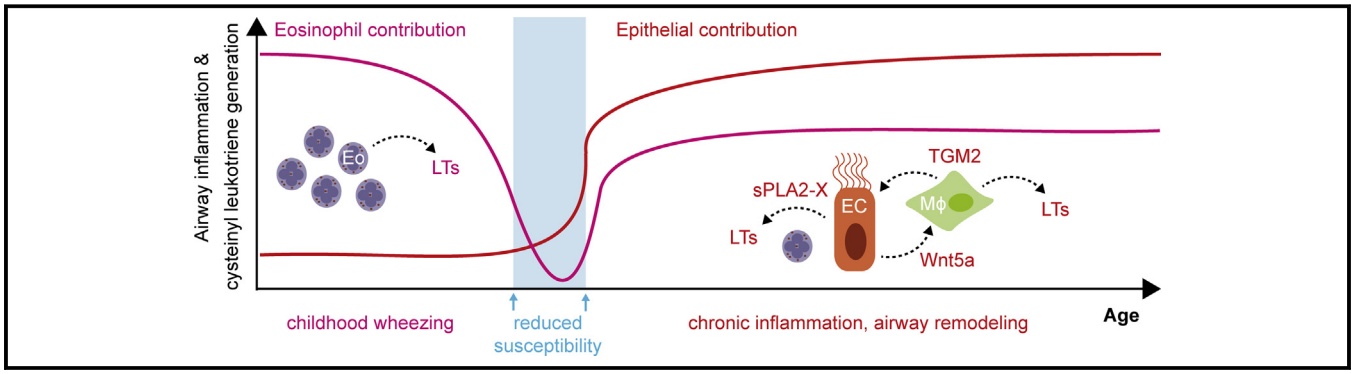
Age dictates a steroid-resistant cascade of Wnt5a, transglutaminase 2, and leukotrienes in inflamed airways

Katharina Dietz, MSc,[a] Marta de los Reyes Jiménez, MSc,[a] Eva S. Gollwitzer, PhD,[b] Adam M. Chaker, MD,[a,c] Ulrich M. Zissler, PhD,[a] Olof P. Rådmark, PhD,[d] Hoeke A. Baarsma, PhD,[e] Melanie Königshoff, MD, PhD,[e] Carsten B. Schmidt-Weber, PhD,[a] Benjamin J. Marsland, PhD,[b] and Julia Esser-von Bieren, PhD[a] *Munich, Germany, Lausanne, Switzerland, and Stockholm, Sweden*

GRAPHICAL ABSTRACT



Background: Airway remodeling is a detrimental and refractory process showing age-dependent clinical manifestations that are mechanistically undefined. The leukotriene (LT) and wingless/integrase (Wnt) pathways have been implicated in remodeling, but age-specific expression profiles and common regulators remained elusive.

Objective: We sought to study the activation of the LT and Wnt pathways during early- or late-onset allergic airway inflammation and to address regulatory mechanisms and clinical relevance in normal human bronchial epithelial cells (NHBEs) and nasal polyp tissues.

Methods: Mice were sensitized with house dust mite (HDM) allergens from days 3, 15, or 60 after birth. Remodeling factors in murine bronchoalveolar lavage fluid, lung tissue, or human nasal polyp tissue were analyzed by means of Western blotting, immunoassays, or histology. Regulatory mechanisms were studied in cytokine/HDM-stimulated NHBEs and macrophages.

Results: Bronchoalveolar lavage fluid LT levels were increased in neonatal and adult but reduced in juvenile HDM-sensitized mice. Lungs of neonatally sensitized mice showed increased 5-lipoxygenase levels, whereas adult mice expressed more group 10 secretory phospholipase A_2, Wnt5a, and transglutaminase 2 (Tgm2). Older mice showed colocalization of Wnt5a and LT enzymes in the epithelium, a pattern also observed in human nasal polyps. IL-4 promoted epithelial Wnt5a secretion, which upregulated macrophage Tgm2 expression, and Tgm2 inhibition

From [a]the Center of Allergy and Environment (ZAUM), Member of the German Center for Lung Research (DZL), Technical University of Munich and Helmholtz Center Munich; [b]the Faculty of Biology and Medicine, University of Lausanne, Service de Pneumologie, Centre Hospitalier Universitaire Vaudois (CHUV), Lausanne; [c]the Department of Otolaryngology, Allergy Section, Klinikum Rechts der Isar, Technische Universität München, Munich; [d]the Division of Physiological Chemistry II, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm; and [e]the Comprehensive Pneumology Center (CPC), Helmholtz Zentrum München, Member of the German Center for Lung Research (DZL) and Ludwig-Maximilians-Universität, University Hospital Grosshadern, Munich.

Supported by the German Center for Lung Research (DZL; 82DZL00302) and by the Else Kröner-Fresenius-Stiftung (grant 2015_A195). C.B.S.-W. received grant support from Allergopharma, PLS Design as well as Zeller AG and received speaker honoraria from Allergopharma. A.M.C. reports giving lectures sponsored by Allergopharma, ALK-Abelló, and GlaxoSmithKline and received grant support or consultant arrangements via Technical University of Munich with Allergopharma, ALK-Abelló, HAL-Allergy, Mundipharma, Lofarma, Zeller AG, and Novartis.

Disclosure of potential conflict of interest: K. Dietz has received grants from the German Center for Lung Research ([DZL]82DZL00302). A. M. Chaker has received consultancy fees from Allergopharma, ALK-Abelló, HAL-Allergy, Mundipharma, and Lofarma; grants from Allergopharma, German Federal Environmental Agency, Zeller AG, Novartis AG, Allergopharma, ALK-Abelló, and GlaxoSmithKline; payment for manuscripts from Bayerisches Ärzteblatt; funds for travel from the European Academy of Allergy and Clinical Immunology (EAACI), DGAKI, and SMI. O. P/ Rådmark is employed by the Karolinska Institute and has received grants from the Swedish Research Council. H. A. Baarsma has received grants from Helmholtz Postdoc Programme (PD-135[2014-2017]) and a European Respiratory Society (ERS) long-term research fellowship (ERS LTRF 79-2012). M. Königshoff has received a grant from a European Research Council Starting Grant (ERC-StG-LS7, grant no. 261302). C. B. Schmidt-Weber has received board membership from the Norwegian Research Council, consultancy fees from LETI Pharma and GLG Consultancy, employment from PLS Design, grants from Allergopharma and PLS Design, and travel expenses paid by EAACI invitations. J. Esser-von Bieren has received grants from Else Kröner-Fresenius-Stiftung (2015_A195) and support for travel from EAACI. The rest of the authors declare that they have no relevant conflict of interests.

Received for publication January 19, 2016; revised July 12, 2016; accepted for publication July 26, 2016.

Available online August 20, 2016.

Corresponding author: Julia Esser-von Bieren, PhD, Center of Allergy and Environment (ZAUM), Technische Universität München and Helmholtz Zentrum München, 80802 Munich, Germany. E-mail: Julia.esser@tum.de.

The CrossMark symbol notifies online readers when updates have been made to the article such as errata or minor corrections

0091-6749/$36.00

http://dx.doi.org/10.1016/j.jaci.2016.07.014

in turn reduced LT release. Tgm2, group 10 secretory phospholipase A_2, and LT enzymes in NHBEs and nasal polyps were refractory to corticosteroids.
Conclusion: Our findings reveal age differences in LT and Wnt pathways during airway inflammation and identify a steroid-resistant cascade of Wnt5a, Tgm2, and LTs, which might represent a therapeutic target for airway inflammation and remodeling. (J Allergy Clin Immunol 2017;139:1343-54.)

Key words: *Age, airway remodeling, airway inflammation, allergy, asthma, bronchial epithelial cells, house dust mite, leukotrienes, lipid mediators, nasal polyps, secretory phospholipase A_2, transglutaminase 2, Wnt5a*

Asthma affects more than 300 million patients worldwide, and airway remodeling, characterized by persistent changes in airway structure, airway hyperresponsiveness, and lung function impairment, is a major cause of morbidity that cannot be reversed by currently available therapies.[1,2] Thus airway remodeling represents a major unmet clinical need, which is insufficiently addressed because underlying mechanisms are incompletely understood.[3] Recently, age differences in the susceptibility and clinical picture of airway inflammation and remodeling have emerged.[4,5] Consistent with the increase in markers of airway eosinophilia in human neonates,[6] rodent models of airway inflammation have shown that airway eosinophilia is increased in neonates.[7-9] However, despite increased eosinophilia at a young age, changes in airway structural cells are more severe later in life.[7,9]

Leukocytes, which infiltrate the respiratory tissue of allergic and asthmatic patients, produce cytokines, such as IL-4, IL-13, IL-5 and TGF-β1, which promote inflammation and remodeling.[10] In addition, myeloid cells produce lipid mediators, such as leukotrienes (LTs), which perpetuate inflammatory cell recruitment and remodeling.[11,12] Although cell infiltration was traditionally assumed to correlate with airway remodeling, recent evidence suggests that these might be parallel rather than causally related processes.[13,14] This has led to an increasing appreciation of airway epithelial cells (AECs) as central players in airway inflammation and remodeling.[15,16] AECs can produce IL-25, IL-33, and thymic stromal lymphopoietin, which activate type 2 inflammation.[15] In mice sensitized with house dust mite (HDM) allergens from day 3 after birth, IL-33 levels were shown to increase with age and to be crucial for remodeling, whereas lung IL-13 levels peaked early after HDM sensitization, suggesting an early neonatal T_H2 bias, followed by increased production of epithelial remodeling factors during persistent allergen exposure.[17,18] AECs also respond to type 2 cytokines and bioactive lipids, such as cysteinyl leukotrienes (cysLTs), by producing eotaxins, matrix metalloproteinases, and TGF-β1.[19-21] In response to inflammatory stimuli, such as bradykinin, LPS, or HDM extract, human bronchial epithelial cells upregulate enzymes of the LT biosynthetic cascade (5-lipoxygenase [5-LO], 5-lipoxygenase activating protein, leukotriene C_4 synthase [LTC_4S], and LTA_4 hydrolase), resulting in the capacity to produce LTs.[22-24]

Transglutaminase 2 (Tgm2) has been identified as another epithelium-expressed enzyme that was crucial for the development of airway inflammation in mice.[25] Tgm2 was found to be overexpressed in the airways of asthmatic patients and to regulate cysLT production through activating group 10 secretory phospholipase A_2 (sPLA2-X), another hallmark enzyme of asthma severity and remodeling.[26-28] Increased expression of a different sPLA2 (group IID) has recently been shown to confer increased susceptibility to respiratory tract infection associated with aging,[29] but age-dependent regulation of lipid mediator pathways in allergic airway inflammation has remained elusive.

A pathway that has only recently emerged as being involved in asthma is the wingless/integrase (Wnt) pathway, which in human subjects and mice comprises 19 secreted glycolipoproteins.[30,31] Wnt5a in particular is essential for lung development and expressed in the epithelial and mesenchymal compartment in the neonatal lung.[32,33] Wnt5a is abundant in airway smooth muscle cells (ASMCs), is potently upregulated by TGF-β1, and promotes collagen and fibronectin expression, thus implicating Wnt5a in remodeling in asthmatic patients.[34-36] In hematopoietic stem cells Wnt5a expression was observed to increase with age,[37] but potential age-dependent fluctuations of Wnt5a levels during airway inflammation are unclear.

Recent work from our own group has shown that Tgm2 and Wnt5a are among the most prominently IL-4–induced genes in normal human bronchial epithelial cells (NHBEs).[38] It is also known that Wnt signaling is defective in the adipose tissue of Tgm2-deficient mice.[39] However, whether regulatory loops between Tgm2 and Wnt signaling occur in the respiratory tract was unknown. Moreover, potential links between the LT and Wnt pathways during airway inflammation and remodeling have not been investigated.

Here we show that airway LT, Tgm2, Wnt5a, and sPLA2-X profiles show age-dependent alterations during HDM-induced allergic airway inflammation and that AECs abundantly express these factors in settings of airway inflammation. Finally, we identify a regulatory network of Tgm2, Wnt5a, and LTs, which might contribute to the steroid resistance of remodeling processes in the setting of asthma and nasal polyposis.

Abbreviations used

AEC:	Airway epithelial cell
ASMC:	Airway smooth muscle cell
BALF:	Bronchoalveolar lavage fluid
CRSwNP:	Chronic rhinosinusitis with nasal polyps
cysLT:	Cysteinyl leukotriene
DMSO:	Dimethyl sulfoxide
FZD:	Frizzled
GC:	Glucocorticosteroid
HDM:	House dust mite
5-LO:	5-Lipoxygenase
LTC_4S:	Leukotriene C_4 synthase
MDM:	Monocyte-derived macrophage
NHBE:	Normal human bronchial epithelial cell
αSMA:	α Smooth muscle actin sPLA2-X, Group 10 secretory phospholipase A_2
STAT6:	Signal transducer and activator of transcription 6
Tgm2:	Transglutaminase 2
Wnt:	Wingless/integrase
ZO-1:	Zonula occludens 1

METHODS

Animal experiments were performed according to institutional guidelines and Swiss federal and cantonal laws on animal protection.

Mouse model of allergic airway inflammation

Allergic airway sensitization was induced, as previously published.[8] Additional information can be found in the Methods section in this article's Online Repository at www.jacionline.org.