

Immunomodulation of allergic responses by targeting costimulatory molecules

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Purpose of review

This review focuses on putative targets, including costimulatory and additional pathways involving T regulatory cells, that may be critical for modifying allergic responses.

Recent findings

Multiple costimulatory signals including CD28/CTLA4: CD80/CD86, ICOS: ICOSL, OX40:OX40L and PD-1: PD-L1/PD-L2 have been identified and implicated in the regulation of immune disorders. Recent studies indicate that T regulatory cells may also suppress T cell costimulation by the secretion of TGF- β and IL-10, suggesting an important role of T regulatory cells in the regulation of allergic disorders.

Summary

Immune-mediated disorders, including allergic diseases, have been increasing in prevalence. Unravelling these immune pathways may suggest new targets for immunomodulation.

Keywords

allergy, autoimmunity, costimulatory molecules, T regulatory cells

Introduction

Immune-mediated disorders are increasing in severity and prevalence [1]. Current understanding of the pathogenesis of allergy, autoimmune diseases and transplantation rejection emphasizes their inflammatory nature. T-cell activation, which is essential in promoting allergen-dependent inflammation, requires a first signal involving an antigen-specific T-cell receptor (TCR) ligand and second signals provided by costimulatory molecules [2]. Activation of these two signals leads to full T-cell activation, including proliferation and acquisition of effector function. T-cell receptor activation in the absence of costimulatory signals leads to the loss of antigen responsiveness, delineated as T-cell clonal anergy, critical for tolerance induction [3]. Approaches that promote tolerance in autoimmunity and transplantation models are based on interference with T-cell activation and costimulatory pathways. In addition to positive costimulatory signals, negative signals for T-cell activation include cytotoxic T-lymphocyte antigen 4 (CTLA4). CTLA4 deficiency results in a fatal systemic autoimmunity and may be also essential for tolerance induction [4].

Targeting costimulatory molecules, in particular CTLA4, is of therapeutic interest, especially in the areas of autoimmune disorders and transplantation. This review focuses on the role of costimulatory molecules as immunomodulators, particularly in the development of allergic disorders. We discuss the recent literature regarding several costimulatory pathways including CD28/CTLA4: CD80/CD86, ICOS: ICOSL, OX40:OX40L and PD-1: PD-L1/PD-L2, as well as immunomodulator pathways including T regulatory (Treg) cells.

Costimulatory molecules

The costimulatory molecules can be grouped into two major families: the immunoglobulin superfamily and the tumor-necrosis factor receptor (TNFR) superfamily.

Immunoglobulin superfamily

CD28 is a surface molecule expressed by T cells [5] and binds to its ligands CD80 and CD86, which are primarily expressed on the surface of antigen-presenting cells (APCs) [6] (Fig. 1). CTLA4, also a ligand for CD80 and CD86, is expressed by T cells upon activation, providing a negative feedback signal that limits the immune responses [7]. Inducible T-cell costimulator

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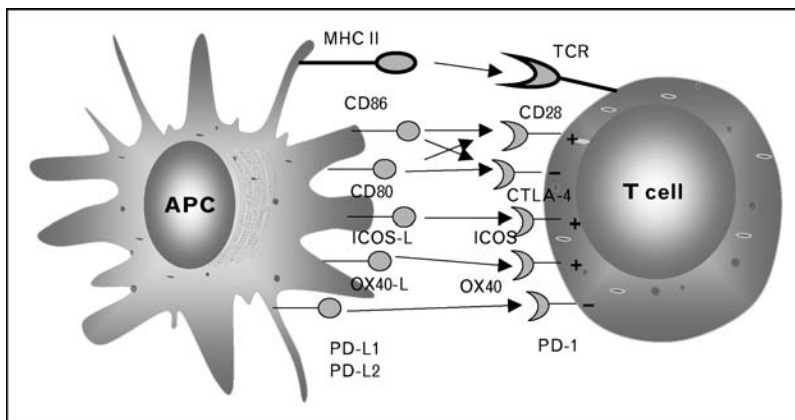
Abbreviations

APC	antigen-presenting cell
CTLA4	cytotoxic T-lymphocyte antigen 4
ICOS	inducible T-cell costimulator system
Treg	T regulatory cell

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Figure 1 Costimulatory molecules on T cells interact with specific ligands on antigen-presenting cells

APC, antigen-presenting cell; MHC, major histocompatibility complex; TCR, T cell receptor; CTLA4, cytotoxic T lymphocyte antigen 4; ICOS, inducible T cell costimulator system; PD-1, program death 1. Black arrow indicates ligation; (–) negative costimulatory signal; (+) positive costimulatory signal.



system (ICOS) is structurally and functionally very close to CD28. Unlike CD28, ICOS requires induction on the cell surface by antigen stimulation, suggesting that ICOS plays a role in activating preactivated T cells rather than naïve T cells [8,9]. PD-1 is a second costimulatory molecule from the CD28 family that delivers an inhibitory signal to T and B cells by the engagement of its two ligands: PD-1 ligand 1 (PD-L1) and PD-1 ligand 2 (PD-L2). PD-L1 is expressed on hematopoietic and parenchymal cells while PD-L2 is expressed only in macrophages and dendritic cells [10].

Tumor-necrosis factor receptor superfamily

OX40, a member of the tumor-necrosis factor (TNF) receptor family, is mainly expressed on activated CD4+ Th2 and CD8+ T cells [11]. The ligand of OX40 (OX40L) is expressed on dendritic cells as well as B cells, vascular endothelial cells, natural killer cells, and mast cells [12]. Signaling through OX40 occurs after CD28 signals and allows the survival and continuous proliferation of the effector T cells [11].

Disease models: targeting costimulation in autoimmunity and transplantation

Autoimmunity is associated with aberrancies in positive or negative costimulatory pathways. For example, CTLA4 polymorphisms are associated with T cell-mediated autoimmune diseases [13]. Deletion of CTLA4 interferes with the induction of T-cell anergy, and thus leads to systemic autoimmunity [14]. Autoimmune disease exacerbation has been reported in several murine models with anti-CTLA4 antibodies. Also, the beneficial effects of blockade of CD28:CD80/CD86-mediated T-cell activation have been demonstrated in models of autoimmune diseases using gene-deleted mice, anti-CD80 and/or anti-CD86 antibodies, or soluble fusion proteins of CD28. The balance between CD28-mediated T-cell costimulation and CTLA4-mediated inhibition is

critical in determining the outcome of the immune response. Recent structural analysis confirms the low binding affinity of CD28 for its ligand during T cell-APC interactions compared with CTLA4, which suggests that soluble fusion proteins of CTLA4 rather than CD28 may be well suited in blocking CD28:CD80/CD86-mediated T-cell activation [15^{••}]. These advances have led to investigation of effects of a soluble fusion protein of CTLA4 (CTLA4 Ig), which is presumed to function by binding to CD80/CD86. CTLA4 Ig inhibits costimulation, both through CD28, as well as negative signaling via CTLA4. Administration of CTLA4 Ig in patients with psoriasis resulted in symptom improvement [16]. Indeed, CTLA4 Ig has recently received Food Drug Administration (FDA) approval for use in rheumatoid arthritis [17^{••}]. To date, details regarding the specific mechanisms of action of costimulation blockade remain to be elucidated relative to the treatment of autoimmune disorders.

Manipulation of costimulatory signals is also a potential target to prevent allograft rejection. In murine transplantation models, graft acceptance is prolonged by blockade of CD28 interaction with CD80 and CD86 when using CTLA4 Ig or anti-CD80/CD86 mAb [18–20,21[•]]. Furthermore, in an intestinal allograft rejection model, blockade of the CD28-CD80/CD86 interactions with CTLA4 Ig reduces rejection in wild type and CD28-deficient mice [22]. In a cardiac allograft model, CTLA4 Ig also leads to the permanent acceptance of heart allografts [23]. CTLA4 Ig administration reduces proliferation and enhances the susceptibility to apoptosis of dividing cells, suggesting pathways for CTLA4 Ig induced allograft survival in transplantation models [24]. Other pathways of interest include CD45, a tyrosine phosphatase, which can induce CTLA4, the negative regulator, and lead to prolonged allograft survival [25,26^{••}].

Disease models: targeting costimulation in allergy

Murine models of allergic inflammation have examined the contribution of costimulation, utilizing stimulating and blocking monoclonal antibodies, fusion proteins or genetically modified mice.

CD28:CD80/CD86 and cytotoxic T-lymphocyte antigen 4

Blocking the ligation of costimulatory molecules CD28 with CD80/CD86 leads to a decrease in airway inflammation. Systemic administration of CTLA4-Ig during allergen sensitization inhibited the ligation of CD28:CD80/CD86, reducing airway inflammation [27,28]. This inhibition during the primary immune response also significantly decreased bronchoalveolar lavage fluid IL-4 and serum of IgE [29]. Moreover, inhibition of CD28:CD80/CD86 ligation by systemic or intranasal administration of CTLA4 Ig attenuated production of Th2 cytokines and increased Th1 cytokines [30,31]. Administration of anti-CD86 mAb during both allergen sensitization and airway challenge reduced allergen-specific IgE, Th2 cytokine production and airway hyperresponsiveness [32–34]. CD80 is also crucial in the initiation and maintenance of allergic immune responses [32,35,36], indicating that both costimulatory molecules are effective in regulating allergic immune responses. Analysis of CD80 and CD86-deficient mice revealed findings consistent with the blocking experiments described above [36]. In both CD80/CD86 double-deficient and CD28-deficient mice, production of IgE, eosinophilic airway inflammation and development of airway hyperresponsiveness were absent following allergen sensitization and airway challenge [37,38].

Further findings in atopic human subjects indicate that blockade of CD28 signaling may play a role in decreasing allergic inflammation. For example, a recent study showed increased expression of the costimulatory molecules CD28, CD86 and CTLA4 in patients with allergic asthma compared with normal subjects [39]. In atopic asthmatic subjects, allergen-induced secretion of IL-5 and IL-13 was inhibited by CTLA4 Ig, suggesting the requirement of CD80/CD86 for Th2 cytokine secretion [40]. In addition, CTLA4 Ig inhibited allergen-specific Th2 cytokine production from T cells isolated from bronchial biopsies from asthmatics [41].

Inducible T-cell costimulator system

Application of anti-ICOS mAb during allergen sensitization was effective in reducing airway allergic responses [42]. In addition, when anti-ICOS antibody was administered during allergen challenge, Th2 cytokine and serum IgE production were decreased [43,44]. Adoptive transfer of ICOS+ T cells into naïve mice induced airway inflammation upon airway challenge [45], suggesting that

ICOS costimulation is important for the establishment of allergic disorders.

PD-1

PDL-1, expressed in pulmonary dendritic cells, macrophages and B cells, was enhanced after allergen sensitization and airway challenge. Also, anti-PD-L2 mAb administration during allergen challenge increased airway hyperresponsiveness, lung eosinophilia and levels of Th2 cytokines in bronchoalveolar lavage fluid. In addition, no effect on the inflammatory response was demonstrated when PD-L2 was blocked during sensitization, suggesting that PD-L2 function may primarily influence the effector phase of allergic responses [46].

OX40

Allergen-sensitized and challenged OX40L-deficient mice showed decreased airway hyperreactivity, Th2 cytokine production and serum IgE levels [47]. In addition, administration of anti-OX40L mAb abolished development of airway inflammation only when administered during allergen sensitization [48]. Moreover, blocking of the OX40 pathway at the time of allergen challenge suppressed memory T effector cell accumulation in lung-draining lymph nodes and lung, and prevented allergic responses [49]. These findings indicate that the recall response of memory T cells may be OX40 signal dependent, suggesting that targeting the OX40 pathway may modulate ongoing allergic disorders.

Costimulation in allergy: role of T regulatory cells

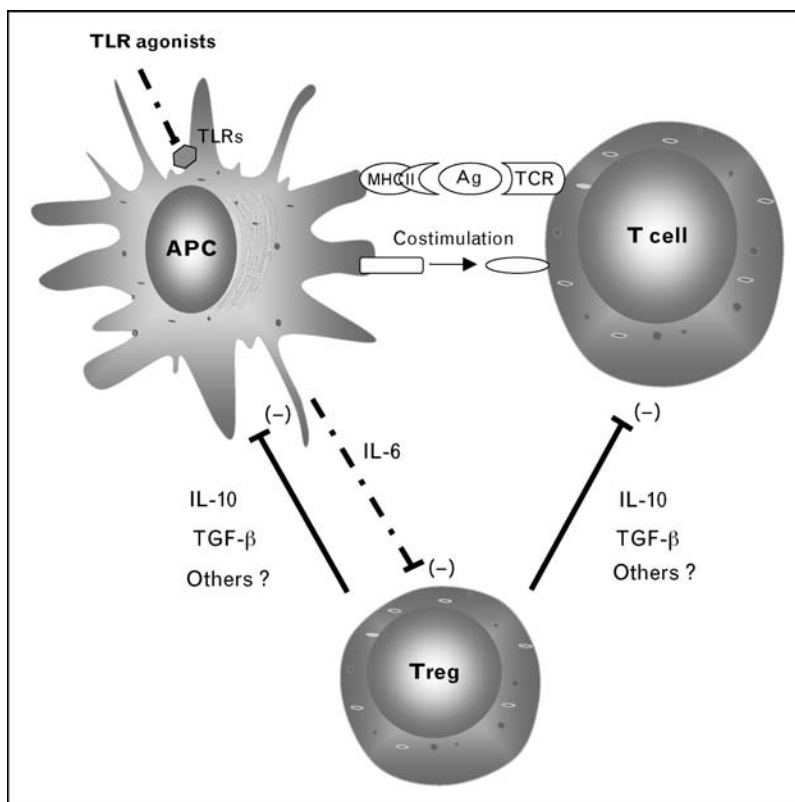
A cardinal event in the development of allergic immune responses is the activation of CD4+ T cells. Once activated, these cells produce Th2 cytokines, inducing IgE production by B cells and recruitment of eosinophils [50]. In addition to CD4+ T cells, T regulatory cells are an additional subtype of T cells, with immunosuppressive function that regulates both Th1 and Th2 cells [51].

Tregs can be divided into two subtypes: thymus-derived CD4+CD25+ regulatory cells, also known as naturally occurring Tregs, and adaptive Tregs, which are inducible *in vivo* or *in vitro*. Naturally occurring CD4+CD25+ Tregs express a high-affinity IL-2 receptor α chain (CD25). In addition to CD25, Tregs may also express CTLA4 and GITR on their surface and the transcription factor FOXP3, a hallmark for CD4+CD25+ Tregs [52].

Tregs can suppress T cells and APCs via secretion of cytokines including IL-10 and TGF- β [51,53**] (Fig. 2). IL-10 can suppress T-cell proliferation by inhibiting CD28 costimulation [54], suggesting that IL-10 may also play a role in suppressing other costimulatory molecules. In addition, membrane-associated TGF- β on the surface of Tregs may also play a role in the suppressor activity

Figure 2 Pathways for T regulatory cell suppressive function

T regulatory cells can suppress T cells or APCs by secreting IL-10 and TGF- β . Activation of Toll-like receptors (TLRs) on antigen-presenting cells (APCs) induces production of IL-6 that renders T regulatory cells (Tregs) ineffective. MHC, major histocompatibility complex; TCR, T cell receptor; Ag, antigen. Costimulation (representing pathways shown in Fig. 1); (–) negative signals.



mediated by cell-cell contact [55]. In a murine model of asthma, TGF- β -producing Tregs inhibit the development of allergic immune responses [56] (Fig. 2).

Tregs may play an essential role in the suppression of Th2 immune responses in both humans and murine models. In murine models of asthma, inhibition of Tregs led to an increase of airway hyperresponsiveness [57,58]. Proinflammatory cytokine IL-6 has been shown to inhibit Tregs function. Blocking of IL-6 resulted in an increase of lung Tregs [58]. In addition, activation of innate Toll like receptors (TLRs) on APC induces production of IL-6 and abrogates Treg suppressor activity [59] (Fig. 2).

Treg suppressor function was examined in seasonal atopic patients compared with nonatopic control subjects. Tregs from the atopic group appear to be less effective in suppressing T-cell proliferation when compared to a nonatopic group, suggesting that an imbalance between Tregs and T effector cells results in the development of allergic disorders [60]. Furthermore, allergen immunotherapy, which involves subcutaneous injections of increasing doses of allergen [61], increased Tregs in grass pollen-stimulated peripheral blood mononuclear (PBMC) cell cultures. These data suggest that allergen immunotherapy has a role in increasing the number of

Tregs that modulates immune responses [62]. In support of this finding, immunotherapy in patients with house-dust mite allergy demonstrated increased IL-10-producing Tregs in the peripheral blood posttherapy [51].

Synthetic peptides derived from amino acid sequences of allergens have also been evaluated for the treatment of allergic individuals. Intranasal administration of house-dust mite peptides inhibited T-cell response [63]. In addition, administration of oral peptides to rheumatoid arthritis patients was associated with a decrease in the PBMC production of IL-2, IFN- γ and TNF- α and an increase in the production of IL-10 and expression of mRNA encoding Foxp3 [64], suggesting that peptide immunotherapy may also increase Tregs.

Although no current study shows direct evidence that Foxp3 suppresses allergic immune responses, a recent study showed that glucocorticoids upregulate Foxp3 in asthmatic patients [65]. Interestingly, glucocorticoids also inhibit the development of Tregs, leading to exacerbation of allergic airway diseases [66••]. A relationship between innate immunity, Tregs and atopic disease has also been shown. TLR2 stimulation decreased IL-10 and Foxp3 in cord blood mononuclear cells from atopic compared with nonatopic mothers [67••].

Conclusion

Targeting of costimulatory molecules by using specific monoclonal antibodies or fusion proteins may be a promising therapeutic approach for allergic diseases. The murine models of allergy, transplantation and autoimmunity showed disease amelioration after costimulatory blockade with CTLA4Ig. CTLA4Ig has also been approved by the FDA for the treatment of rheumatoid arthritis [17^{••}]. Some issues include accessibility of costimulatory blocking reagents to targeted tissues and their nonspecific interactions. Also, blockade of a single costimulatory molecule may not be effective and a multi-target approach may be more efficacious [17^{••},68]. Other exciting future therapeutic approaches under investigation include the application of Tregs. Further investigation of costimulation, Tregs and innate immunity are required. The determination of the mechanisms of costimulatory activation or suppression is crucial for designing therapies directed against allergic diseases.

References and recommended reading

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- of outstanding interest

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