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Original article

Up-regulation of serum periostin and squamous cell carcinoma antigen levels in infants with acute bronchitis due to respiratory syncytial virus

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Abbreviations:
SCCA, squamous cell carcinoma antigen; RSV, respiratory syncytial virus; mAPI, modified Asthma Predictive Index; Th 2, T-helper 2; IL, interleukin; ATS-DLD, American Thoracic Society-Division of Lung Disease; AD, atopic dermatitis; mPIS, modified Pulmonary Index Score; ELISA, enzyme-linked immunosorbent assay; RT-PCR, reverse transcription-polymerase chain reaction; TSLP, thymic stromal lymphopoietin

ABSTRACT

Background: Periostin and squamous cell carcinoma antigen (SCCA) are involved in the pathogenesis of asthma. Acute bronchitis due to respiratory syncytial virus (RSV) infection during infancy exhibits an asthma-like pathogenesis, suggesting that it may be associated with the subsequent development of asthma. However, the mechanism by which RSV infection leads to development of asthma has not yet been fully elucidated.

Methods: Infants younger than 36 months were enrolled and classified into three groups. Group I included patients hospitalized with RSV-induced bronchitis. These patients were further stratified into two sub-groups according to whether the criteria for the modified Asthma Predictive Index (mAPI) had been met: Group I consisted of mAPI (+) and mAPI (−) patients; Group II included patients with food allergy as a positive control group; and Group III included children with no allergy as a negative control group. Serum periostin and SCCA levels were measured in the groups. This study was registered as a clinical trial (UMIN000012339).

Results: We enrolled 14 subjects in Group I mAPI (+), 22 in Group I mAPI (−), 18 in Group II, and 18 in Group III. In Group I, the serum periostin and SCCA levels were significantly higher during the acute phase compared with the recovery phase. However, no significant differences were found between Group I mAPI (+) and mAPI (−).

Conclusions: The serum periostin and SCCA levels increased during acute RSV bronchitis. Both periostin and SCCA may play a role in the pathogenesis of acute bronchitis due to RSV.

Introduction

Periostin and squamous cell carcinoma antigen (SCCA) are involved in the pathogenesis of asthma. Takayama and Izuhara *et al.* reported for the first time T-helper (Th) 2 cell cytokines, including interleukin (IL)-4 and 13, induced periostin expression in airway

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epithelial cells and fibroblasts.[1] Periostin is an extracellular matrix protein that may play important role in airway remodeling and subepithelial fibrosis in asthma. In fact, a significant expression of periostin has been found in bronchial epithelial cells of children with asthma compared to atopic non-asthmatic and healthy children.[2] SCCA is a protein extracted from cervical cancer,[3] and its expression in response to IL-13 production has also been observed in bronchial epithelial cells. Moreover, the expression of SCCA is increased in the airway tissues of asthmatic children compared to non-asthmatic children.[4] Additionally, increased serum SCCA levels have been found during the acute phase of an asthma exacerbation in children.[5] Thus, the previous studies suggest that both periostin and SCCA are related to the pathogenesis of asthma. However, to the best of our knowledge, there have been no reports indicating that periostin and SCCA play a role in the pathogenesis of viral-induced asthma or the development of asthma in infants.

Acute bronchitis due to respiratory syncytial virus (RSV) in infancy exhibits an asthma-like symptoms suggesting that RSV infection may be associated with the subsequent development of asthma.[6,7] However, the mechanism by which RSV infection facilitates the development of asthma remains poorly understood. We hypothesized that acute bronchitis due to RSV infection during infancy preceded the development of asthma, and further identified the dynamics of serum periostin and SCCA in infants with RSV-induced acute bronchitis.

Methods

Participants

Infants under the age of 36 months were enrolled at Toho University Ohashi Medical Center and Jikei University Daisan Hospital from October 2013 to January 2015.

The subjects were classified into three groups: Group I included patients hospitalized with bronchitis due to RSV infection (RSV rapid test positive, PRIMECHECK®, Alfresa Pharma Corporation, Osaka, Japan, at Toho University Ohashi Medical Center and Imunoace® RSV-Neo, TAUNS, Laboratories, Inc., Shizuoka, Japan, at Jikei University Daisan Hospital). These patients were further stratified into two sub-groups on the basis of whether the criteria for the modified Asthma Predictive Index (mAPI)[8] were met: Group I mAPI (+); and Group I mAPI (−). The criterion for the mAPI was considered at discharge. Group II included patients with food allergy as a positive control group and Group III included children with no allergy as a negative control group. Positive controls are subjects with allergy but without bronchitis due to RSV infection, and negative controls are those with neither allergy nor bronchitis due to RSV infection. There were no patients with food allergy in Groups I and III.

The exclusion criteria consisted of: heart disease[9]; diagnosis of bronchial asthma[4,5,10]; allergic rhinitis[11,12]; kidney disease[13]; bone disease[14]; neuromuscular disease[15]; malignant disease[16]; and the administration of systemic glucocorticosteroids within one month of the study,[17] as these variables may affect the serum periostin and SCCA levels. Bronchial asthma was diagnosed according to the American Thoracic Society-Division of Lung Disease (ATS-DLD) criteria.[18] The previous studies reported increased serum periostin and SCCA levels in patients with atopic dermatitis (AD).[19,20] However, in the present study, we could not exclude AD because the criteria for the mAPI included AD.[8] The severity of dyspnea in the patients from Group I was evaluated using the modified Pulmonary Index Score (mPIS).[21]

This study was approved by the ethics committees of Toho University Ohashi Medical Center (13−83) and Jikei University Daisan Hospital (25-085 7220). Written informed consent was obtained from the parents or legal guardians of each participant. This study was conducted according to the principles expressed in the Declaration of Helsinki. This study was registered as a clinical trial on November 19, 2013 (UMIN000012339).

Measurements of serum periostin, SCCA and IL-13 levels

Blood samples were obtained from all participants. In Group I, the samples were collected on two occasions: 1) during the acute phase of RSV bronchitis (the time of admission); and 2) the recovery phase (at discharge). The serum samples were stored at −80 °C. The serum periostin and SCCA levels were measured using an enzyme-linked immunosorbent assay (ELISA) at the Shino-Test Corporation (Kanagawa, Japan).[22,23] The serum IL-13 levels were measured using a Human IL-13 Quantikine ELISA Kit (R&D Systems, Minneapolis, MN, USA).

Reverse transcription-polymerase chain reaction analysis for RSV

Flocked swabs (FloQSwab™, Copan Flock Technologies, Brescia, Italy) were inserted into the nasopharynx and the specimens were obtained from the patients in Group I. The swabs were placed in cryogenic vials (Nalgene®, Sigma−Aldrich Japan, Tokyo, Japan) with 1.0−1.5 mL RNase inhibitor (RNA later™, Sigma−Aldrich Japan) and stored at −80 °C. Total RNA was extracted and purified from 140 μl of swabs using QIAamp Viral RNA Mini Kit (Qiagen). One microgram of total RNA was reverse transcribed into 50 ng/μL cDNA using SuperScript VILO cDNA Synthesis Kit (Life Technologies) under the recommended conditions. The oligonucleotides used for reverse transcription-polymerase chain reaction (RT-PCR) amplification were based on the published sequences of RSV group A and B strains.[24] PCR was carried out containing 1.25 μL each primer (final concentration of 0.5 μM), 12.5 μL KAPATaq EXtra HotStart Ready-Mix™ with dye (Nippon Genetics, Tokyo, Japan) and 50 ng of cDNA sample. RT-PCR was performed according to the manufacturer's instructions.

Statistical analysis

Data analysis was performed using SAS ver 9.4 (SAS Institute, Cary, NC, USA). Wilcoxon signed rank test and Steel test were used for paired comparison and nonparametric multiple comparison with control groups, respectively. Mann−Whitney U test was also used for comparison between the groups and Spearman's rank correlation coefficient was used for correlation between two variables. A p value < 0.05 was considered to be statistically significant.

At the commencement of this study, we did not have sufficient information concerning serum periostin and SCCA levels in children. However, in adults, serum periostin levels of approximately 28 ng/mL and 8 ng/mL were found in patients with asthma and healthy controls, respectively (the standard deviation was assumed to be 10 ng/mL). With an error of 0.05 and a power of 0.8, the total sample size required was calculated to be 17 patients per group.

Results

Participant characteristics

We enrolled 14 subjects in Group I mAPI (+), 22 in Group I mAPI (−), 18 in Group II, and 18 in Group III. Participant characteristics are summarized in Table 1. The subjects in Group II were 21.7 ± 8.4 months of age and significantly older than those in the other groups (vs. Group I mAPI (−), $p = 0.001$; vs. Group III, $p = 0.007$). The total serum IgE levels in Group II were 404.1 ± 510.2 IU/mL, which was significantly higher than in the

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