

The role of co-stimulation in airway inflammation

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Summary

There is considerable evidence to support an important role for co-stimulatory molecules in regulating the proliferation and activation of T cells in the immune response. Of particular relevance is the interaction between CD28 on T cells and B7 expressed on the surface of antigen presenting cells (APCs). CTLA-4, another molecule present on activated T cells may downregulate T cell activity, but its role remains uncertain. CTLA4-Ig, a fusion protein consisting of the extracellular domain of CTLA4 and the Fc portion of human immunoglobulin G1 (IgG₁), has been useful for studying the role of CD28/B7 interactions in immune responses. A number of studies have shown that CTLA4-Ig can switch off T cell activation. In an ovalbumin sensitive murine model of asthma, CTLA4-Ig treatment suppressed the response to inhaled allergen (increased airway hyperresponsiveness [AHR], IgE production, recruitment of eosinophils into the lungs, production of IL-4, IL-5, and IL-10 and increased IFN γ production from CD3-TCR-activated T cells). Anti B7-2 treatment has similar effects suggesting that interaction of B7-2 with CD28 is important in the development of a Th-2 type inflammatory response in mice. Recent observations have been of relevance to human allergic disease. *In vitro* studies have shown that CTLA4-Ig or anti-B7-2 antibody can inhibit allergen-induced proliferation and cytokine production by peripheral blood mononuclear cells from atopic subjects. The role of co-stimulation has been studied in a human bronchial explant model of asthma. CTLA4-Ig fusion protein effectively blocked allergen-induced production of IL-5 and IL-13 in bronchial explants from atopic asthmatics. These studies confirm the requirement for interaction between co-stimulatory molecules in cytokine production and allergic inflammation, and point to the CD28-B7 pathway as being important to the allergen-induced inflammation in asthma. Studies of organ transplantation in primates suggest that CTLA4-Ig is extremely effective in preventing organ rejection. While phase 1 clinical trials have shown CTLA-4-Ig treatment of patients with psoriasis vulgaris to be well tolerated and to result in clinical improvement, its role in asthma management merits further investigation.

Keywords: co-stimulation, asthma, immune response, CTLA-4, B7, antigen-presenting cell, T lymphocytes, cytokines

Introduction

There are complex cellular interactions in the development of allergic inflammation. Although much is known about the role of eosinophils, mast cells, T cells and IgE in the inflammatory response, relatively little is known about the initial interaction between the antigen presenting cell (APC)

and the T cell. One reason for this is that it has been difficult to study these interactions and to test the various concepts using *in vitro* and *in vivo* models.

Activation of T cells requires two signals. If an APC simply presented antigen in the context of its major histocompatibility (MHC) class II molecules to the T cell receptor, this would result in tolerance, anergy and apoptosis of the T cells (i.e. the immune response would be shut down). A second signal is needed and is given by co-stimulatory molecules, which recognize each other and are expressed on the APC and the T cell (Fig. 1). The presence of this second

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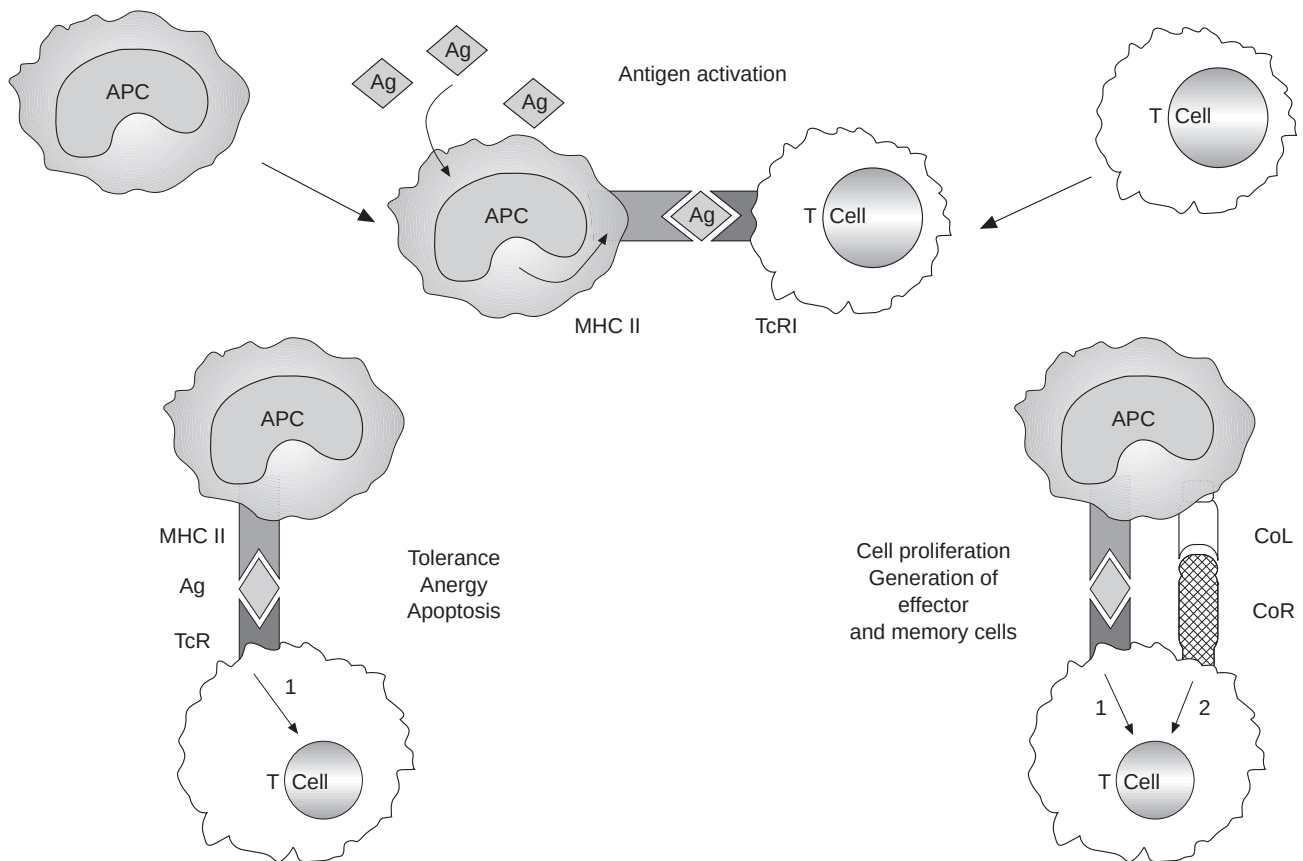


Fig. 1. Activation of T cells requires two signals.

signal results in cell proliferation and generation of effector and memory cells.

B7: CD28/CTLA-4 costimulatory pathway

There is now considerable evidence to support an important role for co-stimulatory molecules in regulating the proliferation and activation of T cells in the immune response. A large number of co-stimulatory molecules have been identified but the two most important are the associations between the B7 family of molecules on APCs and CD28 and its relative homologue CTLA-4 expressed on T cells (Fig. 2). Following interaction between the T cell receptor (TCR) on the T cell membrane with the MHC antigen-peptide complex on the APC, CD28 expressed on the surface of T cells interacts with B7-1 (CD80) and B7-2 (CD86) ligands expressed on the surface of APCs to provide a powerful augmenting stimulus to T cell activation. Studies have suggested that binding of another molecule present on activated T cells, CTLA-4, to B7-1 and B7-2 delivers a negative signal and functions to downregulate T cell activity. However, the role of CTLA4 remains uncertain, with recent

studies suggesting that CTLA-4 might also deliver a positive signal to T cell activation.

CTLA-4 fusion protein

One particular development, which has enhanced our understanding of how these co-stimulatory molecules interact in immune responses, has been the development of the CTLA-4 fusion protein (CTLA-4 Ig). This is composed of the extracellular domain of the CTLA-4 and the constant Fc portion of human immunoglobulin (Ig)G1. Because CTLA-4 has a very high affinity for B7 molecules, it will preferentially bind to B7 molecules on APCs making it very difficult for CD28 (which is low affinity) to bind to these molecules (Table 1). In addition, there are relatively fewer B7 molecules available on the APC and these will preferentially bind to CTLA-4 on T cells giving a negative signal to the T lymphocyte.

Using an ovalbumin sensitive murine model of asthma, Tsuyuki *et al.* [1] showed that treatment with CTLA-4-Ig fusion protein (which blocks the interaction between CD28 and B7) significantly reduced the generation of interleukin

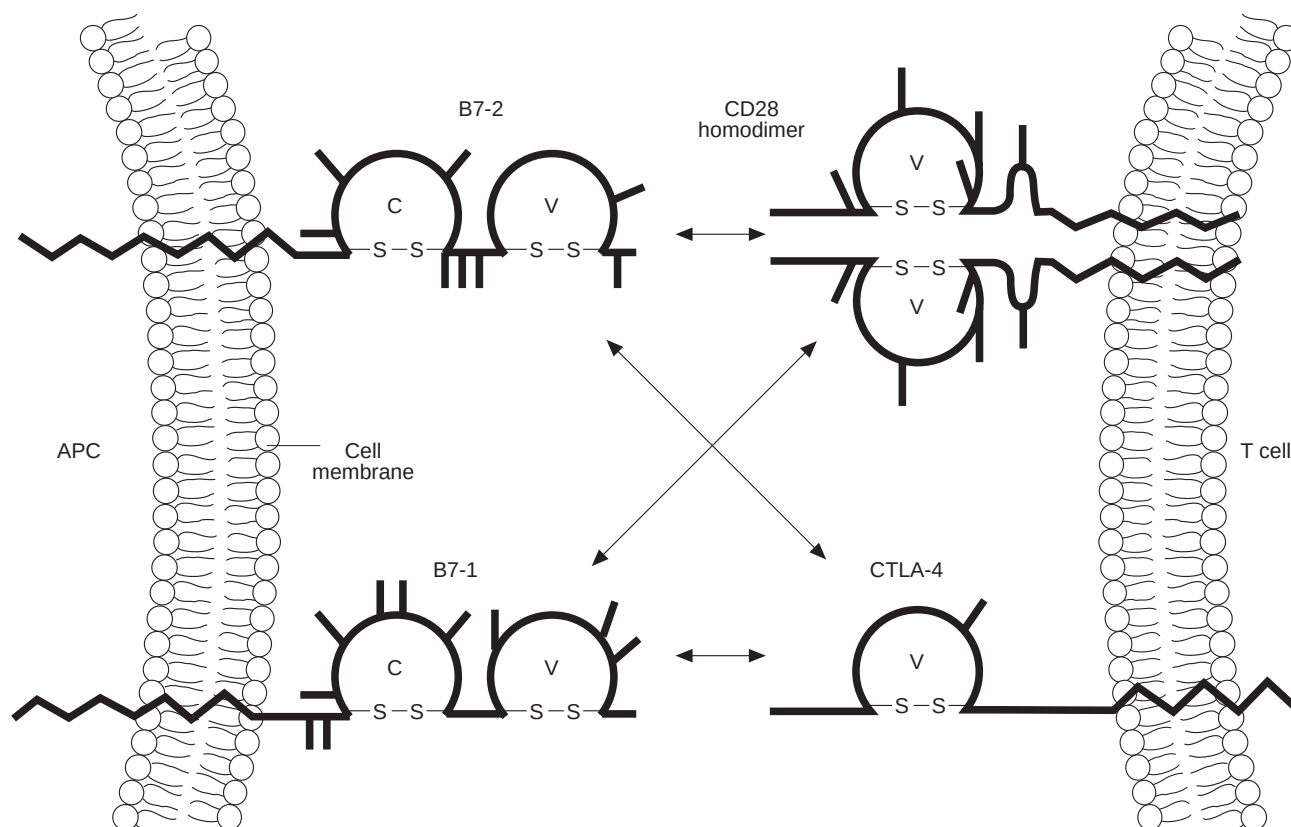


Fig. 2. B7:CD28/CTLA-4 co-stimulatory pathway.

(IL)-4 and IL-5 in response to inhaled allergen. This was associated with an inhibition of IgE generation, a reduction in the eosinophilic inflammation in the lungs and a reduction in the enhanced bronchial hyperresponsiveness that is seen in this model.

Anti-B7-2 treatment has been shown to have effects similar in magnitude on eosinophil infiltration, cytokine production, IgE production and hyperresponsiveness, suggesting that interaction of B7-2 with CD28 is important

in the development of a Th-2 type inflammatory response in mice. This observation has recently been shown to be of relevance to human allergic disease. *In vitro* studies have shown that exposure of naive cord blood mononuclear cells to Der P allergen or Th-2 type cytokines, IL-4 or granulocyte macrophage colony-stimulating factor (GM-CSF), results in increased expression of B7-2 and to a lesser extent B7-1.

There are a number of human diseases in which CD28/B7 co-stimulatory blockade may have therapeutic potential. These include inflammatory bowel disease, multiple sclerosis, rheumatoid arthritis and psoriasis, in which activated T lymphocytes play a major role. In addition, it is hoped that the application of this CTLA-4-Ig fusion protein may be of use in preventing transplant rejection. It remains to be shown whether CTLA-4-Ig fusion protein will have a therapeutic role in asthma. However, a recent phase I study reported the first application of the CTLA-4-Ig fusion protein in human disease in patients with psoriasis vulgaris [2]. The treatment was well-tolerated and resulted in a dose-related improvement in the skin lesions of these patients.

Evidence of the relevance of this co-stimulatory pathway in human allergic disease comes from three compartments:

Table 1. B7:CD28/CTLA-4 co-stimulatory pathway

	CD28	CTLA-4
General features		
Expression	Constitutive	Inducible
Avidity for ligand	Low	High
Abundance	High	Low
Effects on T cells		
Proliferation	Increased	Decreased
Cytokine production	Increased	Decreased

peripheral blood, bronchoalveolar lavage (BAL) and bronchial biopsies. Larche *et al.* [3] took peripheral blood mononuclear cells (PBMCs) from atopic asthmatics and normal subjects and stimulated them *in vitro* with allergen to induce IL-5 production. Application of the CTLA-4-Ig fusion protein effectively blocked the generation of IL-5. In addition, anti-CD86 (a monoclonal antibody directed against B7-2) abrogated IL-5 production whereas anti-CD80 (a monoclonal antibody against B7-1) did not inhibit allergen-induced IL-5 production from PBMCs. This suggests that allergen-induced T cell activation can be blocked by agents that interfere with B7-2 co-stimulation. It is not known whether similar responses will be seen with bronchial tissue from asthmatic subjects.

Bronchial explant model

The role of co-stimulation was recently studied in a human bronchial explant model of asthma. Bronchial biopsies obtained at bronchoscopy from house dust mite (HDM) sensitive atopic asthmatics were cultured for 24 h in the presence of house dust mite allergen extract. In this bronchial explant model, allergen stimulated the production of cytokines IL-5 and IL-13. Addition of CTLA4-Ig fusion protein to the culture medium effectively blocked production of both IL-5 and IL-13 found on stimulation *ex vivo* with house dust mite antigen (Fig. 3) [4]. These studies confirm the requirement for interaction between co-stimulatory molecules in cytokine production and allergic inflammation, and point to the CD28/B7 pathway as being important to the allergen-induced inflammation in asthma.

The bronchial explant model was also used to investigate the mechanisms that may be responsible for the recruitment of cells into the airways. House dust mite allergen (Der P) stimulated the generation and release of RANTES and IL-16 (two cytokines important in the recruitment of T lymphocytes and eosinophils) into the supernatant of bronchial biopsy tissue taken from asthmatic individuals but not from control subjects. In addition, the culture supernatant from the bronchial explants of asthmatic individuals produced pronounced upregulation of T cell chemotactic activity. The fusion protein CTLA-4-Ig significantly reduced the generation of IL-16 and RANTES and reduced T cell chemotactic activity following stimulation with allergen [5].

Conclusions

There is now good evidence that T cell co-stimulation is important for T cell proliferation and effector function. The CD28/B7 pathway is involved in several T cell-mediated inflammatory diseases. CTLA-4-Ig fusion protein has

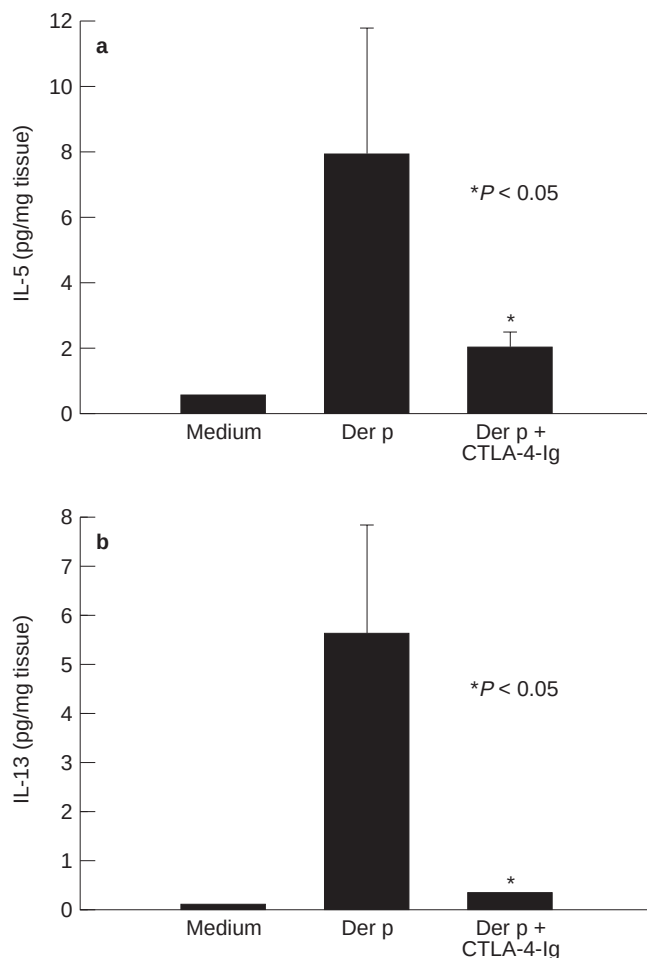


Fig. 3. CTLA-4-Ig inhibits allergen-induced (a) IL-5 and (b) IL-13 expression in asthmatic patients. Reprinted with permission of the American Thoracic Society from ref [4].

already been shown (in a number of animal models) to be of relevance to the control of autoimmune disease and transplant rejection. There is now some encouraging data in relation to psoriasis, but we have yet to see whether CTLA-4-Ig is a potential therapeutic strategy for bronchial asthma. Nevertheless, the CD28/B7 pathway is emerging as an important mechanism in the pathogenesis of bronchial asthma.

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