

Activin, neutrophils, and inflammation: just coincidence?

Paschalis Sideras · Eirini Apostolou · Athanasios Stavropoulos ·
Alexandros Sountoulidis · Arianna Gavriil ·
Anastasia Apostolidou · Evangelos Andreakos

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Abstract During the 26 years that have elapsed since its discovery, activin-A, a member of the transforming growth factor β super-family originally discovered from its capacity to stimulate follicle-stimulating hormone production by cultured pituitary gonadotropes, has been established as a key regulator of various fundamental biological processes, such as development, homeostasis, inflammation, and tissue remodeling. Deregulated expression of activin-A has been observed in several human diseases characterized by an immuno-inflammatory and/or tissue remodeling component in their pathophysiology. Various cell types have been recognized as sources of activin-A, and plentiful, occasionally contradicting, functions have been described mainly by *in vitro* studies. Not surprisingly, both harmful and protective roles have been postulated for activin-A in the context of several disorders. Recent findings have further expanded the functional repertoire of this molecule demonstrating that its ectopic overexpression in mouse airways can cause pathology that simulates faithfully human acute respiratory distress syndrome, a disorder characterized by strong involvement of neutrophils. This finding when considered together with the recent discovery that neutrophils constitute an important source of activin-A *in vivo* and earlier observations of upregulated activin-A expression in diseases characterized by strong activation of neutrophils may collectively imply a more intimate link between activin-A expression and neutrophil reactivity. In this review, we provide an outline of the functional repertoire of activin-A and suggest that this growth

factor functions as a guardian of homeostasis, a modulator of immunity and an orchestrator of tissue repair activities. In this context, a relationship between activin-A and neutrophils may be anything but coincidental.

Keywords Activin · TGF- β · Inflammation · Remodeling · Neutrophils

Introduction

The transforming growth factor β (TGF- β) super-family encompasses a large group of structurally related polypeptide growth factors including TGF- β isoforms, activins, bone morphogenetic proteins (BMPs), growth differentiation factors (GDFs), nodal, and Müllerian inhibiting substance [1]. Numerous studies utilizing genetically modified transgenic or knock-out mouse lines have demonstrated that whereas normal signaling by this system plays a pivotal role during early development, adult homeostasis, and tissue injury–repair, its deregulation can contribute to development of pathology.

Activins together with structurally related inhibins form a subgroup within the TGF- β -super-family. Although activin-A, the prototype member of this subfamily, was initially identified on the basis of its ability to stimulate the release of follicle-stimulating hormone (FSH) from pituitary gonadotropes *in vitro* [2], numerous studies that followed have demonstrated that it is actually a remarkable pluripotent growth factor essential for embryonic development [3–8] and adult tissue homeostasis [9–14].

Activins and inhibins are homo- or heterodimeric proteins formed by the interaction of “ α ” and “ β ” subunit which are encoded by distinct genes [15]. One gene encoding for the “ α ” subunit (inhibin- α) and four genes encoding for “ β ” subunits (inhibin- β A, inhibin- β B, inhibin- β C, and inhibin- β E) have been characterized in mammals [13,

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P. Sideras (✉) · E. Apostolou · A. Stavropoulos ·
A. Sountoulidis · A. Gavriil · A. Apostolidou · E. Andreakos
Centre for Immunology and Transplantations, Biomedical
Research Foundation Academy of Athens, Soranou Efessiou 4,
11527 Athens, Greece
e-mail: sideras@bioacademy.gr

16–18]. Activins are formed by homo- or heterodimerization of inhibin- β subunits. So far, the homodimeric $\beta A/\beta A$ (activin-A) and $\beta B/\beta B$ (activin-B) and the heterodimeric $\beta A/\beta B$ (activin-AB) dimers have been purified from biological fluids and their activity assessed, with activin-A receiving the greatest attention till now. Heterodimerization of the inhibin- α subunit with the inhibin- βA or - βB subunits results in the production of the naturally occurring activin antagonists inhibin-A and inhibin-B, respectively (reviewed by de Kretser et al. [10]). The functions of activin-A, activin-B, and activin-AB appear to be very similar; however, the expression pattern of the βA and βB subunits differs [19] and mice deficient for either of these subunits exhibit different phenotypes [20, 21] suggesting that these activin forms may perform overlapping functions, without however being fully redundant. Absence of any detectable phenotype in βC or βE knockout mice led initially to the conclusion that their function can be compensated [18]. However, subsequent studies demonstrated that the βC subunit can form homodimers or heterodimers with the βA or βB subunits and it was postulated that these forms might compete for receptor occupancy providing thus an antagonistic regulatory mechanism [22, 23]. Consistently, it was shown that recombinant activin-C could antagonize activin-A in vitro and transgenic animals overexpressing inhibin- βC were found to develop numerous abnormalities in testis, liver, and prostate [24].

TGF- β super-family ligands signal via ternary receptor complexes that are formed by two types II and I receptor polypeptides [25]. Both types of receptors possess serine-threonine kinase activity. The type II receptors are constitutively active and thus upon interaction with the ligand come in proximity with the type I receptors, phosphorylate them and activate their kinase activity. Activated type I receptors phosphorylate and activate receptor-activated Smads (R-Smads), the intracellular effector molecules of this system. TGF- β super-family signaling can be classified into two branches: the TGF- β branch, represented by ligands, such as TGF- β , activin, nodal, or myostatin, which leads to activation of Smad2 and Smad3; and the BMP branch, represented by ligands, such as BMPs and GDFs, which leads to activation of Smad1, Smad5, and Smad8. Phosphorylated R-Smads form complexes with the common mediator, Smad4 and enter the nucleus where they bind to DNA and interact with transcription factors to regulate gene expression [26]. Finally, the inhibitory Smads (I-Smads) Smad6 and Smad7 are induced upon TGF- β superfamily ligand-mediated signaling and exert a negative feedback effect by competing with R-Smads for receptor interaction and by marking the receptors for degradation [26]. TGF- β super-family ligands can also signal via Smad-independent intracellular pathways that involve mitogen-activated protein kinases and are referred to as “non-canonical” [27].

Activins utilize for signaling the type I receptors activin-like kinases (ALKs), ALK-2, ALK-4, and ALK-7 and the type II receptors Act-RIIA and Act-RIIB [28–31]. Activin-A binds to the type II receptor and cooperatively recruits the type I receptor [32].

Activins, like other TGF- β superfamily proteins, are synthesized as large precursor proteins that contain N-terminal pro-regions and C-terminal mature domains [33]. The pro-domains guide correct folding and dimerization and contribute to biological activity of mature activins [34–36]. The dimeric precursors are proteolytically cleaved and activins are secreted from the cell noncovalently associated with their pro-peptides. Outside the cell, the pro-domain of activin-A can target it to heparin sulfated proteoglycans [37] thus forming gradients around the cells that produce it and also protecting it from proteolytic attack [38]. Interestingly, activin-B lacks the critical residues required for binding to heparin sulfated proteoglycans and may diffuse more easily away from the source of its production, affecting the relative local concentration of mature activin-A and activin-B in a tissue [39]. Whereas TGF- β isoforms bind their pro-peptides with very high affinity and therefore are secreted from cells as latent complexes [40], the affinity of the βA pro-peptide for mature activin-A is very low (Kd 100 nM). Although this affinity can support sequestration of activin-A within tissues, the pro-peptide can be easily displaced by the much higher affinity of the mature peptide to activin type II receptors (Kd 200 pM), and therefore, activin-A is secreted in the extracellular spaces “practically” in an active form [36].

Levels of bioavailable activins are tightly regulated

The function of activins is tightly regulated at several levels. Inhibin-A and inhibin-B, sharing the same β -subunits as activin-A and activin-B are able to compete for the activin receptors acting thus as extracellular regulators of activin signaling [41]. Furthermore, follistatin and follistatin-like protein-3 (FSTL-3) [42–44], are synthesized and secreted independently, can bind to activins with high affinity and induce their rapid endocytosis and proteolytic degradation modulating thus the amounts of bioavailable activins in the tissues [45]. Follistatin was initially identified due to its ability to suppress FSH release by the rat pituitary [46], however, it was later demonstrated that this was mediated via blocking activin’s access to its receptors [47].

Two follistatin variants generated through alternative splicing of a primary mRNA have been described, namely, follistatin-288 (FS-288), and follistatin-315 (FS-315) [48]. FS-288 possesses a heparan binding sequence that enables association with heparan sulfate proteoglycans. It normally associates with activins at the cell surface and facilitates

their internalization and degradation [49]. However, it can be released into the circulation by heparin [46, 50]. FS-315 on the other hand is the predominant follistatin form in the circulation. It can bind to heparin sulfate only after interaction with activins due to a conformational change that exposes its heparan sulphate binding segment [51, 52]. A third isoform, FST-303 has been identified as a proteolytic by-product of FST-315 [53]. FSTL-3 lacks heparan binding segment and thus behaves like FS-315 [54]. The crystal structures of activin-A in complex with FSTL-3 [55] and FST-288 [56] have been solved. In both these models, two follistatin molecules encircle the activin dimer, such that one quarter of the activin surface is buried. Both forms of follistatin contact two discontinuous surfaces of activin, shielding both the types I and II receptor binding sites [55, 56]. Interestingly, follistatin binds to activin-B with an affinity 10-fold lower than to activin-A [45, 57] indicating a possibility for differential regulation of these two activins. Furthermore, follistatin can inhibit other TGF- β ligands such as myostatin and GDF-11 [39] and likewise, FSTL-3 can bind myostatin [58, 59] and some members of the BMP subfamily [39, 43, 60]. Thus, follistatin variants and follistatin-like proteins provide a versatile mechanism for local or systemic regulation of the levels of bioavailable activins and some other members of the TGF- β superfamily. Bioavailability of activin-A can be modulated by the action of additional molecules, such as α 2-macroglobulin, cerberus, and lipovitelin (reviewed by Philips [14]).

Activin-A expression is upregulated in disorders characterized by immuno-inflammatory and/or tissue remodeling pathophysiology

One of the biological processes with which deregulated activin-A expression and function were implicated early on as contributor of pathogenesis, was fibrosis. Hedger et al. demonstrated that activin-A could stimulate mitosis of 3 T3 fibroblasts in vitro [61]. Although, its capacity to stimulate fibroblasts was a fraction of that of TGF- β , this study provided an early link between activin-A and fibrosis. Expression of activin-A protein was detected by immunofluorescence in bronchiolar epithelium and smooth muscle cells of veins in both control and bleomycin-treated mice and increased activin-A secretion was observed in cultures of alveolar macrophages isolated from bleomycin-treated animals [62]. Although the bleomycin model resembles more acute lung injury (ALI) than *bona fide* fibrosis, a subsequent report describing increased activin-A immunostaining in metaplastic epithelium, hyperplastic smooth muscle cells, desquamated cells, alveolar macrophages, and smooth muscle cells, admittedly in a very small group of patients with different forms of interstitial lung disease, firmly-associated

activin-A with the fibrotic process in the literature [63]. This association was further strengthened by the observations that: (a) activin-A could increase fibroblast migration and their differentiation to myofibroblasts in vitro [64], (b) prophylactic administration of follistatin could ameliorate bleomycin-induced pathology in mice [65], (c) increased levels of activin-A produced by hepatic stellate cells upon carbon tetrachloride-induced liver injury were contributing to development of hepatic fibrosis, the severity of which could be diminished by the co-administration of follistatin [66], and (d) the detection of increased serum levels of activin-A, in patients with systemic sclerosis and the demonstration that activin-A could upregulate in vitro collagen production by fibroblasts isolated from patients with systemic sclerosis [67]. Detection of skin abnormalities in animals deficient for either activin-A or follistatin and development of hyper-keratotic skin in the later provided the first evidence pointing towards involvement of the activin-A/follistatin system in skin remodeling [20, 68]. Consistently, substantial increase of activin-A and activin-B expressions was detected after skin injury in mice [69]. In situ hybridization studies revealed different spatiotemporal expression pattern for the two activins, with activin-A being expressed in the granulation tissue below the wound and activin-B in the hyper-proliferative epithelium. While both activins were upregulated during the first 7 days post-injury, after 13 days, activin-A expression was back to baseline levels while activin-B expression persisted [69]. Overexpression of activin-A in the epidermis of transgenic animals under the control of the keratin-14 promoter caused dermal fibrosis, epidermal hyper-thickening, and enhanced wound repair [70]. Conversely, transgenic animals where follistatin was overexpressed using the same promoter as above, had a mild dermal and epidermal atrophy and after injury exhibited a severe delay in wound healing. However, upon healing the wounds presented substantially reduced scar formation [71, 72]. It should be noted that the aforementioned studies constitute the earliest direct demonstration of the detrimental effect of deregulated activin-A expression in vivo. Interestingly, local upregulation of activin-A has been detected by immunohistochemistry during the inflammatory and repair phases that occur in burnt skin as well [73]. Although collectively these reports have implicated activin-A in the processes associated with tissue repair and fibrosis, its actual role in the context of these processes must be quite complex. Activin-A levels were found to be upregulated by several profibrotic agents such as TGF- β 1, tumor necrosis factor- α (TNF- α), endothelin, interleukin (IL)-13, thrombin, and angiotensin, and conversely activin-A itself was found to be able to stimulate production of pro-fibrotic factors, including TNF- α , connective tissue growth factor (CTGF), endothelin, type 1 collagen, tissue inhibitor of metalloproteinase-1, and plasminogen activator inhibitor-1 (PAI-1) (reviewed by de Kretser et al. [74]).

Activin-A has been implicated in the development of allergic airway inflammatory conditions and thus in the pathophysiology of asthma. Rosendahl et al. [75] using an experimental model of ovalbumin (OVA)-induced airway inflammation demonstrated a dramatic increase in the numbers of inflammatory and structural cells expressing nuclear phosphorylated Smad2 within the allergen-challenged lungs that was accompanied by substantial increase in the mRNA levels of inhibin- β A, inhibin- β B, inhibin- β C, inhibin- β E, and ALK4 (the activin type I receptor) and thus linked for the first time activin-A with respiratory inflammation [75]. The observation that the rat basophilic leukemia (RBL-2H3) [76] and human mast cells (MC) [77] could produce activin-A upon stimulation with ionomycin or ionomycin plus PMA respectively, the colocalization of activin-A with MC in biopsies from human asthmatics, and the defective production of activin-A upon allergen challenge in MC-deficient mice [77] linked activin-A with MC function and suggested that these cells were an important source of activin-A in the inflamed airways. Increased activin-A levels were detected in the serum of patients with severe asthma compared with levels of patients with moderate asthma and healthy control subjects and increased mRNA levels were detected in T cells from patients with moderate asthma. Expression of activin-A by infiltrating lymphocytes and structural cells of the lung was detected in tissue sections from OVA challenged mice [78] or human asthmatic patients by immunohistochemistry [78, 79] and activin-A was found to promote proliferation of human airway smooth muscle cells, and regulate migration and differentiation of bone marrow-derived MC progenitors [80]. Concurrent release of activin-A and follistatin in the lungs of OVA challenged mice with maximal BAL concentrations coinciding with maximal airway eosinophilia and frequency of IL-4, IL-5 and IL-13 producing cells in mediastinal lymph nodes was reported by Hardy et al. [81]. Immunohistochemical staining pointed towards airway epithelial cells as the source of activin-A and follistatin, and decline of immunostaining for both molecules that paralleled goblet cell metaplasia was interpreted to postulate that these molecules were pre-stored and released upon allergen stimulation [81]. Although upregulation of activin-A in the context of airway inflammation is well documented, conflicting views have been put forward regarding the role of this molecule in asthma pathophysiology based on studies in the mouse OVA allergic airway inflammation model. Hardy et al. [81] observed that neutralization of the allergen-induced activin-A with exogenous follistatin had a beneficial effect [81], however, Semitekoulou et al. [82] using the same animal model observed exacerbation of allergen-induced pathology upon neutralization of activin-A using specific antibodies and attenuation of pathology upon treatment of the allergen challenged animals with

recombinant activin-A. An interesting twist in the interplay of activin-A with allergic airway inflammation is the recent report demonstrating that activin-A, like TGF- β , could drive in vitro generation of T(H)9 cells [83]. Importantly, in vivo inhibition of T(H)9 differentiation induced by allergen and reduction of airway hyper-reactivity and collagen deposition could be accomplished only by blocking both activin-A and TGF- β [83]. Thus, although activin-A levels are increased during airway inflammatory responses, the actual role of this molecule and consequently its potential therapeutic utility for asthma therapy definitely needs further substantiation.

Serendipitously, while studying the effect of castration on the levels of circulating follistatin in rams, Philips et al. [84] observed a robust elevation in the levels of circulating follistatin both in castrated and sham-operated rams. This surprising finding demonstrated that tissues other than the gonads could produce follistatin and also associated upregulation of follistatin levels with the inflammatory response associated with the surgery [84]. This notion was further substantiated by replicating similar follistatin release patterns in sheep injected with lipopolysaccharide (LPS) [85], sheep injected with recombinant IL-1 β or in lambs subjected to intra-thoracic injection of yeast [52]. A few years later, the development of activin-A-specific immunoassays allowed Jones et al. [86] to monitor activin-A levels in the serum of LPS-injected sheep and demonstrate that activin-A was released within 40 min from LPS injection, slightly earlier than TNF- α and significantly earlier than IL-6 [86]. The observation that serum activin-A levels exhibited a biphasic response provided the basis for the hypothesis that the initial release of activin-A was due to pre-stored material released from responding cells, whereas the latter release was due to newly synthesized material [86]. Similar results were obtained in the mouse system where upon LPS injection a rapid initial rise of serum activin-A was followed by a secondary rise 3–8 h later [87]. Survival and other pathology-associated parameters in LPS-treated mice improved upon prophylactic administration of follistatin [87] or prophylactic and therapeutic (24-h post-LPS injection) administration of an activin neutralizing fusion protein composed of the extracellular portion of ActRIIB fused to the Fc portion of the human IgG1 molecule [88]. Detection of increased levels of activin-A in the serum of patients with sepsis [89] and increased activin-A levels in cerebrospinal fluid of rabbits [90] and human patients [91] with bacterial meningitis raised the possibility that activin-A could be a crucial player in the pathophysiology of sepsis.

Detection of increased levels of activin-A mRNA in mouse models of inflammatory bowel disease [92] and in biopsies of Crohn's disease or ulcerative colitis patients [93] suggested that activin-A may play an important role in the pathophysiology of inflammatory bowel disease. The demonstration by Dohi et al. [92] that prophylactic and therapeutic treatment

with follistatin could improve the survival rate of mice in three mouse colitis models, attenuate several pathology-associated parameters and upregulate proliferation of intestinal epithelial cells and tissue repair, improving thus the barrier function of the colonic mucosa, strongly supported the association between activin-A and colitis pathophysiology [92]. It should be noted that the study of Dohi et al. is among the very few activin-A related studies where activin-A upregulation within the diseased tissues was verified by *in situ* mRNA hybridization and the beneficial effect of follistatin was demonstrated in a therapeutic protocol with activin-A neutralization commencing after the onset of pathology.

Several studies have implicated activin-A in the pathophysiology of inflammatory arthropathies. High levels of activin-A, in some reports exceeding 30 ng/ml were detected in synovial fluid of patients with rheumatoid arthritis and gout [94–96]. Activin-A was detected in fibroblastoid synovial cells and CD68⁺ macrophage-lineage cells in the proliferative reactive synovial membranes obtained from rheumatoid arthritis patients, as well as in and the smooth muscle and the endothelial layer of the arteries in these vascularized proliferative tissues [94, 96]. Furthermore production of activin-A by synoviocytes and chondrocytes was found to be upregulated *in vitro* by IL-1 β , TGF- β , interferon- γ (IFN- γ), and IL-8 [94, 96].

In patients with heart failure, serum levels of activin-A were found to be significantly elevated with magnitudes correlating with disease severity, and increased levels of inhibin- β A mRNA were detected in T cells but not monocytes [97]. Furthermore, in a rat model of heart failure induction of activin-A expression by cardiomyocytes and concerted increase in activin types I and II receptor mRNA was observed after myocardial infarction. Moreover, treatment of neonatal rat cardiomyocytes with activin-A was found to induce expression of mediators involved in infarction healing and myocardial remodelling such as matrix metalloproteinase-9, tissue inhibitor of metalloproteinase-1, transforming growth factor-beta-1, and monocyte chemo-attractant protein-1. The above findings were interpreted to suggest involvement of activin-A in the pathogenesis of heart failure.

Collectively, overexpression of activin-A has been observed in experimental models and patients suffering from acute inflammatory disorders (sepsis, meningitis, and endotoxemia), autoimmune disorders (rheumatoid arthritis, inflammatory bowel disease, and systemic lupus erythematosus), traumatic injuries (skin burns and surgery) and diseases with a strong tissue remodeling element (skin, liver, lung, and kidney fibrosis). The beneficial effect of activin-A neutralization via prophylactic, and, in some cases, therapeutic administration of follistatin in disease relevant animal models strongly suggested that activin-A was not a simple bystander but rather a key component of the pathogenetic mechanisms [98], raising

hope that the activin-A signaling pathway could constitute an attractive target molecular system for development of therapeutic interventions.

Activin-A is produced by various cell types and stimulates numerous biological responses *in vitro*

Given the strong association of activin-A to human pathology, special effort has been allocated for the identification of the cellular sources of this growth factor in the context of the different physiological and pathological conditions with which it has been associated with, the characterization of the cellular responses triggered by activin-A and more importantly, the identification of the activin-A-induced responses which, when deregulated, could cause pathology.

Hence, numerous studies have provided information regarding the secretion of protein or the synthesis of mRNA for activin-A, in tissues and isolated cell subpopulations of immune and non-immune origin. Thus, it was shown that human peripheral blood monocytes produce activin-A upon stimulation with LPS [99], granulocyte-macrophage colony-stimulating factor (GM-CSF), IFN- γ [100], or TREM-1 [101]. Expression of mRNAs for activin types I and II receptors (ActRI, ActRIB, ActRII, and ActRIIB) and secretion of activin-A by mouse peritoneal macrophages was demonstrated after stimulation with agonists of Toll-like receptor (TLR) 2, 4, and 9 [102, 103]. Production of activin-A by activated microglia and infiltrating macrophages was demonstrated in an animal model of experimental meningitis induced by inoculation of *Streptococcus pneumoniae* in the CSN [90]. Human monocyte-derived dendritic cells and CD1c⁺ and CD123⁺ peripheral blood dendritic cell (DC) populations were found to express both types I and II activin receptors and secrete high levels of activin-A after exposure to bacteria, specific TLR agonists, or CD40 ligand (CD40L) [104–106]. Interestingly, blood plasmacytoid DC did not synthesize activin-A upon stimulation with influenza virus [105]. Human MC upon stimulation with PMA plus calcium ionophore (A23187) and cultured murine bone marrow MC (BMMC) upon stimulation by cross-linking the IgE receptors were found to produce activin-A as well [77]. Anti-CD3 stimulated CD4^{pos}CD25^{neg} T cells, and not CD4^{pos}CD25^{pos} regulatory T cells, especially when cultured under Th2 polarizing condition were found to secrete substantial amounts of activin-A in the culture medium, including thus T cells among the putative sources of bioactive activin-A [107]. Finally, activated B cells were also found to express types I and II activin receptors and to secrete activin-A [108].

The studies discussed earlier by Jones et al. in sheep [86] demonstrated that upon LPS administration activin-A levels in the serum increased displaying a biphasic response. The

biphasic activin-A response was interpreted as providing support for the hypothesis that the early response was due to the release of pre-stored activin-A, whereas the second delayed response was due to de-novo synthesis. Activin-A and follistatin were detected by immunohistochemical staining in mouse airways [81]. The reduction of this immunostaining upon challenge of OVA sensitized animals with allergen correlated with increased levels of activin-A and follistatin in the BAL and development of allergic airway inflammation in the airways. These findings were used to further support the notion that preformed activin-A was released in the early stages of an inflammatory response [81]. Analysis of freshly isolated human neutrophils demonstrated that, compared to blood mononuclear cells, they contained 20-fold more activin-A which they could release rapidly upon stimulation with $\text{TNF}\alpha$, introducing thus neutrophils as an important source of activin-A. The fact that $\text{TNF}\alpha$ stimulated neutrophils in vitro released activin-A within 1 h although corresponding mRNA levels were not increased until 12 h of culture supported the “pre-stored activin-A” concept [109]. Expression of activin-A by neutrophils and stimulation of its secretion by $\text{TNF}\alpha$ was also confirmed with murine bone marrow derived neutrophils [110]. Finally, in a comprehensive study, Wu et al. demonstrated that basically all analyzed tissues from untreated or LPS-treated mice contained detectable activin-A and follistatin mRNA and protein [111]. The levels of activin-A in the different tissues analyzed ranged substantially indicating that homeostatic levels of activin-A differ among different tissues. The highest mRNA expression was found in the liver, and the highest concentration of activin-A protein in the bone marrow. Upon LPS treatment, activin-A and follistatin mRNA levels did not change significantly within the first hour after LPS treatment in any of the analyzed tissues, however, a 35 % decrease and a 5-fold increase in activin-A protein level was observed in bone marrow and lung respectively. These changes were associated with migration of bone marrow-derived, activin-A-loaded, neutrophils into the lung parenchyma. Again the authors interpreted these observations as suggesting that the rapid increase in circulating activin-A during LPS-induced inflammation is regulated at the posttranscriptional level, from newly translated and/or stored protein. Interestingly, despite the implication of neutrophils as important activin-A producers, so far little is known regarding the effect of activin-A on the functionality of these cells.

We have isolated epithelial, endothelial, inflammatory, and mesenchymal cells from enzymatically digested control and OVA-challenged animals and analyzed the mRNA levels for activin-A and follistatin. Activin-A mRNA was detected in all cell types in the untreated lungs, however, follistatin mRNA was found exclusively in the mesenchymal fraction, i.e., $\text{EpCAM}^{\text{neg}}/\text{CD31}^{\text{neg}}/\text{CD45}^{\text{neg}}$ cells (unpublished observations). Upon

allergen challenge, the increased activin-A mRNA (>10-fold) was mainly in the CD45^{pos} fraction and again upregulation of follistatin mRNA was detected exclusively in the mesenchymal fraction (unpublished observations). Although these observations do not exclude the possibility that some pre-stored activin-A or follistatin could be released in the airways, they are more compatible with the notion that the bulk of these molecules, at least in the OVA-induced allergic airway inflammation model, is most likely newly synthesized.

Activin-A was not only found to be produced by different cells of the immune system, as outlined above, it was also shown to affect their function in various ways depending on the activation state of the target cells and the coexistence of other stimulatory agents. Thus, activin-A could stimulate the migration of immature murine DCs [112], acting as a pro-inflammatory agent. However, human monocyte derived DCs and CD1c^+ peripheral myeloid DCs, when stimulated in vitro with CD40 ligand (CD40L) in the presence of follistatin to block the autocrine action of activin-A, produced higher levels of DC cytokines (IL-6, IL-10, IL-12p70, and $\text{TNF}\alpha$) and chemokines (IL-8, IP-10, RANTES, and MCP-1) [104] suggesting thus indirectly a pro-inflammatory effect of activin-A on DCs. Moreover, in the same study, it was shown that neutralization of the DC-derived activin-A significantly enhanced the capacity of DCs pulsed with chemically inactivated whole *flu* particles to stimulate expansion of viral-antigen-specific effector CD8^+ T cells in vitro [104]. These findings combined with the demonstration that activin-A could inhibit maturation of DCs [104, 113] and the fact that interaction of T cells with immature DCs could produce an immunosuppressive or tolerogenic response [114], could collectively indicate a potential, activin-A-mediated, autocrine feedback attenuation of DC-mediated responses.

Equally complex appeared to be the effect of activin-A on macrophage functions. Thus, It has been shown that activin-A can stimulate monocytes/macrophages to produce inflammatory mediators, such as IL-1 β , $\text{TNF}\alpha$, IL-6, nitric oxide (NO), prostaglandin E2, and thromboxanes [115–118], enhance the phagocytic capacity of mouse and rat peritoneal macrophages in vivo and in vitro [115, 117], and promote human monocyte to Langerhans cell (LC) differentiation and migration in vitro [119]. In contrast, other studies demonstrated that activin-A could have anti-inflammatory effects on macrophages that were already activated or in a primed state. Thus, activin-A was shown to attenuate NO release, phagocytic and pinocytic capacity and expression of CD14 and MHC II by LPS activated mouse peritoneal macrophages [120]. Moreover, decreased secretion of IL-1 β and NO as well as IL-1 β and iNOS mRNA, and reduced expression of CD68, CD14, and TLR4 was observed upon LPS stimulation in the mouse macrophage cell line RAW264.7 [121]. Likewise, using activated human monocytic cell lines THP-1 and U-937 it was shown that activin-A could inhibit the production of IL-1 β and enhance at the same time

the production of IL-1 receptor antagonist, resulting thus in attenuation of IL-1 β biological activity [122]. Using the murine microglial cell line MG6 it was shown that activin-A could mitigate its activation by LPS and attenuate upregulation of pro-inflammatory mediators including IL-18, IL-6 and iNOS [123]. Likewise, using primary rat microglia cell cultures, it was shown that activin-A was able to decrease IFN- γ -induced synthesis of NO and LPS-induced TNF- α , IL-6, inducible NO synthase, and IL-1 β [124].

Based on the observation that activin-A could stimulate peritoneal macrophages to express arginase-1 (Arg-1), one of the markers of alternatively activated macrophages (M2), and could also inhibit the IFN- γ -induced expression of inducible NO synthase, one of the markers of inflammatory (M1) macrophages, Ogawa et al. proposed that activin-A promotes differentiation of macrophages toward the M2 phenotype [107]. However, a recent study by Sierra-Filardi et al. demonstrated that activin-A was expressed preferentially by M1 macrophages and more importantly, promoted differentiation of macrophages toward the M1 phenotype [125]. Neutralization of activin-A with specific antibodies in cultures of macrophages that were treated with GM-CSF (conditions that normally lead to M1 polarization) led to reduced expression of M1 markers (*CCL17*, *ECSCR*, and *CCR2*) and increased expression of M2 markers (*MAFB*, *ETS2*, *FOLR2*, *IL10*, *IGF1*, and *SERPINB2*), further supporting the notion that activin-A is an inducer of M1 macrophages [125]. Although the above two studies appear to contradict each other, they might actually present a variation of the same theme, namely, that activin-A affects in different ways naïve and activated or primed cells. The peritoneal macrophages used by Ogawa et al. [107] were murine and were collected after intra-peritoneal injection of thioglycolate, certainly not a resting macrophage population; whereas the macrophage populations analyzed in the study of Sierra-Filardi et al. [125] originated from human peripheral blood monocytes and were polarized using appropriate culture conditions in vitro.

Activin-A was also found to affect T cell function. Early studies demonstrated that recombinant activin-A could inhibit phytohaemagglutinin (PHA)-induced rat thymocyte proliferation in vitro [61]. More importantly, activin-A was shown to enhance in vivo the conversion of mouse peripheral naïve T cells to Foxp3⁺ T regulatory cells (Tregs) and amplify the capacity of TGF- β 1 to promote in vitro development of Foxp3⁺ Tregs [126]. Induction of another IL-10 producing, however, Foxp3⁻ T regulatory population by activin-A has been described by Semitekolou et al. [82]. Thus, activin-A appears to attenuate T cell responses in two ways, either by inhibiting directly T cell responsiveness or indirectly by promoting the generation of T regulatory cells. Natural killer (NK) cells have also been listed among the inflammatory cells affected by activin-A. Robson et al. [127] demonstrated that NK cells express types I and II activin receptors

and that activin-A directly regulates NK cell functions by downregulating expression of the T-box transcription factor T-bet, secretion of IFN- γ , and expression of CD25 [127]. Finally, B lymphocytes have not been exempt from the regulatory influences of activin-A as it has been shown that through direct action on resting B cells, activin-A could enhance IgG and IgE production [108], induce IgA production from mouse mesenteric lymph node cells [128] and decrease proliferation and autoantibody production from B cells of individuals with pulmonary alveolar proteinosis [129].

In accordance with the broad expression pattern of activin-A described in the study of Wu et al. [111], other cell types of non-immune origin have been described earlier as sources of activin-A and follistatin. For example, it has been shown that bovine aortic endothelial cells upon LPS stimulation upregulate activin-A and follistatin mRNA synthesis and protein secretion [130]. Bone marrow-derived stromal fibroblasts were found to produce activin-A, and this was enhanced by IL-1 β and LPS and inhibited by IFN- γ . Interestingly, the opposite was observed with follistatin secretion, namely, IL-1 β and LPS exhibiting an inhibitory and IFN- γ a stimulatory effect on follistatin secretion by the same type of cells [131]. Production of activin-A has also been demonstrated in cultures of synoviocytes and chondrocytes upon stimulation with IL-1 β , TGF- β , IFN- γ , and IL-8 [94], consistent with the detection of high activin-A levels in the synovial fluid of patients with rheumatoid arthritis and gout. Moreover, production of activin-A was demonstrated upon serum stimulation in cultures of quiescent, skin derived keratinocytes and fibroblasts, and again activin-A production was found further enhanced by IL-1 β and TNF- α [132]. Hepatocytes and hepatic stellate cells have also been described as activin-A producers [66].

Collectively, the existing information indicates that activin-A mRNA and protein are synthesized continuously in healthy tissues. In the context of immuno-inflammatory responses, different types of inflammatory cells and injured or stressed tissue-resident cells, such as endothelial, epithelial, and mesenchymal cells can contribute to activin-A production. Activin-A depending on its concentration, context, and the previous history of its cellular targets or the presence of other regulatory signals in the micro-environment can stimulate sometimes apparently counteracting responses.

Ectopic overexpression of activin-A in mouse airways induces severe and chronic respiratory pathology

Despite the long list of putative functions that have been attributed to activin-A primarily from in vitro studies, and the continuously expanding list of diseases with which activin-A has been associated, until recently, little direct in vivo demonstration of the pathogenic consequences of deregulated activin-A

expression has been produced [20, 68–70]. Therefore, to validate directly the consequences of deregulated activin-A expression in vivo, we overexpressed activin-A in the mouse airways using adenovirus-mediated gene transfer and meticulously monitored functional and structural alterations in the respiratory system for up to 4 months, the time period post-viral instillation required for the activin-A-induced response to unfold fully [88]. These studies demonstrated that ectopic overexpression of activin-A in mouse airways could cause severe and chronic respiratory pathology, which even four months after virus instillation was not fully resolved. Virus-derived activin-A was detected in the broncho-alveolar lavage (BAL) fluids of the experimental animals reaching maximum after seven days, and persisting at increased levels up to day 56. Follistatin mRNA levels were upregulated with a relatively delayed kinetic reaching maximum levels around days 15–24 and remaining high until day 35.

The sequence of events associated with the development of activin-A-induced pathology is illustrated in Fig. 1. The first stage (days 0–15) was characterized by a dramatic and transient wave of cell death, substantial increase in high-mobility group box-1 (HMGB1) immunostaining, appearance of two waves of inflammatory cells in the BAL (the first consisting primarily of neutrophils and macrophages and the second, more robust wave, of neutrophils, macrophages and lymphocytes),

secretion of numerous TH1, TH2, and TH17 cytokines (with pro-inflammatory cytokines dominating early on and anti-inflammatory cytokines later), development of hyaline membranes, a gradual and eventually dramatic reduction of lung compliance accompanied by decline of surfactant protein-C (SpC), SpB, and SpA expression levels, development of a hyper-coagulant state accompanied by substantial upregulation of tissue factor (TF) mRNA levels and upregulation of CTGF mRNA levels. The second stage (days 15–35) was characterized by persistence of HMGB1 immunostaining, dramatic thickening of the alveolar septa and development of honeycomb-like histopathology, upregulation of collagens I and III mRNA levels, and increased deposition of collagen in the tissues. The third and final stage (days 35–4 months) was characterized by upregulation of tissue matrix metalloproteases (MMP2 and MMP9), gradual removal of the collage that was deposited earlier and eventual development of emphysematous lesions. Although until stage II, the process appeared to move towards the development of an interstitial fibrosis-like phenotype, during stage III the process shifted completely direction leading eventually to the development of emphysema like pathology. The sequence of events triggered by the ectopic activin-A expression bears striking similarity to the pathophysiology of human acute lung injury/acute respiratory distress syndrome (ALI/ARDS) [133], and consistently, dramatically

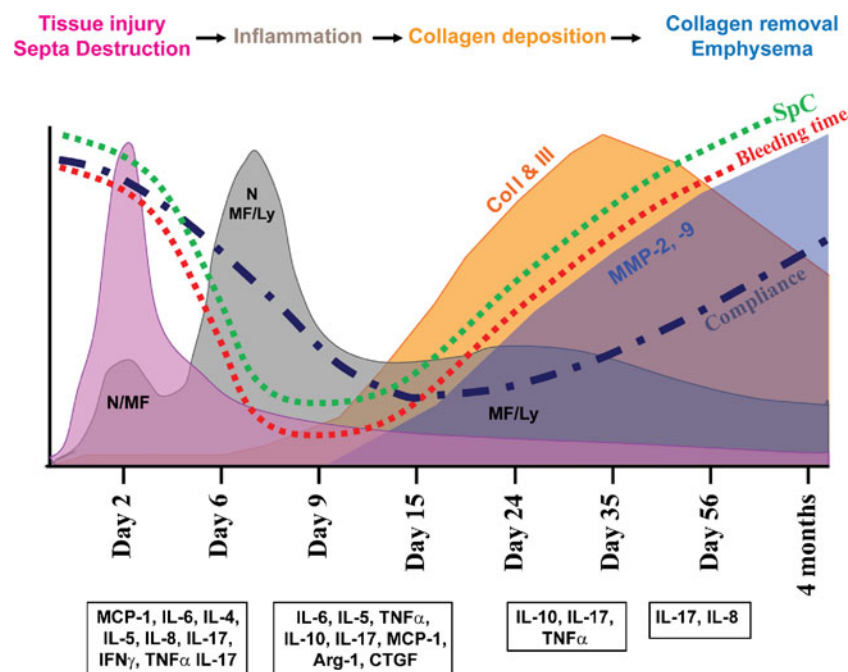


Fig. 1 Schematic illustration of the sequence of events triggered by the ectopic upregulation of activin-A in the mouse lung. The process starts with an acute wave of necroptotic cell death (*pink diagram*) and proceeds via the sequential induction of different waves of inflammatory cells (*gray diagram*), upregulation of collagens I and III mRNA levels and collagen deposition in the lung tissue (*orange diagram*), and finally, MMP-2 and MMP-9 mRNA upregulation and development of

emphysematous lesions (*blue diagram*). The decline in surfactant expression and lung compliance and the development of a hyper-coagulant state are indicated with *green*, *blue*, and *red lines*, respectively. The cytokines and chemokines upregulated at the mRNA level at different stages of the pathogenic process are indicated below the illustration. *N* neutrophils, *MF* macrophages, *Ly* lymphocytes

increased levels of activin-A were detected in BAL fluids from ARDS patients, strongly supporting the notion that activin-A could play a key role in the pathophysiology of this disorder [88]. The association of activin-A with the pathophysiology of ALI/ARDS is consistent with and complementary to earlier studies that have implicated activin-A in the pathophysiology of sepsis [86, 87]. ALI/ARDS and sepsis are distinct disease entities; however, they are intimately related [133–136]. They both represent typical examples of severe pathology triggered by overzealous and inappropriately controlled immune reactivity. ALI/ARDS represent fulminate respiratory conditions in which injury of the lung epithelium and endothelium triggers an uncontrolled inflammatory response and leads to often fatal functional and structural deterioration of the lung [136]. Despite the wide variety of recognized precipitating causes such as pneumonia and aspiration of gastric contents, the leading cause of ARDS is severe sepsis [136, 137]. This “cause-effect” relationship could indicate that common harmful effector mechanisms are mobilized during their development, with activin-A being one of them.

The histopathology induced by ectopic expression of activin-A in murine lungs shares some features with that induced by overexpression of other molecules such as TGF- β , IL-1 β , and TNF- α , although it is overall quite unique to activin-A. Whereas the inflammatory response that consist of neutrophils, macrophages, and lymphocytes, resembles the one induced by IL-1 β overexpression [138], the histopathology resembles the one described in transgenic animals overexpressing TNF- α in the lung [139]. Despite the similarities, however, the TNF- α -induced phenotype requires considerably longer time to develop compared with the ~25 days for the activin-A adenovirus treated animals. Interestingly, the phenotype in the activin-A treated animals is different from the one induced by TGF- β overexpression in the lung using either adenovirus in rats or transgene-mediated expression in mice [140, 141]. Both TGF- β and activin-A utilize the same Smad proteins and in the genetic background of the C57BL6 mouse they both induce transient waves of cell death, mobilize macrophages, induce Egr-1 and CTGF expression [141, 142], and seem to affect surfactant homeostasis [143] and the coagulation system [144]. However, the final outcome in the case of TGF- β overexpression is a more robust fibrotic response whereas in the case of activin-A a transient fibrotic response followed by development of emphysema [88]. Remarkably, the respiratory pathology bearing the highest similarity to the one induced by activin-A overexpression is caused by transient overexpression of gremlin, an inhibitor of BMP-mediated signaling [145]. This surprising finding strongly suggests that basal levels of BMP signaling are part of the mechanism that maintains homeostasis in the adult lung tissue, and thus suppression of basal BMP signaling may cause pathology. Interestingly, treatment of healthy animals by intra-tracheal

instillation of a follistatin expressing adenovirus leads to upregulation of SpC and CC10 expression, improvement of lung compliance and extension of tail-bleeding time, strongly suggesting that basal levels of activin-A signaling as well is continuously regulating lung homeostasis (unpublished observation). The development of pathology upon stimulation of the activin-A or suppression of the corresponding BMP signaling pathway, and the improvement of lung function parameters upon suppression of basal activin-A levels by the ectopic expression of follistatin, could indicate that a constant and dynamic balance between basal activities of BMP and activin-A signaling maintains homeostasis in the healthy lung. It appears that these pathways are constantly hardwired in the physiology of the lung and for this reason most likely abrupt changes in their concentration precipitate immediate and robust responses. The presence of activin-A protein and mRNA in all the tissues of healthy animals analyzed by Wu et al. [111] might indicate that activin-A may play a similar role in maintaining homeostasis in other tissues as well.

Abrupt increases in activin-A levels in a tissue can self-inflict acute tissue injury

The earliest change detected in the lungs upon activin-A overexpression was an acute and transient wave of alveolar cell death that reached maximum levels around 48 h post-viral instillation. Interestingly, a similar transient wave of alveolar cell death, with maximum levels again 48 h post transgene activation, has been observed in lung-specific inducible TGF- β transgenic animals [141] suggesting that cell death might be a common characteristic of abrupt changes in the levels of TGF- β super-family ligands. Importantly, the transient TGF- β -triggered wave of cell death was crucial since its prevention with different anti-apoptotic reagents could ameliorate development of pathology [141]. Although, apoptotic cells in the TGF- β transgenic animals exhibited typical TUNEL⁺ morphology, the TUNEL⁺ staining induced by activin-A overexpression involved fragmented cell-debris either attached to basement membranes of alveolar-septa or present in clumps in airspaces, indicating a rapid transition of cells undergoing apoptosis into secondary necrosis and cellular rupture (Fig. 2a). The exact mechanism of activin-A-induced cells death has not been clarified as yet, however, it is very likely that the combination of the acute recruitment of neutrophils in the lung and probably the release of powerful mediators they carry, the activation of the coagulation cascade directly by the effect of activin-A on epithelial cells [88] or indirectly through the activity of neutrophils [146, 147], the suppression of surfactant expression and possibly other responses that have not been analyzed as yet, such as activation of the complement system, could collectively lead to the tissue damage and

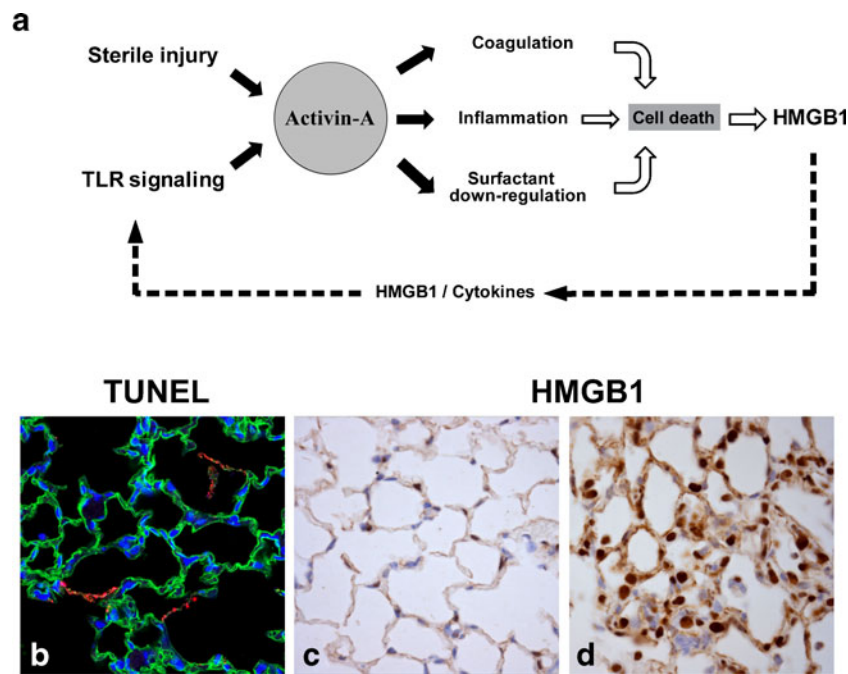


Fig. 2 a Activation of inflammatory cells through TLR stimulation or sterile injury of tissue resident cells can lead to abrupt increase in the levels of bioavailable activin-A within a tissue. The combination of aggravated innate immunity, activation of the coagulation system, and alterations in molecular systems crucial for homeostasis, such as the surfactants in the lung, can collectively lead to acute necroptotic cell death which leads to the release of highly pro-inflammatory complexes of DAMPs and cytokines that can activate via a feed-forward like

response further activation of the TLR signaling pathway and further production of activin-A. If gone out of control, this feed-forward system can deteriorate into a vicious-cycle and cause pathology. **B** Confocal image of the necroptotic response caused 48-h post-activin-A adenovirus instillation in the mouse lung. Staining for type I pneumocytes is shown with green and TUNEL signal is shown with red. **c, d** HMGB1 immunostaining of lung tissue section analyzed 48-h post-instillation of a control (**c**) or an activin-A-producing adenovirus (**d**)

enhanced cell death (Fig. 2). TLR signaling appears to be a major inducer of activin-A production [74, 87, 102]. Interestingly, high levels of activin-A induce sustained release of HMGB1 (Fig. 2d) and pro-inflammatory cytokines and chemokines, such as IL-6, MCP-1, KC, IL-1 β , IL-4, IL-5, and TNF- α [88]. HMGB1/cytokine complexes that are formed under these conditions are known to activate TLRs [148] establishing thus a feed-forward regulatory loop that can sustain activin-A production as long as the infectious agents or signs of tissue injury persist in the affected tissues (Fig. 3a). This feed-forward loop can be turned on not only by infection but also as a consequence of “sterile” disturbance of the homeostatic balance like in the case of sterile tissue injury or other forms of stress [98].

It is evident that such a feed-forward system, if gone out of control, can deteriorate into a vicious cycle and cause pathology. Surely, activin-A was not selected evolutionary to cause damage. The activities induced by the acute upregulation of activin-A are powerful protective mechanisms that are beneficial for the majority of individuals facing an infection or a severe injury and obviously the collateral tissue damage is an inevitable trade-off for efficient protection [149].

Abrupt deviations from the homeostatic levels of activin-A in a tissue could be interpreted as a strong “danger” signal mobilizing thus appropriate protective mechanisms. Activin-A

is a “morphogen”, i.e., designed evolutionary to stimulate different spectra of biological responses depending on its concentration, context, the previous history of its cellular targets or the presence of other regulatory signals in the micro-environment, and thus ideally suited to tailor appropriate tissue responses to different stress situations [14]. Different thresholds of activin-A in a tissue may reflect different levels of deviation from the homeostatic balance and could act as “yellow” or “red-alert”-like signals mobilizing to different extends the defense and/or repair mechanisms in the affected tissues [88]. Evidently, aberrant activation or loss of appropriate control under the influence of other co-morbidities or genetic predisposition could unnecessarily activate a “red-alert” response causing senseless collateral tissue damage and pathology.

Activin-A regulates the balance between innate and adaptive immunity

Correct balance between innate and adaptive immunity is a prerequisite for effective defense against infectious intruders. Although, innate immunity provides a rapid and efficient first line of defense, many of its powerful components do not discriminate between self and non-self and

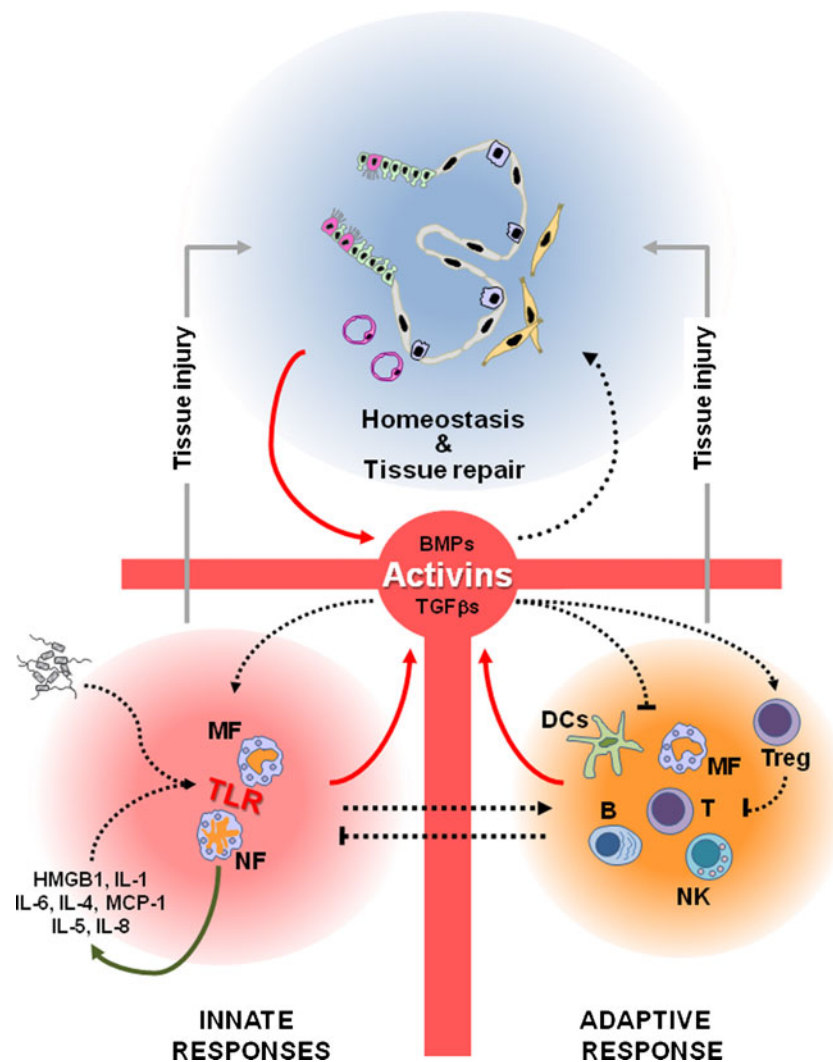


Fig. 3 Activin-A: guardian of homeostasis, coordinator of innate and adaptive immunity, and orchestrator of tissue repair activities. Activin-A is produced by numerous resident cells to maintain homeostatic balance in a tissue (the lung tissue is used as an example in the current cartoon). Upon disturbance of homeostasis due to infection, injury, or tissue malfunction, high levels of activin-A can be produced by cells of either the innate or the adaptive immune system. Positive and negative effects on different immune cells contribute to the coordination of immune reactivities and balance the relative involvement of innate

and/or adaptive immunity. Potentially, both innate and adaptive responses can cause collateral tissue injury. Activin-A may orchestrate the tissue repair activities needed for restitution of tissue integrity and homeostasis. Other members of the TGF- β super-family, such as TGFs and BMPs may also regulate some of the outlined interactions. *Red lines* indicate sources of activin-A; *dotted lines* indicate positive (\rightarrow) or negative (\leftarrow) influences on the indicated populations; *MF* macrophages, *B* B lymphocytes, *T* T lymphocytes, *DCs* dendritic cells, *NK* natural killer cells, *Treg* T regulatory cells, *TLRs* toll-like receptors

consequently collateral tissue damage is inevitable, especially in the context of prolonged and robust innate responses [149, 150]. Therefore, at the right point, innate responses must be contained and give way to the more sophisticated and specific effector mechanisms of adaptive immunity. Failure to regulate correctly the transition between the innate and adaptive responses can lead to chronic inflammation, a condition associated with numerous inflammatory and autoimmune disorders. Upregulation of activin-A expression appears to favor innate immunity in two ways, namely, by acting as a generic amplifier of innate inflammatory responses and by exerting inhibitory effects on

adaptive responses. As discussed earlier, activin-A induces *in vitro* expression of numerous pro-inflammatory mediators, and it seems from the studies of Apostolou et al. [88] that either directly or indirectly it can stimulate these activities *in vivo* as well. Thus, its capacity to upregulate TNF- α , IL-6, IL-1 β , and HMGB1 production, and thus establish a feed-forward amplification loop by further activating the TLR pathway might explain the overt activation of innate responses by activin-A. On the other hand, the capacity of activin-A to inhibit maturation of DCs [104, 113], proliferation of T cells, induction of cytotoxic T cells, induction of Foxp3 negative [82] and via a TGF- β synergism, Foxp3-

positive regulatory T cells [126] illustrates some of the mechanisms by which activin-A could exert its negative effect on adaptive immune function. The dramatically prolonged survival of adenovirus infected cells in animals treated with activin-A expressing adenoviruses [88] may reflect an inhibitory effect on the adaptive mechanisms that normally eliminate virus infected cells. Interestingly, we have observed that lymphopenic RAG-1-deficient animals are much more sensitive to activin-A overexpression than wild-type animals of the same genetic background (unpublished observation). This may indicate that the adaptive system is not just a passive receiver of negative signals from activin-A but rather raises a counter regulatory response in an attempt to temper the innate activities. Such a dynamic balance between innate and adaptive responses could minimize the risk for collateral damage by an overzealous early innate response [151, 152]. In conclusion, as previously argued [88, 98], activin-A together with follistatin (and other activin inhibitors) occupy a key position in the inter-phase of innate and adaptive immunity. High levels of bioavailable activin-A may intensify innate immunity, whereas, upregulation of follistatin or other activin-A modulating molecules and containment of activin-A reactivity could catalyze a shift towards adaptive-immune and tissue-repair activities.

Activin-A orchestrates tissue repair activities in an attempt to reconstitute homeostasis

Like a molecular “Swiss army knife,” activin-A appears to play an important role regulating repair of tissue injury as well. Activation of fibroblasts and modulation of the extracellular matrix turnover either by stimulating collagen mRNA synthesis or affecting the expression of MMPs and tissue inhibitors of metalloproteases (TIMPs) [64, 107, 153, 154] are among the tissue repair related activities that can be modulated by activin-A. In the adenovirus-mediated activin-A overexpression in the lung, commencement of tissue-repair-related activities appears to correlate with upregulation of follistatin [88] and is characterized by a decline in cytokine production with high IL-17 mRNA levels being the only remnant of the previous “cytokine storm” response, the appearance of “alternatively activated” like macrophages, the upregulation of collagens I and III mRNA levels and transient increase in the collagen deposition in the lung parenchyma. Indeed, alternatively activated macrophages [155], IL-17 [156], and HMGB1, in the absence of other cytokines [148, 157], have been suggested to support tissue repair, and thus the late activation of these processes by activin-A are consistent with the notion that this molecule not only turns on activities that compromise lung functionality, but also coordinates the ensuing necessary remedial activities. Due to the specially delicate architecture of the

lung parenchyma clearance of collagen deposits that correlate with a shift in the protease/anti-protease balance in favor of the former may under certain circumstances lead to another form of tissue remodeling, namely development of emphysematous lesions.

Activin-A and neutrophils, an overlooked affair

Neutrophils constitute the most abundant white blood cell type in circulation and are the first inflammatory cells that are recruited from the circulation into tissues where homeostasis has been compromised either by infection, injury or other forms of stress. They have at their disposal an impressive arsenal of armaments, which, due to their nondiscriminatory nature, can be as hazardous to the host cells as to their intended targets, namely, the microbial intruders. Neutrophils can phagocytize, release the content of their granules in the environment, or utilize a remarkable strategy known as NETosis, an active form of cell death that involves the controlled release of chromatin decorated with antimicrobial granular and cytoplasmic proteins into the extracellular space [158, 159]. Extensive coverage of the biology of these extraordinary cells is beyond the scope of this review. Without any doubt, many of the articles in this issue of “*Seminars in Immunopathology*” are providing adequate coverage, as numerous reviews in the literature have done previously [160–163]. However, steadily accumulating evidence may imply a special relationship between neutrophils and activin-A, and consequently, this facet of neutrophil biology certainly deserves highlighting herein. Neutrophils have been implicated in the pathophysiology of sepsis, ARDS, rheumatoid arthritis, inflammatory bowel disease, systemic lupus erythematosus, pre-eclampsia, coronary syndrome, and different forms of tissue injury (reviewed in Refs. [164–168]) and NETosis has been demonstrated in the context of some of these diseases and has been implicated in their pathophysiology. Thus, NETs have been detected in biopsies from human asthmatics that were characterized by infiltration of neutrophils [169], in the lungs of experimental animals that were challenged with sublethal doses of influenza A virus H1N1 [170] or LPS [171] and developed ARDS-like pathology, in the context of transfusion-related acute lung injury in humans and mice [172], and evidence for development of NETs has been produced in systemic lupus erythematosus [173]. Interestingly, the abovementioned disorders are also characterized by upregulated expression of activin-A [133, 134, 174–178] and most probably this correlation is not coincidental. The studies by Chen et al. and Wu et al. [109–111] suggest that this correlation could stem from the fact that neutrophils are major producers of activin-A and consequently diseases characterized by neutrophilia might also exhibit enhanced

activin-A production. However, recent observations in our laboratory may indicate that the relationship between neutrophils and activin-A is more complex than that. In the OVA model of allergic airway inflammation, the levels of activin-A in the BAL fluid of the OVA-challenged animals ranges from 1 to 3 ng/ml and the great majority of the inflammatory cells in the BAL are eosinophils (80–90 %). We have observed that raising the levels of activin-A in the airways of the OVA-challenged animals using adenoviruses to levels in the BAL ~5–10 ng/ml could completely shift the eosinophil-dominated airway inflammatory response into a neutrophil-dominated (<1 % eosinophils and 60 % neutrophils) (unpublished observation). Although it is not known for the moment whether this preferential recruitment of neutrophils is caused by a direct action of activin-A on neutrophils or is mediated indirectly via the induction of TNF- α , IL-8, IFN- γ , IL-17, or any other pro-inflammatory mediator, it is quite clear that a very special, complex and dynamic relationship must link neutrophils and activin-A. Indeed, Kambas et al. have recently demonstrated that neutrophils from patients with sepsis could release large amounts of NET-borne TF that could generate thrombin and activate platelets [179]. Therefore, neutrophils could activate the coagulation cascade indirectly, via an activin-A-mediated stimulation of TF production by epithelial cells [88] and directly by releasing TF-loaded NETs in the tissues [179]. Furthermore, Saffarzadeh et al. have recently demonstrated that NETs can directly induce epithelial and endothelial cell death [171], raising thus the possibility of activin-A-mobilized, neutrophil-derived NETs could contribute to the acute wave of alveolar cell death observed in mice upon intra-tracheal instillation of activin-A-producing adenoviruses. Remarkably, very little is known so far regarding the effect of activin-A signaling in neutrophils. In fact, a search for activin-A and neutrophils in the current literature resulted only in a handful of publications, with a tiny fraction of them directly addressing the consequences of activin-A-mediated signaling on neutrophils function [109]. Thus, tantalizing questions regarding the possible involvement of activin-A in the attraction of neutrophils in a tissue and/or the involvement of activin-A in the modulation of neutrophil functions, such as the formation and release of NETs, remain still unanswered. A more comprehensive scrutiny of the neglected relationship between neutrophils and activin-A is therefore highly warranted.

Concluding remarks

Activin-A has been known for almost 26 years and still the list of putative functions for this cytokine is steadily growing. The functional repertoire of this remarkable molecule includes numerous and occasionally contradicting pro-

inflammatory, anti-inflammatory, and tissue remodeling activities. Although there is still a great deal to learn, some features of activin-A biology are becoming better understood and hence a sketchy map that positions activin-A in the context of immuno-inflammatory processes can be drawn (Fig. 3). Thus, it is now recognized that different levels of activin-A mRNA and protein are continuously produced in almost any normal tissue analyzed [111]. These “basal” activin-A levels of production/activity are most likely important for the fine tuning of essential homeostatic functions. Abrupt changes in the levels of bioavailable activin-A in a tissue rapidly activate a number of biological responses which, although they are meant to be protective, if not managed properly, they can cause severe tissue injury and pave the way to pathology (Fig. 3). The potentially tissue-damaging responses triggered by activin-A include among others exuberant activation of the innate immune system, with neutrophils and macrophages being among the first to be recruited in tissues upon increase in activin-A expression, activation of the coagulation system that could lead to disseminated micro-vascular thrombosis, and in the respiratory system dramatic reduction of surfactant biosynthesis. The rationale behind the trade-off between protection and collateral tissue damage has been very eloquently discussed in review articles in the literature [149, 150]. One can envisage situations where the crisis is so critical that a robust innate response that will restrict the spread of the intruder by activating the coagulation and complement systems and recruiting a robust neutrophil response could provide an effective protection, although at the cost of collateral tissue damage. It seems that activin-A does not function only during the acute phase of an inflammatory response, but as the response unfolds it may function as a coordinator of innate and adaptive immune responses, amplifying the former and suppressing directly or indirectly through induction of regulatory T cells the later. Consequently, aberrant activin-A function in this context can lead to improper balance between innate and adaptive responses with potentially detrimental consequences. Finally, activin-A regulates activities that are related to remediation of the tissue injury caused either by infection or by collateral damage inflicted by an overzealous or protracted activation of the immune system. Thus, it has become evident that activin-A plays key roles at the interface of homeostasis, innate and adaptive immunity and tissue repair (Fig. 3).

Although the rich functional repertoire of activin-A might appear confusing at a first glance, it becomes more palpable if we recognize that the different and occasionally counteracting activities that have been described by *in vitro* studies for activin-A, constitute *in vivo* integral components of a dynamic continuum in which the time factor is of key importance. It is very clear that as an inflammatory response unfolds, both the cells and the microenvironment involved

evolve continuously (Fig. 3). The tissue resident and the recruited inflammatory cells go through different stages of activation, express new receptors and co-receptors and often desensitize some, and different cytokines are produced at each stage from the onset till the eventual resolution of the response [88, 149, 150]. Consequently, in vivo, activin-A signaling targets “different” cells in the context of “different” microenvironments as the response unfolds time-wise. Thus, pro-inflammatory activities of activin-A predominate during the acute, early phase of the response targeting primarily naïve cells, whereas, anti-inflammatory and matrix remodeling activities are manifested later in the response and target primed and activated cells. It is very likely that early and late activin-A-induced responses are linked with a “cause–effect” relationship, interlink in the fabric of a master homeostasis restitution program. As mentioned earlier, similar cause–effect relationship has been described between the transient apoptotic response induced by ectopic overexpression of TGF- β in the lung and the fibrotic response that followed much later [141].

Although this review has focused primarily on activin-A and its role in immuno-inflammatory and tissue-remodeling-associated processes, if one considers the extensive functional overlap between activin-A, activin-B, activin-AB, and TGF- β s, and the apparently antagonistic action of activin-C, activin-E, and BMPs, the need of studying the TGF- β -superfamily signaling system in an integrated manner becomes an absolute necessity. The therapeutic action of activin-A neutralizers, such as follistatin or a fusion protein composed of the extracellular portion of ActRIIB, the type II receptor for activins, linked to the Fc portion of the human IgG1 antibody, in colitis animal models [92] and in the exogenous activin-A or LPS-driven ALI/ARDS animal models [88] clearly indicate that even after establishment of pathology, neutralization of activin-A can still attenuate pathology associated parameters. This raises expectations for the clinical application of activin-A inhibitors and highlight the need for further research to further unwind the principles that govern and modulate its in vivo actions. Likewise, the intimate relation between neutrophil biology and activin signaling that is slowly emerging necessitates a more thorough investigation. Given their direct involvement in safeguarding and remediating homeostasis, such a relationship is most likely anything but coincidental.

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References

- Shi Y, Massague J (2003) Mechanisms of TGF-beta signaling from cell membrane to the nucleus. *Cell* 113(6):685–700
- Vale W, Rivier J, Vaughan J, McClintock R, Corrigan A, Woo W, Karr D, Spiess J (1986) Purification and characterization of an FSH releasing protein from porcine ovarian follicular fluid. *Nature* 321(6072):776–779
- Brown CW, Houston-Hawkins DE, Woodruff TK, Matzuk MM (2000) Insertion of *Inhbb* into the *Inhba* locus rescues the *Inhbanull* phenotype and reveals new activin functions. *Nat Genet* 25(4):453–457
- Ferguson CA, Tucker AS, Christensen L, Lau AL, Matzuk MM, Sharpe PT (1998) Activin is an essential early mesenchymal signal in tooth development that is required for patterning of the murine dentition. *Genes Dev* 12(16):2636–2649
- Vassalli A, Matzuk MM, Gardner HA, Lee KF, Jaenisch R (1994) Activin/inhibin beta B subunit gene disruption leads to defects in eyelid development and female reproduction. *Genes Dev* 8(4):414–427
- Smith JC, Price BM, Van Nimmen K, Huylebroeck D (1990) Identification of a potent *Xenopus* mesoderm-inducing factor as a homologue of activin A. *Nature* 345(6277):729–731. doi:10.1038/345729a0
- Green JB, New HV, Smith JC (1992) Responses of embryonic *Xenopus* cells to activin and FGF are separated by multiple dose thresholds and correspond to distinct axes of the mesoderm. *Cell* 71(5):731–739
- Cancilla B, Jarred RA, Wang H, Mellor SL, Cunha GR, Risbridger GP (2001) Regulation of prostate branching morphogenesis by activin A and follistatin. *Dev Biol* 237(1):145–158
- Matzuk MM, Finegold MJ, Mather JP, Krummen L, Lu H, Bradley A (1994) Development of cancer cachexia-like syndrome and adrenal tumors in inhibin-deficient mice. *Proc Natl Acad Sci U S A* 91(19):8817–8821
- de Kretser DM, Loveland KL, Meehan T, O’Byrne MK, Phillips DJ, Wreford NG (2001) Inhibins, activins and follistatin: actions on the testis. *Mol Cell Endocrinol* 180(1–2):87–92
- Li Q, Kumar R, Underwood K, O’Connor AE, Loveland KL, Sehra JS, Matzuk MM (2007) Prevention of cachexia-like syndrome development and reduction of tumor progression in inhibin-deficient mice following administration of a chimeric activin receptor type II-murine Fc protein. *Mol Hum Reprod* 13(9):675–683
- Zhou X, Wang JL, Lu J, Song Y, Kwak KS, Jiao Q, Rosenfeld R, Chen Q, Boone T, Simonet WS, Lacey DL, Goldberg AL, Han HQ (2010) Reversal of cancer cachexia and muscle wasting by ActRIIB antagonism leads to prolonged survival. *Cell* 142(4):531–543
- Massague J (1990) The transforming growth factor-beta family. *Annu Rev Cell Biol* 6:597–641
- Phillips DJ (2000) Regulation of activin’s access to the cell: why is mother nature such a control freak? *Bioessays* 22(8):689–696
- Barton DE, Yang-Feng TL, Mason AJ, Seeburg PH, Francke U (1989) Mapping of genes for inhibin subunits alpha, beta A, and beta B on human and mouse chromosomes and studies of jsd mice. *Genomics* 5(1):91–99
- Fang J, Yin W, Smiley E, Wang SQ, Bonadio J (1996) Molecular cloning of the mouse activin beta E subunit gene. *Biochem Biophys Res Commun* 228(3):669–674
- Ht G, Neidhardt H, Schneider C, Pohl J (1995) Cloning of a new member of the TGF-beta family: a putative new activin beta C chain. *Biochem Biophys Res Commun* 206(2):608–613
- Lau AL, Kumar TR, Nishimori K, Bonadio J, Matzuk MM (2000) Activin betaC and betaE genes are not essential for mouse

- liver growth, differentiation, and regeneration. *Mol Cell Biol* 20 (16):6127–6137
19. Feijen A, Goumans MJ, van den Eijnden-van Raaij AJ (1994) Expression of activin subunits, activin receptors and follistatin in postimplantation mouse embryos suggests specific developmental functions for different activins. *Development* 120(12):3621–3637
 20. Matzuk MM, Kumar TR, Vassalli A, Bickenbach JR, Roop DR, Jaenisch R, Bradley A (1995) Functional analysis of activins during mammalian development. *Nature* 374(6520):354–356
 21. Thompson TB, Cook RW, Chapman SC, Jardetzky TS, Woodruff TK (2004) Beta A versus beta B: is it merely a matter of expression? *Mol Cell Endocrinol* 225(1–2):9–17
 22. Mellor SL, Cranfield M, Ries R, Pedersen J, Cancilla B, de Kretser D, Groome NP, Mason AJ, Risbridger GP (2000) Localization of activin beta(A)-, beta(B)-, and beta(C)-subunits in human prostate and evidence for formation of new activin heterodimers of beta(C)-subunit. *J Clin Endocrinol Metab* 85 (12):4851–4858
 23. Mellor SL, Ball EM, O'Connor AE, Ethier JF, Cranfield M, Schmitt JF, Phillips DJ, Groome NP, Risbridger GP (2003) Activin betaC-subunit heterodimers provide a new mechanism of regulating activin levels in the prostate. *Endocrinology* 144 (10):4410–4419
 24. Gold E, Jetly N, O'Bryan MK, Meachem S, Srinivasan D, Behuria S, Sanchez-Partida LG, Woodruff T, Hedwards S, Wang H, McDougall H, Casey V, Niranjana B, Patella S, Risbridger G (2009) Activin C antagonizes activin A in vitro and overexpression leads to pathologies in vivo. *Am J Pathol* 174(1):184–195
 25. Greenwald J, Groppe J, Gray P, Wiater E, Kwiatkowski W, Vale W, Choe S (2003) The BMP7/ActRII extracellular domain complex provides new insights into the cooperative nature of receptor assembly. *Mol Cell* 11(3):605–617
 26. Moustakas A, Souchevlytskiy S, Heldin CH (2001) Smad regulation in TGF-beta signal transduction. *J Cell Sci* 114(Pt 24):4359–4369
 27. Moustakas A, Heldin CH (2005) Non-Smad TGF-beta signals. *J Cell Sci* 118(Pt 16):3573–3584
 28. Bernard DJ (2004) Both SMAD2 and SMAD3 mediate activin-stimulated expression of the follicle-stimulating hormone beta subunit in mouse gonadotrope cells. *Mol Endocrinol* 18(3):606–623
 29. Bernard DJ, Lee KB, Santos MM (2006) Activin B can signal through both ALK4 and ALK7 in gonadotrope cells. *Reprod Biol Endocrinol* 4:52
 30. Suszko MI, Balkin DM, Chen Y, Woodruff TK (2005) Smad3 mediates activin-induced transcription of follicle-stimulating hormone beta-subunit gene. *Mol Endocrinol* 19(7):1849–1858
 31. Xia Y, Schneyer AL (2009) The biology of activin: recent advances in structure, regulation and function. *J Endocrinol* 202(1):1–12
 32. Attisano L, Carcamo J, Ventura F, Weis FM, Massague J, Wrana JL (1993) Identification of human activin and TGF beta type I receptors that form heteromeric kinase complexes with type II receptors. *Cell* 75(4):671–680
 33. Mason AJ, Farnworth PG, Sullivan J (1996) Characterization and determination of the biological activities of noncleavable high molecular weight forms of inhibin A and activin A. *Mol Endocrinol* 10(9):1055–1065
 34. Gray AM, Mason AJ (1990) Requirement for activin A and transforming growth factor-beta 1 pro-regions in homodimer assembly. *Science* 247(4948):1328–1330
 35. Husken-Hindi P, Tsuchida K, Park M, Corrigan AZ, Vaughan JM, Vale WW, Fischer WH (1994) Monomeric activin A retains high receptor binding affinity but exhibits low biological activity. *J Biol Chem* 269(30):19380–19384
 36. Walton KL, Makanji Y, Wilce MC, Chan KL, Robertson DM, Harrison CA (2009) A common biosynthetic pathway governs the dimerization and secretion of inhibin and related transforming growth factor beta (TGFbeta) ligands. *J Biol Chem* 284 (14):9311–9320
 37. Li S, Shimono C, Norioka N, Nakano I, Okubo T, Yagi Y, Hayashi M, Sato Y, Fujisaki H, Hattori S, Sugiura N, Kimata K, Sekiguchi K (2010) Activin A binds to perlecan through its pro-region that has heparin/heparan sulfate binding activity. *J Biol Chem* 285(47):36645–36655
 38. Macri L, Silverstein D, Clark RA (2007) Growth factor binding to the pericellular matrix and its importance in tissue engineering. *Adv Drug Deliv Rev* 59(13):1366–1381
 39. Walton KL, Makanji Y, Harrison CA (2012) New insights into the mechanisms of activin action and inhibition. *Mol Cell Endocrinol* 359(1–2):2–12
 40. Bottinger EP, Factor VM, Tsang ML, Weatherbee JA, Kopp JB, Qian SW, Wakefield LM, Roberts AB, Thorgeirsson SS, Sporn MB (1996) The recombinant proregion of transforming growth factor beta1 (latency-associated peptide) inhibits active transforming growth factor beta1 in transgenic mice. *Proc Natl Acad Sci U S A* 93(12):5877–5882
 41. Mathews LS, Vale WW (1991) Expression cloning of an activin receptor, a predicted transmembrane serine kinase. *Cell* 65 (6):973–982
 42. Hayette S, Gadoux M, Martel S, Bertrand S, Tigaud I, Magaud JP, Rimokh R (1998) FLRG (follistatin-related gene), a new target of chromosomal rearrangement in malignant blood disorders. *Oncogene* 16(22):2949–2954
 43. Tsuchida K, Arai KY, Kuramoto Y, Yamakawa N, Hasegawa Y, Sugino H (2000) Identification and characterization of a novel follistatin-like protein as a binding protein for the TGF-beta family. *J Biol Chem* 275(52):40788–40796
 44. Schneyer A, Tortoriello D, Sidis Y, Keutmann H, Matsuzaki T, Holmes W (2001) Follistatin-related protein (FSRP): a new member of the follistatin gene family. *Mol Cell Endocrinol* 180(1–2):33–38
 45. Harrison CA, Gray PC, Vale WW, Robertson DM (2005) Antagonists of activin signaling: mechanisms and potential biological applications. *Trends Endocrinol Metab* 16(2):73–78
 46. Nakamura T, Takio K, Eto Y, Shibai H, Titani K, Sugino H (1990) Activin-binding protein from rat ovary is follistatin. *Science* 247 (4944):836–838
 47. Harrington AE, Morris-Triggs SA, Ruotolo BT, Robinson CV, Ohnuma S, Hyvonen M (2006) Structural basis for the inhibition of activin signalling by follistatin. *EMBO J* 25(5):1035–1045
 48. Shimasaki S, Koga M, Esch F, Cooksey K, Mercado M, Koba A, Ueno N, Ying SY, Ling N, Guillemain R (1988) Primary structure of the human follistatin precursor and its genomic organization. *Proc Natl Acad Sci U S A* 85(12):4218–4222
 49. Hashimoto O, Nakamura T, Shoji H, Shimasaki S, Hayashi Y, Sugino H (1997) A novel role of follistatin, an activin-binding protein, in the inhibition of activin action in rat pituitary cells. Endocytotic degradation of activin and its acceleration by follistatin associated with cell-surface heparan sulfate. *J Biol Chem* 272(21):13835–13842
 50. Phillips DJ, Jones KL, McGaw DJ, Groome NP, Smolich JJ, Parsson H, de Kretser DM (2000) Release of activin and follistatin during cardiovascular procedures is largely due to heparin administration. *J Clin Endocrinol Metab* 85(7):2411–2415
 51. Lerch TF, Shimasaki S, Woodruff TK, Jardetzky TS (2007) Structural and biophysical coupling of heparin and activin binding to follistatin isoform functions. *J Biol Chem* 282(21):15930–15939
 52. Phillips DJ, de Kretser DM (1998) Follistatin: a multifunctional regulatory protein. *Front Neuroendocrinol* 19(4):287–322
 53. Sugino K, Kurosawa N, Nakamura T, Takio K, Shimasaki S, Ling N, Titani K, Sugino H (1993) Molecular heterogeneity of follistatin, an activin-binding protein. Higher affinity of the carboxyl-terminal truncated forms for heparan sulfate proteoglycans on the ovarian granulosa cell. *J Biol Chem* 268(21):15579–15587

54. Sidis Y, Tortoriello DV, Holmes WE, Pan Y, Keutmann HT, Schneyer AL (2002) Follistatin-related protein and follistatin differentially neutralize endogenous vs. exogenous activin. *Endocrinology* 143(5):1613–1624
55. Stamler R, Keutmann HT, Sidis Y, Kattamuri C, Schneyer A, Thompson TB (2008) The structure of FSTL3:activin A complex. Differential binding of N-terminal domains influences follistatin-type antagonist specificity. *J Biol Chem* 283(47):32831–32838
56. Thompson TB, Lerch TF, Cook RW, Woodruff TK, Jardetzky TS (2005) The structure of the follistatin:activin complex reveals antagonism of both type I and type II receptor binding. *Dev Cell* 9(4):535–543
57. Schneyer A, Schoen A, Quigg A, Sidis Y (2003) Differential binding and neutralization of activins A and B by follistatin and follistatin like-3 (FSTL-3/FSRP/FLRG). *Endocrinology* 144(5):1671–1674
58. Hill JJ, Davies MV, Pearson AA, Wang JH, Hewick RM, Wolfman NM, Qiu Y (2002) The myostatin propeptide and the follistatin-related gene are inhibitory binding proteins of myostatin in normal serum. *J Biol Chem* 277(43):40735–40741
59. Lee SJ, McPherron AC (2001) Regulation of myostatin activity and muscle growth. *Proc Natl Acad Sci U S A* 98(16):9306–9311
60. Otsuka F, Moore RK, Iemura S, Ueno N, Shimasaki S (2001) Follistatin inhibits the function of the oocyte-derived factor BMP-15. *Biochem Biophys Res Commun* 289(5):961–966
61. Hedger MP, Drummond AE, Robertson DM, Risbridger GP, de Kretser DM (1989) Inhibin and activin regulate ³H]thymidine uptake by rat thymocytes and 3 T3 cells in vitro. *Mol Cell Endocrinol* 61(1):133–138
62. Matsuse T, Fukuchi Y, Eto Y, Matsui H, Hosoi T, Oka T, Ohga E, Nagase T, Orimo H (1995) Expression of immunoreactive and bioactive activin A protein in adult murine lung after bleomycin treatment. *Am J Respir Cell Mol Biol* 13(1):17–24
63. Matsuse T, Ikegami A, Ohga E, Hosoi T, Oka T, Kida K, Fukayama M, Inoue S, Nagase T, Ouchi Y, Fukuchi Y (1996) Expression of immunoreactive activin A protein in remodeling lesions associated with interstitial pulmonary fibrosis. *Am J Pathol* 148(3):707–713
64. Ohga E, Matsuse T, Teramoto S, Katayama H, Nagase T, Fukuchi Y, Ouchi Y (1996) Effects of activin A on proliferation and differentiation of human lung fibroblasts. *Biochem Biophys Res Commun* 228(2):391–396
65. Aoki F, Kurabayashi M, Hasegawa Y, Kojima I (2005) Attenuation of bleomycin-induced pulmonary fibrosis by follistatin. *Am J Respir Crit Care Med* 172(6):713–720
66. Patella S, Phillips DJ, Tchongue J, de Kretser DM, Sievert W (2006) Follistatin attenuates early liver fibrosis: effects on hepatic stellate cell activation and hepatocyte apoptosis. *Am J Physiol Gastrointest Liver Physiol* 290(1):G137–G144
67. Takagi K, Kawaguchi Y, Kawamoto M, Ota Y, Tochimoto A, Gono T, Katsumata Y, Takagi M, Hara M, Yamanaka H (2011) Activation of the activin A-ALK-Smad pathway in systemic sclerosis. *J Autoimmun* 36(3–4):181–188
68. Matzuk MM, Lu N, Vogel H, Sellheyer K, Roop DR, Bradley A (1995) Multiple defects and perinatal death in mice deficient in follistatin. *Nature* 374(6520):360–363
69. Hubner G, Hu Q, Smola H, Werner S (1996) Strong induction of activin expression after injury suggests an important role of activin in wound repair. *Dev Biol* 173(2):490–498
70. Munz B, Smola H, Engelhardt F, Bleuel K, Brauchle M, Lein I, Evans LW, Huylebroeck D, Balling R, Werner S (1999) Overexpression of activin A in the skin of transgenic mice reveals new activities of activin in epidermal morphogenesis, dermal fibrosis and wound repair. *EMBO J* 18(19):5205–5215
71. Wankell M, Kaesler S, Zhang YQ, Florence C, Werner S, Duan R (2001) The activin binding proteins follistatin and follistatin-related protein are differentially regulated in vitro and during cutaneous wound repair. *J Endocrinol* 171(3):385–395
72. Wankell M, Munz B, Hubner G, Hans W, Wolf E, Goppelt A, Werner S (2001) Impaired wound healing in transgenic mice overexpressing the activin antagonist follistatin in the epidermis. *EMBO J* 20(19):5361–5372
73. McLean CA, Cleland H, Moncrieff NJ, Barton RJ, de Kretser DM, Phillips DJ (2008) Temporal expression of activin in acute burn wounds—from inflammatory cells to fibroblasts. *Burns* 34(1):50–55
74. de Kretser DM, O’Hehir RE, Hardy CL, Hedger MP (2012) The roles of activin A and its binding protein, follistatin, in inflammation and tissue repair. *Mol Cell Endocrinol* 359(1–2):101–106
75. Rosendahl A, Checchin D, Fehniger TE, ten Dijke P, Heldin CH, Sideras P (2001) Activation of the TGF-beta/activin-Smad2 pathway during allergic airway inflammation. *Am J Respir Cell Mol Biol* 25(1):60–68
76. Funaba M, Ikeda T, Ogawa K, Abe M (2003) Calcium-regulated expression of activin A in RBL-2H3 mast cells. *Cell Signal* 15(6):605–613
77. Cho SH, Yao Z, Wang SW, Alban RF, Barbers RG, French SW, Oh CK (2003) Regulation of activin A expression in mast cells and asthma: its effect on the proliferation of human airway smooth muscle cells. *J Immunol* 170(8):4045–4052
78. Karagiannidis C, Hense G, Martin C, Epstein M, Ruckert B, Mantel PY, Menz G, Uhlig S, Blaser K, Schmidt-Weber CB (2006) Activin A is an acute allergen-responsive cytokine and provides a link to TGF-beta-mediated airway remodeling in asthma. *J Allergy Clin Immunol* 117(1):111–118
79. Kariyawasam HH, Pegorier S, Barkans J, Xanthou G, Aizen M, Ying S, Kay AB, Lloyd CM, Robinson DS (2009) Activin and transforming growth factor-beta signaling pathways are activated after allergen challenge in mild asthma. *J Allergy Clin Immunol* 124(3):454–462
80. Funaba M, Ikeda T, Ogawa K, Murakami M, Abe M (2003) Role of activin A in murine mast cells: modulation of cell growth, differentiation, and migration. *J Leukoc Biol* 73(6):793–801
81. Hardy CL, O’Connor AE, Yao J, Sebire K, de Kretser DM, Rolland JM, Anderson GP, Phillips DJ, O’Hehir RE (2006) Follistatin is a candidate endogenous negative regulator of activin A in experimental allergic asthma. *Clin Exp Allergy* 36(7):941–950
82. Semitekolou M, Alissafi T, Aggelakopoulou M, Kourepini E, Kariyawasam HH, Kay AB, Robinson DS, Lloyd CM, Panoutsakopoulou V, Xanthou G (2009) Activin-A induces regulatory T cells that suppress T helper cell immune responses and protect from allergic airway disease. *J Exp Med* 206(8):1769–1785
83. Jones CP, Gregory LG, Causton B, Campbell GA, Lloyd CM (2012) Activin A and TGF-beta promote T(H)9 cell-mediated pulmonary allergic pathology. *J Allergy Clin Immunol* 129(4):1000–1010, e1003
84. Phillips DJ, Hedger MP, McFarlane JR, Klein R, Clarke IJ, Tilbrook AJ, Nash AD, de Kretser DM (1996) Follistatin concentrations in male sheep increase following sham castration/castration or injection of interleukin-1 beta. *J Endocrinol* 151(1):119–124
85. Klein R, Clarke IJ, Hedger MP, Robertson DM (1996) Plasma follistatin concentrations increase following lipopolysaccharide administration in sheep. *Clin Exp Pharmacol Physiol* 23(8):754–755
86. Jones KL, de Kretser DM, Clarke IJ, Scheerlinck JP, Phillips DJ (2004) Characterisation of the rapid release of activin A following acute lipopolysaccharide challenge in the ewe. *J Endocrinol* 182(1):69–80
87. Jones KL, Mansell A, Patella S, Scott BJ, Hedger MP, de Kretser DM, Phillips DJ (2007) Activin A is a critical component of the inflammatory response, and its binding protein, follistatin,

- reduces mortality in endotoxemia. *Proc Natl Acad Sci U S A* 104 (41):16239–16244
88. Apostolou E, Stavropoulos A, Sountoulidis A, Xirakia C, Giaglis S, Protopapadakis E, Ritis K, Mentzelopoulos S, Pasternack A, Foster M, Ritvos O, Tzelepis GE, Andreacos E, Sideras P (2012) Activin-A overexpression in the murine lung causes pathology that simulates acute respiratory distress syndrome. *Am J Respir Crit Care Med* 185(4):382–391
 89. Michel U, Ebert S, Phillips D, Nau R (2003) Serum concentrations of activin and follistatin are elevated and run in parallel in patients with septicemia. *Eur J Endocrinol* 148(5):559–564
 90. Michel U et al (2003) Increased activin levels in cerebrospinal fluid of rabbits with bacterial meningitis are associated with activation of microglia. *J Neurochem* 86(1):238–245
 91. Ebert S, Phillips DJ, Jenzewski P, Nau R, O'Connor AE, Michel U (2006) Activin A concentrations in human cerebrospinal fluid are age-dependent and elevated in meningitis. *J Neurol Sci* 250 (1–2):50–57
 92. Dohi T, Ejima C, Kato R, Kawamura YI, Kawashima R, Mizutani N, Tabuchi Y, Kojima I (2005) Therapeutic potential of follistatin for colonic inflammation in mice. *Gastroenterology* 128(2):411–423
 93. Hubner G, Brauchle M, Gregor M, Werner S (1997) Activin A: a novel player and inflammatory marker in inflammatory bowel disease? *Lab Invest* 77(4):311–318
 94. Yu EW, Dolter KE, Shao LE, Yu J (1998) Suppression of IL-6 biological activities by activin A and implications for inflammatory arthropathies. *Clin Exp Immunol* 112(1):126–132
 95. Gribo R, Tanaka T, Harper-Summers R, Yu J (2001) Expression of activin A in inflammatory arthropathies. *Mol Cell Endocrinol* 180 (1–2):163–167
 96. Ota F, Maeshima A, Yamashita S, Ikeuchi H, Kaneko Y, Kuroiwa T, Hiromura K, Ueki K, Kojima I, Nojima Y (2003) Activin A induces cell proliferation of fibroblast-like synoviocytes in rheumatoid arthritis. *Arthritis Rheum* 48(9):2442–2449
 97. Yndestad A, Ueland T, Oie E, Florholmen G, Halvorsen B, Attramadal H, Simonsen S, Froland SS, Gullestad L, Christensen G, Damas JK, Aukrust P (2004) Elevated levels of activin A in heart failure: potential role in myocardial remodeling. *Circulation* 109(11):1379–1385
 98. Phillips DJ, de Kretser DM, Hedger MP (2009) Activin and related proteins in inflammation: not just interested bystanders. *Cytokine Growth Factor Rev* 20(2):153–164
 99. Eramaa M, Hurme M, Stenman UH, Ritvos O (1992) Activin A/erythroid differentiation factor is induced during human monocyte activation. *J Exp Med* 176(5):1449–1452
 100. Shao L, Frigon NL Jr, Sehy DW, Yu AL, Lofgren J, Schwall R, Yu J (1992) Regulation of production of activin A in human marrow stromal cells and monocytes. *Exp Hematol* 20 (10):1235–1242
 101. Dower K, Ellis DK, Saraf K, Jelinsky SA, Lin LL (2008) Innate immune responses to TREM-1 activation: overlap, divergence, and positive and negative cross-talk with bacterial lipopolysaccharide. *J Immunol* 180(5):3520–3534
 102. Ebert S, Zeretzke M, Nau R, Michel U (2007) Microglial cells and peritoneal macrophages release activin A upon stimulation with Toll-like receptor agonists. *Neurosci Lett* 413(3):241–244
 103. Ogawa K, Funaba M, Mathews LS, Mizutani T (2000) Activin A stimulates type IV collagenase (matrix metalloproteinase-2) production in mouse peritoneal macrophages. *J Immunol* 165 (6):2997–3003
 104. Robson NC, Phillips DJ, McAlpine T, Shin A, Svobodova S, Toy T, Pillay V, Kirkpatrick N, Zanker D, Wilson K, Helling I, Wei H, Chen W, Cebon J, Maraskovsky E (2008) Activin-A: a novel dendritic cell-derived cytokine that potently attenuates CD40 ligand-specific cytokine and chemokine production. *Blood* 111 (5):2733–2743
 105. Scutera S, Riboldi E, Daniele R, Elia AR, Fraone T, Castagnoli C, Giovarelli M, Musso T, Sozzani S (2008) Production and function of activin A in human dendritic cells. *Eur Cytokine Netw* 19(1):60–68
 106. Yu J, Shao LE, Frigon NL Jr, Lofgren J, Schwall R (1996) Induced expression of the new cytokine, activin A, in human monocytes: inhibition by glucocorticoids and retinoic acid. *Immunology* 88(3):368–374
 107. Ogawa K, Funaba M, Chen Y, Tsujimoto M (2006) Activin A functions as a Th2 cytokine in the promotion of the alternative activation of macrophages. *J Immunol* 177(10):6787–6794
 108. Ogawa K, Funaba M, Tsujimoto M (2008) A dual role of activin A in regulating immunoglobulin production of B cells. *J Leukoc Biol* 83(6):1451–1458
 109. Chen Y, Wu H, Winnall WR, Loveland KL, Makanji Y, Phillips DJ, Smith JA, Hedger MP (2011) Tumour necrosis factor- α stimulates human neutrophils to release preformed activin A. *Immunol Cell Biol* 89(8):889–896
 110. Wu H, Chen Y, Winnall WR, Phillips DJ, Hedger MP (2013) Regulation of activin A release from murine bone marrow-derived neutrophil precursors by tumour necrosis factor- α and insulin. *Cytokine* 61:199–204
 111. Wu H, Chen Y, Winnall WR, Phillips DJ, Hedger MP (2012) Acute regulation of activin A and its binding protein, follistatin, in serum and tissues following lipopolysaccharide treatment of adult male mice. *Am J Physiol Regul Integr Comp Physiol* 303 (6):R665–R675
 112. Salogni L, Musso T, Bosio D, Mirolo M, Jala VR, Haribabu B, Locati M, Sozzani S (2009) Activin A induces dendritic cell migration through the polarized release of CXC chemokine ligands 12 and 14. *Blood* 113(23):5848–5856
 113. Segerer SE, Muller N, Brandt J, Kapp M, Dietl J, Reichardt HM, Rieger L, Kammerer U (2008) The glycoprotein-hormones activin A and inhibin A interfere with dendritic cell maturation. *Reprod Biol Endocrinol* 6:17
 114. Jonuleit H, Schmitt E, Schuler G, Knop J, Enk AH (2000) Induction of interleukin 10-producing, nonproliferating CD4(+) T cells with regulatory properties by repetitive stimulation with allogeneic immature human dendritic cells. *J Exp Med* 192 (9):1213–1222
 115. Nusing RM, Barsig J (1999) Induction of prostanoid, nitric oxide, and cytokine formation in rat bone marrow derived macrophages by activin A. *Br J Pharmacol* 127(4):919–926
 116. Nusing RM, Mohr S, Ullrich V (1995) Activin A and retinoic acid synergize in cyclooxygenase-1 and thromboxane synthase induction during differentiation of J774.1 macrophages. *Eur J Biochem* 227(1–2):130–136
 117. Wang Y, Cui X, Tai G, Ge J, Li N, Chen F, Yu F, Liu Z (2009) A critical role of activin A in maturation of mouse peritoneal macrophages in vitro and in vivo. *Cell Mol Immunol* 6(5):387–392
 118. Yamashita N, Nakajima T, Takahashi H, Kaneoka H, Mizushima Y, Sakane T (1993) Effects of activin A on IgE synthesis and cytokine production by human peripheral mononuclear cells. *Clin Exp Immunol* 94(1):214–219
 119. Musso T, Scutera S, Vermi W, Daniele R, Fornaro M, Castagnoli C, Alotto D, Ravanini M, Cambieri I, Salogni L, Elia AR, Giovarelli M, Facchetti F, Girolomoni G, Sozzani S (2008) Activin A induces Langerhans cell differentiation in vitro and in human skin explants. *PLoS One* 3(9):e3271
 120. Zhou J, Tai G, Liu H, Ge J, Feng Y, Chen F, Yu F, Liu Z (2009) Activin A down-regulates the phagocytosis of lipopolysaccharide-activated mouse peritoneal macrophages in vitro and in vivo. *Cell Immunol* 255(1–2):69–75
 121. Wang SY, Tai GX, Zhang PY, Mu DP, Zhang XJ, Liu ZH (2008) Inhibitory effect of activin A on activation of lipopolysaccharide-stimulated mouse macrophage RAW264.7 cells. *Cytokine* 42 (1):85–91

122. Ohguchi M, Yamato K, Ishihara Y, Koide M, Ueda N, Okahashi N, Noguchi T, Kizaki M, Ikeda Y, Sugino H, Nisihara T (1998) Activin A regulates the production of mature interleukin-1beta and interleukin-1 receptor antagonist in human monocytic cells. *J Interferon Cytokine Res* 18(7):491–498
123. Sugama S, Takenouchi T, Kitani H, Fujita M, Hashimoto M (2007) Activin as an anti-inflammatory cytokine produced by microglia. *J Neuroimmunol* 192(1–2):31–39
124. Wilms H, Schwark T, Brandenburg LO, Sievers J, Dengler R, Deuschl G, Lucius R (2010) Regulation of activin A synthesis in microglial cells: pathophysiological implications for bacterial meningitis. *J Neurosci Res* 88(1):16–23
125. Sierra-Filardi E, Puig-Kroger A, Blanco FJ, Nieto C, Bragado R, Palomero MI, Bernabeu C, Vega MA, Corbi AL (2011) Activin A skews macrophage polarization by promoting a proinflammatory phenotype and inhibiting the acquisition of anti-inflammatory macrophage markers. *Blood* 117(19):5092–5101
126. Huber S, Stahl FR, Schrader J, Luth S, Presser K, Carambia A, Flavell RA, Werner S, Blessing M, Herkel J, Schramm C (2009) Activin A promotes the TGF-beta-induced conversion of CD4+ CD25- T cells into Foxp3+ induced regulatory T cells. *J Immunol* 182(8):4633–4640
127. Robson NC, Wei H, McAlpine T, Kirkpatrick N, Cebon J, Maraskovsky E (2009) Activin-A attenuates several human natural killer cell functions. *Blood* 113(14):3218–3225
128. Lee HJ, Kim PH (2009) Further characterization of activin A-induced IgA response in murine B lymphocytes. *Immune Netw* 9(4):133–137
129. Bonfield TL, Bama BP, John N, Malur A, Culver DA, Kavuru MS, Thomassen MJ (2006) Suppression of activin A in autoimmune lung disease associated with anti-GM-CSF. *J Autoimmun* 26(1):37–41
130. Wilson KM, Smith AI, Phillips DJ (2006) Stimulatory effects of lipopolysaccharide on endothelial cell activin and follistatin. *Mol Cell Endocrinol* 253(1–2):30–35
131. Abe M, Shintani Y, Eto Y, Harada K, Kosaka M, Matsumoto T (2002) Potent induction of activin A secretion from monocytes and bone marrow stromal fibroblasts by cognate interaction with activated T cells. *J Leukoc Biol* 72(2):347–352
132. Hubner G, Werner S (1996) Serum growth factors and proinflammatory cytokines are potent inducers of activin expression in cultured fibroblasts and keratinocytes. *Exp Cell Res* 228(1):106–113
133. Meduri GU (1996) The role of the host defence response in the progression and outcome of ARDS: pathophysiological correlations and response to glucocorticoid treatment. *Eur Respir J* 9(12):2650–2670
134. Khadaroo RG, Marshall JC (2002) ARDS and the multiple organ dysfunction syndrome. Common mechanisms of a common systemic process. *Crit Care Clin* 18(1):127–141
135. Costa EL, Schettino IA, Schettino GP (2006) The lung in sepsis: guilty or innocent? *Endocr Metab Immune Disord Drug Targets* 6(2):213–216
136. Tsushima K, King LS, Aggarwal NR, De Gorordo A, D'Alessio FR, Kubo K (2009) Acute lung injury review. *Intern Med* 48(9):621–630
137. Bernard GR, Artigas A, Brigham KL, Carlet J, Falke K, Hudson L, Lamy M, Legall JR, Morris A, Spragg R (1994) The American-European Consensus Conference on ARDS. Definitions, mechanisms, relevant outcomes, and clinical trial coordination. *Am J Respir Crit Care Med* 149(3 Pt 1):818–824
138. Kolb M, Margetts PJ, Anthony DC, Pitossi F, Gauldie J (2001) Transient expression of IL-1beta induces acute lung injury and chronic repair leading to pulmonary fibrosis. *J Clin Invest* 107(12):1529–1536
139. Lundblad LK, Thompson-Figueroa J, Leclair T, Sullivan MJ, Poynter ME, Irvin CG, Bates JH (2005) Tumor necrosis factor-alpha overexpression in lung disease: a single cause behind a complex phenotype. *Am J Respir Crit Care Med* 171(12):1363–1370
140. Sime PJ, Xing Z, Graham FL, Csaky KG, Gauldie J (1997) Adenovector-mediated gene transfer of active transforming growth factor-beta1 induces prolonged severe fibrosis in rat lung. *J Clin Invest* 100(4):768–776
141. Lee CG, Cho SJ, Kang MJ, Chapoval SP, Lee PJ, Noble PW, Yehualaesht T, Lu B, Flavell RA, Milbrandt J, Homer RJ, Elias JA (2004) Early growth response gene 1-mediated apoptosis is essential for transforming growth factor beta1-induced pulmonary fibrosis. *J Exp Med* 200(3):377–389
142. Bonniaud P, Martin G, Margetts PJ, Ask K, Robertson J, Gauldie J, Kolb M (2004) Connective tissue growth factor is crucial to inducing a profibrotic environment in “fibrosis-resistant” BALB/c mouse lungs. *Am J Respir Cell Mol Biol* 31(5):510–516
143. Beers MF, Solarin KO, Guttentag SH, Rosenbloom J, Kormilli A, Gonzales LW, Ballard PL (1998) TGF-beta1 inhibits surfactant component expression and epithelial cell maturation in cultured human fetal lung. *Am J Physiol* 275(5 Pt 1):L950–L960
144. Jablonska E, Markart P, Zakrzewicz D, Preissner KT, Wygrecka M (2010) Transforming growth factor-beta1 induces expression of human coagulation factor XII via Smad3 and JNK signaling pathways in human lung fibroblasts. *J Biol Chem* 285(15):11638–11651
145. Farkas L, Farkas D, Gauldie J, Warburton D, Shi W, Kolb M (2011) Transient overexpression of Gremlin results in epithelial activation and reversible fibrosis in rat lungs. *Am J Respir Cell Mol Biol* 44(6):870–878
146. Ritis K, Doumas M, Mastellos D, Micheli A, Giaglis S, Magotti P, Rafail S, Kartalis G, Sideras P, Lambris JD (2006) A novel C5a receptor-tissue factor cross-talk in neutrophils links innate immunity to coagulation pathways. *J Immunol* 177(7):4794–4802
147. Kambas K, Markiewski MM, Pneumatikos IA, Rafail SS, Theodorou V, Konstantonis D, Kourtzelis I, Doumas MN, Magotti P, Deangelis RA, Lambris JD, Ritis KD (2008) C5a and TNF-alpha up-regulate the expression of tissue factor in intra-alveolar neutrophils of patients with the acute respiratory distress syndrome. *J Immunol* 180(11):7368–7375
148. Klune JR, Dhupar R, Cardinal J, Billiar TR, Tsung A (2008) HMGB1: endogenous danger signaling. *Mol Med* 14(7–8):476–484
149. Medzhitov R (2008) Origin and physiological roles of inflammation. *Nature* 454(7203):428–435
150. Serhan CN (2007) Resolution phase of inflammation: novel endogenous anti-inflammatory and proresolving lipid mediators and pathways. *Annu Rev Immunol* 25:101–137
151. Kim KD, Zhao J, Auh S, Yang X, Du P, Tang H, Fu YX (2007) Adaptive immune cells temper initial innate responses. *Nat Med* 13(10):1248–1252
152. Palm NW, Medzhitov R (2007) Not so fast: adaptive suppression of innate immunity. *Nat Med* 13(10):1142–1144
153. Yamashita S, Maeshima A, Kojima I, Nojima Y (2004) Activin A is a potent activator of renal interstitial fibroblasts. *J Am Soc Nephrol* 15(1):91–101
154. Yoshinaga K, Mimori K, Inoue H, Kamohara Y, Yamashita K, Tanaka F, Mori M (2008) Activin A enhances MMP-7 activity via the transcription factor AP-1 in an esophageal squamous cell carcinoma cell line. *Int J Oncol* 33(3):453–459
155. Mosser DM, Edwards JP (2008) Exploring the full spectrum of macrophage activation. *Nat Rev Immunol* 8(12):958–969
156. O'Connor W Jr, Zenewicz LA, Flavell RA (2010) The dual nature of T(H)17 cells: shifting the focus to function. *Nat Immunol* 11(6):471–476
157. Bianchi ME, Manfredi AA (2007) High-mobility group box 1 (HMGB1) protein at the crossroads between innate and adaptive immunity. *Immunol Rev* 220:35–46
158. Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, Weinrauch Y, Zychlinsky A (2004) Neutrophil extracellular traps kill bacteria. *Science* 303(5663):1532–1535

159. Brinkmann V, Zychlinsky A (2007) Beneficial suicide: why neutrophils die to make NETs. *Nat Rev Microbiol* 5(8):577–582
160. Segal AW (2005) How neutrophils kill microbes. *Annu Rev Immunol* 23:197–223
161. Nathan C (2006) Neutrophils and immunity: challenges and opportunities. *Nat Rev Immunol* 6(3):173–182
162. Borregaard N (2010) Neutrophils, from marrow to microbes. *Immunity* 33(5):657–670
163. Mantovani A, Cassatella MA, Costantini C, Jaillon S (2011) Neutrophils in the activation and regulation of innate and adaptive immunity. *Nat Rev Immunol* 11(8):519–531
164. Amulic B, Cazalet C, Hayes GL, Metzler KD, Zychlinsky A (2012) Neutrophil function: from mechanisms to disease. *Annu Rev Immunol* 30:459–489
165. Kovach MA, Standiford TJ (2012) The function of neutrophils in sepsis. *Curr Opin Infect Dis* 25(3):321–327
166. Nemeth T, Mocsai A (2012) The role of neutrophils in autoimmune diseases. *Immunol Lett* 143(1):9–19
167. Vinten-Johansen J (2004) Involvement of neutrophils in the pathogenesis of lethal myocardial reperfusion injury. *Cardiovasc Res* 61(3):481–497
168. Wright HL, Moots RJ, Bucknall RC, Edwards SW (2010) Neutrophil function in inflammation and inflammatory diseases. *Rheumatology (Oxford)* 49(9):1618–1631
169. Dworski R, Simon HU, Hoskins A, Yousefi S (2011) Eosinophil and neutrophil extracellular DNA traps in human allergic asthmatic airways. *J Allergy Clin Immunol* 127(5):1260–1266
170. Narasaraju T, Yang E, Samy RP, Ng HH, Poh WP, Liew AA, Phoon MC, van Rooijen N, Chow VT (2011) Excessive neutrophils and neutrophil extracellular traps contribute to acute lung injury of influenza pneumonitis. *Am J Pathol* 179(1):199–210
171. Saffarzadeh M, Juenemann C, Queisser MA, Lochnit G, Barreto G, Galuska SP, Lohmeyer J, Preissner KT (2012) Neutrophil extracellular traps directly induce epithelial and endothelial cell death: a predominant role of histones. *PLoS One* 7(2):e32366
172. Thomas GM, Carbo C, Curtis BR, Martinod K, Mazo IB, Schatzberg D, Cifuni SM, Fuchs TA, von Andrian UH, Hartwig JH, Aster RH, Wagner DD (2012) Extracellular DNA traps are associated with the pathogenesis of TRALI in humans and mice. *Blood* 119(26):6335–6343
173. Bouts YM, Wolthuis DF, Dirkx MF, Pieterse E, Simons EM, van Boekel AM, Dieker JW, van der Vlag J (2012) Apoptosis and NET formation in the pathogenesis of SLE. *Autoimmunity* 45(8):597–601
174. Fournier BM, Parkos CA (2012) The role of neutrophils during intestinal inflammation. *Mucosal Immunol* 5(4):354–366
175. Guasti L, Dentali F, Castiglioni L, Maroni L, Marino F, Squizzato A, Ageno W, Gianni M, Gaudio G, Grandi AM, Cosentino M, Venco A (2011) Neutrophils and clinical outcomes in patients with acute coronary syndromes and/or cardiac revascularisation. A systematic review on more than 34,000 subjects. *Thromb Haemost* 106(4):591–599
176. Gupta AK, Hasler P, Holzgreve W, Hahn S (2007) Neutrophil NETs: a novel contributor to preeclampsia-associated placental hypoxia? *Semin Immunopathol* 29(2):163–167
177. Knight JS, Kaplan MJ (2012) Lupus neutrophils: ‘NET’ gain in understanding lupus pathogenesis. *Curr Opin Rheumatol* 24(5):441–450
178. Menezes GB, Mansur DS, McDonald B, Kubes P, Teixeira MM (2011) Sensing sterile injury: opportunities for pharmacological control. *Pharmacol Ther* 132(2):204–214
179. Kambas K, Mitroulis I, Ritis K (2012) The emerging role of neutrophils in thrombosis—the journey of TF through NETs. *Front Immunol* 3:385