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MINI-SERIES "T-CELL CO-STIMULATORY MOLECULES"
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Master switches of T-cell activation and differentiation

K.C. Beier, T. Kallinich and E. Hamelmann

ABSTRACT: T-cells play a central role in allergic airway diseases such as bronchial asthma. The imbalance between allergen-specific pro-inflammatory and pro-allergic T-cell responses on one hand and regulatory or suppressive T-cell responses on the other may best explain the development of unwanted immune responses against environmental allergens, which lead to immunoglobulin E production and airway inflammation. A key role in the fine tuning of any T-cell response is provided by the engagement of so-called co-stimulatory molecules that are required for the full activation of T-cells and the recognition of antigens *via* the antigen-specific T-cell receptor. Many of these co-stimulatory molecules have been identified only recently, leading to a fundamental change in the overall understanding of T-cell regulation.

Due to their pivotal impact on T-cell differentiation and control, co-stimulatory molecules are promising targets for therapeutic intervention in T-cell-regulated or -mediated immune disorders, including allergic diseases and asthma. In the present article, an attempt is made to summarise the current knowledge on the basic concept of co-stimulation, the presently known co-stimulatory molecules and their various functions on T-cell activation or suppression. The mini-series will be completed by two more articles describing the recent experimental studies and preliminary clinical findings regarding the role of co-stimulatory molecules in allergic disorders and bronchial asthma, and a discussion regarding the feasibility of co-stimulatory molecules as potential targets for the treatment of allergic airway disease.

Although it is too early for any clinical implication or utilisation at this moment, the authors are convinced that a better understanding of co-stimulation in the context of allergic asthma will finally provide novel and promising approaches for treatment and prevention.

KEYWORDS: Allergic immune response, allergic inflammation, bronchial asthma, co-stimulation, immune modulation, T-cell

T-cells play a central role in allergen-mediated airway inflammation and disease. The prerequisite to any immune response against an allergen is its specific recognition, which, in the case of T-cell responses, is mediated by the antigen-specific T-cell receptor (TCR). However, the TCR-transmitted signal alone is insufficient to induce T-cell responses; it requires additional modulation by a constantly growing number of co-receptors with positive or negative regulatory function. Many of these co-stimulatory molecules have been identified only recently, leading to a fundamental change in the understanding of T-cell regulation.

Considering their pivotal impact on T-cell regulation, co-stimulatory molecules are promising targets for therapeutic intervention in T-cell-regulated or -mediated immune disorders, including allergic diseases. The aim of this mini-series of three articles is: 1) to give an overview of the current knowledge of the various co-stimulatory molecules; 2) to summarise the recent experimental studies and preliminary clinical findings regarding a role of co-stimulatory molecules in allergic disorders and bronchial asthma; and 3) to discuss the feasibility of co-stimulatory molecules as potential targets for the treatment of allergic airway disease.

THE PRINCIPLES OF CO-STIMULATION

Antigen-mediated immune responses are controlled and driven by the specific activation of T-cells. The ''first signal'' delivered by the TCR

AFFILIATIONS
Charité – Universitätsmedizin Berlin, Dept of Paediatric Pneumology and Immunology, Campus Virchow-Klinikum, Berlin, Germany.

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

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mediates the specificity of a T-cell response *via* the recognition of specific epitopes of a given antigen presented in combination with the major histocompatibility complex on antigen-presenting cells (APCs). However, activation of T-cells by this receptor interaction alone fails to induce cytokine production and sustained proliferation, but rather results in T-cell apoptosis, or the induction of specific nonresponsiveness (anergy) to subsequent stimulation with the same antigen [1].

To become fully activated, T-cells require additional signals delivered by so-called co-stimulatory molecules (fig. 1a). These molecules are transmembrane proteins that induce an intracellular signalling cascade *via* their cytoplasmic tail that modifies the TCR-mediated signal. Co-stimulatory molecules cannot activate T-cells without concomitant TCR crosslinking. According to this definition, adhesion molecules such as intercellular adhesion molecule-1 are not co-stimulatory because they enhance T-cell activation merely by facilitating the contact between T-cell and APC. Furthermore, T-cell co-stimulation refers to a signal that is delivered to the T-cell exclusively. In this respect, the CD40/CD40L interaction is also not considered as co-stimulatory, although this is an important receptor/ligand pair in T/B cooperation.

The dependence of T-cell activation on this ''second signal'' delivered by co-stimulators adds a second line of regulation to antigen-specific T-cell responses that reaches far beyond a mere ''on–off'' command. With a growing number of both stimulatory and inhibitory co-stimulatory molecules being identified, the classic concept of co-stimulation as a two-signal event has changed. T-cells simultaneously express an adjustable spectrum of co-stimulatory molecules. Today, T-cell co-stimulation is recognised as an integrating process of various positive and negative signals that determine the mode of T-cell activation (as reviewed previously [2]; fig. 1b).

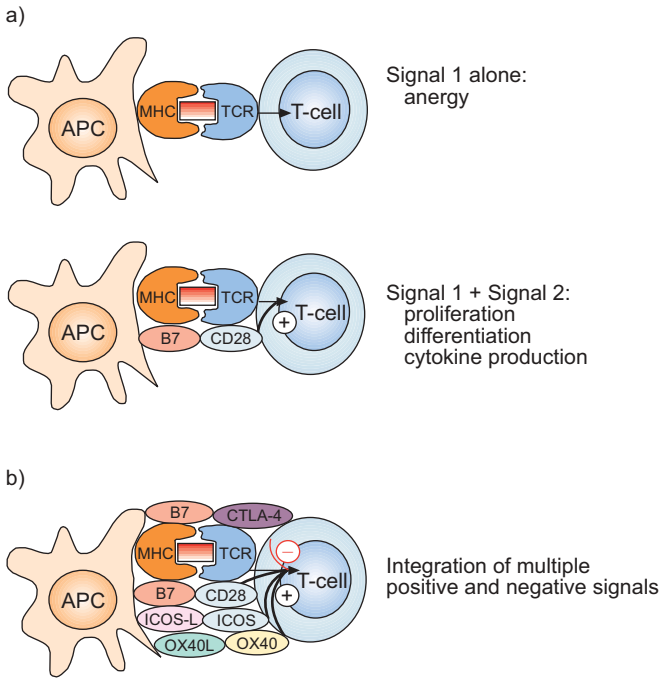

FIGURE 1. a) Historic view and b) modern concept of co-stimulation. APC: antigen-presenting cell; MHC: major histocompatibilty complex; TCR: T-cell receptor; CTLA: cytotoxic T-lymphocyte antigen; ICOS: inducible co-stimulator; L: ligand.

Co-stimulatory molecules can be categorised according to particular characteristics as follows.

Expression pattern

Only a few co-stimulatory molecules (CD28, CD27, herpes virus entry mediator (HVEM) and B- and T-lymphocyte attenuator (BTLA)) are expressed constitutively on both unstimulated and stimulated cells (constitutive expression). The majority of T-cell co-stimulators are induced upon T-cell activation (inducible expression; table 1). Some co-stimulatory molecules are downregulated upon repetitious stimulation. Therefore, T-cells, such as naïve, recently activated, or memory T-cells express a unique combination of co-stimulatory receptors depending on their history of activation and their state of differentiation.

Expression density

The number of copies of a co-stimulatory molecule expressed on the T-cell surface may have an impact on the modulating effect of T-cell effector function. For inducible co-stimulator (ICOS), for instance, it was demonstrated that a high-density expression was specifically associated with high production of interleukin (IL)-10, while an intermediate ICOS-expression density was correlated with the predominant production of T-helper cell (Th)2-type cytokines, IL-4, IL-5 and IL-13 [3].

Positive versus *negative signal*

Co-stimulatory molecules can modulate the TCR-mediated T-cell activation either negatively or positively (table 1). Most members of the constitutively expressed co-stimulators show positive amplifying effects, whereas the group of inducible co-stimulators contains both positive (ICOS, CD30, OX40, 4-1BB, signalling lymphocyte activation molecule (SLAM)) as well as negative (cytotoxic T-lymphocyte antigen (CTLA)-4, programmed death (PD)-1) regulators (table 1). Positive and negative regulatory co-stimulators may be expressed on the same cells either simultaneously or consecutively, which contributes to the complexity of this integral process.

Co-stimulatory effect

Co-stimulatory signals modify the effector phase of T-cell activation by inducing or suppressing the production of cytokines, by influencing cell survival or apoptosis, or by the upregulation of other cell surface molecules. Generally, the signalling can be regarded as one-way through the receptor on the T-cell side [2]. In recent years, it has been reported that for many co-stimulatory receptor–ligand pairs signals are also delivered through the ligand. However, the *in vivo* significance of this so-called back-signalling is poorly understood [4]. The different effects of the various co-stimulatory molecules on T-cell activation and function are discussed below in more detail.

Availability of ligands

The availability of the specific ligands for co-stimulatory molecules varies at different sites of T-cell activation. Some ligands, such as B7-1 and B7-2, which are the common ligands

TABLE 1 Currently known co-stimulatory molecules and their ligands

Expression	Signal	Co-stimulatory molecule	Ligand	Superfamily
Constitutive	Positive	CD28	B7-1, B7-2 (CD80, CD86)	CD28/B7
		CD27	CD70	TNF/TNFR
		HVEM	LIGHT, BTLA	TNF/TNFR, CD28/B7
	Negative	BTLA	HVEM	CD28/B7, TNF/TNFR
Inducible	Positive	ICOS	ICOS-L	CD28/B7
		OX40 (CD134)	OX40L	TNF/TNFR
		4-1BB (CD137)	4-1BBL	TNF/TNFR
		CD30	CD30L (CD153)	TNF/TNFR
		SLAM (CD150)	SLAM (CD150)	CD2
	Negative	CTLA-4 (CD152)	B7-1, B7-2 (CD80, CD86)	CD28/B7
		PD-1	PD-L1, PD-L2	CD28/B7
		Unknown	B7-H4	CD28/B7
	Obscure	Unknown	B7-H3	CD28/B7

TNF: tumour necrosis factor; R: receptor; HVEM: herpes virus entry mediator; LIGHT: homologous to lymphotoxin, inducible expression, competing for GpD of herpes virus, expressed on activated T-lymphocytes; BLTA: B- and T-lymphocyte attenuator; ICOS: inducible co-stimulator; L: ligand; SLAM: signalling lymphocyte activation molecule; CTLA: cytotoxic T-lymphocyte antigen; PD: programmed death; B7-H4 and -H3: B7 homologues 4 and 3. Modified from [2]

for CD28 and CTLA-4, are mostly restricted to specialised APCs such as dendritic cells, B-cells and macrophages. Other ligands can also be found on endothelial, epithelial and other nonlymphoid tissues in the periphery. This suggests a basic difference in T-cell co-stimulation in lymphoid organs *versus* in the periphery (as reviewed previously [2]).

CO-STIMULATORY MOLECULES

With the rapid increase in knowledge of the function of a growing number of specific co-stimulatory molecules, co-stimulation is now starting to be recognised as the master switch for T-cell activation and modulation of T-cell function. In the following section, the latest facts about the currently known co-stimulatory molecules will briefly be summarised, with a focus on expression pattern, ligand interaction and function, if available.

The CD28/B7 superfamily

A common characteristic of the CD28 homologues is a single extracellular immunoglobulin (Ig) variable-like domain followed by a short cytoplasmic tail. The genes for CD28, CTLA-4 and ICOS are clustered in close proximity on chromosome 2q33 [5], suggesting that they evolved from gene duplication. Sequence analysis showed that all three have an unpaired cysteine that allows them to homodimerise on the T-cell surface. In contrast, the genes for PD-1 and BTLA are separately located (on 2q37 and 3q13, respectively). PD-1 was shown to exist as a monomer on the cell surface and BTLA is likely to be expressed as a monomer because it also lacks the unpaired cysteine residue (fig. 2) [6].

Members of the CD28/B7 superfamily have key roles in regulating T-cell activation and tolerance. The constitutively expressed CD28 and the inducible molecule ICOS provide critical positive second signals that promote and sustain T-cell responses. CTLA-4, PD-1 and BTLA deliver negative second signals attenuating or terminating an ongoing T-cell response. CD28 and CTLA-4 share the ligand pair B7-1 and B7-2, while ICOS and PD-1 bind to their own distinct ligands. BTLA is the only member of the Ig superfamily that binds to a member of the tumour necrosis factor receptor (TNFR) superfamily, namely HVEM (fig. 2). Furthermore, the identification of two orphan B7 homologues, B7-H3 and B7-H4, indicates that there are more pathways in the CD28/B7 family that are as yet unidentified.

CD28

CD28 is the most extensively studied member of the group of co-stimulatory molecules.

Expression

CD28 is constitutively expressed on almost all resting human CD4+ T-cells and 50–80% of all CD8+ T-cells. Chronic stimulation leads to downregulation of CD28 expression, suggesting a negative feedback mechanism limiting an overwhelming immune response [7].

Ligands

CD28 has two ligands, B7-1 (CD80) and B7-2 (CD86). Both are constitutively expressed at low levels on a subpopulation of specialised APCs and are rapidly upregulated on almost all APCs upon activation [8]. Both B7-1 and B7-2 can be expressed on activated T-cells [9]. However, the significance of their expression on T-cells is not yet well understood.

Function

Co-stimulation *via* CD28 is the most important pathway for the initial activation of naïve T-cells. Ligation of CD3 and CD28 promotes the production of IL-4 and IL-5 and seems to be unique in its ability to induce very high levels of IL-2 production, which enhances proliferation in an autocrine manner. Moreover, it provides resistance to apoptosis and induces long-term expansion of T-cells [10–12].

Cytotoxic T-lymphocyte antigen-4

CTLA-4 (CD152) is a potent inhibitor of T-cell activation.

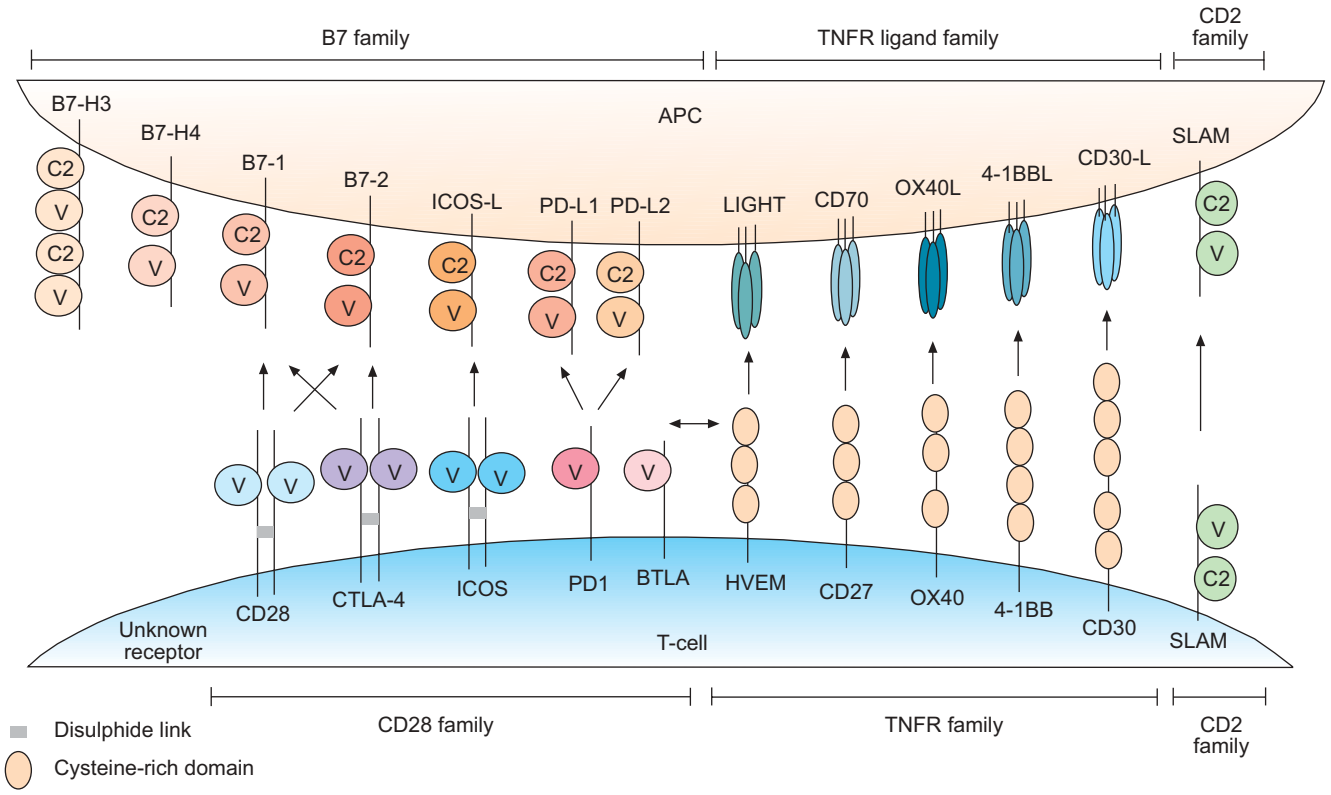

FIGURE 2. Currently known co-stimulatory molecules and their ligands. TNFR: tumour necrosis factor receptor; APC: antigen-presenting cell; B7-H3 and -H4: B7 homologues 3 and 4; ICOS: inducible co-stimulator; L: ligand; PD: programmed death; LIGHT: homologous to lymphotoxin, inducible expression, competing for GpD of herpes virus, expressed on activated T-lymphocytes; V: immunoglobulin-like variable domain; C2: constant type-2 immunoglobulin-like domain; SLAM: signalling lymphocyte activation molecule; CTLA: cytotoxic T-lymphocyte antigen; BTLA: B- and T-lymphocyte attenuator; HVEM: herpes virus entry mediator.

Expression

In contrast to CD28, CTLA-4 is only expressed after T-cell activation. It is usually located intracellularly and moves rapidly to the cell surface at the site of T-cell/APC interaction where it can inhibit the earliest events of T-cell activation [13, 14].

Ligands

Like CD28, CTLA-4 binds to the two ligands B7-1 and B7-2. CTLA-4 has higher affinity to B7-1 and B7-2 than CD28 and forms a lattice structure upon engagement with these ligands, which limits the ability of CD28 to interact with them [15]. CTLA-4 produces potent inhibitory effects on T-cell activation even at low expression levels.

Function

Besides ligand competition, CTLA-4 counteracts the positive signal of CD28 by the delivery of a negative signal that restricts autocrine IL-2 production and inhibits cell cycle progression. CTLA-4-deficient mice develop a fatal lymphoproliferative disease within the first few weeks of life, showing the importance of this molecule in the restriction of T-cell-mediated immune responses [16].

Inducible co-stimulator

ICOS was the third member of the CD28 family to be discovered and is a positive regulator like CD28 [17, 18].

Expression

Unlike the constitutively expressed CD28, ICOS has to be induced by antigen stimulation, suggesting that ICOS provides signals to recently activated or memory rather than to naïve T-cells [17–19]. CD28 co-stimulation enhances ICOS expression, indicating that some of the functions ascribed to CD28 might be due in part to ICOS signalling [19–21]. However, ICOS expression is not entirely dependent upon CD28 signals, because CD28- CD8+ T-cells are able to express ICOS upon TCR stimulation [19]. ICOS is upregulated on both Th1 and Th2 cells during the initial phase of differentiation; expression levels remain high on Th2 cells but decrease on Th1 cells upon repetitive stimulation [20, 21].

Ligand

In contrast to CD28 and CTLA-4, ICOS binds to its own ligand, ICOS-L. ICOS-L is constitutively expressed at high density by B-cells, dendritic cells (DC) and macrophages, and also at low levels on T-cells. Moreover, ICOS-L is expressed on peripheral tissues (endothelium, brain, heart, liver and kidney) where it is upregulated by a variety of inflammatory signals [22–24].

Function

Like CD28, ICOS co-stimulation augments the production of effector cytokines such as IL-4, IL-5, interferon (IFN)-γ, and tumour necrosis factor (TNF)-α. It is superior to CD28 in the

enhancement of IL-10 production [17, 19]. Interestingly, ICOS co-stimulation promotes these effector-cell functions without enhancing IL-2 production. This lack of IL-2 production might provide a self-limiting mechanism to ICOS co-stimulation [25]. Besides its effects on T-cell responses, the ICOS/ICOS-L pathway seems to be most important for T-/B-cell cooperation regulating the production of IgG_1, IgG_{2a} and IgE in mice [20, 26, 27], and the maintenance of the B-memory cell pool in humans [28]. This effect on B-cells seems to be due to the induction of cytokine secretion and the expression of other cell surface molecules on the T-cell by ICOS co-stimulation, rather than by direct signalling through ICOS-L.

Programmed death-1

PD-1 is the second negative regulatory member, besides CTLA-4, of the CD28/B7 family.

Expression

In contrast to other co-stimulatory molecules, PD-1 is not restricted to T-cells. It is also expressed on B-cells and myeloid cells following activation [29].

Ligands

PD-1 can bind two different ligands, PD-L1 (B7-H1) [30] and PD-L2 (B7-DC) [31, 32]. PD-L1 is constitutively expressed on freshly isolated T-cells, B-cells, macrophages and DCs, and was detected in the thymus as well as nonlymphoid tissues such as heart, kidney and liver. In contrast, PD-L2 is only expressed on activated macrophages and DCs [33]. 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.

Function

Despite its name, PD-1 is associated with downmodulation of T-cell responses rather than apoptosis. It was shown that PD-1 leads to arrest in cell cycle phase G0–G1 [29]. CD28 co-stimulation or exogenous IL-2 can overcome the inhibitory effect of PD-1 on proliferation and cytokine production. Therefore, PD-1 effectively suppresses T-cell responses in situations in which IL-2 production is limited, as in the case of ICOS co-stimulation [34]. The importance of PD-1-mediated T-cell suppression for peripheral tolerance is indicated by the observation that PD-1-deficient mice develop auto-immune diseases [35, 36]. In contrast to CTLA-4-deficient mice, symptoms of auto-immunity develop later in life. 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 auto-immune processes in the periphery [37].

The B- and T-lymphocyte attenuator

BTLA is another inhibitory molecule in the CD28/B7 family with similarities to CTLA-4 and PD-1.

Expression

BTLA is expressed by nearly all lymphoid cells, including T-cells, B-cells and DCs [38, 39]. It is upregulated on T-cells upon activation [40].

Ligand

Recently, it has been shown that the receptor for BTLA is HVEM [41, 42]. Remarkably, this is the only CD28/B7 family member interacting with a member of the TNFR family.

Function

Co-stimulation by BTLA leads to downregulation of IL-2 production *in vitro*. Proliferation of BTLA-deficient T-cells is increased, and BTLA-deficient mice have increased specific antibody responses and enhanced sensitivity to experimental auto-immune encephalomyelitis. After polarisation of T-cells, BTLA is mainly expressed on Th1 cells, suggesting a role for BTLA in controlling Th1-biased immune responses [40].

B7 homologue 3

B7-H3, a recently identified member of the human B7 family, is an orphan ligand sharing ~25% amino acid identity with other B7 family members.

Expression

B7-H3 is not detectable on peripheral blood mononuclear cells, although mRNA is found in various peripheral tissues and in several tumour cell lines. Expression of B7-H3 is induced on DCs and monocytes after activation by inflammatory cytokines or mitogens *in vitro* [43].

Ligand

Soluble B7-H3 protein binds a putative ligand on activated CD4+ and CD8+ T-cells that is distinct from CD28, CTLA-4, ICOS and PD-1 [43].

Function

The function of B7-H3 and its receptor is still unknown. Originally, the ligand for B7-H3 was described as co-stimulating the proliferation of both CD4+ and CD8+ T-cells, enhancing the induction of cytotoxic T-cells, and increasing IFN-γ production by activated T-cells *in vitro.* However, analysis of B7-H3-deficient mice indicated an inhibitory effect on T-cell function [44].

B7 homologue 4

Also known as B7x and B7S1, B7-H4 is yet another (and the most recently described) member of the B7 family of co-stimulatory proteins.

Expression

B7-H4 has a very broad tissue distribution. It is expressed on epithelial cells and has now been discovered to be inducible on T-cells, B-cells, DCs, and monocytes upon *in vitro* stimulation [45].

Ligand

Studies of the B7-H4 fusion protein showed that the putative receptor of B7-H4 is upregulated on activated T-cells [46].

Function

Triggering of the B7-4 ligand on T-cells with soluble B7-H4 fusion protein has a profound inhibitory effect on growth, cytokine secretion and the development of cytotoxicity. Administration of B7-H4 Ig into mice impairs antigen-specific

T-cell responses, whereas the blockade of endogenous B7-H4 by specific monoclonal antibody promotes T-cell responses [46].

The TNFR superfamily

Several members of the TNF/TNFR superfamily have co-stimulatory function and influence T-cell activation and differentiation in a number of ways. A common feature of the members of this family of transmembrane glycoproteins is cysteine-rich repeats of ~40 amino acids in the extracellular amino terminal. Some sequence similarities in the cytoplasmic regions may also occur (fig. 2). Some members of this family possess a region of homology in their intracellular domains known as the death domain. This domain derives its name from the fact that it is involved in signalling processes that ultimately lead to programmed cell death by apoptosis. Molecules of this family are either induced or highly upregulated on the T-cell surface after recognition of antigen. Thus, they seem to regulate cell proliferation and/or prevent unnecessary cell death after initial induction of the immune response. This process of co-stimulation may, therefore, control the absolute number of effector T-cells and regulate the frequency of memory T-cells that subsequently develop during a given antigen-mediated response. The constitutively expressed molecules CD27 and HVEM, as well as the inducible OX40 (CD134), CD30 and 4-1BB (CD137) will be introduced as follows.

OX40 (CD134)

Expression

OX40 is primarily expressed on activated T-cells of the CD4 phenotype with a bias for Th2 cells [47]. Antigen-experienced T-cells re-express OX40 rapidly within 4 h upon re-stimulation [48].

Ligand

The ligand for OX40, OX40L, is expressed on activated B-cells, DCs and endothelial cells [49].

Function

OX40 ligation induces cytoplasmic expression of B-cell leukaemia/lymphoma 2 gene product (Bcl)-xL and Bcl-2, thus delivering anti-apoptotic signals and playing a role in T-cell survival [50]. Several studies of OX40- and OX40L-deficient mice have been performed, showing that OX40/OX40L signalling plays a major role in T-cell function in antiviral, as well as allergen-mediated immune responses (as previously reviewed [51]). It was shown that OX40 co-stimulation is not only necessary to accumulate effector T-cells during primary immune responses but also influences subsequent formation of memory cells [48].

CD30

Expression

CD30 is expressed on activated T-cells with a strong bias for Th2 [47], and on B-cells, natural killer (NK) cells and eosinophils [52].

Ligand

CD30L is expressed in inflamed peripheral tissues and resting B-cells, as well as on activated T-cells so that interaction among T-cells can occur *via* CD30/CD30L [52]. Like OX40L, CD30L is found in areas of T-cell–B-cell contact, where it appears to maintain the survival of primed and memory T-cells [52].

Function

CD30 is inducible by TCR signalling in a CD28-dependent manner or by activation *via* IL-4 [53]. In terms of its preferential expression on Th2 cells, CD30 is similar to OX40. Triggering of T-cells *via* CD30 results in the predominant generation of Th2 cytokines [54]. Like OX40, CD30 seems to be important for T-cell memory and the humoral immune response [55].

4-1BB (CD137)

Expression

4-1BB is rapidly induced after activation of T-cells, B-cells, monocytes and nonlymphoid tissues [56].

Ligand

4-1BBL is expressed on B-cells, monocytes and DCs upon activation [57]. *In vitro*, 4-1BBL can activate both CD4 and CD8 T-cells. *In vivo*, however, agonistic anti-4-1BB antibodies show a preferential effect on CD8 T-cells [52].

Function

A major effect of 4-1BB appears to be directed towards sustaining T-cell survival. 4-1BB engagement prevents activation-induced cell death by induction of Bcl-xL and Bfl-1 (a Bcl-2 homologue first described in human foetal liver), two pro-survival members of the Bcl-2 family [52].

CD27

Expression

CD27 is expressed constitutively on T-cells, NK cells and B-cells [58]. In humans, CD27 expression increases transiently with activation and is subsequently downregulated on T-effector cells after several rounds of cell division, in a similar manner to CD28 [52].

Ligand

CD70, which is the ligand for CD27, is found on activated T-cells, B-cells and DCs. CD27 signalling in B-cells also plays a direct role in B-cell memory commitment and differentiation and contributes to germinal centre formation [59].

Function

CD27 engagement does not co-stimulate high levels of IL-2 production by T-cells, but rather induces TNF at comparable levels to that induced by CD28 engagement. CD27 promotes the development of cytotoxic T-lymphocyte effectors, and enhances T-cell survival [60] (as reviewed previously [59]). CD27 expression may divide the memory T-cell population into two groups: effector cells lacking CD27 display a high antigen recall response, whereas CD27+ memory T-cells require additional co-stimulation for T-cell receptor triggering [61]. Mice overexpressing the natural CD27-ligand, CD70, showed a progressive conversion of naïve T-cells into effector-memory cells, which finally led to the loss of naïve T-cells in lymph nodes and the spleen [62].

HVEM

Expression

HVEM is expressed on T-cells constitutively and may be expressed upon stimulation by DCs, NK cells and on memory and naïve B-cells from peripheral blood or the tonsils, but not on germinal centre B-cells.

Ligand

HVEM transmits a positive co-stimulatory signal by interaction with LIGHT (homologous to lymphotoxin, inducible expression, competing for GpD of herpes virus, expressed on activated T-lymphocytes), a molecule of the TNFR ligand family. Interestingly, it may also interact with the inhibitory molecule BTLA, which belongs to the CD28/B7 family [41, 42] and can bind secreted lymphotoxin [63].

Function

The HVEM–LIGHT interaction is thought to deliver a positive co-stimulatory signal for the cell expressing HVEM; this is based on the signalling profile of HVEM and the ability of soluble LIGHT to augment T-cell proliferation and cytokine secretion [64]. Due to spatial binding characteristics of BTLA *versus* LIGHT, it might be possible that only the binding of LIGHT induces a co-stimulatory signal, while BTLA binds competitively, reducing positive signals through HVEM and inducing inhibitory signals through BTLA [64]. Stimulation of B-cells with LIGHT-expressing T-cells increased B-cell proliferation induced by the CD40/CD40L interaction and IgG and IgM (but not IgA) secretion [65].

Other co-stimulatory molecules

The signalling lymphocyte activation molecule

SLAM (CD150) is a self-ligand cell surface glycoprotein and belongs to a new family of co-stimulatory molecules [66].

Expression

SLAM is expressed on activated T-cells, B-cells, macrophages and DCs [66]. Cell-surface expression is upregulated with rapid kinetics in activated T-cells and lipopolysaccharide/IFN-γ-activated macrophages.

Ligand

Interestingly, SLAM interacts with the identical molecules on partner cells in a homotypic way.

Function

It was initially shown that SLAM co-stimulation promotes proliferation and production of Th1 cytokines *in vitro* [67, 68]. *In vivo* experiments with cell lines, however, led to conflicting results and require further investigation in knock-out mice [66].

CONCLUDING REMARKS

The modern concept of co-stimulation has shed new light on the mechanisms involved in the regulation of T-cell activation and differentiation. The identification of several new co-stimulatory molecules and their receptors supports the idea of fine-tuning T-cell functions *via* a multitude of simultaneously or consecutively expressed T-cell molecules. Not only does the joint action of various co-stimulatory receptors on T-cells add to the complexity of this mechanism but also to the availability of ligands on different cell types and in different organs. The identification of the orphan ligands B7 homologue 3 and B7 homologue 4 shows that there may be more co-stimulatory molecules to be identified. Table 2 categorises the currently known co-stimulatory molecules that were introduced in the article according to functional aspects. With a growing insight into co-stimulatory mechanisms, a vast array of potential targets for the modulation and redirection of T-cell responses becomes available. Accordingly, many experimental studies using animal models have explored the efficacy of targeting co-stimulatory molecules for the inhibition, or even prevention of, the development of the different diseases. Among these, allergen-induced sensitisation and airway disease are of the most interest, because they are clearly mediated by misled T-cell responses against common environmental antigens. Most of the aforementioned molecules have been studied in murine models of allergic airway inflammation. The following article of this mini-series will give, therefore, an overview on these studies in murine models as well as discuss preliminary data in the human system. Finally, the third article will discuss the utilisation and feasibility of co-stimulatory molecules for novel treatment strategies of allergic airway disease.

TABLE 2 Functional classification of co-stimulatory molecules

Functional aspect	Characterisation	Co-stimulatory molecule
Expression pattern	Constitutively	CD28, CD27, HVEM, BTLA
	Inducible	ICOS, CTLA-4, PD-1, OX40, 4-1BB, CD30, SLAM, putative receptors for B7-H3 and B7-H4
T-cell modulation	Positive/enhancement	CD28, ICOS, OX40, CD27, 4-1BB, CD30, HVEM, SLAM
	Negative/inhibition	CTLA-4, PD-1, BTLA, putative receptor for B7-H4
T-cell differentiation	Th1	4-1BB, SLAM, BTLA
	Th2	ICOS, OX40, CD30
	Treg	ICOS, CTLA-4, PD-1
T-cell function	T effector/helper	ICOS, CTLA-4, PD-1, OX40
	T memory	OX40, CD30, CD27
Location of action	Central	CD28, HVEM, CTLA-4
	Central and Peripheral	ICOS, PD-1, OX40, 4-1BB

HVEM: herpes virus entry mediator; BTLA: B- and T-lymphocyte attenuator; ICOS: inducible co-stimulator; CTLA: cytotoxic T-lymphcyte antigen; PD: programmed death; SLAM: signalling lymphocyte activation molecule; B7-H3 and -H4: B7 homologues 3 and 4; Th: T-helper cell; Treg: regulatory T-cell.

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