

Could relative abundance of airway lipoxins be the clue to restore corticosteroid sensitivity in severe asthma?

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Severe asthma is not a frequent condition because its prevalence lies within 5% to 10% of the general asthma population, or even lower.¹ However, there are substantial direct and indirect costs associated with the treatment of severe asthma due to extensive use of specialized medical care, frequent hospitalization, and missed school/workdays.^{2,3} Severe asthma requires treatment with either high-dose inhaled corticosteroids or systemic corticosteroids.² Despite escalated treatment, severe asthma may still remain poorly controlled or even uncontrolled in some individuals.²

The roots of severe asthma are presently not completely understood. Research may partly be convoluted by patient heterogeneity because the current definition of severe asthma is phenotype-based, and reliable biomarkers to stratify patients are still being sought after. In some patients, asthma may be aggravated by poor adherence to corticosteroid therapy.⁴ In other, therapy-compliant patients it was documented that airway inflammation persists even under treatment with corticosteroids. This was shown by cytokine and cellular composition of airway secretions, which is qualitatively and quantitatively different from that in well-controlled asthma. Various mechanisms explaining persistent airway inflammation and corticosteroid insensitivity have been proposed, including abnormal profiles of lipid mediators, such as overabundance of proinflammatory leukotrienes and deficiency of anti-inflammatory lipoxins. Yet, the understanding of severe asthma pathogenesis lags far behind our appreciation of the impact that this condition exerts on patients and the health care system, and this incomplete knowledge fuels further research on this subject.

The current issue of the *Journal* presents an article from a European collaboration led by Gagliardo.⁵ The authors quantified airway levels of lipoxin (LX) A₄ and leukotriene (LT) B₄ in

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children with severe asthma, and attempted to uncover the interactions between the corticosteroid signaling pathway and LXA₄. Previous studies have already shown that systemic and airway LXA₄ levels can be low in individuals with asthma. However, those studies focused on adult patients, whereas Gagliardo et al for the first time tested LXA₄ levels in induced sputum from children with severe asthma.

Studies with pediatric patients are interesting for adult allergists and respirologists from the perspective that severity of childhood asthma appears to be a strong determinant of asthma severity in the adulthood.⁶ In this regard, better control of childhood asthma may help decrease the burden of adult asthma in a particular individual. An essential step toward better asthma control is establishment of proper therapy adherence, which, as mentioned above, is often inadequate in patients with severe asthma. An advantage of the study by Gagliardo et al is that they ensured proper treatment adherence of study patients by verifying inhalation techniques and assessing basal plasma cortisol levels. This should rule out improper treatment adherence as a potential study confounder.

The study was well done in most aspects. The authors obtained induced sputum samples to quantify the levels of LXA₄ and LTB₄. This permitted assessment of airway abundance of LXA₄ relative to that of LTB₄. Gagliardo et al did not observe in severe asthma a true deficiency of airway LXA₄. Rather, children with severe asthma presented with low abundance of this lipoxin relative to the levels of proinflammatory LTB₄. In contrast, both absolute levels of LXA₄ and its abundance relative to LTB₄ were more favorable in children with well-controlled asthma. Furthermore, in children with severe asthma, lower airway LXA₄ levels were associated with more frequent asthma exacerbations during the study's follow up. Therefore, low relative abundance of airway LXA₄ appears to be associated with more severe and labile asthma.

Furthermore, this study quantified the number of induced sputum cells expressing the LXA₄ receptor (formyl peptide receptor 2), FRP2/ALX. The number of cells positive for FRP2/ALX was decreased in severe asthma, and this could further aggravate the relative LXA₄ deficiency.

For experiments on LXA₄/corticosteroid interactions, Gagliardo et al used primary cells, which are valuable because they represent a better model than immortalized cell lines to explore clinically relevant phenomena. The authors demonstrated that supplementation of exogenous LXA₄ potentiates DNA binding of glucocorticoid receptor in corticosteroid-treated primary cells.

But the study also has some shortcomings. Thus, the expression of FRP2/ALX in induced sputum cells was assessed by immunostaining. Had the authors complemented immunostaining

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with quantitative PCR analysis of FRP2/ALX mRNA expression, they could have gained insights into the mechanism of decreased percentage of FRP2/ALX positive cells. The FRP2/ALX receptor as a protein appears to be translated only when a specific promoter region of its gene is activated,⁷ and this cannot be evaluated by immunostaining. Another limitation is that quantification of LXA₄ was done by Gagliardo et al using an ELISA. Ideally, these quantifications should be performed using liquid chromatography/mass spectrometry to warrant higher specificity of lipoxin detection.

Some of these shortcomings stem from utilization of primary cells. With all the advantages of primary cells as the model for disease-pertinent processes, their use bears its own limitations, including lower cell numbers, extensive culture-to-culture variability, and high associated costs, just to name a few. Overall, despite the limitations, Gagliardo et al's effort was impressive to complement the LXA₄ and FRP2/ALX quantifications in induced sputum with some mechanistic studies to uncover the significance of LXA₄ for corticosteroid signaling.

What causes low relative LXA₄ abundance in severe asthma reported by Gagliardo et al? A recent study⁸ may have uncovered the mechanisms behind this abnormality. It was documented that LXA₄ synthesis can be decreased by lipid peroxidation products.⁸ The latter are generated under oxidative stress, which is likely to be present in severe asthma.

Given the low relative LXA₄ abundance, it is logical to ask whether augmentation of airway lipoxins levels would restore corticosteroid sensitivity in individuals with severe asthma. The study by Gagliardo et al provides some support for this assumption by demonstrating that DNA binding of glucocorticoid receptor is enhanced by exogenous LXA₄. It is reasonable to assume that increased DNA binding will lead to higher transcription of corticosteroid-responsive anti-inflammatory genes. Still, future studies will need to directly demonstrate whether cell responses to corticosteroids are truly potentiated in the presence of LXA₄, such as by testing the expression of corticosteroid-responsive genes. In addition to increased gene transcription, corticosteroids exert posttranscriptional and transcription-independent (also called "nongenomic") effects.^{9,10} We cannot extrapolate from DNA-binding experiments

how these corticosteroid effects will be modulated by LXA₄, and this prompts further research.

Should lipoxins be tested as a corticosteroid-potentiating therapy for severe asthma, they may not need to be supplied directly. As mentioned above, lipid peroxidation is a modifier of lipoxin abundance,⁸ and diminishing oxidative stress may beneficially modulate the lipoxin levels. Furthermore, lipoxin biosynthesis can be amplified by pharmacological manipulation of lipid metabolism.⁸ In theory, both these approaches may be useful to augment the lipoxins indirectly. Therefore, more research needs to be done to assess the usefulness and practicality of direct lipoxin supplementation and/or indirect manipulation of lipoxin synthesis to restore corticosteroid sensitivity and reign over malevolent airway inflammation in severe asthma.

REFERENCES

1. Hekking PP, Wener RR, Amelink M, Zwinderman AH, Bouvy ML, Bel EH. The prevalence of severe refractory asthma. *J Allergy Clin Immunol* 2015;135:896-902.
2. Chung KF, Wenzel SE, Brozek JL, Bush A, Castro M, Sterk PJ, et al. International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. *Eur Respir J* 2014;43:343-73.
3. Fitzpatrick AM. Severe asthma in children: lessons learned and future directions. *J Allergy Clin Immunol Pract* 2016;4:11-9.
4. Gamble J, Stevenson M, McClean E, Heaney LG. The prevalence of nonadherence in difficult asthma. *Am J Respir Crit Care Med* 2009;180:817-22.
5. Gagliardo R, Gras D, La Grutta S, Chanez P, Di Sano C, Albano GD, et al. Airway lipoxinA4/FRP2-ALXR levels in severe childhood asthma. *J Allergy Clin Immunol* 2016;137:XXX-XXX.
6. Tai A, Tran H, Roberts M, Clarke N, Gibson AM, Vidmar S, et al. Outcomes of childhood asthma to the age of 50 years. *J Allergy Clin Immunol* 2014;133:1572-8.e3.
7. Waechter V, Schmid M, Herova M, Weber A, Gunther V, Marti-Jaun J, et al. Characterization of the promoter and the transcriptional regulation of the lipoxin A4 receptor (FPR2/ALX) gene in human monocytes and macrophages. *J Immunol* 2012;188:1856-67.
8. Ono E, Dutilleul S, Kazani S, Wechsler ME, Yang J, Hammock BD, et al. Lipoxin generation is related to soluble epoxide hydrolase activity in severe asthma. *Am J Respir Crit Care Med* 2014;190:886-97.
9. Dauletbaev N, Eklove D, Mawji N, Iskandar M, Di Marco S, Gallouzi IE, et al. Down-regulation of cytokine-induced interleukin-8 requires inhibition of p38 mitogen-activated protein kinase (MAPK) via MAPK phosphatase 1-dependent and -independent mechanisms. *J Biol Chem* 2011;286:15998-6007.
10. Ayroldi E, Cannarile L, Migliorati G, Nocentini G, Delfino DV, Riccardi C. Mechanisms of the anti-inflammatory effects of glucocorticoids: genomic and nongenomic interference with MAPK signaling pathways. *FASEB J* 2012;26:4805-20.