IL-27 and type 2 immunity in asthmatic patients: Association with severity, CXCL9, and signal transducer and activator of transcription signaling

Min Xie, MD,[a,b] Anthony T. Mustovich, DO,[b] Yi Jiang, MD,[b] John B. Trudeau, BA,[b] Anuradha Ray, PhD,[b] Prabir Ray, PhD,[b] Haizhen Hu, BS,[b] Fernando Holguin, MD, MPH,[b] Bruce Freeman, PhD,[b] and Sally E. Wenzel, MD[b]

Pittsburgh, Pa, and Wuhan, China

Background: Severe asthma (SA) can involve both innate and type 2 cytokine–associated adaptive immunity. Although IL-27 has been reported to potentiate T_H1 responses (including the chemokine CXCL9) and suppress T_H2 responses, its function in asthmatic patients is unknown.

Objective: We sought to evaluate IL-27 expression in human asthma alone and in combination with type 2 immunity to determine the relationship to disease severity and CXCL9 expression. We also sought to model these interactions *in vitro* in human bronchial epithelial cells.

Methods: Bronchoalveolar lavage cells from 87 participants were evaluated for IL-27 mRNA and protein alone and in association with epithelial CCL26 (a marker of type 2 activation) in relation to asthma severity and CXCL9 mRNA. Human bronchial epithelial cells cultured at the air-liquid interface and stimulated with IL-27 (1-100 ng/mL) with or without IL-13 (1 ng/mL) were evaluated for CXCL9 expression by using quantitative real-time PCR and ELISA. Phosphorylated and total signal transducer and activator of transcription (STAT) 1/3 were detected by means of Western blotting. Small interfering RNA knockdown of STAT1 or STAT3 was performed.

Results: Bronchoalveolar lavage cell IL-27 mRNA and protein levels were increased in asthmatic patients. Patients with evidence for type 2 pathway activation had higher IL-27 expression ($P = .02$). Combined IL-27 and CCL26 expression associated with more SA and higher CXCL9 expression ($P = .004$ and $P = .007$ respectively), whereas IL-27 alone was associated with milder disease. *In vitro* IL-13 augmented IL-27–induced CXCL9 expression, which appeared to be due to augmented STAT1 activation and reduced STAT3 activation.

Conclusions: IL-27, in combination with a type 2/CCL26 signature, identifies a more SA phenotype, perhaps through combined effects of IL-27 and IL-13 on STAT signaling. Understanding these interactions could lead to new targets for asthma therapy. (J Allergy Clin Immunol 2015;135:386-94.)

Key words: *Asthma, IL-27, IL-13, CXCL9, epithelial cells, signal transducer and activator of transcription 1, signal transducer and activator of transcription 3*

Asthma is a heterogeneous disease consisting of multiple phenotypes. Although different phenotyping schemes have been suggested, the most prominent current scheme relates to the presence or absence of a type 2 cytokine signature.[1,2] However, a type 2 signature can be present in patients with a range of disease severity and clinical characteristics, suggesting that additional cytokine pathways also control the biologic and clinical presentation.[1,3,4]

IL-27, a novel cytokine sharing subunits with IL-6 families, is primarily produced by activated antigen-presenting cells, specifically dendritic cells but also pulmonary macrophages.[5,6] Functionally, IL-27 is characterized by pleiotropic effects on T_H cell function, differentiation, and development.[7,8] Recent studies have reported levels of IL-27 to be increased in response to allergen, to be present in human eczematous skin lesions, and to be genetically associated with asthma susceptibility.[9-11] IL-27 has also been reported to induce steroid resistance, suggesting it could contribute to more complex asthma phenotypes.[6] However, with its contradictory proinflammatory and anti-inflammatory effects,[12-17] its biologic function in asthmatic patients is unclear.

A large body of evidence suggests that alterations in the respiratory epithelium play a crucial role in both the development and persistence of asthma.[18,19] Indeed, pioneering studies to divide asthma into T_H2-high versus T_H2-low phenotypes evaluated the expression of 3 IL-13–induced genes in the airway epithelium of patients with mild asthma compared with that of healthy control subjects (HCs).[1] CCL26/eotaxin-3 similarly represents a gene highly induced by IL-13, which can be used to differentiate "T_H2-high versus T_H2-low" asthma.[1,3] Like IL-13, IL-27 can also affect human bronchial epithelial cells (HBECs).[20] Previous *in vitro* studies have suggested that signal transducer and activator of transcription (STAT) 1 and STAT3

From [a]the Department of Respiratory and Critical Care Medicine, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, and [b]the University of Pittsburgh Asthma Institute@UPMC, Pulmonary Allergy Critical Care Medicine Division, Department of Medicine, University of Pittsburgh.

Supported by the National Institutes of Health (HL069174, HL064937, AI-40600, and RR024153) and the National Natural Science Foundation of China (No. 81470002).

Disclosure of potential conflict of interest: This study was funded by the National Institutes of Health. A. Ray is a member of the board of the American Thoracic Society, is employed by the University of Pittsburgh, and has received payment for delivering lectures from Yale University and Northwestern University. P. Ray is employed by the University of Pittsburgh and has received payment for delivering lectures from Yale University and the American Academy of Allergy, Asthma & Immunology (AAAAI). B. Freeman receives money from patents from Complexa, where he is also a shareholder. S. E. Wenzel has received funding from Amgen, ARRAY, AstraZeneca, GlaxoSmithKline, Merck, Novartis, Sanofi Aventis, Genentech, UptoDate, ICON, and Pfizer. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication April 9, 2014; revised July 23, 2014; accepted for publication August 20, 2014.

Available online October 11, 2014.

Corresponding author: Sally E. Wenzel, MD, Pulmonary Allergy Critical Care Medicine Division, University of Pittsburgh Asthma Institute@UPMC/UPSOM, NW628 Montefiore Hospital, 3459 Fifth Ave, Pittsburgh, PA 15213. E-mail: wenzelse@upmc.edu.

0091-6749/$36.00

© 2014 American Academy of Allergy, Asthma & Immunology

http://dx.doi.org/10.1016/j.jaci.2014.08.023

Abbreviations used
ALI: Air-liquid interface
BAL: Bronchoalveolar lavage
FENO: Fraction of exhaled nitric oxide
GAPDH: Glyceraldehyde-3-phosphate dehydrogenase
HBEC: Human bronchial epithelial cell
HC: Healthy control subject
ICS: Inhaled corticosteroid
IHC: Immunohistochemistry
qRT-PCR: Quantitative real-time PCR
SA: Severe asthma
SARP: Severe Asthma Research Program
siRNA: Small interfering RNA
STAT: Signal transducer and activator of transcription

are the main signaling pathways controlling IL-27's effects on epithelial cells.[21] In myeloid cells STAT3 activation appears to counterbalance STAT1 activation, limiting the overall expression of STAT1-dependent pathways.[22] However, the functional effect of IL-27 on HBECs from asthmatic patients and involved pathways remains poorly understood, particularly in the presence of a background type 2 (IL-13) signature.

Therefore we hypothesized that IL-27, in association with a type 2 (IL-13) gene signature (high CCL26 expression) identifies a more severe asthma (SA) phenotype than the presence of either cytokine or its signature alone. We hypothesized that the mechanisms for this increased severity would include synergistic augmentation of epithelial expression of the type 1 chemokine monokine induced by IFN-γ (CXCL9) by IL-13 and IL-27 through alterations in the balance of STAT1 and STAT3. Bronchoscopic samples from a range of asthmatic and HC participants were analyzed for the presence of a type 2 signature (based on epithelial CCL26 expression) alone and in association with bronchoalveolar lavage (BAL) cell IL-27 expression in association with asthma severity and CXCL9 expression to evaluate this. To determine potential mechanisms for these effects, the effect of IL-27 alone and in combination with IL-13 on primary HBEC activation of STAT1 and STAT3 was evaluated to determine their role in the synergistic increase in CXCL9 expression.

METHODS

Subjects

Participants were 18 to 65 years old and enrolled in the Severe Asthma Research Program (SARP) or the Electrophilic Fatty Acid Derivatives in Asthma study.[23] All studies were approved by the University of Pittsburgh's Institutional Review Board, and all participants provided informed consent. Subjects were nonsmokers in the last year, and all had a smoking history of less than 5 pack years. SA was defined by using the 2001 American Thoracic Society definition.[24] Patients with mild asthma not receiving inhaled corticosteroids (ICSs; mild/no ICS group) had a prebronchodilator FEV_1 of 80% of predicted value or greater. Patients with mild-to-moderate asthma receiving low- to moderate-dose ICSs (mild-mod/ICS group) had an FEV_1 of greater than 60% of predicted value.[25] HCs had no history of chronic respiratory disease and normal lung function but could be atopic. All participants were extensively characterized, as previously described, including clinical questionnaires, spirometric measures before and after bronchodilator, fraction of exhaled nitric oxide (FENO), complete blood counts, allergy skin prick testing, and IgE measurement.[25,26]

Bronchoscopy and sample processing

Epithelial brushings and BAL cells were obtained bronchscopically from fourth- to fifth-generation airways, as previously published and shown in the SARP Manual of Procedures.[27,28] Cells were placed in Qiazol (Qiagen, Valencia, Calif) for extraction of RNA.

Quantitative real-time PCR

Epithelial and BAL cell RNA was extracted in Qiazol (Qiagen), and mRNA expression was determined by using quantitative real-time PCR (qRT-PCR). Primers and probes were purchased from Applied Biosystems (Foster City, Calif; Assays on Demand: IL-27 p28, Hs00377366_m1; CCL26, Hs00171146_m1; and CXCL9, Hs00171065_m1). qRT-PCR was performed on the ABI PRISM 7900 sequence detection system (Applied Biosystems) at the core facilities of the University of Pittsburgh. The levels of each marker were determined relative to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) by using the Δ cycle threshold method.

Immunohistochemistry

BAL cell cytospin preparations were fixed in 2% paraformaldehyde. Cytospin preparations were rinsed, blocked, and incubated overnight with goat polyclonal anti-human IL-27 antibody (R&D Systems, Minneapolis, Minn) at a 1:50 dilution. Secondary antibody staining alone and isotype controls were used to confirm primary antibody specificity. Biotinylated secondary rabbit anti-goat antibody was added, and the cytospin preparations were incubated with ABC reagent (Vector Laboratories, Burlingame, Calif), developed with 3-amino-9-ethylcarbazole, counterstained with hematoxylin, and overlaid with Crystal Mount (Electron Microscopy Sciences, Hatfield, Pa). IL-27^+ cells were counted in a blind manner by 2 independent observers from 500 cells to obtain the percentage of IL-27^+ cells.

Primary air-liquid interface epithelial cell culture and small interfering RNA transfection

Primary HBECs obtained from bronchoscopic brushings were cultured at the air-liquid interface (ALI), as previously described.[28,29] From day 0 of the

TABLE I. Baseline demographic characteristics (n = 87)

	Subject group				
Demographics	**HCs (n = 26)**	**Mild/no ICS (n = 16)**	**Mild-moderate/ICS (n = 16)**	**SA (n = 29)**	**Overall difference (*P* value)**
Age (y)	27 (24-35)	24 (20-31)	31 (25-44)	43 (34-55)	<.001
Sex (male/female)	15/11	5/11	3/13	8/21	.038
Race (CA/AA/other)	20/1/5	11/2/3	7/7/2	23/4/2	.12
BMI (kg/m^2)	24 (22-29)	27 (24-31)	27 (26-34)	32 (26-37)	.001
Exhaled NO (ppb)	22 (15-40)	40 (26-54)	25 (16-35)	38 (22-75)	.01
Blood eosinophils (/μL)	100 (100-200)	200 (100-300)	200 (100-300)	300 (100-500)	.04
Baseline FEV_1 (% predicted)	95 (90-105)	89 (85-97)	89 (66-104)	59 (45-71)	<.001
Serum IgE (kU/L)	31 (15-83)	98 (52-334)	142 (42-430)	172 (38-600)	<.001

Categorical analyses were performed with Pearson χ^2 tests. Continuous variables were analyzed by using Kruskal-Wallis tests and presented as medians (25th-75th percentiles).
AA, African American; *BMI*, body mass index; *CA*, white; *NO*, nitric oxide.

Download English Version:

https://daneshyari.com/en/article/6063346

Download Persian Version:

https://daneshyari.com/article/6063346

Daneshyari.com