

EDITORIAL

Clinical and Experimental Allergy

Putting fat on the fire? Lysophospholipid mediators in bronchoalveolar lavage fluid after allergen challenge

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The emergence of the structurally related, biologically active lysophospholipids, lysophosphatidic acid (LPA) and sphingosine 1-phosphate (S1P) as mediators in lung disease, including allergy [1, 2], has sparked interest in whether LPA and S1P are present in bronchoalveolar lavage fluid (BALF). The levels of expression of LPA and its potential biological relevance in exacerbation or amelioration of disease is the focus of a paper by Georas et al. [3] in this issue. They demonstrated that LPA is present in BALF of allergy subjects, and that levels increase following allergen challenge. They suggest that LPA has a role to play in the maintenance of epithelial barrier integrity.

LPA and S1P exert multiple effects on immune cell growth, migration and survival, mainly via binding to the 'EDG' family of G-protein-coupled receptors [4]. LPA binds to receptors LPA₁, LPA₂ and LPA₃, and to the structurally distinct receptor LPA₄, while S1P binds to S1P₁, S1P₂, S1P₃, S1P₄ and S1P₅. Each receptor couples to separate intracellular pathways and hence to different biological outcomes. Thus, the EDG receptor expression profile of a cell largely determines its response to LPA or S1P. EDG receptors are widely expressed on airway cells associated with allergy, such as smooth muscle cells [5, 6], primary bronchial epithelial cells [7], neutrophils, eosinophils and mast cells [8–13]. LPA can induce mast cell chemokine production [11] and histamine release [12, 13].

The multiple biological effects of LPA and S1P and widespread expression of EDG receptors in airway cells suggest that changes in the local levels of these mediators in allergy and other lung diseases would have important consequences for factors including barrier integrity, inflammatory cell influx and airway remodelling and obstruction. The development of reliable methods of measurement of LPA and S1P in BALF is therefore of importance for assessing changes in the levels of these

mediators in the lung in airway disease, for example following allergen challenge in allergic subjects.

Georas et al. [3] used liquid chromatography and mass spectroscopy to measure LPA species in human BALF. They demonstrated the presence of LPA species in the lung in allergic subjects. Significantly, they showed that 18 h following allergen challenge, LPA levels increased approximately threefold, with a greater increase for some polyunsaturated species of LPA. S1P is also increased in BALF of asthma patients following allergen challenge [14]. Thus, allergen challenge in allergic patients results in increased levels of both LPA and S1P in the lung.

What are the potential consequences of this? *In vitro* studies have indicated that LPA induces eosinophil and neutrophil migration [8, 10, 15]. Furthermore, animal studies have suggested a role for LPA-induced leucocyte recruitment *in vivo*, in which LPA mediated Rho-dependent migration of eosinophils and neutrophils to the lung [16]. Therefore, one obvious potential consequence of increased LPA levels in the lung would be increased recruitment of leucocytes, including eosinophils and neutrophils. Eosinophils express LPA receptors LPA₂ and LPA₃ [10]. Neutrophils of healthy control subjects express LPA₂, while the neutrophils of pneumonia patients during acute exacerbation additionally express LPA₁ [8]. However, Georas et al. [3] could not observe any significant correlation between BALF levels of LPA and either total leucocyte or eosinophil or neutrophil numbers.

We have observed that LPA induces significantly greater migration of isolated peripheral blood neutrophils from pneumonia patients compared with control subjects, possibly due to expression of LPA₁ [8]. Thus, it would be important to consider the LPA receptor profile of eosinophils and neutrophils pre- and post-allergen challenge, in peripheral blood and in BALF. This would be relevant to the direct effects of raised local levels of LPA on granulocyte migration or on other aspects of their function at local inflammatory sites. In contrast to LPA, S1P levels in BALF correlate with eosinophil recruitment in asthma patients following allergen challenge [14]. S1P, via the S1P₁ receptor, is essential for T lymphocyte migration

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between blood, lymph nodes and tissue [17, 18], implying that the chemoattractant properties of S1P are central to its immunological function. Thus, while the levels of both LPA and S1P increase in the human lung following allergen challenge, their function in leucocyte recruitment varies.

The results of Georas et al. [3] suggest that the significance of increased LPA in the lung following allergen challenge lies in a direction other than its chemoattractant potential. Increased local levels of LPA may have effects on already migrated eosinophils and neutrophils, for example on their degranulation, adhesion or survival. Exogenous LPA induces degranulation of peripheral blood neutrophils via phospholipase D activation [19]. LPA also induces reactive oxygen metabolite production, calcium mobilization and transient actin polymerization in eosinophils [10], resulting in integrin up-regulation and hence increased eosinophil adhesiveness. Also, LPA may still be important in leucocyte recruitment in lung diseases other than allergy, as LPA induces IL-8 production from human bronchial epithelial cells [20] and maturing dendritic cells [21].

Georas et al. [3] did show that the BALF protein content negatively correlated with LPA levels in the absence of allergen challenge, implying that, under these non-inflammatory conditions, LPA promotes the integrity of the epithelium as a barrier. They demonstrated the expression of receptors LPA₁ and LPA₂ on primary airway epithelial cells, and showed that LPA dose-dependently increased resistance in an electrical cell substrate impedance system. However, following allergen challenge, there was only a modest correlation between LPA levels and BALF protein content, suggesting loss of epithelial barrier resistance. The authors suggest that the increase in LPA levels following allergen challenge may be an attempt to overcome this loss in resistance as inflammatory conditions subside. Thus, the effects of LPA in allergy may be anti-inflammatory.

LPA may also influence the pathogenesis of asthma in a number of ways, for example by stimulation of growth of smooth muscle, thereby contributing to airway remodelling and bronchoconstriction. LPA and epidermal growth factor (EGF) induce a Rho-dependent synergistic up-regulation of smooth muscle cell mitogenesis [2, 22], partly due to an LPA-induced increase in EGF receptor [2, 22]. This synergistic promotion of cell growth may be mediated by the synergistic activation of the AP-1 transcription factor by LPA and EGF [23]. LPA also stimulates the contraction of airway smooth muscle [24]. Thus, increased LPA levels in the lung following allergen challenge may enhance airway smooth muscle mitogenesis and contribute to repair in the airways, further adding to its putative repair and homeostasis role. On the other hand, increased LPA may also contribute to bronchospasm during an asthmatic attack or promote long-term airway remodelling. LPA receptor expression and LPA

functional effects on airway smooth muscle will be an important area for further investigation of LPA effects in allergy.

Georas et al. [3] point out that the source of LPA in the late-phase allergic response is currently unclear. Activated platelets are a major source of both LPA and S1P [25], and intravascular platelet activation has recently been implicated in allergic asthma [26]. However, the source of increased LPA following allergen challenge remains to be determined categorically.

It is important that methods for measurement of LPA in BALF continue to be refined and that, in concert, the expression of receptors for LPA on relevant cells is carefully considered in airway diseases. Local levels of LPA as well as receptor expression represent a potentially exciting field for development of novel therapies. These drugs may affect leucocyte recruitment and activation, barrier integrity and airway remodelling and hyper-reactivity and so play an important role in the control of asthma.

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