Eur Respir J 2005; 26: 1069–1073
DOI: 10.1183/09031936.05.00106104

SHORT REPORT

Involvement of eicosanoids and surfactant protein D in extrinsic allergic alveolitis

A. Higashi*,#, N. Higashi*,#, T. Tsuburai*, Y. Takeuchi*, M. Taniguchi*, H. Mita*, A. Saito*, K. Takatori¶, K. Arimura# and K. Akiyama*

ABSTRACT: The pathophysiology of extrinsic allergic alveolitis (EAA) involves oxidative lung damage as well as interstitial and alveolar inflammation. Macrophages and mast cells are inflammatory components of EAA that produce both leukotrienes (LTs) and prostaglandin D_2 (PGD_2). In addition, PGD_2 is also produced by the free-radical-catalysed peroxidation of arachidonic acid during oxidative stress. Urinary 8-iso prostaglandin $F_2\alpha$ (8-iso$PGF_2\alpha$) and serum surfactant protein D (SP-D) are considered appropriate biomarkers of oxidative stress and interstitial lung disease activity, respectively. The present study aimed to assess the association of these biomarkers with the pathophysiology of EAA.

Two cases of acute EAA caused by the inhalation of fungi spores were reported. Eight asthmatic patients and six healthy control subjects were also enrolled in the current study.

The serum SP-D and urinary eicosanoid (LTE_4, PGD_2 metabolite (9α,11βPGF_2), 8-iso$PGF_2\alpha$) concentrations markedly increased during the acute exacerbation phase. These concentrations decreased following corticosteroid therapy in the EAA patients. There was a significant correlation between serum SP-D and urinary 9α,11βPGF_2 concentrations in the EAA patients.

In conclusion, although the present study proposes that serum surfactant protein-D and urinary eicosanoids are new biomarkers involved in the various immunological responses in extrinsic allergic alveolitis, further large-scale studies are needed to investigate the role of these compounds, not just as biomarkers, but also as biological potentiators of extrinsic allergic alveolitis.

KEYWORDS: Extrinsic allergic alveolitis, 8-iso prostaglandin $F_2\alpha$, prostaglandin D_2, surfactant protein D

Acute extrinsic allergic alveolitis (EAA) is characterised by oxidative lung damage [1]. During oxidative stress, prostaglandin D_2 (PGD_2) is nonenzymatically produced by the free-radical-catalysed peroxidation of arachidonic acid (isoprostane pathway) [2]. Briefly, isoprostanes are a unique series of PG-like compounds formed by the random oxidation of tissue phospholipids by oxygen radicals [2]. Thus, isoprostanes contain racemic mixtures of E-, D-, F-type and thromboxane-type prostane rings [3, 4]. The racemic D-ring isoprostane (12-isoPGD_2) subsequently undergoes rapid epimerisation to racemic PGD_2 [2].

In contrast, alveolar macrophages and mast cells produce cysteinyl-leukotrienes (CysLTs) and cyclooxygenase-dependent PGD_2 [5, 6]. Alveolar macrophages play a key role in acute EAA [7]. There is also a persistent increase in the number of alveolar mast cells in EAA patients [8]. EAA is categorised as a T-helper1-type disease and interferon (IFN)-γ plays a pivotal role in granuloma formation in EAA [7]. Interestingly, mast cells, which express the Fcγ receptor I after incubation with IFN-γ, can produce PGD_2 and CysLTs even in response to immunoglobulin (Ig)G stimulation [9].

Urinary leukotriene E4 (LTE_4) is now considered to be the most reliable analytical parameter for monitoring the endogenous synthesis of CysLTs [10, 11]. Similarly, urinary 9α,11β prostaglandin F_2 (9α,11βPGF_2) is a relatively stable PGD_2 metabolite and an appropriate indicator of mast cell activation [5]. Of the isoprostanes, 8-iso prostaglandin $F_2\alpha$ (8-iso$PGF_2\alpha$) is the best-characterised isomer and urinary 8-iso$PGF_2\alpha$ is considered the most accurate indicator of oxidant stress [2]. Taking this into account, it was

AFFILIATIONS
*Clinical Research Center, National Sagamihara Hospital, Sagamihara, #Dept of Neurology and Geriatrics, Kagoshima University Graduate School of Medical and Dental Science, Kagoshima, and ¶Division of Microbiology, National Institute of Health Sciences, Tokyo, Japan.

CORRESPONDENCE
N. Higashi
Dept of Allergy
Kagoshima Univeristy Graduate School of Medical and Dental Science
8-35-1 Sakuragaoka
Kagoshima 890-8520
Japan
Fax: 81 992657164
E-mail: Noritaka.Higashi@ki.se

Received:
September 10 2004
Accepted after revision:
August 15 2005

European Respiratory Journal
Print ISSN 0903-1936
Online ISSN 1399-3003

hypothesised that patients with acute EAA show increased concentrations of urinary eicosanoids (CysLTs, 9α,11βPGF$_2$ and 8-isoPGF$_2$α).

Surfactant protein D (SP-D), which is produced by alveolar type II and Clara cells [12], is an important regulatory molecule in both pulmonary surfactant homeostasis and first-line defence mechanisms against microbial or allergen challenges [13]. Krebs von den Lungen-6 (KL-6) is also produced by alveolar type II cells [14]. The measurements of serum SP-D and KL-6 concentrations contribute to the early diagnosis of interstitial lung disease (ILD) [12, 14]. EAA is an acute ILD [7]. Previous studies have demonstrated significant increases in SP-A and KL-6 concentrations in the bronchoalveolar lavage fluid (BALF) of acute EAA patients [15]. Although plasma SP-D concentration is one of the most appropriate prognostic parameters of acute respiratory distress syndrome (ARDS) [16], knowledge of the serum SP-D profile of acute EAA patients is limited [17].

The present study aims to assess the association of these biomarkers with the pathophysiology of EAA.

METHODS

Case reports

Case 1

A 61-yr-old, nonsmoking female suffered from summer-type EAA caused by *Trichosporon asahii*, the most prevalent cause of EAA in Japan [18]. Cell counts revealed that 58.9% of total BALF cells were lymphocytes and the CD4/CD8 ratio of lymphocyte surface markers was 0.6. In addition, transbronchial lung biopsy specimens exhibited lymphocytic alveolitis with granulomas. The subject was diagnosed positive for precipitin to *T. asahii* by double immunodiffusion analysis. The positive findings were confirmed during a provocation test, following which the patient was allowed to return home.

Case 2

A 48-yr-old, nonsmoking female suffered from occupational EAA caused by *Aspergillus niger*, predominantly isolated from house dust in her workplace (a linen room). In addition to being strongly positive for precipitins to both house dust and *A. niger* extract, determined by double immunodiffusion analysis, the patient was also positive for a serum-specific IgG antibody against *A. niger* (10.8 mg·dL^{-1}). High-resolution computed tomography of the patient's chest revealed supportive radiographic findings [7]. The results from both cases are shown in table 1.

Both EAA patients fulfilled the American-European Consensus Conference criteria for ARDS (table 1) [19]. Intensive corticosteroid treatment (*i.v.* administration of 1,000 mg·day^{-1} methylprednisolone for 3 days, followed by oral administration of 0.5 mg·kg^{-1} prednisone) was tapered over the 7-week period, resulting in gradual improvements of both clinical symptoms and radiographic findings.

Control subjects

Eight (six female) stable asthmatic patients (mean age (range) 58 (33–73) yrs) were enrolled as diseased control subjects. Six (three female) healthy control subjects (44 (29–58) yrs) were also enrolled for comparative analysis of urinary eicosanoid data. All the subjects were nonsmokers. Permission to conduct the study was obtained from the Ethics Committee of the National Sagamihara Hospital (Japan) and all participating subjects gave informed consent.

Measurements

Serum and spot urine samples were collected between 09:00–11:00 h. In the case of subjects with acute EAA, urine samples were collected on admission and following therapy. The samples were analysed for KL-6, SP-D and eicosanoid concentrations by methods previously described [10, 12, 17, 20]. Double immunodiffusion analysis of precipitating antibodies against 18 different fungal species was performed according to the Ouchterlony method. A specific IgG antibody against *A. niger* was performed utilising the liquid-phase immunoassay AlaSTAT microplate system (Diagnostic Products Corporation, Los Angeles, USA).

Data analysis

Serum and urinary data were expressed as mean and median, respectively. Relationships were analysed using Spearman's rank correlation test. A p-value <0.05 was regarded as statistically significant.

RESULTS

Serum KL-6 and SP-D concentrations were markedly higher in the acute EAA patients than in the eight asthmatic patients (table 1). As shown in figure 1, urinary eicosanoid concentrations in the acute EAA patients (LTE$_4$: 420 and 185 pg·mg^{-1}-creatinine; 9α,11βPGF$_2$: 658 and 382 pg·mg^{-1}-creatinine; 8-isoPGF$_2$α: 393 and 537 pg·mg^{-1}-creatinine) were markedly higher than in the healthy control subjects (LTE$_4$: 45 pg·mg^{-1}-creatinine; 9α,11βPGF$_2$: 43 pg·mg^{-1}-creatinine; 8-isoPGF$_2$α: 187 pg·mg^{-1}-creatinine). Median values

TABLE 1 Serum Krebs von den Lungen-6 (KL-6) and surfactant protein D (SP-D) concentrations on admission

Subject	Causative organism	$P_{a,O_2}/F_{i,O_2}$ ratio	Serum	
			KL-6 U·mL^{-1}	SP-D ng·mL^{-1}
Case 1	*Tricosporon asahii*	91.4	6090	428
Case 2	*Aspergillus niger*	200	5770	396
Asthma group#			252±70	39±14

Data are presented as mean±SD. P_{a,O_2}: partial pressure of arterial oxygen; F_{i,O_2}: inspiratory oxygen fraction. #: n=8.

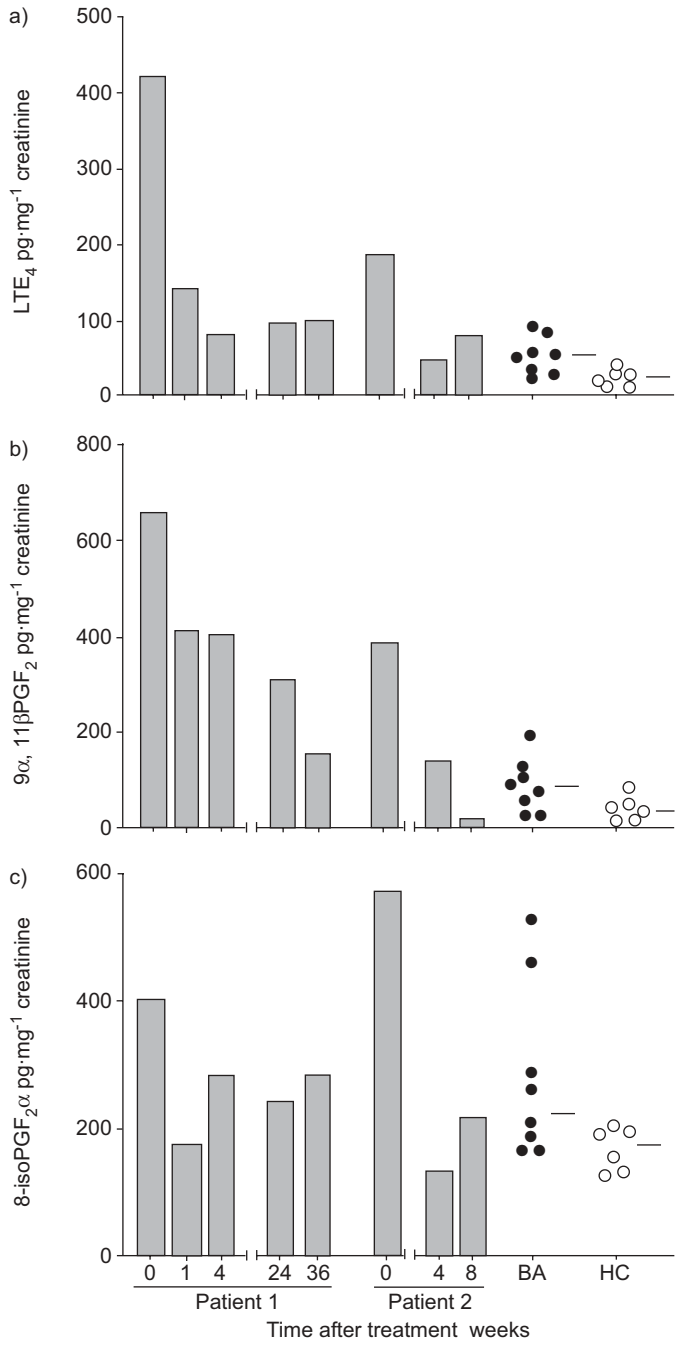

FIGURE 1. a) Urinary leukotriene E_4 (LTE_4), b) 9α,11β prostaglandin F_2 (9α,11βPGF_2), and c) 8-iso prostaglandin F_2α (8-isoPGF_2α) concentrations in the extrinsic allergic alveolitis patients. BA: asthmatic patients; HC: healthy control subjects. Horizontal bars indicate median values.

of urinary LTE_4, 9α,11βPGF_2 and 8-isoPGF_2 concentrations in the asthmatic patients were 55, 79 and 235 pg·mg^{-1}-creatinine, respectively. The serum SP-D, KL-6 and urinary eicosanoid concentrations decreased following corticosteroid therapy in the EAA patients (figs 1 and 2). There was a significant correlation between serum SP-D and urinary 9α,11βPGF_2 concentrations in the EAA patients ($p<0.05$, $rs=1$; fig. 2).

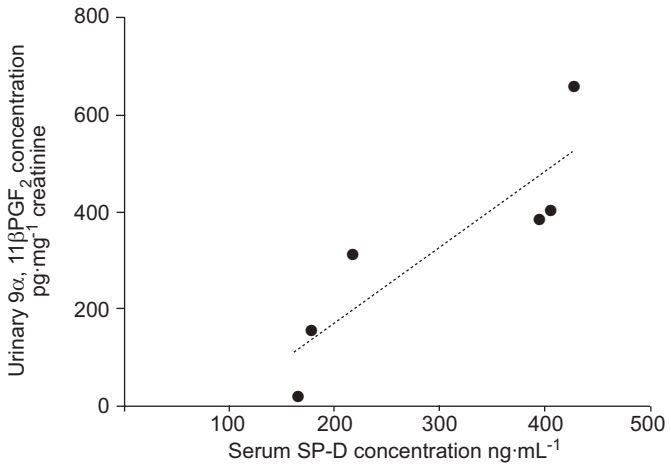

FIGURE 2. A significant correlation between serum surfactant protein D (SP-D) and urinary 9α,11β prostaglandin F_2 (9α,11βPGF_2) concentration was found in the extrinsic allergic alveolitis patients. $rs=1$; $p<0.05$.

DISCUSSION

The present authors demonstrated, for the first time, that acute EAA is characterised by eicosanoid overproduction. Although increased urinary LTE_4 concentration has been reported in patients with ARDS [21], to the current authors' knowledge this is the first report demonstrating a PGD_2 overproduction in patients with ARDS or even acute EAA. The CysLT and PGD_2 overproduction may well be associated with the increased cyclooxygenase activity of both alveolar macrophages [6] and mast cells [5]. However, the higher urinary 9α,11βPGF_2 concentrations in EAA patients are mainly considered to be a reflection of mast cell activation [5, 22]. Interestingly, mast cells activated by aggregated IgG, following IFN-γ-induced up-regulation of Fcγ receptor I, can produce both PGD_2 and CysLTs [9], which is consistent with the pathophysiology of EAA.

8-isoPGF_2α is an accurate biomarker of oxidative stress *in vivo* [2]. The present study demonstrated that urinary 8-isoPGF_2α concentration is increased in acute EAA patients, suggesting a central role for oxidant stress in the pathogenesis of acute EAA. The isoprostane pathway also contributes to the PGD_2 overproduction in acute EAA [2], although the current approach was unable to ascertain its relative contribution to PGD_2 production. It has recently been discovered that isoprostanes containing D- or E-type prostane rings are excreted into the urine as conjugates with *N*-acetyl cysteine sulfoxide, suggesting that these metabolites may be used as biomarkers to estimate whole-body production of D- or E-type isoprostanes [23]. Future experiments using this methodology will hopefully provide even more answers.

Increased 8-isoPGF_2α concentrations have been reported in the breath condensate of patients with ARDS [24], ILD [25] and asthma [26]. Recently, WOOD *et al.* [27] demonstrated that despite high variability, sputum 8-isoPGF_2α concentrations were significantly increased in patients with severe persistent asthma. The present study also demonstrated similar findings in that the two asthmatic patients with extremely high urinary 8-isoPGF_2α concentrations (458 and 525 pg·mg^{-1}-creatinine,

respectively) were characterised by severe persistent asthma and hypereosinophilia (11.2 and 17.2%, respectively).

These eicosanoids are also known to possess various other biological activities, such as being potent constrictors of pulmonary vascular smooth muscle and causing plasma exudation [4–6]. Although the full extent of the biological activity of the eicosanoids in acute EAA remains to be determined, the various components of the eicosanoid metabolic pathways may become therapeutic targets in acute EAA.

Consistent with a previous case report by TANAKA *et al.* [17], serum SP-D concentrations in the acute EAA patients were markedly increased. In contrast, serum SP-D concentrations in the asthmatic patients were low, which is in accordance with data by KOOPMANS *et al.* [28] Interestingly, the serum SP-D concentrations subsequently decreased and showed a significant correlation with urinary $9\alpha,11\beta PGF_2$ concentrations in the EAA patients. Serum SP-D is a biomarker of ILD activity [12] and SP-D plays a protective role in pulmonary inflammation [13]. PGD_2 and its metabolite, 15-d-PGJ_2, have the potential to serve as downregulators of lung injury induced by bleomycin [29]. Taken together, these findings suggest that both SP-D and PGD_2 appear to be important regulatory factors in the pathophysiology of EAA.

In conclusion, the present study proposes that serum surfactant protein D and urinary eicosanoids are new biomarkers involved in various immunological responses in extrinsic allergic alveolitis. Further large-scale studies are needed to investigate the role of these compounds, not just as biomarkers, but also as potentiators of extrinsic allergic alveolitis.

REFERENCES

1 Calhoun WJ. Enhanced reactive oxygen species metabolism of air space cells in hypersensitivity pneumonitis. *J Lab Clin Med* 1991; 117: 443–452.
2 Morrow JD, Hill KE, Burk RF, Nammour TM, Badr KF, Roberts LJ 2nd. A series of prostaglandin F2-like compounds are produced *in vivo* in humans by a non-cyclooxygenase, free radical-catalyzed mechanism. *Proc Natl Acad Sci USA* 1990; 87: 9383–9387.
3 Morrow JD, Chen Y, Brame CJ, *et al.* The isoprostanes: unique prostaglandin-like products of free-radical-initiated lipid peroxidation. *Drug Metab Rev* 1999; 31: 117–139.
4 Morrow JD, Roberts LJ. The isoprostanes: their role as an index of oxidant stress status in human pulmonary disease. *Am J Respir Crit Care Med* 2002; 166: S25–S30.
5 Dahlen SE, Kumlin M. Monitoring mast cell activation by prostaglandin D_2 *in vivo*. *Thorax* 2004; 59: 453–455.
6 Holgate ST, Peters-Golden M, Panettieri RA, Henderson WR Jr. Roles of cysteinyl leukotrienes in airway inflammation, smooth muscle function, and remodeling. *J Allergy Clin Immunol* 2003; 111: Suppl. 1, S18–S34.
7 Patel AM, Ryu JH, Reed CE. Hypersensitivity pneumonitis: current concepts and future questions. *J Allergy Clin Immunol* 2001; 108: 661–670.
8 Laviolette M, Cormier Y, Loiseau A, *et al.* Bronchoalveolar mast cells in normal farmers and subjects with farmer's lung. Diagnostic, prognostic, and physiologic significance. *Am Rev Respir Dis* 1991; 144: 855–860.
9 Woolhiser MR, Brockow K, Metcalfe DD. Activation of human mast cells by aggregated IgG through FcgammaRI: additive effects of C3a. *Clin Immunol* 2004; 110: 172–180.
10 Higashi N, Taniguchi M, Mita H, Osame M, Akiyama K. A comparative study of eicosanoid concentrations in sputum and urine in patients with aspirin-intolerant asthma. *Clin Exp Allergy* 2002; 32: 1484–1490.
11 Kumlin M. Measurements of leukotrienes in the urine: strategies and applications. *Allergy* 1997; 52: 124–135.
12 Honda Y, Kuroki Y, Matsuura E, *et al.* Pulmonary surfactant protein D in sera and bronchoalveolar lavage fluids. *Am J Respir Crit Care Med* 1995; 152: 1860–1866.
13 Ikegami M, Hull WM, Yoshida M, Wert SE, Whitsett JA. SP-D and GM-CSF regulate surfactant homeostasis *via* distinct mechanisms. *Am J Physiol Lung Cell Mol Physiol* 2001; 281: L697–L703.
14 Kohno N, Awaya Y, Oyama T, *et al.* KL-6, a mucin-like glycoprotein, in bronchoalveolar lavage fluid from patients with interstitial lung disease. *Am Rev Respir Dis* 1993; 148: 637–642.
15 Hamm H, Luhrs J, Guzman y Rotaeche J, Costabel U, Fabel H, Bartsch W. Elevated surfactant protein A in bronchoalveolar lavage fluids from sarcoidosis and hypersensitivity pneumonitis patients. *Chest* 1994; 106: 1766–1770.
16 Eisner MD, Parsons P, Matthay MA, Ware L, Greene K. Plasma surfactant protein levels and clinical outcomes in patients with acute lung injury. *Thorax* 2003; 58: 983–988.
17 Tanaka H, Sugawara H, Saikai T, Tsunematsu K, Takahashi H, Abe S. Mushroom worker's lung caused by spores of *Hypsizigus marmoreus* (Bunashimeji): elevated serum surfactant protein D levels. *Chest* 2000; 118: 1506–1509.
18 Matsunaga Y, Usui Y, Yoshizawa Y. TA-19, a novel protein antigen of *Trichosporon asahii*, in summer-type hypersensitivity pneumonitis. *Am J Respir Crit Care Med* 2003; 167: 991–998.
19 Bernard GR, Artigas A, Brigham KL, *et al.* The American-European Consensus Conference on ARDS. Definitions, mechanisms, relevant outcomes, and clinical trial coordination. *Am J Respir Crit Care Med* 1994; 149: 818–824.
20 Morrow JD, Zackert WE, Yang JP, *et al.* Quantification of the major urinary metabolite of 15-F_2t-isoprostane (8-iso-PGF_2alpha) by a stable isotope dilution mass spectrometric assay. *Anal Biochem* 1999; 269: 326–331.
21 Bernard GR, Korley V, Chee P, Swindell B, Ford-Hutchinson AW, Tagari P. Persistent generation of peptido leukotrienes in patients with the adult respiratory distress syndrome. *Am Rev Respir Dis* 1991; 144: 263–267.
22 Cai Y, Bjermer L, Halstensen TS. Bronchial mast cells are the dominating LTC4S-expressing cells in aspirin-tolerant asthma. *Am J Respir Cell Mol Biol* 2003; 29: 683–693.
23 Milne GL, Gao L, Porta A, Zanoni G, Vidari G, Morrow JD. Identification of the major urinary metabolite of the highly reactive cyclopentenone isoprostane 15-A_{2t}-isoprostane *in vivo*. *J Biol Chem* 2005; 280: 25178–25184.

24 Carpenter CT, Price PV, Christman BW. Exhaled breath condensate isoprostanes are elevated in patients with acute lung injury or ARDS. *Chest* 1998; 114: 1653–1659.
25 Montuschi P, Ciabattoni G, Paredi P, *et al.* 8-Isoprostane as a biomarker of oxidative stress in interstitial lung diseases. *Am J Respir Crit Care Med* 1998; 158: 1524–1527.
26 Montuschi P, Corradi M, Ciabattoni G, Nightingale J, Kharitonov SA, Barnes PJ. Increased 8-isoprostane, a marker of oxidative stress, in exhaled condensate of asthma patients. *Am J Respir Crit Care Med* 1999; 160: 216–220.
27 Wood LG, Garg ML, Simpson JL, *et al.* Induced sputum 8-isoprostane concentrations in inflammatory airway diseases. *Am J Respir Crit Care Med* 2005; 171: 426–430.
28 Koopmans JG, van der Zee JS, Krop EJ, Lopuhaa CE, Jansen HM, Batenburg JJ. Serum surfactant protein D is elevated in allergic patients. *Clin Exp Allergy* 2004; 34: 1827–1833.
29 Ando M, Murakami Y, Kojima F, *et al.* Retrovirally introduced prostaglandin D_2 synthase suppresses lung injury induced by bleomycin. *Am J Respir Cell Mol Biol* 2003; 28: 582–591.