Are TGF-β gene polymorphisms associated with asthma risk?

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Abstract

Introduction

Asthma is an inflammatory disease, leading to airway obstruction, hyper-responsiveness, heightened mucus production, and it can affect remodelling of the airway wall. Almost 300 million people in the world are estimated to be affected by asthma. Several studies have shown that regulatory T cells (Treg cells) have a key role in controlling allergic diseases and chronic inflammation on asthma. Thus, recently, attention has been given to Treg cells producing interleukin-10 (IL-10) and transforming growth factor-β (TGF-β). TGFβ-1 is an important replicated asthma candidate gene, and few studies have evaluated the direct association of TGF-β polymorphisms and risk to allergic diseases. Thus, the aim of this article was to critically review the main polymorphisms on TGF-β gene described so far and depict the role of such polymorphisms on asthma development.

Conclusion

Although these data seem controversial, polymorphisms on TGF-β1 may be an interesting marker for asthma since it is related to an increase on TGF-β1 levels, and it may be related to tissue remodelling. However, more studies are required to better understand the results observed so far.

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Introduction

Asthma and its pathophysiology

Asthma is the most common chronic disease that affects childhood and is the main cause of morbidity in adults. Epidemiological data show that this illness affects 4%–17% children and 7.7% adults in the United States. Furthermore, almost 300 million people worldwide could be affected by asthma. In Brazil, Salvador has extremely high rates of up to 24.6% among school-age children (13–14 years).

Studies have shown that asthma develops during the intrauterine foetal programming. Atopic asthma is most frequent in children because there is a predominant immunological Th2 response at this stage of life. This condition is an inflammatory disease, leading to airway obstruction, hyper-responsiveness, heightened mucus production, and it can affect remodelling of the airway wall. The increasing number of studies about this pathology allowed a paradigm shift in immunology and molecular biology that led to an extensive analysis of inflammatory cells and mediators involved in the pathophysiology of asthma. The pathophysiological characteristics of asthma are not limited to the Th2 response. Studies have shown that the immune response also involves T helper cells (Th1), Th9, as well as the Th1 and Th3 profile.

The Th2 response is characterised by the production of Th2 cytokines; interleukin-4 (IL-4) and IL-13 are main cytokines responsible for the stimulation of plasma cells to secrete IgE and continue the inflammatory process. IL-4 also stimulates increased expression of IgE receptors on mast cells, eosinophils and basophils. Other mediators are also involved in the inflammatory process, such as Tumour Necrosis Factor (TNF) and nitric oxide released by macrophages which produce neutrophil elastase. Rather than Th2-type cytokines, it has been well documented that IL-2, IL-12, interferon-γ (IFN-γ), TNF-α and transforming growth factor-β (TGF-β) produced by CD4+ T helper 1 cells (Th1) are implicated in the pathogenesis of asthma. Epithelial cells in repair phase produce TGF-β, fibroblast growth factor and endothelin, which regulate fibroblasts and myofibroblasts to release collagen, elastic fibre, proteoglycan and glycoprotein, and these substances induce airway wall thickening. Antigen-presenting cells, especially dendritic cells (DCs), play a critical role in initiating and regulating early inflammatory events at epithelial surfaces and control the recruitment and activation of Th2 cells. A cooperation of airway epithelium and DCs controls asthma development, and Th2 activation requires DC-mediated antigen presentation. Thus, cytokines such as IL-33 are induced. IL-33 is a member of the IL-1 family that can induce activation of DCs, mast cells, eosinophils and basophils, ultimately leading to increased expression of Th2-associated cytokines and IgE. In addition, IL-33 administration provokes hyper trophy of bronchial epithelial cells, as well as mucus secretion. Studies have shown that IL-33 is an attractive candidate for therapeutic intervention, either by its soluble receptor,
ST2, targeting the lung or by its small molecule inhibitors that could act systemically as a central regulator of the inflammatory response characteristic of asthma. Based on the central role of TGF-β, especially on its ability to modulate various immunological profiles, the aim of this review was to discuss whether TGF-β gene polymorphisms are associated with asthma risk.

**Discussion**

The authors have referenced some of their own studies in this review. These referenced studies have been conducted in accordance with the Declaration of Helsinki (1964), and the protocols of these studies have been approved by the relevant ethics committees related to the institution in which they were performed. All human subjects, in these referenced studies, gave informed consent to participate in these studies.

**TGF-β**

TGF-β is a pleiotropic and multifunctional growth factor released by several immunologic cells (epithelial cells, eosinophils, Th2 lymphocytes, macrophages and fibroblast) showing key roles in immune response on homeostasis, infections and inflammatory diseases through its immune modulatory and fibrogenic activity. The TGF-β superfamily consists of more than 33 members, all holding a similar prodomain fold, including bone morphogenetic proteins (BMPs), growth and differentiation factors (GDFs), activins and inhibins. The TGF-β family has three different TGF-β isoforms in mammals: TGF-β1, the most prevalent isoform, TGF-β2 and TGF-β3 that have similar properties in vitro. TGF-β is the main regulator of the immune response and exerts potent anti-inflammatory activity by inhibiting the differentiation of immune cells (Th1, Th2, cytotoxic T cells and B cells) as well as inhibition of cytokine production (IFN-γ and IL-2). Furthermore, it acts as an important differentiating factor for some cells that exert powerful immunosuppressive effects, such as regulatory T cells (Treg cells) FOXP3+.

Additionally, TGF-β1 plays an important role in extracellular matrix remodelling and fibrosis through the induction of target genes such as connective tissue growth factor (CTGF), α-smooth muscle actin (α-SMA), collagen 1α2 (col1a2) inducing proliferation and chemoattraction of fibroblasts and their differentiation into myofibroblasts, which finally will induce fibrosis and contraction of the extracellular matrix.

**TGF-β and asthma immune response**

Allergic diseases are caused by inappropriate immunological responses to allergens. Several studies have shown that regulatory T cells (Treg cells) have a key role in controlling allergic diseases and chronic inflammation in asthma. A defect on immune regulation leads to an exacerbation of Th2 response. Treg cells are essential in the maintenance of immunological tolerance to self-antigens and in the regulation of the immune response to infectious organisms and represent a major pathway proposed to keep immune response in check.

**Figure 1:** TGF-β on asthma immune response. TGF-β is crucial to induce expression of the main regulatory transcription factor Foxp3 and to regulate the development, function and IL-10 production from of CD4+CD25+ Treg cells. Moreover, TGF-β can down-regulates the activation of Th2 lymphocytes, suppress the inflammatory response and airway hyperreactivity and inhibits IgE release. TGF-β also seems to be involved in the airway remodelling and, consequently, severity of asthma.

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homeostasis in the airways. Thus, recently, attention has been given to Treg cells producing IL-10 and TGF-β. These immune modulatory cytokines can down regulate the production of both Th1 and Th2 cytokines and suppress the inflammatory response on asthma (Figure 1).23–25

Accordingly, TGF-β is crucial in the development and function of CD4+ CD25+ Treg and induces expression of the master regulatory transcription factor Foxp3, a critical gene regulator for Treg cells development. TGF-β inhibits IgE, the main antibody associated with allergic diseases and asthma26 and has been shown to induce IL-10 expression in T cells (Figure 1).27

Hansen et al. have demonstrated that T cells producing TGF-β are able to reduce inflammation and airway hyperreactivity in a mouse model.28 It was also demonstrated that blocking the TGF-β signalling pathway in T lymphocytes, an increasing inflammation and airway hyperreactivity is observed, suggesting that TGF-β-induced immune regulation reduces the pulmonary inflammatory response in vivo (Figure 1).29

In contrast, several studies have shown that TGF-β is associated to an increased airway remodelling by inducing apoptosis of airway epithelial cells and is potentially involved in the regulation of epithelial cells adhesion leading to tissue damage (Figure 1).30 The neutralisation of TGF-β in two different models of chronic allergen challenge-reduced airway remodelling.31 Asthmatic patients showed increased TGF-β expression in both bronchial biopsy sections and bronchoalveolar lavage in comparison with normal subjects and expression correlated with the lung fibrosis degree.32

These data reinforce the idea that TGF-β acts to regulate immune response in the lungs and that perturbations in the level of expression of that cytokine or even in one of the TGF-β signalling pathway molecules may have severe consequences for maintenance of pulmonary homeostasis. However, many studies have investigated the rationale that increased TGF-β expression may be associated with asthma severity by increasing airway remodelling.

TGF-β gene polymorphisms and allergic disease risk
Polymorphisms in gene sequences can affect the expression of proteins in various ways: levels of gene transcription, splicing, stability and levels of mRNA translation.33 Polymorphisms in genes that participate on immunity may influence the development of several diseases. Susceptibility to many diseases is associated with a particular ‘pro-inflammatory’ profile, which can be explained by individual genetic determinants. TGF-β1 is an important replicated asthmatic candidate gene, and few studies have evaluated the direct association of TGF-β1 polymorphisms and risk to allergic diseases, in particular, asthma.34–36 The TGF-β gene is located on chromosome 19q13.1–13.3, and some polymorphisms were shown in this gene and can be found in exons, introns and promoter gene sequences. The most studied polymorphisms on TGF-β gene are described in Table 1. Below we present some of them and discuss their role on cytokine levels as well as on asthma development:

rs1800469 (-509 C > T)
The -509 C > T polymorphism is located in the promoter region of TGBF1 and can modulate TGF-β1 function and circulating TGF-β1 levels (Table 1). The T allele has been associated with higher TGF-β1 plasma levels. An interesting Genome-wide association study (GWAS) previously reported that -509 C > T was associated with asthma in a Mexican population.37 These results were consistent with several authors.34,38 In contrast, other authors found no association between -509 polymorphisms and risk to clinically manifested atopic asthma or other allergies.33,39 Although these data seem controversial, this single-nucleotide polymorphism (SNP) can be an interesting marker for allergic diseases. However, more studies are needed to better understand the results obtained so far. Lack of associations can be achieved when sample size is small and therefore may have lacked power to detect statistically significant associations. Moreover this polymorphism was associated with IgE levels. One study described phenotypic association between total IgE levels in serum and -509 C > T(39) and also association with persistent IgE-mediated cow’s milk allergy in children.40

rs2241712 (-10807 G > A)
The rs2241712 (-10807) is located in the promoter region of the TGF-β1 gene and has been associated with some respiratory diseases such as asthma and chronic obstructive pulmonary disease (COPD) (Table 1). Studies have analysed associations between rs2241712 and asthma. In one of these studies, the authors have shown an association between rs2241712 and asthma in atopic subjects. The G/A genotype was associated with decreasing asthma risk; however, this association was not statistically significant.38 Furthermore, in a study conducted in Costa Rica revealed an association of the A allele with increased airway responsiveness.42 However, another study showed an association between the G allele with asthma risk, but this difference was not statistically significant.38 It seems that this SNP may have an important role in the respiratory disease development. Therefore more studies are needed to elicit this association. Although the relationship between rs2241712 and TGF-β1 serum levels has not been evaluated, this polymorphism presents score 2b in the RegulomeDB database being likely to affect binding to transcription factor and, potentially, change the TGF-β1 production (Table 1).

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rs1800468 (-800 G > A)
We found only one study that analysed the SNP-800 in association with asthma and serum IgE levels. Moreover, the authors found no association between the SNP-800 and clinical manifestation of atopic diseases and serum IgE levels (Table 1).

rs1800470 (869 T > C)
The rs1800470 is located in codon 10 of exon 1 and is related to change the amino acid sequence, leucine to proline in the signal peptide. Codon 10 polymorphism has been studied in several diseases as nephropathy in Type 1 diabetes, allergic rhinitis and asthma. The 869 polymorphism was found to be associated with an increased circulating TGF-β1 concentration, increased production of TGF-β1 in vitro and increased risk of asthma (Table 1).

rs1800471 (codon 25)
The 915 G > C polymorphism results in an amino acid change (arginine to proline) at codon 25; however, the functional impact of this polymorphism is unknown (Table 1). In allergic diseases, no association was found for this SNP. Although, the haplotype analysis including rs1800471 (codon 10) was associated with a history of parental asthma.

Conclusion
Several SNPs on TGF-β1 have been studied in asthma diseases. Some SNPs were positively associated with asthma but not always replicated by all authors. Although these data seem controversial, polymorphisms on TGF-β1 may be an interesting marker for asthma since it is related to an increase on TGF-β1 levels, and it may be related to tissue remodelling. However, more studies are required to better understand the results observed so far. It is important to point out that in some studies, lack of associations can be related to small sample size and consequently no power to detect statistically significant associations. Moreover, the prevalence of these polymorphisms may vary from population to population, which can also interfere with these controversial results. Greater sample size and studies using a considerable number of informative ancestry markers are required to confirm these findings and also to identify populations, where TGF-β1 variants are mediating the causal pathway of allergic diseases, in special, asthma.

Abbreviations list
TGF-β, Transforming growth factor-β; TNF, Tumour Necrosis Factor; GDFs, Growth and differentiation factors; CTGF, Connective tissue growth factor; α-SMA, α-smooth muscle actin; GWAS, Genome-wide association study; SNP, Single-nucleotide polymorphism.

References