

REVIEW

Transforming growth factor- β in allergic inflammatory disease of the upper airways: friend or foe?

R. J. Salib^{*†} and P. H. Howarth^{*}

^{*}Division of Infection, Inflammation and Repair, School of Medicine, Southampton General Hospital, Southampton SO16 6YD, UK and [†]Department of Otorhinolaryngology, Southampton University Hospitals NHS Trust, Southampton SO16 6YD, UK

Clinical & Experimental Allergy

Summary

TGF- β is a multi-functional cytokine with a huge array of effects on a variety of cell types. It is rapidly emerging as a key major player in the way the airway epithelium behaves and its ability to repair itself. This is not only of relevance to allergic airway diseases such as asthma and allergic rhinitis, which are increasing in prevalence worldwide, but in many other diseases. The full impact any disruption of TGF- β signalling may have in the development and persistence of allergic inflammatory airway diseases is yet to be fully realized and remains the subject of ongoing research. There has been a recent revival of interest in the role of regulatory T cells in controlling allergic inflammation. Evidence is emerging of a significant contribution by TGF- β to this regulatory process. This review aims to summarize current knowledge relating to TGF- β in relation to allergic inflammatory upper airways disease, and attempts to clarify some of the discrepancies and inconsistencies in this area. It also considers the therapeutic implications of novel TGF- β therapy, including potential future applications in the treatment of nasal polyposis and reduction of post-operative scar tissue formation following endoscopic sinus surgery.

Correspondence:

Dr Rami J. Salib, Department of Otorhinolaryngology, Southampton University Hospitals, Tremona Road, Southampton SO16 6YD, UK.
E-mail: rjs4@soton.ac.uk

Introduction

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 its ability to induce a transformed or tumour cell characteristic in normal cells. Subsequently, it was shown that TGF- β inhibits epithelial cell proliferation and regulates a diverse array of cellular functions unrelated to cellular transformation. Nevertheless, the name transforming growth factor has persisted to the present day. Today, the TGF- β superfamily is recognized as one of the largest families of secreted multi-functional 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, and bone morphogenic proteins.

TGF- β family members are produced as large precursor dimeric protein, with a signal sequence and a large pro-domain, that are processed proteolytically into mature proteins [3]. 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]. The Smad proteins have been identified as the main family of intracellular mediators whose function is the regulation of TGF- β signalling from the cell surface to the nucleus [13, 14]. Up to date reviews have been published regarding TGF- β activation, receptor signalling, and signal transduction [15–17].

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 with mutations in the TGF- β signalling pathway having been implicated in such conditions. These include mutations in receptors which can cause hereditary haemorrhagic telangiectasia, primary pulmonary hypertension, persistent müllerian duct syndrome, hereditary non-polyposis colon

cancer, and juvenile polyposis syndrome, to name but a few [18]. Smad mutations have also been linked with cancers, particularly those of the colon and gastrointestinal tract [19, 20]. As such, one of the main challenges faced in molecular medicine will be to further our understanding of the TGF- β signalling pathway to the stage where molecular strategies can be devised in order to improve disease control [18].

TGF- β is an important fibrogenic and immunomodulatory factor that may play a role in the structural changes observed in the asthmatic airways. Inconsistent, and at times contradictory, evidence has emerged regarding the role and nature of TGF- β expression in the lower airways, and in particular asthma. This subject has, however, been comprehensively reviewed in the literature [21–24] and will not be addressed any further in this article. In comparison, the equivalent picture in upper airway allergic disease, and in particular allergic rhinitis and nasal polyposis, has received less attention in the literature and as such will constitute the primary focus of this review.

Transforming growth factor- β in inflammatory cell chemotaxis

The attraction of leucocytes to tissues represents a key step in the genesis and maintenance of the inflammatory response. This process is mediated through an increasingly appreciated number of chemotactic cytokines, otherwise known as chemokines. Allergic rhinitis, an inflammatory mucosal disorder of the nose, is characterized by the epithelial cell accumulation of mast cells [25]. This phenomenon can be linked to the local generation and release of chemotactic factors. TGF- β is recognized as a potent mast cell chemoattractant, with maximum chemotaxis *in vitro* being evident at the femtomolar level, which is several log orders less than the picomolar concentrations of eotaxin, regulated upon activation normal T cell expressed and secreted, and stem cell factor that are required to induce mast cell chemotaxis under the same circumstances [26]. Human gastrointestinal mast cells have been shown to express TGF- β receptors, and the ability of TGF- β to promote mast cell chemotaxis has previously been demonstrated *in vitro* with both a human mast cell line and cultured primary gut mast cells [26]. This chemotactic response has been considered to be mediated by the TGF- β serine/threonine type I and type II receptors [26]. The findings of a recent study of enhanced epithelial TGF- β immunoreactivity in rhinitis which correlated with intraepithelial mast cell numbers, and the colocalization 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 [27]. In femtomolar concentrations, TGF- β 1 has also been shown to be

strongly chemotactic for neutrophils, T cells, monocytes, and fibroblasts [11, 12, 26, 28].

Immunomodulatory role of transforming growth factor- β

The TGF- β family members play an important role in the regulation of the immune system [29]. This is clearly evident in TGF- β 1 null mice, which develop extensive multi-organ inflammation and die shortly after birth [30]. Furthermore, mutations of TGF- β 2 or TGF- β 3 in a similar model have also been reported to result in embryonic lethality [31, 32]. *In vitro*, all three TGF- β isoforms appear to exert similar immunoregulatory effects [33]. These include an inhibitory effect on T cell proliferation [34], and differentiation [35]. With the prevalence of allergic diseases such as asthma, atopic dermatitis, and rhinitis on the increase worldwide, interest has been recently revived in the role of CD24⁺CD25⁺ regulatory T cells (Tregs) in allergic disease. Regulatory T cells have been shown to be critical in maintaining the balance between tolerance and immunity, a process crucial to immune homeostasis and prevention of autoimmune diseases. There are two populations of Tregs: natural (constitutive) and inducible (adaptive). The inducible Tregs include type I regulatory cells (Tr-1) and T helper (Th)3 with the latter secreting high levels of TGF- β resulting in suppression of Th1 and Th2 responses. In addition, there are CD8⁺ T cells with regulatory activity, which exert their function through IL-10 or TGF- β [36].

While the role of Tregs in allergic disease is not well understood, there is compelling evidence emerging that Tregs can regulate Th2 immune responses, which characterize allergic disorders, by attenuation of allergic sensitization and prevention in progression of allergic diseases [36]. The immunosuppressive effects of Tregs are thought to be mediated through cell-contact-dependent mechanisms involving membrane-bound TGF- β interacting with CD4⁺CD25⁺ responder cells through TGF- β receptor II [37]. Not only can TGF- β mediate cell–cell suppression between Tregs and CD4⁺, CD25⁺, or CD8⁺ cells, but it is thought to play a role in the conversion of CD4⁺CD25⁺ T cells, in the presence of T cell receptor antigenic stimulation, into the regulatory phenotype. In its role as an immunoregulatory cytokine, TGF- β can also directly suppress T cell proliferation and macrophage activation [37]. Factors which contribute to the regulation of TGF- β in Tregs include CD28, cytotoxic T lymphocyte antigen-4, glucocorticoid-induced TNF receptor, and forkhead/winged helix or forkhead box P3 encoded by Foxp3 [37].

Clinical improvement following allergen immunotherapy is associated with induction of TGF- β -producing Tr-1 cells with resultant suppression of the Th2 cytokine profile [38]. As we improve our understanding of regulatory mechanisms involved in the development of allergic

senitization, the targeting of natural and TGF- β -producing Tregs holds promise as the basis for development of future immunotherapies for more effective control and prevention of allergic inflammatory diseases [36, 38–40].

Transforming growth factor- β and tissue repair

Advances in molecular and cell biology have led to an expansion in our knowledge and understanding of the processes involved in wound healing. The actual process of wound healing involves a coordinated sequence of biological events, beginning with platelet-induced haemostasis, followed by an influx of inflammatory cells including fibroblasts, the formation of new extracellular matrix and blood vessels (granulation tissue), and finally the proliferation of cells to reconstitute the tissue. TGF- β plays an important role in the coordination of these events. Inactive TGF- β 1, bound locally to the extracellular matrix, is activated following injury, and thereafter acts as a potent stimulus for the deposition of extracellular matrix, in addition to regulating cell migration to sites of inflammation. TGF- β exhibits a bell-shaped concentration–response curve, inducing proliferation at low concentrations while inhibiting it at higher concentrations. TGF- β is also a potent stimulus for fibroblast proliferation and extracellular matrix deposition [2]. The extracellular matrix is a dynamic superstructure of self-aggregating macromolecules including fibronectin, collagen, and proteoglycans, to which cells attach by means of surface receptors known as integrins [41]. TGF- β 1 induces the deposition of extracellular matrix by simultaneously stimulating cells to increase the production of matrix proteins, decrease the production of matrix-degrading proteases, increase the production of inhibitors of these proteases, as well as modulating the expression of integrins so as to make cells adhere more to the matrix [4].

The sustained production of TGF- β can, however, lead to an unbalanced deposition of extracellular matrix, which underlies the development of tissue fibrosis. The fibrogenic potential of TGF- β has been realized following repeated injections of higher doses. Two weeks of intravenous injections of TGF- β 1 produced marked fibrosis in the kidneys and liver of rats [42]. Severe cachexia and generalized tissue fibrosis developed in mice given TGF- β 1 intraperitoneally for 10 days [43]. TGF- β has also been implicated in the pathogenesis of fibrosis in kidney, liver, and lung diseases. In normal tissue repair, the production of TGF- β and extracellular matrix is terminated by as yet unknown mechanisms, as the tissue repairs itself. In chronic disease states, either repeated tissue injury, or a defect in TGF- β regulation, or both could potentially lead to the uncontrolled activation/production of TGF- β with corresponding deposition of extracellular matrix, leading eventually to tissue fibrosis [5].

Upper airways and paranasal sinuses

Nasal polyposis

Our current understanding of the role of TGF- β in the pathogenesis of chronic inflammatory conditions of the upper airway and sinuses remains in its infancy. The vast majority of conducted research has so far focused on the involvement of TGF- β in nasal polyposis, with allergic rhinitis receiving less attention in this respect.

Nasal polyposis is a chronic inflammatory disease of the paranasal sinuses, characterized by a benign growth of the nasal and paranasal sinus mucosa in the region of the middle/superior meati and sphenoethmoidal recesses. Histopathologically, nasal polyps consist of oedematous connective tissue covered with respiratory epithelium. The subepithelial area is characterized by eosinophilic infiltration in >80% of cases; however, non-eosinophilic nasal polyposis also exists and should be differentiated [44]. The pathogenesis of nasal polyposis remains unclear with the eosinophil thought to be the major orchestrator of the inflammatory response in nasal polyps. How this eosinophilic inflammation leads to polyp formation remains largely unclear. Furthermore, there is little agreement as to whether increased fibrosis and basement membrane thickening are consistent histological features of nasal polyps. Studies investigating the histological features specific to nasal polyps have reported varying degrees of fibrosis, basement membrane and epithelial thickening associated with these conditions [45, 46]. However, these findings have been readily contested in other studies [47, 48].

Similarly variable results have been reported relating to the distribution and likely actions of TGF- β in nasal polyposis. Some studies have suggested an up-regulation of TGF- β levels in nasal polyps compared with normal tissue [49]. This is particularly the case for TGF- β 1 [50–55]. Using Northern blot analysis, *in situ* hybridization and immunohistochemistry, a study found TGF- β 1 to be present only in inflamed nasal polyp and not in normal mucosa [56]. The TGF- β 1 was further localized specifically to eosinophils, the extracellular matrix, and the basement membrane. Similar findings have been reported for TGF- α in nasal polyposis [48]. This is significant as TGF- α must be present along with TGF- β in order to transform fibroblasts [57]. Other studies, however, have suggested the opposite trend with lower levels of TGF- β activity in nasal polyps compared with normal tissue [44, 58–61]. It is thought that the resultant loss of the anti-inflammatory action of TGF- β is partly responsible for the propagation of the inflammatory response resulting ultimately in the formation and growth of the polyps [60].

TGF- β is thought to contribute to some of the pathological features observed in nasal polyps such as thickening of the epithelial basement membrane and stromal fibrosis [54,

55, 62]. One study has suggested a dual effect for TGF- β 1 expression in nasal polyps with an anti-inflammatory role through its ability to inhibit pro-inflammatory mRNA and protein production, as well as a pro-fibrotic effect promoting remodelling and cell growth [63]. TGF- β -induced fibroblast proliferation and myofibroblast differentiation in nasal polyps has been shown to be inhibited *in vitro* by the corticosteroid fluticasone propionate [64]. Budesonide, another corticosteroid, has also been shown to reduce TGF- β 1 expression levels in nasal polyps [65]. Other evidence, however, has suggested that corticosteroids used in the treatment of nasal polyps may exert their beneficial effect through the up-regulation of anti-inflammatory genes including TGF- β [66].

These opposing findings have thus led to different theories regarding the possible roles of TGF- β in nasal polyposis and the effects of corticosteroids on its expression.

Allergic rhinitis

While there has been a sizeable body of work focusing on the involvement of TGF- β in nasal polyposis, allergic rhinitis has received scant attention in this respect. Similarly conflicting findings, as in nasal polyposis, exist regarding the nature and extent of the remodelling response in the upper airway in allergic rhinitis [67–69]. Some observers believe that remodelling changes, and in turn TGF- β expression levels, may fluctuate in the nasal mucosa depending on the degree of inflammatory cellular infiltration, with respect to whether the tissue was extracted during seasonal exacerbations or periods of remission (e.g. in and out of the pollen season) [70]. This might at least partly account for some of the discrepant findings in various studies. It is fair to state, however, that the overall balance of evidence available seems to suggest that there is usually some discernible evidence of remodelling in the rhinitic nasal mucosa [69].

Evidence has been provided of enhanced epithelial TGF- β immunoreactivity in allergic rhinitis, including correlation with intraepithelial mast cell numbers, and the co-localization of TGF- β receptors to mast cells [27], 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. 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 and house dust mite to up-regulate both TGF- β gene expression and protein release. It is presumed, therefore, that house dust mite-activated mast cells in suitably sensitized individuals can, possibly through TNF- α release, up-regulate TGF- β expression in epithelial cells. With the presence of TGF- β receptor expression on mast cells, this

would suggest the possibility of bi-directional interaction, which may be fundamental to the process of epithelial immunoregulation.

However, *in vivo* consequences of TGF- β -induced effects are difficult to infer from local concentrations, because TGF- β -induced effects depend on a complex system of regulatory proteins and receptors. This is best illustrated by the contrasting results of one study linking TGF- β to fibrosis in asthmatic airways [71], with another study failing to show any significant difference in TGF- β expression between asthmatic and healthy controls [72]. An explanation for this has been put forward by a study on pulmonary fibrosis in mice. Mice that were deficient in TGF- β -activating protein (α v β 6 integrin) were protected from fibrosis, although TGF- β expression remained the same as in the fibrotic mice [73]. This highlights that TGF- β effects depend not only on its local concentrations, but also on regulatory proteins.

A study employing DNA microarray analysis techniques investigated 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 [74]. The study also looked at the nasal lavage fluid levels of TGF- β 1 in both groups. No significant differences in TGF- β 1 protein or mRNA levels were found between patients and the controls. In contrast, low expression levels of TGF- β -inducible transcripts were found in the disease group compared with 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. In a mouse model of allergic rhinitis, dexamethasone has been shown to up-regulate the levels of TGF- β suggesting a possible mechanism for its anti-inflammatory action [75]. Our group employed a real-time quantitative PCR (TaqMan RT-PCR) to examine whole biopsy gene expression levels of TGF- β isotypes and receptors in seasonal and perennial allergic rhinitis in the natural setting [76]. Increased gene expression levels were found for TGF- β RI and - β RII in seasonal allergic rhinitis compared with controls. No corresponding significant differences were found in relation to TGF- β isoform levels.

Potential therapeutic applications and future challenges

As a potent growth factor involved in wound healing in the body, TGF- β has been used to accelerate wound healing through topical, subcutaneous, or incisional application [77]. Furthermore, TGF- β 2 has been used to repair retinal holes with TGF- β 3 appearing to accelerate wound healing [4, 78–80]. Under certain conditions, TGF- β 1 can also induce angiogenesis *in vivo* causing endothelial cells to organize into tubular structures [81]. TGF- β has also been recently targeted in an attempt to decrease

or inhibit the scarring response. A potentially useful strategy for modifying the scarring response has been the use of fully humanized neutralizing monoclonal antibodies to TGF- β . Human neutralizing antibody to TGF- β 2 (CAT-152) was found significantly to reduce conjunctival scarring in a rabbit model of glaucoma filtration surgery with a resultant improvement in the surgical outcome both *in vitro* and *in vivo* [82]. Similar results were found in patients 1 year after undergoing primary trabeculectomy for glaucoma [83].

With the apparent success of this modality, the next logical step was to test specifically designed antisense oligonucleotides (OGN) against TGF- β isoforms. OGN represent a promising therapeutic modality, which has already been successfully applied in oncological, inflammatory and viral infective diseases [84–86]. They represent a potentially novel and more direct approach to inhibit the progression of aberrant tissue repair and regeneration. Antisense technology has as its basis the selective impairment of protein synthesis in the cytoplasm through the use of antisense OGN sequences. The use of novel antisense OGN targeting TGF- β 1 in two different animal models of glaucoma filtration surgery has been shown to significantly reduce post-operative scarring and improve surgical outcome [87]. The usefulness of this approach is further underlined by its apparent long-lasting effects following only a single administration at the time of surgery, thus making it particularly attractive in the clinical context. Promising effects have also been observed in cataract surgery [88].

Apart from its use in the eye, this novel concept could have applications virtually anywhere in the body where modulation of the wound healing response is deemed to be both desirable and beneficial. These applications include the potential use of these modalities in allergic inflammatory lower airway diseases such as in asthma where remodelling changes including subepithelial fibrosis [89–91] have been linked to the pro-fibrotic effect of TGF- β [92]. Other chronic inflammatory diseases which could also benefit include chronic obstructive pulmonary disease and chronic bronchitis, both of which display histological evidence of extracellular matrix deposition associated with fibroblast proliferation, as well as pulmonary fibrosis where TGF- β has been linked to the dysregulated repair process [93]. However, one potential problem in utilizing such a therapeutic approach could be that while attempting to suppress or even reverse these structural changes in the airway, the resultant loss of TGF- β 's immunosuppressive effect, may indirectly lead to the up-regulation of the Th2 inflammatory response.

It would be difficult at this point in time to speculate on any equivalent potential application and likely benefit of this novel anti-scarring therapy in the upper airways and sinuses, particularly as our current knowledge of the expression, action, and regulation of TGF- β is currently

limited and at best controversial. Nevertheless, one potential application of antisense OGN which target TGF- β in the nose could be in the adjunctive treatment of nasal polyps, particularly those which display histological features consistent with increased fibrosis and basement membrane thickening. Furthermore, and in the surgical context, the outcome of endoscopic sinus surgery can often be significantly compromised by the occurrence of excessive post-operative scarring. TGF- β has been recently implicated in the healing process following such surgery [94]. Thus the design and use of future therapeutic modalities capable of modifying TGF- β expression and activity, particularly those that can be applied at the time of surgery, could help reduce the degree of post-operative scar tissue formation, with a consequent improvement in the surgical outcome.

It can be argued, however, that this represents far too simplistic an approach in view of the pleiotropic actions of TGF- β on virtually every cell type in the body. For example, using TGF- β as an immunosuppressive agent to treat allergic inflammatory disease would certainly represent a very attractive therapeutic option, but how can the unwanted pro-fibrotic effects be avoided? The ideal scenario would entail the ability to harness TGF- β 's desirable therapeutic effects on a particular cell type, while preventing any unwanted complications or side effects elsewhere in the body [77]. This is likely to represent a formidable, yet rewarding, challenge for the future.

Conclusions

The TGF- β story remains in its infancy and largely unexplored. TGF- β has many faces and a host of tantalizing properties. In particular, its wide-ranging effects on cells of the immune system can be bewildering and even contradictory. This cytokine can stimulate cellular growth, differentiation, migration, and cytokine production but can also inhibit these very same processes. TGF- β stimulatory or inhibitory effects can depend on the cell type and in some cases the level of differentiation of a particular cell. The role of TGF- β in allergic inflammatory upper airway disease remains highly controversial and further research is needed to clarify the many discrepancies and inconsistencies that have emerged to date. Table 1 summarizes some of TGF- β 's effects on a variety of cell types in allergic inflammatory airway disease. As far as the answer to the question posed in the title of this review, we feel on balance that TGF- β in allergic inflammatory upper airway disease could be both friend and foe. It might be easiest to think of TGF- β as a double-edged sword with one facet responsible for persistent epithelial activation, mast cell chemotaxis, and structural changes, and the other exerting an immunomodulatory role by inhibition of T cell activation and down-regulation of the inflammatory response. It is perhaps the balance between

Table 1. Some of the effects of TGF- β on different cell types in allergic inflammatory airway disease

Target cell type	Effect of TGF- β
Mast cells, monocytes, neutrophils, and T cells	Chemotactic effect
Naïve T cells	Inhibition of proliferation and pro-inflammatory cytokine production
Regulatory T cells	Enhancement of suppressive ability against T-helper (Th)1 cell-mediated autoimmunity and Th2-mediated allergic inflammation
Epithelium	Persistent epithelial activation with inhibition of epithelial proliferation and suppression of proliferative signals. Prevention of full repair following damage.
Fibroblast	Chemotactic effect. Deposition of extracellular matrix (collagen), basement membrane thickening and subepithelial fibrosis

these two facets which will ultimately determine the end result. Evidently, the unravelling of the function of the TGF- β signalling pathway is likely to require a significant amount of work and will undoubtedly represent a significant challenge for the future. The main obstacle in translating these findings therapeutically, however, is likely to stem from the multi-faceted nature of TGF- β 's regulatory role.

Acknowledgements

This work was supported by the Louis Alexander and Augustus Newman Foundation Research Fellowships from the Royal College of Surgeons of England, and an equipment grant from the Section of Laryngology and Rhinology at the Royal Society of Medicine.

References

- Moses HL, Branum EL, Proper JA, Robinson RA. Transforming growth factor production by chemically transformed cells. *Cancer Res* 1981; **41**:2842–8.
- Roberts AB, Anzano MA, Lamb LC, Smith JM, Sporn MB. New class of transforming growth factors potentiated by epidermal growth factor: isolation from non-neoplastic tissues. *Proc Natl Acad Sci USA* 1981; **78**:5339–43.
- Flaumenhaft R, Kojima S, Abe M, Rifkin DB. Activation of latent transforming growth factor beta. *Adv Pharmacol* 1993; **24**:51–76.
- Border WA, Noble NA. Transforming growth factor beta in tissue fibrosis. *N Engl J Med* 1994; **331**:1286–92.
- Massague J. The transforming growth factor-beta family. *Annu Rev Cell Biol* 1990; **6**:597–641.
- Sporn MB, Roberts AB. Transforming growth factor-beta: recent progress and new challenges. *J Cell Biol* 1992; **119**:1017–21.
- Wahl SM. Transforming growth factor beta: the good, the bad, and the ugly. *J Exp Med* 1994; **180**:1587–90.
- Lawrence DA. Transforming growth factor-beta: a general review. *Eur Cytokine Netw* 1996; **7**:363–74.
- Reibman J, Meixler S, Lee TC *et al.* Transforming growth factor beta 1, a potent chemoattractant for human neutrophils, bypasses classic signal-transduction pathways. *Proc Natl Acad Sci USA* 1991; **88**:6805–9.
- Brandes ME, Mai UE, Ohura K, Wahl SM. Type I transforming growth factor-beta receptors on neutrophils mediate chemotaxis to transforming growth factor-beta. *J Immunol* 1991; **147**:1600–6.
- Postlethwaite AE, Keski-Oja J, Moses HL, Kang AH. Stimulation of the chemotactic migration of human fibroblasts by transforming growth factor beta. *J Exp Med* 1987; **165**:251–6.
- Wahl SM, Hunt DA, Wakefield LM *et al.* Transforming growth factor type beta induces monocyte chemotaxis and growth factor production. *Proc Natl Acad Sci USA* 1987; **84**:5788–92.
- Heldin CH, Miyazono K, ten Dijke P. TGF-beta signalling from cell membrane to nucleus through SMAD proteins. *Nature* 1997; **390**:465–71.
- Wrana JL. Regulation of Smad activity. *Cell* 2000; **100**:189–92.
- ten Dijke P, Hill CS. New insights into TGF-beta-Smad signalling. *Trends Biochem Sci* 2004; **29**:265–73.
- Shi Y, Massague J. Mechanisms of TGF-beta signaling from cell membrane to the nucleus. *Cell* 2003; **113**:685–700.
- Derynck R, Zhang YE. Smad-dependent and Smad-independent pathways in TGF-beta family signalling. *Nature* 2003; **425**:577–84.
- Zhu HJ, Burgess AW. Regulation of transforming growth factor-beta signaling. *Mol Cell Biol Res Commun* 2001; **4**:321–30.
- Massague J, Chen YG. Controlling TGF-beta signaling. *Genes Dev* 2000; **14**:627–44.
- de Caestecker MP, Piek E, Roberts AB. Role of transforming growth factor-beta signaling in cancer. *J Natl Cancer Inst* 2000; **92**:1388–402.
- Bosse Y, Rola-Pleszczynski M. Controversy surrounding the increased expression of TGF beta 1 in asthma. *Respir Res* 2007; **8**:66.
- Makinde T, Murphy RF, Agrawal DK. The regulatory role of TGF-beta in airway remodeling in asthma. *Immunol Cell Biol* 2007; **85**:348–56.
- Howell JE, McAnulty RJ. TGF-beta: its role in asthma and therapeutic potential. *Curr Drug Targets* 2006; **7**:547–65.
- Duvernelle C, Freund V, Frossard N. Transforming growth factor-beta and its role in asthma. *Pulm Pharmacol Ther* 2003; **16**:181–96.
- Bradding P, Feather IH, Wilson S *et al.* Immunolocalization of cytokines in the nasal mucosa of normal and perennial rhinitic subjects. The mast cell as a source of IL-4, IL-5, and IL-6 in human allergic mucosal inflammation. *J Immunol* 1993; **151**:3853–65.
- Olsson N, Piek E, ten Dijke P, Nilsson G. Human mast cell migration in response to members of the transforming growth factor-beta family. *J Leukoc Biol* 2000; **67**:350–6.
- Salib RJ, Kumar S, Wilson SJ, Howarth PH. Nasal mucosal immunoexpression of the mast cell chemoattractants TGF-beta,

- eotaxin, and stem cell factor and their receptors in allergic rhinitis. *J Allergy Clin Immunol* 2004; 114:799–806.
- 28 Roberts AB, Joyce ME, Bolander ME, Sporn MB. Transforming growth factor-beta (TGF- β): a multifunctional effector of both soft and hard tissue regeneration. In: Westermarck B, Betsholtz C, Hokfelt B, eds. *Growth factors in health and disease: basic and clinical aspects*. Amsterdam: Excerpta Medica, 1990; 89–101.
 - 29 Gorelik L, Flavell RA. Transforming growth factor-beta in T-cell biology. *Nat Rev Immunol* 2002; 2:46–53.
 - 30 Kulkarni AB, Huh CG, Becker D *et al*. Transforming growth factor beta 1 null mutation in mice causes excessive inflammatory response and early death. *Proc Natl Acad Sci USA* 1993; 90:770–4.
 - 31 Sanford LP, Ormsby I, Gittenberger-de Groot AC *et al*. TGFbeta2 knockout mice have multiple developmental defects that are non-overlapping with other TGFbeta knockout phenotypes. *Development* 1997; 124:2659–70.
 - 32 Proetzel G, Pawlowski SA, Wiles MV *et al*. Transforming growth factor-beta 3 is required for secondary palate fusion. *Nat Genet* 1995; 11:409–14.
 - 33 Schluesener H, Jung S, Salvetti M. Susceptibility and resistance of human autoimmune T cell activation to the immunoregulatory effects of transforming growth factor (TGF) beta 1, beta 2, and beta 1.2. *J Neuroimmunol* 1990; 28:271–6.
 - 34 Kehrl JH, Wakefield LM, Roberts AB *et al*. Production of transforming growth factor beta by human T lymphocytes and its potential role in the regulation of T cell growth. *J Exp Med* 1986; 163:1037–50.
 - 35 Sad S, Mosmann TR. Single IL-2-secreting precursor CD4 T cell can develop into either Th1 or Th2 cytokine secretion phenotype. *J Immunol* 1994; 153:3514–22.
 - 36 Elkord E. Role of regulatory T cells in allergy: implications for therapeutic strategy. *Inflamm Allergy Drug Targets* 2006; 5:211–7.
 - 37 Wahl SM, Chen W. Transforming growth factor-beta-induced regulatory T cells referee inflammatory and autoimmune diseases. *Arthritis Res Ther* 2005; 7:62–8.
 - 38 Nouri-Aria KT, Durham SR. Regulatory T cells and allergic disease. *Inflamm Allergy Drug Targets* 2008; 7:237–52.
 - 39 Akdis M, Blaser K, Akdis CA. T regulatory cells in allergy: novel concepts in the pathogenesis, prevention, and treatment of allergic diseases. *J Allergy Clin Immunol* 2005; 116:961–8.
 - 40 Taylor A, Verhagen J, Blaser K, Akdis M, Akdis CA. Mechanisms of immune suppression by interleukin-10 and transforming growth factor-beta: the role of T regulatory cells. *Immunology* 2006; 117:433–42.
 - 41 Ruoslahti E. Integrins. *J Clin Invest* 1991; 87:1–5.
 - 42 Terrell TG, Working PK, Chow CP, Green JD. Pathology of recombinant human transforming growth factor-beta 1 in rats and rabbits. *Int Rev Exp Pathol* 1993; 34 (Part B):43–67.
 - 43 Zugmaier G, Paik S, Wilding G *et al*. Transforming growth factor beta 1 induces cachexia and systemic fibrosis without an anti-tumor effect in nude mice. *Cancer Res* 1991; 51:3590–4.
 - 44 Bachert C, Gevaert P, Holtappels G, Cuvelier C, van Cauwenberge P. Nasal polyposis: from cytokines to growth. *Am J Rhinol* 2000; 14:279–90.
 - 45 Cauna N, Manzetti GW, Hinderer KH, Swanson EW. Fine structure of nasal polyps. *Ann Otol Rhinol Laryngol* 1972; 81:41–58.
 - 46 Kakoi H, Hiraide F. A histological study of formation and growth of nasal polyps. *Acta Otolaryngol* 1987; 103:137–44.
 - 47 Bousquet J, Vignola AM, Campbell AM, Michel FB. Pathophysiology of allergic rhinitis. *Int Arch Allergy Immunol* 1996; 110:207–18.
 - 48 Lam SM, Zhu DF, Ahn JM. Transforming growth factor-alpha and rhinitis. *Laryngoscope* 1999; 109:1119–24.
 - 49 Lee CH, Rhee CS, Min YG. Cytokine gene expression in nasal polyps. *Ann Otol Rhinol Laryngol* 1998; 107:665–70.
 - 50 Zaravinos A, Soufla G, Bizakis J, Spandidos DA. Expression analysis of VEGFA, FGF2, TGFbeta1, EGF and IGF1 in human nasal polyposis. *Oncol Rep* 2008; 19:385–91.
 - 51 Rostkowska-Nadolska B, Kapral M, Mazurek U, Gawron W, Pres K. Co-expression of the TGF-beta1 and TGF-beta2 isoforms in nasal polyps and in healthy mucosa. *Postepy Hig Med Dosw* 2007; 61:702–7.
 - 52 Huang L, Liu Z, Lu X, Cui Y. Expression of transforming growth factor beta receptor I and II in chronic rhinosinusitis, nasal polyps and normal nasal mucosa tissues. *Lin Chuang Er Bi Yan Hou Ke Za Zhi* 2006; 20:780–3.
 - 53 Bradley DT, Kountakis SE. Role of interleukins and transforming growth factor-beta in chronic rhinosinusitis and nasal polyposis. *Laryngoscope* 2005; 115:684–6.
 - 54 Wang Q, Wang T, Chen F, Li Z, Xin X. Significance and expression of transforming growth factor beta in human nasal polyp tissue. *Zhonghua Er Bi Yan Hou Ke Za Zhi* 2001; 36:199–202.
 - 55 Guo J, Liu M, Wang X *et al*. Expression and significance of TGF-beta 1 in nasal polyps. *Lin Chuang Er Bi Yan Hou Ke Za Zhi* 2001; 15:206–8.
 - 56 Ohno I, Lea RG, Flanders KC *et al*. Eosinophils in chronically inflamed human upper airway tissues express transforming growth factor beta 1 gene (TGF beta 1). *J Clin Invest* 1992; 89:1662–8.
 - 57 Anzano MA, Roberts AB, Smith JM, Sporn MB, De Larco JE. Sarcoma growth factor from conditioned medium of virally transformed cells is composed of both type alpha and type beta transforming growth factors. *Proc Natl Acad Sci USA* 1983; 80:6264–8.
 - 58 Eisma RJ, Allen JS, Lafreniere D, Leonard G, Kreutzer DL. Eosinophil expression of transforming growth factor-beta and its receptors in nasal polyposis: role of the cytokines in this disease process. *Am J Otolaryngol* 1997; 18:405–11.
 - 59 Rostkowska-Nadolska B, Kapral M, Mazurek U *et al*. The profile of expression of transforming growth factor beta1 and TGFbetaRI, TGFbetaRII and TGFbetaRIII genes in nasal polyps. *Otolaryngol Pol* 2007; 61:944–50.
 - 60 Figueiredo CR, Santos RP, Silva ID, Weckx LL. Microarray cDNA to identify inflammatory genes in nasal polyposis. *Am J Rhinol* 2007; 21:231–5.
 - 61 Hirschberg A, Jokuti A, Darvas Z *et al*. The pathogenesis of nasal polyposis by immunoglobulin E and interleukin-5 is completed by transforming growth factor-beta1. *Laryngoscope* 2003; 113:120–4.
 - 62 Watelet JB, Claeys C, Perez-Novoa C *et al*. Transforming growth factor beta1 in nasal remodeling: differences between chronic rhinosinusitis and nasal polyposis. *Am J Rhinol* 2004; 18:267–72.
 - 63 Little SC, Early SB, Woodard CR *et al*. Dual action of TGF-beta1 on nasal-polyp derived fibroblasts. *Laryngoscope* 2008; 118:320–4.

- 64 Serpero L, Petecchia L, Sabatini F *et al.* The effect of transforming growth factor (TGF)-beta1 and (TGF)-beta2 on nasal polyp fibroblast activities involved upper airway remodeling: modulation by fluticasone propionate. *Immunol Lett* 2006; 105:61–7.
- 65 Chen FQ, Huang WG, Qiao L, Jiang HY. The expression of transforming growth factor alpha and its receptor in nasal polyps. *Lin Chuang Er Bi Yan Hou Ke Za Zhi* 2000; 14: 483–4.
- 66 Benson M. Pathophysiological effects of glucocorticoids on nasal polyps: an update. *Curr Opin Allergy Clin Immunol* 2005; 5:31–5.
- 67 Watelet JB, Van Zele T, Gjomarkaj M *et al.* Tissue remodelling in upper airways: where is the link with lower airway remodeling? *Allergy* 2006; 61:1249–58.
- 68 Bousquet J, Jacot W, Vignola AM, Bachert C, Van Cauwenberge P. Allergic rhinitis: a disease remodeling the upper airways? *J Allergy Clin Immunol* 2004; 113:43–9.
- 69 Salib RJ, Howarth PH. Remodelling of the upper airways in allergic rhinitis: is it a feature of the disease? *Clin Exp Allergy* 2003; 33:1629–33.
- 70 Calderon MA, Lozewicz S, Prior A *et al.* Lymphocyte infiltration and thickness of the nasal mucous membrane in perennial and seasonal allergic rhinitis. *J Allergy Clin Immunol* 1994; 93: 635–43.
- 71 Minshall EM, Leung DY, Martin RJ *et al.* Eosinophil-associated TGF-beta1 mRNA expression and airways fibrosis in bronchial asthma. *Am J Respir Cell Mol Biol* 1997; 17:326–33.
- 72 Hoshino M, Nakamura Y, Sim JJ. Expression of growth factors and remodelling of the airway wall in bronchial asthma. *Thorax* 1998; 53:21–7.
- 73 Kaminski N, Allard JD, Pittet JF *et al.* Global analysis of gene expression in pulmonary fibrosis reveals distinct programs regulating lung inflammation and fibrosis. *Proc Natl Acad Sci USA* 2000; 97:1778–83.
- 74 Benson M, Carlsson B, Carlsson LM *et al.* DNA microarray analysis of transforming growth factor-beta and related transcripts in nasal biopsies from patients with allergic rhinitis. *Cytokine* 2002; 18:20–5.
- 75 Lee SS, Won TB, Kim JW *et al.* Effects of dexamethasone on the expression of transforming growth factor-beta in the mouse model of allergic rhinitis. *Laryngoscope* 2007; 117:1323–8.
- 76 Salib RJ. Transforming growth factor-beta gene expression studies in nasal mucosal biopsies in naturally occurring allergic rhinitis. *Ann R Coll Surg Engl* 2007; 89:563–73.
- 77 Flanders KC, Burmester JK. Medical applications of transforming growth factor-beta. *Clin Med Res* 2003; 1:13–20.
- 78 Sporn MB, Roberts AB, Shull JH *et al.* Polypeptide transforming growth factors isolated from bovine sources and used for wound healing in vivo. *Science* 1983; 219:1329–31.
- 79 Loewen MS, Walner DL, Caldarelli DD. Improved airway healing using transforming growth factor beta-3 in a rabbit model. *Wound Repair Regen* 2001; 9:44–9.
- 80 Wu L, Siddiqui A, Morris DE *et al.* Transforming growth factor beta 3 (TGF beta 3) accelerates wound healing without alteration of scar prominence. Histologic and competitive reverse-transcription-polymerase chain reaction studies. *Arch Surg* 1997; 132:753–60.
- 81 Roberts AB, Sporn MB, Assoian RK *et al.* Transforming growth factor type beta: rapid induction of fibrosis and angiogenesis in vivo and stimulation of collagen formation in vitro. *Proc Natl Acad Sci USA* 1986; 83:4167–71.
- 82 Cordeiro MF, Gay JA, Khaw PT. Human anti-transforming growth factor-beta2 antibody: a new glaucoma anti-scarring agent. *Invest Ophthalmol Vis Sci* 1999; 40:2225–34.
- 83 Siriwardena D, Khaw PT, King AJ *et al.* Human antitransforming growth factor beta(2) monoclonal antibody-a new modulator of wound healing in trabeculectomy: a randomized placebo controlled clinical study. *Ophthalmology* 2002; 109:427–31.
- 84 Tamm I, Dorken B, Hartmann G. Antisense therapy in oncology: new hope for an old idea? *Lancet* 2001; 358:489–97.
- 85 Lang KA, Peppercorn MA. Promising new agents for the treatment of inflammatory bowel disorders. *Drugs R D* 1999; 1:237–44.
- 86 Yacyshyn BR, Bowen-Yacyshyn MB, Jewell L *et al.* A placebo-controlled trial of ICAM-1 antisense oligonucleotide in the treatment of Crohn's disease. *Gastroenterology* 1998; 114:1133–42.
- 87 Cordeiro MF, Mead A, Ali RR *et al.* Novel antisense oligonucleotides targeting TGF-beta inhibit in vivo scarring and improve surgical outcome. *Gene Ther* 2003; 10:59–71.
- 88 Saika S, Yamanaka O, Sumioka T *et al.* Fibrotic disorders in the eye: targets of gene therapy. *Prog Retin Eye Res* 2008; 27:177–96.
- 89 Redington AE, Howarth PH. Airway wall remodelling in asthma. *Thorax* 1997; 52:310–2.
- 90 Roche WR, Beasley R, Williams JH, Holgate ST. Subepithelial fibrosis in the bronchi of asthmatics. *Lancet* 1989; 1:520–4.
- 91 Brewster CE, Howarth PH, Djukanovic R *et al.* Myofibroblasts and subepithelial fibrosis in bronchial asthma. *Am J Respir Cell Mol Biol* 1990; 3:507–11.
- 92 Kovacs EJ, DiPietro LA. Fibrogenic cytokines and connective tissue production. *FASEB J* 1994; 8:854–61.
- 93 Santana A, Saxena B, Noble NA, Gold LI, Marshall BC. Increased expression of transforming growth factor beta isoforms (beta 1, beta 2, beta 3) in bleomycin-induced pulmonary fibrosis. *Am J Respir Cell Mol Biol* 1995; 13:34–44.
- 94 Watelet JB, Gevaert P, Bachert C, Holtappels G, van Cauwenberge P. Secretion of TGF-beta1, TGF-beta2, EGF and PDGF into nasal fluid after sinus surgery. *Eur Arch Otorhinolaryngol* 2002; 259:234–8.