Respiratory Medicine 123 (2017) 34–41

Contents lists available at ScienceDirect

Respiratory Medicine

journal homepage: www.elsevier.com/locate/rmed

Characteristics associated with clinical severity and inflammatory phenotype of naturally occurring virus-induced exacerbations of asthma in adults

Asger Bjerregaard [a, b, c, *], Ingrid A. Laing [b, c], Nadia Poulsen [a, b, c], Vibeke Backer [a], Asger Sverrild [a], Markus Fally [a], Siew-Kim Khoo [b, c], Lucy Barrett [f], Svetlana Baltic [f], Philip J. Thompson [f], Glenys Chidlow [d], Chisha Sikazwe [d, e], David W. Smith [b, d, e], Yury A. Bochkov [g], Peter Le Souëf [c], Celeste Porsbjerg [a]

[a] Respiratory Research Unit, Bispebjerg University Hospital, Copenhagen, Denmark
[b] Telethon Kids Institute, Perth, Australia
[c] School of Paediatrics & Child Health, University of Western Australia, Perth, Australia
[d] Department of Microbiology, PathWest Laboratory Medicine WA, Nedlands, Australia
[e] School of Pathology and Laboratory Medicine, University of Western Australia, Perth, Australia
[f] Institute for Respiratory Health, University of Western Australia, Perth, Australia
[g] Department of Pediatrics, School of Medicine and Public Health, University of Wisconsin, USA

ARTICLE INFO

Article history:
Received 5 October 2016
Received in revised form
5 December 2016
Accepted 16 December 2016
Available online 18 December 2016

Keywords:
Asthma
Exacerbation
Eosinophils
Viral infection
TSLP

ABSTRACT

Background: In experimental studies viral infections have been shown to induce type 2 inflammation in asthmatics, but whether this is a feature of naturally occurring virus-induced asthma exacerbations is unknown. Thymic stromal lymphopoietin (TSLP) released from the airway epithelium in response to damage, has been suggested as a link between viral infection and type 2 inflammation, but the role of TSLP in asthma exacerbations is unknown.

Objective: To assess whether type 2 inflammation, as measured by sputum eosinophils and fractional exhaled nitric oxide (FeNO), is a feature of naturally occurring virus-induced exacerbations of asthma and whether TSLP is associated with this type 2 inflammation.

Methods: Patients presenting to hospital with acute asthma were examined during the exacerbation, and after 4 weeks recovery. The assessments included spirometry, FeNO and induced sputum for differential counts and TSLP mRNA levels. Nasal swabs were collected for viral detection.

Results: Sputum eosinophils and FeNO were similar between virus-positive ($n = 44$) and negative patients ($n = 44$). In virus-positive patients, TSLP expression was lower at exacerbation than follow-up ($p = 0.03$). High TSLP at exacerbation was associated with lower sputum eosinophils ($p = 0.01$) and higher FEV1 ($p = 0.03$). In virus-positive patients, %-predicted FEV1 negatively correlated with both FeNO and sputum eosinophils ($p = 0.02$ and $p = 0.05$, respectively).

Conclusion: Our findings support that type 2 inflammation is present in patients during virus-induced asthma exacerbations, to the same degree as non-viral exacerbations, and correlate negatively with FEV1. However, in virus-positive patients, high TSLP expression during exacerbation was associated with low sputum eosinophils, suggesting that the effect of TSLP *in vivo*, in the setting of an asthma exacerbation, might be different than the type 2 inducing effects observed in experimental studies.

1. Introduction

With an estimated prevalence of nearly 10%, asthma is one of the more frequent chronic diseases in Western countries, and exacerbations are the major cause of morbidity and health care

* Corresponding author. Respiratory Research Unit, Bispebjerg University Hospital, Bispebjerg Bakke 23, 2400 Copenhagen, Denmark.
E-mail address: asger.bjerregaard@dadlnet.dk (A. Bjerregaard).

http://dx.doi.org/10.1016/j.rmed.2016.12.010
0954-6111/

utilization [1]. Respiratory viruses are one of the most common triggers of asthma exacerbations [2], but the underlying mechanisms are not fully understood.

Overall, around half of asthma patients have prominent type 2 inflammation during stable disease [3], characterized by IL-5 driven eosinophilia in both sputum and blood and mucus hypersecretion, airway hyperresponsiveness and increased IgE production, driven by IL-4 and IL-13. This Th2-high phenotype has been associated with more severe disease [4,5] and an increased risk of exacerbations [6]. Whether aggravated type 2 inflammation is a feature of both virus-induced and non-viral exacerbations, is currently unknown and is likely to have implications for the efficacy of emerging biological treatment in reducing the rate and severity of these exacerbations.

Allergen sensitization and exposure is the established cause of increased type 2 inflammation. However, *in vitro* studies have suggested that respiratory viruses are capable of independently inducing type 2 inflammation, by causing a release of pro-inflammatory cytokines, including thymic stromal lymphopoietin (TSLP), from the airway epithelium [7,8]. In this innate immune pathway, TSLP causes release of type 2 cytokines IL-4, IL-5 and IL-13, from T-cells through activation of dendritic cells [9] and by direct action on innate lymphoid cells (ILC) [10]. TSLP is increased in patients with asthma, compared to controls [11] and anti-TSLP antibodies have been shown to reduce airway inflammation in response to an allergen provocation test [12]. Epithelial cells from asthmatics seem to be particular prone to TSLP release following exposure to viral infection *in vitro* [8] but whether TSLP is induced in asthmatics during viral infection *in vivo* and stimulates type 2 inflammation is unknown.

Asthma patients, particularly those with type 2 inflammation, experience more severe symptoms when experimentally infected with respiratory viruses [13,14] and, although controversial, the mechanism is proposed to involve a reduced production of interferons [15–18]. Whether patients with high type 2 inflammation during a real-life viral infection experience more severe exacerbations, is unclear.

The aim of the present study was to test the hypothesis that sputum eosinophilia and increased FeNO are features of naturally occurring virus-induced exacerbations. Secondarily, that the level of sputum eosinophilia and FeNO are associated with the degree of TSLP expression in sputum. Finally we wished to test whether any type 2 inflammation during a virus-induced exacerbation would be associated with more severe exacerbations, measured by FEV1. Exploratory outcomes included rhinovirus subtype and systemic markers of inflammation.

2. Methods

2.1. Study design

The study was a prospective observational study conducted at Bispebjerg University Hospital between July 2013 and September 2015. Patients were recruited both from the hospital Emergency Department, and from the acute asthma clinic within the hospital research unit and were seen within 24 h of contact. Exacerbations were defined as worsening of symptoms surpassing the daily variation that required a change in treatment in line with ERS/ATS recommendations [19]. After 4 weeks, participants were re-examined in the research unit. All patients had a doctor's diagnosis of asthma and had the diagnosis confirmed during the study in line with GINA recommendations [1]. Patients were between 16 and 45 years of age. Pregnant women and patients with pulmonary diseases other than asthma were excluded. Both current and past smokers were included.

The study was approved by the local scientific ethics committee (protocols nr: H-2-2013-046, H-3-2011-121, H-15003691).

2.2. Examinations

Fractional expiratory nitric oxide (FeNO) (NioxMinor, Aerocrine, Sweden) was assessed following the recommendations of the ERS and ATS [20] to use the mean of two measurements. Patients completed spirometry (EasyOne, NDD, Switzerland) according to ERS guidelines [21], using NHANES III for lung function predicted values [22]. Because patients were examined during an exacerbation, they were not asked to withhold beta-2-agonists prior to spirometry, but the time since the last dose of reliever was taken was recorded. Induced sputum was processed as described by Pavord et al. [23]: In brief, after inhalation of 1 mg terbutaline, sputum was induced by inhalation of hypertonic saline in increasing concentrations (3%, 4%, and 5%) for 5 min each (total duration 15 min). Sputum plugs were selected and processed, cytospins were prepared, and a differential cell count of 400 non-squamous cells was completed. The hospital clinical laboratory completed a full white blood cell differential count and measured high-sensitivity C-reactive protein (hsCRP), total IgE and specific IgE to common allergens (grasses, house dust mite, birch, mugwort, dog, cat, horse, *Cladosporium herbarum* and *Alternaria tenuis*). Atopy was defined as the presence of specific IgE (>0.35 IU/ml) to one or more of these allergens. Asthma control was assessed with the 5-item Asthma Control Questionnaire (ACQ) [24].

2.3. Detection of respiratory pathogens

Flocked nasal swabs (Copan, Italy) were used to sample turbinate nasal. Samples were assessed for human adenovirus species B-D; human bocavirus; coronaviruses OC43, 229E, HKU1 and NL63; influenza viruses A, B and C; parainfluenzaviruses 1–4; KI and WU polyomaviruses; respiratory syncytial virus types A and B and human metapneumovirus, using a tandem multiplex real-time PCR assay [25,26].

A PCR directed at picornavirus sequences in the 5′UTR was used to detect RV. The products were identified to species level as RV-A, RV-B or RV-C by sequencing of this 260 bp product and analysed using ClustalX software [27,28]. The local hospital laboratory at Bispebjerg Hospital completed bacterial cultures on spontaneous expectorates collected during the exacerbation [29].

2.4. Quantification of TSLP mRNA expression

Sputum cell pellets were stored in RNAlater (Ambion, USA) and kept at −80 °C until processing. RNeasy Mini kit (Invitrogen, USA) was used to extract mRNA following the manufacturers recommendations. After the RNA extraction, cDNA was synthesised using SSIII reverse transcriptase (Invitrogen, USA) as described by the manufacturer. TSLP was quantified using digital droplet PCR (QX200, Bio-Rad, USA) with TaqMan primers ands probes. GAPDH was used for normalization. For the analyses participants were divided into TSLP high and TSLP low by the median expression.

2.5. Statistical analyses

The data was analysed with SPSS version 22.0 (IBM SPSS, USA). Normally distributed data are reported as mean ± standard deviation and analysed using students t-test for continuous variables, and chi-squared test for categorical variables. Log-transformed variables are reported as geometric mean (GM) with 95% CI. For log-transformation of sputum eosinophil percentage, cases with 0% were set to 0.1% prior to transformation. Non-normally distributed