Transforming growth factor- β gene expression studies in nasal mucosal biopsies in naturally occurring allergic rhinitis

RAMI J SALIB

Allergy and Inflammation Research, Division of Infection, Inflammation and Repair, School of Medicine, Southampton General Hospital, Southampton, UK

ABSTRACT

INTRODUCTION Evidence has been provided of enhanced epithelial transforming growth factor-beta (TGF- β) immunoreactivity in allergic rhinitis, including correlation with intra-epithelial mast cell numbers, and the co-localisation of TGF- β receptors to mast cells, suggesting that the epithelial expression of TGF- β may represent an important biological process involved in either the recruitment or retention of mast cells within the epithelium in naturally occurring allergic rhinitis.

PATIENTS AND METHODS In order to extend the above findings, evaluation was undertaken in whole nasal biopsies from subjects with naturally occurring allergic rhinitis, of levels of TGF- β isotypes and receptors gene expression using real-time quantitative polymerase chain reaction (TaqMan RT-PCR), and the results compared to those for tumour necrosis factor-alpha (TNF- α), as a positive control. The study was also extended to evaluate gene expression for connective tissue growth factor (CTGF) and Smad proteins, as downstream markers of TGF- β bioactivity, in the same populations.

RESULTS There were no significant differences between the rhinitic and non-rhinitic groups in the expression of TGF- β isoforms or Smad-3, Smad-6 and Smad-7 proteins; however, there was increased gene expression for TGF- β RI and TGF- β RII along with CTGF in seasonal allergic rhinitis. TNF- α gene expression was also increased in seasonal allergic rhinitis, consistent with a more acute inflammatory response in this form of rhinitis.

CONCLUSIONS This study advances our understanding of the role of TGF- β in the pathogenesis of the inflammatory response in allergic rhinitis.

KEYWORDS

Transforming growth factor beta – Allergic rhinitis – Gene expression – Tumour necrosis factor-alpha – Smad – Connective tissue growth factor

CORRESPONDENCE TO

Rami J Salib, Allergy and Inflammation Research, Division of Infection, Inflammation and Repair, School of Medicine, F Level South Block (810), Southampton General Hospital, Southampton SO16 6YD, UK

T: +44 (0)23 8079 4155; F: +44 (0)23 8079 8492; E: rjs4@soton.ac.uk

Transforming growth factor-beta (TGF- β) was first identified as a protein secreted from sarcoma cells that promoted normal rat kidney cells to grow in soft agar.^{1,2} It was named transforming growth factor because of this ability to induce a transformed or tumour cell characteristic in normal cells. Subsequently, it was shown that TGF- β inhibited epithelial cell proliferation and regulated a diverse array of cellular functions unrelated to cellular transformation. Nevertheless, the name transforming growth factor has persisted. Today, the TGF- β superfamily is recognised as one of the largest families of secreted prototypic multifunctional peptides exerting an array of biological effects in a large variety of cell types. The superfamily includes TGF- β isoforms, activins and inhibins,

growth and differentiation factors (GDFs), and bone morphogenic proteins (BMPs; Fig. 1).

TGF- β family members are produced as large precursor dimeric proteins, with a signal sequence and a large pro-domain, that are processed proteolytically into mature proteins (Fig. 2).⁵ They are involved in the regulation of processes crucial to the initiation, maintenance and resolution of inflammatory responses.^{4,6} Their effects include inhibition of proliferation of many cell types,⁷ regulation of immune responses, stimulation of extracellular matrix formation,⁸ and chemotaxis for inflammatory cells.^{9–12} For an illustrated model of TGF- β activation, receptor signalling, and signal transduction, refer to Figure 3.

Dysfunction of the TGF- β signalling pathway appears to be intimately linked to many human disorders. Elucidation of the TGF- β superfamily signal transduction pathways has certainly provided further helpful insight into the aetiopathogenesis of certain human diseases. Mutations in the TGF- β signalling pathway have been implicated in various human diseases, both hereditary and spontaneous. These include mutations in receptors which can cause

hereditary haemorrhagic telangiectasia, primary pulmonary hypertension, persistent mullerian duct syndrome, hereditary non-polyposis colon cancer, and juvenile polyposis syndrome, to name but a few.¹⁵ Smad mutations have also been linked with cancers, particularly those of the colon and gastrointestinal tract.^{14,15} Therefore, one of the main challenges we face in molecular medicine will be to further our understanding of the TGF- β signalling pathway

Figure 3 TGF- β : structure, latency, activation, and receptors. LAP (latency-associated peptide); LTBP (latent TGF- β binding protein); M6P/IGFII-R (mannose-6-phosphate/type II insulin-like growth factor receptor; PLg (plasminogen); PL (plasmin); Smad (TGF- β signal transduction proteins); Anti-Smad (antagonistic Smad); Co-Smad (common partner Smad); R-Smad (receptor-regulated Smad); TGase (transglutaminase); TGF- β RI, TGF- β RII, TGF- β RIII (TGF- β receptor types I, II, III); TSP (thrombospondin); uPA (urokinase plasminogen activator); and uPA-R (uPA receptor). TGF- β is secreted in the form of a latent complex (small) that binds to LTBP to form the large complex. This complex may be sequestered in the matrix or activated to release the TGF- β ligand, which can then bind to TGF- β RII. Binding of TGF- β induces association of types I and II receptors. The kinase of TGF- β RII phosphorylates the GS domain of TGF- β RI, thus activating the TGF- β RI kinase, which signals to downstream targets via Smad proteins. Smad-2 becomes phosphorylated and associates with Smad-4, and these complexes accumulate in the nucleus. The Smad-2/4 complex associates with DNA-binding proteins to activate transcription of target genes. Adapted and reproduced with permission from: Fortunel NO, Hatzfeld A, Hatzfeld JA. Transforming growth factor- β : pleiotropic role in the regulation of hematopoiesis. *Blood* 2002; **96**: 2022–36. © Copyright American Society of Hematology.

to the stage where molecular strategies can be devised to improve disease control.¹⁵

Allergic rhinitis, an inflammatory mucosal disorder of the nose, is characterised by the epithelial cell accumulation of mast cells and eosinophils. This phenomenon can be linked to the local generation and release of chemotactic factors. Whilst much attention has focused on chemotactic factors, and in particular chemokines, of relevance to tissue eosinophil accumulation, scant attention has been given within the nose to those factors relevant to epithelial mast cell accumulation. Mast cells play an important pathophysiological role in the clinical expression of allergic rhinitis,¹⁶ and their numbers are increased within the airway epithelium in persistent disease.¹⁷ The process of tissue mast cell accumulation and localisation to the airway epithelium is incompletely understood. Although it is recognised that

there are uncommitted progenitors within the nasal mucosal tissue, in the form of CD34⁺, IgE⁺ and tryptase⁺ cells,¹⁸ which are considered to mature *in situ* under the influence of growth factors such as stem cell factor, the local accumulation within the epithelium is likely to be a reflection of the epithelial expression of mast cell chemotactic factors that attract and immobilise the cell at that site within the tissue. We have previously provided evidence of increased epithelial immunoreactivity for the known mast cell chemo-attractant, TGF- β , in allergic rhinitis and evidence that nasal mast cells express receptors for TGF- β .¹⁷ In that study,¹⁷ the TGF- β isoforms (TGF- β 1, TGF- β 2, and TGF- β 3) all had increased epithelial expression in allergic rhinitis and positive correlations were evident between the extent of immunoreactivity for each of TGF- β 1 and TGF- β 2 and epithelial mast cell numbers. These findings of enhanced

epithelial TGF- β immunoreactivity in allergic rhinitis, the correlation with intra-epithelial mast cell numbers, and the co-localisation of TGF- β receptors to mast cells, suggest that the epithelial expression of TGF- β may represent an important biological process involved in either the recruitment or retention of mast cells within the epithelium in naturally occurring allergic rhinitis. Furthermore, use of the RPMI 2650 nasal epithelial cell line as an *in vitro* model to evaluate the regulation of TGF- β biology has demonstrated the ability of TNF- α to up-regulate both TGF- β gene expression and protein release, indicating that mast cell-epithelial interactions concerning TGF- β are bi-directional, which may be fundamental to epithelial immunoregulation.¹⁹

In vivo consequences of TGF- β -induced effects are difficult to infer from local concentrations, since TGF- β -mediated effects depend on a complex system of regulatory proteins and receptors. The gene expression of TGF- β in naturally occurring allergic rhinitis, including seasonal and perennial disease, using quantitative gene expression methods such as TaqMan RT-PCR has not yet been defined. So far, there has only been one published study examining the expression of TGF- β and related transcripts in seasonal allergic rhinitis with comparison to healthy controls.²⁰ However, this study used the DNA microarray analysis technique, where simultaneous analysis of expression of thousands of genes is possible, and did not include seasonal (out of season) or perennial rhinitis groups.

The aims of this study were to:

1. Use a real time quantitative gene expression method (TaqMan RT-PCR) to examine TGF- β (TGF- β 1, TGF- β 2, TGF- β 3, TGF- β RI, TGF- β RII, TGF- β RIII) gene expression in whole tissue nasal mucosal samples from subjects with naturally occurring seasonal (in and out of season) and perennial allergic rhinitis, and in healthy non-atopic non-rhinitic volunteers for comparison.
2. Determine whether TGF- β signalling was active in the upper airways in allergic rhinitis by examining the downstream effects of TGF- β , and in particular determining the gene expression levels (using TaqMan RT-PCR) of the Smad proteins (Smad-3, Smad-6, and Smad-7) and the TGF- β -inducible gene connective tissue growth factor (CTGF).
3. Determine, using TaqMan RT-PCR, the gene expression levels of TNF- α in allergic rhinitis for use as a positive marker of inflammation in the upper airways.

Patients and Methods

Recruitment and phenotyping of subjects

Subjects with seasonal allergic rhinitis (in and out of season), perennial allergic rhinitis, and non-atopic non-rhinitic volunteers were recruited and phenotyped for this

study. Not all the biopsies could, however, be used for the final analysis as in some either the RNA was degraded or there was insufficient biopsy material for RNA extraction. The final numbers were as follows: normal ($n = 9$); seasonal, out of season ($n = 7$); seasonal, in season ($n = 13$); and perennial ($n = 14$). The study was approved by the Joint Research Ethics Committee of Southampton & South West Hampshire Health Authority and conformed to the Declaration of Helsinki. All participants gave written informed consent.

Nasal biopsy and processing

Under local anaesthesia (topical lignocaine and adrenaline), nasal biopsy specimens were taken under direct vision from the inferior or inferomedial border of the inferior turbinate by using Hartmann's ear forceps (Medicon, Tuttlingen, Germany). The biopsy specimens were flash-frozen in liquid nitrogen and immediately transferred to a liquid nitrogen container for long-term storage. Gene expression studies were evaluated in whole tissue samples.

Quantitative gene expression

For analysis of gene expression, total RNA was extracted from cell lysates using the RNeasy column extraction method (Qiagen, Crawley, UK) according to the manufacturer's protocol. One microgram of total RNA was reverse transcribed using the Reverse Transcription System (Promega, Southampton, UK) according to manufacturer's instructions. Primer and probe sequences were labelled with a 5'-reporter dye FAM (6-carboxy-fluorescein) and a 3'-quencher dye TAMRA (6-carboxy-N,N,N',N'-tetramethyl-rhodamine). Primers directed against the housekeeping genes ubiquitin C (UBC) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were used as the endogenous controls (Eurogentec, Seraing, Belgium). For each sample, measured in duplicate, the PCR reaction contained 25 ng of cDNA template, 3.2 pM fluorogenic probe, 15 pM of forward and reverse primers, 7.5 μ l qPCR mix (Eurogentec), in a final volume of 12.5 μ l. Separate normalising control samples were run which contained 1 μ l of UBC and GAPDH primer and probe mixes (Eurogentec). RT negative samples were used to demonstrate that the signals obtained were RT-dependent and not due to genomic contamination. The PCR protocol was as follows: 95°C, 10 min, followed by 40 cycles of denaturation 95°C, 15 s, and annealing/extension 60°C, 1 min. Thermocycling and real-time detection of PCR products were performed on an IcylerIQ sequence detection system (Bio-Rad, Hercules, CA, USA); following completion of the PCR reaction, the thresholds for fluorescence emission baseline were set automatically at 10 times the background levels on the FAM and Yakima yellow dye layers. Expression levels for the target gene were calculated using the $\Delta\Delta C_T$ method and expressed relative to the geometric mean of the house-keeping genes, GAPDH and UBC. Primer and probe sequences are detailed in Table 1 (MWG-Biotech AG, Milton Keynes, UK).

Statistical analysis

All statistical analyses were performed using SPSS for Windows (SPSS Inc.). The data were presumed not to be normally distributed and, therefore, comparisons between clinical groups for gene expression levels were made using the non-parametric Mann-Whitney U-test.

Results

Assessment of RNA degradation and genomic contamination

Based on representative RNA integrity gels, there was no evidence of any significant degradation in the extracted RNA from the samples. Similarly, any genomic contamination present was negligible.

Table 1 Oligonucleotide sequences for TaqMan RT-PCR

	Oligonucleotide	Sequence
TGF- β 1	Forward (Eurogentec)	TGGACATCAACGGGTCTCACTA
	Reverse (Eurogentec)	AAGCAGGAAAGGCCGGTT
	Probe (Eurogentec)	CGAGGTGACCTGCCACCATTTCATT
TGF- β 2	Forward (Qiagen)	GACCAACCGGCGGAAGA
	Reverse (Qiagen)	CAGCAATTATCCTGCACATTCTAA
	Probe (Eurogentec)	CAATAGGCCGCATCCAAAGCACGT
TGF- β 3	Forward (MWG-Biotech AG)	CAAATTCAAAGGCGTGGACA
	Reverse (MWG-Biotech AG)	TTAGATGAGGGTTGTGGTGATCCT
	Probe (Eurogentec)	TCCACGGCCATGGTCATCCTCAT
TGF- β R1	Forward (MWG-Biotech AG)	GCCACAACCGCACTGTCA
	Reverse (MWG-Biotech AG)	TGAACAAGCAATGGTAAACCTGAG
	Probe (Eurogentec)	CCATCGAGTGCCAAATGAAGAGGACCT
TGF- β R11	Forward (Qiagen)	CTGTGGCCGCTGCACAT
	Reverse (MWG-Biotech AG)	TTGTTGTCACTGACTATCATGTCGTTA
	Probe (MWG-Biotech AG)	CCTCTGGACGCGTATCGGCAGC
TGF- β R111	Forward (MWG-Biotech AG)	CCCCCAGAAGTTGCCTAAGTC
	Reverse (MWG-Biotech AG)	CATCATGGCCAGATTATCGA
	Probe (MWG-Biotech AG)	CTGACGAAGCCTGCACCTCGGTG
CTGF	Forward (MWG-Biotech AG)	GCGGCTTACCGACTGGAA
	Reverse (Qiagen)	GGACCAGGCAGTTGGCTCTA
	Probe (Eurogentec)	CACGTTTGGCCAGACCCAACTATGAT
Smad-3	Forward (Qiagen)	GTGCTCCATCCTGCCTTTTCAC
	Reverse (Qiagen)	ATTTCTCCTCCTGCCGTTCT
	Probe (Eurogentec)	CTCGCCCTTCTTCCAGCCAGCAG
Smad-6	Forward (MWG-Biotech AG)	CGATTCCACATTGTCTTACACTGA
	Reverse (MWG-Biotech AG)	GACATGCTGGCGTCTGAGAA
	Probe (Eurogentec)	ACTCCCTCATCACTGCTCCGGGGTT
Smad-7	Forward (MWG-Biotech AG)	CTCCAGATACCCGATGCATTTTC
	Reverse (MWG-Biotech AG)	CCCTGTTTCAGCGGAGGAA
	Probe (Eurogentec)	CAAACCAACTGCAGACTGTCCAGATGCTT
TNF- α	Forward (MWG-Biotech AG)	AAGAGGGAGAGAAGCAACTACAGA
	Reverse (MWG-Biotech AG)	GGTGGAGCCGTGGTCAG
	Probe (Eurogentec)	CCTCAGACGCCACATCCCC

Figure 4 TGF- β 1, TGF- β 2, TGF- β 3, TGF- β RI, TGF- β RII, and TGF- β RIII gene expression relative to normal controls in nasal mucosal biopsies. The box plots show the median and interquartile range, and the bars show the 10th and 90th percentiles. * $P < 0.05$ versus non-atopic non-rhinitic controls.

TGF- β 1, TGF- β 2, and TGF- β 3

Whilst there were increases in TGF- β 1, TGF- β 2 and TGF- β 3 mRNA levels in the disease groups (most pronounced in the

seasonal group) compared with the control group, these did not reach statistical significance (Fig. 4).

TGF- β RI

There was an increase in TGF- β RI mRNA levels in the disease groups compared to the control group, being statistically significant in the seasonal (in season) group (median [range]; 1.45 [0.92–6.32]) compared with the control group (median [range]; 1.04 [0.45–1.91]; Fig. 4).

TGF- β RII

There was an increase in TGF- β RII mRNA levels in the disease groups being statistically significant in the seasonal (in season) group (median [range]; 1.72 [0.67–5.50]) compared with the control group (median [range]; 0.71 [0.32–2.85]; Fig. 4).

TGF- β RIII

There was an increase in TGF- β RIII mRNA levels in the seasonal (in season) group compared to the control group. However, this did not reach statistical significance (Fig. 4).

Smad-3, Smad-6, and Smad-7

There were no significant changes apparent for Smad-3, Smad-6, and Smad-7 mRNA levels in the disease groups compared with the controls (Fig. 5).

CTGF

There was an increase in CTGF mRNA levels in the disease groups, being statistically significant in the seasonal (in season) group (median [range]; 1.99 [0.71–12.58]) compared with the control group (median [range]; 1.06 [0.19–8.04]; Fig. 6).

TNF- α

There was an increase in TNF- α mRNA levels in the disease groups, being statistically significant in the seasonal (in season) group (median [range]; 4.76 [0.78–86.92]) compared with the control group (median [range]; 1.07 [0.53–2.25]; Fig. 7).

Discussion

Whilst there has been a significant body of work focusing on the involvement of TGF- β in nasal polyposis, allergic rhinitis has received scant attention in this respect. Our own findings

have clearly identified increased epithelial immuno-expression of TGF- β and TGF- β receptors in perennial and seasonal (in-season) allergic rhinitis compared with non-atopic non-rhinitic controls.¹⁷ However, *in vivo* consequences of TGF- β -induced effects are difficult to infer from local

concentrations, since TGF- β -induced effects depend on a complex system of regulatory proteins and receptors. This is illustrated in the contrasting results where TGF- β has been reported and linked to fibrosis in asthmatic airways,²¹ whereas in other studies no differences were reported in TGF- β expression between asthmatic and healthy controls.²² An explanation for this has been put forward by a study of pulmonary fibrosis in mice. Mice that were deficient in TGF- β -activating protein were protected from fibrosis, although TGF- β expression remained the same as in the fibrotic mice.²³ This highlights that the effects of TGF- β depend not only on its local concentrations, but also on regulatory proteins. The gene expression of TGF- β in naturally occurring allergic rhinitis, including seasonal (in and out of season), and perennial disease using RT-PCR has not yet been defined. There has so far only been one published study examining the gene expression levels of TGF- β and related transcripts in seasonal allergic rhinitis with comparison to healthy controls.²⁰ This study employed DNA microarray analysis techniques to study the gene expression levels of transcripts encoding TGF- β , TGF- β -regulatory proteins and receptors in patients with symptomatic seasonal allergic rhinitis and healthy controls. The study also looked at the nasal lavage fluid levels of TGF- β 1 in both groups. In this study, no differences in TGF- β 1 protein or mRNA levels were found between patients and the controls. In contrast, the authors found low expression levels of TGF- β -inducible transcripts in the disease group compared to the controls, suggestive of potentially reduced TGF- β activity. The authors went on to suggest that this potentially decreased activity of this anti-inflammatory cytokine may contribute to the chronicity of the allergic inflammatory reaction.

This study is the first to use a real-time quantitative gene expression method (TaqMan RT-PCR) to quantify TGF- β (TGF- β 1, TGF- β 2, TGF- β 3, TGF- β RI, TGF- β RII, TGF- β RIII) gene expression in nasal mucosal biopsies from subjects with naturally occurring seasonal (in and out of season) and perennial allergic rhinitis, and in healthy non-atopic non-rhinitic volunteers for comparison. Furthermore, and using the same method, the downstream effects of TGF- β were examined by quantifying the gene expression levels of the TGF- β -inducible gene CTGF, as well as the Smad proteins (Smad-3, Smad-6, and Smad-7) as markers of TGF- β signal transduction. There was an increase in TGF- β 1, TGF- β 2, and TGF- β 3 mRNA levels in the disease groups, particularly the seasonal (in season) group, compared with the controls. However, these differences did not reach statistical significance. There was a similar trend for the three TGF- β receptors, with the mRNA levels being increased for the three receptors in the seasonal (in season) group compared to the controls, significantly so for TGF- β RI and TGF- β RII. Overall, the results of the gene expression analysis concur with the epithelial immuno-expression data which showed enhanced immunoreactivity levels for TGF- β isotypes and receptors in the disease groups compared to the

controls.¹⁷ The fact that the increases were only significant for TGF- β RI and TGF- β RII and mainly in the seasonal (in season) group could be due to a number of factors. It is possible that the TGF- β gene levels are particularly up-regulated in relation to an acute allergic/inflammatory reaction as in the seasonal (in season) group, as opposed to a chronic type picture as in perennial rhinitis where the changes were not as impressive. Alternatively, it is feasible that TGF- β mRNA levels are not necessarily related to the protein levels, and that gene transcriptional changes are occurring at a later stage.

Whilst the up-regulation of the TGF- β receptors in the disease group would suggest that TGF- β activity in seasonal (in season) allergic rhinitis might be increased compared to non-atopic controls, nevertheless, and in view of the complex nature of TGF- β function and signalling pathways, it would be presumptuous to make this statement based on the levels of individual transcripts or proteins. Therefore, the downstream effects of TGF- β were investigated by examining the gene expression of the Smad-3, Smad-6, and Smad-7, part of a family of proteins involved in propagating the TGF- β signal from the cell membrane to the nucleus, as well as CTGF, one of the TGF- β -inducible genes.

Recent identification of the Smad family of proteins has advanced our understanding of how TGF- β signals from the membrane to the nucleus.²⁴ The receptor-regulated or R-Smads (Smad-1, Smad-2, Smad-3, Smad-5 and Smad-8) are directly phosphorylated by the type I receptors. The Co-Smads which contain one member, Smad-4, form heteromeric complexes with the R-Smads which accumulate in the nucleus following their phosphorylation. In contrast, the inhibitory Smads (Smad-6 and Smad-7) oppose the effects of R-Smads and, therefore, antagonise TGF- β signalling.²⁵ The activated TGF- β receptors induce phosphorylation of Smad-2 and Smad-3, which form hetero-oligomeric complexes with Smad-4. The complexes then translocate to the nucleus and regulate transcriptional responses, together with DNA binding cofactors. Thus, phosphorylation of Smad-2 and Smad-3 is a key step in the initiation of TGF- β signal transduction and serves as an indicator of active TGF- β signalling. Anti-phosphorylated Smad antibodies have recently been used to demonstrate TGF- β /activin-mediated signalling *in vivo* in antigen-induced airway inflammation and that this activity was associated with remodelling changes within the lower airway in asthma.^{26,27} So far, there has been no published studies relating to Smad expression or function within the upper airways. Thus, the choice of Smads included Smad-3 as a key initiator of the TGF- β transduction process and the two inhibitory Smads (Smad-6 and Smad-7) in order to also establish whether there was antagonism of TGF- β signalling in the absence of activation. CTGF, a known fibroblast mitogen,²⁸ and promoter of collagen deposition, has been shown to work in concert with TGF- β to promote fibrogenesis.²⁹ TGF- β is a recognised potent inducer of CTGF, and disruption of this interaction has been recognised

as having an antifibrotic potential. The only data available relating to the gene expression levels of CTGF come from the DNA microarray analysis of Benson *et al.*;²⁰ their results are suggestive of lower CTGF gene expression levels in patients compared to controls.

Interestingly, there did not appear to be any significant disease-related changes in Smad-3, Smad-6, and Smad-7 gene expression levels, whereas CTGF gene expression appeared to be up-regulated in the seasonal (in season) disease group compared to the controls. Thus the CTGF findings appeared to correlate with the findings for the TGF- β receptors (RI and RII). Despite the lack of change in the Smad proteins, the changes in CTGF in acute seasonal disease coupled with the associated increase in the signalling receptors TGF- β RI and TGF- β RII, would suggest an increased level of TGF- β activity in acute seasonal rhinitis. This is because the Smad signalling pathway is only one of a host of signalling pathways involved in TGF- β signal transduction. It has been suggested that Rho-like guanosine triphosphatases may play a critical role in TGF- β activation and its downstream pathways,³⁰ including CTGF induction in fibrogenesis. In confirmation of this, a recent study has shown that CTGF expression and induction by TGF- β is abrogated by Simvastatin (statin, antilipidaemic agent) via a Rho signalling mechanism.³¹ Therefore, just because the Smad proteins are not apparently changed does not automatically exclude other signalling pathways from being actively involved, such as the Rho signalling pathway in the case of CTGF.

Whilst the magnitude of the changes in gene expression levels of the TGF- β receptors I and II as well as CTGF in seasonal disease are relatively small in absolute terms, nevertheless they were statistically significant. It could be argued, however, that in view of the magnitude of the changes, they could be non-specific. To address this issue, it was considered important to examine another marker which is expected to be up-regulated in acute allergic/inflammatory situations such as in acute seasonal rhinitis, thus acting essentially as a positive marker. Whilst cell adhesion molecules, such as intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1), are known to be up-regulated in seasonal (in season) rhinitis, the degree of increase is relatively small; in addition, these molecules are predominantly epithelially expressed and would not necessarily be of significant benefit in nasal biopsy specimens where the vast majority of the tissue is likely to be submucosal. Whilst TNF- α is known to be a pro-inflammatory cytokine,³² the relationship between TNF- α and allergic rhinitis has not been clarified. TNF- α has been demonstrated within the airway epithelium in nasal biopsies taken from individuals with allergic rhinitis,^{33,34} and has also been shown to contribute to the development of allergic rhinitis in a murine model.³⁵ TNF- α also has actions relevant to eosinophil activation and recruitment. A study which looked at the TNF- α mRNA expression levels in asthma found the levels increased in bronchoalveolar lavage from atopic asthmatics compared to normal controls.³⁶

Furthermore, TNF- α gene expression has been shown to be significantly up-regulated in bronchial biopsies from severe asthmatics compared with mild asthmatics.³⁷ Indeed, the results clearly showed a significant up-regulation of TNF- α mRNA levels in acute seasonal disease compared with the controls. Whilst there was a high degree of variability in the TNF- α mRNA levels in the seasonal (in season) group, this could well be a reflection of the differing levels of inflammation depending on the nature (extent of inflammation at the biopsy site) and site (epithelium versus submucosa) of the biopsy specimen. Despite this, the trend of the increase was clear and confirms that the changes encountered in relation to TGF- β receptor and CTGF gene expression in the acute seasonal group are likely to be genuine, albeit of low magnitude. Whilst displaying lower variability levels in general, there was also a non-significant increase in TNF- α mRNA levels in the perennial rhinitis group compared with the controls. It is feasible that the discrepancy in the levels is related to the level of inflammation which is likely to be more pronounced in the acute seasonal disease compared with a chronic low grade type inflammatory picture such as in the perennial disease variety.

Conclusions

This is the first study to employ a real-time quantitative gene expression assay to examine gene expression levels of TGF- β isoforms and receptors in seasonal and perennial allergic rhinitis in the natural setting. Whole biopsy gene expression studies found no differences between the rhinitic and non-rhinitic groups in the expression of TGF- β isoforms, or in Smad-3, Smad-6 or Smad-7, but did find increased gene expression for TGF- β RI and TGF- β RII along with CTGF in seasonal allergic rhinitis. Whilst the results of this study will help advance our understanding of the role of TGF- β in the pathogenesis of the inflammatory response in allergic rhinitis, nevertheless future studies are urgently needed to shed further light on the role of TGF- β role in the upper airways and paranasal sinuses. Evidently, the unravelling of its function is likely to represent a formidable, yet rewarding, challenge for the future. The main obstacle in translating any future findings therapeutically, however, is likely to stem from the pleiotropic and complex nature of TGF- β 's regulatory role.

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