

REVIEW

Activin-A: a novel critical regulator of allergic asthma

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Summary

Activin-A is a pleiotropic cytokine that belongs to the TGF- β superfamily and plays an important role in fundamental biological processes, such as development and tissue repair. Growing evidence proposes a crucial role for activin-A in immune-mediated responses and associated diseases, with both enhancing and suppressive effects depending on the cell type, the cytokine micromilieu and the context of the response. Several recent studies have demonstrated a striking increase in activin-A expression in experimental models of asthma, as well as, in the asthmatic airway in humans. Importantly, a strong immunoregulatory role for activin-A in allergic airway disease, with suppression of T helper (Th) type 2 cell-driven allergic responses and protection against the development of cardinal features of the asthmatic phenotype was revealed by *in vivo* functional studies. Activin-A-mediated immunosuppression is associated with induction of functional allergen-specific regulatory T cells. In human asthma, although activin-A levels are increased in the airway epithelium and submucosal cells, the expression of its signalling components is markedly decreased, pointing to decreased regulation. Nevertheless, a rapid activation of the activin-A signalling pathway is observed in the airway of individuals with asthma following inhalational allergen challenge, suggestive of an inherent protective mechanism to control disease. In support, *in vitro* studies using human airway epithelial cells have demonstrated that endogenous activin-A suppresses the release of inflammatory mediators, while it induces epithelial repair. Collectively, compelling evidence suggests that activin-A orchestrates the regulation of key events involved in the pathogenesis of allergic asthma. The critical role of activin-A in allergic airway responses places this cytokine as an exciting new therapeutic target for asthma.

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Asthma

Asthma is a chronic heterogeneous inflammatory airway disease that affects up to 300 million people world-wide with increased prevalence and incidence in the last two decades. Clinically, asthma is defined by increased airway hyperresponsiveness (AHR) and reversible airway obstruction which manifests as cough, wheezing and intermittent attacks of breathlessness. A cardinal feature common to most asthma phenotypes is excessive Th2-driven airway inflammatory responses against harmless environmental antigens (allergens) [1]. Emerging evidence suggests that, apart from Th2 cells, activation of other Th effector subsets, such as Th1 and Th17 and IL-17-expressing Th2 cells, along with neutrophils, participates in disease pathogenesis, particularly in relation to severe

asthma [2–4]. Moreover, subsets of recently identified innate immune cells, termed ‘natural helper cells’, ‘nuocytes’ or ‘multipotent progenitor cells’ that respond to IL-25 and IL-33 produced in the airway, may also be involved in the induction of Th2-associated allergic airway inflammation [5]. As multiple and distinct immune cell subsets are being identified as mediators of allergic airway inflammation, the need for identification of factors that can effectively control dysregulated immune responses and protect against asthmatic disease becomes increasingly important.

Asthma is also characterized by airway structural changes, collectively termed, remodelling. These include denuded airway epithelium, increase in the number and size of goblet cells, fibroblasts and airway smooth muscle (ASM) cells along with reticular basement membrane

thickening and increased deposition of extracellular matrix proteins. Although induction of remodelling is related to tissue repair and restoration of normal airway architecture, dysregulation of these processes can lead to increased AHR and/or progressive airway obstruction. While some aspects of airway remodelling are related to structural cell activation, several lines of evidence suggest that other facets are associated with aberrant activation of inflammatory cells infiltrating the airway [1].

Critical controllers of inflammatory responses are suppressive cytokines, such as TGF- β_1 and IL-10, and regulatory T cells (Tregs). Although these factors can suppress allergic airway inflammation and associated remodelling in certain experimental disease models, their expression and/or function is often impaired or overridden in human asthma, especially in the airway, the active disease site [6, 7]. Activin-A is a cytokine with a unique role in key biological processes [8]. Moreover, recent advances underscore the critical impact that activin-A has on Th2-driven airway inflammation and associated asthmatic disease. In the present review, we delineate the biology of activin-A and its effects on the regulation of allergic inflammation and airway repair. We also highlight unanswered questions and discuss the potential of modulating activin-A and its signalling pathway as novel therapeutic tools for asthma.

The transforming growth factor- β superfamily

The TGF- β superfamily is comprised of at least 45 members in humans. On the basis of structural homology, the

ligands can be further divided into distinct subgroups: the TGF- β subgroup, including TGF- β_1 , - β_2 , - β_3 , the bone morphogenetic proteins (BMPs), the nodal and the activins. TGF- β superfamily ligands signal via a constitutively active serine-threonine kinase-specific type II receptor that activates a type I receptor. The receptor complex propagates the signal via phosphorylation-induced activation of receptor-regulated Smads (r-Smads) that subsequently translocate to the nucleus in association with the common-mediated co-Smad 4 to initiate gene transcription ([9], Fig. 1). A remarkable overlap underlies the usage of these intracellular signalling pathways by TGF- β superfamily members (Fig. 1). Despite this, there is a considerable diversity in the functions of these ligands achieved mainly by distinct combinatorial interactions in the receptor complexes that allow differential binding or signalling in response to the same ligand. There is also variation in the affinity of the TGF- β ligands for the different receptor combinations. In addition, Smads interact with multiple transcription factors or co-activators and co-repressors which in turn activate distinct intracellular signalling pathways. Hence, a versatile system is created by which TGF- β superfamily ligands induce diverse responses in a cell and context-specific manner.

Activin-A structure and signalling

Activin-A was originally defined, over two decades ago, as an endocrine factor on the basis of its ability to stimulate the release of follicle stimulating hormone from pituitary gonadotrophs *in vitro* [10]. Since then, several

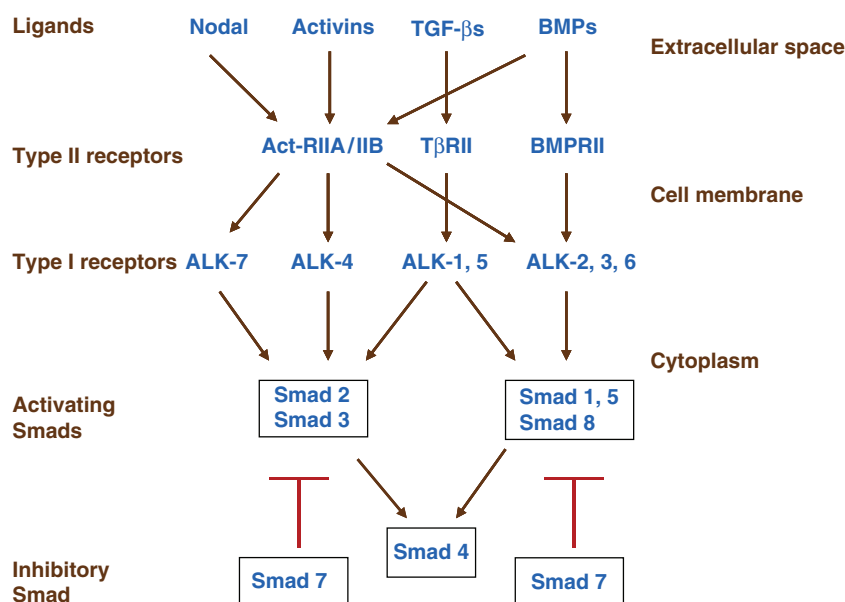


Fig. 1. TGF- β superfamily signalling pathways. TGF- β superfamily ligands bind to the constitutively active type II receptors that subsequently phosphorylate type I receptors. Activation of type I receptors propagates the signal by phosphorylating intracellular r-Smads. Smads associate with the co-Smad 4 and translocate to the nucleus wherein they initiate gene transcription. Smad 7 is induced in response to TGF- β ligands and inhibits downstream signalling. ALK, activin-like kinase; BMP, bone morphogenetic protein.

studies have uncovered essential roles for activin-A in erythropoiesis, mesoderm induction, neuron survival and the maintenance of stem cell pluripotency [8]. *Activin-A*^{-/-} mice are embryonically lethal due to severe craniofacial abnormalities [11], while activin receptor type IIA (*act-RIIA*)^{-/-} mice reach adulthood but have major deficiencies in their reproductive systems [12]. Activin-A is comprised of two β -subunits of which four isoforms have been identified in mammals: β A, β B, β C and β E (β D in *Xenopus laevis*). β A and β B dimerize to form homodimeric activin-A (β A : β A), homodimeric activin-B (β B : β B) and heterodimeric activin-AB (β A : β B). Activin subunits are highly conserved between mammals with nearly 100% homology at the amino acid level. Activin-A is the most widely expressed ligand [13]. Activin-A is expressed as a glycosylated pre-proligand that undergoes enzymatic cleavage to become activated [14]. Unlike TGF- β ₁, activin-A is not secreted or stored in an inactive or latent form, supporting the notion that these two cytokines exhibit a distinct mode of activation.

Activins signal through type I [activin-like kinase (ALK)-2, -4 and -7] and type II receptors (Act-RIIA and Act-RIIB) [8] (Fig. 1). While TGF- β ₁ signals through distinct receptors, BMPs can also bind, albeit with lower affinity, to Act-RIIA and Act-RIIB [15]. Activins signal through phosphorylation of Smads 2 and 3, while BMPs signal via Smads 1, 5 and 8. TGF- β s signal through Smads 1, 2, 3, 5, 8.

Activin-A functions are regulated at several points of the signalling pathway. Naturally occurring circulating proteins, termed inhibins, bind to activins and antagonize their function through effects on ligand synthesis, as well as, receptor binding [16]. Follistatin is another potent physiological inhibitor of activins and, once bound, induces rapid endocytic internalization and proteolytic degradation of activins [16]. Notably, follistatin and inhibins neutralize the effects of all activins and not only those mediated by activin-A. Moreover, follistatin can also inhibit certain functions of other TGF- β superfamily members, including TGF- β ₃, myostatin and the BMPs [8, 17, 18]. Betaglycan, a protein that regulates TGF- β ₁ binding to its type II receptor, promotes the interaction of inhibins with Act-RIIA and Act-RIIB, leading to inhibition of activin signalling [16]. Other decoy receptors, such as BMP and activin membrane-bound inhibitor and Cripto, share sequence homology to activin receptors and antagonize activin functions [16]. Smad 7 inhibits activin-A intracellular signalling through binding to ALK-4 and preventing the recruitment and phosphorylation of Smads 2, 3 (Fig. 1). In addition, Smad 7 interacts with ubiquitin ligases and promotes ALK-4 degradation through proteasomal and lysosomal pathways [19]. The exact factors that regulate Smad levels remain incompletely defined. Unlike r-Smads and Smad 4, which are expressed in most cell types and are activated mainly by TGF- β superfamily

members, Smad 7 expression can be rapidly up-regulated also in response to other, unrelated, cytokines. For example, IFN- γ -mediated activation of the Jak/Stat-1 pathways and TNF- α -induced activation of NF- κ B, EGF and MAPK signalling, influence Smad 7 expression [20, 21]. Following activation, the E3 ubiquitin ligases, Smad-ubiquitination-regulatory factor (Smurf)-1 and -2, regulate Smad levels through proteasome-mediated degradation. Smad 7 also undergoes ubiquitination and degradation but transcriptional induction of Smad 7 by TGF- β superfamily members ensures that a steady supply of this molecule is available. Taken together, it seems that depending on the factors which are present and active in the micromilieu, activating or inhibitory Smad pathways predominate.

Activin-A: a multi-faceted cytokine in immunity and inflammation

Activin-A and its receptors are expressed by a wide array of immune cells and are tightly modulated according to the inflammatory stimulus and the activation status of the cell. Antigen-presenting cells, such as dendritic cells (DCs) and tissue macrophages, are a potent source of activin-A. For example, mouse peritoneal macrophages secrete activin-A following stimulation with lipopolysaccharide (LPS) *in vitro* and *in vivo* [22]. Activin-A is also upregulated in mouse monocytes following co-culture with activated T cells [23]. In humans, CD1c⁺ DCs and plasmacytoid DCs in the peripheral blood spontaneously secrete activin-A, while monocyte-derived DCs up-regulate activin-A following CD40L or toll-like receptor (TLR) ligand stimulation *in vitro* [24]. Tissue cells, such as epithelial cells (in the mouse lung and intestine), fibroblasts and microglia also secrete activin-A following *in vitro* stimulation with inflammatory mediators, such as LPS and IFN- γ [25, 26]. Induction of activin-A in human synoviocytes occurs following *in vitro* stimulation with inflammatory cytokines and, interestingly, TGF- β ₁ itself [27]. Such data predict that activin-A will be rapidly expressed in inflammatory settings.

Indeed, *in vivo* studies have demonstrated that activin-A is one of the earliest cytokines released into the circulation upon systemic LPS administration [28, 29]. Activin-A is secreted within 1 h into the serum alongside TNF- α , followed by IL-6 and follistatin. These findings translate into human diseases as activin-A is significantly increased in the serum during septicemia and in the cerebrospinal fluid and serum during bacterial meningitis [30]. Increased levels of activin-A and its receptors are also observed in epithelial, endothelial cells and infiltrating macrophages in intestinal tissue from inflammatory bowel disease models and in individuals with Crohn's disease and ulcerative colitis, as compared to healthy controls [31, 32]. Moreover, activin-A expression is

increased in infiltrating macrophages and synoviocytes from individuals with rheumatoid arthritis [33].

Activin-A is also up-regulated in the skin following injury and promotes tissue repair and wound healing [34]. In addition, activin-A is induced in alveolar macrophages, bronchial epithelial and endothelial cells and fibroblasts in mouse models of bleomycin-induced lung fibrosis [35] and human interstitial fibrotic lung disease [36]. Activin-A exerts pro-fibrotic effects in this context, as it increases fibroblast migration and their differentiation to myofibroblasts *in vitro* [37]. In support, *in vivo* blockade of activin-A ameliorates bleomycin-induced lung fibrosis [38]. A recent study also demonstrated that activin-A and ALK-4 are significantly increased in the serum of individuals with systemic sclerosis (SSc) and activate collagen production in SSc human fibroblasts [39]. TGF- β_1 and activin-A stimulate each other's expression in fibroblasts, pointing to synergistic effects in the development of fibrosis [40].

Activin-A exerts distinct effects on immune responses ranging from enhancing to inhibitory depending on the cell type, the existing cytokine milieu and the context of the response.

Pro-inflammatory effects

Stimulation of mouse and rat macrophages with recombinant activin-A (r-activin-A) increases TNF- α , IL-1 β and prostaglandin E2 release and enhances their phagocytosis *in vivo* and *in vitro*, pointing to pro-inflammatory effects [41, 42]. Activin-A promotes human monocyte to Langerhans cell (LC) differentiation and enhances LC migration *in vitro* [43]. In addition, activin-A enhances the migration of immature mouse DCs [44] and stimulates the proliferation and IgG and IgE release from mouse B cells *in vitro* [45, 46]. Activin-A also participates in granulocyte macrophage-colony stimulating factor triggered macrophage polarization into a pro-inflammatory M1 phenotype [47]. Finally, *in vivo* studies show that systemic administration of follistatin increases the survival of mice following a lethal dose of LPS, associated with decreased serum levels of TNF- α and IL-1 β [48].

Anti-inflammatory effects

An increasing body of evidence suggests that activin-A can also exert a strong anti-inflammatory role. Early studies demonstrated that r-activin-A inhibits phytohaemagglutinin-induced rat thymocyte proliferation *in vitro* [49]. In contrast to the stimulatory effects of activin-A on resting mouse macrophages, r-activin-A treatment of LPS-stimulated macrophages decreases the up-regulation of CD68, CD14, TLR4 and IL-1 β and reduces their phagocytosis *in vitro* [50, 51]. In addition, r-activin-A inhibits IL-6-driven mouse B cell proliferation and monocyte

phagocytosis *in vitro* [27, 52]. Activin-A increases IgA production from mouse mesenteric lymph node cells, pointing to a role for this cytokine in the maintenance of intestinal immune homeostasis [53]. In humans, *in vitro* studies have demonstrated that treatment of peripheral blood DCs with follistatin considerably enhances CD40L-induced pro-inflammatory cytokine and chemokine release, as well as, DC-driven CD8⁺ T cell proliferation and IFN- γ production [24]. As DCs produce activin-A in inflammatory settings, these findings propose an auto-crine regulatory role for activin-A in attenuating DC responses. Indeed, treatment of DCs with r-activin-A reduces HLA-DR expression and suppresses their T cell stimulatory properties *in vitro* [54]. Activin-A expression is also associated with the tolerogenic capacity of peripheral blood DCs during healthy human pregnancy [55]. Activin-A inhibits the proliferation, maturation, T-bet expression and cytokine release by human natural killer cells [56]. Furthermore, decreased proliferation and auto-antibody production from B cells of individuals with pulmonary alveolar proteinosis is observed following treatment with r-activin-A *in vitro* [57]. Notably, recent studies have shown that activin-A can also affect Treg cell responses as it enhances the conversion of mouse peripheral naïve T cells to forkhead box P3⁺ (Foxp3⁺) Tregs *in vivo* and increases TGF- β_1 -induced Foxp3 expression on T cells *in vitro* [58]. Thus, similar to other immunomodulatory cytokines, activin-A exerts a dual suppressive role: (a) it directly inhibits immune cell responses, and (b) it induces the generation of Treg cells.

From the aforementioned studies, it becomes evident that the functional outcome of activin-A signalling depends on a plethora of parameters, such as, *in vitro* vs. *in vivo* effects, the anatomical location and the cytokine micromilieu, the phase and magnitude of the immune response, the activation status of the target cell and the dose used. The exact intracellular signalling pathways that lead to such distinct functional outcomes are currently elusive.

Activin-A in the regulation of experimental asthma

Recent advances have uncovered a key role for activin-A in the regulation of Th2-mediated allergic responses and tissue remodelling in experimental models of asthma. Studies using a mouse model of ovalbumin-induced acute allergic airway inflammation have revealed that activin-A is highly expressed in the airway [59, 60]. Mouse mast cells (MCs) are a submucosal source of activin-A *in vivo*, while activin-A mRNA is rapidly induced in bone marrow-derived MCs following IgE-cross-linking [60]. MC-derived activin-A induces airway ASM proliferation *in vitro* [60] and this is highly significant considering that MC infiltration of ASM is associated with AHR in asthma [61]. Mouse and human MCs express activin-A receptors

[60] and treatment with r-activin-A promotes the migration and differentiation of mouse MC progenitor cells *in vitro* [62]. T cells and macrophages also produce activin-A in the airway of allergic mice ([59] and our unpublished observations). In fact, differentiated mouse Th2 cells are a dominant source of activin-A [63]. Interestingly, the Th2-associated transcription factor c-Maf interacts with the activin-A promoter and participates in gene transcription [63]. Further evidence for a role of activin-A in Th2 responses comes from studies demonstrating that it induces the alternative activation of mouse macrophages into the M-2 phenotype [63].

An increase in activin-A expression by bronchial epithelial cells has been observed in allergic mice, as compared with alum controls ([59] and our unpublished observations). Notably, another study demonstrated decreased activin-A expression in bronchial epithelium concomitant with an increase in the bronchoalveolar lavage (BAL) upon pulmonary allergen challenge [64]. The observed differences in the aforementioned studies could be due to the distinct experimental models and time-points studied. Not much is known about the activation of activin-A signalling pathways in experimental asthma models. Increased expression of ALK-4 and ActRIIA, along with phosphoSmad (pSmad)-2, in submucosal fibroblasts has been shown, pointing to effects of activin-A on airway repair and remodelling [59].

The role of activin-A in Th2-driven allergic responses and associated airway disease remained, until recently, incompletely defined. Our studies have revealed that activin-A suppresses allergic airway inflammation and protects against AHR and airway disease in mice [65]. Activin-A-mediated Th2 cell suppression is associated with induction of CD4⁺CD25⁻FoxP3⁺ Treg cells that produce IL-10 and inhibit allergen-driven Th2 responses *in vitro* and upon adoptive co-transfer *in vivo*. In addition, transfer of activin-A-induced Tregs into allergic mice confers protection against most disease features. Activin-A-induced suppression is dependent on IL-10 and TGF- β_1 release, pointing to synergistic effects between these immunosuppressive factors. Interestingly, a striking therapeutic effect for activin-A in experimental asthma was also observed. Findings from another study wherein follistatin was administered directly in the airways, pointed to pro-inflammatory effects of activin-A in allergic responses [64]. Indeed, as discussed earlier, it seems that activin-A may exert distinct *in vivo* functions depending on the route of administration or the site of overexpression [65].

The effects of activin-A neutralization during allergic responses were observed even in the presence of endogenous TGF- β_1 , suggesting that these two cytokines, as also shown in other studies, should not be considered as redundant during inflammatory processes [24, 25, 27, 28, 42, 44, 45, 53, 60, 62, 63, 65]. In support, activin-A and

TGF- β_1 induce different Treg subsets, indicating that, at least in the Th2 setting, these factors operate through distinct suppressive mechanisms.

Emerging evidence proposes that activin-A participates also in the induction of structural changes in the airway. An important recent study demonstrated that activin-A is a critical mediator of house dust mite (HDM)-induced airway remodelling in mice [66]. Using a model of pulmonary HDM challenge, Lloyd and colleagues demonstrated that adenoviral-induced overexpression of Smad 2 in airway epithelial cells results in enhanced airway remodelling and AHR. Interestingly, this is accompanied by a striking up-regulation of activin-A and IL-25 expression in the airway. Antibody-mediated neutralization of activin-A *in vivo* attenuates structural changes, concomitant with a decrease in IL-25 expression. Notably, these data propose a novel functional link between activin-A and IL-25 signalling in allergen-driven airway remodelling [66]. Another elegant study demonstrated that intra-tracheal administration of IL-13 in mice results in significantly increased activin-A expression in the BAL, associated with enhanced mucus secretion [67].

Activin-A in the control of airway inflammation and remodelling in human asthma

Although the afore-mentioned studies provide significant functional data regarding the effects of activin-A on experimental asthma, translation of these findings in human disease is essential for identification of clinical relevance and the design of novel therapeutic interventions. To this end, several investigators have examined the effects of activin-A on the asthmatic response in humans. Initial studies have demonstrated that cross-linking of the Fc ϵ RI on peripheral blood monocytes from atopic individuals results in increased activin-A release [68]. Karagiannidis et al. [69] demonstrated elevated serum activin-A levels in individuals with severe asthma, compared with mild asthmatics and controls. Importantly, peripheral blood CD4⁺ T cells from moderate asthmatics were a potent source of activin-A.

Our studies have shown increased activin-A expression in bronchial biopsies from mild asthmatics with lower expression in the normal airway [65]. We found that T cells, MCs and macrophages express activin-A in the airway, consistent with previous studies [60, 65, 69, 70]. Interestingly, neutrophils account for the highest number of infiltrating cells expressing activin-A in asthma [70]. In support, a very recent study demonstrated that peripheral blood neutrophils contain high levels of stored activin-A which is rapidly released upon stimulation with TNF- α *in vitro* [71]. These findings may have important clinical implications with particular relevance to severe, neutrophilic-associated, asthma phenotypes which often fail to respond to corticosteroid therapy [72]. Activin-A is also

highly expressed in the asthmatic epithelium [65, 70]. IL-13 and TNF- α induce activin-A expression in human bronchial epithelial cells (HBECs) [67, 70], while a significant up-regulation of activin-A is observed following respiratory syncytial virus infection *in vitro* [73]. Considering the emerging significance of epithelium in allergic responses and asthma, further understanding of the mechanisms that govern activin-A induction in airway epithelial cells is essential.

Despite increased activin-A levels in the asthmatic airway, the expression pattern of its signalling pathway is markedly different. Our studies have demonstrated that expression of ALK-4, along with Act-RIIA, is significantly decreased in airway submucosal cells of mild asthmatics [65]. Act-RIIA levels are minimal in airway epithelium also, while Act-RIIB is almost absent. In contrast to a previous study, wherein more symptomatic and severe asthmatics were included, our findings demonstrated decreased pSmad 2 levels in the airway of mild asthmatics [70, 74]. Besides, we noticed a marked decrease in inhibitory Smad 7 expression in asthmatics compared with controls (unpublished observations), consistent with previous studies [75]. These altered expression patterns between activin-A and its signalling pathways, especially in the inflammatory compartment, suggest dysregulated signalling in these cells. Decreased ALK-4/Act-RIIA expression may be associated with decreased basal receptor density, as reported for the TGF- β_1 signalling receptor, ALK-5, in experimental asthma models and human disease [59, 76]. It could also be postulated that decreased activin-A signalling, especially in infiltrating immune cells, is caused by activation inflammatory mediators in the local milieu. For example, LPS a pro-inflammatory mediator induced in the airway and implicated in the pathogenesis of experimental asthma, has been shown to decrease mRNA levels of activin-A receptors in monocytes and monocyte-derived DCs *in vitro* [22, 24, 77]. As an effect, inflammatory cells in the airway of asthmatics are either unable to respond to activin-A or respond in an altered manner, a process leading to decreased immune regulation and disordered tissue repair.

Interestingly, allergen inhalational challenge and, thus, disease provocation induces a rapid (within 24 h) increase in pSmad 2 expression in the airway of mild asthmatics [70, 78, 79]. Given that both activin-A and TGF- β_1 signal via Smad 2, the differential modulation of receptor expression allows visualization of the signalling cascade involved [8]. Allergen challenge results in marked epithelial and submucosal increases in ALK-4 and Act-RIIA expression, whereas ALK-5 levels are decreased [70]. ALK-4 strongly co-localizes with infiltrating CD3⁺ T cells and α -smooth muscle actin⁺ fibroblasts, resembling myofibroblasts (Fig. 2). ALK-4 expression is also increased in bronchial epithelium post-allergen challenge (Fig. 2) [70]. We would speculate that increased activin-A signalling,

especially in immune cells, at this phase of the allergic response, may be part of a protective immune regulatory network that attempts to control allergen-driven airway inflammation. An alternative, non-mutually exclusive, explanation is that activation of factors that induce airway remodelling upon allergen challenge [80] counteracts the suppressive effects of inflammatory cytokines on expression of activin signalling components. Further research is needed to evaluate such possible mechanisms.

Epithelial damage with impaired proliferation and incomplete repair are consistent features of the asthmatic phenotype [4]. Our functional *in vitro* studies demonstrate that r-activin-A significantly increases the proliferation of HBECs [70]. In addition, *in vitro* treatment of HBECs with follistatin considerably enhances TNF- α - and IL-13-induced chemokine expression by these cells, pointing to anti-inflammatory effects of activin-A on airway epithelial cell responses. Collectively, it can be postulated that rapid induction of ALK-4 and Act-RIIA expression, along with pSmad 2, in the airway upon allergen inhalational challenge, may be part of an inherent protective

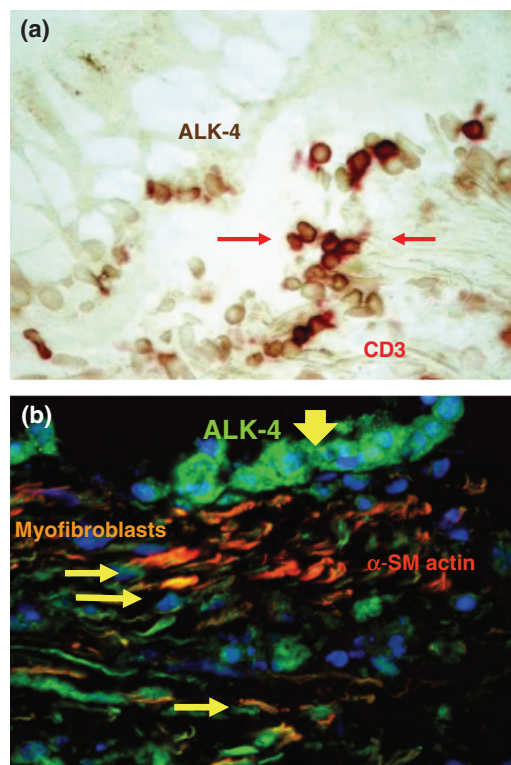


Fig. 2. Activation of activin-A signalling during allergen challenge in asthmatics. (a) Photomicrograph showing CD3⁺ T cells (red) expressing ALK-4 (brown) in the bronchial mucosa of mild asthmatics post-allergen inhalational challenge (arrows). (b) Confocal image showing ALK-4⁺ (green) and α -smooth muscle actin⁺ (red) cells in bronchial mucosa. ALK-4 expressing α -smooth muscle actin⁺ myofibroblasts are depicted in orange (arrows). ALK-4 expression on bronchial epithelium is also observed (arrowhead). ALK, activin-like kinase; SM, smooth muscle.

mechanism to respond to activin-A signalling in order to control inflammation and enhance epithelial repair. Nevertheless, considering the effects of activin-A on the induction of airway structural changes [60, 66], an involvement of the activation of its signalling pathway in the development of allergen-induced AHR is possible and merits further investigation.

We can begin to propose a model that encompasses the role of activin-A in the regulation of immune responses in the normal airway and during allergic asthma (Fig. 3). Upon airway exposure to inflammatory stimuli (i.e. allergen, viruses, pollutants) and/or injury, activin-A and its signalling pathway become rapidly activated in epithelial and infiltrating immune cells, while its secretion in BAL is also enhanced [54]. Increased activin-A expression leads to the suppression of airway epithelial and Th2 cell-mediated allergic responses, concomitant with an induction and/or expansion of Treg cells. At the same time,

activin-A promotes epithelial cell proliferation and repair. As a result, immune homeostasis and normal airway architecture are restored. However, in the strong inflammatory milieu that is characteristic of allergic asthma, a deficiency/dysregulation in activin-A signalling, especially in inflammatory cells, is apparent leading to impaired or dysregulated function. Thus, Th2 inflammation remains uncontrolled and remodelling events are initiated. These processes ultimately lead to the exacerbation of AHR and other features that define asthma and symptoms of disease.

Concluding remarks and future prospects

Growing evidence supports a multi-faceted role for activin-A in immune responses and associated diseases. In relation to asthma, it seems that activin-A acts as a critical molecular switch that controls the balance between

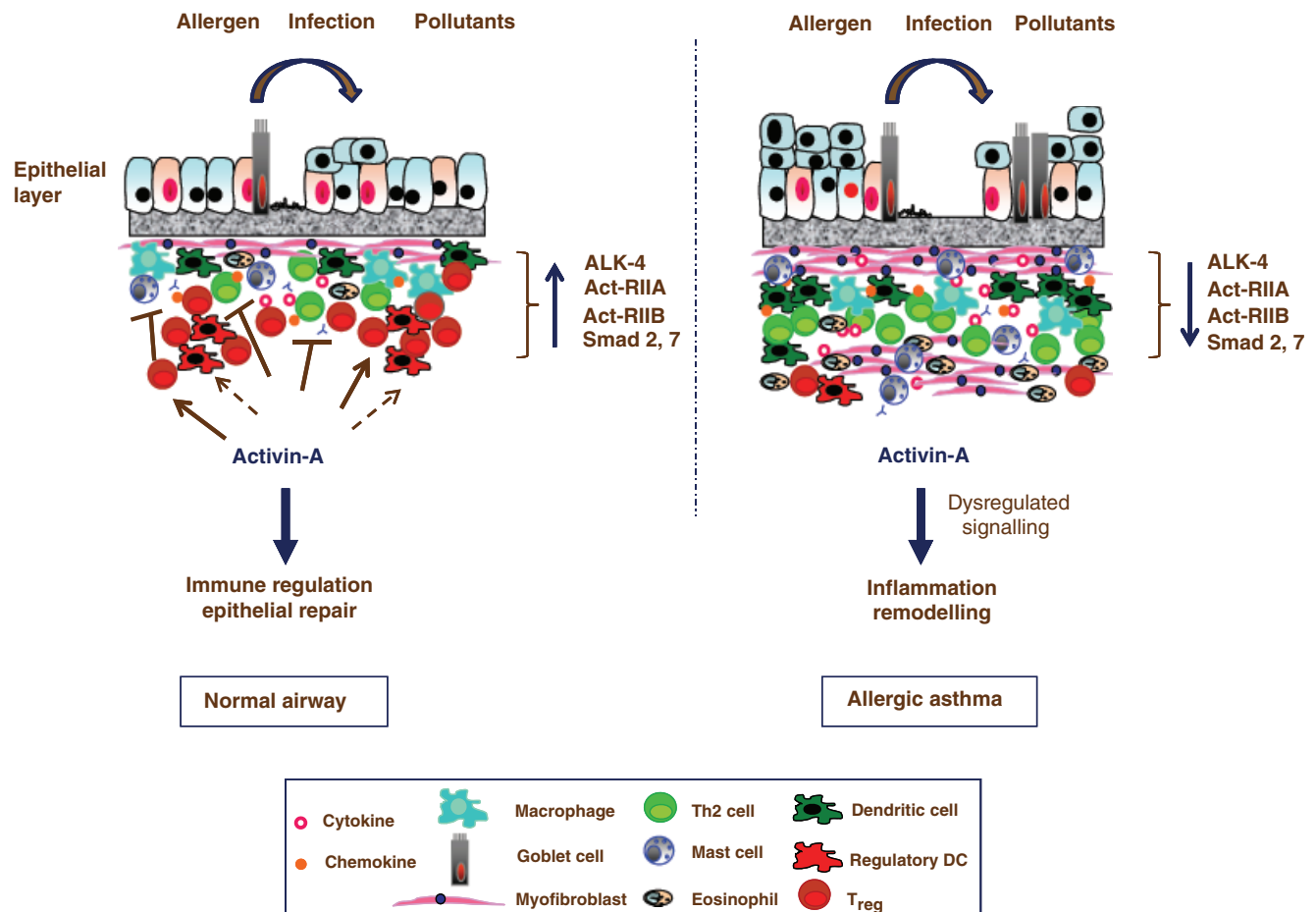


Fig. 3. Proposed model for activin-A's role in allergic asthma. Activin-A and its signalling pathway are rapidly activated following airway exposure to allergens, infectious agents, pollutants. Increased expression of activin-A and its receptors in the airway epithelium, submucosal cells and the BAL inhibits inflammatory cytokine and chemokine release by airway epithelial cells and induces tissue repair. At the same time, activin-A suppresses Th2-mediated effector responses both directly and through induction and/or expansion of Treg cells. An effect of activin-A on the recruitment and/or activation of regulatory DC subsets can be also envisaged (dotted arrows). In allergic asthma, a deficiency in the expression of activin-A receptors and Smads in the airway, especially in submucosal cells, results in dysregulated signalling and, ultimately, in altered function. As a result, inflammatory and remodelling events remain uncontrolled and disease exacerbation occurs. ALK, activin-like kinase; BAL, bronchoalveolar lavage; DC, dendritic cell.

immunity and tolerance in the airway. As activin-A can also promote airway epithelial cell repair, its therapeutic potential for asthma becomes evident. However, in view of the pro-fibrotic role of activin-A, effects on allergen-induced airway remodelling in asthmatics should be clearly defined. Moreover, it is conceivable that activin-A is differentially involved in the pathogenesis or remission in distinct asthma phenotypes and future studies should address this. Overall, it is clear that further work is necessary to dissect the complex role of activin-A in asthma. Considering the suppressive effects of activin-A in experimental asthma models, a role for this cytokine in the regulation of human Th2-driven allergic responses in asthmatics is highly plausible. Hence, the design of strategies which modulate activin-A and its signalling pathway offers the prospect of developing specific im-

munotheapies for asthma that aim not only to treat or reverse airway pathology but also to prevent the induction and progression of disease.

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