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Increased serum ADAM8 concentration in patients with drug-induced eosinophilic pneumonia-ADAM8 expression depends on a the allergen route of entry

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Summary

Background: ADAM8 (a disintegrin and a metalloprotease 8) has been linked to asthma and eosinophilic pneumonia (EP). ADAM8 cleaves a variety of substrates and is a sheddase for CD23, the low affinity IgE receptor. The concentration of soluble ADAM8 (sADAM8) is increased in bronchoalveolar lavage fluid (BALF) from patients with smoking-induced acute eosinophilic pneumonia (AEP) and chronic eosinophilic pneumonia (CEP), but not drug-induced EP (Drug-EP). In AEP, the BALF sADAM8 concentration significantly correlates with the soluble CD23 concentration (sCD23).

Methods: To evaluate the involvement of ADAM8 in the pathogenesis of eosinophilic pneumonia, we measured the concentrations of sADAM8 and its substrate, soluble CD23 (sCD23), in serum from patients with AEP, CEP, and Drug-EP. We also measured the change in the sADAM8 concentration after a provocation test.

Results: In contrast to the BALF findings, serum sADAM8 concentrations were increased in Drug-EP (mean \pm SEM; 639.6 ± 49.15) and serum ADAM8 levels correlated positively with the serum sCD23 levels in patients with Drug-EP ($P = 0.0080$, $R^2 = 0.8465$). Serum sADAM8 concentrations were also increased in AEP (409 ± 76.91) and CEP (644.7 ± 87.03). Serum ADAM8 concentrations were also elevated after the provocation test.

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Conclusion: Serum ADAM8 concentrations were elevated in Drug-EP, although the sADAM8 concentrations were not increased in the BALF in Drug-EP. Thus, the pathogenesis of AEP and Drug-EP may be distinct with regard to allergen exposure; AEP may be caused by the inhalation of antigens, whereas Drug-EP may be caused by bloodstream antigens. These findings indicate that ADAM8 levels reflect the route of eosinophilic inflammation in EP.

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Introduction

Eosinophils infiltrate the lung tissue, thereby impairing gas exchange and inducing symptoms, such as dyspnea, fever, and cough. This process may be secondary to several factors, including exposure to drugs or parasite migration and fungus, or it may be idiopathic. Eosinophilic pneumonia (EP) is defined as the pulmonary infiltration of eosinophils independent of peripheral blood eosinophilia. This pulmonary disorder includes clinically different types of EP, such as acute idiopathic eosinophilic pneumonia (AEP), chronic eosinophilic pneumonia (CEP), and drug-induced eosinophilic pneumonia (Drug-EP). Although these different forms of EP have been clinically characterized,^{1–4} the pathogenesis of these different forms is not known.

ADAM (a disintegrin and a metalloprotease) family members are implicated in the proteolytic processing of membrane-bound precursors, and modulate cell-cell and cell-matrix interactions. ADAM8 has metalloprotease activity *in vitro* and catalyzes CD23, the low affinity IgE receptor.^{5–7} CD23 has an important role in the modulation of allergic pulmonary inflammation, likely mediated by negative and positive signaling.^{8–12}

ADAM8 is specifically induced in experimental asthma and is highly expressed in human eosinophils.^{6,13} We previously reported that soluble ADAM8 (sADAM8) significantly inhibits experimental asthma.¹⁴ We also demonstrated that sADAM8 levels are increased in bronchoalveolar lavage fluid (BALF) samples from patients with cigarette smoking-induced AEP and CEP, but for reasons that are not clear, ADAM8 is not increased in BALF samples from patients with Drug-EP.¹⁵ In addition, ADAM8 levels correlate positively with soluble CD23 (sCD23) levels in BALF from patients with AEP.¹⁵

In the present study, we aimed to extend our earlier study by measuring serum sADAM8 and sCD23 levels in patients with EP. The findings reported in the present study are the first to demonstrate that serum sADAM8 concentrations are increased in EP.

Method

Patients

We retrospectively analyzed 22 patients with EP (6 patients with smoking-induced AEP, 8 patients with CEP, and 8 patients with Drug-EP) who were diagnosed at the Oita University Faculty of Medicine Hospital and related hospitals from 1999 to 2006. Characteristics of the patients with EP are summarized in the Table 1. Pulmonary eosinophilia was detected by bronchoalveolar lavage and transbronchial lung biopsy

specimens. AEP was diagnosed according to the criteria described by Allen.¹⁶ Most patients with AEP showed spontaneous improvement, although two patients required corticosteroid therapy for a few days. One of the six patients with AEP had a history of pediatric asthma. In this study, idiopathic AEP was not included. The diagnosis of CEP was made according to the criteria described by Carrington et al.¹⁷ All patients were treated with corticosteroids. The patients with Drug-EP satisfied the following diagnostic criteria: fever or some respiratory symptoms, diffuse pulmonary infiltrates on chest X-ray films, increased percentage of eosinophils in BALF, prompt improvement after cessation of the causative drug, absence of other possible causes, or recurrence of the symptoms with drug challenge. We also studied a control group of 50 patients with non-eosinophilic lung disease; 10 patients with idiopathic pulmonary fibrosis [IPF], 10 patients with sarcoidosis; and 30 healthy volunteers [HV]. Patients with usual interstitial pneumonia associated with collagen vascular diseases were excluded. In all 10 IPF cases, the diagnosis was pathologically confirmed as usual interstitial pneumonia. Sarcoidosis was diagnosed on the basis of typical clinical features and the presence of epithelioid cell granulomas in biopsy specimens from the lung, skin, or lymph nodes. All tests were performed in the context of routine medical care. None of the patients was treated with glucocorticoids before the serum collection was completed. The healthy volunteers provided informed consent.

Provocation test

According to Yasui's method,^{18,19} provocation tests were performed using the same drug and route of administration as in the initial case. Drug provocation tests were performed with careful observation and under informed consent. These tests were started by administering the smallest dosage of the suspected causal drug that could achieve a response, and increasing the dose step-by-step at daily intervals until reaching a normal daily dose or until symptoms occurred (e.g., 10% of a single dose of medicine, then a single dose of medicine, up to a normal daily dose for 3 days). If the same adverse reaction occurred, drug administration was stopped and the provocation test was considered positive, and the next drug was then started. The drug provocation test was judged positive according to Yasui's criteria,¹⁸ modified especially for EP.^{19,20} The current diagnostic criteria for drug-induced pneumonia are two or more of the following (including (1) or (2) and either (3), (4), or (5)); (1) 1 °C increase in body temperature, (2) skin rash, (3) increase in the alveolar-arterial difference in oxygen tension (A-aDO₂) of more than 10 mm Hg, (4) more than 20% increase in the number of WBC or eosinophilia in the peripheral blood, (5) positive conversion of C reactive protein.

Table 1 Subject characteristics.

	AEP	CEP	Drug-EP	HV
Sex (M/F)	(4/2)	(3/5)	(5/3)	(12/18)
Age	21.17 ± 3.08	67.88 ± 4.12	64.25 ± 6.64	39.90 ± 2.22
ADAM8 (pg/ml)	409 ± 76.91	644.7 ± 87.03	639.6 ± 49.15	239.8 ± 23.87
CD23 (U/ml)	27.63 ± 9.12	46.56 ± 9.84	92.75 ± 34.4	16.14 ± 5.5
BAL				
Total cells (×10 ⁵ /ml)	8.25 ± 2.20	13.64 ± 3.83	5.66 ± 2.15	N.D
Eo (%)	54.55 ± 7.25	53.39 ± 8.44	34.10 ± 2.63	N.D
Lym (%)	17.68 ± 4.73	11.28 ± 6.16	15.06 ± 3.59	N.D
Neut (%)	7.03 ± 5.43	4.98 ± 3.22	10.00 ± 5.35	N.D
CD4/8	1.598 ± 0.33	2.52 ± 0.36	1.72 ± 0.55	N.D

AEP, smoking-induced acute eosinophilic pneumonia; CEP, chronic idiopathic eosinophilic pneumonia; Drug-EP, drug-induced eosinophilic pneumonia; IPF, idiopathic pulmonary fibrosis; Sar, sarcoidosis; HV, healthy volunteers.

Data are expressed as mean ± SEM.

The cause of the Drug-EP in four of eight cases was confirmed by the provocation test, and withdrawal of the causative drugs led to a favorable outcome without specific treatment. The four cases were described in previous papers.^{19,20}

Determination of serum sADAM8 and sCD23 levels

Serum sADAM8 and sCD23 concentrations were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's protocol. A Quantikine kit from R & D Systems (Minneapolis, MN) was used to quantify sADAM8. A human sCD23 ELISA kit (Bender MedSystems, Vienna, Austria) was used to quantify CD23. The minimal detectable levels were 5.27 pg/ml (sADAM8) and 6.8 U/ml (sCD23).

Statistical analysis

The Kruskal–Wallis test was used to compare values between more than two groups. In case of a significant difference between groups, differences between pairs of group were analyzed by a Mann–Whitney U test. Correlation

coefficients were determined by Pearson's linear regression analysis between sADAM8 and sCD23. Correlation coefficients between longitudinal changes in ADAM8 concentrations and the delay in obtaining BALF after the clinical onset and provocation test were determined by Spearman's method. A difference was considered significant when the *p*-value was less than 0.05.

Results

sADAM8 and sCD23 concentrations in the serum from patients with various diffuse lung diseases

The serums ADAM8 and sCD23 concentrations were measured by ELISA. sADAM8 concentrations were not significantly elevated in non-eosinophilic conditions, such as IPF (mean ± SEM; 294.6 ± 52.16), sarcoidosis (295.6 ± 26.43), or in HV (239.8 ± 23.87). The sADAM8 concentrations were significantly elevated in AEP (*p* = 0.0395), CEP (*p* = 0.0001), and Drug-EP (*p* < 0.0001), in comparison with HV (Fig. 1a). There was significant difference in serum ADAM8 concentrations between AEP and Drug-EP (*p* = 0.0426) (Fig. 1a). The CD23 concentrations were significantly

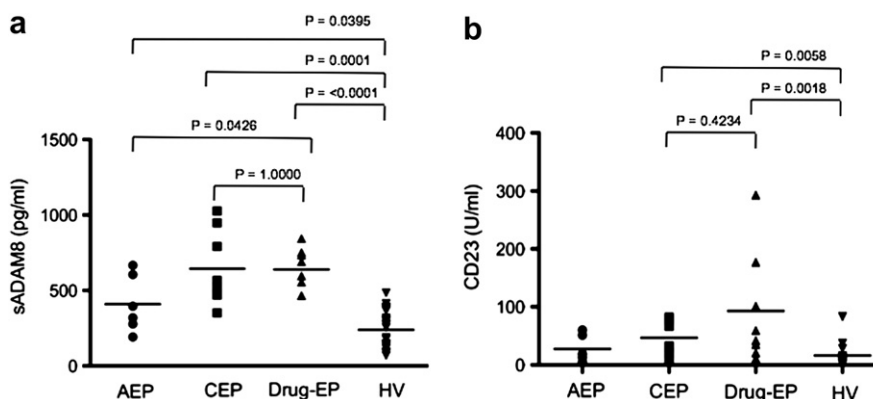


Figure 1 (a) Concentration of ADAM8 in serum obtained from patients with smoking-induced acute eosinophilic pneumonia (AEP), chronic idiopathic eosinophilic pneumonia (CEP), drug-induced pneumonia (Drug-EP), and healthy volunteers (HV). Significant differences are shown at the top. (b) Concentration of CD23 in serum obtained from patients with AEP, CEP, Drug-EP, and HV. Significant differences are shown at the top.

elevated in CEP ($p = 0.0058$) and Drug-EP ($p = 0.0018$), compared with HV (Fig. 1b). Serum CD23 concentrations did not differ between the different types of EP.

Longitudinal changes in ADAM8 concentrations in serum obtained after the clinical onset and provocation test

The serum ADAM8 concentrations differed significantly between the clinical onset and provocation test (Fig. 2). Prednisone treatment could not be responsible for the decline in ADAM8, because all cases improved spontaneously.

Relationship between serum sADAM8 and sCD23 concentrations

There was significant correlation between the sADAM8 and sCD23 concentrations in the serum from patients with Drug-EP ($p = 0.0080$, $R^2 = 0.8465$; Fig. 3), but not AEP and CEP (data not shown), although sADAM8 and sCD23 concentrations were significantly elevated in CEP. These results suggest that ADAM8 was the main sheddase for CD23 in Drug-EP, but not AEP and CEP.

Discussion

ADAM8 is strongly associated with allergic airway inflammation in humans and mice, and recent studies of ADAM8 are beginning to provide insight into its role in asthma and eosinophilic pneumonia pathogenesis. The potential importance of ADAM8 in eosinophil inflammation is also highlighted by a reported microarray analysis of whole lung from mice in which ADAM8 mRNA was upregulated after antigen challenge with ovalbumin.¹³ Eosinophils are a major source of ADAM8 mRNA. Gene-targeted mouse studies revealed that ovalbumin-induced ADAM8 is largely dependent on the signal transduction and activation of STAT-6 and interleukin-4 α -chain.¹³ A recent study identified CD23, L-selectin,²¹ P-selectin glycoprotein ligand-1,²¹ and vascular cell adhesion molecule as specific substrates for the metalloproteinase associated with ADAM8.⁶ We previously reported that sADAM8 has a physiologic role in

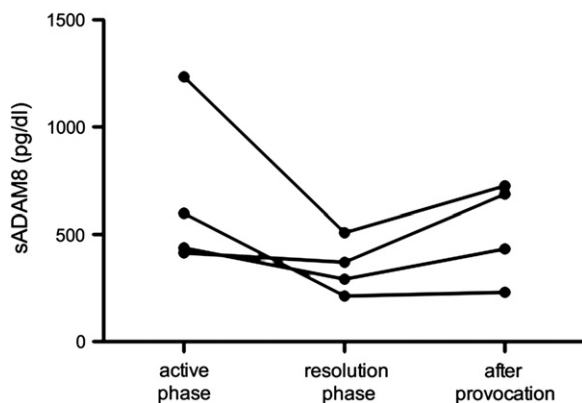


Figure 2 Longitudinal changes in ADAM8 concentration in serum obtained after clinical onset and provocation test in Drug-EP.

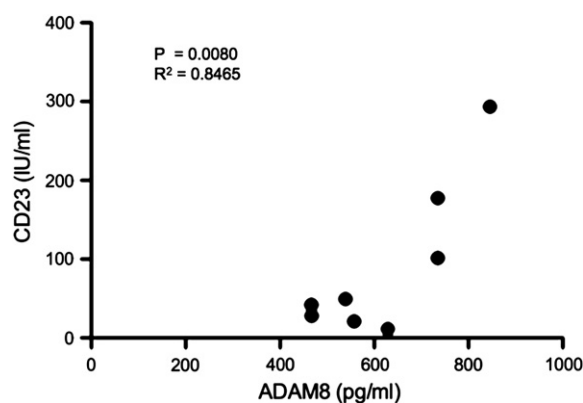


Figure 3 Relationship between ADAM8 and CD23 concentrations in serum from patients with Drug-EP.

protecting against allergic pulmonary disease in experimental murine asthma.¹⁴ ADAM8 concentrations in BALF are significantly elevated in AEP and CEP in comparison with HV.¹⁵ These findings indicate that ADAM8 is associated with allergic lung inflammation in humans.

ADAM8 converts membrane-bound CD23 into a soluble form that might be involved in the regulation of IgE synthesis and the activation of macrophages to release a variety of pro-inflammatory mediators, although the role of soluble CD23 in human allergic lung inflammation is not yet clear. Mice deficient in CD23 produce higher levels of IgE than their wild-type counterparts²² without any effect on B cell or T cell growth and differentiation.^{22–24} Conversely, mice overexpressing membrane CD23 exhibit a weaker IgE response.²⁴ In contrast, CD23 knockout mice do not exhibit an antigen-specific IgE-mediated antibody response.²⁵ Membrane CD23 negatively regulates pulmonary inflammation and acute bronchial hyperresponsiveness.^{8,9} sCD23 acts to upregulate or downregulate the level of IgE produced by activated human B cells, possibly by interacting with or without the CD21/CD19 complex, suggesting a regulatory role for sCD23 in the regulation of IgE levels.^{26,27} sCD23 binding to CD11b/CD18, CD11c/CD18, and α v integrins on monocytes increases their production of pro-inflammatory cytokines.^{10–12}

We previously demonstrated that sADAM8 levels are increased in BALF samples from patients with smoking-induced AEP and CEP, but not in BALF samples from patients with Drug-EP.¹⁵ In contrast to the BALF findings, serum ADAM8 concentrations were significantly higher in Drug-EP compared to AEP. These findings indicate that the regulation of sADAM8 differs between AEP and Drug-EP. Drug-EP is caused by bloodstream allergens, whereas AEP is caused by cigarette smoking (inhalation exposure to chemicals or antigens). The pathogens or allergens related to CEP are unclear. These findings indicate that the induction of ADAM8 expression reflects the route of entry for chemicals or antigens, although it is not clear why certain compounds induce Th2-dominant immune responses. Changes in sADAM8 concentrations were observed longitudinally in serum obtained at clinical onset, during the resolution phase (before the provocation test), and after the provocation test. These results demonstrate that ADAM8 induction parallels drug-induced eosinophilic lung inflammation.

The findings of the present study are the first to demonstrate changes in serum sADAM8 levels after allergen challenge. Drug-EP often has an acute onset and is difficult to distinguish from AEP. Together with these clinical findings, evaluation of ADAM8 in BALF and serum may contribute to distinguishing between Drug-EP from AEP.

We previously demonstrated that sCD23 concentrations are significantly elevated in BALF only in patients with AEP. In addition, the sADAM8 and sCD23 concentrations are significantly correlated in BALF from patients with AEP, but not CEP and Drug-EP.¹⁵ In contrast, in the present study, serum sCD23 concentrations were not elevated in AEP. The serum sCD23 concentrations were significantly elevated in patients with Drug-EP compared with HV. Serum ADAM8 and CD23 concentrations correlated significantly in patients with Drug-EP, but not in those with AEP or CEP. These findings suggest that ADAM8 regulates the degradation of CD23 in the serum in Drug-EP; on the other hand, a protease other than ADAM8, such as ADAM10 or other proteases²⁸ might regulate the degradation of CD23 in AEP and CEP.

The difference in the ADAM8 concentrations between AEP, CEP, and Drug-EP, as well as the relation of ADAM8 to CD23 concentrations indicates that the pathogenesis of AEP, CEP, and Drug-EP is distinct, although the accumulation of eosinophils in the lungs is a common feature. The pathogenesis of AEP and Drug-EP may be distinct with regard to allergen exposure; AEP is caused by inhalation of chemicals or allergens, whereas Drug-EP is caused by bloodstream antigens. ADAM8 levels reflect the pathogenesis of eosinophilic inflammation in Drug-EP.

This study is the first finding to demonstrate that the antigen exposure route, i.e., inhalation versus and bloodstream exposure, differentially activates inflammatory and structural cells in the lung in association with ADAM8 expression in serum and BALF. This study provides further insight into eosinophilic inflammation and opens new avenues for the recognition of allergic pulmonary disease.

Conflict of interest statement

None of the authors have a conflict of interest to declare in relation to this work.

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