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Comment on "An extracellular matrix fragment drives epithelial remodeling and airway hyperresponsiveness"

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Increased airway hyperresponsiveness and epithelial remodeling in asthmatic LTA_4H-KO mice may be mediated by CysLTs rather than elevated tripeptide PGP.

The recent article by Patel *et al.* (*1*) characterizes the biological response of an allergic pulmonary inflammation on the background of leukotriene A_4 hydrolase (LTA_4H)–deficient mice. The authors demonstrate that, although many parameters of inflammation were reduced in the absence of LTA_4H, the mice exhibited increased airway resistance, airway remodeling, and goblet cell activation.

Work from this group previously suggested that accumulation of the tripeptide proline-glycine-proline (PGP) in the absence of LTA_4H would lead to enhanced neutrophilic inflammation in models of pulmonary infection (*2*, *3*); in this model of sterile allergic inflammation, however, no such enhanced neutrophilic inflammation could be observed. Our recent publication demonstrated that PGP is actually not chemotactic for neutrophils in LTA_4H knockout (KO) mice nor in human cells and provides an explanation for the observations by Patel *et al.* on neutrophils in this sterile inflammatory setting (*4*). Therefore, our conclusion was that the enhanced neutrophil response previously observed in infection models is likely a compensatory immune response to the infection in the absence of LTA_4H rather than driven by PGP elevation.

Similarly, we believe that the observations on airway hyperresponsiveness and epithelial remodeling in this recent article by Patel *et al.* may not be due to the elevation of the PGP peptide but could actually be caused by the effects of the cysteinyl leukotrienes (CysLTs) in asthma, which are known to induce bronchoconstriction, airway remodeling, goblet cell activation, and mucus production (*5*). It is well known that inhibition of specific enzymes of the arachidonic acid pathway can lead to the elevation of lipid mediators due to pathway shunting. We have observed that absence of LTA_4H can lead to the elevation of CysLTs after stimulation of immune cells in vitro or in inflammatory settings in vivo in LTA_4H KO mice (Fig. 1). It is tempting to speculate that the same may have also occurred in the experimental setting used by Patel *et al.*, particularly because this was observed even clinically with the LTA_4H inhibitor JNJ40929837 by Johnson & Johnson in an asthma clinical trial (*6*).

The authors did not detect elevated CysLTs in house dust mite (HDM)–challenged LTA_4H KO mice (*1*). However, this measurement was performed 24 hours after allergen challenge. CysLTs are released very rapidly after allergen challenge and become less abundant thereafter (*7*). Hence, the elevation of CysLTs may have been missed by just assessing one single timepoint after the challenge.

To demonstrate that the observed effect on airway resistance, airway remodeling, and goblet cell activation is actually due to PGP and not due to the well-known effects of CysLTs, the authors should have demonstrated that the effect can also be seen in LTA_4H KO mice that were treated with the CysLT receptor antagonist Montelukast or more ideally a 5-lipoxygenase inhibitor. The latter would allow for the study of elevated PGP without the simultaneous elevation of CysLTs in LTA_4H KO mice.

Although the authors demonstrate that PGP is elevated in bronchoalveolar lavage fluid of LTA_4H KO mice after allergen challenge (*1*), they never demonstrate that acetyl-PGP is also elevated. In the paper, it is assumed that PGP gets converted into acetyl-PGP in vivo and that this will have similar effects to PGP. However, because this was not directly shown, we question the use of acetyl-PGP as a surrogate to demonstrate the role of PGP. Indeed, taking into account that acetyl-PGP is not degraded by LTA_4H and that knocking out LTA_4H in mice does not influence its abundance (*4*), the observation that this peptide is not detected in the epithelium of HDM-stimulated mice further validates this concern.

Furthermore, we believe that using the L-arginine-threonine-arginine (RTR) peptide as an antagonist to revert the effects of acetyl-PGP is not an appropriate control either because it has been shown previously that RTR also interferes with the action of other immune mediators signaling via CXCR1 and CXCR2 such as IL-8 (interleukin-8) (*8*). Hence, any observed immune modulation using this peptide will be a result of polypharmacology and, without additional controls, cannot be deconvoluted into a single pathway, particularly given the fact that acetyl-PGP is not even detectable in HDM-stimulated LTA_4H KO mice.

The authors suggest that the failures of LTA_4H inhibitors in the clinic may be due to PGP elevation. We believe that this conclusion is unsubstantiated, as to our knowledge, so far, only two LTA_4H inhibitors were tested in clinical efficacy trials in patients. The first, compound JNJ40929837 by Johnson & Johnson, was tested in an asthma trial and showed lack of efficacy (*6*). In this study, it was clinically demonstrated that CysLTs were elevated by LTA_4H inhibition, providing a possible reason for the lack of efficacy because CysLTs are among the main pathological drivers in asthma. The second, compound Acebilustat from Celtaxsys, was tested in cystic fibrosis (CF) and demonstrated a reduction in markers of neutrophilic inflammation (*9*), as well as a clinically meaningful reduction in inflammatory exacerbations in a 48-week phase 2b study (http://www.celtaxsys.com/2018/08/02/celtaxsys-announces-results-of-phase-2-trial-showing-clinically-meaningful-improvement-in-pulmonary-exacerbations-in-cystic-fibrosis-patients/). Although it was previously demonstrated that the levels of PGP are elevated in CF (*10*),

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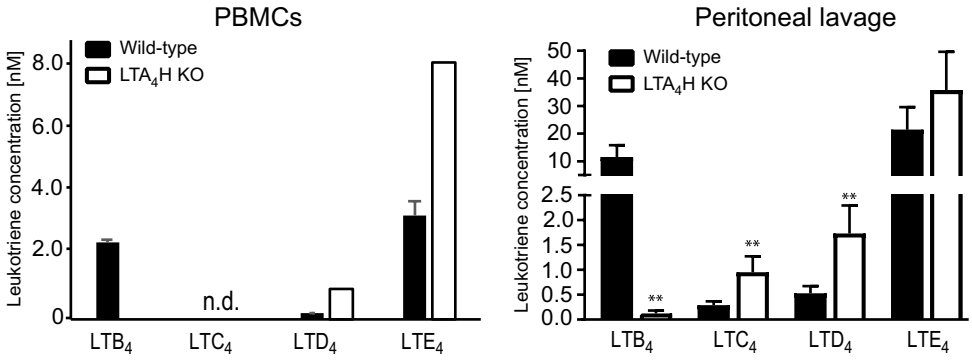

Fig. 1. LTA_4H KO mice generate elevated levels of cysteinyl leukotrienes during inflammation. (**A**) Peripheral blood mononuclear cells (PBMCs) of C57/BL6 wild-type and LTA_4H KO littermate mice were stimulated for 30 min with ionophore A23187, and generation of leukotrienes was determined by liquid chromatography tandem mass spectrometry (LC-MS/MS). PBMCs of a pool of five mice per strain were analyzed. Average ± SEM are shown. (**B**) Acute sterile peritonitis was induced by intraperitoneal administration of zymosan A particles into C57/BL6 wild-type and LTA_4H KO littermate mice. Four hours after the induction of the inflammatory process, lavages were collected and analyzed for content of leukotrienes by LC-MS/MS. Five animals per group were analyzed, and data are depicted as average ± SEM. $^{**}P < 0.01$ using the two-tailed Welch's *t* test comparing wild-type and LTA_4H KO mice. n.d., not detected; LTB_4, leukotriene B_4; LTC_4, leukotriene C_4; LTD_4, leukotriene D_4; LTE_4, leukotriene E_4.

treatment of patients with CF with LTA_4H inhibitors did obviously result in anti-inflammatory effects, indicating that, clinically, there is no relevant effect of PGP elevation as well.

In summary, we believe that the data presented by Patel *et al.* do not unequivocally demonstrate a causative role for PGP in airway hyperresponsiveness and epithelial remodeling. The observed effects may be alternatively explained by the elevation of cysteinyl leukotrienes that are known to cause such effects in airways and can be increased in LTA_4H KO mice. A direct effect of PGP on these lung parameters has not been shown, but instead, acetyl-PGP was used as a surrogate. Acetyl-PGP was not shown to be elevated in HDM-challenged LTA_4H KO mice and is very different from PGP in terms of its biological activity as demonstrated by us and others in the past (*4*).

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Competing interests: We disclose that all authors are current or former employees of and hold company stocks or stock options with Novartis Pharma AG. T.A.R. is a co-author on a published patent on the discovery of LTA_4H inhibitors (US20180170887A.1).

Submitted 17 September 2018
Accepted 29 May 2019
Published 19 June 2019
10.1126/scitranslmed.aav4538

Citation: C. A. Penno, N. Wack, C. Laguerre, F. Hasler, S. Numao, T. A. Röhn, Comment on "An extracellular matrix fragment drives epithelial remodeling and airway hyperresponsiveness". *Sci. Transl. Med.* **11**, eaav4538 (2019).

Science Translational Medicine

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Sci Transl Med **11**, eaav4538.
DOI: 10.1126/scitranslmed.aav4538