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Suppressing allergic immune responses

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TABLE OF CONTENTS

1. ABSTRACT

While most studies are devoted to understanding the activation of cytokine gene transcription, few studies have focused on the silencing of cytokine gene transcription. This review will focus on the molecular mechanisms by which Th1-promoting factors downregulate the *Il4* gene.

2. INTRODUCTION

The incidence and mortality of allergic diseases—such as food allergies, anaphylaxis, allergic rhinitis, atopic dermatitis and asthma—has increased at an alarming rate in Western countries over the past several decades. In the United States alone, over 50 million people suffer from allergic diseases (1, 2). The economic cost of allergic diseases was estimated at a staggering $13 billion in 2006 (3). The Hygiene Hypothesis, based on epidemic studies, was developed to explain such a remarkable increase in the incidence and mortality of allergic diseases (4, 5). Accumulated experimental evidence supports the notion that factors that promote Th1 responses can also have profound effects on suppressing Th2 responses. This review will focus on the molecular mechanisms by which Th1-promoting factors downregulate the *Il4* gene.

3. ALLERGIC INFLAMMATION

Allergic disorders are immune-mediated diseases (6-10). Allergens are environmental substances that include both indoor and outdoor allergens. The most common indoor allergens include house dust mites and cat/dog danders; the most common outdoor allergens include pollens and fungal spores (11). Allergens affect susceptible individuals in ways that do not resemble bacteria and viruses. Generally, allergens do not activate toll-like receptor (TLR), whereas bacteria and viruses do activate TLR. Rather, they trigger certain types of immune responses, known as type-2 immune responses. Effector cells that carry out the type-2 immune responses include T-helper 2 (Th2) cells, eosinophils, mast cells and basophils (6-9). Th2 cells produce interleukin 4 (IL-4), IL-5, IL-9 and IL-13 (6-9). IL-4 and IL-13 are essential for IgE production (12, 13), which can activate eosinophils, basophils and mast cells by crosslinking the FcepislonR1 receptor displayed on their surface (14). Th2-type cytokines are known to play a critical role in recruiting effector cells to the site of allergic inflammation. IL-4 and IL-13 can selectively upregulate the expression of vascular cell adhesion molecule-1 (15, 16). These cytokines also powerfully induce the expression of chemokines such as MDC/CCL22, TCA3/CCL1, and eotaxin/CCL11 (17). IL-4 and IL-13 also upregulate the expression of CCR3, CCR4, and CCR8 (8-10). These upregulated molecules are pivotal in recruiting Th2 cells, eosinophils and basophils to the site of inflammation. Both IL-4 and IL-13 play important roles in goblet cell differentiation and mucus production (18, 19), while IL-5 is essential in development of lung eosinophilia. For instance, mice deficient in IL-5 fail to exhibit eosinophilia (20, 21).

IL-4 acts as the major factor in initiating airway inflammation because of its ability to drive naïve $CD4^+$ T cells to differentiate into Th2 cells (22-25). IL-4 is essential in IgE production, which can activate basophils, eosinophils, and mast cells. Although we have learned much about the transcription of the *Il4* gene, limited studies have focused specifically on the downregulation of Th2 cytokine gene expression.

4. THE IFN-GAMMA-STAT1-IRF OR THE IFN-GAMMA-STAT-T-BET PATHWAY IN SUPPRESSING *IL4* GENE TRANSCRIPTION

It has been reported that regulatory factors, such as T-bet, which are important in *Ifng* gene transcription (26, 27) also possess the ability to suppress *Il4* gene transcription (26, 27). Ectopic expression of T-bet in Th2 cells induced *Ifng* gene transcription and suppressed *Il4* gene transcription. However, different reports have documented various degrees of suppression of *Il4* gene transcription by T-bet in Th2 cells. The reported level of inhibition ranged from 70% inhibition (26) to undetectable inhibition (28). It has also been shown that phosphorylated T-bet was able to bind GATA-3 and thus interaction resulted in sequestration of GATA3 by T-bet (29, 30). The notion that T-bet plays an important role in suppressing the *Il4* gene is further supported by an *in vivo* study using targeted T-bet deletion. T-bet deficient mice developed spontaneous allergic airway inflammation and asthma (31). Polymorphisms in T-bet have been found to correlate with susceptibility to asthma development in humans (32).

An *Il4* gene silencer has been described by Rao and colleagues (33). Deletion of the silencer region in the *Il4* gene promoted IL-4 expression. The authors further demonstrated that T-bet binds to the "silencer" region of the *Il4* gene and recruits Runx3 and Cbfbeta (33, 34). Our own work shows that continuous T-bet expression is required to silence the IL-4-producing potential in Th1 cells (35). We further analyzed signaling pathways that lead to the silencing of the IL-4-producing potential in Th1 cells and demonstrated that the IL-12-STAT4 pathway and the IFN-gamma-STAT1 pathway converge at the point of T-bet. Compared with the IL-12-STAT4 pathway, the IFN-gamma-STAT1-T-bet signaling pathway is the major pathway that leads to silencing of the IL-4-producing potential of Th1 cells (36).

Using an approach that combined traditional genetic and transcriptional profiling analyses, Bix and colleagues have identified another *Il4* gene repressor, Mina-1 (a member of *jumonji* C family) (37). Mina expression levels inversely correlated with the susceptibility to develop Th2-mediated diseases. Mina can repress IL-4 via its binding to NFAT. Overexpression of Mina in transgenic mice suppressed *Il4* expression, whereas knocked down Mina expression resulted in elevated *Il4* expression (37). These findings explain the Th2 bias (the propensity to heighten Th2 immune responses) in certain mouse strains and Th2 disease-susceptible individuals.

In addition to T-bet, it has been reported that IFN-gamma-R$^{-/-}$, STAT4$^{-/-}$, IRF-1$^{-/-}$, and IRF-2$^{-/-}$ mice demonstrated a propensity to mount a Th2-type immune response even to pathogens that were expected to elicit a Th1 response in wild type mice (38-42). These results suggest that Th1-promoting factors are critical in suppressing Th2 responses. It was discovered that IL-4-induced STAT6 phosphorylation was reduced in committed Th1 cells (43, 44). Our group demonstrated that IFN-gamma was the key factor in silencing the *Il4* gene (45). We believe that IFN-gamma can suppress *Il4* gene expression by inducing T-bet (36) and by inhibiting STAT6 recruitment to the IL-4 Receptor (46).

5. THE ROLE OF T REGULATORY CELLS AND IL-10 IN SUPPRESSING TH2 DIFFERENTIATION

IL-10 is a potent regulatory cytokine (8). IL-10 knockout mice had a higher baseline airway reactivity and more eosinophilic airway inflammation after allergen challenges (47), indicating an important role for IL-10 in controlling airway inflammation and responsiveness. IL-10 treatment suppresses airway hyperreactivity, goblet cell hyperplasia, and airway eosinophilia, whereas antibody against IL-10 or IL-10 receptor (IL-10R) enhances asthmatic systems (48). IL-10 acts on many types of cells, including CD4$^+$ T cells (49). Treg inhibition appears to be non-specific to Th2 cells. IL-10 inhibits Th1 and Th2 cell differentiation (50, 51). O'Garra and colleagues have reviewed this topic extensively (52); therefore, I will not cover this topic further. Rather, I want to draw your attention to two recent studies, which use an approach that deletes a gene of interest only in Foxp3 positive Treg. These studies reveal that CTLA-4 and IRF-4 play essential roles in Treg-mediated suppression of autoimmune diseases (53, 54). Notably, these two recent papers have firmly established that Treg cells are critical in keeping IgE serum levels in check under normal physiological conditions (53, 54).

6. IL-27-MEDIATED SUPPRESSION OF TH2 DIFFERENTIATION

IL-27 is a recently discovered member of the IL-12 family (55). It is a heterodimeric cytokine composed of Epstein-Barr virus-induced gene 3 (EBI3) and p28. IL-27 binds to the IL-27 receptor (IL-27R) and gp130 to exert its biological functions (55-57). It has been reported that IL-27 is a potent inducer of Th1 cell differentiation (58-62) as well as a promoter of T regulatory type 1 (Tr1) cell differentiation (63, 64). IL-27 has been shown to suppress Th17 cell differentiation (65, 66) and to suppress Th2 cell differentiation or Th2 immune responses in parasitic infection, allergic asthma, and autoimmune diseases (67-73). It has also been shown that IL-27 acts directly on CD4$^+$ T cells and suppresses the *Il4* gene independent of IFN-gamma (68, 74). However, it is less clear if IL-27 suppresses the *Il4* gene independent of IL-10 or if human IL-27 carries out the same biological effects in a similar manner as its murine counterpart. Our own work suggests that both human and mouse IL-27 suppresses *Il4* gene expression in the absence of IL-10 (our unpublished data). It appears that mouse IL-27 suppresses the *Il4* gene in a STAT-1-dependent and T-bet-independent manner (our unpublished data). Finding the IL-27-induced *Il4* gene repressor would be of interest for future research.

7. THE ROLE OF CHROMATIN STRUCTURE IN SUPPRESSING TH2 CYTOKINE GENE EXPRESSION

It has become well documented that chromatin

structure plays a pivotal role in Th2 cytokine gene expression (75). As naïve $CD4^+$ T cells differentiate into Th1 or Th2 cells, the histone molecules that surround the regulatory regions of Th1 or Th2 cytokine gene loci undergo covalent modifications. These modifications lead to conformational changes in chromatin structure, which allows transcription factors to gain access to cytokine gene loci. Chromatin modifications include the demethylation of CpG islands in the regulatory regions; the acetylation of lysine residues of histone 3 (H3); and the demethylation of lysine 4 of H3 that surrounds the regulatory regions of the cytokine genes. For example, the *Ifng* locus becomes "open" to transcription factors under Th1-inducing conditions (76, 77). Similarly, the *Il4* locus becomes "open" under Th2-inducing conditions (76, 77).

Inactive cytokine genes are often associated with "repressive" chromatin structure, which is a highly condensed structure. Several potential mechanisms have been proposed to explain how a "repressive" chromatin structure can silence the *Il4* locus in Th1 cells. Wilson and colleagues demonstrated that DNA methyltransferase 1 (Dnmt1) plays a critical role in suppressing Th2 cytokine gene transcription (78, 79). Modification of H3 at lysine 9 and 27 has been shown to inversely correlate with gene transcription. Bix and colleagues showed that H3 trimethylation at lysine 27 was detected in Th1 cells that had gone through two rounds of differentiation under Th1-inducing conditions (80). However, this delayed modification might not reflect the initial T-bet-mediated formation of the repressive chromatin-remodeling complex. The group also reported that they did not observe modification of lysine 9 of H3 at the *Il4* locus (80). Thus, the exact histone modifications that lead to the silencing of the *Il4* gene remain to be identified. Since the first histone lysine demethylase KDM1 (LSD1) was discovered in 2004, a large number of histone lysine demethylases have been recognized and shown to play important roles in gene expression as well as cellular differentiation and animal development (81). Among these demethylases, LSD1 may be more likely to suppress the *Il4* gene because this enzyme specifically demethylates the dimethyl modification of H3 at lysine 4 (H3K4me2 is a modification that has been linked to *Il4* gene transcription) (82). It is not clear whether T-bet is involved in recruiting Dnmt1 or LSD1 into the *Il4* regulatory regions of the *Il4* gene.

8. CAN THE KNOWLEDGE GAINED BE USED TO IMPROVE IMMUNOTHERAPY?

The effectiveness of immunotherapy, using allergen extract (a high dose of antigen) to induce a patient's tolerance to allergens (to which they already have developed hypersensitivity) has been well documented; patients who received subcutaneous injections of allergen extracts show remarkable reduction in allergy symptoms (83-85). Immunotherapy has been shown to induce the development of T regulatory cells (Treg). Treg cells suppress allergic immune responses via the production of IL-10 and TGF-beta1 (86, 87). However, current immunotherapy, developed 100 years ago, faces two obstacles: systemic allergic reaction and repeated doctor visits over a long period of time (83).

Based on epidemic studies (the Hygiene Hypothesis) (4) and discovery of Toll-like receptor (88, 89), promoting Th1 responses has been proposed as an effective way to suppress Th2 response. Coffman and colleagues demonstrated that mice immunized with a TLR-9 agonist (CpG-containing immunostimulatory sequences, ISSs) prior to exposure to allergens completely prevented any development of allergic airway inflammation and airway hyperreactivity (90). ISSs conjugated to a major allergic component of ragweed have been shown to be effective in reducing allergic symptoms in several clinical studies (90-92). It has been reported that ISSs can inhibit Th2 response by rendering dendritic lung cells unable to effectively present antigens to Th2 cells and ISSs can inhibit the IgE-dependent release of Th2 cytokines, especially IL-4, from basophils and/or mast cells (93).

Because Th2 inhibitory factors produced by dendritic cells in response to infection can suppress the Th2 response early during immune responses, there is a need to identify and characterize the DC-produced Th2 inhibitory factors. Early-acting Th2 inhibitory factors could be used to develop an improved immunotherapy.

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