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REVIEW

Autotaxin and lysophosphatidic acid signalling in lung pathophysiology

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Abstract

Autotaxin (ATX or ENPP2) is a secreted glycoprotein widely present in biological fluids. ATX primarily functions as a plasma lysophospholipase D and is largely responsible for the bulk of lysophosphatidic acid (LPA) production in the plasma and at inflamed and/or malignant sites. LPA is a phospholipid mediator produced in various conditions both in cells and in biological fluids, and it evokes growth-factor-like responses, including cell growth, survival, differentiation and motility, in almost all cell types. The large variety of LPA effector functions is attributed to at least six G-protein coupled LPA receptors (LPARs) with overlapping specificities and widespread distribution. Increased ATX/LPA/LPAR levels have been detected in a large variety of cancers and transformed cell lines, as well as in non-malignant inflamed tissues, suggesting a possible involvement of ATX in chronic inflammatory disorders and cancer. In this review, we focus exclusively on the role of the ATX/LPA axis in pulmonary pathophysiology, analysing the effects of ATX/LPA on pulmonary cells and leukocytes *in vitro* and in the context of pulmonary pathophysiological situations *in vivo* and in human diseases.

Key words: Autotaxin; Lysophosphatidic acid; Lung; Acute lung injury; Pulmonary fibrosis; Asthma; Lung cancer

Core tip: In the lungs, autotaxin (ATX) is constitutively expressed in the bronchial epithelium, and all pulmonary cell types express some amount of lysophosphatidic acid (LPA) receptor. LPA affects all pulmonary cell types, mainly promoting a pro-inflammatory state. Increased ATX/LPA levels have been detected in various pathophysiological situations, both in mice and humans, including acute, allergic or chronic pulmonary inflammation; fibrosis; and lung cancer. Genetic or pharmacologic interventions targeting the ATX/LPA axis have proved beneficial for modelled disease management in animal models, establishing the ATX/LPA axis as a possible therapeutic target.

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INTRODUCTION

Autotaxin (ATX, ENPP2) is a secreted glycoprotein widely present in biological fluids, including the blood[1,2]. It is a member of the exo/ecto-nucleotide/pyrophosphatase/phosphodiesterase family of ectoenzymes (NPPs) that hydrolyse phosphodiester bonds of various nucleotides and derivatives[3]. However, ATX primarily functions as a plasma lysophospholipase D, and it is largely responsible for the bulk of lysophosphatidic acid (LPA)

production in the plasma and at inflamed and/or malignant sites[2,4,5]. LPA is a phospholipid mediator produced in various conditions both in cells and in biological fluids, and it evokes growth-factor-like responses, including cell growth, survival, differentiation and motility, in almost all cell types[2,4]. The large variety of LPA effector functions is attributed to at least six G-protein coupled LPA receptors (LPARs) with overlapping specificities and widespread distribution[6,7]. Finally, a group of transmembrane lipid-phosphate phosphatases have been suggested to act as negative regulators of LPA metabolism[8,9]. Beyond the well-established role of the ATX/LPA axis in carcinogenesis[10,11], high levels of ATX expression have been observed in non-malignant, inflamed tissues, suggesting a possible involvement of ATX in chronic inflammatory disorders[12,13]. Given the role of the ATX/LPA axis in human disease, a large number of ATX inhibitors and LPAR antagonists are being developed[14-17] in pursuit of a compound with likely therapeutic potential. The reviews cited above summarise the current knowledge on ATX, LPA and LPA receptors and their therapeutic relevance and targeting. In this review, we focus exclusively on the role of the ATX/LPA axis in pulmonary pathophysiology, analysing extensively the effects of ATX/LPA on pulmonary cells and leukocytes *in vitro,* as well as discussing these effects in the context of pulmonary pathophysiology *in vivo* and the pathogenesis of human diseases.

THE ATX/LPA AXIS IN THE HEALTHY LUNG

The gene encoding ATX consists of 27 exons and, through alternative splicing, gives rise to five protein isoforms, designated α-ε, that differ by the presence or absence of sequences encoded by exons 12, 19 and 21[18,19]. All isoforms are catalytically active, and the polybasic insertion in ATXα has been suggested to confer specific binding to cell surface heparin sulphate (HS) proteoglycans[20]. In the absence of proteomic data, ATX mRNA expression analysis indicates that ATXγ is brain specific and that ATXβ is the more abundant isoform, exhibiting a broad tissue distribution that includes the lungs[18-21]. *In situ* hybridisation localised ATX mRNA expression to the basal cells of normal human bronchial epithelium[22], and ATX can be detected in the bronchoalveolar lavage fluid (BALF) of healthy humans (unpublished data). Accordingly, immunohistochemical studies have indicated constitutive ATX expression predominantly in the mouse bronchial epithelium that could also be detected in the BALF of healthy mice[23]. Moreover, with genome-wide linkage analysis coupled with expression profiling, ATX was identified as a candidate gene involved in the control of pulmonary functions (dead space volume, V_D; total lung capacity, TLC; lung compliance, C_L; and diffusing capacity for CO, D_{CO})[24].

Because ATX is a constitutively active enzyme, the biological outcome of ATX's enzymatic action - largely LPA production and signalling - will depend on its expression levels, the local availability of its substrates, and the abundance and activity of the different LPA receptors in the microenvironment.

Lysophosphatidylcholine (LPC), the main substrate of ATX, is a highly abundant bioactive lysoglycerophospholipid present at high concentrations (100-200 μmol/L) in the circulation[25,26] and is predominantly associated with albumin and lipoproteins[12]. LPC can also be detected in the BALF of healthy mice (< 1 μmol/L, unpublished data), whereas phosphatidylcholine (PC; mostly 16:0), one of the main precursors of LPC, is a major constituent of the surfactant, which is a macromolecular complex composed primarily of lipids (90%) and surfactant proteins (SPs A-D) and is largely responsible for maintaining minimal surface tension within the alveolar surfaces[27]. It remains unknown if BALF LPC is synthesised from surfactant (or membrane) PC, if it diffuses from the circulation or if it is transported with albumin. Therefore, and given its abundance, LPC levels are not a limiting factor in ATX's enzymatic activity and LPA production, although it is unknown whether LPC's associations with other molecules (*e.g.*, carrier proteins) are masking the bioavailability of LPC as an ATX substrate.

A 50% reduction or a 100% increase in systemic (and BALF) ATX/LPA levels in genetically modified mice[28,29] does not result in any appreciable effect on gross lung pathology[23], although further pulmonary functional studies are needed. Conditional deletion of ATX from the bronchial epithelium results in significantly reduced (but not completely abrogated) levels of BALF ATX, which do not, however, affect lung development and gross pathophysiology in healthy, non-stimulated mice[23]. Therefore, fluctuations in ATX levels in the lung seem to be well tolerated under normal, healthy conditions.

LPA can also be detected in the BALF of healthy human and mouse lungs[23,30,31], the bulk of which is most likely synthesised from the enzymatic action of ATX on BALF (and most likely membrane) LPC.

LPA receptors are widely expressed throughout the body, and the lungs are no exception. All pulmonary cell types have been reported to express different LPARs, as summarised in Table 1. Compiled data analysis from multiple published works, including Northern analyses, real-time PCR and microarray data, suggest a slightly different expression profile in mouse (LPAR3 > LPAR1, LPAR2, LPAR5; no LPAR4) and human (LPAR3 > LPAR1; no LPAR2, 4, 5) lungs[6]. Therefore, different LPA receptors are expressed in the lung tissue of healthy mice, although detecting their relative abundance in different cell types will have to wait for the emergence of specific antibodies and/or conditional KO mice. Interestingly, LPAR1 has been found to be dually sequestered in caveolin-1 and clathrin subcompartments of plasmalemmal fractions in porcine cerebral microvascular endothelial cells[32], and LPAR1 has been reported to heterodimerise with CD97, an adhesion-linked GPCR[33]. LPAR1 has also been reported to cluster with CD14, the LPS co-receptor, upon treatment of MEL12 pulmonary epithelial cells with LPS,

Table 1 Expression of lysophosphatidic acid receptor in pulmonary cell types and leukocytes

Cell type	LPAR1	LPAR2	LPAR3	LPAR4	LPAR5	LPAR6	Ref.
NHBEs	+++	++	+++	-	+	+	[41]
NHBEs	+++	++	+++	-	++	+	[59]
NHBEs	++	++	++	-	NA	NA	[43]
HBE	++	++	NA	NA	NA	NA	[56]
HBE (BEAS-2B)	++	++	++	+	++	+	[59]
HBE (HBEpCs)	+++	+	++	+	+	NA	[209]
HBE (HBEpCs)	++	++	++	NA	NA	NA	[46]
HBE (HBEpCs)	++	++	++	NA	NA	NA	[49]
HBE (HBEpCs)	++	++	++	NA	NA	NA	[68]
Primary mouse tracheal EpCs	++	+++	++	++	-	NA	[57]
NHBE cells	++	++	++	-	NA	NA	[43]
A549 alveolar epithelial carcinoma	++	NA	NA	NA	NA	NA	[40]
A549 alveolar epithelial carcinoma	+++	+	-	-	NA	NA	[74]
NCI-H522 lung epithelial carcinoma	-	++	+	-	NA	NA	[74]
RLCNR rat lung adenocarcinoma	NA	NA	-	NA	++	NA	[283]
Primary mouse lung fibroblasts	+++	+	+	+	++	NA	[31]
human fetal lung fibroblasts MRC5	+++	++	++	+	+	NA	[226]
Mouse embryonic fibroblasts	+++	++	+	+++	+	NA	[101]
NHLFs CCL-151	+++	+	++	+	+	++	[81]
NHLFs	++	++	NA	NA	NA	NA	[42]
Primary mouse lung endothelial	++	+	-	+++	+	NA	[31]
HPAECs	+	++	+	+	+	+++	[106]
HMVECs	+	++	+	+	+	+++	[106]
HEV ECs	NA	NA	NA	++	NA	++	[116]
Human eosinophils	+	-	+	NA	NA	NA	[148]
Primary mouse neutrophils	-	+++	-	-	+	NA	[31]
Primary mouse alveolar macrophages	-	+	+	+	+	NA	[31]
Human alveolar macrophages	+	++	++	NA	NA	NA	[158]
Rat alveolar macrophages	++	+	-	NA	NA	NA	[158]
Human monocytes	+	-	-	NA	NA	NA	[176]
Mouse DCs	++	++	++	NA	NA	NA	[58]
Human immature DCs	+	+	+	NA	NA	NA	[172]
Human mature DCs	+	+	+	NA	NA	NA	[172]
Human immature DCs	-	+	-	NA	NA	NA	[174]
Human mature DCs	-	+	-	NA	NA	NA	[174]
Mouse immature DCs	++	+	+++	++	+++	NA	[175]
Mouse mature DCs	+++	+	+	++	++	NA	[175]
Jurkat T cells	+	+++	-	NA	NA	NA	[178]
Jurkat T cells	+	+	+	NA	NA	NA	[295]
Human CD4 T cells	+	+++	NA	NA	NA	NA	[177]
Human CD4 T cells	+	+++	-	NA	NA	NA	[176]
Human CD8 T cells	-	-	-	NA	NA	NA	[176]
Mouse CD4 T cells	+	+	+	+	+	NA	[31]
Mouse CD8 T cells	+	+++	+	+	++	NA	[31]
Human B lymphocytes	-	+	+	NA	NA	NA	[176]
Human platelets	+	NA	+	NA	NA	NA	[296]
Human platelets	+	+	+	NA	NA	NA	[295]
Human platelets	+	+	+	+	+	NA	[297]
Human mast cells	+	+	+	+	NA	NA	[199]
Human mast cells	+	+	+	-	NA	NA	[200]
Human mast cells	-	++	+	-	+++	++	[201]
Lung resident mesenchymal stem cells	+++	+	+	NA	NA	NA	[298]

+++: Strong expression; ++: Moderate expression; +: Low expression; -: No expression; NA: Not available; LPAR: Lysophosphatidic acid receptor; NHBEs: Normal human bronchial epithelial; HBE: Human bronchial epithelial; HBEpCs: Human bronchial epithelial cells; NHLFs: Normal human lung fibroblasts; ECs: Endothelial cells; HPAECs: Human pulmonary arterial ECs; HMVECs: Human pulmonary microvascular ECs; HMVECs: Human pulmonary microvascular ECs; HEVs: High endothelial venules; DCs: Dendritic cells.

an interaction abolished upon the disruption of lipid rafts[34]. Further studies are needed to explore possible homo- and hetero-dimerisation of LPARs and the effect of their possible association with other GPCRs within lipid rafts.

Complete genetic deletion of LPAR1-5 does not result in any gross pathological signs in the lungs of non-stimulated mice[6], with the exception of the development of pulmonary hypertension in aged LPAR1 and 2 double KO mice[35], which is consistent with the proven role of ATX/LPA in vascular development[6,28,36-38] and the effects of LPA on endothelial and smooth muscle cells

physiology (see below).

ATX has been shown to be necessary for embryonic development, as complete genetic deletion results in aberrant vascular and neuronal development leading to embryonic lethality[6,28,36-38]. However, preliminary studies with inducible complete genetic deletion of ATX in adult mice or long-term potent pharmacological ATX inhibition indicate no gross pathological signs (unpublished data). Moreover, and according to published reports, fluctuations in ATX/LPA levels and the abrogation of LPA receptor signalling are well tolerated in the lungs, with the exception of aged mice. Therefore, the ATX/LPA axis does not seem to have a major role in the pulmonary physiology of healthy adult mice. However, more studies are needed to determine the effect of lysophospholipid homeostasis on healthy pulmonary functions and vice versa.

LPA EFFECTS ON PULMONARY CELLS

The possible involvement of the ATX/LPA axis in pulmonary pathophysiology has been widely explored in *in vitro* studies, mainly upon LPA treatment of various cell types and lines, primary or established. The main findings, exclusively concerning cells of pulmonary origin, are summarised in Table 2, presented below together with major findings from cells of different origin, and are discussed later in the context of disease pathogenic mechanisms. Notably, all reported effects were observed at LPA concentrations much higher than the physiological LPA levels in the plasma and BALF, and thus, they address possible perturbed functions in pathophysiological situations involving the increased production of LPA at local sites. Finally, differential effects have been observed for different LPA species and in the presence of appropriate carriers (*e.g.*, albumin, gelsolin); however, the mechanisms regulating phospholipid homeostasis and LPA activity are far from being understood.

Epithelial cells

The airway epithelium, the first line of defence of the lungs against inhaled stimuli, plays a protective role through its barrier activity to inhaled insults. Increased epithelial apoptosis in response to injury is believed to play a major role in the initiation of pulmonary pathophysiological disorders, such as fibrosis. Moreover, damaged epithelial cells release a plethora of factors that contribute to repair mechanisms such as growth factors, chemokines, cytokines and prostaglandins[39]. Mouse bronchial epithelial cells have been reported to be the major ATX-producing cell type in the mouse lung[23], and transformed pulmonary epithelial cell lines (A549) have also been reported to express ATX[40]. All pulmonary cell types have been reported (with some controversy) to express at least one LPA receptor, as indicated in Table 1.

LPA signalling through LPAR1 has been reported to induce anchorage-dependent apoptosis in cultured normal human bronchial epithelial cells (NHBEs)[41], and the genetic deletion of LPAR1 or LPAR2 results in a decreased number of TUNEL$^+$ bronchial epithelial cells *in vivo* post-bleomycin (BLM)-mediated lung injury, which specifically targets epithelial cells[41,42]. ATX expression from epithelial transformed A549 cells has been reported to induce their LPA-dependent and LPA-independent migration[40], a crucial step for re-epithelisation and tissue remodelling.

The stimulation of normal human bronchial epithelial cells (HBEpCs) with LPA increases stress fibre formation, reorganises αvβ6 at their ends and leads to increased transforming growth factor-beta (TGF-β) activity *via* LPAR2/Ga_q and RhoA/Rho kinase[43]. TGF-β plays crucial roles in tissue regeneration and cell differentiation, and integrin αvβ6 has been shown previously to bind and activate TGF-β1, a mechanism that was suggested to regulate pulmonary inflammation and fibrosis[44].

LPA induces interleukin-8 (IL-8) expression from HBEpCs, the major chemoattractant of neutrophils, through nuclear factor kappa B (NF-κB)/AP1 and PKCδ/p38/extracellular regulated protein kinases (ERK)/c-Jun N-terminal kinase (JNK) pathways[45,46]. LPA levels and their effects on IL-8 expression have been reported to be regulated intracellularly by acylglycerol kinase (AGK)[47] and extracellularly by lipid phosphatase-1 (LPP1)[48] and ATX[40]. Moreover, the stimulation of IL-8 expression is mediated, at least in part, by LPA-mediated phosphorylation and transactivation of the epidermal growth factor receptor (EGFR)[49]. *In vitro* results were verified *in vivo*, where intratracheal LPA administration to mouse lungs stimulated the expression of MIP-2, the mouse homologue of IL-8, and neutrophil influx[45]. Another pro-inflammatory action of LPA in HBEpCs *in vitro* is the induction of thymic stromal lymphopoietin (TSLP) and chemokine CCL20 through CARMA3-mediated NF-κB activation[50]. TSLP stimulates dendritic cell maturation, leading to antigen presentation to T cells and the initiation of an adaptive immune response to an inhaled antigen[51], whereas CCL20 induces the chemotaxis of T cells and dendritic cells (DCs)[52]. Both cytokines are expressed in the airway of asthmatic patients and contribute to airway inflammation in mouse models of asthma[52,53].

LPA has also been reported to induce IL-13 decoy receptor α2 expression and to inhibit IL-13 signalling in HBEpCs *in vitro*[54]. IL-13 is a Th2 cytokine and a mediator of allergic inflammation and disease, the levels of which were found to be increased in the BALF of asthma patients and ovalbumin-challenged mice[55]. LPA levels were also found to be increased after segmental allergen challenge[56]. Therefore, LPA-induced stimulation of IL-13Rα2 and abrogation of IL-13 signalling would conceivably abrogate the induction of allergic asthma in mice. Heterozygous LPAR2 knockout mice exhibit reduced neutrophil infiltration in the lungs upon treatment with *Schistosoma mansoni* soluble egg antigen (SEA)[57]; however, the adoptive transfer of allergen-pulsed LPAR2$^{-/-}$DCs induce substantially more lung inflammation, pointing to

Table 2 Lysophosphatidic acid effects on different cell types

Cell type	Primary	Species	LPA effect	Receptor	Experiment	Carrier	Ref
Alveolar and bronchial epithelial	Yes	Mouse	Apoptosis	LPAR1	*In vivo*	Biological fluid	[41]
Alveolar and bronchial epithelial	Yes	Mouse	Apoptosis	LPAR2	*In vivo*	Biological fluid	[41,42]
NHBEs	Yes	Human	(anchorage dependent) Apoptosis	LPAR1	*In vitro*	FAF BSA	[41]
NHBEs	Yes	Human	TSLP, CCL20 induction		*In vitro*	No	[50]
NHBEs	Yes	Human	TGF-β activation	LPAR2	*In vitro*	No	[43]
NHBEs	Yes	Human	Induction of Soluble ST2 expression	LPAR1,3	*In vitro*	NA	[61]
Bronchial epithelial (HBEpCs)	Yes	Human	EGFR transactivation, IL-8 secretion		*In vitro*	BSA; BSA	[46,49]
Bronchial epithelial (HBEpCs)	Yes	Human	Induction of IL-13 Ralpha 2	Gαi linked	*In vitro*	No	[54]
Bronchial epithelial (HBEpCs)	Yes	Human	Epithelial barrier integrity enhancement	LPAR1,3	*In vitro*	NA; BSA	[56,209]
Bronchial epithelial (HBEpCs)	Yes	Human	Decrease of EGFR-EGF binding		*In vitro*	BSA	[66]
Bronchial epithelial (HBEpCs)	Yes	Human	COX-2 expression, PGE2 secretion	Gαi linked	*In vitro*	No	[63]
Bronchial epithelial (HBEpCs)	Yes	Human	PDGFR-β transactivation		*In vitro*	BSA	[68]
Bronchial epithelial (HBEpCs)	Yes	Human	c-Met redistribution on the membrane		*In vitro*	No	[70,299]
Bronchial epithelial (BEAS-2B)	No	Human	EGFR transactivation		*In vitro*	BSA	[66]
Bronchial epithelial (BEAS-2B)	No	Human	RANTES inhibition	LPAR1	*In vitro*	FAF BSA	[59]
R3/1 Alveolar epithelial	No	Rat	Inhibition of attachment		*In vitro*	FAF BSA	[41]
Tracheal epithelial	Yes	Mouse	COX-2 expression, PGE2 secretion	LPAR2	*In vitro/vivo*	No	[57]
H292 lung cancer epithelial	No	Human	Decrease of EGFR-EGF binding		*In vitro*	BSA	[66]
A549 alveolar epithelial carcinoma	No	Human	Decrease of EGFR-EGF binding		*In vitro*	BSA	[66]
A549 alveolar epithelial carcinoma	No	Human	p53 decrease		*In vitro*	FAF BSA	[73]
A549 alveolar epithelial carcinoma	No	Human	Cell migration	LPAR1	*In vitro*	BSA; BSA	[40,74]
NCI-H522 lung epithelial carcinoma	No	Human	Cell motility		*In vitro*	BSA	[74]
Fetal lung fibroblasts (HFL1)	No	Human	Chemotaxis		*In vitro*	Biological fluid	[31]
NLFs CCL151	No	Human	Proliferation, EGFR ectodomain shedding	Gi/o linked	*In vitro*	No	[81]
Fetal lung fibroblasts MRC5	No	Human	proliferation	LPAR1,3	*In vitro*	No	[226]
Fetal lung fibroblasts IMR-90	No	Human	Chemotaxis	LPAR1	*In vitro*		[95]
NLFs	Yes	Human	Differentiation, TGF-β expression and signaling	LPAR2	*In vitro*	No	[42]
Lung fibroblasts	Yes	Mouse	Differentiation, TGF-β expression and signaling	LPAR2	*In vitro*	No	[42]
Lung fibroblasts	Yes	Mouse	Chemotaxis	LPAR1	*In vitro*	FAF BSA	[31]
Lung fibroblasts	Yes	Mouse	Protection from apoptosis	LPAR1	*In vitro*	FAF BSA	[41]
NIH 3T3 fibroblasts	No	Mouse	Protection from apoptosis, proliferation	Gi linked	*In vitro*	FAF BSA	[99]
NIH 3T3 fibroblasts	No	Mouse	Migration, protection from apoptosis, proliferation		*In vitro*	BSA	[100]
Rat1/c-Myc fibroblasts	No	Rat	Protection from apoptosis		*In vitro*	FAF BSA	[99]
Lung endothelial	Yes	Mouse	Vascular leak/extravasation	LPAR1	*In vivo*	Biological fluid	[31]
HPAECs pulmonary endothelial	Yes	Human	Increase of the endothelial layer permeability	LPAR6	*In vitro*	FAF BSA	[106]
BPAE pulmonary artery endothelial	No	Bovine	Migration, chemotaxis		*In vitro*	FAF BSA	[127-129]
Smooth muscle cells	Yes	Rabbit, cat	Contraction		*Ex vivo*	BSA	[140]
HASM airway smooth muscle cells	Yes	Human	Proliferation, stimulation of EGFR signaling		*In vitro*	NA; BSA	[139,141]
HASM airway smooth muscle cells	Yes	Human	Activation of TGF-β		*In vitro*	NA	[143]
Dendritic cells	Yes	Mouse	Inhibition of activation	LPAR2	*In vitro, in vivo*	FAF BSA	[58]
Lung resident mesenchymal stem cells	Yes	Human	Migration	LPAR1	*In vitro*	No	[298]

LPA: Lysophosphatidic acid; NHBEs: Normal bronchial epithelial; HBEpCs: Human bronchial epithelial cells; BEAS-2B: Bronchial epithelial cell line; NLFs: Normal lung fibroblasts; HPAECs: Human pulmonary arterial endothelial cells; LPAR: Lysophosphatidic acid receptor; NA: Not available; TSLP: Thymic stromal lymphopoietin; TGF-β: Transforming growth factor beta; EGFR: Epidermal growth factor receptor; BSA: Bovine serum albumin; FAF: Fatty acid free.

an anti-inflammatory role of LPA/LPAR2[58]. Consistent with a potential anti-inflammatory role of LPA signalling, especially in the context of allergic inflammation, LPA has been found to inhibit the tumor necrosis factor (TNF)/interferon (IFN)-γ-induced production of CCL5/RANTES in an established human bronchial epithelial cell line (BEAS-2B)[59]. RANTES is a chemoattractant for eosinophils, monocytes and T-cells and seems to exacerbate asthma[60]. LPA has also been reported to induce the expression of soluble ST2 (sST2) from HBEpCs, a decoy

receptor of IL-33 that attenuates IL-33 and endotoxin-induced inflammatory responses[61]. The increased expression has also been verified *in vivo*, where the intratracheal administration of LPA increased sST2 levels in BALF[61]. However, the physiological relevance of this finding remains unknown, as the abrogation of LPA signalling *in vivo, via* the genetic deletion of LPAR1 or LPAR2, attenuates LPS-induced responses[34,62]. The controversial anti-inflammatory effects of LPA are exemplified by its stimulation of cyclo-oxygenase-2 (COX-2) expression and prostaglandin E2 (PGE2) release from HBEpCs[63]. *In vivo*, LPAR2$^{+/-}$ mice express less COX-2 and secrete lower amounts of PGE_2 compared to wild-type mice upon allergic stimulation[57]. COX-2 and PGE_2 are commonly considered potent proinflammatory mediators and are involved in several inflammatory diseases. However, in the lungs, as opposed to other parts of the body, PGE2 has a role in limiting the immune-inflammatory response and tissue repair processes[64,65]. The generation of conditional knockouts for the different LPA receptors will be instrumental in dissociating the inflammatory effects of LPA in different cell types *in vivo*. Moreover, the possible differential effects of LPA in stromal and innate immune cells, as compared to adaptive immune cells, should be addressed with appropriate bone marrow transfer experiments.

LPA stimulates PGE2 production and IL-8 secretion in HBEpCs through EGFR phosphorylation and transactivation[49,63] introducing the concept that LPA can also activate or modulate structurally distinct receptors. LPA induces a decrease in EGFR binding of EGF both in HBEpCs and established epithelial cell lines (BEAS-2B) *via* different signalling pathways, including transactivation of EGFR[66,67]. The decrease in EGF binding to its receptor is sustained in normal cells but is rapidly reversed in cancer cell lines (H292, A549)[67]. LPA has been found to transactivate receptor tyrosine kinases (RTKs) other than EGFR, such as platelet-derived growth factor receptor-β (PDGF) in lung epithelial cells. Specifically, in primary cultures of HBEpCs, LPA stimulates tyrosine phosphorylation of PDGFRβ and threonine/tyrosine phosphorylation of the downstream molecule ERK1/2, both through PDGFR kinase, suggesting that PDGFR is transactivated by LPA[68]. As PDGF promotes cell proliferation[69], PDGFR transactivation from LPA may have a proliferative role in airway epithelium.

In general, transactivation of RTKs by GPCRs induces tyrosine phosphorylation of RTKs, thereby resulting in further signal transduction. Similarly, LPA induces tyrosine phosphorylation of EGFR and PDGFRβ in HBEpCs[66,68]. By contrast, LPA has no effect on tyrosine phosphorylation of another RTK, c-Met, which is the receptor of hepatic growth factor (HGF)[70]. Rather, LPA in HBEpCs induces serine phosphorylation of c-Met and its redistribution to the plasma membrane[70]. Moreover, LPA has an inverse effect on c-Met compared to the c-Met ligand, HGF. HGF induces tyrosine phosphorylation of c-Met and its internalisation, whereas LPA reverses these effects and promotes the redistribution of the c-Met-E-cadherin complexes on the plasma membrane through PKCδ[70]. The implication of LPA on c-Met signalling, which is involved in tumour invasion and metastasis, could be of importance in lung cancer (LC) in which c-Met is overexpressed[71]. Conversely, the inhibition of HGF signalling by LPA is another link between LPA and fibrosis, in which HGF has an important protective role[72].

The ATX/LPA axis is widely known to be implicated in cancer[10,11]; however, limited studies have addressed the role of ATX/LPA in LC. A549 lung carcinoma epithelial cells express ATX, which localises to perinuclear and exocytotic vesicle-like bodies and is later secreted in the culture medium[40]. ATX has been reported to induce the migration of A549 cells, most likely through the phosphorylation of PKCδ and of the actin-binding protein cortactin, which could be inhibited by an LPAR1/LPAR3 inhibitor or knock-down of LPAR1[40]. Interestingly, mutant ATX or heat-inactivated cell supernatant were also reported to promote cell migration, proving that ATX-induced cell migration does not depend totally on ATX enzymatic activity and LPA[40]. This LPA-independent pathway of cell migration could be mediated by the binding of ATX to cell-surface receptors, such as integrin β4, which, as shown by co-immunoprecipitations, takes place even after ATX has been heat inactivated[40].

Moreover, in the context of carcinogenic epithelial cells, LPA has been shown to decrease the total cellular content of the tumour suppressor p53 in A549 lung epithelial cells, most likely through proteasomal degradation regulated by PI3K, and simultaneously to decrease the nuclear localisation of p53 and the p53-dependent transcription of cell-cycle arrest genes[73]. Overexpression of LPA receptors 1, 2 or 3 in A549 cells was found to be sufficient to cause a severe reduction in p53-dependent transcription[73]. Moreover, LPA protects A549 cells from genotoxic drugs, which normally cause nuclear accumulation of p53 and apoptosis, by reducing the total levels of p53 and preventing apoptosis[73]. This may explain the protection that LPA offers to carcinoma cells against chemotherapeutic agents. In addition, the fact that p53 inhibition regulates transcription by LPA means that LPA suppresses the G_1-S cell cycle arrest induced by p53 and favours tumour cell growth. The same A549 cell line, which predominantly expresses LPAR1, shows induction of cell motility by LPA[74], whereas its migration and invasion are inhibited *in vitro* by 1-bromo-3(*S*)-hydroxy-4-(palmitoyloxy) butyl-phosphonate (BrP-LPA), a dual-function pan-antagonist of LPA receptors and inhibitor of the lysophospholipase D activity of ATX[75,76]. Expression of LPAR1 seems to be crucial for motility, as another lung epithelial carcinoma cell line with no LPAR1 but significant LPAR2 levels is not susceptible to LPA-induced cell motility[74]. Intriguingly, when A549 cells are injected along extracellular matrix (ECM) in nude mice, the resulting tumours are inhibited, and the number of vessels is decreased by BrP-LPA, an inhibitor of ATX

and LPA signalling[76]. Therefore, LPA regulates many of the aspects of A549 cells that promote carcinogenesis, such as cell cycle promotion, migration, invasion and survival.

Taken together, these results show that LPA seems to be involved in different aspects of pulmonary epithelial pathophysiology, including migration, apoptosis, pro-(and anti-) inflammatory gene expression and transactivation of RTK receptors. However, most of the reported LPA effects in epithelial cells described above were examined *in vitro*, in the absence of cell-to-cell interactions and a functional ECM, which are defining events especially in the case of epithelial cells. Their implication on pulmonary pathophysiological situations *in vivo* is discussed below.

Fibroblasts

Fibroblasts are ubiquitous cells found in connective tissue that provide mechanical strength to tissues by providing a supporting framework of ECM[77]. Moreover, fibroblasts are important sentinel cells in the immune system, which have been suggested to play a critical role in the switch from acute inflammation to adaptive immunity and tissue repair[78]. Fibroblasts from different anatomical regions exhibit characteristic phenotypes that are maintained even after prolonged culture *in vitro*, suggesting that many fibroblasts have an imprinted phenotype. They are extremely versatile cells that display a remarkable capacity to differentiate into other components of the connective tissue, such as cartilage, bone, adipocyte and smooth muscle cells. Their differentiation to myofibroblasts, under mechanical pressure from the ECM and/or profibrotic TGF-β stimulation, regulates connective tissue remodelling by combining the ECM-synthesising features of fibroblasts with cytoskeletal characteristics of contractile smooth muscle cells. Myofibroblasts can have multiple origins, regress and disappear by apoptosis on wound epithelialisation, and may persist in fibrotic situations and cause organ dysfunction, such as pulmonary fibrosis[79]. Moreover, fibroblasts are associated with cancer cells (cancer-associated fibroblasts) at all stages of cancer progression, and their functional contribution to this process is beginning to emerge[80].

Fibroblast proliferation is required in wound healing to fill an open wound. In the lungs, LPA has been reported to promote the proliferation of established human normal lung fibroblasts (CCL151), along with ERK phosphorylation and the transcription of *c-fos*, *HB-EGF* and *amphiregulin* genes[81]. In accordance, cell migration, rounding and proliferation in response to LPA are decreased in embryonic fibroblasts from LPAR1-null mice but are not absent, consistent with redundant signalling from LPA receptors[82]. In support of a role of LPA in lung fibroblast proliferation, LPA stimulates proliferation of synovial fibroblasts mediated through the GPCR, ERK, p38 and Rho kinase signalling pathways[83]. The proliferative effects of LPA in synovial fibroblasts correlates with the development of actin stress fibres[83], in agreement with early reports on LPA effects in Swiss 3T3 fibroblasts, also indicating tyrosine phosphorylation of focal adhesion kinase (FAK), paxillin and p130[84-86]. Moreover, LPA-induced cytoskeleton reorganisation in peritoneal mesothelial cells promotes connective tissue growth factor (CTGF) expression, which in turn promotes NIH3T3 fibroblast proliferation, an effect abolished upon silencing of CTGF or LPAR1 in mesothelial cells[87].

LPA has also been shown to augment human foetal lung and human foreskin fibroblast-mediated contraction of collagen gels[88,89] and to promote the contraction of human myofibroblasts, isolated from the palmar aponeurosis of patients with Dupuytren's disease[90]. LPA-mediated contraction of myofibroblasts has been suggested to involve Rho/Rho kinase inhibition of myosin light chain (MLC) phosphatase (MLCP)[90]. In accordance, LPA has been found to increase the phosphorylation and thereby the inhibition of MLCP in Swiss 3T3 fibroblasts in a ROK-dependent manner[91]. This would therefore suggest that MLCP inhibition could play a role in LPA-induced fibroblast contraction by enhancing the effect of MLC kinase (MLCK), leading to prolonged phosphorylation of MLC and, subsequently, an increase in actin/myosin cross-bridging and contraction. Moreover, LPA has also been shown to induce an MLC-independent pathway of cell contraction through rac, p21-activated kinase 1 (PAK1) and cofilin-1-mediated membrane ruffling[92].

In addition to promoting Rho-dependent cell contraction, LPA is also a potent stimulator of Rac, leading to lamellipodia protrusion and cell migration[74,93]. This involves Gi-dependent activation of PI3K that, in turn, activates the Rac-specific guanine nucleotide-exchange factor Tiam1[93]. Moreover, LPA synergises with EGF, PDGF and β1A integrins in the stimulation of cell migration[94]. In the lung, it has been shown that LPA bound to albumin acts through LPAR1 as a chemoattractant for primary mouse lung fibroblasts. Chemotaxis induced by BALFs isolated from fibrotic mice is attenuated by more than 50% when the fibroblasts are deprived of LPAR1, suggesting that LPA is the predominant fibroblast chemoattractant in the airspaces of BLM-treated mice[31]. In addition, a selective inhibitor for LPAR1, AM966, has been found to inhibit the chemotaxis of IMR-90 human lung fibroblasts mediated by LPA[95]. The LPAR1-LPAR3 inhibitor Ki16425 inhibits chemotaxis of human foetal lung fibroblasts induced by BALF from IPF patients, showing the importance of LPAR1 in LPA-induced fibroblast chemotaxis[31]. *In vivo,* LPAR1 deletion protects against BLM-induced fibrosis in mice, most likely due to the observed decrease in fibroblast accumulation. By contrast, fibroblast collagen production and differentiation to myofibroblasts remain unaffected by LPAR1 deletion[31] but have been suggested to be regulated by LPAR2[42]. In human lung fibroblasts, LPA induces the expression of α-smooth muscle actin (αSMA), fibronectin (FN), collagen I a2 and TGF-β1 protein expression, mediated through LPAR2[42]. In support of an effect of LPA on

the differentiation of lung fibroblasts to myofibroblasts, tumour-secreted LPA promotes the differentiation of peritumor fibroblasts to myofibroblasts and accelerates hepatocellular carcinoma progression[96], as well as the expression of αSMA in human adipose tissue-derived mesenchymal stem cells[97,98].

LPA signalling, specifically through LPAR1, has been found to completely suppress the apoptosis of adherent primary mouse lung fibroblasts induced by serum deprivation[41]. Similar anti-apoptotic effects of LPA have also been reported in NIH3T3, Swiss 3T3 and Rat-1 fibroblasts[99], as well as ATX-transfected NIH3T3 fibroblasts[100], further supporting a role of ATX/LPA in mediating pathologic fibroblast accumulation.

Finally, signs of LPA-induced differential expression in fibroblasts can be extrapolated from an expression profiling study of mouse embryonic fibroblasts (MEFs)[101]. LPA induces the transcription of more than 100 immediate-early genes associated with growth and cell cycle progression, growth regulatory kinases and secreted factors such as chemokines, pro-angiogenic factors and pro-fibrotic factors. Also very prominent is the activation of genes related to cytoskeletal organisation and integrin signalling, which is in line with the role of LPA in cell motility. Simultaneously, LPA-downregulated genes are associated with adhesion[101]. Therefore, LPA seems to have a plethora of actions on fibroblasts concerning cell cycle, growth, motility and inflammation. However, when used at low concentrations, LPA enhances mostly genes associated with cell movement rather than cell growth, indicating that LPA acts predominantly as a motility factor than a growth factor at low concentrations[101]. The effects of LPA on differential gene expression in MEFs have been suggested to be mediated by beta-arrestin 2 in an independent study[102].

Endothelial cells

The endothelium is the thin layer of cells that lines the interior surface of blood vessels and lymphatic vessels forming an interface between circulating blood or lymph in the lumen and the rest of the vessel wall. Endothelial cells (ECs) are involved in many aspects of vascular biology, including barrier function, blood clotting, angiogenesis, vasoconstriction and vasodilation[103]. Although endothelial dysfunction, or the loss of proper endothelial function, is a hallmark for vascular diseases and often regarded as a key early event in the development of atherosclerosis and cardiovascular diseases, chronic lung diseases such as COPD, pulmonary hypertension and interstitial lung diseases have all been reported to have a lung vascular disease component[104,105]. Moreover, the interaction of endothelial cells with immune cells is instrumental for the extravasation of inflammatory cells at local sites.

LPA increases the permeability of an endothelial layer consisting of human pulmonary arterial ECs (HPAECs) to FITC-dextran (in transwell assays) and reduces their electrical impedance[106]. The LPA-induced loss of endothelial barrier function is associated with changes in actin stress fibre formation[106]. Similar observations have been made in human umbilical vein ECs, in which it has been shown that LPA induces endothelial hyperpermeability through the activation of RhoA and Rho kinase, master regulators of signals to the cytoskeleton[107-109]. Therefore, and despite some conflicting reports on the effects of LPA on the permeability of other endothelial systems[109-111], the increased levels of LPA in certain pulmonary pathophysiological conditions could increase endothelial permeability, thereby facilitating the influx of inflammatory cells and soluble factors. Indeed, genetic deletion of LPAR1 or LPAR2 and the resulting abrogation of LPA signalling attenuate the BLM-induced vascular leakage observed during the development of modelled pulmonary inflammation and fibrosis[31,42].

Interestingly, ECs of high endothelial venules (HEVs), largely responsible for lymphocyte extravasation into secondary lymphoid organs, have been reported to express and secrete ATX, and chemokine-activated lymphocytes express receptors with enhanced affinity for ATX[112,113]. Moreover, it has recently been shown that HEV-expressed HS plays a role in chemokine presentation and lymphocyte homing[114], while the polybasic insertion in ATXα has been suggested to confer specific binding to cell surface HS proteoglycans[20]. Impressively, intravenous injection of enzymatically inactive ATX attenuates T-cell homing to lymphoid tissues, suggesting that EC-bound-ATX is an adhesive substrate for homing lymphocytes[112]. In the same homing cascade, LPA locally produced by HEV-ATX stimulates the polarisation, motility and transendothelial migration of naïve T-cells[112,115] or the motility of the ECs[116]. Furthermore, LPA stimulates the expression of IL-1β, IL-8 and MCP-1 from human ECs[117-119] *via* the p38 and JNK pathways[119], and it has been reported that LPA activates ECs to secrete chemokines which, in the presence of LPA, might modulate interactions between the endothelium and circulating monocytes[120]. LPA increases ICAM-1 expression in HUVECs, which might also enhance interactions with leukocytes[121,122] through ROCK2[122] in an NF-κB dependent mechanism[121,122]. A similar effect has also been reported in human aortic ECs, in which LPA stimulates E-selectin and VCAM-1 expression and increases the binding of monocytes and neutrophils[123]. It has also been reported that LPA upregulates the expression of pentraxin-3 (PTX3) in a human artificial EC cell line[124]. PTX3 is an acute-phase protein produced at the sites of infection and inflammation by various tissues and cells, in particular innate immunity cells, in response to proinflammatory signals and Toll-like receptor engagement. In addition, it has recently been reported that PTX3 regulates leukocyte recruitment upon acute lung injury (ALI)[125], while genetic variation in PTX3 is associated with primary graft dysfunction after lung transplantation[126]. Although the endothelial cells of HEVs (and other endothelial systems) differ substantially from pulmonary endothelial cells, similar mechanisms may be in play in the lung, further

regulating inflammatory cell influx.

Beyond the effects of LPA in endothelial permeability and the possible regulation of the influx of inflammatory cells, LPA stimulates the migration of some (but not all) pulmonary EC types through ECM-dependent cytoskeletal rearrangements involving focal adhesions[127-129]. The migration, proliferation and differentiation of ECs are all essential steps in angiogenesis, and LPA has been reported to be involved in all processes, in different EC systems and experimental conditions (reviewed in[130]). Notably, LPA dramatically downregulates the surface expression of CD36, the receptor of thrombospondin-1 and other anti-angiogenic proteins in primary microvascular endothelial cells and promotes angiogenesis *via* a PKD-1-dependent signalling pathway[131]. LPA enhances VEGF-C expression in human endothelial cell lines through LPAR1/3, COX-2, NF-κB and EGFR transactivation-dependent mechanisms[132,133]. Therefore, the ATX/LPA axis might also stimulate angiogenesis, thereby exacerbating carcinogenesis and possibly chronic lung diseases that have been suggested to include a vascular component.

Smooth muscle cells

Smooth muscle cells (SMCs) play an important role in mediating a wide range of physiological processes, such as blood pressure regulation and airway responsiveness. Their principle function is to contract or relax in response to stimuli, and they are capable of major phenotypic changes in response to alterations in local environmental cues[134]. LPA has been suggested to be such a phenotypic modulator of SMCs, and its possible involvement in vascular diseases and atherosclerosis have been extensively reviewed elsewhere[13,135], suggesting that isolated vascular SMCs respond to LPA by proliferating and migrating. The early growth response-1 (Egr-1) transcription factor[136,137], which regulates the transcription of a large variety of genes in SMCs implicated in vascular diseases and fibrotic genes in fibroblasts[138], has been proposed to be central to LPA responses of vascular SMCs.

LPA has been reported to stimulate the proliferation of airway SMCs in marked synergism with EGF[139] and to enhance their contraction in response to serotonin and methacholine[140]. Moreover, LPA upregulates the expression of EGF receptors, increases EGF binding[141,142] and induces integrin αvβ5-mediated TGF-β activation[143], suggesting a possible involvement of LPA in asthma and obstructive lung diseases.

Remarkably, LPA has been suggested to target vascular and oncogenic pathways *via* the receptor for advanced glycation end products (RAGE)[144]. LPA has been reported to bind avidly to RAGE, which is required for LPA effects in vascular SMCs, including Akt signalling, proliferation and migration[144]. RAGE is a member of the immunoglobulin superfamily and has been shown to be a pattern recognition receptor that transduces the effects of multiple ligands, including advanced glycation end products (AGEs), advanced oxidation protein products, S100/calgranulins, high-mobility group box-1 (HMGB1) and amyloid-β peptide[145]. RAGE is highly expressed in the lungs, suggesting a potentially important role in lung homeostasis, and the disruption of RAGE levels has been implicated in the pathogenesis of a variety of pulmonary disorders, including ALI, fibrosis and cancer[145]. The discovery that it can be transactivated by LPA opens up novel research directions on the effects of LPA in the lung.

LPA EFFECTS ON LEUKOCYTES

In addition to the different immunomodulatory effects of ATX/LPA in stromal cells presented above, including the modulation of barrier functions of endothelial cells, vascular remodelling and cytokine secretion from epithelial cells, LPA has been reported to have direct effects on leukocytes. As with every cell in the body, primary alveolar leukocytes all express some LPA receptors (Table 1)[31].

Granulocytes

Eosinophils have a unique contribution in initiating inflammatory and adaptive responses due to their bidirectional interactions with DCs and T cells and to their large panel of secreted cytokines and soluble mediators[146]. They are mainly involved in parasite infections and allergic diseases; however, they have significant contributing roles in a wide range of other diseases[147]. LPA exhibits chemotactic activity towards human peripheral blood eosinophils, shown to express LPAR1,3 mRNA, both *in vitro*[148] and in the lung *in vivo*[149]. Moreover, LPA re-arranges the eosinophil actin cytoskeleton, upregulates the expression of the integrin CD11b on their surface and stimulates Ca^{++} mobilisation and the production of reactive oxygen intermediates[148]. The observed effects of LPA in eosinophils, shown pharmacologically to be mediated through LPAR1/3-$G_{i/o}$, are comparable to those obtained from other well-known chemoattractants such as C5α, PAF, CCL5, CCL11 and CCL13[148], suggesting that LPA is a potent chemoattractant and activator of eosinophils.

Like eosinophils, human peripheral blood neutrophils, the most abundant granulocytes or leukocytes in the blood and the major effectors of acute inflammation[150], respond to LPA by calcium flux and oxidative burst[151]. LPA has also been reported to stimulate neutrophil degranulation[152] and to promote neutrophil chemotaxis both *in vitro*[153] and in the lung *in vivo*[149]. Despite the limited studies and some conflicting reports[154], it seems that LPA might have a role in neutrophilic responses and therefore in acute inflammation and lung injury.

Macrophages

Macrophages (MΦs), the most plastic cells of the haematopoietic system and the predominant resident immune cells in the lungs, have well-established roles in lung homeostasis, tissue repair and immunity[155,156]. Peripheral blood monocytes and/or tissue MΦs in mice, humans and rats all express some of the receptors for LPA; how-

ever, different publications, all based on RT-PCR data, report different expression patterns[31,157-159], while bone marrow-derived MΦs were found to express all 5 major LPARs (unpublished data and[83]). All transformed monocytic cell lines (MM6, RAW, THP-1, J774A.1) have also been reported to express LPA receptors[160-162]. However, a systematic study on LPAR expression during the differentiation of monocyte to MΦs and upon inflammatory activation of primary, resident or immigrating cells is still lacking.

The same is true for ATX, as there are limited reports on ATX expression in monocytes/MΦs. LPS-stimulated transformed monocytic THP-1 cells have been reported to express ATX mRNA[163,164] that is inhibited by pharmacological inhibitors of PKR, JNK and p38 MAPK[164]. More importantly, alveolar MΦs from BLM-challenged fibrotic mice and human IPF patients have been shown with immunocytochemistry to express ATX[23]. Macrophage ATX expression has also been noted in LC patients (unpublished data). Therefore, ATX expression from inflammatory or tumour-associated MΦs would stimulate local LPA production and its plethora of effects.

As far as the effects of LPA on MΦs themselves are concerned, LPA has been shown to protect murine primary peritoneal MΦs from apoptosis induced by serum deprivation, suggested to be mediated through PI3K[165]. By contrast, LPA has no effect on macrophage proliferation[165]. In THP-1 cells, LPA significantly increases reactive oxygen intermediates (ROI) production and prostaglandin E2 release[161]. In RAW264.7 cells, LPA stimulates cell survival and induces monocyte lipid accumulation from oxidised low-density lipoprotein (ox-LDL), suggested to be mediated through PPARγ activation, and CD36 scavenger receptor uptake[166]. LPA in J774A.1 cells also induces ox-LDL uptake[162] and IL-1 expression[167]. In MM6 cells, LPA has been reported to increase cytosolic Ca^{++}, a second messenger of cellular activation that regulates diverse biological processes such as the secretion of cytokines and the expression of proinflammatory genes[160]. Therefore, the limited studies on the effects of LPA in MΦs point to a potential pro-survival and pro-inflammatory role of the ATX/LPA axis, although more studies are needed, especially in primary cells, employing flow cytometry analysis of surface expression markers.

Dendritic cells

Dendritic cells (DCs) are the most potent antigen-presenting cells specialised in the activation of naive T-lymphocytes and the initiation of the immune response and are among the major immunological cells residing in the lungs. LPA (50 μmol/L) has been shown to affect the differentiation of peripheral circulating monocytes to DCs *in vitro*, which, however, have impaired immunological functions[168]. Interestingly, LPC, the precursor of LPA and the substrate of ATX, has also been reported to promote dendritic cell maturation from monocytes, with the ability to stimulate IL-2 and IFN-γ production by allogeneic T lymphocytes[169]. Notably, LPC released from apoptotic cells has also been suggested to be a potent chemotactic signal to MΦs *via* the phagocyte receptor G2A[170,171].

Both mature and immature DCs express LPARs[1-3] but respond differently to LPA[172]. LPA induces calcium flux, actin polymerisation and chemotaxis of immature DCs, whereas LPS-exposed mature DCs are insensitive. However, LPA inhibits, in a PTX-insensitive manner, the secretion of IL-12, and TNF and enhances the secretion of IL-10 from LPS-exposed mature DCs[172]. Other groups have suggested a predominance of LPAR2 in DCs[173,174] and reported that LPA induces IL-6 and IL-8 in maturing DCs[174] but does not have these effects in mature DCs[174]. Moreover, LPA does not exert a dominant effect on the ability of DCs to stimulate Th cell polarisation but does inhibit LPS-induced responses[173]. Similarly, unsaturated LPA species (as opposed to saturated ones) are able to induce the chemotaxis of immature but not LPS-exposed mouse bone marrow-derived DCs *in vitro*, attributed to LPAR3[175]. Finally, $LPAR2^{-/-}$ DCs have been reported to induce the proliferation of co-cultured T cells and their IL-13 secretion, more so than in wild-type DCs, suggesting that LPAR2 in DCs has a suppressive role in the Th2 inflammation and airway response to allergens[58]. Indeed, adoptive transfer of $LPAR2^{-/-}$ DCs pulsed with ovalbumin (OVA) enhances lung inflammation in comparison with OVA-pulsed wild-type DCs[58]. However, a different group reported that heterozygous LPAR2 KO mice are partially protected from allergic inflammation[57].

Taken together, and despite the limited available information and observed discrepancies, LPA seems to have a pro-inflammatory role in immature DCs, promoting inflammatory cytokine secretion and their chemotaxis and maturation, whereas in mature cells, LPA has a potential anti-inflammatory role, which might depend on the allergen.

Lymphocytes

Lymphocytes, the major cellular components of the adaptive immune response, all express some LPARs, as assessed with RT-PCR and in some rare case with western blots, but the results have been somewhat conflicting[176-178]. Moreover, the presence of recently identified LPARs remains to be examined. In one such study, LPAR5 was reported to be highly expressed in gastrointestinal lymphocytes[179]. As with all cells, future FACS studies on primary cells are needed to clarify the constitutive and inducible regulation of LPA receptor expression in T- and B-cell subsets. By contrast, no ATX mRNA expression was detected in splenocytes, thymocytes and $CD8^+$ T-cells, even upon their activation with phorbol myristate acetate (PMA) (unpublished data and[83]), consistent with the expression of other NPP family members in these cells[3]. However, a human transformed pre-B-cell line (Nalm-6) was reported to express and secrete ATX, the effects of which on LPA production were suggested to be counteracted by the simultaneous expression

of LPP1[180].

LPA stimulates Jurkat leukemic T cells, leading to calcium flux and proliferation[181]. Similarly, immortalised human B lymphoblasts respond to LPA with calcium flux, MAPK activation, and immunoglobulin production[182]. However, there is a wide variety of responses to LPA regarding calcium flux depending on the cell line[183].

Jurkat cells also respond to LPA *in vitro* by migrating through a matrigel membrane, an experimental connective tissue-like barrier[184]. As mentioned above, and despite opposing findings[185], LPA produced locally by HEV-ATX has been shown to stimulate the polarisation, motility and transendothelial migration of naïve T-cells[115,116], and ATX/LPA has also been shown to affect endothelial permeability and thus the regulation of lymphocyte influx[112,113]. Similar mechanisms may also exist in B-cells, as LPA has been shown to enhance LFA-mediated adhesion of murine follicular and marginal zone B-cells to ICAM-1 *in vitro,* similar to the effects of CXCL12 and CXCL13 chemokines and PMA[186], suggesting that LPA may be involved in B-cell homing within the spleen.

LPA has also been reported to inhibit the apoptosis induced by antibodies to Fas, CD2 or CD3/CD28 of a human T lymphoblast cell line (Tsup-1), accompanied with the suppression of the apoptotic protein Bax[187]. Similarly, LPA protects B-cell lines (BJAB and I-83) and primary chronic lymphocytic leukaemia cells from apoptosis. By contrast, LPA does not protect normal B-cells from fludarabine- and etoposide-induced apoptosis[188]. LPA protects transformed pre-B cells (Nalm-1) from spontaneous or staurosporine-induced apoptosis[180]. However, indirect pro-apoptotic effects of LPA on T-cells have been reported through the upregulation of Fas in ovarian cancer cells[189,190].

LPA, surprisingly in a PTX-insensitive manner, suppresses IL-2 secretion from anti-CD3/CD28-activated $CD4^+$ T-cells, but not similarly activated $CD8^+$ cells or non-activated $CD4^+$ cells[176], although opposing results on IL-2 expression have been reported in Jurkat cells[181]. By contrast, LPA was reported to enhance PMA-induced IL-13 promoter activity and gene expression in Jurkat and human peripheral blood $CD4^+$ lymphocytes *in vitro*, but only under submaximal conditions and not by itself[178]. Therefore, it seems that LPA might co-stimulate the polarisation to Th2 responses, although both cultured human Th1 and Th2 cells responded to LPA by inducing calcium flux and chemotaxis[191].

Platelets and mast cells

Platelets are the principle effector cells in haemostasis and have additional major functions in inflammation, vascular integrity, and tissue repair. In the lungs, platelets contribute to pulmonary vascular barrier function and are required for defence against pulmonary haemorrhage[192]. Increased coagulation and depressed fibrinolysis, as a consequence of the activation of circulating quiescent platelets, result in diffuse alveolar fibrin deposition, which serves to amplify pulmonary inflammation, while the interaction of platelets with endothelial cells and leukocytes is critical in the pathogenesis of ALI[193]. Moreover, asthma is associated with a procoagulant state in the bronchoalveolar space, further aggravated by impaired local activities of the anticoagulant protein C system and fibrinolysis[194]. ATX has been reported to bind to integrins β1 and β3 on the surface of platelets[195], consistent with the integrin-mediated binding of ATX to lymphocytes[112] and insights from the crystal structure[196]. ATX was found to inhibit fibrinogen-dependent platelet aggregation and enhance their thrombin-induced LPA production[195], whereas systemic genetic overexpression of ATX in mice *in vivo* resulted in bleeding diathesis and attenuation of thrombosis[29]. On the other hand, LPA levels in serum prepared from platelet-rich plasma are 5-10-fold higher than in platelet-poor plasma[197], indicating that activated platelets are a major source of LPA in the circulation. Therefore, the recruitment of circulating ATX to the platelet surface could enhance the local LPA production during clotting, which in turn would exert its numerous effects in adjacent cells. The effects of LPA on platelets, which express the five major LPARs, include shape change, fibronectin matrix assembly, platelet-monocyte co-aggregate formation and synergism with other platelet agonists, such as epinephrine and adenosine diphosphate, and have been reviewed elsewhere[135].

Mast cells, potent effector cells of the innate immune system, are mainly implicated in pro-inflammatory responses to allergens but can also contribute to protection against pathogens[198]. LPA potently induces the proliferation and differentiation of mast cells, which also express LPARs[199-201], providing a synergistic signal with the major mast cell growth factor, stem cell factor (SCF)[199]. LPA strongly enhances the formation of secretory granules and the cell-surface expression of kit[199]. Mast cells primed with IL-4 respond to LPA by the production of chemokines, including macrophage inflammatory protein (MIP)-1b, monocyte chemotactic protein (MCP)-1, and IL-8[200]. Moreover, LPA induces histamine release from rat peritoneal mast cells and mouse skin fragments[202], and the subcutaneous administration of LPA increases plasma exudation in the skin[203]. Thus, LPA may both support reactive mastocytosis (a feature observed in several disease states) and serve as an amplifier of mucosal inflammation, in which mast cell hyperplasia is mediated by a Th2 cytokine-based mechanism.

THE ATX/LPA AXIS IN LUNG PATHOPHYSIOLOGY

Acute lung injury

Acute lung injury (ALI), or mild acute respiratory distress syndrome (ARDS), is a diffuse heterogeneous lung injury characterised by arterial hypoxemia, respiratory failure and low lung compliance, non-cardiogenic pulmonary oedema, and widespread capillary leakage leading to alveolar flooding[204]. Bacterial or viral pneumonia is the most common

cause of ALI and ARDS, but sepsis due to non-pulmonary infections, the aspiration of gastric contents, major trauma with shock and/or mechanical ventilation also commonly precipitate this type of injury[204]. Altered permeability of epithelial and endothelial barriers, inappropriate accumulation of leukocytes and uncontrolled activation of coagulation pathways are among the main pathophysiological concepts in ALI and ARDS[204], and LPA seems to affect all of them.

Elevated ATX/LPA levels have been detected in an animal model of LPS-induced ALI (unpublished data[34]), and the genetic deletion of LPAR1 or LPAR2 has been reported to moderately attenuate inflammation but not the epithelial/vascular leakage induced by LPS[62,205]. However, both pulmonary inflammation and vascular leakage in response to BLM are entirely abrogated in the absence of LPAR1 or LPAR2[31,42]. The partial protection of LPAR1- and LPAR2-null mice and attenuation of inflammation from LPS-induced lung injury are consistent with the observed LPA stimulation of IL-8 secretion from pulmonary epithelial cells *in vitro*[45,46]. IL-8 is the major chemoattractant of neutrophils[206], which in turn predominate LPS-induced inflammatory responses in ALI/ARDS[207]. LPA can also directly induce neutrophil chemotaxis *in vitro*, as well as neutrophil activation and degranulation[153,208]. However, the exogenous administration of LPA to the lungs has been reported to both increase and decrease neutrophilic accumulation and LPS-induced lung injury[149,209], highlighting the importance of assessing endogenous local control mechanisms of LPA production. Conclusive insights are expected to be obtained by the ongoing conditional genetic deletion of ATX and an examination of LPS-induced ALI severity, as well as by the creation of conditional knockouts for LPARs.

The ability of LPA to induce integrin-dependent activation of TGF-β in pulmonary epithelial cells[43] points to another pro-inflammatory role of LPA. TGF-β activation has been reported to disrupt the alveolar epithelial barrier integrity, leading to alveolar flooding[210,211]. Moreover, TGF-β is known to induce the expression of plasminogen activator inhibitor-1 (PAI1), a major inhibitor of fibrinolysis, whereas fibrin deposition is a hallmark of ALI[212]. Therefore, by promoting TGF-β activation in the pulmonary epithelium, LPA could indirectly promote epithelial barrier disruption and inhibit fibrinolysis in an environment of high TGF-β content and in this manner promote lung injury.

Moreover, LPA has been shown to increase the permeability of endothelial systems[106-108], which could facilitate the entry of inflammatory cells in the alveolar space, although there is much controversy on the issue[109-111]. *In vivo*, genetic deletion of LPAR1 or LPAR2 attenuated the BLM-induced vascular leak[31,42], indicating that LPA signalling indeed disrupts vascular endothelial barrier integrity, in turn promoting the infiltration of inflammatory cells and possibly ALI.

Idiopathic pulmonary fibrosis

Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive, fibrotic form of diffuse lung disease that occurs mainly in older adults and is characterised by a progressive worsening of lung functions and a poor prognosis[213,214]. Clinically, IPF is characterised by progressive, exertional dyspnoea and non-productive cough, worsening of pulmonary function and radiographically evident interstitial infiltrates (honeycombing). Histologically, IPF is associated with the appearance of Usual Interstitial Pneumonitis (UIP), which is characterised by patchy subpleural and/or paraseptal interstitial fibrosis alternating with areas of mild inflammation and normal lung. The hallmark of IPF/UIP is the presence of hyperplastic reparative epithelium overlying distinctive fibroblastic foci that deposit exuberant ECM components, leading to thickening of alveolar septa and the collapse of normal lung architecture[213]. Although the aetiology and pathogenesis of IPF remain poorly understood, a number of conditions and risk factors are weakly associated with the disease: cigarette smoking, occupational/environmental factors, gastro-oesophageal reflux, latent viral infections, and age/gender/genetic predisposition[213]. To study the pathogenetic mechanisms that govern disease activation and perpetuation, a number of animal models have been developed that reproduce the clinical features of IPF, although it remains unclear if they truly replicate the chronic and progressive forms of the disease[215]. Among them, the BLM model is the most widely used and best characterised model and is responsible, together with the site-specific and/or temporal overexpression or ablation of candidate pathogenic genes, for most of our knowledge concerning IPF pathogenesis[215,216]. In this context, current research suggests that the mechanisms driving IPF reflect abnormal, deregulated wound healing in response to repetitive pulmonary epithelial damage, involving increased vascular permeability of the endothelium, extravascular coagulation, TGF-β activation, fibroblast persistence and differentiation to myofibroblasts, leading to exaggerated collagen deposition[214,217].

Deregulated phospholipid homeostasis seems to be an integral component of pulmonary fibrosis pathogenesis. Early studies have reported the altered composition of phospholipids in IPF[218-221], whereas experiments with genetically modified mice implicate proinflammatory mediators, such as prostaglandins, thromboxanes and leukotrienes, in the pathogenesis of BLM-induced pulmonary fibrosis[222-224]. These mediators derive from arachidonic acid, which is the product of phosphatidylcholine (PC) hydrolysis by PLA2[225], with concurrent release of LPC, the substrate of ATX.

ATX shows strong staining intensity within the alveolar epithelium immediately adjacent to fibroblastic foci and lower intensity in interstitial MΦs, fibroblast-like cells and in areas of bronchiolar metaplasia in IPF lung samples[23]. A similar expression profile was also demonstrated in fibrotic non-specific interstitial pneumonia (fNSIP) samples, a histopathological pattern sharing common pathologic features with UIP. By contrast, ATX has minimal expression within the inflammatory components of

cellular NSIP lung samples and in areas of loose connective tissue, called Masson bodies, representing the pathogenetic hallmark of cryptogenic organising pneumonia (COP). The two latter pathologies represent two forms of idiopathic interstitial pneumonias (IIPs) with favourable prognoses and excellent treatment response to corticosteroids, indicating that ATX upregulation is closely associated with more progressive and irreversible forms of pulmonary fibrosis, such as IPF/UIP and fNSIP[23]. In the mouse BLM-induced fibrotic lung, high constitutive ATX expression has been noted in the bronchial epithelium, the major source of ATX in the lungs, as well as in inflammatory alveolar MΦs, resulting in increased ATX BALF levels[23,31]. However, the increase in ATX BALF closely follows BALF total protein levels, suggesting that additional ATX could be extravasated from the circulation. As a consequence of the increased ATX levels, LPA levels are also increased in the BALFs of fibrotic mouse and human lungs[23,31], even at early time points[41]. Pharmacological inhibition of ATX results in the attenuation of LPA levels, confirming that ATX is solely responsible for LPA production in the lung[23].

Conditional genetic deletion of ATX from the majority of bronchial epithelial cells or MΦs results in the attenuation of BLM-induced pulmonary inflammation and fibrosis, as indicated by the improved lung architecture, reduced inflammation and collagen production, highlighting the importance of local pulmonary ATX production and verifying ATX as a major contributor to disease pathogenesis[23]. Likewise, genetic deletion of either LPAR1 or LPAR2 also results in attenuation of the BLM-induced disease[31,42], suggesting that the ATX/LPA axis is a candidate for therapeutic interventions. Indeed, pharmacological inhibition of either ATX or LPAR1 results in attenuation of BLM-induced disease symptoms[23,95], and pharmacological inhibition of LPAR1/3 alleviates radiation-induced pulmonary fibrosis[226]. However, the relative contribution of each receptor to pulmonary inflammation and fibrosis will have to be evaluated in head-to-head studies, with animals of the same genetic background and in comparison with littermate controls.

The apoptosis of alveolar epithelial cells is found both in the lungs of IPF patients and in animal models of the disease, correlating with the increased expression of "death-inducing" TNF/TNF receptor family members and various apoptotic markers[227]. Furthermore, induction of epithelial apoptosis is sufficient to initiate a fibrotic response in animal models[228], whereas genetic or pharmacological blocking of apoptotic signals can prevent a BLM-induced fibrotic response[229]. These observations have contributed significantly to the prevailing hypothesis that the mechanisms driving IPF reflect abnormal, deregulated wound healing in response to multiple sites of on-going alveolar epithelial injury[214]. LPAR1- and LPAR2-null mice, which are both protected from the development of the BLM-induced disease, exhibit significantly reduced numbers of TUNEL$^+$ epithelial cells[31,42], suggesting that LPA promotes epithelial apoptosis upon lung injury. In agreement, LPA signalling through LPAR1 was reported to induce anchorage-dependent apoptosis in cultured normal human bronchial epithelial cells[41], although the intracellular mechanisms and the role of cell-to-cell and cell-to-ECM contacts need to be defined. Interestingly, BLM-induced, epithelial cells undergoing apoptosis *in vivo* express TNF[230], which has been suggested to stimulate ATX expression[83,231]. Therefore, stimulation of apoptosis in epithelial cells from BLM in mice or unidentified insults in humans can stimulate TNF expression, which in turn promotes ATX expression and the local production of LPA, perpetuating the damage. Moreover, the critical involvement of the cytoskeleton in epithelial apoptosis and BLM-induced disease[232], as well as the reported ability of LPA to rearrange the cytoskeleton of bronchial epithelial cells[43], argue for an additional intracellular pathway mediating the effects of LPA in epithelial cells.

Increased fibroblast accumulation, due to increased fibroblast proliferation and migration and to decreased fibroblast apoptosis, is a hallmark of IPF pathogenesis[233]. Consistent with a role for ATX/LPA in disease pathogenesis, LPA stimulates lamellipodia protrusion and fibroblast cell migration[74,93]. Moreover, LPA acts as a chemoattractant for primary mouse lung fibroblasts, and genetic deletion of LPAR1 attenuates lung fibroblast chemotaxis induced by BALF from fibrotic mice, proving that LPA is the predominant fibroblast chemoattractant in the airspaces of BLM-treated mice[31]. In humans, BALFs from IPF patients with elevated LPA levels induce fibroblast chemoattraction, in contrast with BALF from healthy individuals, an effect abrogated by an LPAR1 inhibitor[31], while LPA also induces the chemotaxis of human lung fibroblasts *in vitro*[95]. Thus, the chemotactic effect of LPA on fibroblasts could be a determining factor for the development of IPF. Moreover, the ability of LPA to promote the proliferation of lung fibroblasts *in vitro*[81,226] and its ability to completely suppress the apoptosis of adherent primary mouse lung fibroblasts[41] or non-lung fibroblasts[99] further indicate that LPA promotes pathologic fibroblast accumulation in the airspaces. Chronic fibrosis is characterised by the persistence of myofibroblasts, which promote tissue remodelling by expressing fibrogenic and extracellular mediators[234]. LPA, through LPAR2, induces the differentiation of human lung fibroblasts to myofibroblasts by inducing αSMA, FN, collagen I a2 and TGF-β1 protein expression[42], whereas LPA-mediated differentiation of peritumor fibroblasts to myofibroblasts in the liver has also been shown[96]. Therefore, the ATX/LPA axis is also implicated in pulmonary fibrosis through fibroblast recruitment, proliferation and differentiation into myofibroblasts.

It is well accepted that inflammatory mediators play a role both in the initiation and progression of pulmonary fibrosis, despite the failure of anti-inflammatory treatments[214]. A prominent effect of LPA in epithelial cells is the production of IL-8[45,46], a potent neutrophil chemoat-

tractant, suggesting that the observed increased levels of LPA in the early phases on BLM-induced lung injury[41] can promote the initiation of the inflammatory cascade. LPA stimulation of ECs also results in the upregulation of inflammatory mediators, such as IL-1β, IL-8 and MCP-1[117-119], and adhesion molecules, such as ICAM-1, VCAM-1 and E-selectin, that might enhance interactions with leukocytes, facilitating their extravasation[119,121,123]. Moreover, EC-bound-ATX has been shown to be an adhesive substrate for homing lymphocytes[112], whereas LPA stimulates the polarisation, motility and transendothelial migration of naïve T-cells[112,115]. Genetic deletion of LPAR1 or LPAR2 results in the attenuation of vascular leakage upon BLM treatment[31,42], whereas LPA increases the permeability of an endothelial layer consisting of human pulmonary arterial ECs[106]. Therefore, LPA can also affect the inflammatory component of pulmonary fibrosis through the stimulation of cytokine production, through the modulation of the endothelial barrier and through the promotion of inflammatory cells extravasation.

TGF-β is the major pro-fibrotic factor in several organs. In the lung, it is produced from a wide variety of cells, including alveolar MΦs and neutrophils, activated epithelial and endothelial cells, fibroblasts and myofibroblasts[235]. When activated, TGF-β is a pleiotropic growth factor with chemotactic and proliferative properties, inducing macrophage and fibroblast recruitment and the secretion of a number of pro-inflammatory and pro-fibrotic cytokines[235]. TGF-β levels are increased in the BALFs of fibrotic lungs in both BLM-challenged mice and human IPF patients[236], whereas adenoviral delivery of TGF-β is sufficient to promote fibrosis in the absence of inflammation[237,238]. LPA induces TGF-β expression in pulmonary fibroblasts *in vitro*[42], and stimulation with LPA leads to increased TGF-β activity through integrin αvβ6 in bronchial epithelial cells[43] and through integrin αvβ5 in smooth muscle cells[143]. Therefore, LPA can indirectly promote pro-fibrotic responses by potentiating TGF-β activation and possibly expression.

HGF is a growth factor for epithelial and endothelial cells. It is activated only in injured tissues, the lungs included, and its expression increases post-lung injury[239]. In patients with IPF, HGF levels in BALF are increased compared to healthy subjects; however, fibroblasts from IPF patients express less HGF and have a decreased activation capability of pro-HGF[239]. In fact, the exogenous administration of HGF alleviates fibrosis and induces lung repair[72,239]. The protective effect of HGF has been suggested to be mediated through the restriction of myofibroblast recruitment, the promotion of proliferation and the survival of lung epithelial and endothelial cells[72] and the induction of myofibroblast apoptosis[239]. Compared to HGF, LPA has the opposite effects on c-Met, the basic receptor of HGF, in normal human bronchial epithelial cells: LPA induces c-Met serine phosphorylation and its redistribution to the cell membrane, and it is also capable of abrogating HGF-induced c-Met activation[70]. Therefore, LPA could indirectly have profibrotic consequences through the inhibition of HGF signalling.

Conclusively, the ATX/LPA axis may promote pulmonary fibrosis in several ways, such as the induction of vascular leakage, fibroblast migration, fibroblast differentiation, epithelial cell apoptosis, inflammatory cell influx, TGF-β signalling and HGF signalling suppression; however, anti-inflammatory effects have also been reported.

Asthma

Asthma, a common chronic inflammatory lung disease that leads to airflow obstruction[53], has an onset usually early in life in association with sensitisation to common aeroallergens. Asthma can be divided into phases, such as acute or chronic, severe or not severe, with the pathophysiology of the disease differing among the distinct phases[240]. Acute asthma, or allergic asthma, is triggered by allergens that lead to IgE reactions and the activation of mast cells located beneath the mucosa of the lower airways of the respiratory tract. Mast cells release their granules, thereby stimulating mucus production and airway smooth muscle contraction, which constricts the airway, causing the characteristic asthmatic wheezing. Furthermore, a Th2 lymphocyte response is also a predominant feature of acute asthma and, together with mast cells, lead to cytokine secretion, thus mediating inflammation in the form of eosinophil and other leukocyte recruitment[240,241]. Eosinophils, key players in asthma, further promote inflammation and enhance airway hyperresponsiveness and airflow obstruction[240]. Chronic asthma is a result of the inflammation obtained from acute asthma. The acquired chronic inflammation leads to mucosal epithelium hypersensitivity so that even simple environmental agents such as smoke can evoke asthma attacks. In persistent asthma, the lung epithelium is injured, and airway smooth muscle becomes hypertrophic[242]; both these tissues secrete inflammatory mediators[240]. Further changes in asthmatic lungs include mucus gland hypertrophy, collagen deposition and thickening of the basal lamina, increased matrix deposition and thickening throughout the airway walls[242,243], all of which contribute to airflow obstruction.

The involvement of the ATX/LPA axis in asthma was first established when it was shown that allergen exposure leads to an increase in the LPA levels in the BALF of humans[56] and a mouse model of asthma[57]. Similar results were obtained more recently: allergen challenge in asthmatic patients leads to an increase in LPA levels, accompanied by an increase in BALF ATX levels[244]. In a triple allergen asthmatic mouse model, ATX expression is localised in terminal bronchial epithelial cells and alveolar MΦs[244]. Transgenic mice overexpressing ATX in the liver, which leads to systemic 100%-200% increases in the ATX levels in the serum[29], develop increased pulmonary inflammation and higher levels of IL-4 and IL-5 in lung homogenates and BALFs upon triple allergen challenge[244]. Accordingly, heterozygous ATX full knockout mice, with a 50% reduction of systemic/serum ATX and

LPA levels[28], exhibit reduced inflammation and IL-4/5 levels upon triple allergen challenge[244], indicating a major role for ATX/LPA in asthma pathogenesis. Pharmacological treatment with an ATX inhibitor attenuates disease development[244], establishing ATX as a potential drug target in the treatment of asthma.

Allergic inflammation in LPAR2$^{-/-}$ knockout mice is also attenuated[244]. Surprisingly, heterozygous LPAR1 or LPAR2 knockout mice have also been reported to develop distinct aberrant responses upon *Schistosoma mansoni* egg sensitisation and challenge[57]. However, and in a different mouse asthma model, using systemic immunisation with ovalbumin and alum, LPAR2$^{-/-}$ knockout mice showed greater allergic sensitisation, higher eosinophilia and Th2 inflammation[58]. These observed discrepancies could be due to the different allergens utilised and/or the genetic backgrounds of the experimental and control groups of mice, urging further comparative, genetic or pharmacological studies.

As mentioned above, LPA has been reported to have mainly pro-inflammatory effects in pulmonary cell types and pulmonary inflammation, but anti-inflammatory effects have also been reported. In support of a pro-inflammatory role of the ATX/LPA axis in the development of asthma, LPA stimulates IL-8 secretion from HBEpCs *in vitro*[45,46] and IL-8 levels are elevated in mouse lungs after intratracheal LPA administration[45]. IL-8 is a major chemoattractant for neutrophils and eosinophils[245], and its levels are elevated in the BALFs of asthma patients[245]. In accordance, intratracheal administration of LPA stimulates neutrophil infiltration in mice[57] and both eosinophil and neutrophil infiltration in guinea pigs[149], although no significant association was found between LPA and eosinophil recruitment in humans[56]. However, LPA has been shown to act chemotactically on human eosinophils *in vitro*[148]. Eosinophils have a primary role in allergic inflammation, releasing upon activation cytokines and leukotrienes and their highly inflammatory granule components injuring the airway and causing persistent inflammation[240]. The mast cell is another important cell type in the initiation and perpetuation of allergic inflammation through the release of leukotrienes and cytokines[240], whereas the release of histamine from their granules activates the endothelium and increases blood vessel permeability. LPA potently induces mast cell proliferation and differentiation, formation of their secretory granules[199], chemokine production[200] and histamine release[202]. Therefore, the LPA-mediated chemoattraction of eosinophils and mast cell activation, the impairment of EC barriers[107-109] and the enhancement of EC-leukocyte interactions[120-123] can all be deteriorating factors in the pathogenesis of asthma.

LPA has also been reported to stimulate lymphocyte homing[112,116] and TSLP and CCL20 secretion from HBEpCs *in vitro*[50]. TSLP is produced from the airway epithelium upon TLR activation and acts on dendritic cell motility and activation[53,246], leading to the Th2 polarisation[53] that is crucial in asthma. CCL20 contributes to airway inflammation in mouse models of asthma[52] and is known to act on the recruitment of DCs and T cells on the airway and other mucosal surfaces[52,247,248]. Therefore, these results suggest that ATX/LPA could also regulate adaptive immune responses in asthma.

Several other findings implicate LPA in other aspects of asthma. LPA is capable of promoting the proliferation of airway smooth muscle cells[139] and enhancing the contraction of airway smooth muscle[140], which could contribute to smooth muscle mass increase and airway hypercontractility, respectively, both of which are key features in asthma. Furthermore, by activating TGF-β in airway smooth muscle cells in an integrin αvβ5-dependent way[143], LPA can again promote asthma, as TGF-β induces airway remodelling, smooth muscle thickening, ECM deposition and mucous production in an asthma model[249]. Moreover, TGF-β is required for the differentiation of the Th17 cells that are linked to asthma[250,251] and Th9[252], a Th2 subtype that participates in the inflammatory and the remodelling aspect of airway allergy[53]. TGF-β also drives the differentiation of fibroblasts to myofibroblasts, leading to the thickening of epithelial basal lamina and airway walls[241] that follows chronic and severe asthma[253]. The observed activation of TGF-β by LPA in the airway epithelium[44] and smooth muscle[143] could, thus, affect many aspects of the disease.

The reported exacerbated allergic (OVA) inflammation in LPAR2$^{-/-}$ knockout mice is correlated with an LPA-LPAR2 suppressive effect on dendritic cell activation, the subsequent T cell proliferation and Th2 allergen response[58]. In support of this proposed anti-inflammatory role of LPA/LPAR2 in asthma development, LPA was found to inhibit the TNF-α/IFN-γ stimulated CCL5/RANTES[241,254-256] production from HBEpCs *in vitro*[59], whereas RANTES was found to increase in BALFs of asthmatic patients[257], and the severity of asthma has been associated with a polymorphism in the promoter of the *RANTES* gene[258]. LPA has also been shown to induce the expression of the decoy receptors for IL-13 and IL-33, IL-13R2 and soluble ST2 in HBEpCs *in vitro*[54,61]. Notably and concerning IL-13, LPA has been shown to have an opposite effect on T cells at submaximal activation, where it actually stimulates its gene expression[178]. Airway IL-13, found at higher levels in BALF of asthma patients[55], is implicated in asthma in many ways: it promotes survival and migration of eosinophils, activation of MΦs, mast cell maturation, permeability of airway epithelial cells, airway hyperresponsiveness, mucus production and transformation of airway fibroblasts to myofibroblasts leading to collagen deposition[53,241,259,260]. In allergy, IL-13 is also necessary for the isotype switching of B cells from IgM to IgE, whereas it restricts the differentiation of Th17 cells, a subtype also implicated in asthma[261], although these processes take place in secondary lymphoid tissues[53]. IL-33 is another cytokine expressed by the airway epithelium upon PRR activation that activates lung DCs and helps sustain the Th2 response in asthma[53,262]. Therefore, LPA could attenuate asthmatic inflammation by suppressing

IL-13 and IL-33 signalling. Finally, LPA has been shown to stimulate PGE2 expression from HBEpCs *in vitro*[63], whereas epithelial cells from asthmatic patients cultured *in vitro* were shown to overproduce PGE2 compared to normal epithelium[263]. In the lung, PGE2 is bronchoprotective and suppressive of inflammation in asthma[64,262,264], although some indications that it promotes Th2 differentiation do exist[265,266]. Therefore, the induction of PGE2 by LPA could have complex consequences, mostly protective of the pathology.

Lung cancer

Lung cancer (LC) is the most prevalent form of malignancy and the major cause of cancer-related deaths worldwide. The prognosis for patients with LC remains dismal, with a five-year survival rate of 14%. Current therapeutic options are limited to classical adjuvant therapy (a combination of radiation and chemotherapy with cytotoxic drugs) following surgery[267,268]. Histopathologically, LC can be divided into two major histopathological groups: non-small-cell LC (NSCLC)[269] and small-cell LC (SCLC)[270]. Approximately 80% of LC are NSCLC, and they are subdivided into adenocarcinomas, squamous cell, bronchoalveolar, and large-cell carcinomas[271]. SCLC, which accounts for close to 18% of all lung tumours, and large-cell neuroendocrine carcinomas both have a very high proliferative and metastatic potential. SCLC and NSCLC show major differences in histopathologic characteristics that can be explained by the distinct patterns of genetic lesions found in both tumour classes[272]. The molecular origins of LC lie in complex interactions between the environment (tobacco smoke and/or inhaled carcinogens) and host genetic susceptibility. Lung tumourigenesis appears to conform to a multistep model in which 1) self sufficiency of growth signals; 2) insensitivity to anti-growth signals; 3) evasion of apoptosis; 4) increased replication potential; and 5) angiogenesis and metastasis dictate the tumorigenic process[273].

ATX was originally isolated as an autocrine motility stimulation factor from the supernatant of highly metastatic melanoma cells[274]. Since then, increased ATX expression has been detected in a large variety of cancers such as neuroblastoma, hepatocellular carcinoma, breast cancer, renal cell carcinoma, glioblastoma, thyroid carcinoma, B-cell lymphomas, and non-small cell LC (reviewed in[10]). Moreover, the plethora of actions of LPA are concordant with many of the 'hallmarks of cancer', including proliferation, the evasion of apoptosis, angiogenesis and metastasis[10,11]. LPA levels are significantly increased in malignant effusions, and its receptors are aberrantly expressed in several human cancers[10]. Notably, overexpression of ATX and/or LPARs in the mammary gland was recently reported to result in spontaneous breast cancer in aged mice[275], whereas the genetic deletion of LPAR2 attenuates tumour formation in an experimental model of colitis-associated cancer[276].

Despite the established role of the ATX/LPA axis in carcinogenesis, little is known about its involvement in LC. Meta-analysis of datasets from seven different microarray studies on NSCLC for differentially expressed genes related to survival time identified ATX as one of the 64 genes predicting potential beneficial effects of aggressive therapy of stage I LC patients[277]. ATX mRNA is overexpressed in poorly differentiated carcinomas in NSCLC patients[22], while the conditional deletion of ATX from the lung attenuates chemically induced or k-ras-driven lung carcinogenesis (unpublished data and[278]), suggesting a major contribution of ATX in lung carcinogenesis, although the related mechanisms are still not fully investigated.

In support of these data, BrP-LPA, a dual function pan-antagonist of LPA receptors and an ATX inhibitor[75,279], inhibited tumour growth and angiogenesis in a engineered three-dimensional tumour xenograft NSCLC model composed of A549 lung carcinoma epithelial cells encapsulated in 3-D ECM injected in nude mice[76]. Similarly, genetic or pharmacologic neutralisation of LPAR1 attenuates mesenchymal stem cell-dependent angiogenesis and tumour growth in a murine xenograft model of A549 human adenocarcinoma[280]. In accordance, ATX was independently reported to induce the migration of A549 cells[40], and, in the same cells, LPA was shown to decrease the nuclear localisation and cellular abundance of p53[73].

The expression of LPA receptors seems to vary in different lung tumour cells (Table 1 and related references[74,281,282]), possibly regulated by methylation[283,284], and LPAR1 mutations were reported in a rat model of lung carcinogenesis correlating with advanced staging[285]. Again, conditional knockout mice for the different LPARs are needed to examine their individual contribution to lung carcinogenesis.

Genetic deficiency of ATX and its associated effects on LPA production results in embryonic lethality due to aberrant circulation and neural tube closure[28,36,38], suggesting a major effect of ATX/LPA in angiogenesis. Supporting *in vitro* studies have suggested that LPA stimulates the expression of a large number of angiogenic genes in different endothelial and cancer cells and regulates endothelial proliferation and migration (see above; reviewed in[130]). The conditional deletion of ATX and/or LPA receptors in different endothelial systems is expected to dissect the involvement of ATX/LPA to angiogenesis, an obligatory component of carcinogenesis.

EGFR is overexpressed and functions aberrantly in various human cancers, including NSCLC in which it enhances cancer invasion and brain metastasis[286], and has been extensively used as a target of therapeutic approaches[287]. LPA has been shown to induce squamous cell carcinoma cell proliferation and motility[288], ovarian cancer cell invasion[289] and prostate cancer cell proliferation[290] through EGFR transactivation, introducing the concept that LPA can amplify carcinogenic growth signals. Likewise, LPA has been reported to affect c-Met signalling[70,291] which was found to be overexpressed and activated in NSCLC cell lines and tumour tissues[292]. In

addition, c-Met has been suggested to provide resistance to EGFR targeted therapies[293]. Therefore, suggested adjuvant therapies targeting simultaneously both EGFR and c-Met for the treatment of NSCLC[294] could be possibly enhanced by inhibitors of the ATX/LPA axis.

CONCLUSION

ATX is a secreted glycoprotein widely present in biological fluids, including BALFs, largely responsible for the bulk of LPA production in the plasma and at inflamed and/or malignant sites. In turn, LPA evokes growth-factor-like responses in almost all cell types, including pulmonary cells, through its abundant GPCR receptors. ATX/LPA have an established role in inflammation and malignant transformation, and increased ATX and/or LPA levels in the lung have been detected in both humans with pulmonary diseases such as acute lung injury, IPF, asthma, and LC and/or the corresponding animal models. Genetic or pharmacologic interventions targeting the ATX/LPA axis have proven to be beneficial for disease management in animal models, establishing the ATX/LPA axis as a possible therapeutic target.

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