

T-cell costimulatory molecules: Optimal targets for the treatment of allergic airway disease with monoclonal antibodies

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Current treatment for chronic allergic airway disease with anti-inflammatory agents is effective but not specific, and is symptomatic rather than curative. The present review article outlines the involvement of T cells by dissecting the various steps in which naive CD4⁺ T cells differentiate to allergen-specific, activated T cells of the T_H2 type, which play a pivotal role in the pathogenesis of chronic allergic airway disease. Aiming at a concept for a highly specific therapy of this disease, various T cell costimulatory molecules (CD28, CD27, HVAM, BTLA, ICOS, OX40, CD30, 4-1BB, SLAM, CTLA-4, and PD-1) and the non-costimulatory molecule CD40L, all of them expressed on activated T_H2 effector T cells, are discussed as potential targets for an antibody-based therapy. Considering various criteria, including T-cell specific expression and expression characteristics on resting versus activated T cells, reasons are given why ICOS and OX40 can be regarded as optimal targets for such an immunotherapy. Furthermore, arguments are put forward that strongly favor an immunodepletion strategy as compared to an immunoblockade approach, when heading for a specific, long-lasting therapy of chronic allergic airway disease. (*J Allergy Clin Immunol* 2005;116:906-9.)

Key words: Allergic airway disease, monoclonal antibodies, depletion therapy, T cells, costimulatory molecules, inducible costimulator, OX40

Current treatment of chronic allergic airway disease heavily relies on the anti-inflammatory action of inhaled corticosteroids. However, steroids not only suppress the allergen-induced inflammation but also dampen the physiologic local immune response to pathogens. Moreover, treatment with local steroids is only symptomatic and results in a flare-up when discontinued.¹

Abbreviations used

BTLA:	B- and T-lymphocyte attenuator
CD40L:	CD40 ligand
DC:	Dendritic cell
HVEM:	Herpesvirus entry mediator
ICOS:	Inducible costimulator
PD-1:	Programmed death-1
SLAM:	Signaling lymphocyte activation molecule

How could the treatment of bronchial asthma be made more specific and at the same time long lasting? These 2 central questions should be discussed with a view to the basic steps leading to a chronic inflammatory airway reaction (reviewed by Ngoc et al²). The allergen is taken up by phagocytic cells located in peripheral lung tissue and subsequently transported by dendritic cells (DCs) to draining lymph nodes (step 1). The allergen is presented in the lymph nodes to T cells (step 2). Naive T cells recognizing the allergen for the first time become activated and undergo a differentiation into T_H2 effector T cells (step 3). The fully activated T_H2 effector T cells initiate and sustain the local inflammatory process through secretion of soluble mediators (step 4). Concomitantly, the T_H2 effector T cells instruct allergen-specific B cells to differentiate to antibody-secreting plasma cells (step 5) and memory B cells (step 6). Differentiated allergen-specific T cells develop into long-lived memory T cells (step 7). Memory T cells are quiescent in the absence of antigen, but on allergen re-exposure, they quickly become activated T_H2 effector T cells again (step 3).

Regarding a therapeutic strategy, a limitation of the uptake of ubiquitous allergens (step 1) will be difficult to achieve. A specific blockade of allergen presentation by lung DCs (step 2) without generally compromising the local immune defense seems impossible. An isolated suppression of allergen-specific B cells and plasma cells or removal of IgE (steps 5 and 6) might not be sufficient because local inflammatory responses to allergens by intact allergen-specific T_H2 effector T cells will not be impaired.^{3,4} Likewise, blockade of mediators directing the local cellular infiltration, like chemokines or adhesion molecules, or effector molecules, like prostaglandins and leukotrienes (step 4), might be either too unspecific or limited by the redundancy of the system.

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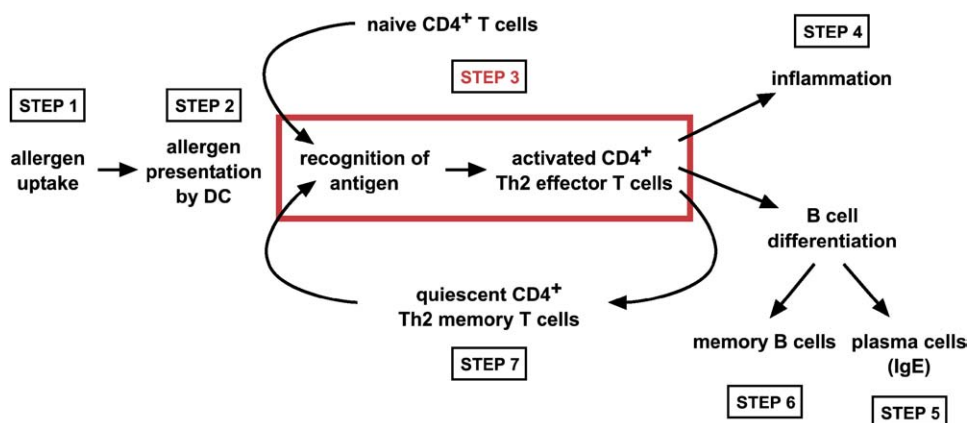


FIG 1. Schematic representation of the development and function of T_H2 effector $CD4^+$ T cells in allergic airway disease.

The optimal target for an immune intervention seems to be the $CD4^+$ T cells recognizing the antigen presented by DCs (step 3) because they orchestrate both the inflammatory process (step 4) and the allergen-specific humoral response (step 5). Moreover, inhibition at this early stage of the immune cascade would also abolish the development of memory T and B cells (steps 6 and 7).

The differentiation of naive T cells or reactivation of memory $CD4^+$ T cells to T_H2 effector T cells (step 3) depends on a precisely orchestrated, hierarchical, multistep, 2-way communication between the antigen-presenting DCs and the T cells and involves soluble factors, as well as cell-surface receptors. The surface receptors on T cells are partly expressed in a constitutive fashion, but most of them are *de novo* induced and further regulated. A greater number of these cell-surface receptors on T cells modulate the T-cell receptor signal in a positive or negative fashion and are therefore designated T-cell costimulators. In the past few years, a series of T-cell costimulators has been identified and at least partially characterized in functional terms (for a review, see Sharpe and Freeman⁵ and Kroczek et al⁶). The activated $CD4^+$ T_H2 effector T cells (Fig 1)⁶ express a pattern of costimulatory molecules that are further reused in the subsequent communication with local lung tissues (step 4, inflammation) and B cells (steps 5 and 6, B-cell differentiation). In addition, $CD4^+$ T_H2 effector T cells are instructed to synthesize and release on demand a set of T_H2 cytokines (eg, IL-4, IL-5, and IL-13) that contribute both to local tissue inflammation and B-cell differentiation into IgE-secreting plasma cells.

Which of the communication molecules used by T cells in the dialogue with DCs and later reused in the effector phase are the optimal targets for the treatment of allergic airway disease? Several criteria have to be considered: cell specificity, expression characteristics, functional aspects, and lack of redundancy. In terms of specificity, the ideal target would be a molecule preferentially or even selectively expressed by $CD4^+$ T_H2 effector T cells. For example, the typical T_H2 cytokines, like IL-4, IL-5, and IL-13, are mainly synthesized by T_H2 effector T cells and

are essential in driving the allergic inflammatory process. However, the T_H2 cytokines are released in small amounts, and they exert their action in tight functional synapses formed between the cytokine-releasing T cells and the target cells and thus might not be easily accessible for blocking antibodies. These aspects and a fair amount of redundancy in the biologic activity of T_H2 cytokines might explain the disappointing results of the first clinical trials that used mAbs against single T_H2 cytokines in bronchial asthma.⁷

Considering the cell-bound receptors and ligands, the receptors on T cells appear attractive. CD40 ligand (CD40L) interacting with CD40 on DCs and B cells was seen for some time as an ideal target because it is nonredundant and appeared to be largely restricted to activated $CD4^+$ T_H2 T cells. Only the later discovery that CD40L is also expressed on (activated) platelets complicated the clinical use of CD40L-specific antibodies in an unforeseen way.⁸ The group of T-cell costimulatory molecules (Table I) consists of constitutively expressed molecules, such as CD28, CD27, herpesvirus entry mediator (HVEM), and B- and T-lymphocyte attenuator (BTLA), which are poor targets because either all T cells (in the case of CD28) or even other immune cells (in the case of CD27, HVEM, and BTLA) would be affected. Of greater interest are the T-cell costimulators, which are *de novo* expressed. This group consists of the positive costimulators inducible costimulator (ICOS), OX40, CD30, 4-1BB, and signaling lymphocyte activation molecule (SLAM) and the negative costimulators cytotoxic T-lymphocyte antigen-4 (CTLA-4) and programmed death-1 (PD-1).⁶ The negative costimulators are unsuitable for blockade, however, because animal studies have shown that an exacerbation of T-cell activation and inflammation has to be expected (because of a lack of negative regulation). Within the positive T-cell costimulators, SLAM appears to be mainly involved in T_H1 reactions⁹ and therefore cannot be regarded as a good target for an antiallergic therapy. In contrast, ICOS, CD30, OX40, and probably also 4-1BB all clearly participate in

TABLE I. Expression and function of T-cell costimulatory molecules

	Expression			T-cell specific	T-cell co-stimulatory function	Comments
	Naive T cells	Activated T _H 2 effector T cells	Resting memory T cells			
CD28	+++	+++	+++	+	Positive	
CD27	++	+++	+++	—	Positive	
HVEM	+++	++	+++	—	Positive?	
BTLA	+++	+++	++	—	Negative	Bias for T _H 1 T cells
ICOS	—	+++	+	+	Positive	Bias for CD4 ⁺ T _H 2 cells
OX40	—	+++	—	+	Positive	Bias for CD4 ⁺ T _H 2 cells
CD30	—	++	—	—	Positive	Bias for CD4 ⁺ T _H 2 cells
4-1BB	—	+++	—	—	Positive	
SLAM	+	+++	+	—	Positive	Bias for T _H 1 T cells
CTLA-4	—	+++	—	+	Negative	Mainly intracellular expression
PD-1	—	+++	—	—	Negative	
CD40L*	—	++	—	+	None	Also on activated platelets

For a more extensive description of the T-cell costimulatory molecules, see KroczeK et al.⁶

HVEM, Herpesvirus entry mediator; BTLA, B- and T-lymphocyte attenuator; CTLA-4, cytotoxic T-lymphocyte antigen-4; PD-1, programmed death-1; SLAM, signaling lymphocyte activation molecule.

*CD40L is not a T-cell costimulatory molecule but is included because of its unusual expression characteristics, which make it a suitable target for depletion therapy.

the development of the T_H2 immune reaction *in vivo*.¹⁰⁻¹⁴ ICOS, preferentially expressed on T_H2 CD4⁺ T cells, substantially contributes to the generation of immunoglobulins other than IgM and IgG3 and is absolutely required for the generation of memory B cells.¹⁵ OX40 and CD30 are similarly biased toward T_H2 CD4⁺ T cells,^{14,16} with OX40 specifically regulating long-term T-cell survival and memory formation.¹³ The role of CD30 is less well defined, but triggering of T cells through CD30 resulted in the generation of T_H2 cytokines.¹² 4-1BB has been originally described as an early costimulator of CD8⁺ T cells enhancing IL-2 and IFN- γ secretion, but more recent data point also to an involvement in CD4⁺ T_H2-mediated responses.¹³

Although the relative contributions of ICOS, OX40, CD30, and 4-1BB for the development and function of T_H2 effector cells is not fully understood yet, it is clear that all these molecules collectively represent the master switches for the interaction of T cells with DCs, B cells, and other cells of the T_H2 immune reaction. It therefore can be expected that a blockade of the ICOS/ICOS ligand, OX40/OX40 ligand, CD30/CD30 ligand, or 4-1BB/4-1BB ligand receptor-ligand pairs would attenuate the T_H2-mediated allergic reaction. It is unclear at present, however, whether the blockade of a single pathway with mAbs would achieve the desired therapeutic effects because some of the functions of these receptor-ligand pairs might be redundant. It is therefore conceivable that an efficient suppression of an exaggerated T_H2 immune response would only be achieved with a simultaneous blockade of several costimulatory pathways involved in this reaction. With such a combinatorial blockade, one would achieve a clearly T_H2-biased and therefore more specific immune suppression compared with that seen with broadly acting agents, such as corticosteroids, rapamycin, or cyclosporin A.

However, the use of mAbs to block T_H2 costimulatory molecules might have major drawbacks. On the one hand, an efficient blockade of these pathways would most likely require a repetitive and continuous therapy. At the same time, a systemic continuous suppression of the T_H2 pathway would also most likely negatively affect the physiologic immune response to T_H2-inducing pathogens, with potentially severe undesired effects.

It is therefore interesting to consider alternative therapeutic strategies based on mAbs. Instead of blocking single T-cell communication pathways or a combination of them, it would be attractive to permanently eliminate the allergen-specific T cells from the immune system. To this end, one would require a target meeting a number of stringent criteria. This target should be expressed as follows: on T cells only (an additional expression on short-lived cells, such as natural killer cells or granulocytes, would be no limitation); in the induction and effector phases of the T_H2 cell immune response only; in a defined window period (7-14 days) of the T_H2 effector phase to allow sufficient time for therapy; and at significant levels to facilitate an efficient binding of depleting antibodies. In addition, its expression would ideally be restricted to T_H2 CD4⁺ T cells (compared with T_H1 T cells) and limited to the primary site of allergen entry (the lung in the case of allergic airway disease).

Probably there is no T cell-specific target that would meet all of the listed criteria in an ideal way. However, a number of molecules on the surface of T cells do come very close. For example, CD40L (which has no costimulatory function for T cells) is still a good candidate for depletion therapy because it is largely restricted to activated CD4⁺ T cells, apart from its expression on (activated) platelets. Furthermore, CD40L is essential for the dialogue of T cells with DCs and B cells. Of the T-cell costimulatory molecules, CD30 and 4-1BB are expressed

on a variety of tissues and therefore cannot be regarded as good targets for a specific elimination of T_H2 T cells. However, ICOS and OX40 are 2 promising candidates. Both of them are T cell specific and are not expressed on naive T cells to a significant degree. Both of them are strongly induced on activated T cells in the effector phase only and are preferentially (but not exclusively) expressed on T_H2 T cells.^{10,11,14} Thus an elimination of ICOS⁺ or OX40⁺ T cells in an actively ongoing allergic reaction could be expected to remove most, if not all, allergen-specific effector T cells. Because these effector T cells (step 3 in Fig 1) are generated both in early and chronic allergic disease, the depletion therapy could be expected to be effective in all stages of the allergic disease. The depletion of CD4⁺ T_H2 effector T cells would eliminate T-cell help for B cells and consequently also downregulate the B-cell reaction toward the allergen or allergens. Thus the allergic inflammatory process would be fully terminated. As long as the patients would not have circumstantial infections at the time of the T-cell depletion treatment (anticipated to last 7-14 days), one could expect that the holes created in the T-cell immune defense would be highly specific for the allergen or allergens. Furthermore, at least in the adult, in whom thymus involution prevents a rapid replenishment of the T-cell pool, this type of therapy can be expected to be long lasting. Finally, because the treatment would only be of short duration and highly specific for allergen-reactive T cells, no impairment of the patient's immune system and reactivity to pathogens would be expected.

It will be exciting to observe whether these predictions made on the basis of recent advances on the biology of T-cell costimulators *in vitro* and in animals¹⁷ will bear out in the development of novel therapies against allergic airway disease.

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REFERENCES

1. Hamelmann E, Schleimer RP. Corticosteroid treatment in bronchial asthma: for better or for worse? *J Allergy Clin Immunol* 2003;111:248-50.
2. Ngoc LP, Gold DR, Tzianabos AO, Weiss ST, Celedon JC. Cytokines, allergy, and asthma. *Curr Opin Allergy Clin Immunol* 2005;5:161-6.
3. Wahn U, Hamelmann E. Anti-IgE therapy. *Clin Allergy Immunol* 2004;18:625-40.
4. Holgate S, Casale T, Wenzel S, Bousquet J, Deniz Y, Reisner C. The anti-inflammatory effects of omalizumab confirm the central role of IgE in allergic inflammation. *J Allergy Clin Immunol* 2005;115:459-65.
5. Sharpe AH, Freeman GJ. The B7-CD28 superfamily. *Nat Rev Immunol* 2002;2:116-26.
6. KroczeK RA, Mages HW, Hutloff A. Emerging paradigms of T-cell co-stimulation. *Curr Opin Immunol* 2004;16:321-7.
7. Leckie MJ. Anti-interleukin-5 monoclonal antibodies: preclinical and clinical evidence in asthma models. *Am J Respir Med* 2003;2:245-59.
8. Kawai T, Andrews D, Colvin RB, Sachs DH, Cosimi AB. Thromboembolic complications after treatment with monoclonal antibody against CD40 ligand. *Nat Med* 2000;6:114.
9. Veillette A, Latour S. The SLAM family of immune-cell receptors. *Curr Opin Immunol* 2003;15:277-85.
10. Löhning M, Hutloff A, Kallinich T, Mages HW, Bonhagen K, Radbruch A, 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.
11. Beier KC, Hutloff A, Löhning M, Kallinich T, KroczeK RA, Hamelmann E. Inducible costimulator-positive T cells are required for allergen-induced local B-cell infiltration and antigen-specific IgE production in lung tissue. *J Allergy Clin Immunol* 2004;114:775-82.
12. Tarkowski M. Expression and a role of CD30 in regulation of T-cell activity. *Curr Opin Hematol* 2003;10:267-71.
13. Croft M. Costimulation of T cells by OX40, 4-1BB, and CD27. *Cytokine Growth Factor Rev* 2003;14:265-73.
14. Lane P. Role of OX40 signals in coordinating CD4 T cell selection, migration, and cytokine differentiation in T helper (Th)1 and Th2 cells. *J Exp Med* 2000;191:201-6.
15. Grimbacher B, Hutloff A, Schlesier M, Glocker E, Warnatz K, Dräger R, et al. Homozygous loss of ICOS is associated with adult-onset common variable immunodeficiency. *Nat Immunol* 2003;4:261-8.
16. Kim MY, Gaspal FM, Wiggett HE, McConnell FM, Gulbranson-Judge A, Raykundalia C, et al. CD4⁺CD3⁻ accessory cells costimulate primed CD4 T cells through OX40 and CD30 at sites where T cells collaborate with B cells. *Immunity* 2003;18:643-54.
17. Kallinich T, Beier KC, Gelfand EW, KroczeK RA, Hamelmann E. Co-Stimulatory Molecules as potential targets for therapeutic intervention in allergic airway disease. *Clin Exp Allergy*. In press 2005.