



OX40/OX40L Ligand Interactions in T-Cell Regulation and Asthma

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The OX40 receptor is preferentially expressed by T cells, and its cognate ligand OX40L is primarily expressed by antigen-presenting cells such as dendritic cells following activation by thymic stromal lymphopoietin (TSLP). TSLP is released by the bronchial epithelium, airway smooth muscle, and some inflammatory cells in response to numerous insults such as allergens, viruses, and physical damage. OX40L is a costimulatory molecule that plays a sentinel role in the adaptive immune response by promoting T helper (Th) 2 polarization of naive T cells within the lymph node. These polarized T cells produce Th2 cytokines such as IL-4, IL-5, and IL-13, which have been implicated particularly in allergic eosinophilic asthma. Animal models have positioned both TSLP and OX40/OX40L as critical in the development of airway inflammation and hyperreactivity. In human disease, there is good evidence that TSLP is upregulated in asthma, but there are limited data to demonstrate overexpression of OX40 or OX40L in disease. Targeting the OX40/OX40L axis or TSLP presents a novel therapeutic strategy that has the potential of modifying the disease process and, therefore, impacting on its natural history. Whether this approach can demonstrate efficacy in established disease rather than at disease onset is unknown. Biologic therapies directed toward OX40/OX40L are in early phases of development, and results from these studies are eagerly awaited.

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Abbreviations: Nod = nucleotide-binding oligomerization domain; Th = T helper; TNF = tumor necrosis factor; TSLP = thymic stromal lymphopoietin

Asthma affects over 300 million people worldwide, and its prevalence is increasing. Asthma is a complex disease characterized by airway hyperresponsiveness, variable airflow obstruction, airway inflammation, varying degrees of subepithelial fibrosis, mucus hyperproduction, and remodeling.¹ Atopic asthma has classically been associated with increased expres-

sion of T helper (Th) 2 cytokines, which are increased in sputum,² bronchoalveolar T cells,³ and bronchial biopsies.⁴ A major effector axis resulting in induction of Th2 polarization is the recognition of allergen presented by dendritic cells in local lymph nodes to CD4⁺ T cells. This axis is an optimal target for drug development because it orchestrates the inflammation and development of an allergen-specific humoral response and the development of T- and B-cell memory. The differentiation of naive T cells or reactivation of memory T cells depends on various costimulatory molecules primarily expressed on the surface of T cells and their cognate ligands. One of the most promising costimulatory targets is OX40 and its ligand, OX40L. OX40L is directly mediated by thymic stromal lymphopoietin (TSLP), which is produced by epithelial cells,⁵ mast cells,⁶ airway smooth muscle,⁷ and dendritic cells,⁸ which are all involved in Th2 responses. TSLP was originally identified as a growth-promoting factor found in cultured supernatants of a thymic stromal cell line in 1994 to support the development of murine B cells.⁹ TSLP plays an important role in many allergic diseases,

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such as atopic dermatitis and asthma. TSLP is also up-regulated in COPD,¹⁰ but its role and relationship to OX40/OX40L signaling in this disease is unclear. TSLP binds to its TSLP receptor and the IL-7 receptor α chain. Dendritic cells play a crucial role in the pathogenesis of allergic disease. TSLP activates immature CD11c⁺ dendritic cells to express OX40L, and these cells then become mature dendritic cells, which migrate to the draining lymph nodes. There they activate the differentiation of naive CD4⁺ T cells by binding to the OX40 receptor, where they become inflammatory cells producing IL-4, IL-5, IL-13, tumor necrosis factor- α (TNF- α), and little or no IL-10 (Fig 1).¹¹

The sentinel roles of the OX40/OX40L axis in the adaptive immune response and TSLP in both the innate and adaptive responses suggest these molecular targets may present attractive novel therapeutic targets. In this article, we consider the evidence that

the OX40/OX40L axis plays a role in asthma, its potential importance as a therapeutic target, and the likely target population.

OX40 AND OX40L

OX40 (ACT35, CD134, TNFRSF4) was identified in 1987 and found to be bound to activated T cells.¹² Since then, it has been cloned in rat, mouse, and human cells. The OX40 receptor is preferentially expressed on the surface of activated regulatory CD4⁺ T cells,¹³ natural killer T cells, natural killer cells, and neutrophils, and more recently, we have found it to be expressed in human airway smooth muscle cells.¹⁴ OX40 signaling strongly regulates T-cell division, survival, and cytokine release.¹⁵ The OX40 ligand (OX40L, CD252, TNFSF4) was originally identified in 1985 as gp34 (GP34) protein on human T cell leukemic virus-transformed cells¹⁶ and is expressed on

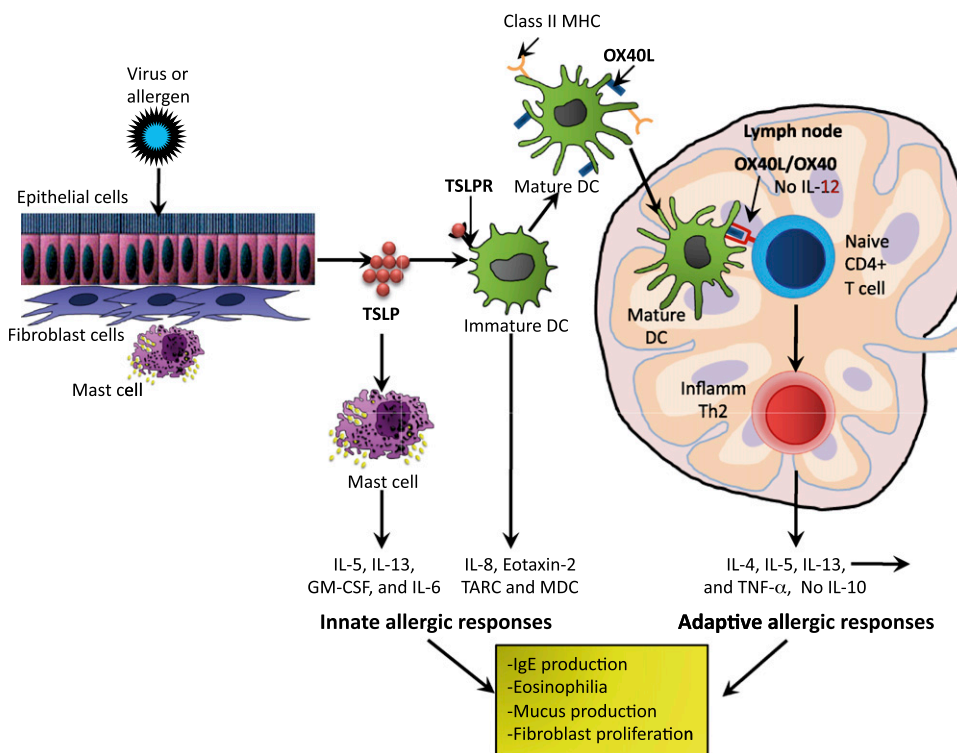


FIGURE 1. Drawing shows the pathophysiologic characteristics of OX40/OX40L and TSLP in allergic inflammation. Cellular damage caused by allergens or viruses triggers mucosal epithelial cells or skin cells (keratinocytes, fibroblasts, and mast cells) to produce TSLP. TSLP initiates the innate phase of allergic immune responses by activating immature DCs by binding to their TSLPR. TSLP/TSLPR-activated DCs produce the chemokines IL-8 and eotaxin-2, TARC, and MDC, and by costimulating mast cells, produce IL-5, IL-13, GM-CSF, and IL-6. The activated immature DCs then mature and produce the OX40L and migrate into the draining lymph nodes, where they trigger the differentiation of allergen-specific naive CD4⁺ T cells by binding to the receptor OX40 and differentiating the CD4⁺ T cells into inflammatory Th2 cells, producing IL-4, IL-5, IL-13, and TNF- α , but no IL-10. These Th2 cytokines then initiate inflammation by triggering IgE production, eosinophilia, mucus production, and fibroblast proliferation. DC = dendritic cell; GM-CSF = granulocyte-macrophage colony-stimulated factor; MDC = macrophage-derived chemokine; MHC = major histocompatibility complex; TARC = T-helper 2 attracting chemokines activation-regulated chemokine; Th = T-helper; TNF = tumor necrosis factor; TSLP = thymic stromal lymphopoietin; TSLPR = thymic stromal lymphopoietin receptor.

antigen-expressing cells, for instance, B cells,¹⁷ dendritic cells,¹⁸ and macrophages¹⁹ as well as airway smooth muscle cells.²⁰

EVIDENCE OF A CRITICAL ROLE FOR OX40/OX40L IN THE PATHOGENESIS OF ASTHMA

Animal Models

In murine asthma models, OX40^{-/-} mice challenged with ovalbumin showed significantly reduced Th2 response, lung inflammation, mucus secretion, 80% to 90% reduction in eosinophilia, decreased goblet cell hyperplasia, and significantly attenuated airway hyperreactivity compared with wild-type mice.²¹ Studies have also demonstrated that OX40L^{-/-} mice sensitized with ovalbumin have significantly reduced total serum IgE, pulmonary eosinophils, cytokines, and pulmonary inflammation compared with wild-type control mice.^{22,23} Inhibition of OX40-OX40L binding via the administration of anti-OX40L mAb in wild-type mice dramatically reduced airway hyperresponsiveness and associated asthma symptoms, compared with mice challenged with isotype control.^{23,24} Mouse splenic CD11c⁺ dendritic cells stimulated for 48 h with TSLP upregulated OX40L expression compared with CD40L or TNF- α and unstimulated cells. A blockade of OX40/OX40L interactions using a specific α -mouse OX40L 4F5 monoclonal antibody significantly inhibited Th2 cytokine production compared with a control antibody. This confirms that OX40L activity on dendritic cells was important for the effects of TSLP in driving Th2 polarization.²⁵ Both in murine and nonhuman primate models of asthma in vivo, a blockade of OX40L inhibited TSLP-mediated Th2 inflammation.²⁵ Studies have demonstrated that OX40 can inhibit the development of adaptive Foxp3⁺ T regulatory cells that differentiate from naive CD4 T-cell populations in response to TGF- β .^{26,27} Foxp3, an X chromosome-encoded fork-head transcription factor family member, is critical for the differentiation of regulatory T cells. These cells have an important role in preventing autoimmunity and pathologic changes inflicted by uncontrolled immune responses to infections. Deficiency or mutation in Foxp3 in humans and mice leads to early onset and susceptibility to diseases such as asthma.²⁸

Targeting OX40L may have the potential to improve the efficacy of immunotherapy to promote tolerance. In wild-type mice exposed to intranasal antigen and specific CD4⁺Foxp3⁺, regulatory T cells were generated, which outnumbered IL-4 and interferon γ -producing CD4 T cells. Inhaled lipopolysaccharide downregulated the regulatory T cells, but up-regulated IL-4⁺ and interferon- γ T cells, and it also increased OX40L expression on dendritic cells

and B cells. Inhibiting OX40/OX40L interactions with an anti-OX40L antibody upregulated regulatory T cells suppressing lipopolysaccharide stimulation.²⁹

Sensitization to fungi such as *Aspergillus fumigatus* and *Alternaria* is associated with poor lung function³⁰ and exacerbations. When bone marrow-derived mouse dendritic cells were stimulated with *Alternaria* for 48 h, upregulation in OX40L expression was detected using flow cytometry,³¹ suggesting that *Alternaria* may play a role in Th2 cytokine release.

The activation of pattern-recognition receptors such as toll-like receptors plays a critical role in Th1 cell differentiation, yet their contribution to the generation of Th2 responses is poorly understood. Interestingly, when mice deficient in either MyD88^{-/-} or TLR4^{-/-} were sensitized intranasally to the common allergen house dust mites and challenged 2 weeks later, they showed diminished Th2 responses as well as fewer OX40Ls presenting dendritic cells in the draining lymph node compared with wild-type mice.³² The activation marker CD30, a member of the TNF receptor family, is expressed on activated T cells. In an acute asthma model, CD30^{-/-} mice developed reduced expression of OX40,³³ whereas in contrast, OX40 expression was not downregulated in a chronic murine asthma model.³⁴ These differences in expression may be the result of the fact that in a chronic asthma model, T cells are able to proliferate, leading to chronic airway inflammation. Airway tolerance is vital for protecting the lung from inflammatory disease-driven allergens, but factors that lead to this susceptibility remain elusive. The pattern recognition receptors nucleotide-binding oligomerization domain (Nod)-like receptors Nod1 and Nod2 are both highly expressed by epithelial cells. Intranasal exposure of Nod2, but not Nod1, induced TSLP-promoting OX40L expression. The generation of these ligands also blocked CD4⁺ fork-head box protein 3⁺ adaptive regulatory T cells and concomitantly drove IL-4-producing CD4 T cells, leading to allergic disease and asthmatic lung inflammation.³⁵

The animal-model data, therefore, present compelling evidence of a central role for OX40/OX40L in the development of Th2 polarization in response to a number of insults that are considered important in asthma. These data support a role for this axis in the immunopathogenesis of asthma.

Human Models

The role of OX40/OX40L in humans is limited compared with murine-model systems. TSLP was preferentially induced in peripheral blood-isolated myeloid dendritic cells from healthy volunteers to express mRNA for OX40L using microarray analysis.¹¹ Blocking OX40/OX40L interactions using a specific

OX40L-neutralizing antibody inhibited the production of Th2 cytokines and TNF- α , but increased the production of IL-10 in CD4⁺ cells cocultured with TSLP-primed dendritic cells.¹¹ Airway smooth muscle cells are critical in the development of bronchoconstriction in asthma, are the major contributors to airway remodeling and persistent airflow obstruction, and release a number of chemokines/cytokines that bind specifically to activated T cells, resulting in increased cellular proliferation. Studies have reported OX40L to be expressed and released by airway smooth muscle cells of people with and without asthma, but with no significant difference.²⁰ However, following TNF- α stimulation, there was an increase in OX40L, and a decrease after stimulation of TNF- α and interferon- γ combined.³⁶ Cells activated with rOX40:Fc over 24 h released IL-6, which was significantly higher in the patients with asthma compared with people without asthma.³⁶ More recently, our group has reported OX40/OX40L expression to be increased in the bronchial submucosa of patients with mild asthma, but not in those with moderate to severe disease, and this was related to the degree of tissue eosinophilia and IL-4 expression.¹⁴

Air pollution, particularly from diesel-exhaust particulates, is associated with worsening in asthma symptoms and control. Bronchial epithelial cells are the first major targets for inhaled pollutants. Bronchial epithelial cells treated with diesel-exhaust particulates express an increase in OX40L expression.³⁷ A recent study also identified TSLP to be highly expressed in isolated nasal epithelial cells from patients with nasal polyposis compared with those without. The TSLP receptor and OX40L receptor were also increased in dendritic cells from the nasal mucosa of patients with nasal polyposis.³⁸

The evidence in humans of a role of OX40/OX40L in asthma is, therefore, circumstantial and the expression data are weak. However, the interaction between OX40/OX40L in Th2 polarization may occur early in the disease pathogenesis and, more importantly, is primarily located in the lymph node rather than the bronchial submucosa. Current strategies to explore this axis have not addressed this compartment, and, therefore, its role has not been fully studied.

TSLP ASTHMA

TSLP is both necessary and sufficient for the development of Th2 cytokine-associated inflammation of the airways in rodents. Mice expressing a TSLP transgene in the airway epithelium develop a spontaneous, progressive inflammatory disease with all the characteristics of human asthma,³⁹ whereas direct intranasal delivery of TSLP (in the presence of antigen)

leads to rapid onset of features similar to severe disease.⁴⁰ Studies have also reported increased TSLP mRNA in bronchial epithelium in asthma in response to allergen, viruses, and other environmental stimuli.⁴¹ In human disease, genetic analysis has shown an association of polymorphisms in *TSLP* with asthma and airway hyperresponsiveness, IgE concentrations, and eosinophilia.⁴²⁻⁴⁴ In addition, patients with asthma have higher concentrations of TSLP in their lungs.^{45,46} The role of TSLP in both the innate and adaptive immune response may suggest that its potential efficacy is broader than targeting the OX40/OX40L axis alone.

TARGETING THE TSLP AND OX40/OX40L AXIS IN ASTHMA

There is an increasing recognition that asthma is a heterogeneous condition.⁴⁷ Complex gene-environment interactions activate several biologic pathways that consequently result in the disordered airway physiologic aspects and symptoms that characterize asthma. The view that asthma is primarily an allergic disease mediated by Th2 cytokines has been challenged because asthma can develop in the absence of atopy. Indeed, allergic sensitization is likely to be more important in early-onset disease and particularly in children, whereas this feature of disease is less prominent in late-onset asthma.⁴⁸ The application of noninvasive measures of airway inflammation has also led us to observe different inflammatory phenotypes, including eosinophilic- and neutrophilic-predominant asthma.⁴⁹ The OX40/OX40L axis is particularly important in allergic sensitization and Th2 polarization. Therefore, predictably, patients with Th2-mediated eosinophilic inflammation are likely to be the most appropriate target population. However, the timing of the intervention is important and may be most effective prior to the onset of allergic sensitization and disease presentation, which obviously is unpredictable, and hence this is not a practical strategy. Ongoing activation of the OX40/OX40L axis may be important in some patients with asthma, but to date, this is uncertain and biomarkers to identify this group are unknown. At present, one clinical trial has been completed using a huMAb OX40L in the prevention of allergen-induced airway in adults with mild asthma. This study was funded by Genentech and completed in January 2011. However, the report from this study is still awaited.⁵⁰

TSLP is likely to be effective in the same population as for OX40/OX40L, but also is critical in the innate immune response, suggesting its potential efficacy may target a broader group of patients with asthma. Critically, the potential efficacy of either approach will need to outweigh the potential side

effects. Importantly, to date, early safety studies suggest that the safety profile of anti-OX40 therapy is good.

CONCLUSION

Current asthma therapies improve symptoms, improve disease control, and reduce exacerbations.¹ None are disease modifying whereby they alter the natural history of the underlying disease. Data from animal models present a compelling argument that targeting the TSLP or OX40/OX40L axis will alter allergic sensitization and T-cell polarization. This presents a tantalizing possibility that this therapeutic approach in asthma may be disease modifying. Patients with asthma and researchers share the ambition to achieve a cure for asthma that can only be achieved by disease modification. Therefore, targeting TSLP or the OX40/OX40L axis presents an exciting opportunity that may provide a step-change in the treatment of asthma. Its fate will become apparent over the forthcoming couple of years as the eagerly awaited clinical trials are reported.

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