Airway lipoxin A_4/formyl peptide receptor 2–lipoxin receptor levels in pediatric patients with severe asthma

Rosalia Gagliardo, PhD,[a] Delphine Gras, PhD,[b] Stefania La Grutta, MD,[a] Pascal Chanez, MD, PhD,[b] Caterina Di Sano, PhD,[a] Giusy D. Albano, PhD,[a] Isabelle Vachier, PhD,[c] Angela M. Montalbano, PhD,[a] Giulia Anzalone, PhD,[a] Anna Bonanno, PhD,[a] Loredana Riccobono, PhD,[a] Mark Gjomarkaj, MD,[a] and Mirella Profita, PhD[a] *Palermo, Italy, and Marseille and Montpellier, France*

Background: Lipoxins are biologically active eicosanoids with anti-inflammatory properties. Lipoxin A_4 (LXA_4) signaling blocks asthmatic responses in human and experimental model systems. There is evidence that patients with respiratory diseases, including severe asthma (SA), display defective generation of lipoxin signals despite glucocorticoid therapy.
Objective: We investigated airway levels of formyl peptide receptor 2–lipoxin receptor (FPR2/ALXR), LXA_4, and its counterregulatory compound, leukotriene B_4 (LTB_4), in patients with childhood asthma. We addressed the potential interplay of the LXA_4-FPR2/ALXR axis and glucocorticoids in the resolution of inflammation.
Methods: We examined LXA_4 and LTB_4 concentrations in induced sputum supernatants from children with intermittent asthma (IA), children with SA, and healthy control (HC) children. In addition, we investigated FPR2/ALXR expression in induced sputum cells obtained from the study groups. Finally, we evaluated *in vitro* the molecular interaction between LXA_4 and glucocorticoid receptor–based mechanisms.
Results: We found that children with SA have decreased LXA_4 concentrations in induced sputum supernatants in comparison with children with IA. In contrast to decreases in LXA_4 concentrations, LTB_4 concentrations were increased in children with asthma independent of severity. LXA_4 concentrations negatively correlated with LTB_4 concentrations and with exacerbation numbers in children with SA. FPR2/ALXR expression was reduced in induced sputum cells of children with SA compared with that seen in HC subjects and children with IA. Finally, we describe *in vitro* the existence of crosstalk between LXA_4 and glucocorticoid receptor at the cytosolic level mediated by G protein–coupled FPR2/ALXR in peripheral blood granulocytes isolated from HC subjects, children with IA, and children with SA.
Conclusion: Our findings provide evidence for defective LXA_4 generation and FPR2/ALXR expression that, associated with increased LTB_4, might be involved in a reduction in the ability of inhaled corticosteroids to impair control of airway inflammation in children with SA. (J Allergy Clin Immunol 2016;137:1796-806.)

Key words: *Childhood asthma, inflammation/resolution, induced sputum, lipoxin A_4, formyl peptide receptor 2–lipoxin receptor, glucocorticosteroids*

Bronchial asthma is defined as a chronic inflammatory disease of the airways linked to disease activity.[1] Although airway inflammation, structural changes of the bronchi, and resolution of inflammation are important features of asthma, little is known about the mechanisms responsible for disease severity in children.[2-4]

The natural resolution of inflammation is an active host response driven in part by decreases in levels of proinflammatory mediators.[5] The promotion of resolution is now recognized as a process, with early signaling pathways engaging biosynthetic circuits for the later formation of counterregulatory mediators as result of a "switch" of eicosanoid class from prostaglandin and leukotriene production to resolvin formation.[5] Lead members of this class of proresolving mediators,[6] lipoxins, are lipoxygenase interaction products derived from arachidonic acid as a result of cellular cooperation[7] with well-described anti-inflammatory and proresolution bioactivities.[8] After tissue injury or inflammation, lipoxins modulate both the innate and adaptive immunity, regulating leukocyte trafficking,[7,9] T-lymphocyte activation,[10] and dendritic cell function.[11]

Lipoxins are produced through cell-cell interactions between leukocytes and resident cells,[12] and they act at specific ALXRs expressed on both leukocytes and airway epithelial cells[13-15] to transduce their anti-inflammatory effects, which include inhibition of the formation and *in vivo* actions of leukotrienes, including the neutrophil chemotactic factor leukotriene B_4 (LTB_4), as well as downregulation of cytokines and mediators of allergic airway inflammation.[16] Lipoxin A_4 (LXA_4) and its synthetic stable analogs attenuate TNF-1α–initiated neutrophil responses and trafficking[17] and downregulate proinflammatory gene expression through inhibition of nuclear factor κB pathways

From [a]the Institute of Biomedicine and Molecular Immunology, Unit of Immunopathology and Pharmacology of the Respiratory System, Italian National Research Council, Palermo; [b]Département des Maladies Respiratoires, AP-HM, Aix Marseille Université; and [c]Med Bio Med, Montpellier.

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Corresponding author: Rosalia Gagliardo, PhD, Istituto di Biomedicina e Immunologia Molecolare, CNR, Via U. La Malfa, 153-90146 Palermo, Italy. E-mail: gagliardo@ibim.cnr.it.

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Abbreviations used

ATS: American Thoracic Society
ERS: European Respiratory Society
FP: Fluticasone propionate
FPR2/ALXR: Formyl peptide receptor 2–lipoxin receptor
GR: Glucocorticoid receptor
GRE: Glucocorticoid response element
HC: Healthy control
IA: Intermittent asthma
ISS: Induced sputum supernatant
LTB4: Leukotriene B_4
LXA_4: Lipoxin A_4
PBG: Peripheral blood granulocyte
p-GR: Phosphorylated glucocorticoid receptor
RT: Room temperature
SA: Severe asthma

in mucosal inflammation.[18] Adults with severe asthma (SA) have decreased peripheral blood and bronchial LXA_4 concentrations,[16,19,20] and low blood concentrations of LXA_4 have been detected in wheezy infants.[21] However, the bioactivity of LXA_4 has not yet been investigated in asthmatic children.

Glucocorticosteroids are the most frequent anti-inflammatory agents used to treat asthma, yet SA in children can be challenging to treat and leads to persistent daily symptoms and exacerbations, contributing to a poor quality of life for the children and their relatives.[22] The reasons for asthma being difficult to treat require detailed investigation. Previously, we demonstrated that children with moderate-to-severe asthma present heterogeneous phenotypes, and although their symptoms are clinically well controlled by inhaled glucocorticosteroid treatment, they are not fully controlled in terms of both bronchial inflammation and disease management.[23,24] Glucocorticosteroids inhibit the synthesis of cytokines and proinflammatory mediators and favor their effects by increasing the expression of specific receptors.[25] In particular, it has been demonstrated that glucocorticosteroids are able to upregulate the formyl peptide receptor 2–lipoxin receptor (FPR2/ALXR),[26] identifying a specific effect of glucocorticosteroids toward the anti-inflammatory receptors. In addition, LXA_4 and its synthetic stable analogs, through G protein–coupled FPR2/ALXR activation signaling, attenuate the induction of proinflammatory gene expression.[8,17,18,27]

In view of the protective actions of lipoxins, the possibility of defective lipoxin counterregulatory signaling in the airways of children with SA can have potentially important pathophysiologic implications for disease severity.

The aim of the present study was to determine whether dysregulated lipoxin biosynthesis and an imbalance in eicosanoid switching during the resolution of inflammation were also present in asthmatic children and to investigate the potential interplay of the LXA_4-FPR2/ALX axis and glucocorticoids in the regulation of airway inflammation.

METHODS

Subjects

Pediatric subjects (age, 6-12 years) were recruited among outpatients attending the Pulmonology/Allergy Clinic of the Italian National Research Council in Palermo. Asthma severity was diagnosed according to European Respiratory Society (ERS)/American Thoracic Society (ATS) guidelines.[28]

Twelve children had intermittent asthma (IA), which was treated with short-acting β_2-agonists as requested during the last 3 months, and 17 children had SA, which was treated with high-dose inhaled corticosteroids (ICSs; ≥500 μg/d fluticasone propionate [FP]) for children less than 12 years of age.[28] The control group consisted of 7 healthy children.

The study was approved by the Institutional Ethics Committee of the Policlinic Hospital of Palermo University and complied with the Helsinki Declaration. Written informed consent was obtained from the parents of the patients enrolled in the study.

Clinical assessment and compliance of patients

Pulmonary function tests were performed, as recommended by the ATS. FEV_1 and forced vital capacity were measured according to ATS guidelines, and the best of 3 technically acceptable and reproducible measurements was retained.[29] Atopic status was assessed based on skin prick test responses[30] to aeroallergens commonly present in the Mediterranean area and total serum IgE measurements. Compliance with treatment was assessed by checking the inhalation technique at each visit. We also measured basal plasma cortisol concentrations at 8 AM by means of electrochemiluminescence twice (visits 1 and 4). Results were expressed in nanomolar concentrations, and adherence to ICSs was considered satisfactory if the cortisol level was less than 100 nmol/L.[31,32] At the beginning of the study, parents were provided with cell phone numbers of physicians who were always on call 24 hours a day for the entire follow-up period to accurately monitor daily symptoms. Parents were instructed to refer to physicians regarding the onset of any symptoms. When symptoms possibly related to exacerbations occurred, on the basis of the physician's evaluation, patients underwent visits on the same or the following day to verify, check, and treat the exacerbations. Moderate and severe exacerbations were defined and differentiated according to the ATS/ERS Task Force on Asthma Control and Exacerbations study.[33] Moderate exacerbations were defined as a 2-day increase in symptoms and signs of asthma requiring an increase in inhaled medications, including ICSs and bronchodilators. Severe exacerbations, which were defined as events requiring urgent action on the part of the patient and physician to prevent a serious outcome, were treated with a short course of oral steroids (1 mg/kg prednisolone for 2-5 days). The number of moderate and severe exacerbations that occurred during the follow-up period was recorded.

Study design

After a 1-month run-in period (started in September), during which FEV_1 values before and after bronchodilation (400 μg of salbutamol) were assessed in eligible children, patients and healthy control (HC) children attended the outpatient clinic (visit 1) and underwent clinical assessment (prebronchodilator and postbronchodilator FEV_1) after 4 (visit 2), 8 (visit 3), and 12 (visit 4) months by the same physician (S.L.G). In patients in whom an upper respiratory tract infection occurred, follow-up visits were postponed 2 weeks. Biomarker levels were measured at visit 1. Total and differential sputum cell counts and LXA_4 and LTB_4 sputum concentrations were measured in 7 HC subjects, 12 children with IA, and 17 children with SA. FPR2/ALX expression was evaluated in group subsets because of limitations in the amount of sputum cells recovered in 5 of 7 HC subjects, 6 of 12 children with IA, and 7 of 17 children with SA.

To better understand the role of LXA_4 in regulation of bronchial inflammation, we evaluated the ability of LXA_4 present in induced sputum supernatants (ISSs) from asthmatic children to direct peripheral blood granulocyte (PBG) migration using a chemotaxis assay. Finally, we investigated the interplay between LXA_4 concentrations and corticosteroids in the resolution of inflammation, assessing the effects of LXA_4 on glucocorticoid receptor (GR)–based mechanisms in PBGs isolated from healthy child donors, children with IA, and children with SA.

Sputum induction and processing

Each subject underwent spirometry before the beginning of the procedure. If FEV_1 was greater than 75% of predicted value at baseline and the child had