Respiratory Medicine 115 (2016) 1–6

Contents lists available at ScienceDirect

Respiratory Medicine

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

Presence of rhinovirus in the respiratory tract of adolescents and young adults with asthma without symptoms of infection

L. Öhrmalm [a, *, 1], A. Malinovschi [b, 1], M. Wong [a], P. Levinson [a], C. Janson [c], K. Broliden [a, 1], K. Alving [d, 1]

[a] Department of Medicine, Infectious Diseases Unit, Center for Molecular Medicine, Karolinska Institutet, Karolinska University Hospital, Solna, Sweden
[b] Department of Medical Sciences: Clinical Physiology, Uppsala University, Uppsala, Sweden
[c] Department of Medical Sciences: Respiratory Medicine and Allergology, Uppsala University, Uppsala, Sweden
[d] Department of Women's and Children's Health, Uppsala University, Uppsala, Sweden

ARTICLE INFO

Article history:
Received 21 January 2016
Received in revised form
10 April 2016
Accepted 13 April 2016
Available online 14 April 2016

Keywords:
Asthma
Rhinovirus
Immunoglobulin E
Type-2 inflammation
Exhaled nitric oxide
Eosinophil cationic protein

ABSTRACT

Background: Viral respiratory infections have been associated with up to 80% of wheezing episodes and asthma exacerbations. However, studies on the role of these viruses in asthmatic patients in the interval between exacerbations are sparse. This study aimed to determine the presence of respiratory viruses, without symptoms of infection, in the airways of young asthmatics as compared to healthy controls.
Material and Methods: Patients 10–35 years of age with stable asthma and a group of healthy controls were analyzed regarding the presence of RNA from common respiratory viruses in nasopharyngeal aspirates by PCR. Self-reported asthma control and quality of life, fraction of exhaled nitric oxide (FeNO), spirometry, and bronchial responsiveness to methacholine were recorded. Blood samples were collected to assess IgE sensitisation and eosinophil cationic protein (ECP) levels.
Results: In 354 patients with asthma and 108 healthy controls, human rhinovirus (HRV) was the only virus detected (4.5% of asthmatics vs. 0.9% of controls; $p = 0.08$). HRV^+ asthma patients had a higher degree of aeroallergen IgE sensitisation (median 37.7 vs. 10.4 kU_A/L, $p = 0.04$), and a tendency for higher levels of serum ECP (median 17.2 vs. 12.6 μg/L, $p = 0.07$), as compared to their HRV^- counterparts.
Conclusions: Absence of symptoms of respiratory tract infection notwithstanding, HRV seems to be more prevalent in the airways of adolescents and young adults with asthma and a high degree of aeroallergen IgE sensitisation than in controls. The presence of HRV seems also to be related to systemic eosinophilic inflammation despite ongoing treatment with inhaled corticosteroids.

1. Introduction

Viral respiratory infections have been associated with up to 80% of wheezing episodes and asthma exacerbations in both children and adults [1–3], and increased rates of hospital admissions for asthma and higher detection rates of respiratory viruses have been reported to coincide [4,5]. However, regarding the role of respiratory viruses in asthmatic patients during stable periods between exacerbations, very little data is available. It has been speculated that human rhinovirus (HRV) has the ability to cause persistent infections in patients with asthma, but only a few small studies have suggested a preponderance of respiratory viruses in non-exacerbating asthmatic individuals compared to healthy controls [6,7].

Viral load and clinical deterioration during acute HRV infections have been shown to be related to reduced interferon response and increased type 2-cytokine (interleukin (IL)-4, IL-5 and IL-13) response in patients with asthma compared to non-asthmatic controls [8,9]. Further, human airway epithelial cells produce the proinflammatory molecule C-X-C motif chemokine 10 (CXCL10) in relation to HRV infection [10], and this response appears to be specific to viral-induced acute asthma [11]. Moreover, Th2 cytokines increase the release of CXCL10 in the presence of rhinovirus infection [12]. Thus, type-2 inflammation in the airways could possibly facilitate viral replication over prolonged periods or, *vice versa*, the infection could promote chronic airway inflammation.

* Corresponding author. Center for Molecular Medicine, Infectious Diseases Unit, L8:01, Karolinska University Hospital, Solna, SE-171 76, Stockholm, Sweden.
E-mail address: lars.ohrmalm@ki.se (L. Öhrmalm).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.rmed.2016.04.002
0954-6111/

In the environment of type-2 inflammation in the airways, inducible nitric oxide synthase is upregulated in the respiratory epithelium and the fraction of exhaled nitric oxide (FeNO) is consequently increased [13,14]. Furthermore, type-2 inflammation is characterised by eosinophil recruitment and viral infections seem to cause systemic activation of eosinophils, at least in subjects with allergic asthma [15,16].

To further elucidate the possible role of persistent virus infections, we have conducted a large cross-sectional study on young patients with asthma (n = 354) and matched controls (n = 108) (the MIDAS cohort) without symptoms of respiratory tract infection. To this end, the prevalence of HRV, respiratory syncytial virus (RSV), influenza viruses A and B, and human coronavirus (hCoV), all known to be associated with asthma exacerbations, was examined in nasopharyngeal aspirates (NPAs), and asthmatics and controls were compared. A secondary aim was to see if the presence of respiratory viruses was related to biomarkers of type-2 inflammation and/or allergic sensitisation.

2. Materials and methods

2.1. Study population

The study was based on the MIDAS cohort, and more details on the methods have been published elsewhere [17–19]. A total of 411 patients between 10 and 35 years of age with physician-diagnosed asthma as reported in their medical records, as well as daily treatment with inhaled corticosteroids (ICS) and/or oral leukotriene-receptor antagonists (LTRA) during at least three months the year before the examination were included in the study. All subjects were interviewed, examined, and sampled while in a stable state of asthma (*i e* no exacerbation within the past two weeks) and having refrained from taking any asthma or allergy medication for 24 h before the study. A total of 122 healthy subjects without any diagnosis of respiratory disease, randomly chosen from the Uppsala population register (Sweden), were sex- and age-matched with the asthma patients and participated as controls. All subjects were told that they should have been free from symptoms of respiratory tract infections (cough, sore throat, runny nose, sneezing, nasal congestion, pink eyes or fever) for at least two weeks on the day of examination. The subject inclusion/exclusion flow chart is shown in Fig. 1.

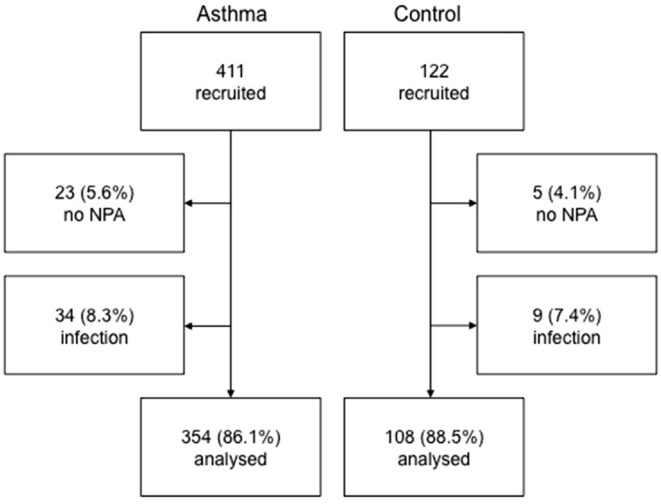

Fig. 1. Flow chart of inclusion in the study. Patients who did not volunteer to undergo sampling of nasopharyngeal aspirate (NPA) or had symptoms of respiratory tract infections during the two weeks preceding sampling were excluded from final analysis.

2.2. Asthma control and quality of life

All subjects with asthma filled in the Asthma Control Test (ACT) and the Juniper Mini Asthma-related Quality of Life Questionnaire (mAQLQ) in order to assess the degree of asthma control and asthma-related quality of life, respectively.

2.3. Respiratory measurements

Dynamic spirometry was performed using a MasterScope spirometer (Erich Jager, Wurzburg, Germany) and the methacholine challenge test was performed using the Aerosol Provocation System (Viasys Healthcare GmbH, Hoechberg, Germany). Exhaled NO was measured according to ATS/ERS recommendations [20] using a chemiluminescence analyser (NIOX Flex; Aerocrine AB, Solna, Sweden).

2.4. Collection of nasopharyngeal aspirates, nucleic acid extraction and real-time PCR methods

NPAs were collected as previously described and stored at −80 °C [21]. Total viral nucleic acids were extracted with QIAamp MinElute Virus Spin Kit (QIAGEN, USA) according to the manufacturer's protocol with 200 μL of NPA eluted into 50 μL of extract. Real-time PCR assays for detection of HRV (including species A, B, and C), hCoV (OC43, 229E, HKU1, and NL63), influenza A and B, and RSV were performed on an ABI 7500 Real-Time PCR System (Applied Biosystems) [22], and both plasmids and viral RNA were used as positive controls.

2.5. Blood analyses

Venous blood samples were drawn for blood cell counts, and for preparation of serum and plasma (EDTA) samples that were stored at −80 °C. For serum, blood was allowed to clot for 60 min at 22 °C. Blood leukocyte counts were determined using routine methods (Cell-Dyn Sapphire, Abbott, IL, USA) at the Department of Clinical Chemistry, Uppsala University Hospital. Measurements of IgE antibodies against a mix of aeroallergens (Phadiatop; cat, dog, horse, *Dermatophagoides pteronyssinus, Dermatophagoides farinae, Cladosporium herbarum*, birch, timothy grass, and mugwort) and food allergens (fx5; egg white, milk, cod fish, wheat, peanut, and soybean) were performed, and subjects were identified as atopic if Phadiatop or fx5 was ≥0.35 kU_A/L. IgE antibodies, total IgE and serum eosinophil cationic protein (S-ECP) were measured using ImmunoCAP System (Thermo Fisher Scientific, Uppsala, Sweden). C-X-C motif chemokine 10 (CXCL10, or IP-10) was measured with a sandwich ELISA (R&D Systems, Minneapolis, MN, USA). CXCL10 measurements were performed in all HRV^+ asthmatics (n = 16) and in 17 HRV^- asthmatics that were matched for sex, age and atopy: females 43.8% *vs* 47.1% (p = 0.85), age 23.4 ± 6.8 (mean ± SD) *vs* 24 ± 6.4 years (p = 0.81), and atopy 87.5% *vs* 88.2% (p = 0.95).

2.6. Statistical analyses

Chi-square test was used to compare proportions. Non-parametric statistics were used in the whole study due to the low number of cases of HRV^+ subjects. The Mann-Whitney *U* test was used to compare medians of continuous variables. A multiple logistic regression model was created to confirm the effect of risk factors for HRV positivity after adjustment for confounding factors. STATA IC 12.1 (StataCorp LP, College Station, Texas, USA) was used for statistical analyses.