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Original article

Elevated uric acid and adenosine triphosphate concentrations in bronchoalveolar lavage fluid of eosinophilic pneumonia

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Abbreviations:
AEP, acute eosinophilic pneumonia; ATP, adenosine triphosphate; BALF, bronchoalveolar lavage fluid; CEP, chronic eosinophilic pneumonia; DAMP, damage-associated molecular pattern molecule; DC, dendritic cell; EDN, eosinophil derived neurotoxin; ELISA, enzyme-linked immunosorbent assay; EP, eosinophilic pneumonia; HP, hypersensitivity pneumonitis; IL, interleukin; ILC2, type 2 innate lymphoid cell; LDH, lactate dehydrogenase; Th, T helper; UA, uric acid

ABSTRACT

Background: Recent evidence has suggested that the innate immune response may play a role in the development of eosinophilic airway inflammation. We previously reported that uric acid (UA) and adenosine triphosphate (ATP), two important damage-associated molecular pattern molecules (DAMPs), activate eosinophil functions, suggesting that these molecules may be involved in the development of eosinophilic airway inflammation. The objective of this study was to measure the concentrations of DAMPs including UA and ATP in the bronchoalveolar lavage fluid (BALF) of patients with eosinophilic pneumonia (EP).
Methods: BAL was performed in patients with EP including acute and chronic eosinophilic pneumonia, and in patients with hypersensitivity pneumonia, and sarcoidosis. UA, ATP, and cytokine concentrations in the BALF were then measured.
Results: The UA concentration was increased in the BALF of EP patients. UA concentrations correlated with eosinophil numbers, and with eosinophil-derived neurotoxin and interleukin (IL)-5 concentrations. Furthermore, the ATP concentration was increased in the BALF of EP patients and ATP concentrations correlated with UA concentrations. Moreover, IL-33 was increased in EP patients and IL-33 concentrations correlated with UA and ATP concentrations.
Conclusions: The UA and ATP concentration was increased in the BALF of EP patients. UA concentrations correlated with eosinophil numbers, and with ATP and IL-33 concentrations. Our findings suggest that DAMPs such as UA and ATP play a role in the pathogenesis of EP.

Introduction

Eosinophilic airway inflammation has been thought to be induced by adaptive immune responses. After antigen presentation by dendritic cells (DCs), T cells are polarized into allergen specific T helper (Th) 2 cells. Th2 cells then produce cytokines such as interleukin (IL)-5 that regulate the recruitment of eosinophils.[1] In support of this mechanism, IL-5-producing T cells are increased in the airway of bronchial asthma.[2,3] However, recent evidences suggested

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that innate immune responses are also involved in the development of eosinophilic airway inflammation. Type 2 innate lymphoid cells (ILC2) and epithelial-related cytokines such as IL-33, IL-25 and thymic stromal lymphopoietin play important roles in this process.[4–6] In mouse models, instillation of IL-33 into the airway can directly induce eosinophilic inflammation even in recombination activating gene 2 knockout mice,[6] suggesting that eosinophilic inflammation can be induced even without adaptive immunity.

Eosinophilic pneumonia (EP) is a disease that is characterized by infiltration of eosinophils into the airway.[7,8] There are several types of eosinophilic pneumonia including acute eosinophilic pneumonia (AEP)[9] and chronic eosinophilic pneumonia (CEP).[10] Although the exact cause and mechanism of EP have not been fully clarified, there are some known causes of EP including certain drugs, chemical fumes, molds, parasites and cigarette smoke.[7,8] Therefore, not only an adaptive immune response but also an innate immune response may play an important role in the pathogenesis of EP.

When cells are stressed or damaged, they release damage-associated molecular pattern molecules (DAMPs), which function as endogenous danger signals that alert the innate immune system to unscheduled cell death and microbial invasion.[11–13] Recently, the role of DAMPs in the activation of eosinophils has also been highlighted.[14–17] Stenfeldt *et al.* reported that damaged epithelial cells can induce eosinophilic migration, degranulation, and cytokine production, probably through the release of DAMPs from damaged cells.[14] Cormier *et al.* reported that signals from necrotic cells can induce eosinophil chemotaxis and degranulation in tumor immunity.[15] Furthermore, we reported that uric acid (UA), an important DAMP, can activate eosinophil functions such as degranulation and cytokine production.[16] We also reported that adenosine triphosphate (ATP), another important DAMP, also induces eosinophil activation.[17] However, the role of these molecules in the pathogenesis of EP has not been fully elucidated.

In this study, we measured the concentration of DAMPs such as UA and ATP in the bronchoalveolar lavage fluid (BALF) of EP patients. We found that the concentration of UA and ATP was increased in the BALF of EP patients. UA concentrations correlated with the number of eosinophils and with eosinophil derived neurotoxin (EDN), IL-5, and eotaxin concentrations. There was also a significant correlation between UA concentrations and ATP and IL-33 concentrations. These findings suggest an important role of DAMPs such as UA and ATP in the pathogenesis of EP.

Methods

Patients

This study included 33 patients with EP (14 with AEP and 19 with CEP), 35 patients with sarcoidosis (who exhibited bilateral hilar lymphadenopathy but not interstitial lung disease), and 6 patients with hypersensitivity pneumonitis (HP) who were admitted to our department from 2003 to 2013 (Table 1, 2). The diagnosis of AEP, as described by Allen *et al.*,[9] was made by the following criteria: acute onset of respiratory failure, diffuse pulmonary infiltrates on chest X-ray films, and an increased number of eosinophils in BALF (>25% of the total cells). The diagnosis of CEP was made according to the criteria by Carrington *et al.*, which included typical clinical features of CEP, dense and multiple foci of consolidation in the peripheral lung fields on chest radiography, increased percentage of eosinophils in BALF, absence of other possible causes, and favorable response to corticosteroid therapy.[10] The time interval from disease onset of AEP and CEP to BAL was 10.3 ± 1.5 days and 36.6 ± 2.9 days, respectively. This study was performed with the approval of the Institutional Review Board of Saitama Medical University Hospital and written informed consent was obtained from patients.

BALF

A bronchofiberscope (Olympus, Tokyo, Japan) was wedged into a subsegmental bronchus of either the right middle lobe or the left lingula, and lavage was performed by a combination of infusion of 50 ml of saline and subsequent gentle aspiration, repeated four times. Fluid recovered from the third and fourth lavages was used as BALF specimens. Cytocentrifuged slides were stained with May-Giemsa to determine cell differential counts. BALF supernatants were immediately stored at −80 °C until analysis. Patients for whom the percentage of recovery of BALF was less than 50% were excluded from this study and therefore, the percentage of recovery of BALF was more than 50% in all patients. There was no difference in recovery rate between EP, sarcoidosis, and HP (data not shown).

Measurement of ATP, UA, albumin, lactate dehydrogenase (LDH), and cytokines/chemokines

ATP concentrations in BALF were measured using an ATP Determination Kit (BioAssay Systems, Hayward, CA, USA) and a luminometer as described previously.[16] UA concentrations were measured by a uricase assay using an automatic clinical chemistry analyzer (LABOSPECT 008, Hitachi, Tokyo, Japan). Albumin concentrations in BALF or serum, and LDH activity in BALF were measured using LABOSPECT 008. IL-5, IL-33, and eotaxin concentrations in BALF were measured using enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN, USA) or Bio-Plex cytokine assay kits (Bio-Rad, Mississauga, Canada). EDN concentrations in BALF were measured using an ELISA (Medical and Biological Laboratory, Nagoya, Japan).

Statistical analysis

Values are expressed as means ± SEM. For statistical analyses, Student's *t*-test or the Mann–Whitney *U*-test was used for paired comparisons, after checking for normality using the Shapiro–Wilk

Table 1
Patient characteristics.

	Sarcoidosis	HP	EP
Number	35	6	33
Age	49.0 ± 1.7	54.7 ± 2.6	47.5 ± 1.8
Sex (man/female)	9/26	4/2	14/19
BAL			
White blood cell ($\times 10^5$/ml)	3.3 ± 0.2	9.4 ± 1.0	11.5 ± 0.8
Macrophage (%)	57.7 ± 1.6	42.1 ± 4.7	23.1 ± 1.8
Neutrophil (%)	2.2 ± 0.3	9.7 ± 3.1	7.0 ± 1.2
Lymphocyte (%)	40.6 ± 1.6	46.9 ± 6.0	27.5 ± 2.2
Eosinophil (%)	0.3 ± 0.0	1.4 ± 0.2	42.0 ± 2.0

Table 2
Characteristics of AEP and CEP patients.

	AEP	CEP
Number	14	19
Age	39.2 ± 22.5	53.0 ± 16.6
Sex (man/female)	8/6	6/13
BAL		
White blood cell ($\times 10^5$/ml)	12.3 ± 1.0	9.8 ± 1.0
Macrophage (%)	24.9 ± 2.3	19.3 ± 2.5
Neutrophil (%)	7.3 ± 1.5	6.4 ± 1.7
Lymphocyte (%)	27.8 ± 2.4	26.8 ± 5.0
Eosinophil (%)	39.6 ± 2.6	47.2 ± 2.5