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Original article

Virus detection and cytokine profile in relation to age among acute exacerbations of childhood asthma

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Abbreviations:
ECP, eosinophil cationic protein; FGF, fibroblast growth factor; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; IL, interleukin; IFN, interferon; IP, interferon-γ-induced protein; MCP, monocyte chemoattractant protein; MIP, macrophage inflammatory protein; PDGF, platelet-derived growth factor; RANTES, regulated on activation, normal T expressed and secreted chemokine; RS, respiratory syncytial; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor

ABSTRACT

Background: Little information is available regarding eosinophil activation and cytokine profiles in relation to age in virus-induced bronchial asthma. We therefore explored the association between age, respiratory viruses, serum eosinophil cationic protein (ECP), and cytokines/chemokines in acute exacerbations of childhood asthma.
Methods: We investigated viruses in nasal secretions from 88 patients with acute exacerbation of childhood asthma by using antigen detection kits and/or RT-PCR, followed by direct DNA sequencing analysis. We also measured peripheral eosinophil counts, and the serum levels of ECP and 27 types of cytokines/chemokines in 71 virus-induced acute asthma cases and 13 controls.
Results: Viruses were detected in 71(80.7%) of the 88 samples. The three major viruses detected were rhinoviruses, RS viruses, and enteroviruses; enteroviruses were found to be dominant in patients aged ≥3 years. There was no change in the levels of rhinoviruses and RS viruses between the two age groups, defined as children aged <3 years and children aged ≥3 years. Serum concentrations of ECP, IL-5, and IP-10 were significantly elevated in virus-induced acute asthma cases compared with controls. Serum ECP values were significantly higher in patients with virus-induced asthma at age ≥3 years compared with those aged <3 years. Among the 27 cytokines/chemokines, serum IP-10 was significantly higher in virus-induced asthma in patients <3 years than in those ≥3 years. Serum ECP and IL-5 production correlated significantly with age, whereas serum IP-10 showed an inverse correlation with age.
Conclusions: Age-related differences in cytokine profiles and eosinophil activation may be related to virus-induced acute exacerbations of childhood asthma.

Introduction

Viral infection induces both the development and exacerbation of bronchial asthma.[1,2] Accumulating evidence suggests that rhinovirus infection is a major cause of acute exacerbations of asthma in both adults[3] and children.[4] Kotaniemi-Syrjänen *et al.* showed that the most significant risk factor for the development of preschool childhood wheezing is the occurrence of symptomatic rhinovirus illness during infancy.[5] The COAST (Childhood Origins of Asthma) study group also reported that wheezing attacks during childhood (2–16 years of age) can be linked to rhinovirus infection with atopy or eosinophilic airway inflammation.[6,7]

Respiratory syncytial (RS) virus is another leading cause of serious lower respiratory tract infection in infants. RS virus infection exacerbates recurrent wheezing attacks in patients with

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established asthma.[8] A number of case–control studies have established at least a statistical correlation between RS virus infection in infancy and the development of recurrent wheezing and asthma in young children (9–14 years old). However, RS virus infection appears unlikely to be a cause of atopic asthma later in life.[9–14]

Heymann *et al.* found that patients of age <3 years with wheezing and positive tests for viruses showed a higher presence of RS virus compared with other viruses. In contrast, rhinovirus was dominant in children aged 3–18 years, suggesting that viral respiratory tract pathogens might differ at different ages in asthmatic children with acute exacerbation.[15]

The purpose of this study was to investigate changes among the viruses detected, peripheral eosinophil counts, serum levels of eosinophil cationic protein (ECP), and several cytokines and chemokines in relation to age in cases of virus-induced acute exacerbation of childhood asthma.

Methods

Patients and study setting

We enrolled 88 subjects attending as outpatients or hospitalized with acute respiratory symptoms (55 boys, 33 girls, mean/median age 3.6/2.8 years) at the Gunma Children's Medical Center between January 1, 2008 and December 31, 2013. All patients had a history of three or more different episodes of recurrent wheezing and documented evidence of wheezing by auscultation. Subjects with asthma were diagnosed according to the criteria of the Japanese guidelines.[16] Briefly, a diagnosis of asthma was confirmed on the basis of a history of recurrent wheezing and dyspnea on at least three independent occasions, and reversible bronchoconstriction.[16] Patients were prescribed short-acting β agonists and/or long-term controller medications. We excluded children with obvious bacterial infections, congenital heart diseases, and chronic lung diseases as well as those who showed the presence of a foreign body, had signs of severe infection, or were immunosuppressed, as these complications can interfere with the assessment of asthma-related outcome measures. The control group included 13 healthy children (8 boys, 5 girls, mean/median age 3.7/4.2 years) with no symptoms of wheezing at the time of examination. Exclusion criteria for the controls included immunosuppression, the presence of other respiratory tract symptoms, or a history of previous wheezing and asthma. Controls and patient cases were age- and sex-matched. This study was approved by the Ethics Committee of Gunma Children's Medical Center. Informed consent was obtained from parents of patients and assent was obtained from the children if they were considered old enough (generally >9-year-old).

Virus detection

Nasal aspirates were obtained from 88 patients during acute exacerbations of asthma as previously reported.[17] Nasal samples were then analyzed using antigen detection kits for RS virus (Becton Dickinson, Fukushima, Japan), influenza virus types A and B (Denka-Seiken, Gosen, Japan), and adenovirus (Tauns, Izunokuni, Japan). The remaining secretions were frozen at −80 °C until examination by reverse transcription-polymerase chain reaction (RT-PCR), followed by direct DNA sequencing analysis as previously reported.[17] Some samples were tested by multiplex PCR (Seeplex RV15 OneStep ACE Detection kit, Seegene, Inc., Seoul, Korea) for the presence of 15 human viral respiratory pathogens (adenovirus A/B/C/D/E, human metapneumovirus, enterovirus, human bocavirus 1/2/3/4, human coronavirus 229E/NL63 and OC43, human parainfluenza virus 1/2/3/4, influenza virus A/B, RS virus A/B, and rhinovirus A/B/C), as reported previously.[18] The amplified PCR products were analyzed by automatic electrophoresis (MCE-202 MultiNA; Shimadzu, Kyoto, Japan).[19]

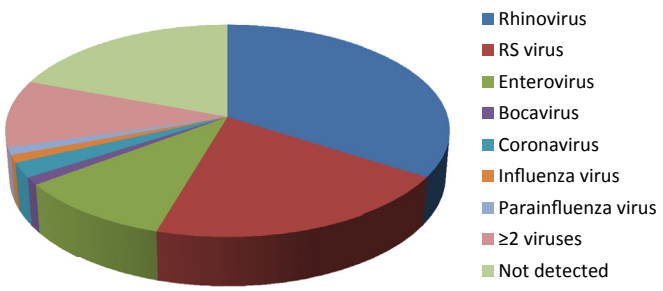

Fig. 1. Virus detection in acute exacerbations of childhood asthma. Among the 88 samples from asthma exacerbation cases, rhinovirus was detected in 30; RS virus in 18; enterovirus in 9; human coronavirus in 2, human bocavirus in 1, influenza virus in 1, human parainfluenza virus in 1, ≥2 viruses in 9; and no viruses were found in 17.

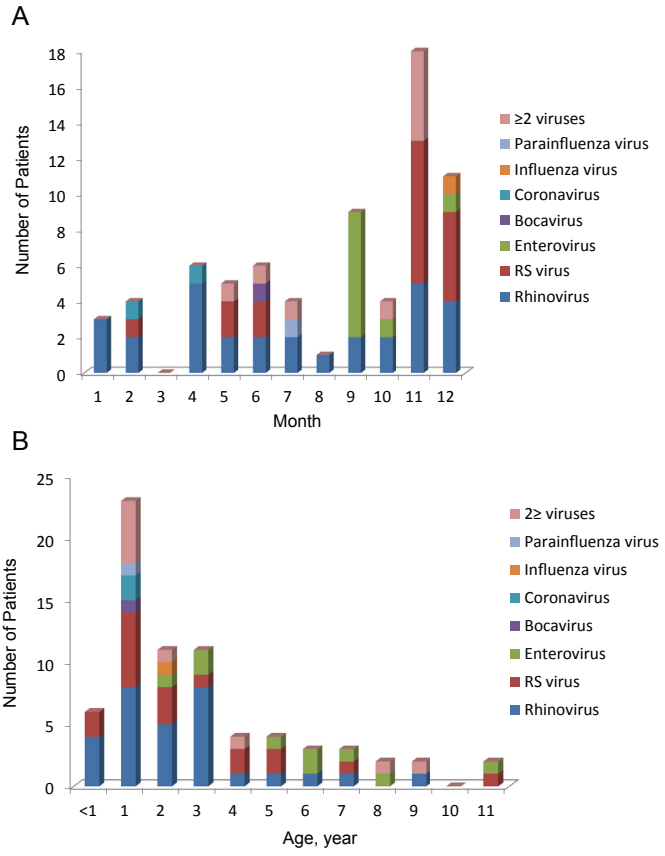

Fig. 2. Monthly (A) and age-dependent (B) changes of virus detection in acute exacerbations of asthma. (A) Two-thirds of the patients were hospitalized or treated for asthma attacks between September and December. RS viruses were frequently detected from May to June and from November to December. Enteroviruses were dominant in September. In contrast, rhinoviruses were detected almost all year round. (B) More than half the patients with acute exacerbations of asthma were <3-year-old. Among the three major detected viruses, rhinovirus, RS virus, and enterovirus, enteroviruses were dominant in patients aged ≥3 years. However, there was no change in the levels of rhinoviruses and RS viruses between the two age groups.