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TGF- β can leave you breathless

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Abstract

Transforming growth factor-beta (TGF- β), a ubiquitous and multifunctional cytokine, is central to the evolution and modulation of host defense. Early on, TGF- β was recognized for its chemotactic and pro-inflammatory properties, but then identification of its powerful suppressive activities focused attention on dissecting its mechanisms of immune inhibition. Just as quickly as TGF- β -mediated regulation of a population of CD4⁺CD25⁺Foxp3⁺ regulatory T cells became the rage, a surprising finding that TGF- β was the impetus behind a subset of pro-inflammatory T helper(Th)17 cells brought back a re-emphasis on its broader ability to dictate inflammatory events. Emerging evidence indicates that much remains to be discovered regarding the complex and intertwined roles of TGF- β in inflammation, T cell lineage commitment, antibody generation, immune suppression and tolerance. While it may appear that TGF- β has multiple, ill-defined, contradictory and overlapping modes of activity that are impossible to unravel, the current excitement for dissecting how TGF- β controls immunity defines a challenge worthy of pursuit. The lung is particularly vulnerable to the influences of TGF- β , which is produced by its immune and non-immune cell populations. In its absence, lung pathology becomes lethal, whereas TGF- β overproduction also has untoward consequences, potentially leaving one breathless, and underscoring the paradoxical, but essential contribution of TGF- β to tissue and immune homeostasis.

Keywords

asthma; inflammation; immune suppression; Th17; Treg; Th2

Introduction

TGF- β is recognized as a staunch supporter of peripheral tolerance to self-antigens and innocuous allergens and in suppression of excessive immune responses detrimental to the host [1]. Discovered nearly 30 years ago [2], TGF- β now represents the prototypic member of a superfamily of structurally and functionally-related peptides that affect many different cellular processes. The three mammalian isoforms of TGF- β (TGF- β 1-3), encoded by separate genes, are naturally occurring in most tissues, but TGF- β 1 is dominant in the immune system. Both structural and immune cells are capable of secreting TGF- β in a latent form and expressing TGF- β receptors. Once activated, a critical step in TGF- β actions, the peptide binds to a heterodimeric receptor complex(T β R) consisting of type I and II transmembrane serine/threonine kinase subunits, activates Smad-dependent signaling pathways, and thus regulates

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target gene expression. Recently, additional proteins, namely phosphatidylinositol-3 kinase, mitogen-activated protein kinases, PP2A phosphatase, Rho family proteins, and epithelial polarity protein Par6, have been linked to Smad-independent TGF- β signaling [3].

The vital role of TGF- β in development and immune regulation is illustrated by the fact that ~50% of TGF- β 1 null mice die *in utero* and the remainder succumb to uncontrolled multifocal lymphoproliferative disorders, including T-helper(Th)1-mediated pulmonary inflammation and compromised respiratory function by 3-5 weeks of age (Fig. 1A,B). Unchecked T cell activation and loss of tolerance are also features of mice with disrupted TGF- β signaling pathways [1,3]. In the absence of TGF- β , its activators [4], its receptors or signaling components, one of the tissues typically affected is the lung, evidence that TGF- β is essential for maintenance of normal lung physiology as well as the host response to injury, infections, or allergens.

TGF- β in lung pathogenesis

Within the mucosal immune system, the surface area of the lung is enormous, encompassing 100-150m² or nearly the size of a volleyball court. The branching bronchial tree brings in oxygen, but also distributes environmental pollutants, antigens and infectious agents to ~300 million alveoli, the walls of which contain a dense capillary network and systemic access[5]. The respiratory epithelium not only functions as a barrier, but through mucociliary clearance, phagocytic cells, and antimicrobial peptides, orchestrates protective innate immunity and if need be, the transition into an adaptive immune response. Continuous bombardment by foreign agents requires a tightly controlled host response and one of the key immunomodulatory agents is TGF- β .

Loss of control in response to environmental and genetic factors can engender pathogenesis, such as allergic asthma, characterized by recurrent inflammation, eosinophilia, airway hyperresponsiveness(AHR), mucus hypersecretion, and remodeling, all of which contribute to a decline in airway function. Approximately 300×10⁶ people worldwide suffer with asthma, a heterogeneous disease affecting all age groups, with increasing prevalence, especially in Western industrialized countries, a potential correlate of the hygiene hypothesis[6]. While multiple inflammatory cells and mediators participate in the pathophysiology of asthma, a biased T-helper(Th)2 lymphocyte response is considered crucial, together with a limited Th1 counterbalancing response, eosinophil, mast cell and basophil activation, IgE production, and more recently, evidence for participation by Th17 and possibly, Th9 cells (Fig. 2).

A role for TGF- β in the wheezing and sneezing associated with allergic asthma stems from direct and circumstantial evidence. Not only have increased TGF- β and TGF- β signaling been detected in asthmatic airways (Fig. 3) and correlated with enhanced eosinophil infiltration, but an association with TGF- β polymorphism has also been reported [7]. Nonetheless, as often occurs with TGF- β , a disconnect appears to exist relevant to these genetic alterations and experimental model studies. Induced increases in TGF- β , whether by oral tolerance or retroviral delivery [8,9], ameliorated airway inflammation, whereas an exaggerated asthmatic phenotype occurred when TGF- β was reduced [10,11].

Aberrant responses to allergens reflect insufficient control of Th2 effector cells and plasma cell immunoglobulin(Ig)E isotype-switch (Fig. 2). APC and multiple local factors polarize a Th2 response, along with recruitment via CCR3, CCR4 and CCR8 ligands. In turn, Th2-derived cytokines mediate and amplify airway inflammation and remodeling, and IL-5, in particular, provokes migration, differentiation and survival of eosinophils, an airway source of TGF- β . In sensitized individuals, allergen-specific IgE binds to mast cell Fc ϵ RI, and likely low affinity Fc ϵ R2(CD23) to engage signaling and release of histamine, proteoglycans, serine proteases,

cytokines and lipid mediators, responsible for early phase reactions. TGF- β may suppress mast cell Fc ϵ RI, promote apoptosis, or induce inhibitor of DNA binding 2(Id2), a transcriptional regulator that negatively regulates B cell differentiation and dissuades IgE class switch recombination[12]. Increased specific IgG that captures allergen before it reaches effector cell IgE and a decreased IgE to IgG4 ratio have been associated with clinical improvement after immunotherapy, which usually coincides with induction of TGF- β and IL-10[13]. Although TGF- β inhibits T-cell proliferation and expression of lineage-associated transcription factors, T-bet and GATA-3, and thereby hobbles Th1 and Th2 differentiation [3], it also represents an essential switch factor in differentiation and maintenance of inducible CD4⁺CD25⁺ regulatory T cells (iTreg), Th17 and putative Th9 populations [14-17](Fig. 4).

TGF- β supports differentiation of Th17 cells

Despite its ability to undermine Th2 responses, accumulating evidence pinpoints TGF- β in molding a population of pro-inflammatory IL-17-producing Th17 cells, which have a propensity to induce CXCL8/IL-8, recruit granulocytes, and enhance bronchial inflammation. Recently identified as a third lineage of T helper cells, distinct from classical Th1 and Th2, Th17 produce IL-17A and IL-17F that appear early, may integrate innate and adaptive immunity, but can serve as pathogenic effectors. The importance of TGF- β in Th17 commitment was first unraveled in mice in 2006 [15,18,19] and subsequently, in humans where TGF- β , combined with one or more cytokines (e.g. IL-6, IL-21, IL-23, IL-1 β) directs the differentiation program of naïve T cells into Th17 [20-22](Fig. 4). In line with *in vitro* observations, overexpression of TGF- β 1 in mouse T cells increased Th17 numbers [18]; conversely, mice deficient for TGF- β 1 [15] or with attenuated T cell TGF- β signaling [23] had constrained Th17 development. RORgammat [24] and likely also RORalpha [25] represent key Th17 transcriptional factors, and their expression in naïve T cells is rapidly enhanced and sustained by TGF- β , together with IL-6. Under these conditions, another small subset that produces IL-10 plus IL-17 emerged [26] and, akin to conventional IL-10-producing Tr1, had protective functions. In an even more complex scenario, a subset of Th17 co-express IFN γ with IL-17, whereas committed Th17 deprived of TGF- β can apparently be re-programmed to T-bet/Stat4-dependent IFN γ -producing Th1 cells in the presence of IL-12 or, to a lesser extent by IL-23, extinguishing their ROR γ t/ α and IL-17 production[27](Fig. 4). Whether this phenotype shift, contingent upon an altered balance between TGF- γ and IL-12/IL-23, occurs in asthmatic inflammation has not been explored, although little evidence for a Th1/IFN γ contribution exists. Nonetheless, these new findings highlight apparent Th17 plasticity, particularly during later stages of development, and remind us that there is much more to be deciphered regarding CD4⁺ T cell lineage commitment and function.

In an amplification arc, IL-17 stimulates bronchial fibroblasts, epithelial and smooth muscle cells to produce chemokines, inflammatory mediators(Fig. 2) and provokes epithelial mucus metaplasia, further contributing to airway pathology. Levels of IL-17 detected in asthmatic airways [28] seem to be linked to severity of disease and in the absence of infection where it serves to mobilize neutrophils and pulmonary defense, IL-17 can potentially be a bad actor. Transgenic mice overexpressing OVA-specific TCR genes in CD4⁺ T cells developed airway inflammation after OVA inhalation, dominated by neutrophils, cohabitants with Th1 and Th17 and their products, IFN γ and IL-17, in bronchoalveolar lavage(BAL). Although IFN γ -depletion exacerbated allergic responses, airway neutrophilia and hyperreactivity were dramatically alleviated in IL-17-deficient mice[29]. In support of this observation, Rag-2-deficient mice adoptively transferred with T-bet-deficient, but not wildtype CD4⁺ T cells, exhibited increased BAL granulocytes after OVA challenge, overridden by anti-IL-17 [30]. Likewise, Smad3 null mice, being defective in TGF- β -elicited Th17 development, exhibit reduced airway inflammatory cell infiltration and less severe tissue damage compared to wildtype when

sensitized and challenged with a clinically relevant allergen, house dust mite(HDM)[31](Qian B. *et al.*, in preparation).

Paradoxically, IL-17 has also been reported to be protective in certain Th2-mediated atopic models, where IL-17 neutralization exacerbated eosinophil recruitment and bronchial hyperreactivity, and exogenous IL-17 reduced symptoms [32]. Although the discrepancy remains unclear, this IL-17 inhibition was attributed to DC suppression and down-regulation of CCL17(TARC), eotaxin, and IL-5. Nonetheless, in humans, IL-17A and IL-17F are both TGF- β -dependent and often considered functionally interchangeable, yet an IL-17F-specific polymorphism has been linked to reduced incidence of asthma[33].

Th9, TGF- β and asthma

Based on rapidly evolving concepts of T cell plasticity, the involvement of TGF- β in phenotype shifting has become a moving target and the situation as it appears today will likely have morphed before the next review. Collectively, the current ensemble of data implicates TGF- β , in the context of changing cytokine cues, as the conductor of a T cell orchestra. In this regard, IL-9, upregulated in asthmatic lungs and a product of Th2 and eosinophils [34], has recently been tied to a T cell subset deviated from the Th2 lineage under the auspices of TGF- β and IL-4 [16,17](Fig. 4). Provisionally termed Th9, a putative additional helper T cell subset, these IL-9⁺ cells can be identified in HDM-induced experimental asthma(Fig. 3)(Qian B., *et al.*, in preparation). Interestingly, HDM is a serine protease and serine protease inhibitors reportedly abolish eosinophil expression of IL-9 through antagonism of 7-transmembrane G protein-coupled protease activated receptors(PAR)2 [34]. IL-9, in turn, accentuates Th2 responses and differentiation of mast cells and hematopoietic progenitors. While clearly in the preliminary stages, elucidation of a link between TGF- β and IL-9 may reveal new avenues of intervention.

TGF- β regulates Treg development and function

With the assistance of TGF- β , the pro-inflammatory functions of Th17 and Th9 could spiral asthmatic pathogenesis out of control. Heroically, however, TGF- β may come to the rescue in the guise of a population of regulatory T cells. Naturally-occurring CD4⁺CD25⁺ Treg are derived from thymus with the early help of TGF- β [35] as a developmentally distinct subset of mature CD4⁺ T cells (5-10%) and, pre-programmed to perform suppressive functions, are critical in controlling immunity. Treg specifically express the transcription factor Foxp3, which masterfully regulates their development, function and survival. Mice devoid of functional Foxp3 develop a lethal autoimmune syndrome [36], rescuable by adoptive transfer of Treg.

In addition to thymically-originated Treg, activated naïve CD4⁺ T cells can acquire Foxp3 and differentiate into iTreg in the periphery, contingent upon TGF- β [1,14,18]. Blockade of TGF- β signaling in naïve T cells ablates conversion to iTreg, which manifests as T cell hyperproliferation and overexuberant inflammation [3,37]. Recent significant findings suggest a reciprocal developmental relationship between Treg and Th17, two TGF- β -inducible CD4⁺ T cell subsets with opposing activities. Mediators, notably IL-2 and retinoic acid [38,39], which positively regulate Treg, suppress Th17 differentiation, while Th17-promoting IL-6 inhibits Treg [18]. In this same vein, their transcriptional factors, ROR γ t and Foxp3, can interact to block each other's function [40]. Similarly, Foxp3 and GATA-3 can antagonize one another, emphasizing the complex contextual regulation of T cell development.

Mechanistically, Treg-mediated immunomodulation is achieved by direct cell-to-cell interactions or release of regulatory cytokines, TGF- β and IL-10 [3,14,37]. Treg are pivotal in maintenance of tolerance to innocuous environmental antigens in health and despite increasing after allergen challenge (Fig. 3), may be functionally inadequate. Delivery of Foxp3⁺ Treg [14] to HDM-challenged mice suppressed inflammatory cell infiltration and collateral damage,

while depletion of Treg before HDM-challenge exacerbated airway responses [41]. Clinically, CD4⁺CD25⁺ Treg from asthmatic children are unable to inhibit either Th2 proliferative responses or cytokines, whereas parallel populations from non-asthmatics were functionally suppressive [42]. In this regard, a point mutation in the Foxp3 gene has recently been identified in an adult patient with both rhinitis and asthma[43]. Importantly, allergen immunotherapy or corticosteroid treatment, which bolster Foxp3-expressing T cells, attenuate asthmatic symptoms [44]. Intersecting resolution pathways managed via resolvins and lipoxins also dampen aspects of allergic inflammation including eosinophil activation [45] and may evoke release of TGF- β [46].

TGF- β , tissue repair and airway remodeling

Persistent inflammation and associated tissue damage trigger injury repair mechanisms, inevitably altering normal structural properties with resultant AHR and declining lung function. Within this milieu, TGF- β potentiates airway remodeling through a plethora of well-known functions in mediating wound healing and fibrosis [1], which exceed the scope of this review. Although elevated TGF- β in epithelial or submucosal cells have been correlated with basement membrane thickness and fibroblast numbers in asthmatic patients, evidence regarding support of TGF- β 's contribution to experimental allergic airway remodeling is conflicting. Within the framework of TGF- β blockade or Smad3-deficiency, allergen(OVA)-induced remodeling is reduced [47,48,49]. Nonetheless, recent data indicate that HDM-induced asthmatic mice treated with neutralizing anti-TGF- β developed airway remodeling comparable to control mice, as did HDM-treated-Smad3 null mice[50], emphasizing, again, that the controlling factors in airway remodeling are not fully defined. This is not a trivial concern in that suggested efforts to manipulate TGF- β levels as a means to suppress allergic inflammation may negatively impact on fibrosis and airway remodeling. As asthma represents such a multifaceted and heterogeneous disease, it may be exceedingly difficult to target a single mediator and expect a miraculous reversal of pathology.

Conclusions

No longer can the case be made that TGF- β 's major role in immunomodulation is strictly immunosuppressive. Rather, it has a constellation of activities that dominate from onset through resolution of innate and adaptive immune events. Conceivably, what is known today represents but the tip of the iceberg in our comprehension of the intertwined connections between TGF- β and a panoply of cells, cytokines, signaling molecules, and transcription factors which coalesce into an immune response appropriate for the inciting agent. From its early release by platelets, its ability to mobilize inflammatory cells directly and indirectly, and its unique orchestration of T cell lineage commitment, TGF- β clearly is fundamental to the nature and outcome of an evolving immune response. Tantamount to a master suppressor, TGF- β defines natural Treg development and iTreg conversion in the periphery. How a single molecule can exert such a diversity of activities is phenomenal, but clearly dependent on environmental cues. Basic discovery remains fundamental to understanding the underlying biology of inflammation and immunopathogenesis, and the unearthing of novel functions for TGF- β represented in the past several years may be but a harbinger of discoveries to come. Although it remains to be established whether targeting TGF- β , either to dampen or embellish selective activities, is appropriate, this approach has the potential to provide therapeutic strategies for multiple immune-based diseases and may help us all breathe a little easier.

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Figure 1. Inflammatory lung pathology in TGF- β -deficient mice

A) Lung tissue from wildtype mouse without evidence of inflammation. **B)** Representative lung tissue from a 3week old TGF- β -/- mouse revealing infiltration of inflammatory cells, particularly around airways and vessels. Infiltrate is dominated by Th1 populations(Tbet⁺) **(C)** with overexpression of IFN γ **(D)** by RT-PCR.

Figure 2. Model of airway inflammation with TGF- β intersecting points

Following breach of the epithelial barrier of the airways by environmental allergens, particularly in genetically susceptible hosts, APC process the allergen and upon presentation to T cells initiate an immune response. Activation of Th2 cells represents the classic allergic pathway, but newly appreciated roles for Th17, Treg and likely Th9 populations confound dissection of the complex immunopathogenic events. Th2-derived cytokines, such as IL-4 and IL-13 influence eosinophil recruitment, mast cell activation, airway smooth muscle cell contraction and proliferation, goblet cell hyperplasia and mucus hypersecretion, monocyte/macrophage CD23 and class II MHC expression, and fibroblast matrix production. IL-5 coordinates eosinophil differentiation from progenitors. TGF- β , which orchestrates suppression of Th1 and Th2, in large part via Treg (TGF- β and IL-10) may exacerbate the response via Th17 commitment and generation of IL-17, which not only recruits and activates granulocytes, but also stimulates epithelial cells to release chemokines (CXCL1/CXCL6/CXCL8/CCL11 eotaxin), inflammatory mediators (TNF α , IL-1 β , IL-6, GM-CSF, G-CSF), as well as mucins. Th2 activation of B cell IgE triggers Fc ϵ RI on mast cells and basophils that release large amounts of inflammatory mediators including histamine, proteases, cytokines and lipid mediators. Putative Th9 secretion of IL-9 together with eosinophil-derived IL-9 dictates IgE, mucus secretion and bronchial hyperresponsiveness. In this milieu, there may be inadequate incentive for recruitment and/or induction of Treg and failed suppression of the host response evoking tissue damage, aberrant repair, airway constriction, obstruction, and remodeling which can eventuate in compromised function.

Figure 3. TGF- β in antigen-induced inflammation of the airways

A) Lung tissue from wildtype mouse treated with PBS. **B)** Lung tissue from wildtype mouse sensitized and challenged with HDM. Note the massive infiltration of inflammatory cells and abundant mucus in airways. **(C-F)** Immunohistochemical staining of TGF- β (**C**), IL-17 (**D**), IL-9 (**E**) and FOXP3 (**F**) in HDM challenged lung. **(G-J)** RT-PCR analyses for indicated cytokines of PBS- and HDM-treated lung tissues of sensitized and challenged mice.

Figure 4. TGF-β directs development of T helper populations

From uncommitted naïve CD4⁺ T lymphocytes, several lineages of CD4⁺ T helper cell populations have been identified. The development and functions of these CD4⁺ T cells are largely influenced by TGF-β, which may be stimulatory or inhibitory depending on context. Whereas TGF-β typically suppresses Th1 and Th2 pathways, it is indispensable for the development of Th17 populations and Treg, which have critical functions in the evolution of inflammation and in the resolution of the response. TGF-β enhances expression of the Th17 transcription factor, RORγt, and polarizes Treg through induction of the transcription factor Foxp3. TGF-β contributes to deviation of a Th9 subset from Th2 populations. In TGF-β-depleted conditions, committed Th17 cells may convert to IFNγ-producing Th1 cells under the influence of IL-12/IL-23.