Beier KC, *et al.* ICOS is an inducible T-cell co-stimulator structurally and functionally related to CD28. *Nature* 1999; 397: 263–266.
113 McAdam AJ, Chang TT, Lumelsky AE, *et al.* Mouse inducible costimulatory molecule (ICOS) expression is enhanced by CD28 costimulation and regulates differentiation of CD4+ T cells. *J Immunol* 2000; 165: 5035–5040.
114 Beier KC, Hutloff A, Dittrich AM, *et al.* Induction, binding specificity and function of human ICOS. *Eur J Immunol* 2000; 30: 3707–3717.
115 Lohning M, Hutloff A, Kallinich T, *et al.* Expression of ICOS *in vivo* defines CD4+ effector T cells with high inflammatory potential and a strong bias for secretion of interleukin 10. *J Exp Med* 2003; 197: 181–193.
116 Akbari O, Freeman GJ, Meyer EH, *et al.* Antigen-specific regulatory T cells develop *via* the ICOS-ICOS-ligand pathway and inhibit allergen-induced airway hyperreactivity. *Nat Med* 2002; 8: 1024–1032.
117 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: 1149–1156.
118 Shilling RA, Pinto JM, Decker DC, *et al.* Cutting edge: polymorphisms in the ICOS promoter region are associated with allergic sensitization and Th2 cytokine production. *J Immunol* 2005; 175: 2061–2065.
119 Tesciuba AG, Subudhi S, Rother RP, *et al.* Inducible costimulator regulates Th2-mediated inflammation, but not Th2 differentiation, in a model of allergic airway disease. *J Immunol* 2001; 167: 1996–2003.
120 Stuber E, Neurath M, Calderhead D, Fell HP, Strober W. Cross-linking of OX40 ligand, a member of the TNF/NGF cytokine family, induces proliferation and differentiation in murine splenic B cells. *Immunity* 1995; 2: 507–521.
121 Agata Y, Kawasaki A, Nishimura H, *et al.* Expression of the PD-1 antigen on the surface of stimulated mouse T and B lymphocytes. *Int Immunol* 1996; 8: 765–772.
122 Nishimura H, Okazaki T, Tanaka Y, *et al.* Autoimmune dilated cardiomyopathy in PD-1 receptor-deficient mice. *Science* 2001; 291: 319–322.
123 Nishimura H, Nose M, Hiai H, Minato N, Honjo T. Development of lupus-like autoimmune diseases by disruption of the PD-1 gene encoding an ITIM motif-carrying immunoreceptor. *Immunity* 1999; 11: 141–151.
124 Matsumoto K, Inoue H, Nakano T, *et al.* B7-DC regulates asthmatic response by an IFN-γ-dependent mechanism. *J Immunol* 2004; 172: 2530–2541.