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Activin-A in the regulation of immunity in health and disease

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ABSTRACT

The TGF-β superfamily of cytokines plays pivotal roles in the regulation of immune responses protecting against or contributing to diseases, such as, allergy, autoimmunity and cancer. Activin-A, a member of the TGF-β superfamily, was initially identified as an inducer of follicle-stimulating hormone secretion. Extensive research over the past decades illuminated fundamental roles for activin-A in essential biologic processes, including embryonic development, stem cell maintenance and differentiation, haematopoiesis, cell proliferation and tissue fibrosis. Activin-A signals through two type I and two type II receptors which, upon ligand binding, activate their kinase activity, phosphorylate the SMAD2 and 3 intracellular signaling mediators that form a complex with SMAD4, translocate to the nucleus and activate or silence gene expression. Most immune cell types, including macrophages, dendritic cells (DCs), T and B lymphocytes and natural killer cells have the capacity to produce and respond to activin-A, although not in a similar manner. In innate immune cells, including macrophages, DCs and neutrophils, activin-A exerts a broad range of pro- or anti-inflammatory functions depending on the cell maturation and activation status and the spatiotemporal context. Activin-A also controls the differentiation and effector functions of Th cell subsets, including Th9 cells, T_{FH} cells, Tr1 Treg cells and $Foxp3^+$ Treg cells. Moreover, activin-A affects B cell responses, enhancing mucosal IgA secretion and inhibiting pathogenic autoantibody production. Interestingly, an array of preclinical and clinical studies has highlighted crucial functions of activin-A in the initiation, propagation and resolution of human diseases, including autoimmune diseases, such as, systemic lupus erythematosus, rheumatoid arthritis and pulmonary alveolar proteinosis, in allergic disorders, including allergic asthma and atopic dermatitis, in cancer and in microbial infections. Here, we provide an overview of the biology of activin-A and its signaling pathways, summarize recent studies pertinent to the role of activin-A in the modulation of inflammation and immunity, and discuss the potential of targeting activin-A as a novel therapeutic approach for the control of inflammatory diseases.

Abbreviations: SMAD, mothers against decapentaplegic; MAPK, mitogen activated protein kinase; ERK, extracellular signal-regulated kinase; JNK, c-Jun *N*-terminal kinase; BAMBI, pseudoreceptor BMP and activin membrane-bound inhibitor homolog; LPS, Lipopolysaccharides; BM, bone marrow; IFN-γ, interferon γ; IL, interleukin; GM-CSF, granulocyte-macrophage colony-stimulating factor; MAF, C-maf proto-oncogene; IGF1, insulin-like growth factor 1; SERPINB2, Plasminogen activator inhibitor 2; F13A1, coagulation factor XIII A chain; PGE2, Prostaglandin E2; HIF-1α, hypoxia-inducible factor 1-alpha; ARDS, acute respiratory distress syndrome; BMP, bone morphogenic protein; GDF, growth and differentiation factor; ActRI, activin-A receptor type I; ACVR1A, activin receptor type-1A; ALK2, activin receptor like kinase 2; ACVR1B, activin receptor type-1B; ALK4, activin receptor like kinase 4; ALK7, activin receptor like kinase 7; ActRIIA, activin receptor type IIA; ActRIIB, activin receptor type IIB; FS, follistatin; I-SMAD, inhibitory SMAD; R-SMAD, receptor-regulated SMAD; TLR, toll like receptor; MMP-2, matrix metalloproteinase-2; NO, nitric oxide; DC, dendritic cell; moDC, monocyte derived dendritic cell; PB, peripheral blood; cDC, conventional dendritic cell; PHA, phytohemagglutinin; PAP, pulmonary alveolar proteinosis; TFH, T follicular helper; Treg, T regulatory; YAP, Yes-Associated Protein; NK, natural killer; fMLP, *N*-formyl-L-methionyl-L-leucyl-phenylalanine; CNS, central nervous system; CSF, cerebrospinal fluid; pPROM, preterm premature rupture of the membranes; HCV, hepatitis C virus; HBV, hepatitis B virus; HCMV, human cytomegalovirus; HRV, human rhinovirus infection; BEC, bronchial epithelial cell; NFAT, nuclear factor of activated T cells; AHR, airway hyperresponsiveness; BAL, bronchoalveolar lavage; OVA, ovalbumin; HDM, house dust mite; SA, severe asthma; MMA, mild-moderate asthmatics; HC, healthy controls; α-SMA, a-smooth muscle actin; VEGF, vascular endothelial growth factor; VEGFR1, VEGF receptor-1; Tr1, Type-1 regulatory; TGF-β, transforming growth factor β; SF, synovial fluid; RA, rheumatoid arthritis; FLS, fibroblast-like synoviocyte; DMBA, 7,12-dimethylbenz[*a*]anthracene; TPA, 12-O-tetradecanoylphorbol 13-acetate; DETC, dendritic epidermal γδ T cell; HPV, human papillomavirus; LLC, Lewis Lung Carcinoma; CFA, Complete Freund's Adjuvant; SLE, systemic lupus erythematosus; BAFF, B-cell activating factor; APRIL, A proliferation-inducing ligand; LPS, lipopolysaccharide

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Table 1
The TGF-β superfamily pathway in humans.

Subfamily	*Ligands*	*Type I receptors*	*Type II receptors*	*R-SMAD*
Transforming growth factor beta (TGF-β)	TGF-β1 TGF-β2 TGF-β3	ALK1 ALK2 ALK5	TβRII	SMAD1 SMAD2 SMAD3 SMAD5 SMAD8
Bone Morphogenic Protein (BMP)	BMP2 BMP8A BMP3 BMP8B BMP4 BMP10 BMP5 BMP11 BMP6 BMP15 BMP7	ALK1 ALK2 ALK3 ALK4 ALK5 ALK6 ALK7	BMPR2 BMPR2B ActR-IIA ActR-IIB	SMAD1 SMAD2 SMAD3 SMAD5 SMAD8
Growth and Differentiation Factor (GDF)	GDF1 GDF7 GDF2 GDF9 GDF3 GDF10 GDF5 GDF11 GDF6 GDF15	ALK1 ALK3 ALK4 ALK5 ALK6	BMPR2 ActR-IIA ActR-IIB	SMAD1 SMAD2 SMAD3 SMAD5 SMAD8
Activin	Activin-A Activin-B Activin-AB	ALK2 ALK4 ALK7	ActR-IIA ActR-IIB	SMAD1 SMAD2 SMAD3 SMAD5 SMAD8
Inhibin	Inhibin-A Inhibin-B	–	ActR-IIA ActR-IIB BMPR2 BMPR2B	–
Nodal	Nodal	ALK4 ALK7	ActR-IIA ActR-IIB	SMAD2 SMAD3
Anti-müllerian hormone (AMH)	AMH	ALK2 ALK3 ALK6	AMHR2	SMAD1 SMAD5 SMAD8
Lefty	Lefty1 Lefty2	–	ActR-IIA ActR-IIB	–

1. Introduction

The TGF-β superfamily consists of more than 45 members including activins, inhibins, myostatin, bone morphogenetic proteins (BMPs), growth and differentiation factors (GDFs) and nodal [1] (Table 1). Activins are found either as homodimers or heterodimers of βA or/and βB subunits linked with disulfide bonds. There are three functional isoforms of activins: activin-A (βAβA), activin B (βBβB) and activin AB (βAβB) [2]. The βC and βE subunits are found in mammals and the βB subunit in *Xenopus laevis* [3]. The principal characteristic of all β subunits is the presence of a cysteine knot in their carboxyl-terminus, a folding domain that contains nine conserved cysteines important for the stabilization and dimerization of the ligands [4]. More specifically, the sixth cysteine is essential for the dimerization, while the other eight form intramolecular disulfide bonds which determine the three-dimensional structure of activins. Transcripts of the βA and βB subunits are detected in nearly every tissue in the human body and exhibit increased expression in the reproductive system, while the βC and βE subunits are predominantly expressed in the liver [3].

Activin-A is a cytokine of approximately 25 kDa and represents the most extensively investigated protein among the family of activins. Activin-A was initially identified as a gonadal protein that induces the biosynthesis and secretion of the follicle-stimulating hormone from the pituitary [1]. It is highly conserved among vertebrates, reaching up to 95% homology between species [5]. Activin-A regulates fundamental biologic processes, such as, haematopoiesis, embryonic development, stem cell maintenance and pluripotency, tissue repair and fibrosis [5,6]. Its importance in developmental processes becomes evident as mice lacking the βA subunit die within 24 h after birth due to severe craniofacial defects, while mice that lack the βB subunit display abnormal development and lowered reproductive capacity [7].

A growing body of evidence has uncovered crucial effects of activin A on innate and adaptive immune responses and the pathophysiology of associated human diseases [5,6]. In this review, we will outline the most recent findings pertinent to the role of activin-A in the regulation of immune responses during infections, allergic and autoimmune disorders and cancer. We will also discuss the effects of dysregulation of activin-A signaling on the initiation and propagation of immune-mediated diseases. Finally, we will contemplate on the potential of targeting activin-A and/or its signaling pathways for the design of more effective personalized immunotherapies.

2. Activin-A signaling and regulation

Activin-A signals through two type I and two type II receptors which, upon ligand binding, assemble the final receptor complex [8]. Type I receptors include Activin receptor type 1A (or Activin receptor Like Kinase 2, ALK2), Activin receptor type 1B (or ALK4) and Activin receptor type 1C (or ALK7) (Table 1,Fig. 1). Activin-A favors ALK4 binding, while it shows lower affinity for ALK2 and ALK7. The type II receptors are the Activin receptor type IIA (ActRIIA) and Activin receptor type IIB (ActRIIB) and are characterized by constitutively-active serine/threonine kinase activity [9]. Notably, the *Acvr2b* gene (encoding ActRIIB) produces 4 alternatively spliced transcripts that exhibit distinct binding affinities for activin-A [8]. Interestingly, crystallography studies of the activin-A - ActRIIB receptor complex discovered that activin-A exhibits a different binding pattern compared to the rest of the TGF-β superfamily members [8]. Once activin-A is bound to its type II receptors, two type I receptors are recruited and become phosphorylated by the type II receptors, an event that leads to the activation of their kinase activity [8]. Subsequently, type I receptors phosphorylate intracellular mothers against decapentaplegic homolog (SMAD) 2 and SMAD3 signaling protein at their carboxyl-terminal SSXS motif, which then form a complex with SMAD4, translocate to the nucleus and activate or silence gene expression [10] (Table 1, Fig. 1). SMAD6 and SMAD7 are main inhibitory SMAD (I-SMAD) proteins that prevent SMAD4 binding to the SMAD2/3 complex and disrupt their transcriptional activity in response to activin-A [8] (Fig. 2).

Activin-A can activate alternative non-canonical, intracellular signaling pathways, including the p38 mitogen activated protein kinase (MAPK), extracellular signal-regulated kinases 1/2 (ERK1/2) and c-Jun *N*-terminal kinases (JNKs) which affect cell migration and differentiation [2,8]. In addition, activin-A, through SMAD2 activation, can activate the canonical Wnt signaling pathway [11] (Fig. 1). Notably, SMAD proteins contain a linker domain comprised of regulatory sites among which are sites for ERK and calcium-regulated kinases [10]. Phosphorylation at this region inhibits SMAD nuclear translocation and transcriptional activity, pointing to a complex regulation of activin-A signal transduction that is characterized by spatiotemporal and cell type dependence [10].

Considering the crucial roles that activin-A plays in biological processes, its mode of action is tightly regulated by a plethora of molecules both at the extracellular and intracellular level. Follistatin (FS) represents the major inhibitor of activin-A as it binds to activin-A with high affinity and neutralizes its functions by preventing activin-A interaction with its type II receptors [12] (Fig. 2). Alternative splicing leads to the formation of two isoforms of FS; the FS288 isoform binds heparan sulphate proteoglycans with high affinity and is considered as a local regulator of activin-A functions, while the FS315 isoform neutralizes circulating activin-A [12]. FS is not a specific inhibitor for activin-A as it neutralizes the functions of all activins, and certain BMPs and myostatin [12].

Inhibins represent soluble heterodimeric proteins and consist of the inhibin α subunit and the activin βA (inhibin A, αβA) or βB subunit (inhibin B, αβB). Inhibins compete for binding to the type II receptors but can also bind directly to activins with variable affinities [12] (Fig. 2). Betaglycan is another protein that binds inhibins and enhances their affinity for ActRII, leading to the inhibition of the interaction

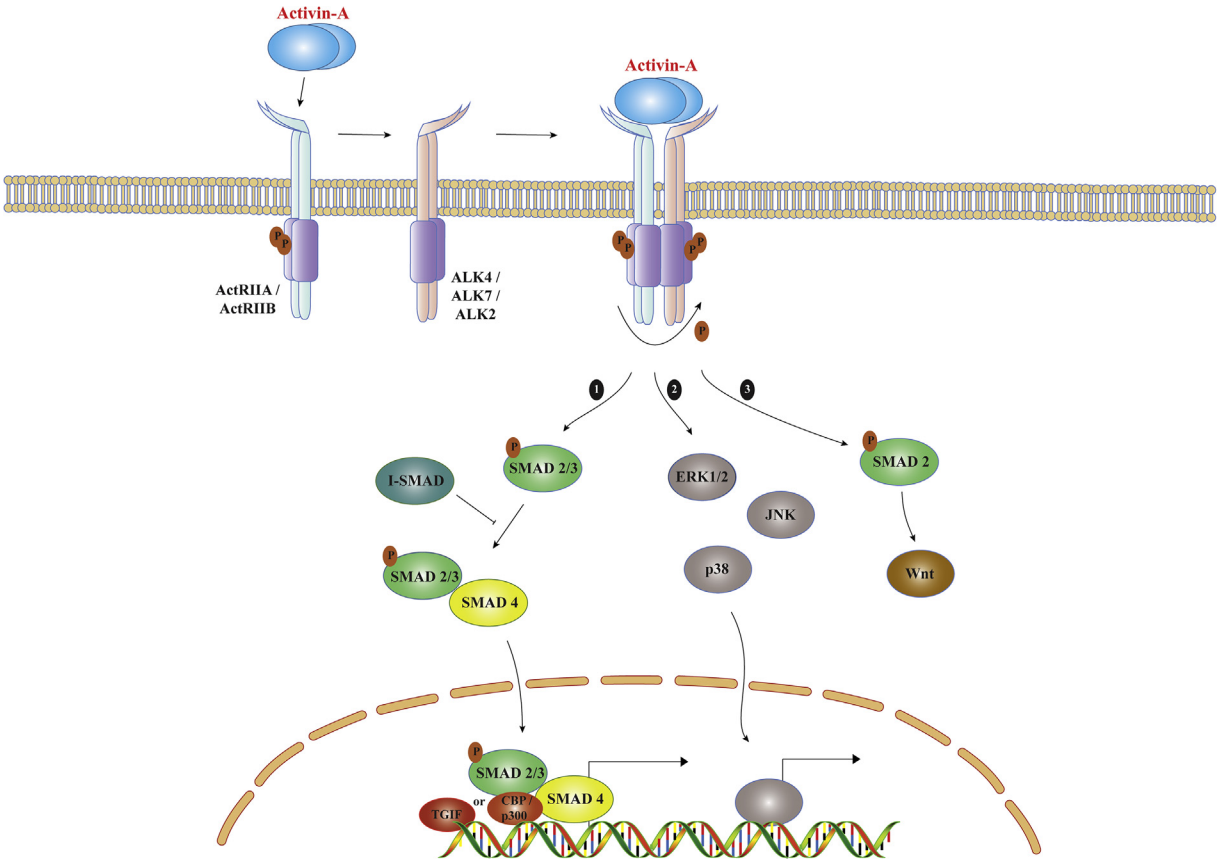

Fig. 1. The signaling pathways of activin-A. Activin-A signals through a heterotetrameric receptor complex. Initially, activin-A binds to the constitutively active ActRII (ActRIIA or ActRIIB). This interaction leads to the recruitment and phosphorylation of the ActRI (ALK4, ALK7 or ALK2). Activated ActRI phosphorylate the intracellular mediators SMAD2/3, which then form a complex with SMAD4 and translocate to the nucleus, where they regulate the transcription of target genes in cooperation with transcription co-factors (e.g CBP/p300 or transforming growth interacting factor (TGIF). In the non-canonical activin-A signal transduction pathway, ActRI activates the ERK, p38 and/or JNK kinases which in turn regulate the transcription of target genes. In addition, activin-A, through SMAD2 activation, can act as a co-activator of the canonical Wnt signaling pathway.

between activins and the type II receptors [12] (Fig. 2). The pseudoreceptor BMP and activin membrane-bound inhibitor homolog (BAMBI) restrains activin-A's signaling by interacting with the type I receptors and inhibiting the formation of the receptor signaling complex. Furthermore, overexpression of Cripto, the co-receptor for nodal ligands inhibits activin-A's signaling, while enhancing nodal signaling by binding to nodal and activin-A receptors [12] (Fig. 2). As mentioned above, activin-A functions are also regulated at the intracellular level. More specifically, the inhibitory I-SMADs bind to type I receptors and mitigate the recruitment and phosphorylation of receptor-regulated SMADs (R-SMADs) (Fig. 2). I-SMADs also promote the binding of the SMAD ubiquitin regulatory factors 1 and 2 to activin-A receptors inducing their ubiquitin-dependent degradation [13].

3. Role of activin-A in the regulation of innate and adaptive immune responses

3.1. Macrophages

Activin-A is produced and secreted in nearly every tissue in the human body. The expression of activin-A is upregulated in mouse peritoneal macrophages and the macrophage cell line RAW264.7 upon binding of Pam3Cys, lipopolysaccharide (LPS) and CpG to their Toll Like Receptors (TLR)2, TLR4 and TLR9, respectively, with LPS showing the strongest induction [14,15]. In addition, LPS-activated macrophages upregulate mRNA levels of Acvr2a and SMAD2/3 [16]. Interestingly, treatment of resting bone marrow (BM)-derived rat and mouse peritoneal macrophages with activin-A enhanced the production of matrix metalloproteinase-2 (MMP-2) and pro-inflammatory mediators, such as, IL-1β, nitric oxide (NO) and prostanoids and increased cell phagocytic activity [16–20]. Moreover, treatment of RAW264.7 cells with activin-A increased their capacity for phagocytosis and pinocytosis, upregulated iNOS and IL-1β at the mRNA level and enhanced IL-1β, IL-6 and NO production in culture supernatants [14,21]. Altogether, these studies suggest that treatment of resting macrophages with activin-A skews them towards a pro-inflammatory M1-like phenotype. In contrast, other studies proposed a suppressive role of activin-A signaling in these cells. Indeed, activin-A increased M2-associated arginase-1 expression in RAW264.7 cells, when administered alone or in combination with interferon γ (IFN-γ) [22]. Other studies have documented that activin-A stimulation did not affect the expression of MHC class I or II molecules on mouse peritoneal macrophages in contrast to RAW264.7 cells, where it upregulated MHCII expression, suggesting that activin-A exhibits different effects on primary cells versus cell lines [20,21]. While, different cell origin may be responsible for these controversial results, it is evident that in both macrophage populations activin-A promotes cell activation [20,21].

The effects of activin-A are different when administered on activated macrophages. Indeed, administration of activin-A on primary mouse macrophages, previously stimulated with LPS, reduced their phagocytic activity, NO secretion, pro-inflammatory cytokine release and MHC II expression in a concentration-dependent manner [14,17,19,23]. *In vivo* studies also demonstrated that activin-A administration in mice, after LPS challenge, reduced the phagocytic activity of macrophages against chicken red blood cells [23]. Activin-A also regulated the function of LPS-activated macrophages through the

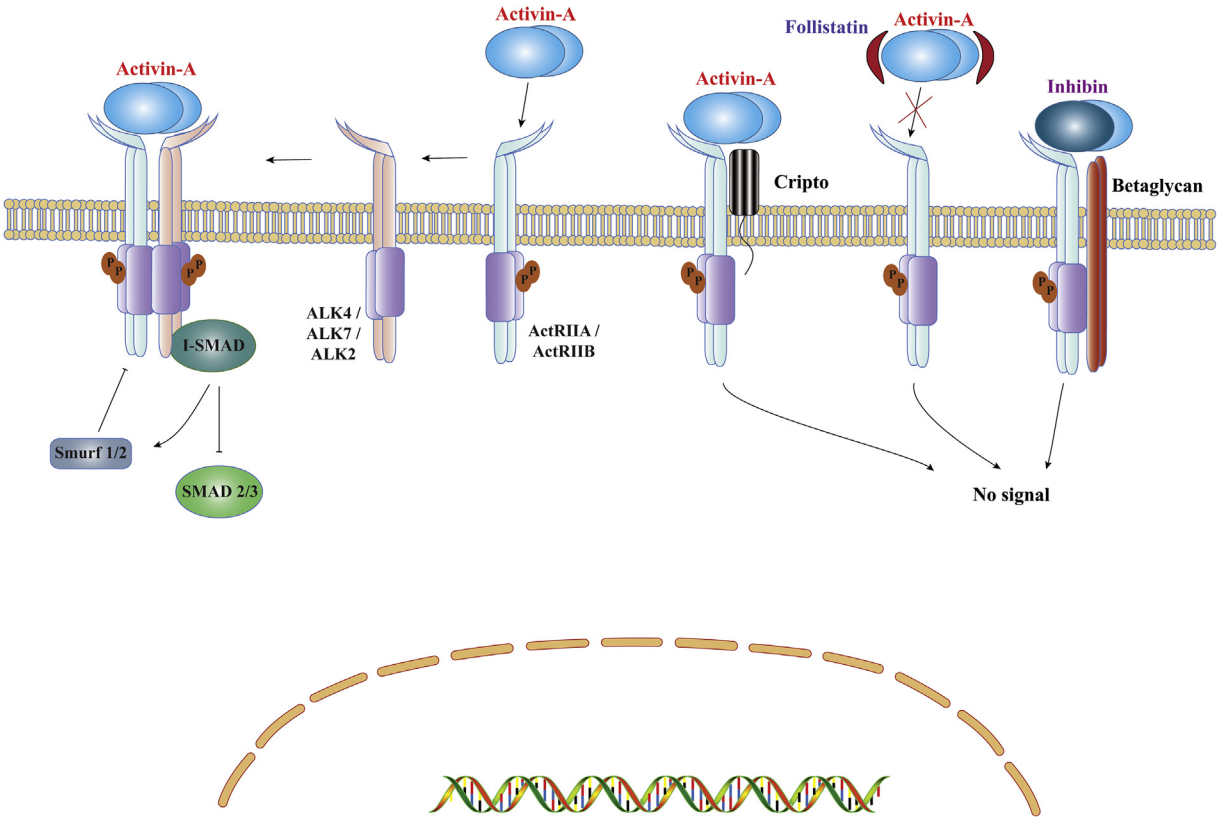

Fig. 2. Regulation of activin-A functions. Activin-A signals through a heterotetrameric receptor complex. Initially, activin-A binds to the constitutively active ActRII (ActRIIA or ActRIIB). This interaction leads to the recruitment and phosphorylation of the ActRI (ALK4, ALK7 or ALK2). Inhibitory SMADs (I-SMADs), SMAD-6 and SMAD-7, are upregulated upon activin-A binding to its receptors and bind to the activated ActRI, inhibiting signal propagation intracellularly. Follistatin (FS) is one of the major inhibitors of activin-A which neutralizes its actions by binding with high affinity to activin-A and preventing its interaction with its type II receptors. Inhibins compete for binding to the type II receptors but can also bind directly to activins with variable affinities. Betaglycan binds inhibins and enhances their affinity for ActRII, leading to the inhibition of the interaction between activins and the type II receptors. Furthermore, Cripto the co-receptor for nodal ligands, inhibits activin-A signaling by binding to activin-A receptors.

suppression of TLR4 and CD14 expression [14,16,23]. Moreover, activin-A suppressed iNOS expression in IFN-γ-stimulated RAW264.7 cells [22]. These data indicate that in activated macrophages activin-A exerts anti-inflammatory functions, generating a negative feedback loop to regulate excessive responses.

Pertinent to humans, tonsil macrophages produced activin-A upon *in vitro* administration of chemical agonists of TLR7 and TLR8 [24]. Moreover, activin-A secretion was upregulated in granulocyte-macrophage colony-stimulating factor (GM–CSF)–treated M1-polarized macrophages and its expression was higher, compared to M–CSF–treated M2 polarized cells both at the mRNA and the protein level [25,26]. Notably, glucocorticoids and all-trans-retinoic acid restrained GM–CSF–mediated activin-A induction in human monocytes [26]. In contrast, stimulation of monocytes with LPS, IFN-γ and GM-CSF enhanced the production of activin-A [27]. T effector cells also induce the production of activin-A by human monocytes, an event that was mostly dependent on CD40/CD40L interactions and IFN-γ and GM-CSF secretion [27].

Interestingly, using an activin-A neutralizing antibody and chemical inhibitors of ALK4 and ALK7, it was demonstrated that activin-A inhibited the expression of M2-type genes, such as, C-maf proto-oncogene (MAF), Insulin-like growth factor 1 (IGF1), plasminogen activator inhibitor 2 (SERPINB2) and coagulation factor XIII A chain (F13A1) and decreased IL-10 secretion in human macrophages polarized with GM-CSF [25]. As GM-CSF upregulates activin-A [26], it is conceivable that GM–CSF–treated macrophages establish the M1 cell phenotype partly through induction of activin-A which suppresses M2 polarization. Interestingly, activin-A expression and SMAD2/3 phosphorylation were increased in human macrophages generated from $CD16^+$ monocytes and stimulated with M-CSF (M16), compared to those originating from $CD14^+CD16^-$ monocytes under the same polarization conditions (M14) [28]. These M16 type macrophages clustered transcriptionally and phenotypically with the pro-inflammatory GM–CSF–differentiated M1 type macrophage populations and blocking activin-A signaling partially reversed this effect [28]. Hence, activin-A plays an important role in the acquisition of GM–CSF–polarized M1 type characteristics by $CD14^+CD16^-$ monocytes even in the presence of the non-inflammatory M-CSF [28]. In contrast, another group demonstrated that M-CSF did not affect activin-A production by human monocytes, but in those studies, there was no distinction between monocytic subpopulations and thus, the effects of M-CSF on $CD16^+$ cells could have been masked [27].

In sharp contrast, other studies demonstrated that treatment of LPS-activated human monocytes with activin-A restrained IL-1β secretion by interfering with its proteolytic maturation [29]. Concomitantly, activin-A increased the production of the IL-1β antagonist, IL-1RA [29]. These effects were observed only when activin-A was administered in the presence of LPS. Thus, similar to the results described in mouse macrophages, activin-A exerts distinct pro- or anti-inflammatory effects depending on the cell activation status and the type of stimulation.

3.2. Dendritic cells

Activin-A affects several aspects of dendritic cell (DC) function. Human monocyte derived dendritic cells (moDCs), generated from BM hematopoietic precursors, and peripheral blood (PB) DCs express

activin-A type I and type II receptors [30–32]. Human tonsil conventional (DCs)-2 secrete high amounts of activin-A upon stimulation with R848, a dual TLR8 and TLR7 synthetic agonist with potent anti-viral activity [24]. A detailed analysis of the kinetics of activin-A receptor expression by human moDCs revealed that early after LPS or CD40L stimulation, *Acvr1b* levels decrease, followed by an upregulation later on [31]. However, the opposite effects were observed for *Acvr2a* expression; *Acvr2b* expression was downregulated by LPS stimulation, while during CD40L activation, *Acvr2b* showed similar expression patterns to those of *Acvr1b* [31]. This complex regulation of activin-A signaling components on moDCs following LPS or CD40L stimulation may have different impacts on their phenotype and/or functions. Activin-A mRNA and protein levels were upregulated in moDCs upon LPS and other TLR agonist stimulation, as well as, upon CD40L ligation or *Bartonella henselae* and *Salmonella thyphimurium* infection but not in the presence of Prostaglandin E2 (PGE_2) or ATP [30,31]. In $CD1c^+$ myeloid DCs, activin-A release was modestly increased after LPS, *Escherichia coli* or CD40L activation compared to moDCs, while plasmacytoid DCs did not express activin-A [30,31]. In BM-derived DCs, cholera toxin enhanced activin-A release [33]. Notably, cholera toxin-treated BM-derived DCs induced the differentiation of $CD4^+$ T cells towards IL-17-producing T cells, at least partly, through the secretion of activin-A [33]. Moreover, treatment of BM-derived DCs with activin-A increased SMAD2 and ERK1/2 phosphorylation, suggesting that activin-A affects the functional properties of DCs [31,32]. Indeed, in a co-culture system wherein BM-derived mouse DCs were used as antigen presenting cells, activin-A enhanced $CD4^+$ and $CD8^+$ T cell proliferation and survival [32]. These effects were mediated through activin A-induced upregulation of the TNF superfamily cytokines, B-cell activating factor (BAFF) and A proliferation-inducing ligand (APRIL) in DCs [32].

Pertinent to the role of activin A in human moDC responses, a study reported that it does not affect cell maturation, as evidenced by no change in the expression of CD83, CCR7 and HLA-DR [30]. Nevertheless, the same study showed that activin-A enhanced monocyte differentiation to DCs [30]. In contrast, other reports demonstrated that activin-A interfered with moDC maturation through the inhibition of cell cytoskeleton rearrangements [34]. Notably, the same studies showed that activin-A-treated moDCs were less potent inducers of T cell proliferation due to impaired antigen presenting capacity [34]. Activin-A-stimulated moDCs also expressed decreased IL-6, IL-10, TNF-α, IL-8, IL-12p70, IL-10, CCL5 and CCL2 levels upon CD40L stimulation [31]. Remarkably, blocking activin-A signaling with FS in CD40L-stimulated human moDCs enhanced their ability to stimulate $CD8^+$ T cell proliferation [31]. Other studies demonstrated that activin-A treatment through induction of CXCL12, CXCL14, MMP-2 and MMP-9, promoted the migration of immature DCs in *in vitro* chemotaxis assays, pointing to a critical role in the recruitment of immature DCs to sites of inflammation [35]. Studies by *Durand M* et al. revealed that activin-A and TGF-β acted synergistically to increase CXCL13 production in co-cultures of human tonsil cDC2 or macrophages with naive PB $CD4^+$ T cells [24]. *In vivo* studies by our group in a model of repeated allergen challenges in the skin, demonstrated that activin-A expression is increased in the skin of atopic individuals and correlates with the cDC infiltration at the inflamed skin [36].

Overall, similar to the results observed in macrophages, activin-A effects on DC responses depend on the cell maturation status and the tissue micromilieu. Still, the *in vivo* role of activin-A in DC maturation and functions remain unexplored and warrant further investigation.

3.3. B cells

Murine B cells upon *in vitro* LPS stimulation produce copious amounts of activin-A [37]. Furthermore, B cells isolated from the spleens of Complete Freund's Adjuvant (CFA)/Ovalbumin (OVA)-immunized mice express increased mRNA levels of activin-A [37]. B cells are also targets of activin-A signaling as evidenced by expression of both type I and II receptors. Nevertheless, upon *in vitro* LPS stimulation, activin-A receptors are decreased and this is also observed in mouse B cells following CFA/OVA immunization *in vivo*, suggesting that activin A acts on resting but not activated B cells [37].

Activin-A does not affect the proliferation or IgG production by LPS-stimulated B cells *in vitro*. Still, treatment of naive B cells with activin-A enhanced their proliferation and IgG production [37,38]. Other studies have shown that pretreatment of either resting or LPS-stimulated B cells with activin-A did not affect IgE levels [37]. In contrast, *in vitro* treatment of murine LPS-stimulated B cells with activin-A led to increased IgA production and this was independent of TGF-β1 signaling [38]. In support, activin-A enhanced IgA secretion by mesenteric lymph node cells *ex vivo*, pointing to a role in the maintenance of gut homeostasis [38]. Of clinical relevance, activin-A inhibited phytohemagglutinin (PHA)-induced proliferation of B cells isolated from healthy individuals and patients with pulmonary alveolar proteinosis (PAP) and diminished the production of autoantibodies against GM-CSF [39].

3.4. T cells

Activin-A and its signaling pathways are expressed by embryonic and adult thymic stromal cells and thymocytes, suggesting that activin-A plays a role in thymic development [40,41]. *In vitro* studies showed that activin-A restrained PHA-induced proliferation of adult rat thymocytes as well as PB $CD4^+$ T cells [42]. In humans, activin-A suppressed the proliferation of polyclonally-stimulated PB $CD4^+$ T cells [43]. Pertinent to T helper cell differentiation, seminal studies unraveled a crucial role for activin-A in human T follicular helper cell (T_{FH}) responses. Briefly, the authors performed an unbiased high-throughput screen of a human extracellular proteome library and each protein was produced as a secreted recombinant molecule and tested for its capacity to regulate the differentiation of naive $CD4^+$ T cells into T_{FH} cells *in vitro*. Strikingly, activin-A emerged as the most potent inducer of the CXCR5 and PD-1 molecules that characterize T_{FH} cells [44]. Activin-A also synergized with IL-12, modulating the human T_{FH} gene program. SMAD2/3 played critical roles downstream of activin-A signaling in the regulation of human TFH cell differentiation [44]. Other studies have also shown that activin-A, in the presence of low doses of TGF-β, drives the generation of mouse Th9 cells *in vitro*, while *in vivo* ablation of activin-A and TGF-β1 signaling restrained Th9 cell differentiation and effector functions [45].

Interestingly, activin-A enhanced TGF-β1-mediated expression of the T regulatory (Treg) cell-associated transcription factor, Foxp3, by mouse $CD4^+CD25^-$ T cells *in vitro* and promoted their conversion to $Foxp3^+$ Treg cells *in vivo* [46]. A recent study also showed that *in vitro* activation of naive $CD4^+$ T cells with activin-A, even in the absence of TGFβ, led to the generation of $Fox3^+$ Treg cells, although combination of activin-A with low doses of TGFβ exhibited synergistic effects on $Foxp3^+$ Treg induction. Activin-A-induced upregulation of Foxp3 was dependent on Yes-Associated Protein (YAP)-mediated, ALK7 expression on T cells. Besides its effects on Treg cell generation, activin-A signaling through ALK7 amplified the suppressive potential of natural and TGF-β-induced Treg cells both *in vitro* and *in vivo* [47].

3.5. Natural killer cells

Human natural killer (NK) cells express both type I and II activin-A receptors [48]. Activin-A inhibits the proliferation, the expression of CD25 and T-bet, and the secretion of IFN-γ, CCL3, CCL4, CXCL8 and CXCL10 by human NK cells *in vitro* [48]. Mouse NK cells also produce and respond to activin-A, as activin type IIA and IIB receptors and SMAD2/3 were expressed in PB NK cells. Activin-A production by mouse NK cells was IL-2 dependent. Activin-A also enhanced IL-2 synthesis by NK cells, illuminating the existence of a positive feedback loop. In addition, activin-A suppressed the ability of NK cells to lyse target cells [49]. Recently, it was discovered that activin-A, acting

through its canonical signaling pathways, inhibited mouse and human NK cell basal glycolysis and oxidative phosphorylation metabolic pathways [(quantified by extracellular acidification rate (ECAR) and oxygen consumption rate (OCR)] and NK cell-mediated cytotoxicity and induced tissue-residency features on NK cells [50]. Altogether, these studies propose an inhibitory role of activin-A on NK cell functions at least in *in vitro* settings.

3.6. Neutrophils

Several studies have shown that mouse and human neutrophils are a potent source of activin-A, the release of which is directly stimulated by TNF-α [51–53]. Peritoneal and PB neutrophils express activin-A signaling pathway components, while stimulation of the later with LPS leads to the production of activin-A. Stimulation of neutrophils with activin-A *in vitro* enhanced reactive oxygen species production, IL-6 release and phagocytosis, but did not affect neutrophil chemotaxis. Furthermore, *in vivo* ablation of SMAD3 attenuated the effects of activin-A on IL-6 release by mouse peritoneal neutrophils [52]. In an *ex vivo* setting, activin-A reduced neutrophil transendothelial migration and chemotaxis towards a gradient of *N*-formyl-L-methionyl-L-leucyl-phenylalanine (fMLP), a potent inflammatory mediator, and these effects were associated with altered calcium signaling [54]. Overall, these studies highlight activin-A as a potent activator of neutrophil functions.

3.7. Microglia

Microglial cells are essential for rapid immune responses to CNS, as well as, systemic infections. Microglia express activin-A and its receptor subunits both at the resting state and upon LPS challenge [15,38,55,56]. Furthermore, *in vitro* LPS stimulation of rat microglia downregulated the expression of SMAD7 [57], suggesting that similar to the findings observed in macrophages, TLR4 stimulation enhances activin-A signaling. Notably, *in vitro* studies demonstrated that activin-A exhibits anti-proliferative effects on mouse and rat primary microglial cells, as well as, on the MG6 microglial cell line [38,56,57]. Still, activin-A administration *in vivo* did not significantly affect microglial populations [58]. These differences in microglia viability may arise from concentration-dependent effects, as it has been shown that activin-A suppressed microglia proliferation in a dose-dependent manner [59]. The contribution of other factors present in the tissue microenvironment in activin-A effects on microglia survival may also explain the divergent results observed in the *in vitro* and *in vivo* studies. In support, *in vitro* studies demonstrated that activin-A stimulation in the presence of IL-34 and cholesterol, promoted microglia survival [60].

Interestingly, activin-A stimulation did not affect the phagocytic activity or TNF-α, IL-6 and CXCL1 production by mouse microglia, even when administered prior to LPS or CPG stimulation [61]. Still, activin-A reduced TNF-α production when administered at a high dose (13 μg/ml) prior to TLR2 or TLR4 stimulation [61]. In contrast, other reports showed that treatment of MG6 murine microglial cells with activin-A, followed by LPS stimulation, decreased the expression of IL-6, IL-18 and iNOS, but had no effect on IL-1β levels [59]. Additionally, activin-A upregulated the expression of the M2-related gene arginase-1 [57]. It was also demonstrated that activin-A production by M2 microglia was essential for oligodentrocyte differentiation and stimulation of myelin production, pointing to an important physiological role for this cytokine in CNS homeostasis [62]. *In vivo* administration of activin-A upon LPS challenge reduced the proliferative effects of TLR4 signaling on microglial cells [56,58]. Notably, activin-A treatment of mouse microglial cell cultures followed by activation with TLR2, 4 and 9 agonists, increased their ability to phagocytose *E. coli K1* strains, pointing to enhancement of anti-microbial responses [61]. Overall, these studies suggest that activin-A endows microglia with an anti-inflammatory phenotype that is also protective against pathogen infections. Still, the role of activin-A in responses elicited by human microglia remains elusive.

4. The function of activin-A in microbial host defense

4.1. Bacterial and parasite infections

Liver cells at the onset of infections and in response to pro-inflammatory cytokine signals produce proteins with immune-modulatory potential, termed acute phase proteins. These proteins play an important role in pathogen clearance and enhance systemic inflammation. *In vitro* studies showed that activin-A suppressed IL-6-mediated induction of three major acute phase proteins, haptoglobin, a1-AGP and fibrinogen [63,64]. Interestingly, *Toxoplasma gondii*-infected human and mouse fibroblasts exhibited increased activation of ALK4, which in turn activated hypoxia-inducible factor 1-alpha (HIF-1α) and promoted cell survival and pathogen replication [65]. This effect was mediated by the activation of a non-canonical ALK4 signaling pathway, involving JNK [65]. Another study in mice were IL-4 signaling was disrupted specifically in $CD11c^+$ DCs showed that lymph node $CD11c^{++}MHCII^{++}$ DCs exhibited increased mRNA expression of activin-A upon infection with *Leishmania major*, and this was associated with impaired Th1, increased Th2 cell responses, exacerbated disease severity and pathogen clearance inability [66] (Fig. 3).

In acute responses to infection, such as, those occurring during LPS administration, activin-A levels are rapidly increased in the serum, reaching a peak within 1 h [67,68]. In mice, activin-A was released concomitantly with TNF-α [67], while in sheep, activin-A increase was detected earlier compared to that of TNF-α and IL-6 [68]. Further experiments using C3H/HeJ mice, characterized by impaired TLR4 signaling due to a point mutation into the coding region of receptor gene, demonstrated a direct link between TLR4 signaling and activin-A production, as LPS challenge did not affect activin-A levels in these mice [67]. It was also suggested that during systemic LPS challenge, activin-A enhances inflammatory responses as *in vivo* administration of FS prolonged survival and reduced circulating TNF-α and IL-1β [67]. Rabbits infected with bacterial meningitis showed a significant increase in activin-A in the cerebrospinal fluid (CSF) which correlated with CSF protein levels (an indication of infection severity and impaired CSF barrier ability) and the number of apoptotic neurons in the dentate gyrus [69] (Fig. 3). Still, no correlations were identified between activin-A expression and leukocyte numbers or bacterial titers in the CSF. The dominant sources of activin-A in the CNS during bacterial meningitis were the activated microglia and CNS-infiltrating macrophages [69] (Fig. 3).

Studies in humans with bacterial meningitis revealed augmented activin-A expression in the CSF which, similar to the results obtained in the rabbit model, did not correlate with leukocyte numbers in the CSF [55,70]. In addition, activin-A levels in the serum were not different between patients and healthy donors [70]. FS was increased in the CSF of patients with meningitis, highlighting the tight regulation of activin-A during infections [71]. In the context of septicemia, increased activin-A and FS levels were detected in the serum of patients, compared to healthy donors [72]. Again, these differences did not correlate with total leukocyte counts in the serum [72]. Studies by our group in neonates with septicemia, have demonstrated a gradual elevation of activin-A in the serum during disease progression [73]. An inverse correlation was also identified between activin-A and pro-inflammatory CXCL8 expression, pointing to immunosuppressive effects of activin A during neonatal infections [73]. Interestingly, activin-A overexpression via adenoviral intratracheal instillation induced a disease that resembles human acute respiratory distress syndrome (ARDS), characterized by alveolar cell death and hyaline membrane formation, reduction of the surfactant proteins, decline of lung compliance and emphysema [74]. *In vivo* neutralization of activin-A using a soluble ActRIIB-Fc fusion-protein attenuated the ARDS-like pathology [74]. Notably, the authors observed enhanced levels of activin-A in the BAL

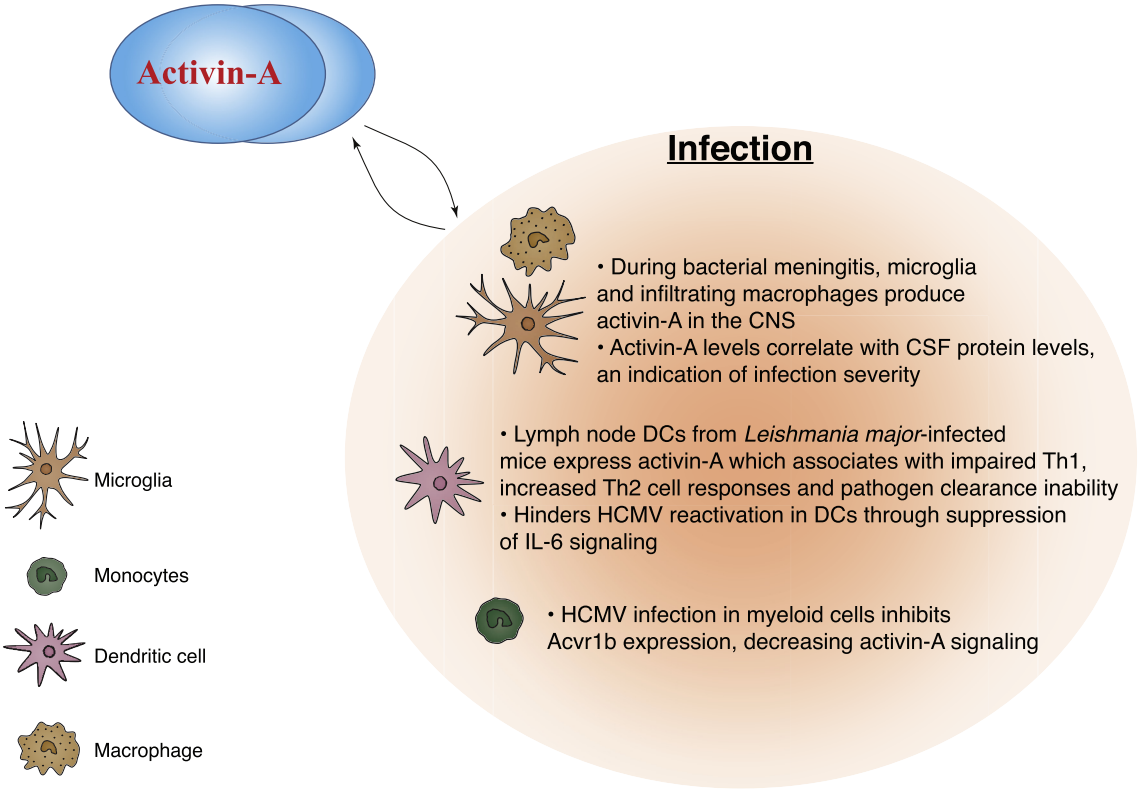

Fig. 3. The pleiotropic effects of activin-A on immune responses in microbial infections. Activin-A effects on immune cells are variable and dependent on the cell type, its activation state and the context of the immune responses. Activin-A enhances protective immune responses during pathogen infections. An overview of the roles of activin-A and its signaling pathways on immune cells in the context of microbial infections is demonstrated.

of patients with ARDS, suggesting that activin-A could be implicated in the mechanisms underlying disease pathogenesis [74].

Activin-A is produced in the human placenta and increases in the serum during the course of pregnancy [75–78]. Furthermore, activin-A production was upregulated in amnion and choriodecidual explants treated with IL-1β and TNF-α, cytokines known to be rapidly produced by gestational tissues in response to bacterial antigens [79,80]. In choriodecidual explants, activin-A was also upregulated following LPS administration [79,81,82]. LPS stimulates the rapid translocation of TLR4 to the basal membrane of the chorioamnion and increased TLR4 membrane localization correlates with the development of chorioamnionitis [83]. As TLR4 and activin-A pathways cross-regulate each other upon LPS challenge, it can be speculated that the interaction of these two pathways plays an important role in disease outcome. In amnion explants, activin-A stimulation induced a concentration-dependent biphasic effect on IL-6, IL-8 and PGE2 production, while it inhibited TNF-α release by choriodecidual and placental explants [84]. Amniochorion explants also exhibited increased MMP-1 and MMP-9 expression in response to activin-A [85].

Preterm labor has been closely associated with inflammatory responses against intrauterine pathogens, initiated by either maternal or fetal gestational tissues [86]. Increased expression of the ActRIIB receptor has been reported in amniotic and chorionic cells of women with preterm labor and upregulated activin-A levels were detected in the amniotic fluid [87]. Periodic serum release of activin-A was identified in both healthy pregnant women and patients with preterm labor [88]. Nevertheless, it was shown that activin-A mean peak amplitude was higher in patients compared to healthy controls [88]. Another study verified increased serum levels of activin-A in patients with preterm labor [89]. Notably, activin-A expression was elevated in the amniotic fluid of patients with intra-amniotic infection or chorioamnionitis [81,85]. Another study demonstrated that activin-A mRNA expression by placental cells was higher in women with preterm premature rupture of the membranes (pPROM), caused by acute chorioamnionitis, compared to those with pPROM but without infection [81]. Finally, in patients with *Chlamydia trachomatis* infection increased expression of activin-A and type II receptor mRNA was detected in the fallopian tube during ectopic pregnancy [90]. These studies illuminate an important role for activin-A in the context of preterm labor and intrauterine infection which seem to contribute to pathogen clearance but also to pregnancy perturbation events. However, further studies are required in order to elucidate the precise role of activin-A in the initiation and development of immune responses during pregnancy.

4.2. Viral infections

During viral infections, such as, hepatitis C virus (HCV) and hepatitis B virus (HBV) infections, activin-A is increased in the serum of patients, compared to uninfected controls [91,92]. In HCV, activin-A levels correlated with IL-6, TNF-α, viral load and AST platelet ratio index, an indicator of liver fibrosis [91]. In the case of HBV, activin-A expression correlated with the viral load and alanine aminotransferase concentration, a marker of hepatic injury [92]. These studies propose that activin-A expression during chronic hepatitis is associated with disease severity and virus-induced fibrotic events. Severe influenza A (H1N1) infection was characterized by increased activin-A in the serum that correlated with IL-6 levels the second day following infection [93]. Activin-A levels were also increased in the CSF during viral meningitis [70].

Notably, *in vitro* studies reported that activin-A restrained HCV replication in the OR6 hepatocellular carcinoma cell line [94]. In the case of Zika virus infection, treatment with activin-A alone or in combination with IFN-α, inhibited virus replication in the A549 lung epithelial cell line [94]. Moreover, *in vitro* administration of activin-A in DCs infected with the human cytomegalovirus (HCMV), inhibited virus reactivation through the suppression of IL-6 signaling [95] (Fig. 3).

Interestingly, the HCMV-encoded miR-UL148D expressed during latent infection inhibited the translation of *Acvr1b*, leading to decreased activin-A signaling in primary myeloid cells [96] (Fig. 3). It was speculated that this effect favors the restriction of pro-inflammatory cytokine secretion that leads to viral reactivation but may also suppress activin-A-associated protective immune responses against the virus [96]. On the other hand, HBV pX encoded oncoprotein propagated activin-A signaling through direct interaction with SMAD4, leading to SMAD2/3/4 complex stabilization, increased nuclear localization and enhanced transcriptional activity [97]. As activin-A signaling affects the expression of extracellular matrix genes involved in fibrotic processes, it is conceivable that HBV infection utilizes this pathway to promote liver fibrosis in the context of chronic infection. In agreement, another study demonstrated that human rhinovirus infection (HRV-16) led to increased production of activin-A in primary human bronchial epithelial cells (BECs) and the BEAS-2B human bronchial epithelial cell line [98]. The same study suggested that HRV infection during childhood affects airway remodelling and asthma predisposition partly through activin-A upregulation [43,99].

Collectively, these findings support the notion that, in the context of viral infections, activin-A signaling initially enhances protective antiviral immunity, while at later disease stages, it promotes fibrotic events, associated with tissue damage and disease chronicity.

5. Activin-A in the regulation of allergic diseases

An increasing body of evidence has proposed fundamental roles for activin-A in Th2-cell mediated allergic responses and airway remodelling. Murine Th2-skewed cells represent a major source of activin-A *in vitro*. In fact, the Th2 cell-related transcription factor c-Maf, regulates activin-A expression as it cooperates with the nuclear factor of activated T cells (NFAT) and transactivates the *activin βA* promoter [22]. Mast cells also produce activin-A which triggers the proliferation of airway smooth muscle cells *in vitro*, implying a crucial role for activin-A in airway hyperresponsiveness (AHR) and remodelling [100,101]. IgE-cross-linking on BM-derived mast cells leads also to a rapid increase of activin-A mRNA [101]. Mouse and human mast cells express type I and II activin-A receptors and activin-A enhances the differentiation and recruitment of BM-derived mast cell progenitors *in vitro* [102] (Fig. 4).

In line with the *in vitro* studies, a constellation of studies using experimental asthma models have revealed that activin-A is involved in the regulation of allergic airway inflammation. Activin-A is expressed in the bronchoalveolar lavage (BAL), lung tissue and submucosal mast cells in a mouse model of OVA-induced acute allergic airway inflammation [101,103]. In a recent study, it was found that maternal D-γ-tocopherol supplementation before OVA challenge increased the expression of activin-A, amphiregulin, IL-5, CCL11, CCL24 and GM-CSF in the lungs and BAL of pups [104]. Other investigators reported that activin-A expression was downregulated in the bronchial epithelium, while its levels were increased in the BAL after allergen challenge [105]. Moreover, intratracheal administration of IL-13, a cytokine critical for mucus production, increased activin-A secretion in BAL and this correlated with heightened mucus secretion [106]. Submucosal fibroblasts also expressed elevated levels of ALK4 and ActRIIA, along with phosphorylated SMAD2, upon allergen challenge, supporting a role for activin-A in airway repair and remodelling [103]. As mentioned above, seminal studies have demonstrated that activin-A induced the differentiation of Th9 cells *in vitro*, while *in vivo* ablation of activin-A and TGF-β1 signaling restrained Th9 cell-mediated allergic responses and ameliorated allergic airway inflammation [45] (Fig. 4). Notably, in mice wherein SMAD2 is overexpressed in the airway epithelium, activin A and IL-25 levels were markedly up-regulated in the lungs after house dust mite (HDM) exposure, while *in vivo* neutralization of activin-A inhibited IL-25 increase and diminished collagen deposition and AHR [107]. In support, *in vivo* administration of FS attenuated lung fibrosis in a mouse model of chronic OVA challenge [108]. In addition, the same group, using a mouse model of cystic fibrosis, demonstrated that intranasal delivery of FS in newborn mice inhibited lung inflammation and pathology [109].

Studies by our group have uncovered important protective functions of activin-A in allergic airway disease in mice [110]. Briefly, *in vivo* neutralization of endogenously-produced activin-A during pulmonary allergen challenge, aggravated Th2 cell-mediated allergic responses and exacerbated allergic airway disease phenotype [110] (Fig. 4). In support, *in vivo* systemic administration of activin-A ameliorated cardinal features of allergic disease, including AHR, pulmonary inflammation and allergen-specific IgE levels. Importantly, the protective effects of activin-A on experimental asthma were mediated through the generation of $CD4^+Foxp3^-IL\text{-}10^+$ Treg cells that suppressed allergen-driven Th2 cell responses *in vitro* and upon transfer *in vivo* [110] (Fig. 4). In contrast to our findings, a study by Hardy and colleagues revealed that *in vivo* administration of FS in the airways inhibited Th2-cell mediated responses, suggesting that activin-A is pro-inflammatory [105] (Fig. 4). The discrepancies observed between our studies and those of Hardy and colleagues may be due to the different activin-A inhibitors used; we administered a specific activin-A neutralizing antibody, whereas *Hardy C.L.* et al. administered FS, known to neutralize all activins and BMPs [12,111]. The distinct routes of activin-A neutralization, i.e. in the airways versus systemically, may have also affected the effects of activin-A. Taken together, these studies highlight the notion that, similar to other cytokines, activin-A exhibits both pro and anti -inflammatory functions depending on the spatiotemporal context.

Our subsequent studies explored whether activin-A-induced Treg cells enhance the generation of DCs with tolerogenic properties (act-Treg-modified DCs). Indeed, through a series of adoptive transfer experiments we discovered that activin-A-induced Treg cells gave rise to DCs with suppressive functions towards experimental asthma [112]. Act-Treg-modified DCs exhibited an immature phenotype, reflected by low MHC-II and co-stimulatory molecules expression, an impaired capacity to uptake antigen and traffic to the draining lymph nodes and attenuated cytokine release in response to LPS challenge *in vivo*. Moreover, act-Treg-modified DCs displayed poor immunostimulatory potential, exemplified by a decreased ability to prime allergen-specific T cell responses *in vitro* and *in vivo*. Importantly, administration of act-Treg-modified DCs protected against experimental asthma both in preventive and therapeutic protocols through the *de novo* generation and expansion of $Foxp3^+$ Tregs. Notably, disruption of PD-1 signaling hindered the capacity of activin-A-induced Treg cells to generate tolerogenic DCs, highlighting the involvement of the PD-1/PDL-1 immunoregulatory pathway [112]. Another interesting study implicated activin-A in the suppression of Th2-cell mediated allergic responses in the skin. Using a mouse model of OVA-induced cutaneous allergic inflammation in mice overexpressing activin-A in keratinocytes (K14-Activin Tg mice), the authors demonstrated decreased Th2 cytokine expression in the skin and dampened OVA-specific IgE levels, emphasizing the notion that activin-A participates in the maintenance of skin homeostasis [113].

A growing body of evidence has proposed that activin-A is involved in the regulation of human asthmatic responses. Activin-A expression by macrophages was demonstrated in lung tissues of patients with asthma [101] (Fig. 4). Increased activin-A expression was also observed in T cells, macrophages and mast cells in bronchial biopsies from MMA patients, compared to HCs [43,101,110]. Interestingly, neutrophils were identified as the most potent source of activin-A in the asthmatic airways [117] (Fig. 4). IgE receptor cross-linking on PB monocytes from atopic individuals also led to heightened activin-A secretion [114]. Heightened activin-A expression was detected in PB $CD4^+$ T cells from MMAs administered with corticosteroids in comparison to untreated MMAs and HCs [43]. Studies by our group and others have shown that activin-A levels are elevated in the serum and BAL of patients with severe asthma (SA), compared to mild-moderate asthmatics (MMA) and healthy controls (HCs) [43,115]. Another group showed that activin A

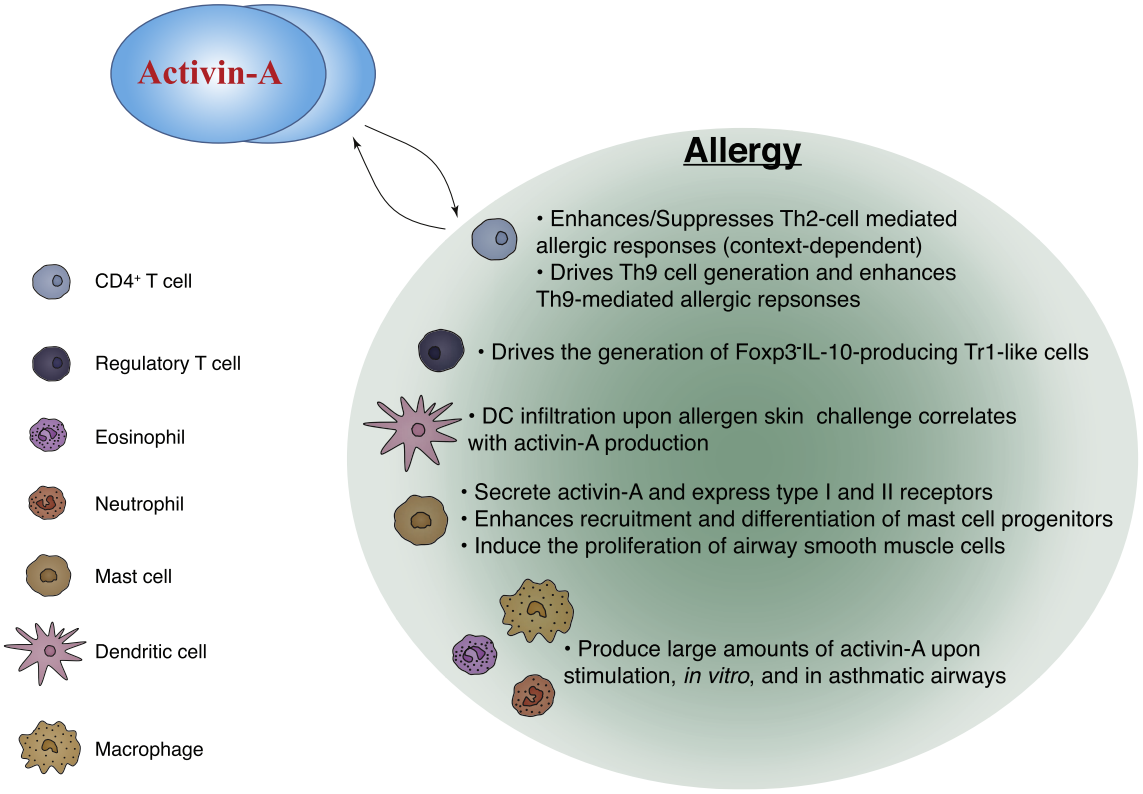

Fig. 4. Activin-A affects several aspects of immune responses in allergic diseases. Activin-A exerts fundamental roles in allergic responses and tissue remodelling in human allergic diseases, including allergic asthma and atopic dermatitis. The effects of activin-A on major immune cell players involved in the development of allergic responses are depicted.

expression was higher in the sputum and BAL of patients with SA, compared to MMA and correlated with eosinophil numbers and reticular basement membrane thickness [116]. *In vitro* studies demonstrated that activin-A expression was upregulated in primary human BECs upon IL-13 administration [106]. Interestingly, a recent study revealed that PB eosinophils obtained from allergic individuals produced significant amounts of activin-A upon *in vitro* stimulation with IL-3 and TNF-α [116] (Fig. 4). Moreover, increased activin-A mRNA levels were detected in eosinophils isolated from the BAL of allergic individuals after *in vivo* allergen challenge [116]. Studies by our groups have also demonstrated that activin-A was upregulated after *in vivo* allergen challenge in patients with nasal polyps, while its signaling pathways were decreased. Moreover, *ex vivo* polyclonal stimulation of nasal mucosa cells obtained from patients with nasal polyps, enhanced activin-A expression which, along with TGF-β1, synergized to increase IL-5 release [118].

Regarding the expression of activin-A signaling pathway components in the asthmatic airways, ALK4 and actRIIA were found to be decreased in airway submucosal cells of MMA patients, compared to HCs. More specifically, actRIIA levels were low in the airway epithelium, while actRIIB levels were nearly absent [110,115]. In addition, no pSMAD2 expression was detected in the airways of MMAs, as opposed to another study wherein more symptomatic and SA patients were included [117,119]. A recent study detected no differences in the expression of actRIIB in bronchial epithelial cells between SA and MMA patients [120].

Notably, studies using primary BECs obtained from asthmatic children revealed that depletion of activin A with shRNA decreased a-smooth muscle actin expression and fibroblast-to-myofibroblast transition, suggesting that activin-A is involved in airway remodelling in humans [121]. Studies by our groups demonstrated that *in vitro* treatment of human BECs with activin-A enhanced their proliferation and promoted epithelial cell repair [122]. Furthermore, blockade of activin-A, using FS, increased TNF-α and IL-13 -induced chemokine release by BECs, suggesting that activin-A exerts an anti-inflammatory role on airway epithelial cell responses [122]. Using a well-established *in vivo* model of repeated allergen skin challenges, we demonstrated that activin-A is increased in the skin of atopics, and its expression correlates with the numbers of infiltrating cDCs (Fig. 4). In fact, activin-A was implicated in retaining cDCs at the inflamed skin site, at least partly, through controlling CCR10/CCR4 expression [35]. Our studies also highlighted novel anti-angiogenic functions for activin-A. Indeed, we found that activin-A restrained vascular endothelial growth factor (VEGF)-induced human pulmonary endothelial cell proliferation and angiogenesis *in vitro* and led to increased production of the anti-angiogenic soluble VEGF receptor-1 (VEGFR1) and IL-18, concomitantly with decreased production of the pro-angiogenic VEGFR2 and IL-17 [119].

Our recent findings have uncovered a key role for activin-A in controlling human Th2 cell-mediated allergic responses. Interestingly, activin-A induced the differentiation of Type-1 regulatory T cell (Tr1)-like cells (act-A-iTr1) cells that produced copious amounts of IL-10 and inhibited T cell responses from asthmatic individuals through IL-10, ICOS and transforming growth factor β (TGF-β) -dependent pathways. Importantly, act-A-iTr1 cells protected against allergic asthma in a humanized mouse model. Mechanistic studies revealed that interferon regulatory factor 4, along with aryl hydrocarbon receptor and c-Maf, were strongly upregulated in human $CD4^+$ T cells in response to activin-A and functionally co-operated in *IL10* and *ICOS* promoters, instructing Tr1 cell differentiation. These findings illuminated activin-A as a novel inducer of human Tr1 cells that can be utilized in future cell therapies for the suppression of airway inflammation and the re-establishment of tolerance [122].

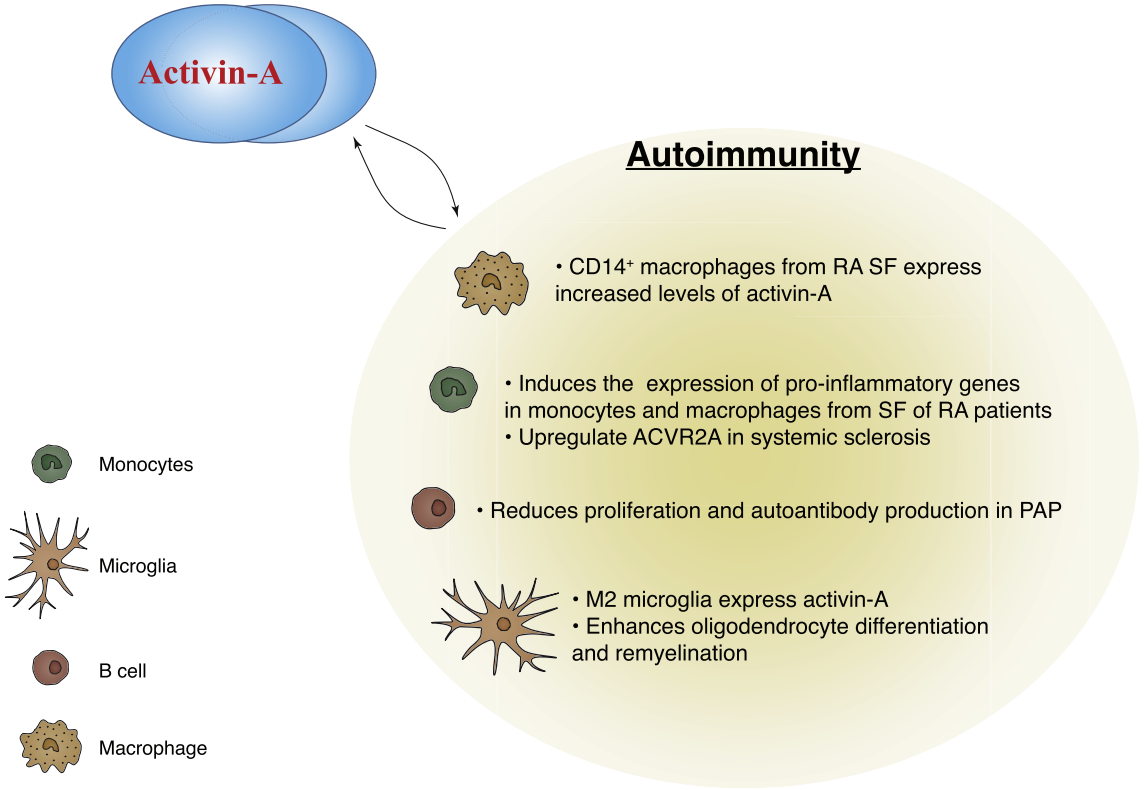

Fig. 5. The role of activin-A in the pathophysiology of autoimmune diseases. Activin-A is implicated in the immunopathology of human autoimmune diseases, including RA, PAP and SSc. An overview of the roles of activin-A and its signaling pathways on immune cell responses in the context of autoimmunity is demonstrated.

6. Activin-A effects on the pathogenesis of autoimmune disorders

Activin-A has been also implicated in the immunopathology of human autoimmune diseases. In the case of systemic lupus erythematosus (SLE), activin-A levels were found elevated in the serum [123,124]. Additionally, activin-A was increased in the synovial fluid (SF) and the serum of patients with rheumatoid arthritis (RA) and a positive correlation was identified between serum activin-A levels and disease activity parameters, including morning stiffness, Ritchie index, erythrocyte sedimentation rate and C-reactive protein [64,123]. In contrast, a negative correlation was observed with haemoglobin levels [64,123]. Notably, $CD14^+$ macrophages obtained from RA patients SF expressed increased activin-A mRNA levels, compared to cells obtained from normal synovium (Fig. 5). Additionally, transcriptomic analyses revealed a close similarity of SF macrophages to the GM–CSF–induced M1 pro-inflammatory cells [25,125]. Importantly, pro-inflammatory gene expression was induced in healthy monocytes and macrophages conditioned with SF from RA patients, and this was dependent on activin-A signaling [125]. These cells exhibited also high levels of pSMAD2 [125] (Fig. 5). $CD68^+$ mononuclear cells and fibroblast-like synoviocytes (FLS) from patients with active RA also expressed activin-A and actRIIA mRNA [126] (Fig. 5). *In vitro* studies on FLS showed that activin-A promoted their proliferation [126]. B cell subpopulations and von-Willebrand factor-expressing endothelial cells expressed also actRIIA in RA synovium [126]. Altogether, these findings propose that activin-A signaling is upregulated in RA patients and correlates with disease activity; still it's *in vivo* effects on RA immunopathology has not been explored.

In PAP, wherein autoantibodies neutralize GM-CSF activity and lead to dysregulated surfactant metabolism in the airways, loss of activin-A signaling was identified as a key event involved in disease pathogenesis. Studies in BAL samples demonstrated reduced activin-A levels in PAP patients, compared to HCs [39]. Moreover, PAP macrophages expressed reduced activin-A [39]. Importantly, activin-A stimulation inhibited the proliferation and autoantibody production from B cells of PAP patients [39] (Fig. 5). Later studies by the same investigators showed that the vitamin D active metabolite, calcitriol, upregulated activin-A expression in human alveolar macrophages and that calcitriol levels were reduced in PAP patients [127]. The authors proposed a mechanism wherein PAP immunopathogenesis is associated with impaired vitamin D signaling leading to reduced activin-A expression and increased B cell proliferation and auto-antibody production, highlighting potent immunosuppressive effects of activin-A on this autoimmune disease [127].

In the context of CNS inflammation, a key study revealed that activin-A signaling is important for oligodendrocyte differentiation and remyelination [128]. Activin-A production by M2-polarized microglia and macrophages was essential for oligodendrocyte differentiation during remyelination [62] (Fig. 5). More specifically, using oligodendrocyte-specific *Acvr2a* conditional knockout mice, the authors demonstrated that the expression of *Acvr2a* is essential for myelin synthesis [128]. Moreover, using a model of lysolecithin-induced demyelination, *Acvr2a* was upregulated and expressed at higher levels compared to *Acvr2b* in sites of active myelination/remyelination, in contrast to non-remyelinating areas where the opposite phenomenon occurred [128]. Interestingly, Acvr2A and Acvr2B receptors were expressed by oligodendrocytes, microglia and macrophages also in remyelinated lesions from patients with multiple sclerosis (MS) [128]. These studies suggest that activin-A exerts a neuroprotective and CNS-repairing role; still, its effects on the regulation of pathogenic Th1 and Th17 cell-associated autoimmune CNS inflammation, critical for MS pathogenesis, remain unexplored.

Finally, in patients with cutaneous systemic sclerosis, PB mononuclear cells expressed increased mRNA levels of *ACVR2A* both at basal levels and following stimulation with type I collagen, although the effects of activin-A upregulation in disease pathogenesis were not defined [129].

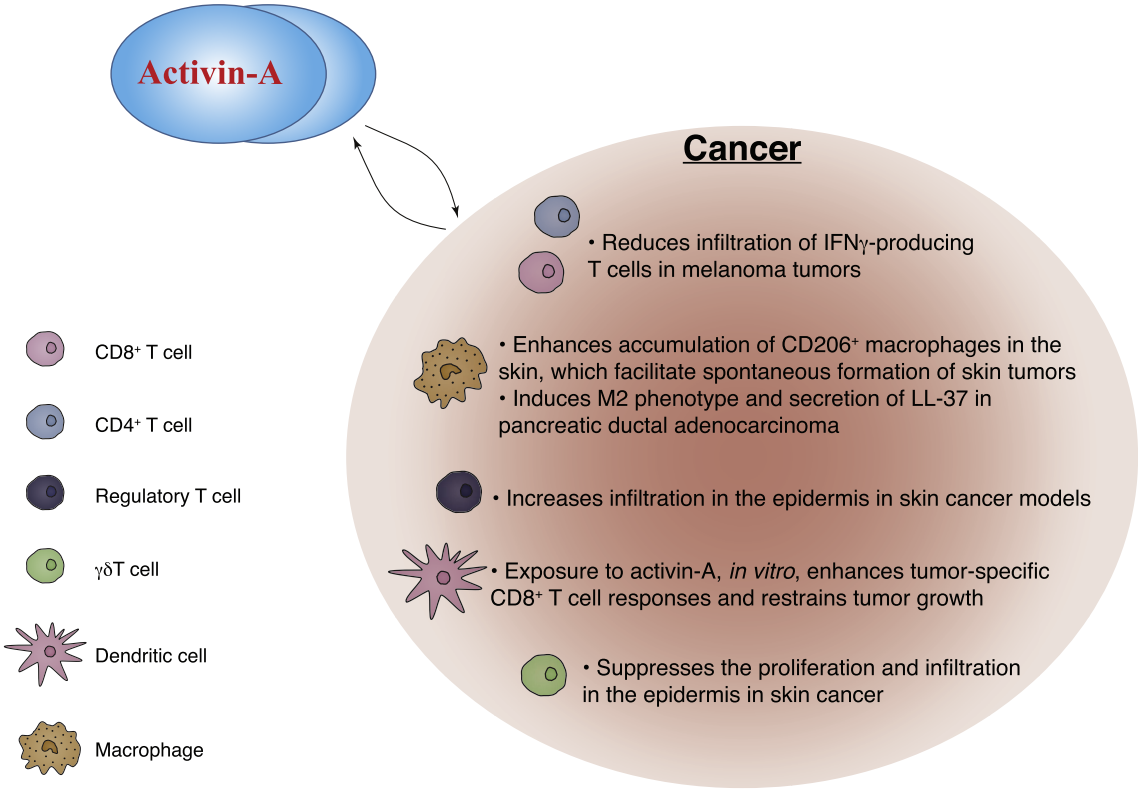

Fig. 6. Pro and anti -tumorigenic functions of activin-A in cancer. Activin-A exerts both pro-tumorigenic functions, promoting immunosuppressive activities of macrophages and Treg cells and anti-tumorigenic effects boosting $CD4^+$ and $CD8^+$ T effector responses and DC antigen presenting functions. These divergent effects of activin-A depend on the type of cancer and the context of the immune response and are summarized in the figure.

7. Divergent effects of activin-A on cancer

The role of activin-A in the various facets of cancer development and progression is cancer type-dependent and has been discussed in detail elsewhere [130]. Here, we focus on the diverse effects that activin-A on distinct immune cell types involved in anti-tumor immunity.

In a non-melanoma skin cancer model, wherein tumors were induced by topical treatment with 7,12-dimethylbenz[*a*]anthracene (DMBA) and 12-O-tetradecanoylphorbol 13-acetat (TPA), activin-A inhibited responses induced by dendritic epidermal γδ T cells (DETCs), cells that confer protection against the development of skin tumors in mice [131]. Specifically, activin-A suppressed the proliferation of DETCs and transgenic animals overexpressing activin-A in keratinocytes exhibited reduced frequencies of DETCs upon DMBA/TPA-induced skin carcinogenesis (Fig. 6). Simultaneously, the frequency of $CD4^+Foxp3^+$ Treg cells infiltrating the epidermis increased in activin-A-overexpressing animals developing skin cancer, while the percentages of activated $CD86^+$ Langerhans cells remained unaltered [132] (Fig. 6).

In another model of skin cancer, induced by the human papillomavirus (HPV) 8, increased levels of activin-A conferred a pro-tumorigenic program on macrophages which subsequently facilitated the progression of skin carcinogenesis [133]. In this model, γδ T cell-infiltration was reduced in the ear epidermis of mice overexpressing activin-A in keratinocytes, leading to increased tumor development (Fig. 6). In contrast, $CD4^+$ T effector cells and $CD4^+Foxp3^+$ Treg cells accumulated in the ear epidermis.Fig. 6). Additionally, activin-A overexpression enhanced the accumulation of $CD206^+$ macrophages in the skin, independently of HPV8 expression and tumor induction, due to enhanced migration of $CCR2^+$ monocytes towards increased activin-A concentrations (Fig. 6). Gene expression analyses further demonstrated that skin macrophages expressed *Acvr1*, *Acvr1b*, *Acvr2a* and *Acvr2b* and responded to activin-A by exhibiting the signature of M1 type macrophages. Still, Arg*1* (encoding arginase 1), considered a characteristic M2 marker, was also upregulated in these macrophages [134]. The depletion of macrophages in HPV8-infected, activin-A-overexpressing mice led to a significant delay in the spontaneous formation of skin tumors, suggesting that activin-A pro-tumorigenic effects were predominantly mediated by the induction of tumor-promoting macrophages [133].

In a mouse melanoma model induced by the intradermal injection of the B16F1 melanoma cell line, lentiviral-induced activin-A overexpression by melanoma cells reduced tumor infiltration by $CD8^+$ cytotoxic T-lymphocytes [135] (Fig. 6). Additionally, an exploration into the TCGA Skin Cutaneous Melanoma database revealed that, among melanoma patients those with the highest levels of activin-A, demonstrated reduced infiltration of cells belonging to the lymphocytic and the myeloid lineage [135]. In another study, using the B16 mouse melanoma model, blocking the effects of activin-A either through the use of a neutralizing antibody or disrupting its signaling through ALK7 decreased the frequencies of $CD4^+Foxp3^+$ Treg cells infiltrating the tumors, concomitant with increased tumor infiltration by IFNγ-producing $CD8^+$ and $CD4^+$ T cells and slower melanoma tumor growth [47] (Fig. 6). In sharp contrast, in other studies in the B16 mouse model of melanoma and the Lewis Lung Carcinoma (LLC) model of lung cancer, *in vivo* administration of DCs, previously exposed to activin-A, enhanced tumor-specific cytotoxic $CD8^+$ T cell responses, inhibited tumor growth and prolonged survival (Fig. 6). These effects were mediated by the expression of the cytokines BAFF and APRIL by activin-A-treated DCs, as knockdown of these genes deteriorated DC anti-tumor potential [32].

In the context of pancreatic ductal adenocarcinoma, *in vitro* studies demonstrated that cancer stem cells secreted activin-A which, in turn, induced the expression of the anti-microbial peptide LL-37 by human M1-polarized, monocyte-derived macrophages [136] (Fig. 6) Specifically, when M1-polarized macrophages were treated with conditioned

media obtained from cancer stem cells, they were polarized towards an M2 cell phenotype, accompanied by increased LL-37 mRNA and protein levels, dependent on activin-A signaling. Notably, LL-37 secreted by macrophages sustained pancreatic ductal adenocarcinoma growth, establishing a pro-tumorigenic link between cancer stem cells and macrophages that was partly mediated by activin-A [136].

8. Concluding remarks

The TGF-β superfamily of cytokines possesses pleiotropic functions governing the initiation of inflammatory responses but also providing immunosuppressive signals that restrain chronic inflammation, protect tissues from ongoing damage and induce regenerative processes. Activin-A affects several aspects of immune responses and growing evidence has unraveled pivotal roles for this cytokine in the pathophysiology of human diseases. Although activin-A has received the lion's share of attention, other activins also seem to play a role in immune responses; still, their functions, particularly those of activin-C (βCβC) and activin-E (βEβE), have barely been explored and merit further investigation.

Activin-A and its signaling pathway components are expressed by a wide variety of immune cells. Activin-A exerts pro-inflammatory effects on resting cells and during the onset of immune responses, while at later time points or during stimulation of activated cells, activin-A exhibits immunosuppressive functions. This is more evident in cells of the innate immune system. Activin-A also controls the differentiation and effector functions of Th cell subsets, including Th9 cells, T_{FH} cells, Tr1 Treg cells and Foxp3$^+$ Treg cells. Moreover, activin-A affects B cell responses, enhancing mucosal IgA and inhibiting the secretion of pathogenic autoantibodies.

Considering that T and B cells are drivers of immunopathogenesis in autoimmune disorders, the critical role of activin-A in the development and propagation of disease pathophysiology becomes evident. Still, there are considerable gaps in our understanding of the functions of activin-A in autoimmune diseases in humans, as well as, in animal models and this is expected to become an area of extensive future investigations. In addition, the signaling pathways and transcriptional programs underlying the functions of activin-A remain only partly defined. These open questions impact not only on the immunopathology of autoimmune diseases but also on the translation of activin-A as a novel therapeutic agent. With the advent of sophisticated high-throughput tools, such as, single-cell transcriptomics, mass cytometry, proteomics and metabolomics, the next years will no doubt open new directions in the field of activin-A and reveal how its functions can be exploited to control the balance between tolerance and autoimmunity.

Pertinent to cancer, activin-A exerts both pro-tumorigenic functions, promoting immunosuppressive activities of macrophages and Treg cells and anti-tumorigenic effects boosting CD4$^+$ and CD8$^+$ T effector responses and DC antigen presenting functions. Again, these divergent effects of activin-A depend on the type of cancer and the context of the immune response. However, several questions remain. Can targeting activin-A functions affect the development of cancer? What are the effects of activin-A on the metastatic potential of cancer cells? Can targeting activin-A be combined with checkpoint blockade immunotherapies? Finally, although the role of activin-A in allergic disorders has been extensively studied, a number of questions remain unanswered. For instance, we don't know whether and how activin-A regulates responses elicited by type 2 innate lymphoid cells. Moreover, the effects of activin-A on several aspects of innate inflammatory responses, including inflammasome activation and autophagy, have not been explored.

Further dissection of the role of activin-A in immune response regulation and of the molecular mechanisms underlying its signaling pathways will pave the way for the development of novel immunotherapies for autoimmune, allergic, infectious diseases and cancer. Still, given the pleiotropic nature of activin-A, limitations pertinent to possible side-effects during systemic administration, along with the investigation of the safest and most efficacious delivery route, need to be addressed before translation to the clinic.

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