

In vivo expression of signal transducer and activator of transcription factor 6 (STAT6) in nasal mucosa from atopic allergic rhinitis: effect of topical corticosteroids

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Summary

Background The allergen-induced late nasal response is associated with a high local expression of interleukin (IL) -4, a TH2-type cytokine implicated in immunoglobulin (Ig) E production, tissue eosinophilia and other events considered to be relevant to allergic inflammation. Interaction of IL-4 with its receptor activates at least two distinct signalling pathways that culminate in the transcription of specific target genes. One pathway involves the activation of a transcription factor termed signal transducer and activator of transcription factor 6 (STAT6).

Objective To investigate the expression of STAT6 in the allergen-induced late nasal response and to examine the effect of local steroid treatment on STAT6 expression.

Methods Inferior turbinate biopsies were obtained from subjects with allergic rhinitis out of the allergen season. Subjects were then randomized into topical steroid- ($n=6$) and placebo-treated ($n=6$) groups in a double-blind fashion. After a 6-week treatment period, a second nasal biopsy was performed 24 h after local challenge with allergen. STAT6 immunoreactivity was examined in biopsy specimens by immunocytochemistry using a specific monoclonal antibody. Numbers of inflammatory cells ($CD3^+$ T cells and MBP^+ eosinophils) and IL-4 mRNA⁺ cells were investigated by immunocytochemistry and *in situ* hybridization, respectively.

Results STAT6 immunoreactivity was detected in all biopsies studied and localized predominantly to inflammatory tissue of the nasal mucosa. After allergen challenge, expression of STAT6 was markedly increased in placebo-treated patients ($P<0.01$). By confocal microscopy, STAT6 was localized to the cytoplasm and the nucleus of positively-staining cells. The allergen-induced increase in STAT6 immunoreactive cells was not observed in the steroid-treated patients. The change in STAT6 immunoreactivity after allergen challenge correlated significantly with the change in numbers IL-4 mRNA⁺ cells ($r=0.74$, $P=0.006$) and $CD3^+$ T cells ($r=0.76$, $P=0.004$), but not MBP^+ eosinophils.

Conclusion This study provides the first evidence of increased STAT6 expression *in vivo* in human allergic inflammation. The results support a role for STAT6 and IL-4 in the pathogenesis of late nasal response and show that decreases in STAT6 expression parallel the reduction in IL-4 expression that occurs with topical steroid treatment.

Keywords: allergic rhinitis, interleukin-4, interleukin-4 receptor, late nasal response, STAT6, steroids, TH2 cells

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Introduction

Numerous human and animal studies of antigen-induced late-phase allergic responses have ascribed a critical regulatory role to CD4⁺ T lymphocytes [1–3]. This function is likely delivered through selective expression of cytokines within the TH2 subset [1,2,4]. TH2-type cytokines possess a number of activities that are relevant to allergic inflammation, including the ability to elicit tissue eosinophilia and IgE production. We and others have previously used the allergen-induced late nasal response as an *in vivo* model for assessing local inflammatory changes that follow specific allergen challenge in humans. In subjects with allergic rhinitis, the clinical response to allergen challenge (nasal blockage, sneezing, rhinorrhoea) is accompanied by the recruitment of T cells and eosinophils to the nasal mucosa within 24 h of nasal allergen provocation [5]. The inflammatory response is associated with a high local expression of interleukin (IL) -4, IL-5, and IL-13; TH2-type cytokines localized predominantly to T cells and suppressed in parallel with topical glucocorticoid treatment [6–11].

Information from *in vitro* studies and animal models of allergic inflammation have suggested that the actions of IL-4 are critical in the pathophysiology of allergic inflammation. For example, mice in which the gene for IL-4 is disrupted or animals in which the cytokine or its receptor are blocked *in vivo* do not develop eosinophilic inflammation, immunoglobulin (Ig) E production, or airway hyperresponsiveness normally observed after local allergen challenge in these models [12–14]. *In vitro*, IL-4 is essential for the induction of IgE synthesis by B lymphocytes [15], and pivotal in the generation of T cells producing TH2-type cytokines, such as IL-4 and IL-5 [16]. The latter cytokine has been suggested to play a direct and obligatory role in the development of tissue eosinophilia by increasing the level of circulating eosinophils, upregulating their adhesive and migratory properties and prolonging their survival in distal tissue sites [17]. IL-4 also has the capacity to regulate the adhesion of eosinophils to the endothelium by augmenting the expression of vascular cell adhesion molecule (VCAM) -1 on endothelial cells [18]. In the airway epithelium, IL-4 may be important in the production of mucus [19], in the generation of mediators such as nitric oxide [20] and in the secretion of pro-allergic cytokines [21] and chemokines [22]. Despite ample evidence of enhanced IL-4 gene expression in human allergic inflammation, the intracellular signalling pathways governing the activity of IL-4 on target cells remains virtually unexplored *in vivo*.

The actions of IL-4 are elicited through a cell surface receptor composed of an α chain – also a component of the IL-13 receptor – and the common γ c chain used by a number of other cytokines [23]. Like IL-4, the α subunit of the IL-4 receptor (IL-4R α) is increased in the airway

mucosa of subjects with allergen-induced rhinitis [24] and allergic asthma [25]. Interaction of IL-4 with its receptor activates at least two distinct signal transduction pathways that culminate partly in the transcription of specific target genes [23]. One pathway is associated with the activation of Janus kinase (Jak) 1 and Jak3 which in turn phosphorylate signal transducer and activator of transcription factor 6 (STAT6) [26,27]. Phosphorylated STAT6 forms biologically active homodimers that move from the cytoplasm to the nucleus and bind specific promoter sequences regulating target gene transcription. A second pathway involves the phosphorylation of IL-4-induced phosphotyrosine substrate/insulin receptor substrate 2 (4PS/IRS2) [28]. Despite the existence of multiple signalling pathways, it appears that many of the key IL-4-induced responses that are potentially implicated in allergic inflammation are mediated by STAT6, particularly the generation of TH2-type cytokine-secreting T cells and B-cell production of IgE [29–31]. The importance of the STAT6 pathway was recently demonstrated in mice deficient in this transcription factor; STAT6 knockout mice were protected from developing airway hyperresponsiveness and producing excess mucus in response to allergen challenge, and failed to mount allergen-specific IgE responses [32,33]. The objective of the present study was to investigate STAT6 immunoreactivity and its association with CD3⁺ T cells, major basic protein (MBP)⁺ eosinophils and IL-4 mRNA⁺ cells in the late nasal response and, in a double-blind trial, to examine the influence of topical corticosteroids on STAT6 expression.

Methods

Subjects and study design

This study was approved by the Ethics Committee of the Sir Mortimer B. Davis-Jewish General Hospital (McGill University, Montreal). Twelve subjects with a clinical history of seasonal hayfever were recruited with informed written consent. Inclusion criteria for this study were: (i) a history of moderate to severe summer hayfever for at least 2 years; (ii) a positive skin-prick test (weal >5 mm) to ragweed allergen extract; and (iii) negative skin-prick tests to a panel of common perennial allergens in the presence of a positive histamine control [5–10,24]. Patients were excluded if they had received topical or oral medication in the previous 6 months or immunotherapy in the previous 5 years. All subjects were studied outside the local ragweed season at a time when they were asymptomatic. Baseline nasal inferior turbinate biopsies (2.5 mm) were obtained from all subjects using Gerritsma forceps after local anaesthesia with oxymetazoline and xylocaine. After 1 week, patients were randomized to receive either a 6-week treatment with topical nasal corticosteroid (budesonide 200 μ g QD) or matched

placebo nasal spray in a double-blind fashion. At the end of the 6-week treatment period, all subjects underwent nasal allergen provocation using ragweed extract (1000 PNU aerosol per nostril) [24]. The presence of a significant clinical response to allergen challenge was confirmed by monitoring numbers of sneezes and degree of nasal obstruction during the 24-h period following nasal ragweed challenge [24]. A second nasal inferior turbinate biopsy was taken 24 h after the allergen challenge. Tissues were processed for immunocytochemistry and *in situ* hybridization as described previously [7,24].

Immunocytochemistry

Cryostat sections of nasal biopsies processed for immunocytochemistry were separately stained with an antihuman CD3 monoclonal antibody (leu-4; Becton-Dickinson, Mississauga, Ontario, Canada) and an antihuman eosinophil MBP antibody (BMK13: a kind gift from Dr Redwan Moqbel, University of Alberta, Canada). Positive cells were detected using the alkaline phosphatase anti-alkaline phosphatase method as described before [5]. STAT6 was detected using a monoclonal antibody (anti-IL-4 Stat: Transduction Laboratories, Lexington, KY, USA) with the avidin-biotin complex method [34]. In all cases, negative controls involved replacement of the primary antibody with an irrelevant species- and isotype-matched antibody, and none exhibited positive staining. To colocalize the immunoreactivity at the sub-cellular level, we used confocal microscopy to examine sections that were stained as previously described [34] and enhanced by silver [35].

In situ hybridization

Cells expressing IL-4 mRNA in the nasal mucosa were enumerated by *in situ* hybridization. Briefly, cDNA for IL-4 was inserted into pGEM vectors and linearized with the appropriate enzymes prior to *in vitro* transcription. Transcription was performed in the presence of ^{35}S -UTP and the appropriate RNA polymerases to generate antisense and sense riboprobes. Ten micrometre sections of nasal inferior turbinate tissue were permeabilized by immersion in 0.3% Triton X-100 in PBS for 10 min followed by exposure to proteinase K solution (1 mg/mL in 20 mM TRIS-HCl and 1 mmol/L EDTA, pH 7.2) for 30 min at 37 °C. The slides were then prehybridized with 50% formamide in $2 \times \text{SSC}$ for 15 min at 37 °C. Hybridization was performed using [^{35}S]UTP-labelled riboprobes (either antisense or sense) for 16 h at 42 °C. Post-hybridization washings were carried out using SSC solutions ($4 \times \text{SSC}$ – $0.1 \times \text{SSC}$) followed by RNase treatment to remove unhybridized single stranded RNA. The preparations were dehydrated, immersed in emulsion and then subjected to autoradiography for

14 days. The autoradiograms were developed and subsequently counter-stained with haematoxylin. As a negative control, sections were hybridized with the sense probe or were pretreated with RNase prior to hybridization with the antisense probe, neither giving a positive signal.

Quantification

For immunocytochemistry and *in situ* hybridization, slides were counted blind in coded random fashion using a light microscope with an eye-piece graticule (0.202 mm^2). At least two sections were immunostained or hybridized from which three to four fields were counted. The graticulate was orientated along the epithelial basement membrane and the results were expressed as the mean number of positive cells per field $\pm \text{SEM}$. The within-observer coefficient of variation for repeated counts of immunostaining was 8% and for hybridized sections less than 5%. Comparison of the mean counts of positive cells at baseline and after allergen was performed using a paired *t*-test in each study group (placebo- and steroid-treated group). Correlations were performed using linear regression analysis and using Spearman's rank method. Significance was accepted at the 5% level of confidence. Statistical tests were performed using a standard computer package (Systat version 5.03, Systat Inc. Evanston, IL USA).

Results

Immunohistochemical analysis of CD3⁺ T cells and MBP⁺ eosinophils in the nasal mucosa before and after nasal allergen challenge

Immunohistochemistry of baseline (pretreatment and pre-challenge) nasal biopsies confirmed that the numbers of CD3⁺ T cells and MBP⁺ eosinophils did not differ significantly between subjects that were subsequently randomized in steroid (mean $\pm \text{SEM}$: 23.17 ± 2.0 [CD3⁺ cells], 1.33 ± 0.49 [MBP⁺ cells]) or placebo (mean $\pm \text{SEM}$: 25.17 ± 2.29 [CD3⁺ cells], 2.33 ± 1.12 [MBP⁺ cells]) groups ($P > 0.05$). At 24 h after allergen provocation, CD3-immunoreactive cells were markedly increased in the nasal mucosa of placebo-treated subjects (mean $\pm \text{SEM}$: 38.0 ± 4.46 , $P < 0.01$) compared with baseline (Fig. 1). In contrast, numbers of CD3⁺ T cells did not increase significantly in steroid-treated subjects after challenge (mean $\pm \text{SEM}$: 20.17 ± 2.02 , $P > 0.05$). Similarly, MBP⁺ eosinophil numbers were significantly increased in the nasal mucosa of placebo- (mean $\pm \text{SEM}$: 18.33 ± 3.16 , $P < 0.01$), but not steroid-treated (mean $\pm \text{SEM}$: 1.50 ± 0.50 , $P > 0.05$) allergic rhinitic subjects 24 h after nasal allergen provocation compared with numbers of MBP-immunoreactive cells before challenge (Fig. 2).

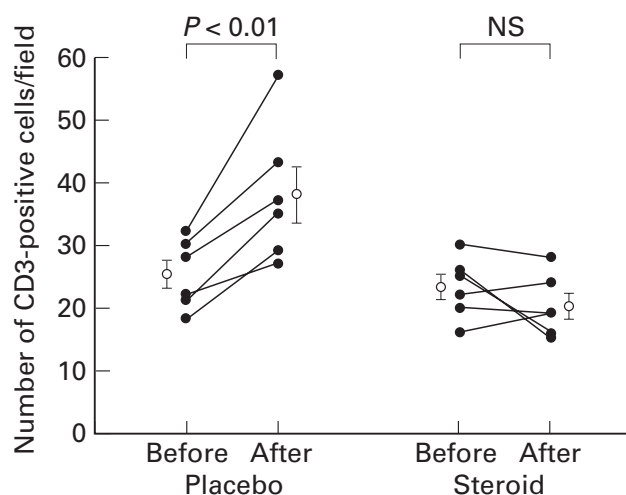


Fig. 1. Number of CD3-positive cells within the submucosa of nasal biopsy specimens obtained before and 24 h after challenge in patients with allergic rhinitis either given placebo or treated with steroids. Results are expressed as the mean number of positive cells per field

STAT6 immunoreactivity in the nasal mucosa before and after allergen challenge

At baseline, STAT6 immunoreactive cells were readily detected in nasal biopsy sections from all 12 subjects studied (range: five to 17 positive cells/field). Subjects in placebo- and steroid-treatment groups showed no significant difference in STAT6 immunoreactivity at baseline ($P > 0.05$). As shown in Fig. 3(a), local allergen challenge was associated with a significant increase in the numbers of STAT6-positive cells at 24 h in placebo-treated subjects (mean $\pm$ SEM: 10.67 ± 1.61

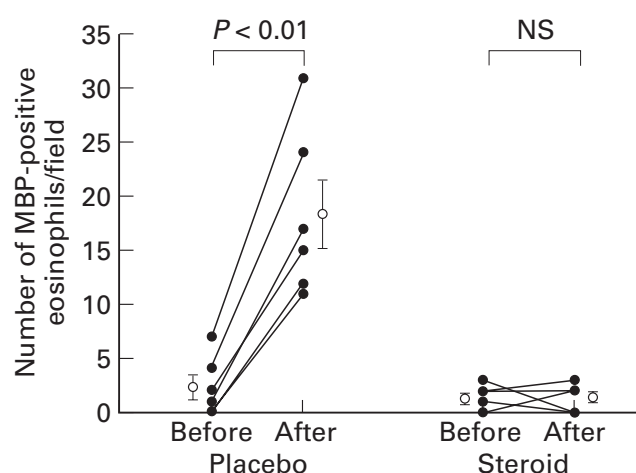


Fig. 2. Number of cells expressing MBP immunoreactivity in the submucosa of nasal biopsy specimens obtained before and 24 h after challenge in patients with allergic rhinitis either given placebo or treated with steroids. Results are expressed as the mean number of positive cells per field

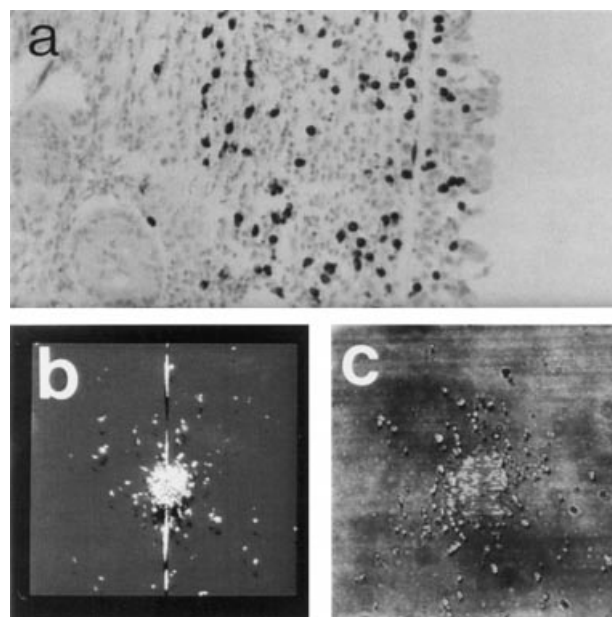


Fig. 3. (a) Immunocytochemistry of STAT6 of a nasal biopsy following antigen challenge showing strong immunoreactivity predominantly in the upper part of the mucosa. (b) Confocal microscopy of STAT6-immunoreactive cells that were enhanced with silver. Three-dimensional image of a lymphocyte expressing STAT6 immunoreactivity. (c) Confocal image showing the localization of the silver granules in the nucleus and cytoplasm

[prechallenge], 30.33 ± 3.62 [post-challenge], $P < 0.01$, Fig. 4). Positive STAT6 signals localized mainly to infiltrating cells in the subepithelial region but were also observed in structural cells (epithelial and endothelial cells). However,

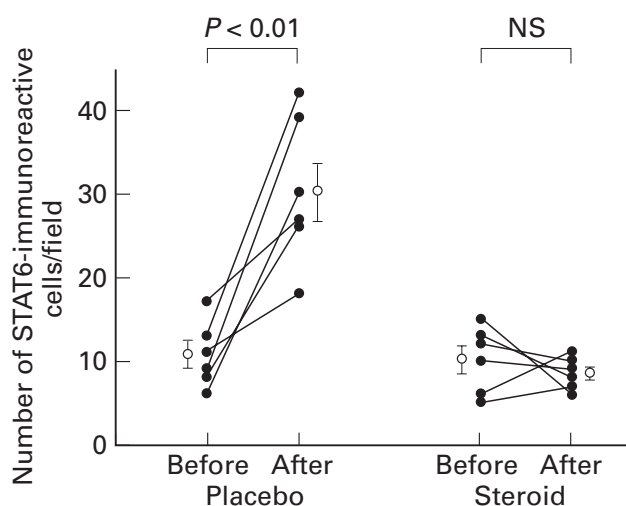


Fig. 4. STAT6 expression in nasal biopsy specimens obtained before and 24 h after challenge in patients with allergic rhinitis either given placebo or treated with steroids. Results are expressed as the mean number of positive cells per field

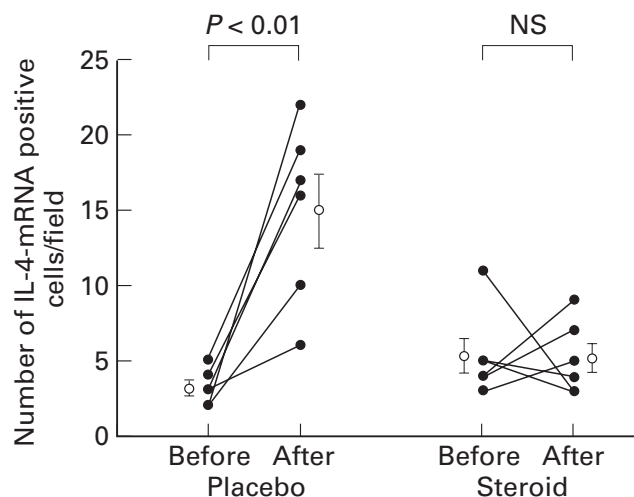


Fig. 5. Number of IL-4 mRNA-positive cells in nasal biopsy specimens obtained before and 24 h after challenge in patients with allergic rhinitis either given placebo or treated with steroids. Results are expressed as the mean number of positive cells per field

there was no significant increase in STAT6 immunoreactivity in steroid-treated subjects 24 h after local allergen challenge compared with baseline (mean $\pm$ SEM: 10.17 $\pm$ 1.62 [prechallenge], 8.50 $\pm$ 0.76 [post-challenge], $P > 0.05$, Fig. 4). STAT6 immunoreactivity was localized in the nucleus and cytoplasm of the infiltrating cells (Figs 3b, 3c).

Local IL-4 gene expression before and after allergen challenge

In vitro, STAT6 activation is induced by the interaction of IL-4 with its cell surface receptor in target cells [23,27]. We therefore examined the expression of IL-4 mRNA in the same allergic rhinitic subjects before and after allergen challenge by *in situ* hybridization using antisense radiolabelled riboprobes. At baseline, allergic rhinitics subsequently randomized to steroid and placebo treatment groups had no significant difference in IL-4 mRNA expression ($P > 0.05$). As shown in Fig. 5, IL-4 gene expression was significantly increased in nasal mucosa of placebo-treated allergic rhinitic subjects 24 h after allergen challenge (mean $\pm$ SEM: 15.00 $\pm$ 2.42) compared with prechallenge levels (mean $\pm$ SEM: 3.17 $\pm$ 0.48, $P < 0.01$). Numbers of IL-4 mRNA⁺ cells were not significantly changed 24 h after nasal allergen challenge (mean $\pm$ SEM: 5.17 $\pm$ 0.98) compared with baseline (mean $\pm$ SEM: 5.33 $\pm$ 1.17, $P > 0.05$) in subjects treated with topical steroids.

Association of STAT6-immunoreactive cells with numbers of T cells and IL-4 mRNA⁺ cells

For linear regression analyses, data were expressed as delta values to reflect the change in immunoreactivity or mRNA

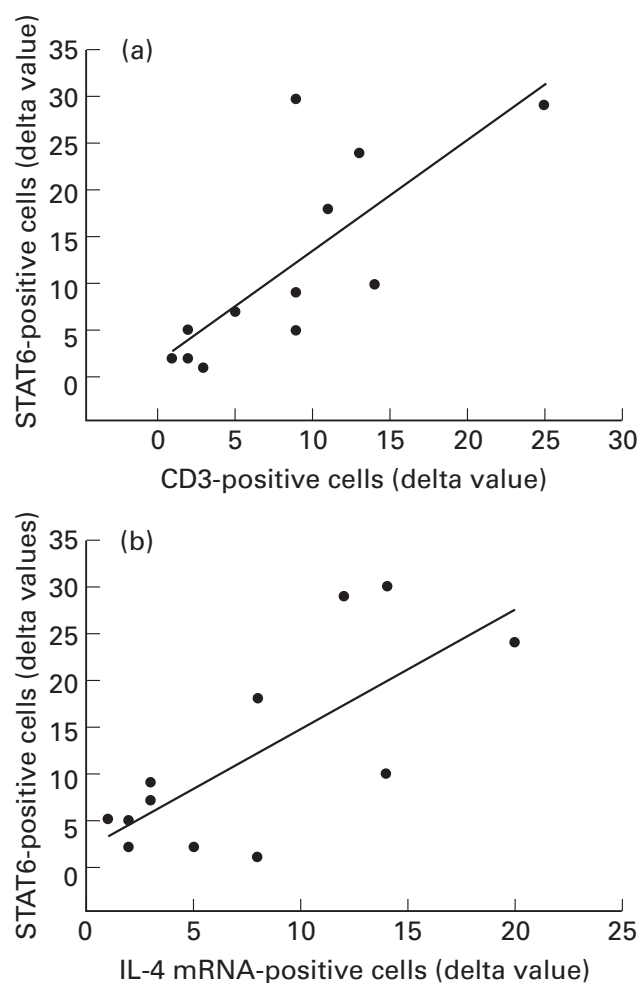


Fig. 6. (a) Correlation of STAT6 immunoreactivity with CD3⁺ T-lymphocyte infiltrate. Linear regression analysis indicated significant correlations between the change in STAT6-immunoreactivity and the change in CD3⁺ T cell numbers ($r = 0.76$, $P = 0.004$). (b) Correlation of STAT6 immunoreactivity with IL-4 mRNA⁺ cells. Linear regression analysis indicated significant correlations between the change in the numbers of STAT6-immunoreactive cells with the change in the numbers of IL-4 mRNA⁺ cells ($r = 0.74$, $P = 0.006$)

expression post-challenge compared with prechallenge. Statistical analyses indicated significant correlations between the change in STAT6-immunoreactivity and the change in CD3⁺ T-cell numbers ($r = 0.76$, $P = 0.004$, Fig. 6a), but not in MBP⁺ eosinophils ($P > 0.05$). The change in the numbers of STAT6 immunoreactive cells also correlated significantly with the change in the numbers of IL-4 mRNA⁺ cells ($r = 0.74$, $P = 0.006$, Fig. 6b).

Discussion

The IL-4 receptor complex consists of IL-4R α and γ c

subunits, both members of the type I cytokine receptor super-family [23]. IL-4R α is relatively specific, being shared only with the receptor for the functionally similar cytokine IL-13, while γ_c is shared by IL-2, IL-7, IL-9, and IL-15 receptors. Ligand binding to the IL-4 receptor complex causes dimerization of the receptor subunits [23,26,27]. This leads to phosphorylation and activation of the protein tyrosine kinases Jak1 and Jak3 that are bound to the membrane proximal region of the cytoplasmic domain of IL-4R α and γ_c , respectively. The activated Jak kinases, in turn, phosphorylate tyrosines on the receptor subunits which can then act as docking sites for 4PS/IRS2 and STAT6 [23,26–28]. The consequent activation of 4PS/IRS2 permits its interaction with signalling mediators containing a Src homology 2 domain, such as the p85 subunit of phosphatidylinositol 3-kinase [27,28]. The tyrosine phosphorylation of STAT6 that occurs through its association with the IL-4 receptor subunits results in the formation of STAT6 homodimers and their immediate translocation to the nucleus [23,26–28]. In the nucleus, STAT6 binds specific DNA sequences in the promoters of IL-4-responsive genes – including those coding for CD23, MHC class II, IL-4R α and IgE – and activates their transcription [29–31]. STAT6 can also repress the transcription of other genes such as that of interferon regulatory factor-1 [36].

In the present study, we examined the expression of the STAT6 transcription factor *in vivo* in human allergic inflammation. STAT6 immunoreactivity was constitutively expressed in the nasal mucosa of subjects with allergic rhinitis (out of the allergen season) and was significantly increased 24 h after local allergen provocation. Expression of STAT6 was localized predominantly to infiltrating cells and, to a lesser extent, to structural cells of the nasal mucosa. On a sub-cellular level, STAT6 immunoreactivity was detected both in the cytoplasm and nucleus of positively staining cells, indicating that the transcription factor was present in inactivated and activated forms, respectively. In contrast with placebo-treated subjects, 6 weeks of treatment with topical corticosteroids was not associated with an increase in STAT6 immunoreactivity 24 h after allergen challenge. We also found that allergen-induced STAT6 expression was significantly correlated with changes in the numbers of CD3⁺ T cells and IL-4 mRNA⁺ cells. Collectively, these results complement and extend findings of increased IL-4 and IL-4R α gene expression in the allergen-induced late nasal response of placebo- but not steroid-treated allergic rhinitic subjects, and enhance our understanding of the putative mechanisms through which IL-4 may exert its effects *in vivo* in allergic inflammation.

Two variants of IL-4R α were recently shown to be strongly related to atopy or atopic asthma; an Arg576Gln substitution associated with impaired binding of a negative regulatory molecule was identified in excess in individuals

with hyper-IgE syndrome and severe eczema [37], and a Ile50Val variant associated with augmented STAT6 activation correlated with atopy and atopic asthma [38]. The latter suggests the potential importance of a single transcription factor, STAT6, in the overall manifestation of allergy and asthma. Two naturally occurring dominant negative and attenuated forms of human STAT6 generated by differential splicing have also been identified and were designated STAT6b and STAT6c [39]. STAT6b, possessing a N-terminal deletion of at least 110 amino acids, was associated with decreased IL-4-induced promoter binding and transcriptional activation relative to STAT6. Unexpectedly, STAT6c – which lacks a portion of the Src homology 2 domain – markedly inhibited IL-4-mediated functions, possibly via the inhibition of STAT6 homodimer formation. Although the upper airways were not studied, STAT6, STAT6b and STAT6c were highly expressed in the lungs, with STAT6 transcripts consistently present at the highest levels. The STAT6 fragment used as an immunogen for the generation of the monoclonal antibody employed in our study appears to be wholly present in STAT6c and partly present in STAT6b [39], suggesting that some of the positive signal that we observed may have originated from the detection of these attenuated isoforms.

In agreement with previous studies of the late nasal response, numbers of T lymphocytes and eosinophils in the nasal mucosa were significantly increased following allergen provocation in placebo-treated allergic rhinitics [5,6]. It is therefore possible that some augmented STAT6 expression may have been a consequence of cellular recruitment rather than *in situ* induction. Our previous studies in the inflamed airway mucosa have identified T lymphocytes as the predominant site of IL-4R α expression [25]. Notably, the allergen-induced changes in T-cell numbers alone cannot account for the magnitude of the change in the numbers of STAT6 immunoreactive cells in the nasal mucosal subepithelium. The correlation of STAT6 with the change in CD3⁺ T-cell infiltration suggests that T cells are indeed a major site of allergen-induced STAT6 expression, that STAT6 is involved in the recruitment of T cells, and/or that T cells play a role in the increase in STAT6 expression after allergen challenge. The last possibility is supported by the correlation between numbers of allergen-induced IL-4 mRNA⁺ cells (of which T cells comprise the largest proportion [8]) and STAT6-positive cells, but further work is required to clarify these and other potential explanations. The association of IL-4 mRNA⁺ cells with STAT6-immunoreactive cells also suggests a role for IL-4 in stimulating the expression of STAT6 *in vivo* in addition to mediating its activation and nuclear translocation. Aside from IL-4 and IL-13, factors capable of causing STAT6 activation include angiotensin II and, in B cells, antigen receptor or CD40-triggering [40,41].

The generation of STAT6-deficient mice has highlighted the importance of this transcription factor in IgE production and in the development of TH2-type cytokine-secreting T cells [29,31]. In contrast with identically treated wild-type litter-mates, allergen-sensitized and challenged STAT6^{-/-} mice were shown to have an absence of mucus-containing cells in the airway epithelium [32]. Such findings are consistent with studies in transgenic mice that overexpressed murine IL-4 selectively in the lungs [19]. Compared with transgene-negative controls, these animals showed a high expression of the MUC5AC mucin gene in the lungs and an accumulation of mucus glycoprotein in the airway epithelium. It is therefore possible that one role of epithelial STAT6 expression in the nasal mucosa of allergic rhinitis is in the production of mucus. Epithelial cells are also believed to be an important source of chemokines, some of which are upregulated by stimulation with IL-4 [22], possibly through the action of STAT6.

The role of STAT6 in endothelial expression of VCAM-1 is not certain, since VCAM-1 expression appears to be unchanged in the airways of STAT6-deficient BALB/c mice in a model of asthma [32]. Allergen-induced airway eosinophilia was significantly reduced but not abolished in STAT6^{-/-} mice compared with wild-type mice, suggesting the existence of STAT6-dependent and independent pathways of eosinophilia. This is consistent with the lack of association between allergen-induced changes in eosinophil numbers and numbers of STAT6-positive cells in the nasal mucosa of allergic rhinitis.

Other transcription factors have also been suggested to play a role in allergic inflammation. For example, nuclear factor κ B (NF κ B) may regulate a large number of genes encoding products that are believed to be involved in inflammatory responses, including cytokines, chemokines and adhesion molecules [42]. One mechanism that has been proposed to account for many of the anti-inflammatory actions of glucocorticoids involves direct interaction of the glucocorticoid receptor with NF- κ B [42]. To our knowledge, there have not been any studies examining the effect of glucocorticoids on STAT6 expression. Our finding that the allergen-induced increase in STAT6 immunoreactivity is attenuated in the nasal mucosa of steroid-treated allergic rhinitis could have a number of potential explanations. Whether this observation is related to the steroid-dependent inhibition of allergen-induced IL-4 production [10], IL-4R α expression [24], and/or increases in inflammatory cells [5] is not known. It is possible that glucocorticoids directly inhibit the activation of STAT6, although this is speculative.

In conclusion, this study provides the first evidence of increased STAT6 expression *in vivo* in human allergic inflammation. The results support a role for STAT6 and IL-4 in the pathogenesis of the late nasal response and show

that decreases in STAT6 expression parallel the reduction in IL-4 expression that occurs with topical steroid treatment.

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