

REVIEW

Co-stimulatory molecules as potential targets for therapeutic intervention in allergic airway disease

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Summary

Airway inflammation is a characteristic feature of allergic asthma. Central to the initiation and progression of the inflammatory process are allergen-specific T lymphocytes that attract eosinophils, mast cells, and B cells to the airways by the secretion of specific cytokines. The direction of T cell responses is influenced by co-stimulatory signals that modulate the antigen-specific signal delivered by the T cell receptor. In addition to the prototypic co-stimulatory molecule, CD28, a number of newly identified co-stimulatory molecules and their ligands have now been characterized. Over the past 5 years, the role of these molecules in the pathophysiology of allergen-mediated sensitization and airway inflammation has been extensively studied in animal models of allergic asthma. The aim of this review is to provide a detailed overview on recent studies in mice and preliminary findings in man and to discuss the potential therapeutic and preventive treatment strategies offered by interactions with co-stimulatory molecules for patients with allergic airway diseases.

Keywords asthma, co-stimulation, T cells, therapeutical targets

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Introduction

Allergic bronchial asthma is characterized by airway obstruction, airway hyper-responsiveness (AHR) and remodelling based on allergen-induced airway inflammation. Thus, suppression of acute and chronic inflammation is a primary goal of asthma therapy. The established anti-inflammatory therapy with corticosteroids is effective in controlling asthmatic symptoms for the majority of patients. A proportion of patients, however, suffer some impairment of daily activities despite frequent intake of high doses of inhaled or even systemic steroids or endure the various side-effects of these treatments. The only curative approach for the treatment of allergic asthma, specific immune therapy, does normally not lead to full control of the symptoms and is limited because of limitations of eligible patients and the possibility of severe side-effects. Therefore, novel therapeutic strategies with a more specific blockade of the inflammatory cascade are required to optimize asthma therapy and minimize adverse reactions. The recent progress in understanding of the cellular and molecular pathways underlying the development of allergen-induced sensitization and airway disease offers the opportunity to establish new forms of anti-allergic therapy.

The pathophysiology of allergen-mediated lung inflammation has been extensively studied in murine models of allergic airway disease. While these studies revealed that certain cells

and mediators like mast cells [1], B cells and even immunoglobulin (Ig)E [2] may be dispensable for the induction of an allergen-induced inflammatory process, there is evidence for an obligatory role for activated CD4⁺ T helper-2 (Th2) cells or their cytokine products in the development of allergic immune responses in the lung [3–6]. CD4⁺ T cells infiltrating the airways of patients with atopic asthma predominantly produce cytokines of the Th2 type, including IL-4, IL-5, and IL-13 [7]. These cytokines attract and activate other effector cells, such as eosinophils, B cells, and mast cells into the airways, thus triggering the progress of the early and late asthmatic reaction [8, 9].

T cells undergo a number of differentiation steps to become Th effector cells. The antigen-specific signal generated by the T cell receptor (TCR) itself is insufficient for optimal T-cell activation. Full activation requires additional stimulation through co-stimulatory molecules interacting with corresponding ligands on antigen presenting cells (APC). Only the amplification and modification of the TCR signal by the co-stimulatory signal enables the antigen-specific pre-activated T lymphocyte to proliferate, secrete cytokines, and express cell-surface molecules for further cell–cell interactions [10]. The prototypic co-stimulatory molecule is CD28, but a substantial number of new co-stimulatory molecules and their ligands have been identified during the past 5 years [11–13] (Fig. 1, Table 1). T cell activation is now understood as an integrational process of positive as well as negative co-stimulatory signals. The T cell response is further fine tuned by receptor density, antigen type, and concentration as well as time of engagement [13, 14].

The critical role of co-stimulatory molecules for the regulation of T cell responses makes these molecules attractive

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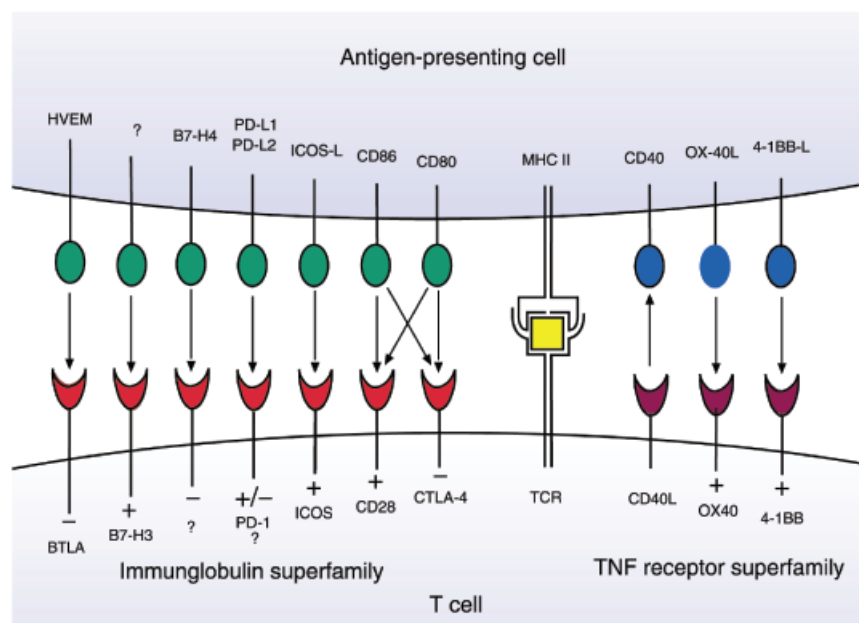


Fig. 1. Co-stimulation of T cells: Co-stimulatory molecules on T cells interact with specific ligands on antigen-presenting cells. MHC, major histocompatibility complex; TCR, T cell receptor

Table 1. Expression of co-stimulatory molecules

	Expression	Counter-receptors	Expression of counter-receptors
<i>Immunoglobulin superfamily</i>			
CD28 [16]	Constitutively on T cells [17]	CD80 (B7-1) and CD86 (B7-2) [18]	Both expressed on APCs, CD80 slower expression kinetic after stimulation than CD86 [19]
CTLA-4 (CD152) [33]	Activated T cells [33]	CD80 (B7-1) and CD86 (B7-2) [18]	Both expressed on APCs, CD80 slower expression kinetic after stimulation than CD86 [19]
ICOS [61]	Activated T cells, memory T cells [61]	B7RP-1 (ICOS-L, B7-H2, hLICOS, B7h, GL50) [62]	APCs, T cells, endothelial cells, up-regulated by inflammatory signals in peripheral tissues [67–69]
PD-1 [93]	Activated T cells, B cells, myeloid cells [94]	PD-L1 (B7-H1) [95] and PD-L2 [96]	Activated APCs, T cells [95], endothelial cells [132]
Orphan ligand	Activated T cells [110]	B7-H3 [110]	Activated APCs [110]
Orphan ligand	?	B7-H4 (B7S1, B7x) [115]	Broad expression, found mostly on epithelial cells [116]
BTLA [117]	T cells, B cells, dendritic cells, g/d T cells [118, 119]	HVEM [121, 133]	Multiple human tissues, high expression by T cells, B cells and monocytes [134]
<i>TNF receptor superfamily</i>			
OX40 (CD134) [135]	Activated T cells [82]	OX40L-ligand [136]	Activated APCs [137]
4-1BB (CD137) [123]	Activated T cells [124, 127], APCs, B cells, NK cells, eosinophils [125]	4-1BB-ligand [126]	Activated APCs, B cells [127]

APC, antigen presenting cells; ICOS, inducible T cell co-stimulator; HVEM, herpes virus entry mediator.

targets for novel therapeutic strategies. A substantial number of studies in murine models provided insight into the contribution of co-stimulation to the pathophysiology of allergen-mediated airway inflammation. When analysing the results obtained in these models and extrapolating to the human situation, several aspects need to be considered. The inflammatory response may vary greatly depending on the type and dosage of antigen, the use of adjuvant, the route of application, and the mouse strain [15]. Furthermore, different approaches utilizing stimulating or blocking monoclonal antibodies (mAb), fusion proteins, or genetically modified mice complicate direct comparisons of the findings from various studies. The goal of this review is to provide detailed analyses of the numerous murine studies and to offer quick reference tables summarizing central aspects such

as mouse strains, blocking reagents, time-points of blockade, and effects on various parameters of airway inflammation. Moreover, we try to outline current understanding of the function of the specific co-stimulatory molecules for allergic diseases in man. Integrating these findings, we discuss the potential of each particular co-stimulatory molecule as a target for novel therapeutic strategies in the treatment of allergic airway disease.

The CD28 system

To date, the most intensively investigated co-stimulatory system for T cell activation is the CD28 system (Fig. 1). The surface molecule CD28 [16] is constitutively expressed by

T cells [17] and binds to its ligands, CD80 (B7.1) and CD86 (B7.2) [18], which are expressed on the surface of APCs [19]. CD86 is constitutively expressed at low levels and is rapidly up-regulated after primary antigen recognition, whereas CD80 exhibits delayed expression kinetics [19]. In murine models for allergic airway inflammation, a rapid and profound up-regulation of CD86 was detected on dendritic cells in the lung [20] and spleen [21].

Ligation of CD28 with B7 molecules leads to a decreased activation threshold and an increased production of IL-2, IL-4, and IL-5. CD28 engagement also induces the expression of anti-apoptotic factors of the bcl-family, thus leading to a clonal expansion and prolonged survival of activated T cells [22–24]. In contrast, the effector functions of previously activated T cells seem to be less dependent on the engagement of CD28/CD80:CD86 [14, 25]. Whereas B7 molecules control the induction of IL-4 synthesis, this dependence is lost after initial priming [26]. In accordance with these *in vitro* findings, blockade of CD28 during the primary Th2-dominated response to the gastrointestinal nematode parasite, *Heligmosomoides polygyrus*, abrogated the development of IL-4 producing cells. In contrast, blocking of CD28 during an established immune response did not alter IL-4 gene expression, protein secretion, or elevation of serum IgE levels [27, 28]. Some studies have supported the hypothesis that differential signalling through the CD28 receptor can have distinct effects on the synthesis of Th1 and Th2 cytokines. The early differentiation of naïve T cells into IL-4 producers was shown to be mainly CD28 dependent [29, 30]. The concept that co-stimulation mediated by CD86 provides the initial signal to induce naïve T cells to differentiate into IL-4 producing Th2 cells, whereas CD80 interaction induces a rather neutral impulse or even direct differentiation towards the Th1 phenotype is currently a matter of debate [31, 32].

Another ligand for CD80 and CD86 is CTLA-4, which is expressed by T cells upon activation [33], and provides a negative feedback signal limiting the immune response [34, 35]. CTLA-4 is not constitutively expressed, but rapidly up-regulated after T cell activation [33]. The observation that CTLA-4-deficient mice develop a lethal lymphoproliferative disease within the first few weeks of life reflects the importance of this molecule in the restriction of T cell-mediated immune responses [36].

CD28 in murine models for asthma

In order to analyse the role of the CD28 co-stimulatory pathway in the pathogenesis of allergen-induced airway inflammation, different approaches were taken (Table 2). The following analyses mostly utilized murine models, in which intraperitoneal sensitization was followed by repeated airway allergen challenges, thus inducing airway inflammation, AHR and the synthesis of Th2-cytokines and IgE. Different approaches were used to interfere with CD28-mediated signalling, including the use of blocking fusion proteins, mAbs, or genetically deficient mice.

Blocking CD28 signalling by fusion protein

The first series of studies was published in 1996 and 1997. In these experiments, ligation of CD28–CD80 and CD86 was

inhibited by systemic administration of a blocking fusion protein, CTLA-4-Ig. When applied during the course of allergen sensitization, airway inflammation and the development of AHR were drastically reduced [37, 38]. Furthermore, blocking CD28 during the primary immune response significantly decreased IL-4 concentrations in the bronchoalveolar lavage (BAL) and the systemic concentrations of ovalbumin (OVA)-specific IgG1, IgE (augmented by IL-4), and IgG2a (augmented by IFN- γ) [37]. In contrast, the production of the Th1-cytokine IFN- γ remained unaltered by administration of CTLA4-Ig [37]. Some of these profound effects can be explained by the complete inhibition of the germinal centre reaction after blocking of the CD28/CD86 signal during the primary immune response [39].

The next question was whether blockade of CD28 was able to alter the immune response upon rechallenge with the allergen. For these studies, the ligation of CD28–CD80 and CD86 was inhibited by systemic or intranasal administration of CTLA-4-Ig during allergen challenge of previously sensitized mice. Following this treatment protocol, cells from the local draining lymph nodes of the airways and the pulmonary compartment exhibited an attenuated production of the Th2 cytokines IL-4, IL-5, and IL-10, whereas production of the Th1-cytokine IFN- γ was increased or remained unchanged [40, 41]. Concomitantly, development of airway inflammation and AHR were also drastically reduced in CTLA-4-Ig treated animals [40–42]. Only one report showed contrasting results with no effect on AHR and cellular infiltration after administration of CTLA4-Ig at the time of allergen challenge [38]. In a more recent study of mild allergen-induced airway inflammation by Deurloo et al. [43], treatment with CTLA4-Ig diminished OVA-specific IgE synthesis, IL-5 levels and numbers of eosinophils in the BAL. However, employing a protocol that induced a more robust airway inflammatory response with adjuvant (alum), the same authors found that CTLA-4-Ig had less pronounced effects [43]. Thus, the interruption of the CD28 co-stimulatory signal seems also to inhibit the secondary immune response, at least in some protocols of murine allergen airway inflammation. Not unexpectedly, a continuous application of CTLA-4 throughout the whole protocol led to a stronger suppression of airway inflammation [44].

Blocking CD28 with monoclonal antibodies

To investigate the role of the individual CD28 ligands for the induction and maintenance of allergic airway inflammation, mAb directed to CD80 or CD86 have been used. In contrast to the studies utilizing CTLA-4-Ig, this approach allowed independent evaluation of the two different CD28 ligands.

After administration of anti-CD86 mAb during the sensitization phase, a clear reduction of all relevant disease parameters, including allergen-specific Ig production, Th2-cytokine production, and development of airway inflammation and AHR was observed [45]. Administration of anti-CD86 mAb during sensitization and airway allergen challenge also reduced symptoms [46], whereas alteration of the inflammatory response after anti-CD80 was only observed in one report [47]. In contrast, in two other experimental settings the administration of anti-CD80 during the effector phase did not lead to a suppression of airway

Table 2. CD28 in mouse models for allergen induced airway disease

Approach	Mouse strain	Blocking reagent	Time of blockade	Outcome				IFN- γ	AHR	IgE	Reference
				Eosinophils in BAL	Pulmonary infiltration	IL-4	IL-5				
Fusion protein	A/J	CTLA4-Ig	Sensitization	↓	ND	↓	↓	↔	↓	↓	[37]
	C57BL/6	CTLA4-Ig	Sensitization	↓	↓*	ND	ND	ND	↓	ND	[38]
	Balb/c	CTLA4-Ig	Effector phase	ND	↓*	ND	ND	ND	↓*	ND	[42]
	Sv129	CTLA4-Ig	Effector phase	↓	ND	↓	↓	↑	↓	↓	[40]
	Balb/c	CTLA4-Ig	Effector phase	↓	ND	↓	ND	↔	↓	↓	[41]
	A/J	CTLA4-Ig	Effector phase	↓	ND	↓	↓	↔	↓	↓	[37]
	Balb/c	CTLA4-Ig	Effector phase	↓	ND	ND	ND	ND	ND†	↓	[43]
	C57BL/6	CTLA4-Ig	Effector phase	↔	↔*	ND	ND	ND	ND	↔	[38]
	C57BL/6	CTLA4-Ig	Continuously	↓	↓	ND	ND	ND	ND	ND	[44]
	C57BL/6	CTLA4-Ig	Continuously	↓	↓	ND	ND	↑	↓	↓	[52]
	C57BL/6J	Y100F-Ig	Continuously	↓	ND	ND	ND	ND	ND	↔	[48]
	B10.A → 5.C.7‡	Y100F-Ig	Continuously	↓	ND	↓	↓	↓	ND	ND	[49]
	A/J	α -CD80 (16-10A1)	Sensitization	↓	ND	↔	↔	↔	↔	↔	[45]
	A/J	α -CD86 (GL-2)	Sensitization	↓	ND	↓	↓	↔	↓	↓	[45]
	A/J	α -CD80/CD86	Sensitization	↓	ND	↓	↓	↔	↓	↓	[45]
Monoclonal Antibody	BALB/cByJ	α -CD80	Continuously	↔	↔	↔	↔	↔	↔	↔	[46]
	BALB/cByJ	α -CD86	continuously	↓	↓	↓	↓	↔	↓	↓	[46]
	C57BL/6	α -CD80 (16-10A1)	Continuously	↓	↓	ND	ND	↔	↓	↓	[47]
	C57BL/6	α -CD86 (GL-1)	Continuously	↓	↓	ND	ND	↔	ND	↓	[47]
	C57BL/6	α -CD80/ α -CD86	Continuously	↓	↓	ND	ND	↔	ND	↓	[47]
	Sv129	α -CD80 (16-10A1)	Continuously	↓	↓	ND	ND	↓	ND	↔	[40]
	Sv129	α -CD86 (GL-2)	Effector phase	(↓)	ND	↔	↔	↔	↔	↔	[40]
	Sv129	α -CD80/CD86	Effector phase	↓	ND	↓	↓	↑	↓	↓	[40]
	C57BL/6	α -CD80 (16-10A1)	Effector phase	↓	ND	↓	↓	↑	↓	↓	[40]
	C57BL/6	α -CD86 (GL-1)	Effector phase	↔	↔	↔	↔	↔	ND	↔	[47]
	C57BL/6	α -CD80/CD86	Effector phase	↔	↔	↔	↔	↔	ND	↔	[47]
	Balb/cByJ	α -CD86	Effector phase	(↓)	ND	(↓)	(↓)	↔	↔	↔	[46]
	Balb/c	α -CTLA-4 (UC10-4F10-11)	Effector phase	↔	ND	↔	↔	ND	↔	↔	[138]
	Balb/c	α -CTLA-4 (UC10-4F10-11)	Sensitization	↑	ND	↑	↑	↔	↑	↑	[138]
Germline deficient mice	C57BL/6	α -CTLA-4 (UC10-4F10-11)	Sensitization	↔	ND	↔	↔	↔	↔	↔	[138]
	C57BL/6 CD28 ^{-/-}	+ CTLA4-Ig		↓	↓	↓	↓	↔	ND	↓	[47]
	C57BL/6 CD28 ^{-/-}			↓	ND	↓	↓	↔	ND	↓	[47]
	129Sv/Jae CD80 ^{-/-}			↓	↓	↓	↓	↑	↓	↓	[50]
	129Sv/Jae CD86 ^{-/-}			↓	↓	↓	↓	↑	↓	↓	[50]
	129Sv/Jae CD80/CD86 ^{-/-}			↓	↓	↓	↓	↑	↓	↓	[50]
	C57BL/6 CD28 ^{-/-}			↓	↓	↓	↓	↓	ND	↓	[44]
	C57BL/6 C.J	+ CTLA4-Ig		↓	↓	↓	↓	↓	ND	↓	[44]
	Balb/c CD28/CTLA4 ^{-/-}			↓	ND	ND	↓	↑	↓	↓	[51]
	Balb/c CD80/CD86 ^{-/-}			↓	ND	ND	↓	↑	↓	↓	[51]
				↓	ND	ND	↓	↑	↓	↓	[51]
				↓	ND	ND	↓	↑	↓	↓	[51]
				↓	ND	ND	↓	↑	↓	↓	[51]
				↓	ND	ND	↓	↑	↓	↓	[51]
				↓	ND	ND	↓	↑	↓	↓	[51]

Mice were sensitized with OVA^{+/+} Alum i.p. and subsequently challenged with OVA via the intranasal or inhalative route. In two studies [47, 52] mice were sensitized by intraperitoneal application of *S. mansoni* eggs followed by intranasal or intratracheal application of soluble egg antigen. One study used an adoptive transfer models in which 5C.7 TCR transgenic cells were injected into B10.A mice followed by immunization with tobacco horn worm moth cytochrome c (MCC) [49].

*Data not shown. †Repetitive OVA challenge did not result in AHR in untreated mice. ‡Transfer of TCR-transgenic T cells from 5C.7 mice into B10.A mice. §Persisting lymphocytic infiltrate with restored IFN- γ production.

¶CTLA4-Ig restored recruitment of lymphocytes but not eosinophils to the BAL. ||Increased production by lung cell culture upon α -CD3 restimulation.

BAL, bronchoalveolar lavage; APC, antigen presenting cells; OVA, ovalbumin; AHR, airway hyper-responsiveness; TCR, T cell receptor.

inflammation [40, 47], whereas in one study anti-CD86 was sufficient to block the inflammatory response [40]. Administration of a combination of both anti-CD80 and anti-CD86 mAb was only in one experimental setting superior to anti-CD86 mAb alone [47].

Taken together, these studies point towards a major contribution of CD86 in the development of allergen-mediated airway inflammation. The anti-inflammatory effect was most likely due to the suppression of Th2-dominated immune response to allergen, as CD86 blockade was associated with a marked decrease in Th2-cytokine synthesis [45–47]. The role of CD80 for the induction or the maintenance of Th2 reactions remains questionable, as selective blockade of the CD80 pathway resulted in suppressed [48, 49], unaltered [40, 45], or even enhanced [29, 32] *in vivo* Th2-type responses.

To further address this question, the group of F. Ronchese utilized a mutated form of CTLA-4, Y100F, which selectively blocks CD28–CD80 interactions, leaving CD28 signals delivered through CD86 intact [48]. On the one hand, the administration of this protein had no effects on the number of eosinophils in the peripheral blood or on the systemic Ig production, but on the other hand it significantly reduced eosinophilic infiltration into the lungs [49]. These observations pointed towards a major role of CD86 signals especially for local immune responses.

CD28 and CD28L knock-out mice

A third approach to study the impact of the CD28 system on allergen-induced airway diseases is the use of mice with germline deletions of co-stimulatory molecules. A disadvantage of this approach is that the co-stimulatory molecule is absent throughout all phases of the immune response and it is not possible to differentiate between effects on sensitization or allergen challenge. Furthermore, the early development and differentiation of T cells might be affected.

Consistent with the results from blocking experiments, CD86-deficient mice developed less airway inflammation in response to allergen sensitization and airway challenge than CD80-deficient animals [50]. In both, CD28-deficient mice and CD80/CD86 double knock out mice, production of IgE, eosinophilic airway inflammation, and development of AHR were virtually absent following allergen sensitization and airway challenge [44, 50, 51]. This confirmed the critical role of CD28 in allergen-induced Th2 immune reactions. Interestingly, in these mice the defect in CD28 expression had no impact on the lymphocytic infiltration into BAL fluid following sensitization and airway challenge, illustrating that this co-stimulatory signal may not be essential for the initial recruitment of mononuclear cells to lung tissue [47].

The inability of CD28-deficient mice to develop an inflammatory response to allergen may be attributed to either the loss of the positive CD28 signal or the consequential predominance of the negative CTLA-4 signal. Indeed, treatment of CD28-deficient mice with CTLA-4-Ig partially restored the inflammatory response with recruitment of lymphocytes into the BAL fluid [44] without re-establishment of Th2-cytokine production or IgG1 class switching.

These results, obtained in CD28- and CD28L-deficient mice, are in good concordance to the conclusions from the

experiments using blocking agents against CD28 and its ligands [41, 47, 52].

CD28 in human asthma

In the past few years, several studies on T cells obtained from BAL fluid and bronchial biopsies from asthmatic patients were performed [53–56]. Proliferative responses of BAL-derived T cells from atopic asthmatics stimulated *in vitro* with allergen in the presence of autologous APC were inhibited by the addition of anti-CD86, but not of anti-CD80 mAb to the culture [56], underscoring the critical role for CD86 and the minor role of CD80 in allergen-induced T cell responses.

CTLA-4-Ig inhibited the production of IL-5, IL-13, IL-16, and RANTES upon allergen-specific *in vitro* stimulation of T cells isolated from bronchial biopsies from asthmatic subjects [54, 55]. A more detailed analysis of these experiments revealed that allergen-mediated Th2-cytokine production in bronchial biopsies was dependent on signalling via both CD80 and CD86 [53].

Given its critical role in an immune balance, it is tempting to speculate that single nucleotide polymorphisms (SNP) in the CTLA-4 gene that have impact on the expression or function of CTLA-4 may influence the development of allergic diseases. The +49 A/G SNP was demonstrated to be associated with increased total IgE serum levels [57] and bronchial hyper-responsiveness (BHR) [58]. The latter study also demonstrated a significant association of the –1147 C/T SNP with BHR and association of the –318 C/T polymorphism with asthma severity [58]. In contrast, two other studies found no association for the +49 A/G and –318 C/T SNPs in different cohorts of asthmatic patients [59, 60].

The inducible T cell co-stimulator system

The recently identified inducible T cell co-stimulator (ICOS) is structurally and functionally closely related to CD28. Unlike constitutively expressed CD28, ICOS has to be induced onto the cell surface by antigen stimulation, which suggests that ICOS provides signals to pre-activated rather than to naïve T cells [61–63]. *In vitro*, ICOS expression is influenced by both TCR and CD28 signals. CD28 co-stimulation enhances ICOS expression, and ICOS up-regulation is markedly reduced in the absence of CD80 and CD86, indicating that some of the functions ascribed earlier to CD28 might be in part because of ICOS signalling [63–65]. However, ICOS expression is not entirely dependent upon CD28 signals, because CD8⁺ T cells that do not express CD28 can express ICOS following TCR stimulation [63]. Like CD28, ICOS co-stimulation augments the production of effector cytokines such as TNF- α , IFN- γ , IL-4, and IL-5 [61, 63]. In contrast to CD28, ICOS co-stimulation does not induce significant production of IL-2. At the same time, co-stimulation via ICOS is more proficient for the release of IL-10 [61], most likely because of high ICOS levels on terminally differentiated effector T cells [66].

ICOS does not bind to CD80 or CD86 but binds to its own ligand, ICOS-L (also named B7-H2, B7RP-1, hLICOS, B7h, or GL50). ICOS-L is constitutively expressed by APCs and T cells and can be up-regulated in peripheral tissues

(endothelium, brain, heart, liver, kidney) by inflammatory signals [67–69] (Fig. 1). The high amount of ICOS-L expression on these tissues during inflammation indicates a role of ICOS in the interaction of effector T cells with non-lymphoid tissues [13], which is less understood at present.

Inducible T cell co-stimulator in murine models for asthma

Similar to the experimental studies carried out for analyzing the impact of CD28 in allergic airway disease, the role of ICOS was delineated using genetically deficient mice and blocking mAb and fusion proteins. Not all of these experiments were intended to study allergic immune reactions, but overall these studies allow a number of predictions about the role of ICOS in allergen-mediated diseases (Table 3).

Inducible T cell co-stimulator and inducible T cell co-stimulator-ligand knock-out mice

Indicative for the possible involvement of the ICOS/ICOS-L system in the development of atopic diseases were the findings from ICOS knock-out mice on the C57BL/6 background [70–72] (Table 3). In the naïve state as well as following mitogen or allergen stimulation, ICOS^{-/-} mice showed reduced serum levels of immunoglobulin IgG1, IgG2a, and IgE compared with wild-type mice [70–72]. Moreover, germinal centre formation during primary immune responses was severely impaired [71, 72]. Consistently, IL-4 and, to a lesser extent, IL-13 production by T cells was impaired in ICOS^{-/-} mice, while IFN- γ production was increased when compared with wild-type animals [70, 71]. ICOS^{-/-} mice were susceptible to induction of allergen-mediated lung inflammation in OVA-primed mice with no differences in

lung histology compared with wild-type mice, but were unable to mount IgE responses [70, 71].

Two years later, these findings were confirmed in studies of ICOS-L knock-out mice [73–75]. ICOS-L-deficient mice were able to mount normal immune responses to both type I and type II T cell-independent antigens [73, 74]. Upon secondary and tertiary challenges, however, they had marked deficits in their response to T cell-dependent antigens in terms of production of antigen-specific class-switched antibodies (IgG1, IgG2a, and IgA) [73], and were unable to produce antigen-specific IgE upon sensitization and allergen airway challenge [74].

Blocking of inducible T cell co-stimulator signalling by fusion protein or monoclonal antibodies

Several studies investigated the effects of ICOS blockade in murine models for allergen-mediated airway inflammation. Blockade of ICOS in OVA-sensitized C57BL/6 mice showed that application of ICOS-Ig or anti-ICOS mAb (12A8) at the time of allergen sensitization had little effect on the development of airway inflammation, whereas blockade of CD28 with CTLA-4-Ig lead to significant reduction of cell numbers in the BAL [38]. In contrast, blockade of ICOS signalling in already sensitized mice significantly reduced the formation of lung inflammation, whereas blockade of the CD28-B7 pathway had no effect. The study demonstrated further that IL-4, IL-5, IL-10 as well as IgE production were impaired when ICOS signalling was interrupted during the effector phase.

Using adoptive transfer of T cells from OVA-sensitized into naïve BALB/c mice followed by airway allergen challenge, the

Table 3. ICOS in mouse models for allergen-induced airway disease

Approach	Mouse strain	Blocking reagent	Time of blockade	Outcome									
				AHR	Eosinophils in BAL	Pulmonary infiltration	IL-4	IL-5	IL-10	IL-13	IFN- γ	IgE	Reference
Fusion protein	C57BL/6	ICOS-Ig	Sensitization	ND	ND	ND	ND	ND	ND	ND	ND	ND	[38]
			Effector phase	↓	↓	↓	ND	ND	ND	ND	ND	ND	
	C57BL/6*	ICOS-Ig	Continuously		↓	↓	↓	↓	↓	ND	ND	↔	[76]
	Balb/c†	ICOS-Ig	Effector phase	↓	↓	ND	ND	↓	ND	ND	ND	ND	[65]
	Balb/c‡	ICOS-Ig	Sensitization and early effector phase	ND	↓	ND	ND	ND	ND	ND	ND	ND	[77]
	Balb/c	ICOS-L-Ig§ (B7RP-1-Fc)	Sensitization and effector phase	ND	↑	ND	↑	ND	↑	ND	↑	(1)	[139]
Monoclonal antibody	C57BL/6	Anti-ICOS (12A8)	Sensitization	(↓)	↔	↔	↔	↔	↓	↔	↔	(1)	[38]
			Effector phase	↓	↓	↓	↓	↓	↓	↓	↔	↓	
	Balb/c‡	Anti-ICOS (12A8)	Sensitization and early effector phase	ND	↓	ND	↔	↓	↑	↔	ND	↑	[77]
			Late effector phase	ND	↔	ND	ND	ND	ND	ND	ND	↔	
Germline-deficient mice	ICOS ^{-/-} (C57BL/6)			ND	ND	ND	↓	ND	↓	ND	↑	↓	[72]
	ICOS ^{-/-} (C57BL/6)			ND	↓	ND	↓	(1)	ND	↓	ND	↓	[70]
	ICOS ^{-/-} (C57BL/6)			ND	ND	ND	↓	ND	ND	ND	↑	↓	[71]
	ICOS-L ^{-/-} (C57BL/6)			ND	ND	ND	↓	ND	↓	ND	↑	ND	[73]
	ICOS-L ^{-/-} (C57BL/6)			ND	↓	↓	↓	ND	↓	ND	↑	↓	[74]

Unless indicated otherwise, mice were sensitized with OVA^{+/+} Alum i.p. and subsequently challenged with OVA via the intranasal or inhalative route.

*Heat inactivated *Schistosoma* eggs used as allergen. †Denovirus conditioned Th2 model. ‡Adoptive transfer of highly polarized Th2 cell line. §ICOS-stimulating reagent. BAL, bronchoalveolar lavage; OVA, ovalbumin; AHR, airway hyper-responsiveness; ICOS, inducible T cell co-stimulator; Th2, T helper 2; i.p., intraperitoneally; IgE, immunoglobulin E.

Table 4. OX40 in mouse models for allergen-induced airway disease

Approach	Mouse strain	Blocking reagent	Time of blockade	Outcome									
				AHR	eosinophils in BAL	Pulmonary infiltration	IL-4	IL-5	IL-10	IL-13	IFN- γ	IgE	Reference
Monoclonal antibody	Balb/c*	Anti-OX40L (RM134L)	Continuously	ND	ND	ND	↓	ND	↓	↓	↔	↓	[90]
	Balb/c	Anti-OX40L (RM134L)	Sensitization	↓	↓	↓	↓	↓	ND	↓	↓	↓	[91]
			Effector phase	↔	↔	↔	(↓)	(↓)	ND	(↓)	ND	ND	
	C57BL/6	Anti-OX40L (RM134L)	Effector phase	↓	↓	↓	↓	↓	ND	↓	ND	↓	[92]
Germline-deficient mice	OX40 ^{-/-} (C57BL/6)			ND	ND	ND	↓	↓	ND	ND	↓	ND	[81]
	OX40L ^{-/-} (Balb/c)			ND	ND	ND	↓	ND	ND	ND	↓	ND	[87]
	OX40 ^{-/-} (C57BL/6)			↓	↓	↓	↓	↓	ND	ND	ND	↓	[85]
	OX40L ^{-/-} (Balb/c)			↓	↓	↓	↓	ND	ND	↓	↓	↓	[86]
	OX40L ^{-/-} (Balb/c)			↓	↓	↓	↓	↓	ND	↓	ND	↓	[91]

Unless indicated otherwise, mice were sensitized with OVA^{+/+} Alum i.p. and subsequently challenged with OVA via the intranasal or inhalative route.

*Leishmania infection.

BAL, bronchoalveolar lavage; OVA, ovalbumin; AHR, airway hyper-responsiveness; 2; i.p., intraperitoneally; IgE, immunoglobulin E.

same group demonstrated that blocking of ICOS distinctively inhibited Th2-, but not Th1-biased airway inflammation [65]. ICOS blockade with ICOS-Ig reduced lung inflammation, lung eosinophilia and airway hyper-reactivity after adoptive transfer of highly polarized Th2 lines. In contrast, the neutrophilic infiltrate induced by transfer of highly polarized Th1 cells was reduced by CD28 blockade but not ICOS blockade. Another study showed that ICOS blockade (with ICOS-Ig) reduced Th2-associated airway inflammatory responses to *Schistosoma mansoni* eggs (effector phase), but did not attenuate the priming of T cells to Th2 cells [76]. Wiley et al. further analysed the question of differential role of ICOS in Th1 vs. Th2-biased immune reactions. They utilized a model of inhaled allergen-induced primary immune reaction that was biased to a Th1- or Th2-type through the local delivery of cytokines. Using this protocol, they demonstrated that a blockade with anti-ICOS mAb (12A8) intraperitoneally or ICOS-Ig i.v. during sensitization inhibited the early inflammatory cell influx and IL-5 production in the lungs in the Th2, but not the Th1 model [77]. Blockade of ICOS in the Th2 model significantly reduced the numbers of infiltrating CD44^{high} CD62^{low} memory/effector T cells in the lungs, suggesting that these cells were responsible for the early influx of other inflammatory cells. In contrast, blocking of ICOS on re-challenge showed no effect on cell numbers in the BAL fluid or on OVA-specific IgE production.

Another hint for the role of ICOS in allergic immune reactions was obtained by analysing the cytokine profile of ICOS-positive CD4⁺ T helper cells in lymphatic tissue of non-immunized mice. It was shown that there is a link between the density of ICOS cell surface expression and the type of cytokine produced [66]. Intermediate ICOS expression was associated with the production of Th2-cytokines, while high ICOS expression was associated with an IL-10-dominated cytokine profile. Employing this information in a model of adoptive transfer, the authors demonstrated that only ICOS⁺, but not ICOS⁻ T cells transferred into naïve BALB/c mice-induced striking airway inflammation upon subsequent airway challenge.

Collectively, one may hypothesize that CD28 is mainly required during the priming phase of an immune response, while ICOS signalling plays a pivotal role during the effector phase of an already established allergic immune response.

Regarding the strong association between high cell surface ICOS expression and the capacity to release IL-10 [66] there is a possible role for ICOS on regulatory T cells [78].

Inducible T cell co-stimulator in human patients

Little is known about the precise role of ICOS in human immune reactions. Initial decisive data were obtained from ICOS-deficient patients. In these patients, the lack of ICOS expression was associated with a humoral immunodeficiency resembling the phenotype of common variable immunodeficiency (CVID) with hypogammaglobulinemia, low numbers of B cells, and recurrent infection of the respiratory and gastrointestinal tracts [79]. Remarkably, in these patients a profound reduction in the number of CD27⁺ IgM⁻ IgD⁻ switched memory B cells was observed. Taken together, the findings in ICOS-deficient patients led to the conclusion, that ICOS expression on T cells is important for late B cell differentiation, class switching, B cell memory formation, and sustained Ig production.

The OX40 system

OX40, a member of the TNF-receptor family, is a co-stimulatory antigen mainly expressed on activated CD4⁺ Th2 T cells, but also on activated CD8⁺ T cells. The expression of OX40 on other cell types is less well defined [80] (Table 1). Expression can be observed on naïve T cells beginning 12–24 h after initial stimulation with an expression peak after 2–3 days and subsequent down-regulation. *In vivo*, OX40 is expressed in the T cell zones of spleen and lymph nodes several days after the first immunization [81]. Antigen-experienced, pre-activated T cells re-express OX40 rapidly within 4 h upon re-stimulation [82], whereas T cells deficient in OX40 undergo apoptosis 4–5 days after *in vitro* activation. This is explained by the requirement of OX40 for cytoplasmic expression of Bcl-xl and Bcl-2 which deliver anti-apoptotic signals for pre-activated T cells [83].

The ligand for OX40, OX40L, is expressed on activated B cells, dendritic cells, and endothelial cells. In the case of B cells and dendritic cells, OX40L expression is promoted by LPS, CD40, and Fc-receptor cross-linking [84].

OX40 in murine models for asthma

The role of OX40 in the induction and maintenance of antigen- or allergen-mediated immune responses was analysed in several experimental approaches, including the use of genetically deficient mice and blockade of OX40 signalling by mAb (Table 4).

OX40- and OX40L-deficient mice

Several studies performed in OX40- and OX40L-deficient mice showed that this system plays a major role for T cells in anti-viral as well as allergen-mediated immune responses (reviewed in [80]). OX40-deficient mice failed to generate significant numbers of antigen-specific CD4 T cells in the late primary response up to 5 weeks, a time-point when the T cell memory population is formed [81]. This indicated that OX40/OX40L interactions were not only necessary to accumulate effector T cells during primary immune responses but also influenced subsequent formation of memory cells. When applied to the murine model of allergen-induced airway inflammation, many prominent features of the disease, such as airway hyper-reactivity (AHR), Th2 cytokine production in BAL fluid and increased total serum IgE levels were significantly reduced in OX40- [85] and OX40L-deficient mice [86]. Similarly, when analysed *in vitro*, OX40L-deficient dendritic cells were less able to stimulate T cell cytokine production [87].

Blocking of OX40 signalling by monoclonal antibodies

In vitro experiments provided the first indication for the involvement of the OX40/OX40L pathway in the synthesis of Th2 cytokines [88, 89]. *In vivo*, in the *Leishmania major* infection model, blocking of OX40 with mAb resulted in the decreased synthesis of Th2 cytokines (IL-4, IL-10, and IL-13), whereas levels of IL-2 and IFN- γ were unimpaired [90]. Furthermore, total IgE levels were significantly decreased in animals treated with anti-OX40 mAb, indicating a direct role for OX40 in Th2-associated IgE production and allergic inflammation. There is ongoing controversy about the importance of OX40-signalling regarding the kinetics of the allergic immune response. Recent work [91] showed that the effect of OX40 is limited to the induction phase of an immune response. Administration of anti-OX40L mAb abolished subsequent development of airway inflammation upon airway allergen challenge only when administered during allergen sensitization, but not when given to already sensitized mice. In contrast to this result, another recently published paper [92], using a different mouse strain but the same blocking antibody, demonstrated that OX40L blockade during airway challenge abolished allergic airway inflammation (cellular infiltration, AHR, allergen-specific IgE production, Th2 cytokine synthesis) also at very late time-points (days 120–124). In this model OX40 was expressed on a significant number of CD44^{hi} memory T cells in immunized mice, and was clearly up-regulated on T cells from the lung and the draining lymph nodes after restimulation through inhaled allergen challenge. Furthermore, T cells from OX40-deficient TCR transgenic mice (OTII OX40^{-/-}) showed deficient expansion or survival when adoptively transferred

into recipients that were challenged with OVA. Taken together, these studies point towards the important role of the OX40/OX40L pathway during the primary and also, as demonstrated more recently, during the secondary immune response in mouse models of allergic airway inflammation.

The PD-1 system

After CTLA-4, PD-1 was identified as the second negative co-stimulatory molecule in the CD28 family. It shares 24% amino acid homology with CTLA-4 in the extracellular domain [93], but lacks the motif required for binding to CD80 and CD86. In contrast to CTLA-4, CD28, and ICOS, PD-1 is not restricted to T cells but is also expressed on B cells and myeloid cells following activation [94].

PD-1 delivers an inhibitory co-signal to T and B cells by engagement of its ligands PD-L1 (B7-H1) [95] and PD-L2 (B7-DC) [96, 97]. PD-L1 is constitutively expressed on freshly isolated T cells, B cells, macrophages, and DCs and was detected in thymus as well as in non-lymphoid tissues such as heart, kidney, and liver. It is further up-regulated on macrophages and DCs by IFN- γ , GM-CSF, or IL-4 [95, 98] and on T cells by CD3 ligation and might play a role in the suppressive function of regulatory T cells [99]. In contrast, PD-L2 is only expressed on macrophages and DCs after stimulation with the cytokines mentioned above [100]. These variations in tissue distribution and expression patterns suggest that the two PD-1 ligands play different roles at various stages of an immune response.

Despite its name, programmed death 1, the molecule is associated with T cell activation rather than apoptosis: It was shown that transfection of PD-1 into cells or cross-linking with mAbs did not affect programmed cell death, but lead to arrest in cell cycle phase G0–G1 [94]. The inhibitory effect of PD-1 on TCR-mediated proliferation and cytokine production can be overcome by CD28 co-stimulation or exogenous IL-2. Therefore, PD-1 effectively suppresses T cell responses in situations in which IL-2 is limiting, e.g. ICOS co-stimulation [101]. However, some groups showed a positive effect for PD-1 stimulation. Resting T cells stimulated with anti-CD3 mAb and B7-DC-Ig (PD-L2-Ig fusion protein) showed enhanced proliferation and IFN- γ production [97]. Another group reported enhanced proliferation and IFN- γ , GM-CSF, and IL-10 production by T cells stimulated with low doses of anti-CD3 mAb and B7-H1-Ig (PD-L1-Ig fusion protein) [95, 102]. It remains unclear, whether these conflicting results are because of a true positive stimulatory effect, to blockade of the negative PD-1 signal by the fusion proteins or to a putative positive signal delivered by an as yet to be identified receptor other than PD-1 [103]. A strong argument against the last hypothesis is provided by the fact that PD-L1-Ig plus anti-CD3 mAb fail to stimulate PD-1-deficient T cells [98].

PD-1 knock-out mice

The importance of PD-1 mediated T cell suppression for peripheral tolerance is supported by the observation that PD-1-deficient mice develop autoimmune diseases. The type of disease is dependent on the genetic background, as BALB/c mice develop dilated cardiomyopathy [104], while C57BL/6 mice develop autoimmune glomerulonephritis and arthritis

[105]. In these mice, antibody responses to T cell independent antigens are enhanced, thus resulting in increased serum levels of IgG2b, IgG3, and IgA [106]. In contrast to CTLA-4-deficient mice, symptoms of autoimmunity develop later in life and seem not to be controlled by the action of CTLA-4. This supports the hypothesis that CTLA-4 is the primary negative co-stimulatory signal within the germinal centre reaction, whereas PD-1 controls the induction and maintenance of autoimmune processes in the periphery [11, 107].

Furthermore, PD-1-deficient mice present with increased numbers of CD4-CD8 double-positive T cells and reduced numbers of single-positive CD8 T cells, suggesting a role for this pathway in thymocyte differentiation [106].

PD-1 in murine models for allergic reactions

First support for a role of the PD-1 pathway in allergic immune reactions was found in a mouse model for hapten-induced contact hypersensitivity [108]. Treatment with anti-PD-1 (J43) or anti-PD-L1 mAb (MIH5, MIH6, MIH7) during sensitization led to increased and prolonged ear swelling and increased numbers of T cells in the draining lymph nodes. In those lymph nodes a particular subset of PD-L1⁺ PD-L2⁻ DC was found, indicating that PD-L1, and not PD-L2, may play a unique inhibitory role in cutaneous sensitization to allergens.

A recently published study analysed the distinctive contribution of PD-L1 or PD-L2 in the development of OVA-induced murine airway inflammation [109]. A substantial number of pulmonary DCs, macrophages, and B cells isolated from the lungs of naïve mice did express PD-L1, which was further enhanced after allergen sensitization and airway challenge. Despite this abundant expression of PD-L1 by various APCs in inflamed lung tissues, treatment with blocking antibodies was not effective. In contrast, expression of PD-L2 was extremely low on lymphocytes of unchallenged mice, and only moderately enhanced on DCs and macrophages after allergen challenge of sensitized mice. Treatment with anti-PD-L2 mAb (TY25) during allergen challenge increased the development of AHR and lung eosinophilia significantly and led to increased levels of IL-5 and IL-13 in BAL fluid. The enhanced allergic symptoms were shown to be IFN- γ dependent, as depletion of IFN- γ with mAb (R4-6A2) abrogated this effect. No effect on the inflammatory response was demonstrated when PD-L2 was blocked during sensitization, indicating that the inhibitory function of PD-L2 is limited to the effector phase (airway challenge).

Other co-stimulatory molecules

Little if any data exist on the roles of B7-H3, B7-H4, BTLA, and 4-1BB in allergen-mediated airway inflammation as the molecules were identified only recently.

B7-H3

B7-H3 is an orphan member of the B7 family whose receptor has not been identified yet. Originally, the molecule was described to activate T cells *in vitro* [110], but analyses of B7-H3-deficient mice indicated an inhibitory effect on T cell function [111]. Using a model of airway inflammation in

which a Th1- or Th2-biased immune reaction was induced by allergen inhalation with concomitant local application of GM-CSF plus IL-12 or GM-CSF alone, a clear augmentation of Th1-biased inflammation responses was observed in B7-H3-deficient animals, whereas Th2 immune reactions were unaltered [111]. Contradictory findings were reported in an independent B7-H3-deficient mouse model used as a recipient for cardiac and islet allografts. Here, the authors found evidence for a co-stimulatory function of B7-H3 [112]. At present the reasons for these divergent findings are not determined yet.

B7-H4

B7-H4 (also designated B7S1, B7x) is another orphan member of the B7 family whose receptor has not been identified yet [113–115]. B7-H4 has a very broad tissue distribution and is found mostly on epithelial cells [116]. The reported expression on some cells of hematopoietic origin has to be confirmed yet. The current experimental data suggest that B7-H4 is a negative regulator of various T cell functions.

BTLA

The inhibitory molecule BTLA [117] is expressed by nearly all lymphoid cell subsets including T cells, B cells, dendritic cells, and γ/δ T cells [118, 119] and is as a member of the B7 family structurally related to CTLA-4 and PD-1 [120]. Cross-linking of BTLA with the TCR leads to co-receptor phosphorylation, phosphatase recruitment and down modulation of IL-2 production *in vitro*. T and B cells development was normal in BTLA-deficient mice, but T and B cells exhibited an increased rate of proliferation when stimulated *in vitro* [117]. After polarization of T cells, BTLA is mainly expressed on Th1 cells, suggesting a role for BTLA in controlling Th1-biased immune responses [117]. After immunization, these mice responded with an exaggerated humoral response to T cell-dependent antigens [117]. On comparison with PD-1- and CTLA-4-deficient mice, BTLA appears to be dispensable for the control of autoreactive T cells as no spontaneous autoimmune disease or lymphadenopathy was reported in these animals. However, BTLA-deficient mice are more susceptible to experimental autoimmune encephalitis (EAE). Very recently, it has been shown that the receptor for BTLA is the herpes virus entry mediator (HVEM) [121, 122]. Interestingly, this observation is the first example of a B7 family member interacting with a member of the TNF-receptor family.

4-1BB

4-1BB (CD137) [123], a member of the TNF-receptor superfamily, is rapidly induced after activation on T cells [124], B cells, monocytes, and non-lymphoid tissues [125]. 4-1BB-ligand [126] is expressed on B cells, monocytes, and DCs upon activation [127]. Functional studies focused on the role of this interaction in the regulation of cytotoxic T cell responses [128, 129] and the control of expansion and survival of CD8⁺ T cells [130]. Recently, it was shown that co-stimulation of CD4⁺ T cells through 4-1BB enhanced the survival and favoured the development of Th1 cytokine producing T cells with a sustained increase in INF-producing cells and

suppression of IL-4 and TNF production [131]. No studies on the role of this molecule interaction in the pathogenesis of allergic airway inflammation have been published.

Summary and conclusion

In past few years we have gained exciting new insights into the fundamental role of co-stimulatory molecules in the development of allergic diseases, many of which were based on the use of gene-defective animals. In other cases, the contribution of particular co-stimulators to the allergic cascade *in vivo* was studied by targeting with specific mAb or fusion proteins. When analysing and interpreting the *in vivo* data obtained with these latter reagents, some word of caution is necessary. In most of these studies essential questions regarding the mode of action of the blocking reagents have not been addressed in detail. Thus the issues of avidity, bioavailability, terminal half-life, and accessibility of the blocking agents to the targeted tissues were usually not determined. Moreover, possible unspecific interactions of the blocking reagents with other cells of the immune system, e.g. via the Fc-receptors, were usually not considered. Therefore, more extensive blocking studies of candidate molecules in animal models are warranted before given co-stimulatory molecules can be chosen as targets for treatment of patients. Nevertheless, from the information gained from studies in animal models and man, several basic conclusions can be made:

1. The successful modulation of ongoing inflammatory process requires a precise knowledge of the participating molecules, their kinetics and site of action. Substantial data are available for some well-characterized molecules like CD28, ICOS, and OX40. For the more recently described 'new' co-stimulators, a definitive statement on their potential use as therapeutic targets cannot be made yet.
2. Negative co-stimulatory molecules physiologically dampen primary immune reactions and are critical for the limitation of the immune responses against self-antigens. The available animal data clearly indicates that blocking of these negative T cell co-stimulators will result in an exacerbation of immune responses. For this reason, it is unlikely that the negative co-stimulators will become targets for the treatment of allergic airway diseases.
3. Optimal target criteria for a co-stimulatory molecule in the prevention and treatment of allergic immune reactions are: (i) specificity for T cells, the main regulatory cells in the allergic cascade; (ii) association with the Th2 effector response, which is critical for the immune deviation underlying the allergic response; and (iii) dependency of the expression/function of the co-stimulatory molecule upon cell activation. These three criteria are best met by ICOS and OX40. CD28 and other co-stimulatory molecules that are constitutively expressed on T cells or even on non-lymphoid tissues are unlikely to be ideal targets. In particular, a general suppression of primary immune responses by CD28 blockade is a noteworthy concern.
4. An alternative concept for the therapy of allergic immune reactions is the elimination of T cells bearing a given co-stimulatory molecule on the cell surface. Ideally, such a target molecule would be inducible, T cell-specific, and

Th2-associated, as mentioned above. Under optimal conditions, T cells specifically activated during an allergic immune response would be permanently removed from the immune system following a short course of treatment (for an extensive discussion of the depletion strategy see [140]).

The last decade has brought us an expanding knowledge on the contribution of co-stimulatory molecules for the shaping and fine-tuning of immune responses. We are now on the brink of translating these findings into first experimental and pre-clinical studies to treat unwanted immune responses. In this context, inducible co-stimulatory molecules with a preference for Th2-biased allergic immune reactions, such as ICOS and OX40, are promising new targets for novel treatment strategies in allergen-mediated airway disease.

References

- 1 Takeda K, Hamelmann E, Joetham A et al. Development of eosinophilic airway inflammation and airway hyperresponsiveness in mast cell-deficient mice. *J Exp Med* 1997; 186:449–54.
- 2 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; 21:480–9.
- 3 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; 142:1407–13.
- 4 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; 10:587–93.
- 5 Maddox L, Schwartz DA. The pathophysiology of asthma. *Annu Rev Med* 2002; 53:477–98.
- 6 Hamelmann E, Oshiba A, Paluh J et al. Requirement for CD8⁺ T cells in the development of airway hyperresponsiveness in a murine model of airway sensitization. *J Exp Med* 1996; 183:1719–29.
- 7 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.
- 8 Durham SR, Ying S, Varney VA et al. 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.
- 9 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; 88:661–74.
- 10 Mueller DL, Jenkins MK, Schwartz RH. Clonal expansion versus functional clonal inactivation: a costimulatory signalling pathway determines the outcome of T cell antigen receptor occupancy. *Annu Rev Immunol* 1989; 7:445–80.
- 11 Carreno BM, Collins M. The B7 family of ligands and its receptors: new pathways for costimulation and inhibition of immune responses. *Annu Rev Immunol* 2002; 20:29–53.
- 12 Sharpe AH, Freeman GJ. The B7-CD28 superfamily. *Nat Rev Immunol* 2002; 2:116–26.
- 13 Kroccek RA, Mages HW, Hutloff A. Emerging paradigms of T-cell co-stimulation. *Curr Opin Immunol* 2004; 16:321–7.

- 14 London CA, Lodge MP, Abbas AK. Functional responses and costimulator dependence of memory CD4⁺ T cells. *J Immunol* 2000; 164:265–72.
- 15 Lloyd CM, Gonzalo JA, Coyle AJ, Gutierrez-Ramos JC. Mouse models of allergic airway disease. *Adv Immunol* 2001; 77:263–95.
- 16 Aruffo A, Seed B. Molecular cloning of a CD28 cDNA by a high-efficiency COS cell expression system. *Proc Natl Acad Sci USA* 1987; 84:8573–7.
- 17 Gross JA, Callas E, Allison JP. Identification and distribution of the costimulatory receptor CD28 in the mouse. *J Immunol* 1992; 149:380–8.
- 18 Linsley PS, Greene JL, Brady W, Bajorath J, Ledbetter JA, Peach R. Human B7-1 (CD80) and B7-2 (CD86) bind with similar avidities but distinct kinetics to CD28 and CTLA-4 receptors. *Immunity* 1994; 1:793–801.
- 19 Hathcock KS, Laszlo G, Pucillo C, Linsley P, Hodes RJ. Comparative analysis of B7-1 and B7-2 costimulatory ligands: expression and function. *J Exp Med* 1994; 180:631–40.
- 20 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; 29:405–9.
- 21 Cheng X, Wang C, Qian G, Zhu B. CD80, but not CD86 were up-regulated on the spleen-derived dendritic cells from OVA-sensitized and challenged BALB/c mice. *Immunol Lett* 2003; 89:31–8.
- 22 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; 356:607–9.
- 23 Jenkins MK, Taylor PS, Norton SD, Urdahl KB. CD28 delivers a costimulatory signal involved in antigen-specific IL-2 production by human T cells. *J Immunol* 1991; 147:2461–6.
- 24 Rulifson IC, Sperling AI, Fields PE, Fitch FW, Bluestone JA. CD28 costimulation promotes the production of Th2 cytokines. *J Immunol* 1997; 158:658–5.
- 25 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; 159:1055–8.
- 26 Schweitzer AN, Sharpe AH. Studies using antigen-presenting cells lacking expression of both B7-1 (CD80) and B7-2 (CD86) show distinct requirements for B7 molecules during priming versus restimulation of Th2 but not Th1 cytokine production. *J Immunol* 1998; 161:2762–71.
- 27 Gause WC, Lu P, Zhou XD et al. polygyrus: B7-independence of the secondary type 2 response. *Exp Parasitol* 1996; 84: 264–73.
- 28 Lu P, Zhou XD, Chen SJ et al. Requirement of CTLA-4 counter receptors for IL-4 but not IL-10 elevations during a primary systemic *in vivo* immune response. *J Immunol* 1995; 154:1078–87.
- 29 Lenschow DJ, Herold KC, Rhee L et al. CD28/B7 regulation of Th1 and Th2 subsets in the development of autoimmune diabetes. *Immunity* 1996; 5:285–93.
- 30 Corry DB, Reiner SL, Linsley PS, Locksley RM. Differential effects of blockade of CD28-B7 on the development of Th1 or Th2 effector cells in experimental leishmaniasis. *J Immunol* 1994; 153:4142–8.
- 31 Natesan M, Razi-Wolf Z, Reiser H. Costimulation of IL-4 production by murine B7-1 and B7-2 molecules. *J Immunol* 1996; 156:2783–91.
- 32 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; 80:707–18.
- 33 Brunet JF, Denizot F, Luciani MF et al. A new member of the immunoglobulin superfamily – CTLA-4. *Nature* 1987; 328: 267–70.
- 34 Walunas TL, Lenschow DJ, Bakker CY et al. CTLA-4 can function as a negative regulator of T cell activation. *Immunity* 1994; 1:405–13.
- 35 Perez VL, Van Parijs L, Biuckians A, Zheng XX, Strom TB, Abbas AK. Induction of peripheral T cell tolerance *in vivo* requires CTLA-4 engagement. *Immunity* 1997; 6:411–7.
- 36 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; 3:541–7.
- 37 Keane-Myers A, Gause WC, Linsley PS, Chen SJ, Wills-Karp M. B7-CD28/CTLA-4 costimulatory pathways are required for the development of T helper cell 2-mediated allergic airway responses to inhaled antigens. *J Immunol* 1997; 158:2042–9.
- 38 Gonzalo JA, Tian J, Delaney T et al. ICOS is critical for T helper cell-mediated lung mucosal inflammatory responses. *Nat Immunol* 2001; 2:597–604.
- 39 Han S, Hathcock K, Zheng B, Kepler TB, Hodes R, Kelsoe G. Cellular interaction in germinal centers. Roles of CD40 ligand and B7-2 in established germinal centers. *J Immunol* 1995; 155:556–7.
- 40 Tsuyuki S, Tsuyuki J, Einsle K, Kopf M, Coyle AJ. Costimulation through B7-2 (CD86) is required for the induction of a lung mucosal T helper cell 2 (TH2) immune response and altered airway responsiveness. *J Exp Med* 1997; 185:1671–9.
- 41 Van Oosterhout AJ, Hofstra CL, Shields R et al. Murine CTLA4-IgG treatment inhibits airway eosinophilia and hyperresponsiveness and attenuates IgE upregulation in a murine model of allergic asthma. *Am J Respir Cell Mol Biol* 1997; 17:386–92.
- 42 Krinzman SJ, De Sanctis GT, Cernadas M et al. Inhibition of T cell costimulation abrogates airway hyperresponsiveness in a murine model. *J Clin Invest* 1996; 98:2693–9.
- 43 Deurloo DT, van Esch BC, Hofstra CL, Nijkamp FP, Van Oosterhout AJ. CTLA4-IgG reverses asthma manifestations in a mild but not in a more 'severe' ongoing murine model. *Am J Respir Cell Mol Biol* 2001; 25:751–60.
- 44 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; 24:563–8.
- 45 Keane-Myers AM, Gause WC, Finkelman FD, Xhou XD, Wills-Karp M. Development of murine allergic asthma is dependent upon B7-2 costimulation. *J Immunol* 1998; 160:1036–43.
- 46 Haczku A, Takeda K, Redai I et al. Anti-CD86 (B7.2) treatment abolishes allergic airway hyperresponsiveness in mice. *Am J Respir Crit Care Med* 1999; 159:1638–43.
- 47 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.
- 48 Harris N, Peach R, Naemura J, Linsley PS, Le Gros G, Ronchese F. CD80 costimulation is essential for the induction of airway eosinophilia. *J Exp Med* 1997; 185:177–82.
- 49 Harris NL, Peach RJ, Ronchese F. CTLA4-Ig inhibits optimal T helper 2 cell development but not protective immunity or memory response to *Nippostrongylus brasiliensis*. *Eur J Immunol* 1999; 29:311–6.
- 50 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; 22:265–71.
- 51 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; 33:1297–304.
- 52 Padrid PA, Mathur M, Li X et al. CTLA4Ig inhibits airway eosinophilia and hyperresponsiveness by regulating the development of Th1/Th2 subsets in a murine model of asthma. *Am J Respir Cell Mol Biol* 1998; 18:453–62.
- 53 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 alphabeta, but not gammadelta, T cells. *J Immunol* 1999; 163:6283–91.
- 54 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: 153–62.
 - 55 Hidi R, Riches V, Al Ali M et al. Role of B7-CD28/CTLA-4 costimulation and NF-kappa B in allergen-induced T cell chemotaxis by IL-16 and RANTES. *J Immunol* 2000; 164: 412–8.
 - 56 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; 161:6375–82.
 - 57 Howard TD, Postma DS, Hawkins GA. Fine mapping of an IgE-controlling gene on chromosome 2q: analysis of CTLA4 and CD28. *J Allergy Clin Immunol* 2002; 110:743–51.
 - 58 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: 171–6.
 - 59 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; 17:179–84.
 - 60 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.
 - 61 Hutloff A, Dittrich AM, Beier KC et al. ICOS is an inducible T-cell co-stimulator structurally and functionally related to CD28. *Nature* 1999; 397:263–6.
 - 62 Yoshinaga SK, Whoriskey JS, Khare SD et al. T-cell co-stimulation through B7RP-1 and ICOS. *Nature* 1999; 402:827–32.
 - 63 Beier KC, Hutloff A, Dittrich AM et al. Induction, binding specificity and function of human ICOS. *Eur J Immunol* 2000; 30:3707–17.
 - 64 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; 165:5035–40.
 - 65 Coyle AJ, Lehar S, Lloyd C et al. The CD28-related molecule ICOS is required for effective T cell-dependent immune responses. *Immunity* 2000; 13:95–105.
 - 66 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; 197:181–93.
 - 67 Mages HW, Hutloff A, Heuck C et al. Molecular cloning and characterization of murine ICOS and identification of B7h as ICOS ligand. *Eur J Immunol* 2000; 30:1040–7.
 - 68 Ling V, Wu PW, Finnerty HF et al. Cutting edge: identification of GL50, a novel B7-like protein that functionally binds to ICOS receptor. *J Immunol* 2000; 164:1653–7.
 - 69 Khayyamian S, Hutloff A, Buchner K et al. ICOS-ligand, expressed on human endothelial cells, costimulates Th1 and Th2 cytokine secretion by memory CD4+ T cells. *Proc Natl Acad Sci USA* 2002; 99:6198–203.
 - 70 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.
 - 71 Tafuri A, Shahinian A, Bladt F et al. ICOS is essential for effective T-helper-cell responses. *Nature* 2001; 409:105–9.
 - 72 McAdam AJ, Greenwald RJ, Levin MA et al. ICOS is critical for CD40-mediated antibody class switching. *Nature* 2001; 409: 102–5.
 - 73 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: 1381–8.
 - 74 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; 4:765–2.
 - 75 Iiyama R, Kanai T, Uraushihara K et al. The role of inducible co-stimulator (ICOS)/B7-related protein-1 (B7RP-1) interaction in the functional development of Peyer's patches. *Immunol Lett* 2003; 88:63–70.
 - 76 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.
 - 77 Wiley RE, Goncharova S, Shea T, Johnson JR, Coyle AJ, Jordana M. Evaluation of inducible costimulator/B7-related protein-1 as a therapeutic target in a murine model of allergic airway inflammation. *Am J Respir Cell Mol Biol* 2003; 28: 722–30.
 - 78 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; 8:1024–32.
 - 79 Grimbacher B, Hutloff A, Schlesier M et al. Homozygous loss of ICOS is associated with adult-onset common variable immunodeficiency. *Nat Immunol* 2003; 4:261–8.
 - 80 Croft M. Costimulation of T cells by OX40, 4-1BB and CD27. *Cytokine Growth Factor Rev* 2003; 14:265–73.
 - 81 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; 165:3043–50.
 - 82 Gramaglia I, Weinberg AD, Lemon M, Croft M. OX-40 ligand: a potent costimulatory molecule for sustaining primary CD4 T cell responses. *J Immunol* 1998; 161:6510–7.
 - 83 Rogers PR, Song J, Gramaglia I, Killeen N, Croft M. OX40 promotes Bcl-xL and Bcl-2 expression and is essential for long-term survival of CD4 T cells. *Immunity* 2001; 15: 445–55.
 - 84 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; 2:507–21.
 - 85 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: 387–92.
 - 86 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; 32:2874–80.
 - 87 Chen AI, McAdam AJ, Buhmann JE et al. OX40-ligand has a critical costimulatory role in dendritic cell: T cell interactions. *Immunity* 1999; 11:689–98.
 - 88 Flynn S, Toellner KM, Raykundalia C, Goodall M, Lane P. CD4 T cell cytokine differentiation: the B cell activation molecule, OX40 ligand, instructs CD4 T cells to express interleukin 4 and upregulates expression of the chemokine receptor, Blr-1. *J Exp Med* 1998; 88:297–304.
 - 89 Ohshima Y, Yang LP, Uchiyama T et al. OX40 costimulation enhances interleukin-4 (IL-4) expression at priming and promotes the differentiation of naive human CD4(+) T cells into high IL-4-producing effectors. *Blood* 1998; 92:3338–45.
 - 90 Akiba H, Miyahira Y, Atsuta M et al. Critical contribution of OX40 ligand to T helper cell type 2 differentiation in experimental leishmaniasis. *J Exp Med* 2000; 191:375–80.

- 91 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; 33:861–9.
- 92 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.
- 93 Ishida Y, Agata Y, Shibahara K, Honjo T. Induced expression of PD-1, a novel member of the immunoglobulin gene superfamily, upon programmed cell death. *EMBO J* 1992; 11: 3887–95.
- 94 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; 8:765–72.
- 95 Dong H, Zhu G, Tamada K, Chen L. B7-H1, a third member of the B7 family, co-stimulates T-cell proliferation and interleukin-10 secretion. *Nat Med* 1999; 5:1365–9.
- 96 Latchman Y, Wood CR, Chernova T et al. PD-L2 is a second ligand for PD-1 and inhibits T cell activation. *Nat Immunol* 2001; 2:261–8.
- 97 Tseng SY, Otsuji M, Gorski K et al. B7-DC, a new dendritic cell molecule with potent costimulatory properties for T cells. *J Exp Med* 2001; 193:839–46.
- 98 Freeman GJ, Long AJ, Iwai Y et al. Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. *J Exp Med* 2000; 192:1027–34.
- 99 Baecher-Allan C, Brown JA, Freeman GJ, Hafler DA. CD4+CD25 high regulatory cells in human peripheral blood. *J Immunol* 2001; 167:1245–53.
- 100 Yamazaki T, Akiba H, Iwai H et al. Expression of programmed death 1 ligands by murine T cells and APC. *J Immunol* 2002; 169:5538–45.
- 101 Bennett F, Luxenberg D, Ling V et al. Program death-1 engagement upon TCR activation has distinct effects on costimulation and cytokine-driven proliferation: attenuation of ICOS, IL-4, and IL-21, but not CD28, IL-7, and IL-15 responses. *J Immunol* 2003; 170:711–8.
- 102 Tamura H, Dong H, Zhu G et al. B7-H1 costimulation preferentially enhances CD28-independent T-helper cell function. *Blood* 2001; 97:1809–16.
- 103 Subudhi SK, Zhou P, Yerian LM et al. Local expression of B7-H1 promotes organ-specific autoimmunity and transplant rejection. *J Clin Invest* 2004; 113:694–700.
- 104 Nishimura H, Okazaki T, Tanaka Y et al. Autoimmune dilated cardiomyopathy in PD-1 receptor-deficient mice. *Science* 2001; 291:319–22.
- 105 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; 11:141–51.
- 106 Nishimura H, Minato N, Nakano T, Honjo T. Immunological studies on PD-1 deficient mice: implication of PD-1 as a negative regulator for B cell responses. *Int Immunol* 1998; 10:1563–72.
- 107 Khoury SJ, Sayegh MH. The roles of the new negative T cell costimulatory pathways in regulating autoimmunity. *Immunity* 2004; 20:529–38.
- 108 Tsushima F, Iwai H, Otsuki N et al. Preferential contribution of B7-H1 to programmed death-1-mediated regulation of hapten-specific allergic inflammatory responses. *Eur J Immunol* 2003; 33:2773–82.
- 109 Matsumoto K, Inoue H, Nakano T et al. B7-DC regulates asthmatic response by an IFN-gamma-dependent mechanism. *J Immunol* 2004; 172:2530–41.
- 110 Chapoval AI, Ni J, Lau JS et al. B7-H3: a costimulatory molecule for T cell activation and IFN-gamma production. *Nat Immunol* 2001; 2:269–74.
- 111 Suh WK, Gajewska BU, Okada H et al. The B7 family member B7-H3 preferentially down-regulates T helper type 1-mediated immune responses. *Nat Immunol* 2003; 4:899–906.
- 112 Wang L, Fraser CC, Kikly K et al. B7-H3 promotes acute and chronic allograft rejection. *Eur J Immunol* 2005; 35: 428–38.
- 113 Zang X, Loke P, Kim J, Murphy K, Waitz R, Allison JP. B7x: a widely expressed B7 family member that inhibits T cell activation. *Proc Natl Acad Sci USA* 2003; 100:10388–92.
- 114 Prasad DV, Richards S, Mai XM, Dong C. B7S1, a novel B7 family member that negatively regulates T cell activation. *Immunity* 2003; 18:863–73.
- 115 Sica GL, Choi IH, Zhu G et al. B7-H4, a molecule of the B7 family, negatively regulates T cell immunity. *Immunity* 2003; 18:849–61.
- 116 Tringler B, Zhuo S, Pilkington G et al. B7-h4 is highly expressed in ductal and lobular breast cancer. *Clin Cancer Res* 2005; 11:1842–8.
- 117 Watanabe N, Gavrieli M, Sedy JR et al. BTLA is a lymphocyte inhibitory receptor with similarities to CTLA-4 and PD-1. *Nat Immunol* 2003; 4:670–9.
- 118 Han P, Goularte OD, Rufner K, Wilkinson B, Kaye J. An inhibitory Ig superfamily protein expressed by lymphocytes and APCs is also an early marker of thymocyte positive selection. *J Immunol* 2004; 172:5931–9.
- 119 Hurchla MA, Sedy JR, Gavrieli M, Drake CG, Murphy TL, Murphy KM. B and T lymphocyte attenuator exhibits structural and expression polymorphisms and is highly induced in anergic CD4+ T cells. *J Immunol* 2005; 174:3377–85.
- 120 Carter LL, Carreno BM. Cytotoxic T-lymphocyte antigen-4 and programmed death-1 function as negative regulators of lymphocyte activation. *Immunol Res* 2003; 28:49–59.
- 121 Sedy JR, Gavrieli M, Potter KG et al. B and T lymphocyte attenuator regulates T cell activation through interaction with herpesvirus entry mediator. *Nat Immunol* 2005; 6:90–8.
- 122 Gonzalez LC, Loyet KM, Calemene-Fenaux J et al. A coreceptor interaction between the CD28 and TNF receptor family members B and T lymphocyte attenuator and herpesvirus entry mediator. *Proc Natl Acad Sci USA* 2005; 102:1116–21.
- 123 Kwon BS, Weissman SM. cDNA sequences of two inducible T-cell genes. *Proc Natl Acad Sci USA* 1989; 86:1963–7.
- 124 Pollok KE, Kim YJ, Zhou Z et al. Inducible T cell antigen 4-1BB. Analysis of expression and function. *J Immunol* 1993; 150: 771–81.
- 125 Schwarz H, Valbracht J, Tuckwell J, von Kempis J, Lotz M. ILA, the human 4-1BB homologue, is inducible in lymphoid and other cell lineages. *Blood* 1995; 85:1043–52.
- 126 Goodwin RG, Din WS, Davis-Smith T et al. Molecular cloning of a ligand for the inducible T cell gene 4-1BB: a member of an emerging family of cytokines with homology to tumor necrosis factor. *Eur J Immunol* 1993; 23:2631–41.
- 127 Futagawa T, Akiba H, Kodama T et al. Expression and function of 4-1BB and 4-1BB ligand on murine dendritic cells. *Int Immunol* 2002; 14:275–86.
- 128 DeBenedette MA, Wen T, Bachmann MF et al. Analysis of 4-1BB ligand (4-1BBL)-deficient mice and of mice lacking both 4-1BBL and CD28 reveals a role for 4-1BBL in skin allograft rejection and in the cytotoxic T cell response to influenza virus. *J Immunol* 1999; 163:4833–41.
- 129 Tan JT, Whitmire JK, Ahmed R, Pearson TC, Larsen CP. 4-1BB ligand, a member of the TNF family, is important for the generation of antiviral CD8 T cell responses. *J Immunol* 1999; 163:4859–68.
- 130 Cooper D, Bansal-Pakala P, Croft M. 4-1BB (CD137) controls the clonal expansion and survival of CD8 T cells *in vivo* but does not contribute to the development of cytotoxicity. *Eur J Immunol* 2002; 32:521–9.

- 131 Kim YJ, Kim SH, Mantel P, Kwon BS. Human 4-1BB regulates CD28 co-stimulation to promote Th1 cell responses. *Eur J Immunol* 1998; 28:881–90.
- 132 Eppihimer MJ, Gunn J, Freeman GJ et al. Expression and regulation of the PD-L1 immunoinhibitory molecule on microvascular endothelial cells. *Microcirculation* 2002; 9: 133–45.
- 133 Montgomery RI, Warner MS, Lum BJ, Spear PG. Herpes simplex virus-1 entry into cells mediated by a novel member of the TNF/NGF receptor family. *Cell* 1996; 87: 427–36.
- 134 Kwon BS, Tan KB, Ni J et al. A newly identified member of the tumor necrosis factor receptor superfamily with a wide tissue distribution and involvement in lymphocyte activation. *J Biol Chem* 1997; 272:14272–6.
- 135 Paterson DJ, Jefferies WA, Green JR et al. Antigens of activated rat T lymphocytes including a molecule of 50 000 Mr detected only on CD4 positive T blasts. *Mol Immunol* 1987; 24: 1281–90.
- 136 Baum PR, Gayle RB III, Ramsdell F et al. Molecular characterization of murine and human OX40/OX40 ligand systems: identification of a human OX40 ligand as the HTLV-1-regulated protein gp34. *EMBO J* 1994; 13: 3992–4001.
- 137 Ohshima Y, Tanaka Y, Tozawa H, Takahashi Y, Maliszewski C, Delespesse G. Expression and function of OX40 ligand on human dendritic cells. *J Immunol* 1997; 159:3838–48.
- 138 Hellings PW, Vandenberghe P, Kasran A et al. Blockade of CTLA-4 enhances allergic sensitization and eosinophilic airway inflammation in genetically predisposed mice. *Eur J Immunol* 2002; 32:585–94.
- 139 Guo J, Stolina M, Bready JV et al. Stimulatory effects of B7-related protein-1 on cellular and humoral immune responses in mice. *J Immunol* 2001; 166:5578–84.
- 140 Kroczeck RA, Hamelmann E. T-cell co-stimulatory molecules: optimal targets for the treatment of allergic airway disease with monoclonal antibodies. *J Allergy Clin Immunol* 2005; 116: 906–9.