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IL-4 Is a Critical Determinant in the Generation of Allergic Inflammation Initiated by a Constitutively Active Stat6

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IL-4 is required for the pathogenesis of atopic diseases and immune regulation. Stat6 is critical for IL-4-induced gene expression and Th cell differentiation. Recently, we have generated mice expressing a mutant Stat6 (Stat6VT) under control of the CD2 locus control region that is transcriptionally active independent of IL-4 stimulation. To determine whether active Stat6 in T cells is sufficient to alter immune regulation in vivo, we mated Stat6VT transgenic mice to IL-4-deficient mice. Stat6VT expression in IL-4-deficient lymphocytes was sufficient to alter lymphocyte homeostasis and promote Th2 differentiation in vitro. HyperTh2 levels in Stat6 transgenic mice correlated with an atopic phenotype that manifested as blepharitis and pulmonary inflammation with a high level of eosinophilic infiltration. In the absence of endogenous IL-4, Stat6VT transgenic mice were protected from allergic inflammation. Thus, in mice with hyperTh2 immune responses in vivo, IL-4 is a critical effector cytokine. The Journal of Immunology, 2008, 180: 3551–3559.
Materials and Methods

Generation of Stat6VT transgenic mice

The generation of Stat6VT transgenic mice was previously described (28). Transgene positive founders (CD2:Stat6VT (78) line), where the human Stat6 gene with V625 and T626 mutated to alanine is under transcriptional control of the CD2 locus control region, were backcrossed to C57BL/6 mice (Harlan Bioproducts for Science). IL-4-deficient mice were purchased from The Jackson Laboratory and mated to Stat6VT transgenic mice to generate IL-4-deficient transgene positive mice. Mice were used between 6 and 12 wk of age. All mice were maintained in specific pathogen-free conditions and experiments were approved by the Indiana University Institutional Animal Care and Use Committee.

Flow cytometry

Splenocytes, thymocytes, and bone marrow cells (1 × 10^3–3 × 10^5 per sample) were washed and stained in PBS with 2% BSA and 0.1% NaCl (FACS buffer). Cells were first incubated with anti-FcγR Abs, clone 2.4G2 (BD Pharmingen), for 10 min. All staining was done at 4°C, followed by one wash with FACS buffer, then fixed in PBS with 2% BSA, 0.1% NaN₃, and 0.5% formaldehyde. Samples were stained with Abs directly conjugated to FITC, PE, CyChrome, or allophycocyanin (BD Pharmingen), or Abs to IgM, IgE, or CD23 (eBioscience) for 15 min. Abs to CD4, CD8, CD3, B220, CD44, CD62L, IgM, IgD, CD43, MHC class II, CD23, CD86, CD11c, CD3, B220, and CCR3 were all obtained from BD Pharmingen. Analysis of lymphocyte populations was performed using a forward scatter (size) gate, and the expression of CD44 and CD62L were determined on CD4+ cells. The cellular composition of bronchoalveolar lavage (BAL) for the populations of eosinophils, neutrophils, T cells, B cells, dendritic cells, and macrophages was performed as described by a flow cytometric method (29). Briefly, eosinophils, neutrophils, T cells, B cells, and mononuclear cells were all distinguished by cell size and the expression of CD3, B220, CCR3, CD11c, and MHC class II.

Total serum IgE measurement

Sera obtained from the indicated mice were analyzed for the levels of IgE by ELISA using IgE Abs (BD Pharmingen) according to manufacturer’s recommendation.

In vitro T cell stimulation

Splenic and lymph node CD4+ T cells were purified with rat anti-mouse CD4 microbeads (Milteny Biotec) according to Manufacturer's protocol. The purified cells were then stimulated in media at 1 × 10^5 cells/ml, with 4 μg/ml anti-CD3 (clone 2C11) and 1 μg/ml anti-CD28 (clone 37.51; BD Pharmingen). After 3 days, supernatants were harvested and cultured with 4 μg/ml anti-CD28 (clone 37.51; BD Pharmingen). After 6 days, cells were washed and incubated with goat anti-rat IgG magnetic beads (Poly-science) for 30 min at 4°C. Purified cells were plated at 4 × 10^5 cells/ml and stimulated with 4 μg/ml anti-CD3 (2C11) and 1 μg/ml anti-CD28 (clone 37.51; BD Pharmingen). After 6 days, cells were harvested and plated at 200,000 cells/0.5 ml, and re-stimulated with 4 μg/ml anti-CD3. After 48 h, supernatants were harvested and cytokine production was analyzed by ELISA. For the real time PCR, differentiated CD4+ T cells were restimulated for 6 h, and cell extracts were prepared in TRIzol. RNA was isolated and cDNA was synthesized by using a First-Strand Cloned AMV kit (Invitrogen Life Technologies). Message levels of the genes Gata3, Thb21, Ccr4, Ccr8, and Ccl22 were determined by Taqman real time PCR. Reagents were obtained from Applied Biosystems. Cycle number of duplicate samples were normalized to the expression of an endogenous control, β2-microglobulin.

Immunoprecipitation and affinity purification of DNA-binding proteins

For DNA-binding assays, a 5'-biotinylated oligonucleotide, whose sequence (TGTTAATTCGTGTGAATTATG) was isolated by binding site selection as described previously (23), was coupled to streptavidin-conjugated agarose beads. Per sample, 3 μg of biotinylated oligonucleotide was incubated with 40 μl of 50 μg/ml streptavidin-conjugated bead slurry in a total volume of 100 μl of a lysis buffer comprised of 50 mM Tris- HCl (pH 8.0), 0.5% IGEPAI, 15 mM NaCl, 0.1 mM EDTA, 10% glycerol, 10 mM NaF, 1mM Na2VO3, 1 mM PMSF, 1 mM DTT, 1 μg/ml aprotinin, 1 μg/ml leupeptin, and 1 μg/ml chymostatin for 2 h at 4°C. A total of 400 μg of whole cell extract was incubated with 40 μl of DNA-coupled agarose beads in lysis buffer at a total volume of 500 μl for 3 h at 4°C. Complexes were washed once in lysis buffer before elution by boiling in reducing loading buffer and used for Western analysis.

Evaluation of inflammation

Measurement of cytokines in the BAL fluid were determined using ELISA. Lung or peri-ocular tissue was dissected and fixed in 10% buffered formalin, embedded in paraffin, sectioned, and stained with H&E.

Results

Expression and activation of Stat6VT in IL-4-deficient mice

Earlier studies from our laboratory have demonstrated the ability of constitutively active Stat6 to skew Th cell differentiation to a Th2 phenotype in the absence of IL-4 stimulation. To determine whether the effects of increased Th2 differentiation in Stat6VT transgenic mice were due solely to the expression of Stat6VT or due to an indirect effect of a hyperTh2 cytokine environment in vivo, we mated mice expressing a constitutively active Stat6 directed by a CD2 locus control region (CD2:Stat6VT) to IL-4-deficient mice to generate transgenic IL-4-deficient mice (Il4−/− CD2:Stat6VT). To verify the presence of activated Stat6VT in Il4−/− CD2:Stat6VT mice, dimerized Stat6 molecules were precipitated from thymic cell lysates of wild-type, CD2:Stat6VT, Il4−/−, and Il4−/− CD2:Stat6VT mice with a high affinity Stat6 binding site and immunoblotted with an anti-Stat6 Ab (Fig. 1A). The presence of Stat6 in extracts from unstimulated CD2:Stat6VT mice and Il4−/− CD2:Stat6VT cells, but not unstimulated wild-type or Il4−/− cells, demonstrates that Stat6VT is constitutively active even in the absence of endogenous IL-4 production.

Our earlier studies indicated a normal development of T lymphocytes in the thymus of CD2:Stat6VT transgenic mice. To determine whether thymocyte development is altered in Il4−/− CD2:Stat6VT mice, thymocytes from wild-type, CD2:Stat6VT, Il4−/−, and Il4−/− CD2:Stat6VT were analyzed by flow cytometry following staining for CD4 and CD8 T cell subsets. No differences were observed in the single or double positive thymic populations in CD2:Stat6VT mice as compared with wild-type mice, consistent with our earlier observations (Fig. 1B). However, we observed an increase in both CD4 and CD8 single positive thymic populations concomitant with a decrease in CD4/CD8 double positive population in Il4−/− CD2:Stat6VT mice as compared with Il4−/− mice (Fig. 1B). The increased development of single positive cells was observed in thymi of mice aged between 4 and 24 wk (data not shown). Despite these alterations, Stat6VT did not alter total thymic cell numbers (Fig. 1B). Therefore, in the absence of endogenous IL-4, Stat6VT increases the proportion of single positive cells in the thymus.

Expression of Stat6VT in IL-4-deficient mice is sufficient to alter lymphocyte homeostasis

Expression of Stat6VT alters lymphocyte homeostasis in the spleen (28). To determine whether we see similar effects in Il4−/− CD2:Stat6VT mice, we analyzed the lymphocyte populations within the spleens of wild-type, CD2:Stat6VT, Il4−/−, and Il4−/− CD2:Stat6VT mice. Our data reveal a 2-fold increase in the percentage of B220+ cells and a 5-fold decrease in the percentage of CD3+ cells in CD2:Stat6VT mice, as compared with wild-type mice with decreases in both CD4+ and CD8+ subsets (Fig. 2A). In CD2:Stat6VT mice on an IL-4-deficient background, we see a similar increase in the percentage of B220+ cells and a decrease in CD3+ cells although the effects were less pronounced.
for 45 min. Activated Stat6 molecules were precipitated from 250 μg/H9262 mice are greater than wild-type cell numbers (Fig. 2).

The T cell nation of splenic cell numbers in these mice reveal that the total B0.001); 14.9 in Il4 transgenic mice (1.8 in wild type vs 8.2 in CD2:Stat6VT (A)). The ratios of CD4:CD8 T cells are significantly increased in Stat6VT as compared with wild-type mice (Fig. 2B). However we did not observe any changes in the B cell subsets in transgenic mice on an IL-4-deficient background.

In the spleen, although no differences were observed in the populations of marginal zone, T1, or follicular B cells, an increase was evident in the population of T2 B cells in CD2:Stat6VT mice compared with wild-type mice (wild type, 2 ± 0.3%; CD2:Stat6VT, 9 ± 0.1%). In contrast, the splenic B cell compartments remained unaltered in transgenic mice on an IL-4-deficient background (Fig. 3B). These results suggest that endogenous IL-4 is required for Stat6VT to alter B cell development in the bone marrow and spleen, but that increased numbers of multiple B cell subsets in the spleen can accumulate in the absence of IL-4.

Endogenous IL-4 is required for the effects of the Stat6VT transgene on B cells

Although we have shown that activated Stat6 expression is present in T cells, expression of the Stat6 transgene in B cells is very low (data not shown). This would suggest that increased gene expression and IgE production observed in Stat6VT transgenic mice may be an indirect effect of increased IL-4 production in the hyperTh2 environment. To test this directly, we examined the expression of MHC class II, CD23, and CD86 on B lymphocytes from wild-type, CD2:Stat6VT, and Il4−/− CD2:Stat6VT mice. In contrast to B cells from CD2:Stat6VT transgenic mice, B cells from Il4−/− CD2:Stat6VT mice did not have increased levels of MHC class II, CD23, or CD86 (Fig. 4A). Analysis of serum levels of IgE demonstrated a similar pattern, indicating that endogenous IL-4 was required for the increased class switching to IgE in Stat6VT transgenic mice (Fig. 4B). These results suggest that the B cell phenotype in Stat6VT transgenic mice is an indirect effect of the hyperTh2 environment.

Expression of Stat6VT in IL-4-deficient mice promotes Th2 differentiation

Because the B cell phenotype in Stat6VT transgenic mice is an indirect effect of IL-4, we next wanted to examine Th2 development in the absence of endogenous IL-4. Purified CD4+ T cells were stimulated for 5 days with anti-CD3 and anti-CD28 under unskewed conditions, then harvested and re-stimulated with anti-CD3 for 24 h. Supernatants were then harvested and analyzed by ELISA for the presence of cytokines (Fig. 5A). Although wild-type T cells secreted very low levels and IL-4-deficient T cells secreted undetectable levels of IL-5 and IL-13, CD2:Stat6VT and Il4−/− CD2:Stat6VT T cells secreted elevated levels of both cytokines. Secretion of IFN-γ was also increased by transgene expression in these cultures compared with nontransgenic mice, though levels were still lower than those routinely observed in Th1 cultures (Fig. 5A). To further examine the Th2 phenotype in these cultures, we assessed the relative expression of the Gata3, Tbx21, chemokine receptors Ccr4 and Ccr8, and the Th2 chemokine Ccl22.
RNA was isolated from anti-CD3-activated unskewed Th cultures and used to determine relative gene expression using RT-PCR. Expression of *Gata3*, *Ccr4*, and *Ccr8* were similar in wild-type and CD2:Stat6VT cultures, though they were greatly decreased in IL-4-deficient cultures. However, Stat6VT expression was sufficient to rescue expression of these genes in the absence of IL-4 (Fig. 5B). Expression of *Tbx21* was modestly decreased in CD2:Stat6VT cultures, compared with wild-type cultures, but expression was not affected in *Il4−/−* cultures (Fig. 5B). Ccl22 expression was greatly increased by Stat6VT expression, though this induction was dependent upon IL-4 (Fig. 5B). Thus, expression of Stat6VT in IL-4-deficient mice is sufficient to promote Th2 differentiation and also rescue the expression of some of the Th2 associated genes.

**Allergic inflammation in Stat6VT transgenic mice**

The in vivo, hyperTh2 environment of Stat6VT transgenic mice may predispose these mice toward an atopic phenotype. Therefore, we monitored Stat6VT transgenic mice on both the wild-type and IL-4-deficient background for allergic disease. We observed that ~75% of Stat6VT transgenic mice <12 wk of age and all transgenic mice older than 12 wk develop blepharitis (Fig. 6, A and B) similar to that observed in IL-4-transgenic mice (30) and NFATc2/NFATc4 double-deficient mice (31). Blepharitis in...
Stat6VT transgenic mice was observed as early as 4 wk of age. Histological examination of tissue from around the eyes demonstrated hyperkeratoses, as well as thickening of the dermis and epidermis. Cellular infiltration of the dermis included lymphocytes and eosinophils, indicative of allergic inflammation (Fig. 6C). In contrast, blepharitis was observed only in older Il4−/− Stat6VT transgenic mice and the limited occurrence was less severe than in transgenic mice on the wild-type background (Fig. 6, A–C).

We next wanted to determine whether there is any evidence of pulmonary infiltration in Stat6VT transgenic mice. BAL and lung tissue were examined for infiltration of leukocytes. Cells from the BAL of these mice were stained with Abs to CCR3, CD3, CD220, CD11c, and MHC class II, and the indicated cell populations were identified by cell size and lineage-specific markers using flow cytometry (29). The populations of eosinophils, neutrophils, T cells, B cells, and macrophages/dendritic cells were all higher in Stat6VT transgenic mice as compared with wild-type, Il4−/− Stat6VT mice (Fig. 7A). Moreover, we observed that Stat6VT transgenic mice developed spontaneous pulmonary inflammation (Fig. 7B). On higher magnification, peribronchial and perivascular inflammation in Stat6VT transgenic mice revealed a predominant infiltration of eosinophils. Eosinophils counted in 10 high-power fields (600×) per lung, from the lungs of seven CD2:Stat6VT mice, was 44±14. In contrast, eosinophils could not be identified in Il4−/− Stat6VT transgenic mice did not contain eosinophils and were largely composed of lymphocytes and neutrophils (Fig. 7B). Analysis of BAL cytokines demonstrated only a modest increase in IL-5 and IL-13 levels in CD2:Stat6VT mice, compared with mice of the other genotypes (Fig. 7C). However, IFN-γ levels were decreased in CD2:Stat6VT mice on either wild-type or Il4−/− background compared with nontransgenic mice, suggesting that there is an alteration in the Th1:Th2 ratio in the lung (Fig. 7C). These results suggest that a hyperTh2 immune system is sufficient to lead to allergic
inflammation in multiple tissues. However, IL-4 is required for the ability of Stat6VT to predispose mice to the development of blepharitis and allergic pulmonary inflammation.
IL-4 promotes Th2 development and stimulates B cells to undergo class switching to IgE, each important in the development of atopic responses. Although studies with Stat6-deficient mice have shown that Stat6 is required for many IL-4-induced functions, a requirement for other signaling pathways is unclear. In this report, using a constitutively active Stat6 mutant, we addressed the ability of this transcription factor to alter immune homeostasis, mediate IL-4-stimulated B and T cell functions, and induce an atopic phenotype in the absence of endogenous IL-4. The phenotypes in Il4/H11002/H11002 transgenic mice may reflect either a requirement for Stat6-independent IL-4 signaling or a requirement for IL-4 within the immune response to activate cells that do not express Stat6VT.

Our previous results with CD2:Stat6VT transgenic mice indicated that expression of Stat6VT did not alter thymic development (28) (Fig. 1). We describe here that Stat6VT expression on a background of IL-4-deficiency increases the percentages of mature single-positive cells, with the most dramatic effects on the CD4+ T cells (Fig. 1). This phenotype suggests that endogenous IL-4 is paradoxically interfering with the effects of Stat6VT and may indicate that additional IL-4 activated pathways are having an effect on thymocyte development. This phenotype is distinct from that in mice transgenic for expression of IL-4, which increases the percentages of single-positive cells much more dramatically (30, 32). Whether the increased CD4+ T cells in the H4-/-CD2:Stat6VT thymus are a result of increased development or decreased emigration to the periphery is still unclear.

We previously described that mice expressing the Stat6VT transgene in T cells have altered immune homeostasis, particularly increasing the percentages of splenic B cells and decreasing splenic T cells (28). In this report, we show that IL-4 deficiency mitigates the effects of Stat6VT expression while also decreasing the overall spleen size from the splenomegaly observed in CD2:Stat6VT mice. Compared with CD2:Stat6VT mice, T cell numbers

**FIGURE 7.** Airway inflammation in CD2:Stat6VT transgenic mice. A. Comparison of major BAL cell types, by flow cytometry. Eosinophils (CD3+ B220+ CCR3+), neutrophils (CD3+ B220+ CCR3+), T cells (CD3+ MHCII+), B cells (B220+ MHCII-), and mononuclear cells (CD3+ B220- CD11c+ MHCII+) were identified as described in Materials and Methods. Data represent the mean ± SE of 4–8 mice. B. Histological analysis of lungs from wild-type, CD2:Stat6VT, H4-/-, and H4-/-CD2:Stat6VT mice. Representative photomicrographs of H&E-stained lung sections are shown. Tissue from an H4-/-CD2:Stat6VT mouse with neutrophilic lung inflammation is shown on the far right. Arrows indicate the eosinophils in high power micrographs. Magnification, ×100 (top); ×600 (bottom). C. Measurement of BAL cytokine levels in mice of the indicated genotypes. Results are expressed as mean ± SEM from three mice.
in the Il4−/− CD2:Stat6VT spleen are significantly increased, though numbers are still lower than in wild-type mice. The activated/memory phenotype of Stat6VT transgenic T cells is also maintained in the absence of IL-4. The reason for this phenotype is unclear. It is possible that Stat6VT is directly altering the expression of these genes. It is also possible that the phenotype could result from a homeostatic expansion of peripheral T cells in response to the T lymphopenia. Regulatory T cells may regulate peripheral T cell numbers, and the increased regulatory T cells in Stat6VT transgenic mice may also contribute to the T cell phenotype (33, 34). In contrast to the T cell alterations, neither B cell percentages nor cell numbers in Il4−/− CD2:Stat6VT spleens are decreased compared with cells in wild-type CD2:Stat6VT mice. The increase in splenic B cells in wild-type CD2:Stat6VT transgenic mice is coincident with a decrease in mature recirculating B cells in the bone marrow, which may further indicate the ability of IL-4 to promote immigration to the spleen at the expense of other peripheral compartments (35). However, the increased B cell numbers in the spleen of Il4−/− CD2:Stat6VT mice suggests that the ability of IL-4 to promote recruitment to the spleen is an indirect effect of IL-4 and Stat6 signaling on T cell function.

Previous studies, using retroviruses expressing a tamoxifen-inducible Stat6-ER fusion protein or Stat6VT, have demonstrated that Stat6 is sufficient to promote Th2 differentiation in an in vitro system (36, 37). With the Stat6VT transgenic mice, we have further shown that an active Stat6 is sufficient to generate Th2 cells in vivo (28). In this report, we have further shown that Th2-like cells secreting IL-5 and IL-13 can develop in CD2:Stat6VT cultures in the absence of endogenous IL-4. We also observed that IFN-γ secretion was increased by transgene expression, though levels were still below those routinely observed in Th1 cultures (Fig. 5A and data not shown). Increased IFN-γ production did not correlate with Tbx21 expression, suggesting that Stat6VT was directly affecting IFN-γ production either directly by altering transcription of Ifng or indirectly by altering protein secretion. It is not clear whether this is a physiological function of Stat6 or a result of overexpression of the constitutively active Stat6. We further examined the expression of additional Th2 genes in the cells that develop from Stat6VT transgenic cells and demonstrated that Stat6VT was sufficient, in the absence of endogenous IL-4, to promote expression of Gata3, Ccr4, and Ccr8 but not Ccl22 (Fig. 5). This suggests that there is a requirement for Stat6-independent IL-4 signaling for some aspects of Th2 development, possibly including the contribution of Irs-2 to Th2 development (38). Although several other transcription factors contribute to the Th2 phenotype, including Irf4, e-maf, and Jun family members, it is not clear whether any of these regulate Ccl22 expression. We have previously shown that Pu.1 may regulate Ccl22 in Th2 cells (39), and it is possible that Pu.1 expression in Th2 cells requires Stat6-independent IL-4 signaling. Further analysis of Ccl22 regulation is required to discern these possibilities.

The development of spontaneous allergic inflammation in Stat6VT mice demonstrates that a hyperTh2 immune response, in the absence of specific Ag priming, is sufficient to initiate atopic symptoms in multiple tissues. The bilateral blepharitis in Stat6VT mice is a common phenotype observed in mice with a hyperTh2 cytokine production, including IL-4–transgenic mice and NFATc2/c4-double-deficient mice (30, 31). We observe early occurrence, usually by the time of weaning. The spontaneous pulmonary inflammation in Stat6VT develops somewhat slower but is readily apparent by 12–16 wk of age. A similar propensity for pulmonary inflammation has been observed in other strains, including Tbx21-deficient mice and in mice overexpressing multiple Th2 cytokines (40, 41). The pulmonary inflammation that develops in Tbx21-deficient mice is dependent upon IL-13, a major effector cytokine in promoting allergic lung infiltration (40–43). In that respect, the requirement for IL-4 in the development of the allergic pulmonary inflammation is noteworthy. In previous studies, Ag-induced allergic pulmonary inflammation was observed in IL-4-deficient mice, although this varied with the route of allergen exposure (44, 45). Because Il4−/− CD2:Stat6VT transgenic T cells are capable of becoming Th2-like, identified by the secretion of IL-5 and IL-13, the lack of allergic inflammation in these mice suggests that IL-4 plays a critical role as an effector cytokine. It is still unclear whether IL-13 plays a role in Stat6VT-induced allergic disease. IL-13 was increased only slightly in BAL fluid from transgenic mice, compared with control mice (Fig. 7C), and preliminary experiments suggest that IL-13 neutralization did not affect pulmonary infiltration in CD2:Stat6VT mice. This might reflect a different etiology in the chronic inflammation that occurs in transgenic mice, compared with the development of acute inflammation observed in most of the murine airway inflammation models. Mating of Stat6VT transgenic mice to other gene-deficient mice will help to define how the mechanisms of pulmonary infiltration compare in mice with chronic inflammation to those with inducible disease.

The effector role of IL-4 in the development of allergic disease is still unclear. It is possible that Stat6-independent IL-4 signaling is required in T cells to develop some aspect of the Th2 phenotype. For example, as discussed above, we have shown that expression of Ccl22 is diminished in Il4−/− CD2:Stat6VT cultures. Stat6-independent signaling mechanisms, such as a pathway through Irs-2 (38), have not been explored in detail. We have recently shown that IL-4 can activate alternative NF-κB signaling in B cells but not in T cells (46). It is not known whether other signaling pathways are engaged by IL-4 signaling. It is equally possible that IL-4 is required as an effector cytokine in the tissue or required for the ability of other inflammatory cells that do not express Stat6VT, such as B cells and myeloid cells, that require stimulation by IL-4 to mediate the development of allergic inflammation. IL-4 clearly plays such a role in B cells (Fig. 4) and could further alter the function of innate immune cells either systemically or locally during the development of inflammation. Future experiments will distinguish these possibilities.

In summary, our results provide evidence for the role of endogenous IL-4 in mediating Stat6 functions. Importantly, generation of an atopic phenotype in Stat6VT transgenic mice, but not in the transgenic mice on an IL-4-deficient background, suggests that endogenous IL-4 is necessary for Stat6VT to promote an atopic phenotype. Continuing work will define the cells involved in the allergic inflammation of Stat6VT mice and the utility of this model in understanding the development of human allergic disease.

Disclosures
The authors have no financial conflict of interest.

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