

Differential Airway Inflammatory Responses in Asthma Exacerbations Induced by Respiratory Syncytial Virus and Influenza Virus A

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Key Words

Bronchial asthma · Cysteinyl leukotrienes · Influenza virus · Respiratory syncytial virus

Abstract

Background: Although respiratory viral infections cause acute exacerbations of asthma, the inflammatory responses vary depending on the causative virus. The purpose of this study was to compare the inflammatory responses in the airways of acute exacerbations of asthma induced by respiratory syncytial virus (RSV) and influenza A virus. **Methods:** Sputum induction was performed in asthmatic patients with acute exacerbations induced by RSV (n = 6), influenza A (n = 7), and non-upper respiratory infection (URI)-related factors (n = 8). Sputum concentrations of cysteinyl leukotrienes (cysLTs), TNF- α and IFN- γ were measured. **Results:** Sputum cysLTs were significantly higher in RSV-induced exacerbations than in influenza A- and non-URI-induced exacerbations. Sputum TNF- α was significantly higher in influenza A-induced exacerbations than in RSV- and non-URI-induced exacerbations. Sputum IFN- γ was significantly lower in RSV-induced exacerbations than in the others. **Conclusions:** RSV and influenza A cause acute exacer-

bations and have different effects on airway inflammation in asthmatic patients. RSV significantly increased cysLTs, while influenza A significantly increased TNF- α in the airway. The underlying mechanism in virus-induced asthma might depend on the viral species.

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Introduction

Bronchial asthma is a chronic respiratory disease that has recently been increasing worldwide and has a large socioeconomic impact. Several studies have shown that allergic airway inflammation is a key component of asthma [1].

Viral respiratory tract infections represent the most common trigger of the acute exacerbation of asthma in both children and adults [2, 3]. Although the underlying mechanism in virus-induced asthma exacerbations remains unknown, it could be that respiratory viruses increase allergic airway inflammation, wherein a variety of inflammatory molecules and cells are critically involved [4]. Among these molecules, cysteinyl leukotrienes (cysLTs) have attracted attention because they cause

bronchoconstriction, eosinophil migration and an increase in vascular permeability, all of which potentially exacerbate allergic airway inflammation. Several reports have shown that numbers of cysLTs in the airway increase following viral respiratory infections in normal subjects [5, 6] and in subjects with asthma [7]. Respiratory syncytial virus (RSV) is a lower respiratory tract pathogen in infants and in the elderly [8–10]. RSV also frequently exacerbates and potentially induces the development of asthma [11–13]. Influenza virus is another representative lower respiratory tract pathogen that causes severe respiratory infection. In comparison with RSV, influenza exacerbates asthma less frequently in children [14]. This indicates that although both influenza and RSV enhance airway inflammation, they could differ in how they induce asthma, through a pathway that is currently unknown. Previous research showed that some respiratory viruses can produce a distinct cytokine that exacerbates allergic airway inflammation [15]. Since RSV infection produces cysLTs, we hypothesized that RSV produces more cysLTs than influenza in asthmatic patients with acute exacerbations. The purpose of this study was to compare the inflammatory responses in induced sputum obtained from naturally occurring RSV- and influenza-induced acute exacerbations of asthma.

Materials and Methods

Subjects

The eligibility criteria for our study included the presence of a mild asthma exacerbation triggered by either an RSV- or an influenza-confirmed upper respiratory infection (URI) episode or factors other than a URI with no detectable virus. The diagnosis and severity of bronchial asthma were based on the Global Initiative for Asthma (GINA) guidelines [16]. Atopy was defined by a positive skin-prick test using 10 common aeroallergens and/or IgE (RAST). A URI was defined by the presence of two or more symptoms suggestive of upper respiratory tract viral infection, e.g. a runny or stuffy nose, sneezing, a sore throat, hoarseness, red or watery eyes, headache or earache, feeling unwell, malaise, muscle aches, chills, cough, painful swollen neck glands, increased use of handkerchiefs and chest tightness [3]. Mild exacerbation of asthma was defined as an increase in one or more of: wheeze, chest tightness and breathlessness, and the patients showed oxygen saturation >95% on room air by pulse oximetry. Patients were clinically classified as having URI-induced mild asthma exacerbation when they had symptoms of a URI and a mild exacerbation of asthma. Subjects were excluded from the study if they were taking regular oral corticosteroids or leukotriene receptor antagonists, had pulmonary infiltration suggestive of pneumonia or had a contraindication for sputum induction. Between June 2007 and September 2009, 21 subjects diagnosed as having a mild exacerbation of asthma at the outpatient clinics of 4 institutions in the Nagasaki

Prefecture, Japan, agreed to participate in the study. Of these, 13 subjects were further defined as having URI-induced asthma, while the remaining 8 were defined as having non-URI-induced asthma as they had mild exacerbations of asthma with neither URI-related symptoms nor positive results for viral tests. As the study focused on two winter viruses, i.e. influenza and RSV, all URI-induced asthma cases were recruited during the late autumn and winter seasons. In contrast, non-URI-induced asthma cases were not recruited during winter (5 during summer and 3 during spring). The exacerbation of asthma was successfully treated by increasing the doses of rapidly acting β_2 agonists and by parenteral corticosteroids in all subjects. The study was approved by the ethics committee of the Nagasaki University Hospital and written informed consent was obtained from each patient.

Study Design

When subjects attended the hospital, a clinical history was taken and a physical examination performed. Thereafter, in all institutions, serum, nasopharyngeal aspirates (NPA) and induced sputum were obtained from eligible subjects within 2 h of arriving at the hospital. Subjects were retrospectively divided into RSV, influenza or non-URI groups based on the diagnostic criteria described in the next section. Finally, baseline characteristics and concentrations of inflammatory products in induced sputum were compared among the 3 groups.

Diagnosis of RSV and Influenza Infections

RT-PCR was performed to amplify RSV RNA, and influenza viral antigen was detected from NPA. In brief, total RNA was isolated from NPA when the patients were first examined using TRIzol® (Life Technologies Inc., Rockville, Md., USA). Complementary DNA was synthesized using a SuperScript® one-step RT-PCR system with Platinum® Tag DNA polymerase (Invitrogen Life Technologies Inc., Carlsbad, Calif., USA) and amplified using 200 ng of cDNA, with primers complementary to the sequence of RSV nucleocapsid protein mRNA (sense: 5-GCG ATG TCT AGG TTA GGA AGA A-3, antisense: 5-GCT ATG TCC TTG GGT AGT AAG CCT-3). Influenza viral antigen was determined using an immunochromatographic assay (RapidTest® Flu II, Sekisui Medical Co. Ltd., Tokyo, Japan) for influenza A and B. For RSV infection, acute and convalescent serum samples were also tested (SRL Co., Tokyo, Japan). A greater than 4-fold rise in antibody titers was considered to indicate infection.

Sputum Induction and cysLT and Cytokine Assays

For sputum induction, all subjects were instructed to gargle with tap water before sputum collection to reduce the possible effect of contamination. Those who could not cough up sputum received nebulized saline via an ultrasonic nebulizer (NE-U12; OMRON Co, Tokyo, Japan) to induce sputum production. Subjects were instructed to cough deeply after 3- to 5-min intervals, and sputum samples were obtained from each patient. Salivary contamination was minimized by microscopic selection of viscid mucocellular portions of sputum. The sample was diluted with 2 ml of Hank's balanced salt solution containing 1% dithiothreitol (Sigma Chemicals, Poole, UK) and gently vortexed at room temperature. Samples were then centrifuged at 400 g for 15 min at 4°C, and the supernatant was kept at –80°C for subsequent measurements. Sputum levels of cysLTs were measured by enzyme immunoassay (EIA, Cayman Chemical Company, Ann Arbor, Mich., USA), and

Table 1. Patient characteristics

Characteristics	RSV	Influenza A	Non-URI
Number	6	7	8
Age, years*	48.0 (39.4–56.8)	42.0 (34.1–56.9)	44.0 (38.5–50.2)
Sex, male	5 (83.3%)	3 (42.9%)	2 (25.0%)
Atopy	3 (50.0%)	3 (42.9%)	4 (50.0%)
Disease duration, years*	25 (18.8–30.1)	21.0 (12.7–36.8)	24.0 (18.0–27.5)
Maintenance ICS FP equivalent, µg/day*	400.0 (385–477.5)	400.0 (385.6–471.1)	400.0 (200.0–410.0)
Time from symptom onset to first assessment, days*	2.5 (2.1–3.2)	3.0 (2.9–3.9)	3.0 (2.0–3.8)

FP = Fluticasone propionate; ICS = inhaled corticosteroid.
 * Values are shown as medians (IQR).

those of TNF- α and IFN- γ were measured by enzyme-linked immunosorbent assays (ELISA, R&D Systems, Inc., McKinley Place, Nebr., USA). The lower detection limits of cysLTs, TNF- α and IFN- γ were 13, 15.6 and 15.6 pg/ml, respectively. It was originally planned to keep the sputum cellular components snap-frozen in each institute and analyze them later. Unfortunately, the preservation process caused significant cellular damage, and few data were available.

Statistics

Because of the non-normal distributions of the measurements and the relatively small sample size, the parameters are summarized by medians with interquartile ranges (IQR), unless otherwise indicated. Group comparisons were conducted using the Kruskal-Wallis test with Dunn's post hoc test for continuous data, and Fisher's exact test for categorical data. A *p* value of <0.05 was taken as a significant difference.

Results

Patient Characteristics

Among the 13 URI-induced asthma exacerbations, serum antibody titers against RSV increased by more than 4-fold in 6 subjects who were enrolled as having RSV-induced asthma. RSV RNAs were detected in 3 of them and influenza A antigens were detected in 7 subjects who were enrolled as having an influenza A-induced asthma exacerbation. Influenza B antigens were not detected. In the 8 non-URI-induced subjects, 1 was induced by passive cigarette smoking, 2 were induced by cold air and 5 had unknown triggers. RSV RNA and significant increases in serum antibodies against RSV and influenza A and B antigens were not detected in the 8 non-URI-induced subjects. Consequently, the subjects consisted of 21 adult asthmatic patients [6 RSV, 7 influenza A and 8 non-URI;

male:female = 10:11; age 44.0 (37.5–51.7) years]. Importantly, all participants had used inhaled corticosteroids and had received annual influenza vaccines before entry. The baseline characteristics of these patients were comparable, as shown in table 1 (*p* > 0.1 for all parameters).

Sputum cysLT and Cytokine Concentrations

RSV infection significantly increased cysLT concentrations compared to influenza A infection and non-URI triggers [(fig. 1a) RSV 705.0 (446.5–927.0) pg/ml, influenza A 260.0 (224.5–310.3) pg/ml and non-URI 235.0 (213.9–333.4) pg/ml]. In contrast, influenza A infection significantly increased sputum TNF- α compared to RSV infection and non-URI triggers [(fig. 1b) RSV 155.5 (120.5–182.9) pg/ml, IV 220.0 (189.8–281.6) pg/ml and non-URI 135.0 (102.3–188.2) pg/ml]. Sputum IFN- γ was significantly lower with RSV infection than with influenza A infection and non-URI triggers [(fig. 1c) RSV 3,750.0 (3,290.0–4,220.0) pg/ml, influenza A 6,050.0 (5,005.0–6,667.5) pg/ml and non-URI 6,950.0 (4,700.0–7,866.7) pg/ml]. Taken together, these results show that asthmagenic RSV and nonasthmagenic influenza A induce distinct inflammatory molecules in the asthmatic airway. RSV induces cysLTs with weak IFN- γ production, both of which could enhance allergic airway inflammation while influenza A induces proinflammatory TNF- α .

Discussion

The major finding of the study was that two representative respiratory viruses, RSV and influenza A, affected airway inflammation differently in asthmatic patients

and caused acute exacerbations. RSV significantly increased cysLTs, while influenza A significantly increased TNF- α in the airway. Respiratory viral infection is a cause of acute exacerbations of asthma, but the underlying mechanism might vary depending on the causative virus. In a study of children, acute asthma episodes were not increased during most influenza periods, but they were substantially increased during RSV periods [14]. In an elderly population, patients with RSV infections were more likely than those with influenza infections to have bronchospasm requiring bronchodilator therapy [17]. An in vitro study indicated that respiratory viruses, which could induce acute asthma exacerbations (e.g. RSV, parainfluenza virus and rhinovirus), produced more IL-11 from pulmonary stromal cells than other viruses and bacteria that rarely exacerbate asthma [15]. This study also suggests that cysLTs as well as IL-11 could be determinants of virus-induced asthma exacerbations. Although the underlying mechanism of RSV-induced cysLT production has yet to be determined, one in vitro study demonstrated that RSV enhances 5-lipoxygenase expression in human airway epithelium and thus increases LT production [18]. It has also been suggested that RSV might amplify mast cell-nerve interactions to release cysLTs in a rat model [19]. The influenza virus included in this study was only influenza A. Although the subtype of influenza was not identified, it is likely that 2009 pandemic H1N1 was not included, since this study was completed before that spread in Japan. In contrast to seasonal influenza, the 2009 pandemic H1N1 greatly increased the risk of asthma attacks [20]. It would thus be interesting to compare our results with those of influenza B or 2009 pandemic H1N1.

Identification of inflammatory response patterns in induced sputum may reveal the mechanism of virus-induced acute exacerbations of asthma. Irrespective of viral species, Grissell et al. [21] reported that a significant increase in IL-10, an immunoregulatory cytokine, in induced sputum was a feature of viral infections in asthmatic patients. In terms of differential cytokine response among viral species, Sung et al. [22] compared cytokine concentrations in infants with RSV and influenza A infections and found that NPA TNF- α was significantly higher in influenza A than in RSV infections. In agreement with this report, our study showed that TNF- α in induced sputum was significantly higher in influenza A than in RSV infections in adult asthmatics with acute asthma. Thus, while significant increases in the production of cysLTs were observed in patients with RSV infection, proinflammatory cell responses were predominant with

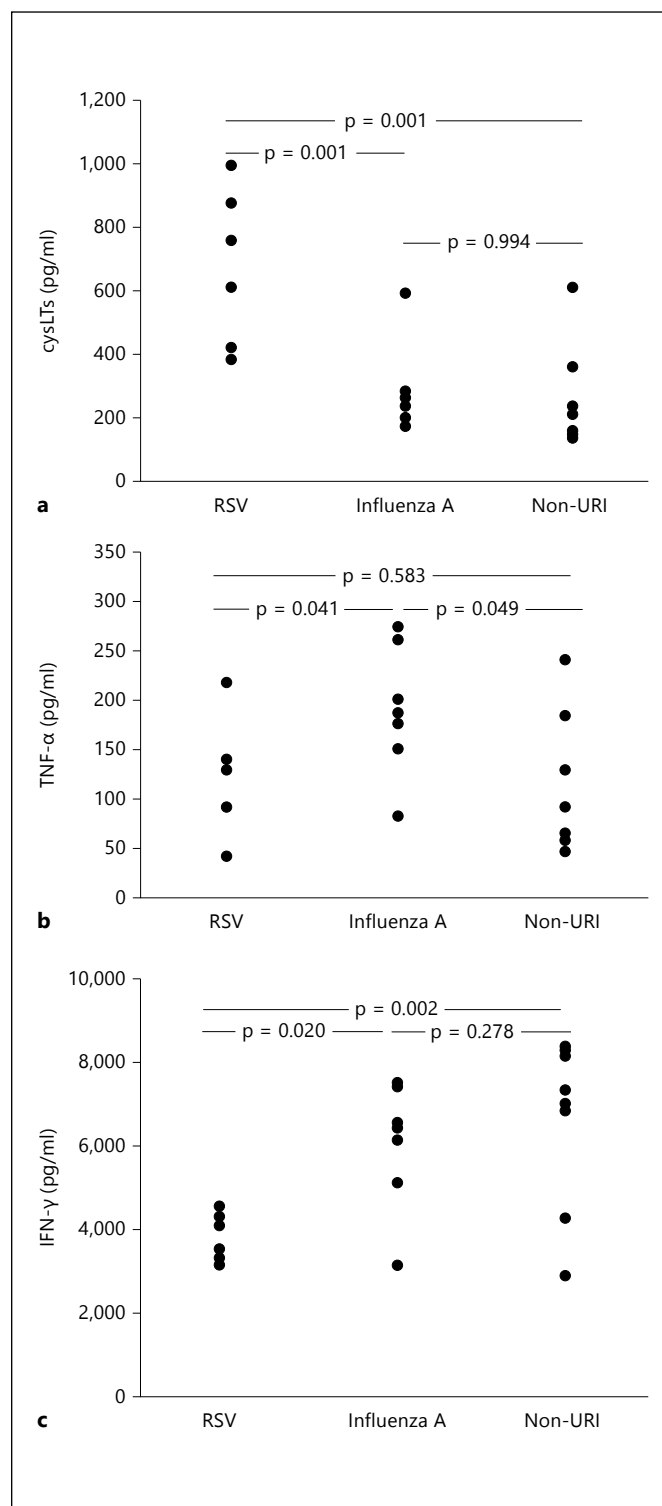


Fig. 1. Inflammatory products in induced sputum. Concentrations of cysLTs (upper), TNF- α (middle) and IFN- γ (lower) in RSV (n = 6), influenza A (n = 7), and non-URI (n = 8) groups.

influenza A infection. Several reports indicated that peripheral blood mononuclear cells isolated from RSV-proven bronchiolitis exhibit a Th2-like cytokine profile and thus promote the development of allergic disorders [23]. Compared with influenza, RSV only weakly induces IFN production in the infected host [24, 25]. In our study, RSV infection also induced a weaker IFN response than influenza A.

The study had several critical limitations. First, with only eligible subjects being included, the number of subjects was small. Second, only RSV and influenza A and B were identified; there might have been simultaneous infection by other respiratory viruses, especially rhinovirus. Third, since almost all participants had received an annual influenza vaccine before entry, the influenza viral

loads could have been smaller than those of RSV, which maybe resulted in lower cysLT production. Finally, and most importantly, only cysLT and limited cytokine concentrations were determined, with a technical problem meaning cellular components could not be determined (as mentioned in the Methods section). More viruses, other inflammatory molecules and cellular components which could be involved in the pathogenesis of allergic airway inflammation should be examined in future research.

In conclusion, despite several critical limitations and a small number of subjects, our study showed that respiratory viruses can exacerbate allergic inflammation in the asthmatic airway through virus-specific pathways. Thus, virus-specific prevention and treatment may be important for virus-induced acute exacerbations of asthma.

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