

Images in Allergy and Immunology

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Transcription factors in allergic diseases

Editor's note: This feature, Images in allergy and immunology, is designed to highlight current concepts of the immunopathology of allergic diseases and other common immunologically mediated diseases. The presentation will appear as sets of images that involve cross-pathology, histopathology, and molecular pathology and will cover a range of topics of interest to allergists and immunologists.

Coordination of genetic expression during allergic inflammation is mediated by a specific set of transcription factors. Although a complete description of all the actions of the transcription factors is lacking, it is clear that these control many aspects of allergic inflammation. Specific transcription factors, known as master regulators of T helper lineage determination, are associated with specific subsets of cytokine-producing T_H cells; these include genes encoding T-box expressed in T cells (T-bet), GATA-binding protein 3 (GATA-3), and Forkhead box P3 (FOXP3), which are associated with T_H1 cells, T_H2 cells, and regulatory T (Treg) cells, respectively. In allergic T_H2-type inflammation, other transcription factors come into play, such as signal transducer and activator of transcription 6 (STAT6), which collaborates with GATA-3. Transcription factors regulate expression of many proinflammatory and anti-inflammatory cytokines, effector proteins, and other transcription factors. As central coordinators of inflammation, they are excellent targets for therapeutic modulation of allergic inflammation.

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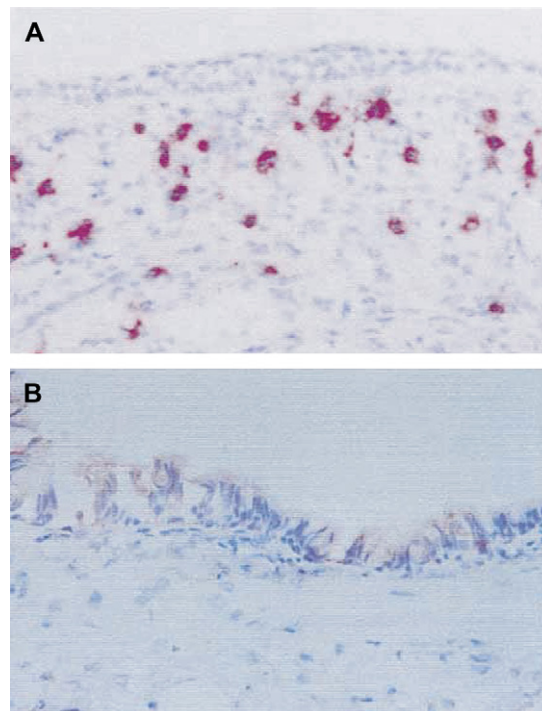


FIG 1. Immunocytochemistry for STAT6 was performed on bronchial biopsy specimens taken from a patient with atopic asthma (**A**) and from a healthy control subject (**B**). STAT6 expression was revealed in red by means of alkaline phosphatase-based immunocytochemistry.

STAT6 is an effector transcription factor of allergic inflammation because it directly modulates the expression of proinflammatory mediators. Its expression is increased in allergic rhinitis and atopic asthma (Fig 1). *STAT6* might also be a risk factor for atopy because certain polymorphisms have been shown to significantly contribute to serum IgE levels.^{1,2} IL-4 and IL-13 receptor signal transduction leads to phosphorylation and activation of STAT6. Activated STAT6 binds to DNA at STAT6-response elements, leading to activation of allergic mediators, such as chemokines, mucus, and IgE. Novel genes associated with allergic inflammation have also been shown to be upregulated by STAT6, including *arginase-1*.³ Although activating many genes, STAT6 can also inhibit expression of many genes; STAT6 can potentially inhibit *IFN-γ*, *IL-12*, and *TNF-α*. Recent evidence points to STAT6 playing a regulatory role in innate immune responses by inhibiting the expression of innate immunity Toll-like receptor 4.⁴

GATA-3 is known as the “master regulator” of T_H2 lineage determination, regulating the expression of major allergy-associated cytokines. Recent single nucleotide polymorphisms in the *GATA-3* locus showed an initial association to asthma-related phenotypes.² GATA-3 is highly expressed in T_H2 cells but not in T_H1-committed cells. In initial steps of T_H2 lineage determination, IL-4, through STAT6, can upregulate

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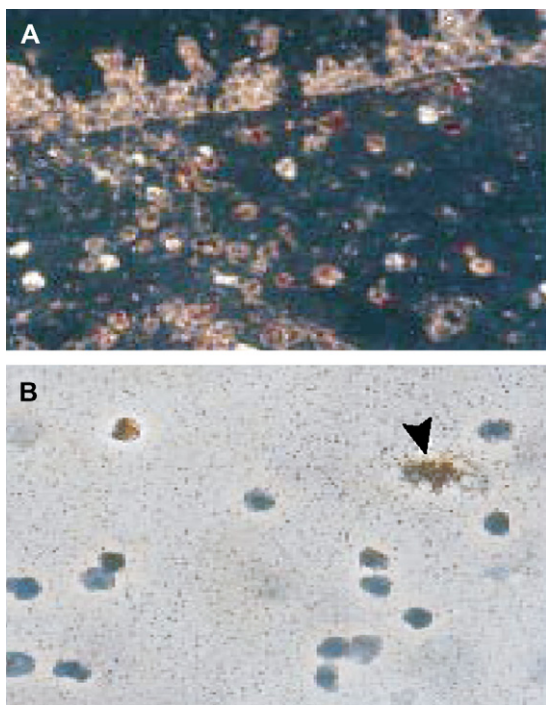


FIG 2. A, *In situ* hybridization was performed for GATA-3 mRNA by using ^{35}S -labeled riboprobes in bronchial biopsy specimens from an atopic asthmatic subject. Dark-field illumination was used to visualize the brightly staining positive cells. **B,** Bronchoalveolar lavage fluid from atopic asthmatic subjects was probed with GATA-3 ^{35}S -labeled riboprobes (silver granule deposition from autoradiography) combined with IL-5 biotin-labeled riboprobes (brown staining from diaminobenzidine) to colocalize the 2 markers. The arrowhead points to an example of a GATA-3/IL-5 mRNA-positive cell.

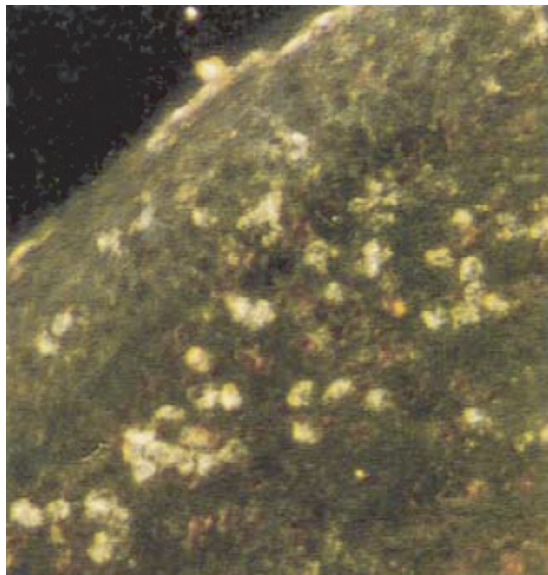


FIG 3. *In situ* hybridization for GATA-3 mRNA with ^{35}S -labeled riboprobes in the nasal tissue explant. The biopsy specimens were obtained from an allergic subject and were cultured for 24 hours with ragweed allergen before processing.

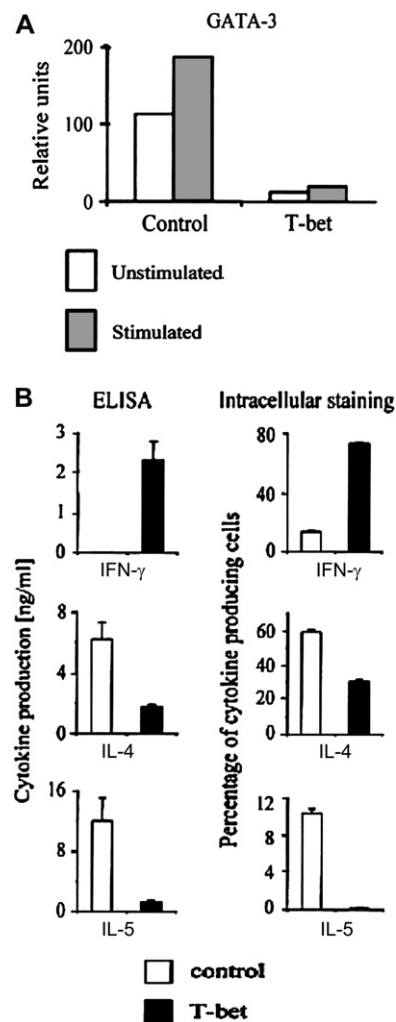


FIG 4. Sustained T-bet expression reduced GATA-3 mRNA levels, as measured by means of quantitative real-time PCR (**A**), and $\text{T}_\text{H}2$ cytokine levels, as measured by means of ELISA and intracellular FACS staining (**B**). T-bet-transfected activated $\text{T}_\text{H}2$ cells isolated from the skin of a patient with atopic dermatitis were either stimulated or unstimulated with anti-CD3 and anti-CD28 mAbs before quantitative PCR analysis. Cells were stimulated overnight before ELISA and 5 hours before FACS analysis. Notice the increased IFN- γ production in T-bet-transfected $\text{T}_\text{H}2$ cells.

expression of GATA-3. GATA-3 promotes the expression of $\text{T}_\text{H}2$ cytokines but at the same time downregulates $\text{T}_\text{H}1$ development by repression of IL-12 receptor β_2 and STAT4⁵ expression. At the same time, GATA-3 augments its own expression through positive feedback autoregulation promoting lineage commitment. Although it is not essential for IL-4 expression, GATA-3 promotes its expression by opening the *IL-4* locus through remodeling of the chromatin structure. GATA-3 also increases the expression of IL-5 and IL-13 by directly binding to their respective promoters. GATA-3 mRNA level, present in lower airway tissue of asthmatic patients (Fig 2, A), correlates with IL-5 mRNA expression (Fig 2, B). GATA-3

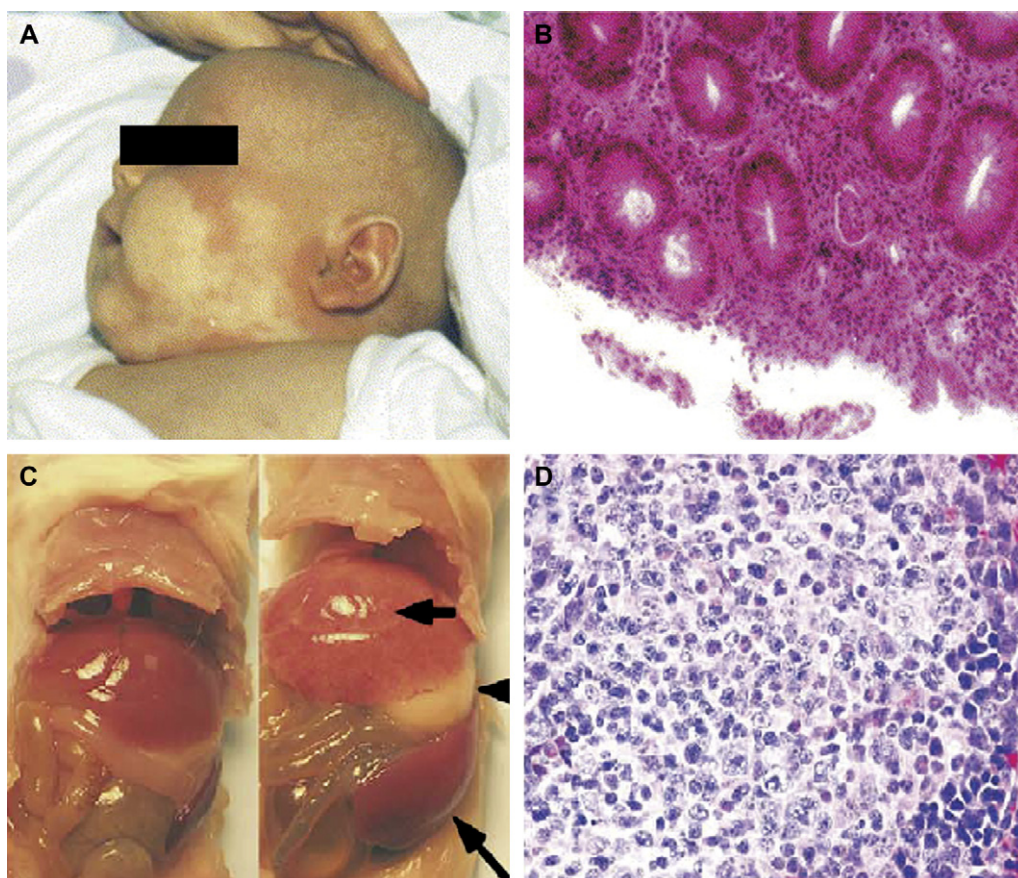


FIG 5. In human subjects, FOXP3 deficiency causes immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome. Disease manifestations include eczema-like skin lesions (**A**) and inflammatory cell infiltration of the colon causing enteritis-like symptoms in a child (**B**) hematoxylin and eosin staining). A BALB/c Foxp3-deficient mouse displays gross abdominal pathology compared with that of its normal littermate (**C**): massive splenomegaly (long arrow), inflamed liver (short arrow), and engorged stomach (arrowhead) caused by inflamed stomach walls (ie, gastroparesis) are seen. Indeed, the spleen (**D**) displays intense periarteriolar infiltration by inflammatory cells (hematoxylin and eosin staining, original magnification $\times 400$).

expression is also increased after allergen challenge in nasal biopsy specimens from allergic patients (Fig 3).

Despite the uncertainty of its mechanism of action, T-bet remains the transcription factor most closely related to the development of the T_H1 phenotype. T-bet, downstream of the IFN- γ receptor signaling, is associated with IFN- γ production by T cells and natural killer cells because its expression is induced through STAT1-dependent signaling downstream of the IFN- γ receptor complex. Aside from some controversy about the mechanisms by which T-bet drives T_H1 gene expression, it has been shown to repress the T_H2 phenotype through an antagonistic effect on GATA-3.⁵ T-bet limits GATA-3 expression at the transcriptional level (Fig 4, A) and reduces T_H2 cytokine production (Fig 4, B).⁶ Although some studies have shown that T-bet induces IFN- γ production and IL-12R β 2 expression, recent data argue that the effects of T-bet are not through direct modulation of transcription. It would seem that T-bet might act more upstream in T_H1 lineage determination by blocking GATA-3,

whereas other transcription factors, such as STAT4, would control T_H1 gene expression.⁵

Interest in FOXP3 has emerged from the growing evidence of the role of Treg cells in inhibiting effector T lymphocytes in allergic disease. FOXP3 and *Foxp3* mutations in both murine models and human subjects, respectively, lead to severe autoimmune and allergic dysregulation featuring intense lymphoproliferation, myeloproliferation, and multiorgan infiltration (Fig 5).^{7,8} As a member of the Fox family of transcription factors, FOXP3 is mostly expressed by thymus-derived CD4⁺CD25⁺ Treg cells, although recent data have raised the possibility that FOXP3-expressing T cells with suppressive activity could be induced in naive T cells in the periphery.⁷ The FOXP3 protein is the key switch of the regulatory phenotype because it suppresses the activity of nuclear factor-associated T cells and nuclear factor κ B, thus interfering with the expression of genes encoding for IL-2, IL-4, and IFN- γ .⁹ Foxp3-deficient scurfy mice display increased peripheral cytokine concentrations, as well as increased GATA-3 and T-bet transcript levels, compared with

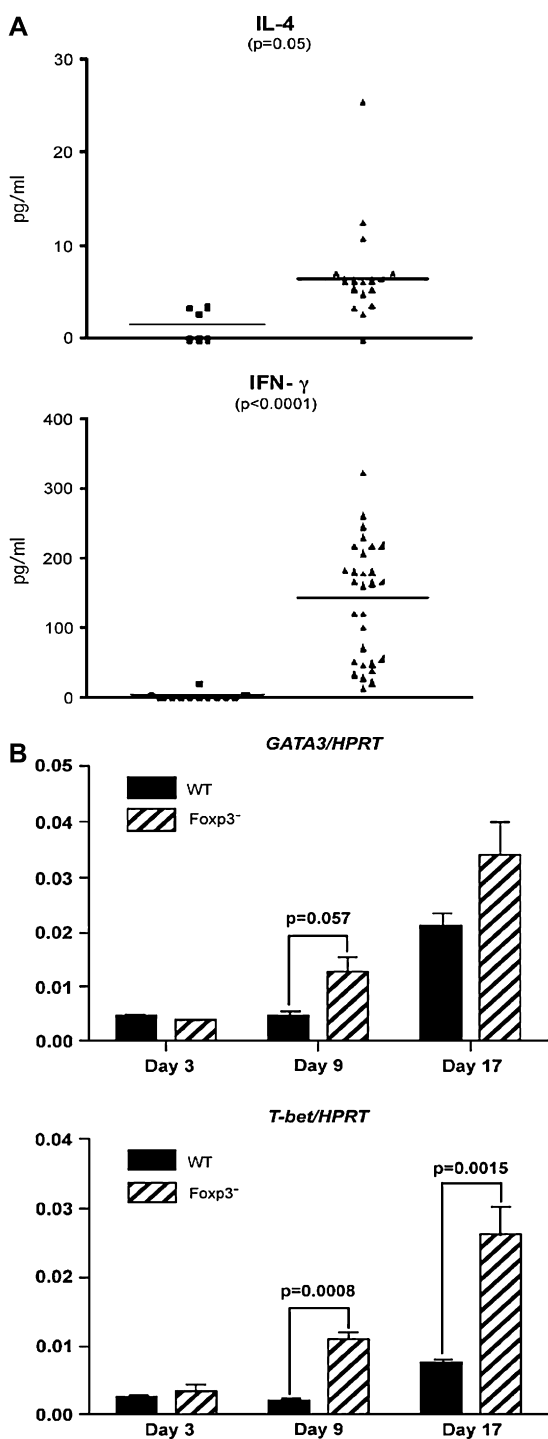


FIG 6. A, Serum concentration of T_H1 and T_H2 cytokines in Foxp3-mutant mice relative to littermate control animals, as determined by using the Cytoflex Bead Array.⁸ B, Levels of GATA-3 and T-bet transcripts in unfractionated splenocytes from wild-type and Foxp3-deficient littermate mice at ages 3, 9, and 17 days. Transcript levels were determined by means of real-time quantitative PCR, and results were normalized to hypoxanthine phosphoribosyl-transferase (HPRT) transcripts.

their wild-type littermates (Fig 6).⁸ Interestingly, T_H2 inflammation seen in Foxp3-deficient mice appeared to be STAT6 dependent.⁹ Once committed, Treg cells would suppress effector T cells by mean of immunoregulatory cytokines (IL-10 and TGF- β), as well as through partially defined cell interactions with antigen-presenting cells, target cells, or both. Whether FOXP3 expression is sufficient for T-cell development of a suppressive phenotype still remain controversial.¹⁰

Further research is certainly warranted for transcription factor-based therapeutics using antisense oligonucleotides, and small interfering (si)RNA or decoy DNA technologies could antagonize T_H2 lymphocyte activation and secretion of proinflammatory mediators. Moreover, promotion of Treg cell suppressive activity could contribute to limiting T_H2 inflammation.

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