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Therapeutic approaches for control of transcription factors in allergic disease

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Activity Objectives

1. To review the role of inflammatory cytokines in the promotion of various T helper phenotypes.
2. To understand the mechanisms of action of transcription factors in the development of allergic disease.
3. To review the therapeutic approaches for control of transcription factors in allergic disease.
4. To understand the mechanisms of glucocorticoid therapy in allergic disease.

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The inflammatory response observed in allergic disease involves multiple cell types but is orchestrated in part by the T_H2 cytokines IL-4, IL-5, and IL-13. In recent years, the transcription factors that control the expression and function of these cytokines have been elucidated, including signal transducer and activator of transcription 6, GATA3, nuclear factor of activated T cells, and nuclear factor κB. These molecules are attractive targets for therapeutic intervention because they regulate the expression of numerous effector molecules and functions simultaneously. For instance, the immunosuppressive agents glucocorticoids and cyclosporin A both function by repressing the activity of transcription factors through a variety of mechanisms. In this review we examine the role of each transcription factor in allergic disease and discuss approaches that have been taken to therapeutically interfere with transcription factor function in allergic disease. (J Allergy Clin Immunol 2008;121:803-9.)

Key words: *Transcription factors, asthma, atopic dermatitis, signal transducer and activator of transcription 6, GATA3, T_H2, nuclear factor of activated T cells, nuclear factor κB, glucocorticoid receptor*

Allergic diseases, including asthma, are characterized by inflammation driven by $CD4^+$ T_H lymphocytes of the T_H2 phenotype.[1] In allergic individuals exposure to allergen results in activation of antigen-specific T_H2 cells and secretion of a variety of cytokines and inflammatory mediators that provoke the inflammatory response. Of the numerous molecules secreted by activated T_H2 cells, the cytokines IL-4, IL-5, and IL-13 are responsible for many of the features of allergic inflammation.[1-3] T_H2 cell expression of IL-4 has multiple effects, including differentiation of naive T cells toward the T_H2 lineage and induction of B-cell isotype switching to produce IgE. IL-5 production is critical for the development of the eosinophilia observed in allergic disease. IL-13 is responsible for numerous features of T_H2 inflammation, especially in the asthmatic lung, such as goblet cell hyperplasia, mucus hypersecretion, and bronchial hyperresponsiveness. IL-13 can also alter the phenotype of structural cells in the airway, promoting the expression of proinflammatory mediators by airway smooth muscle.[4]

Given the central role played by T_H2 cells in allergic disease, the mechanisms that control T_H2 differentiation and activation

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Abbreviations used
AP-1: Activator protein 1
CsA: Cyclosporin A
GR: Glucocorticoid receptor
IκB: Inhibitor of NF-κB
IL-4Rα: IL-4 receptor α
NFAT: Nuclear factor of activated T cells
NF-κB: Nuclear factor κB
p38: p38 mitogen-activated protein kinase
PPAR: Peroxisome proliferators-activated receptor
RXR: Retinoid X receptor
SEGRA: Selective glucocorticoid receptor agonist
siRNA: Short interfering RNA
STAT: Signal transducer and activator of transcription
TTP: Tristetraprolin

have been the subject of intense scrutiny.[5,6] This work has identified several transcription factors that perform critical functions in regulating T_H2 cell function, including signal transducer and activator of transcription (STAT) 6, GATA3, c-Maf, nuclear factor of activated T cells (NFAT), nuclear factor κB (NF-κB), glucocorticoid receptor (GR), and peroxisome proliferator-activated receptors (PPARs). Although the roles played by many of these transcription factors were elucidated in murine T_H2 cells, several studies have shown that human T_H2 cells are controlled by similar mechanisms.[7-10] These transcription factors perform numerous roles in T_H2-mediated inflammation in both antigen-specific T_H2 cells and innate immune and structural cells that respond to T_H2 cytokines. Furthermore, several of these transcription factors (nuclear factor κB [NF-κB], GR, and PPARs) can regulate other genes during the chronic phases of allergic inflammation, which can be characterized by the expression of proinflammatory molecules that are not T_H2 specific. Because transcription factors can control the expression of numerous target genes and also affect cell differentiation, they have become attractive targets for therapeutic intervention.

In this review we describe the role of these transcription factors in allergic disease and report on studies aimed at therapeutic modulation of transcription factor function.

STAT6

The T_H2 cytokines IL-4 and IL-13 have overlapping but nonredundant functions caused by the shared use of a common receptor subunit, IL-4 receptor α (IL-4Rα). Binding of IL-4 or IL-13 to their receptors induces activation of the receptor tyrosine kinases Jak 1, Jak 3, and Tyk 2, which phosphorylate tyrosine residues on the IL-4Rα subunit. This phosphorylation causes recruitment of cytoplasmic STAT6 to the receptor, where it is phosphorylated at tyrosine 641. Once phosphorylated STAT6 dimerizes and translocates to the nucleus to regulate IL-4– and IL-13–dependent gene expression (Fig 1). IL-4 plays a critical role in driving T_H2 cell differentiation, and thus STAT6 is a critical transcription factor in this process. STAT6 knockout mice do not have T_H2 cells and do not induce class switching to IgE, thus demonstrating the essential role of STAT6.[11-13] The use of mice deficient in STAT6 in models of allergic disease has shown that it is critical for the development of T_H2-mediated inflammation. In murine models of asthma, STAT6 knockout mice do not have bronchial hyperresponsiveness, eosinophilia, or mucus overproduction.[14,15] STAT6 deficiency also abrogates IL-4– and IL-13–dependent inflammation in murine models of contact hypersensitivity and atopic dermatitis, with decreased eosinophilia, edema, and IgE responses in both models.[16,17] The effects of STAT6 deficiency in these models of allergic disease depend on 2 aspects of STAT6 function. First, it is critically required for the development of T_H2 cells, and therefore STAT6 knockout mice mount a diminished T_H2 response to allergen challenge. Second, its role in IL-4 and IL-13 signaling in structural cells is also critically important. Elegant studies with mice with restricted expression of STAT6 have shown that T cell–specific expression is not sufficient for pathogenesis and that epithelial cell–specific expression is sufficient for at least some of the features of experimental asthma.[18-20]

The central role of STAT6 in IL-4 and IL-13 signaling in allergic disease suggests that it would be an excellent therapeutic target, and several approaches have been taken to block STAT6 function. Antisense phosphorthioate oligodeoxynucleotides targeting STAT6 have been used *in vitro* to suppress STAT6 signaling in airway smooth muscle and epithelial cell lines.[21,22] In each case STAT6 protein levels were efficiently downregulated, and functional blockade of signaling was observed. Double-stranded oligodeoxynucleotides containing a STAT6-binding site have been used in a model of atopic dermatitis as a decoy for STAT6 binding.[17] In this study *in vivo* transfection of the STAT6 decoy into the skin by using liposomes resulted in amelioration of the IgE-mediated late-phase reaction, with reduced edema and neutrophil and eosinophil infiltration. More recently, a study by Rippmann et al[23] used a short interfering RNA (siRNA) approach to knock down STAT6 levels. The authors obtained complete knockdown of STAT6 function in the human epithelial cell line BEAS-2B and blocked the production of CCL26 by these cells.

Two peptide-based approaches have also been used to block STAT6 function. Stolzenberger et al[24] used a short peptide derived from the IL-4Rα STAT6-binding site fused to a cell-permeable peptide derived from the antennapedia homeodomain to inhibit STAT6 phosphorylation. The peptide treatment blocked STAT6 activity in a variety of cell types; however, the effect was very transient, with STAT6 phosphorylation returning to normal levels within 30 minutes. A more recent study used a dominant-negative peptide derived from the STAT6 dimerization domain coupled to the protein transduction domain 4 from the HIV-TAT protein. Using this chimeric peptide, McCusker et al[25] demonstrated blockade of STAT6 in murine splenocytes, resulting in decreased T_H2 cytokine production *in vitro*. Furthermore, they went on to show that intranasal administration of the STAT6 inhibitory peptide inhibited ovalbumin-induced lung inflammation and mucus production in murine models of rhinitis and asthma. They also observed reduced eosinophilia and airway hyperresponsiveness, suggesting that local administration of this peptide to the airway might have potential as a therapeutic treatment for allergic diseases.

Given the role of STAT6 in all aspects of IL-4 and IL-13 signaling, local blockade of STAT6 function remains a very promising approach for the treatment of allergic disease. Systemic treatment would likely be inappropriate because diminished T_H2 immunity would be a possible side effect.

GATA3

The zinc-finger transcription factor GATA3 is the master regulator of T_H2 cell differentiation. GATA3 expression is