Allergol Immunopathol (Madr). 2017;xxx(xx):xxx-xxx

Allergologia et immunopathologia

Sociedad Española de Inmunología Clínica, Alergología y Asma Pediátrica

www.elsevier.es/ai

ORIGINAL ARTICLE

Increased sputum levels of thymus and activation-regulated chemokine in children with asthma not eosinophilic bronchitis☆

M.J. Kim[1], H.S. Lee[1], I.S. Sol, M.N. Kim, J.Y. Hong, K.E. Lee, Y.H. Kim, K.W. Kim, M.H. Sohn*, K.-E. Kim

Department of Pediatrics, Severance Hospital, Institute of Allergy, Brain Korea 21 PLUS Project for Medical Science, Yonsei University College of Medicine, Seoul, Republic of Korea

Received 16 June 2016; accepted 3 December 2016

KEYWORDS
Asthma;
Biomarkers;
Bronchitis;
Chemokine;
Child;
Sputum;
Thymus and activation-regulated chemokine

Abstract
Background: Thymus and activation-regulated chemokine (TARC), a member of the CC chemokine family, plays a crucial role in Th2-specific inflammation. We aimed to determine the concentration of sputum TARC in children with asthma and eosinophilic bronchitis (EB) and its relation with eosinophilic inflammation, pulmonary function, and bronchial hyperresponsiveness.
Methods: In total, 90 children with asthma, 38 with EB, and 45 control subjects were enrolled. TARC levels were measured in sputum supernatants using an ELISA. We performed pulmonary function tests and measured exhaled fractional nitric oxide, eosinophil counts in blood, and sputum and serum levels of total IgE in all children.
Results: Sputum TARC levels were significantly higher in children with asthma than in either children with EB ($p = 0.004$) or the control subjects ($p = 0.014$). Among patients with asthma, sputum TARC concentration was higher in children with sputum eosinophilia than in those without sputum eosinophilia ($p = 0.035$). Sputum TARC levels positively correlated with eosinophil counts in sputum, serum total IgE levels, exhaled fractional nitric, and the bronchodilator response. Negative significant correlations were found between sputum TARC and FEV1/FVC (the ratio of forced expiratory volume in one second and forced expiratory vital capacity) or PC_{20} (the provocative concentration of methacholine causing a 20% decrease in the FEV_1).
Conclusion: Elevated TARC levels in sputum were detected in children with asthma but not in children with EB. Sputum TARC could be a supportive marker for discrimination of asthma from EB in children showing characteristics of eosinophilic airway inflammation.

☆ This study was approved by the Institutional Review Board of Severance Hospital (Seoul, Korea) (protocol No. 4-2004-0036).

* Corresponding author.
E-mail address: mhsohn@yuhs.ac (M.H. Sohn).

[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.aller.2016.12.004

Please cite this article in press as: Kim MJ, et al. Increased sputum levels of thymus and activation-regulated chemokine in children with asthma not eosinophilic bronchitis. Allergol Immunopathol (Madr). 2017. http://dx.doi.org/10.1016/j.aller.2016.12.004

Introduction

Asthma and eosinophilic bronchitis (EB) are considered the most common causes of chronic cough, accounting for ~25% and 13% of cases, respectively.[1,2] Asthma is a chronic inflammatory disorder of the airways characterised by recurrent episodes of airflow obstruction, airway hyper-responsiveness (AHR), and bronchodilator reversibility (BDR).[3] Structural alterations of the airway, namely remodelling, can be frequently observed in patients with asthma, even in children.[4] EB is defined as chronic cough in patients with no symptoms of objective airflow obstruction, sputum eosinophilia, and without AHR.[1] Sputum eosinophilia defined as greater than 3% of all non-squamous cells is always present in EB. Similar to asthma, EB shows eosinophilic infiltration of the epithelium and submucosa and remodelling features on an endobronchial biopsy.[5] Thymus and activation-regulated chemokine (TARC or CCL17) is a CC family chemokine that acts as a ligand of Th2-dominant CC chemokine receptor 4 (CCR4).[6] It has been proven that the CCR4-TARC interaction plays a role in allergic inflammation. CCR4/TARC expression can be increased by stimulation of Th2 cytokines, and TARC can induce selective migration of Th2 but not Th1 cells by triggering CCR4.[7,8] Elevated serum TARC levels have been described in atopic dermatitis and are suggested as a useful clinical biomarker for assessing severity, disease activity, and response to treatment in patients with atopic dermatitis.[9–11] TARC has also been studied in asthma, eosinophilic pneumonia, and allergic bronchopulmonary aspergillosis.[12,13]

In the present study, we hypothesised that TARC expression in induced (see Methods) sputum is elevated in patients with asthma or EB compared to control subjects. We also analysed the possible correlation of sputum TARC concentration with eosinophilic inflammation, pulmonary function, and AHR in children.

Materials and methods

Subjects

A total of 173 children were enrolled in this study; 90 had a diagnosis of asthma in accordance with American Thoracic Society criteria.[14] Thirty-eight children had a diagnosis of EB based on the following criteria: chronic cough lasting more than four weeks without any clinical symptoms related to reversible airway obstruction, such as recurrent wheezing or dyspnoea; no reversible airway obstruction that could be demonstrated by a negative response to a short-acting bronchodilator (change in forced expiratory volume in 1 s [FEV_1] < 12%); the absence of bronchial hyper-reactivity in a methacholine challenge test (PC_{20}; the provocative concentration of methacholine causing a 20% decrease in the FEV_1 > 16 mg/mL); sputum eosinophilia > 3%; and no lung parenchymal aberrations seen on a simple chest radiograph.[15,16] Children treated with systemic corticosteroids due to asthma exacerbation in the preceding month were excluded from the study. The control group consisted of 45 children who had visited the hospital for a general health workup or vaccination and had no history of wheezing, recurrent or chronic diseases, or infection in the preceding two weeks, or hyper-responsiveness to methacholine. Total serum immunoglobulin E (IgE) levels and peripheral blood eosinophil counts were determined at the initiation of the analyses. A specific IgE test was performed with six allergens common in Korea: *Dermatophagoides pteronyssinus*, *Dermatophagoides farina*, egg white, cow milk, German cockroach, and *Alternaria alternata*. Atopy was defined as above 0.7 KUa/L specific IgE to more than one allergen, or 150 IU/mL total IgE. Atopy was also defined as more than one positive skin test result among 12 common aeroallergens, including two types of house dust mites, cat and dog epithelium, and mould and pollen allergens.[17,18] A saline solution was used as a negative control, and a 0.5% histamine HCl solution was used as a positive control. The wheal diameter was measured after 15 min, and a positive reaction was defined as a wheal diameter >3 mm.[19] This study was approved by the Institutional Review Board of Severance Hospital (Seoul, Korea; protocol No. 4-2004-0036). Informed written consent for participation was obtained from parents, with verbal assent from children.

Sputum induction and processing

These procedures were performed as previously described by Yoshikawa et al.[20] All children were instructed to wash their mouths thoroughly with water. They then inhaled a 3% saline solution nebulised in an ultrasonic nebuliser (NE-U12; Omron Co., Tokyo, Japan) at maximum output at room temperature. The children were encouraged to cough deeply at 3-min intervals thereafter. After sputum induction, spirometry was repeated. If FEV_1 fell, the child was required to wait until FEV_1 returned to baseline values. Sputum samples were kept at 4 °C for no longer than 2 h before further processing. A portion of the samples was diluted with a phosphate-buffered saline solution containing 10 mmol/L of dithiothreitol (WAKO Pure Chemical Industries, Ltd., Osaka, Japan) for cell counting and was gently vortexed at room temperature for 20 min. After centrifugation at 400 × *g* for 10 min, the cell pellet was resuspended. We performed a sputum viability assay with the trypan blue exclusion method to ensure adequate viability. Total cell counts were obtained with a haemocytometer, and slides were prepared with cytospin (Cytospin3; Shandon, Tokyo, Japan) and stained with the May–Grunwald–Giemsa stain) for differential cell counts. The latter were performed by two observers who were blinded to the clinical details and who counted 400 non-squamous cells.

Quantification of blood eosinophils, serum total IgE, and sputum TARC

Eosinophils were counted automatically (NE-8000 system; Sysmex; Kobe, Japan) in peripheral blood, and the serum total IgE levels were measured (CAP system; Pharmacia-Upjohn; Uppsala, Sweden). Concentration of TARC in induced sputum was individually detected with enzyme-linked immunosorbent assay kits (R&D Systems; Minneapolis, MN, USA). The lower detection limit of the assay was 7.81 pg/mL.

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