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Plasma fibronectin in asthmatic patients and its relation to asthma attack.

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Abstract

This study investigated the relation between asthma attacks and levels of plasma fibronectin (FN) and serum eosinophilic cationic protein (ECP) in patients with bronchial asthma in order to clarify the role of FN in the airway inflammation of bronchial asthma. Plasma levels of FN were significantly higher ($P < 0.025$) in patients with bronchial asthma than in healthy controls. They were also significantly higher ($P < 0.05$) in non-atopic asthmatics than in atopic asthmatics. Furthermore, plasma FN was lower during the attack than the non-attack stage ($P < 0.025$), and a significant increase of plasma FN was noted ($P < 0.05$) in asthmatics who had more severe and more frequent attacks. Serum levels of ECP were significantly higher during the attack than the non-attack stage ($P < 0.005$). An increase of plasma FN in the non-attack stage after attacks showed a significant correlation ($P < 0.05$) with a decrease of serum ECP. These observations clearly indicate that the decrease in plasma FN associated with attacks is closely related to aggravation of airway inflammation, and that the increase in plasma FN in the non-attack stage reflects chronic airway inflammation. These results suggest that the fluctuation in plasma levels of FN may be one of the factors affecting allergic inflammation and attacks in bronchial asthma.

KEYWORDS: adhesion molecule, asthma attack, bronchial asthma, eosinophil cationic protein, fibronectin

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Original Article

Plasma Fibronectin in Asthmatic Patients
and its Relation to Asthma AttackMasashi Ohke*, Shinya Tada, Mikio Kataoka, Kiyoshi Matsuo,
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This study investigated the relation between asthma attacks and levels of plasma fibronectin (FN) and serum eosinophilic cationic protein (ECP) in patients with bronchial asthma in order to clarify the role of FN in the airway inflammation of bronchial asthma. Plasma levels of FN were significantly higher ($P < 0.025$) in patients with bronchial asthma than in healthy controls. They were also significantly higher ($P < 0.05$) in non-atopic asthmatics than in atopic asthmatics. Furthermore, plasma FN was lower during the attack than the non-attack stage ($P < 0.025$), and a significant increase of plasma FN was noted ($P < 0.05$) in asthmatics who had more severe and more frequent attacks. Serum levels of ECP were significantly higher during the attack than the non-attack stage ($P < 0.005$). An increase of plasma FN in the non-attack stage after attacks showed a significant correlation ($P < 0.05$) with a decrease of serum ECP. These observations clearly indicate that the decrease in plasma FN associated with attacks is closely related to aggravation of airway inflammation, and that the increase in plasma FN in the non-attack stage reflects chronic airway inflammation. These results suggest that the fluctuation in plasma levels of FN may be one of the factors affecting allergic inflammation and attacks in bronchial asthma.

Key words: adhesion molecule, asthma attack, bronchial asthma, eosinophil cationic protein, fibronectin

FN is a glycoprotein with various biological activities, synthesized and secreted by fibroblasts, and widely formed by various cells *in vivo* [1, 2]. Its various biological activities include cell adhesion, accelerated chemotaxis of cells, control of cell differentiation and repair of tissues [3-6]. In its role as a dimer of the 250-kd peptide chain united by a disulfide bond, FN exists at concentrations of 300-350 $\mu\text{g/ml}$ in plasma [7]. In regard to respiratory diseases, increased formation of FN in the lung and the close relation between FN and fibrosis have been reported in numerous cases of interstitial pneumonia [4, 8, 9]. FN is also known to be

deposited on the basement membrane of bronchial mucosa in bronchial asthma [10]. In this study, the plasma level of FN was determined in patients with bronchial asthma, which is characterized by allergic inflammation of the respiratory tract in relation to asthma attacks. In addition, serum ECP was determined simultaneously as an index of eosinophil activation.

Materials and Methods

Subjects. Ninety-five patients with bronchial asthma, including 45 atopic (25 males and 20 females; mean age, 37) and 50 non-atopic (23 males and 27 females; mean age, 56) asthmatics were subjected to this study. The diagnosis of bronchial asthma was based on, first, clinical history of episodic wheeze, breathlessness,

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chest tightness, or cough and, second, on documented variable airflow obstruction from a 20% change in FEV₁ either spontaneously after inhalation of β_2 -agonist or PD₂₀ to methacholine inhalation challenge testing of 12 μ mol. Atopy was defined by positive reaction (> 3 mm skin wheal) to one or more common inhalant aeroallergens on a skin prick test or by positive reaction on a radioallergen sorbent test for specific IgE. Twenty-eight patients were treated with oral corticosteroid, 95 patients were treated with oral theophylline, and 65 patients were treated with β -stimulant. A total of 95 attack episodes, including 68 mild, and 27 moderate and severe attack episodes, were examined [11]. Sixty normal volunteers without allergic diseases were studied as controls (Table 1). The study was approved by the Human Research Committee of Okayama University Medical School. Written consent was obtained from each subject.

Table 1 Subjects

Asthmatic patients	
No. of cases	95
Age (range)	48 years (17-75 years)
Male/female	48/47
Atopy/non-atopy	45/50
Age of atopy/non-atopy	38/56
Current smokers	13
Previous pharmacological treatment	
Prednisolone	28
Theophylline	95
β -stimulant	65
Intensity of asthma attack	
Mild	68
Moderate and severe	27
Frequency of asthma attack	
\leq 1/week	55
> 1/week	40
Severity of asthma	
Mild	71
Moderate and severe	24
Healthy controls	
No. of cases	60
Age (range)	48 years (17-79 years)
Male/female	32/28
Current smokers	15

Blood samples. Peripheral venous blood was taken for measurement of FN and ECP during both attack and non-attack stages. The non-attack stage was

defined by the absence of any clinical manifestation of wheeze, breathlessness, chest tightness, or cough for at least 2 weeks. Three milliliters of blood was collected with a vacutainer containing 4.5 mg of EDTA-2Na and 1,500 units of aprotinin, and then plasma was immediately separated at 4 °C, frozen, and stored at -70 °C until measurement for FN. Using a vacutainer tube with SST gel (Becton Dickinson, Franklin Lakes, NJ, USA), blood was collected and allowed to stand for 60-120 min at room temperature. After centrifugation at 1,500 \times g for 10 min, serum was separated and stored at -70 °C until measurement for ECP.

Determination of FN in plasma. The plasma level of FN was determined using a FN Test Kit (Boehringer Mannheim Co., Mannheim, Germany). Standard solutions in 6 steps from 0 to 1,000 μ g/ml of FN or 10 μ l of sample plasma and 1 ml of anti-FN serum were incubated, and absorbance (O.D. 340 nm) was determined at 1 and 31 min after the start of incubation. Plasma FN concentrations were obtained simultaneously. The results were used to prepare a calibration curve

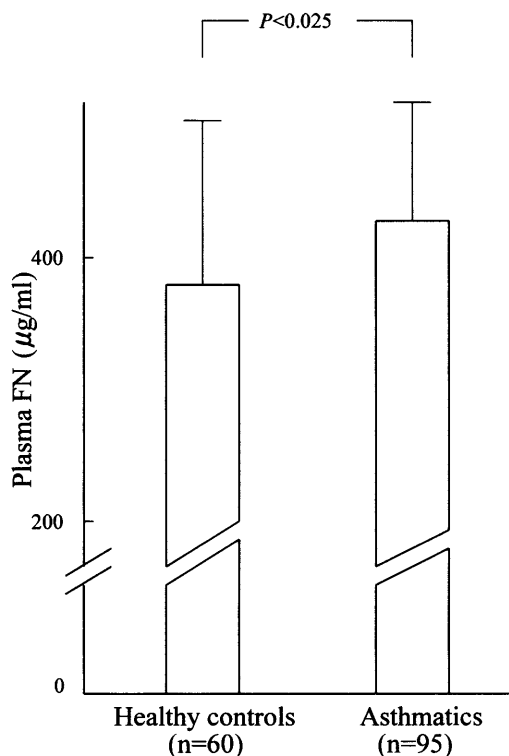


Fig. 1 Plasma FN concentration (mean \pm SD) of asthmatics in the non-attack stage was significantly higher than that of healthy controls ($P < 0.025$).

indicating the differences among the steps of the standard solution.

Serum ECP assay. Serum ECP levels were measured using an ECP RIA Kit (Pharmacia, Uppsala, Sweden). The mixture of ^{125}I -labelled human ECP, $50\ \mu\text{l}$ of serum, and $50\ \mu\text{l}$ of human ant-ECP antiserum was allowed to stand for 3 h at room temperature. Then, 2 ml of the secondary antibody suspension was pipetted to each tube, and the tubes were allowed to stand for 30 min at room temperature, then centrifuged for 10 min at $1,500 \times g$ at 4°C . After removal of the supernatant, the radioactivity of the deposit was detected for 1 min in a gamma counter. The level of ECP in the sample was thus determined in reference to the standard curve.

Statistical analysis. All data are presented as the mean \pm SD (standard deviation). The unpaired Student's *t*-test was used to compare mean values, and correlation coefficients were determined by Pearson's method. Probability values greater than 0.05 were considered to indicate statistical significance.

Results

The plasma level of FN from patients with bronchial

asthma during the non-attack stage was $430 \pm 113\ \mu\text{g}/\text{ml}$, which was significantly higher than that of healthy controls ($385 \pm 113\ \mu\text{g}/\text{ml}$) (Fig. 1, $P < 0.025$).

The plasma level of FN from patients during the non-attack stage was evaluated in regard to intensity and frequency of attacks of bronchial asthma [11]. In patients who had asthmatic attacks more than once a week, the plasma level of FN was significantly higher than that in patients who had less frequent attacks ($P < 0.05$). In regard to the intensity of attacks, the plasma level of FN was higher in asthmatics with moderate and severe attacks than in healthy controls, but was not higher than that in asthmatics with mild attacks (Fig. 2).

The plasma level of FN of asthmatic patients was $375 \pm 89\ \mu\text{g}/\text{ml}$ in the attack, and $430 \pm 113\ \mu\text{g}/\text{ml}$ in the non-attack stage ($P < 0.025$). The plasma level of FN during attacks was significantly lower than that during the non-attack stage. The fluctuation of the plasma FN level between the attack and non-attack stage in each case was about $57\ \mu\text{g}/\text{ml}$ (Fig. 3).

In regard to types of asthma, the plasma level of FN from non-atopic asthmatics ($455 \pm 116\ \mu\text{g}/\text{ml}$) was significantly higher than that from atopic asthmatics ($403 \pm 103\ \mu\text{g}/\text{ml}$) ($P < 0.05$). The plasma level of FN

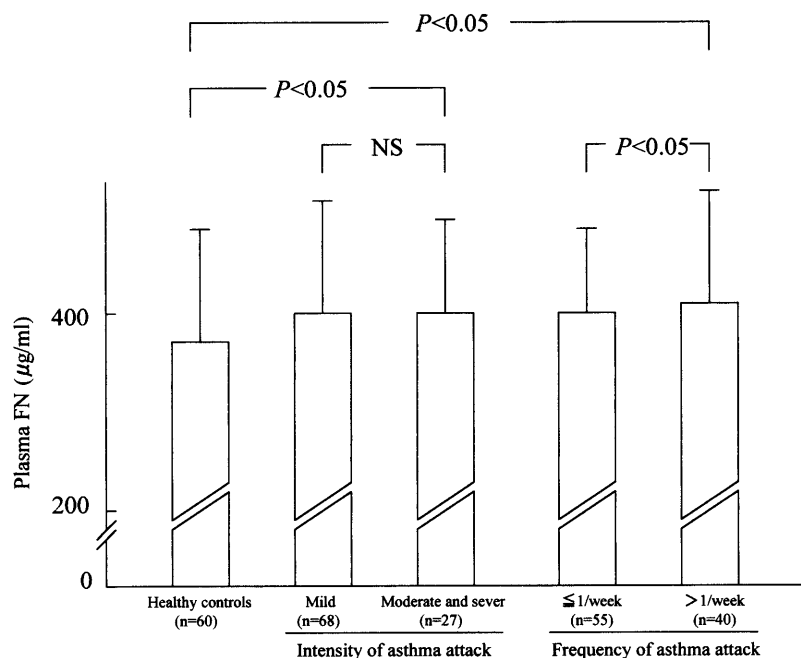


Fig. 2 Plasma FN concentration was higher in asthmatics with moderate and severe attacks than in controls ($P < 0.05$), but was not increased in asthmatics with mild attacks. In patients who had asthmatic attacks more than once a week, plasma FN was significantly higher than in those with less frequent attacks ($P < 0.05$).

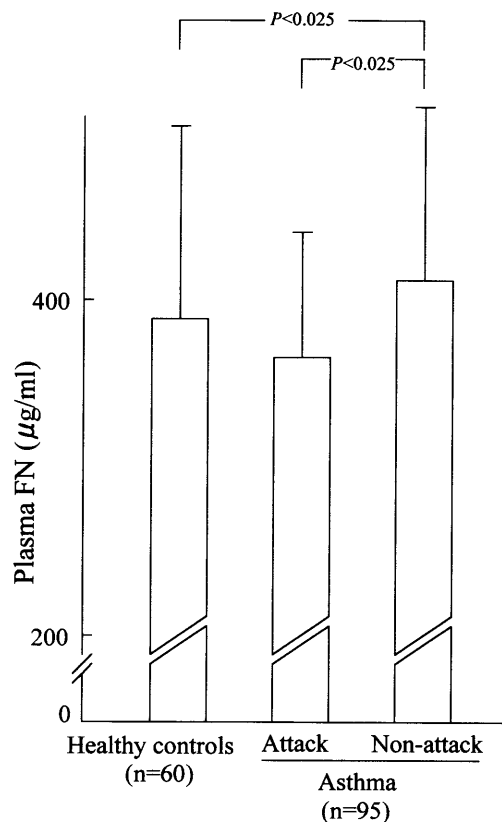


Fig. 3 Plasma FN concentration during attacks was significantly lower than that during the non-attack stage ($P < 0.025$).

from non-atopic asthmatics decreased from 455 ± 116 $\mu\text{g/ml}$ in the non-attack stage to 383 ± 94 $\mu\text{g/ml}$ during attacks (Fig. 4, $P < 0.05$), while there was no significant difference in plasma levels of FN between the attack and non-attack stage in atopic asthmatics.

In asthmatics with moderate and severe attacks, the plasma level of FN decreased significantly from 447 ± 100 $\mu\text{g/ml}$ in the non-attack stage to 347 ± 83 $\mu\text{g/ml}$ during attacks ($P < 0.05$), but no decrease in the plasma level of FN was seen during attacks in asthmatics with mild attacks. Asthmatics with more frequent attacks showed a significant decrease from 460 ± 133 $\mu\text{g/ml}$ during the non-attack stage to 372 ± 104 $\mu\text{g/ml}$ during attacks ($P < 0.05$), but asthmatics with less frequent attacks did not show such a significant decrease.

Serum ECP levels were determined in 27 asthmatics concomitantly with plasma levels of FN during both the attack and non-attack stages. The serum ECP levels (25.5 ± 14.4 $\mu\text{g/l}$) during attacks were significantly higher than those (17.7 ± 13.3 $\mu\text{g/l}$) during the non-

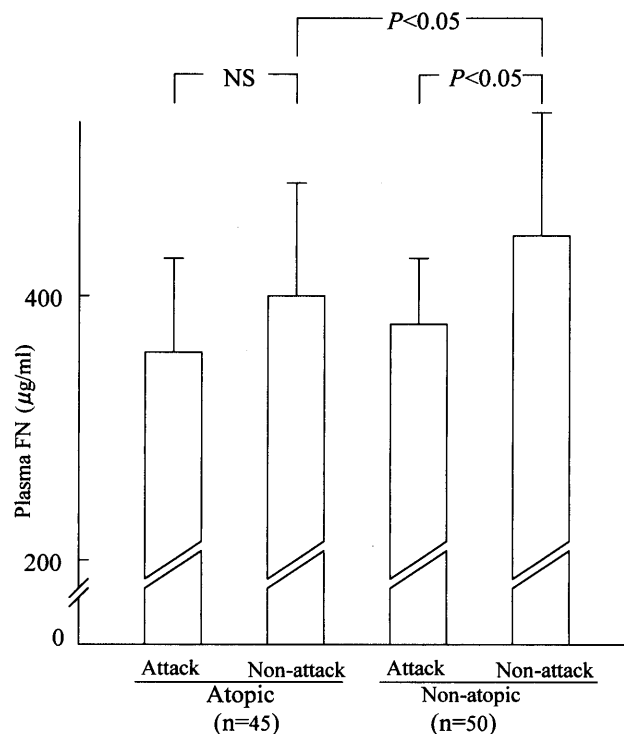


Fig. 4 Plasma FN concentration of non-atopic asthmatics was significantly higher than that of atopic asthmatics ($P < 0.05$). The plasma FN level of non-atopic asthmatics was significantly lower during attacks than in the non-attack stage ($P < 0.05$).

attack stage (Fig. 5, $P < 0.005$).

Relative changes in the levels of plasma FN and serum ECP between the attack and non-attack stages showed a significantly positive correlation (Fig. 6, $P < 0.05$).

Discussion

FN has been reported to be one of the plasma proteins and to exist on the cell surface as a glycoprotein related to modification of cancer cells [1]. Expression of FN on the cell surface is controlled by TGF- β [12], suggesting its participation in the regulation of inflammation. It has been demonstrated that FN is actively involved in eosinophil activation and prolongation of survival time via adhesion of FN with a ligand of VLA-4 [13, 14]. FN also plays roles in the acceleration of LTC₄ formation [15] and the degranulation of mast cells [16, 17]. It has been reported that plasma FN transiently decreased at the critical stage of pneumonia in an animal model, while pneumonia was improved with administration of FN [18,

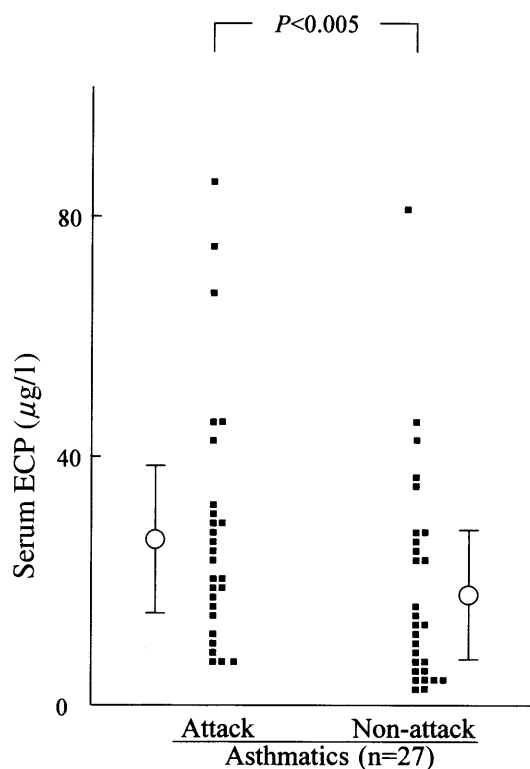


Fig. 5 Serum ECP concentrations in the non-attack stages were significantly higher than those during attacks ($P < 0.005$).

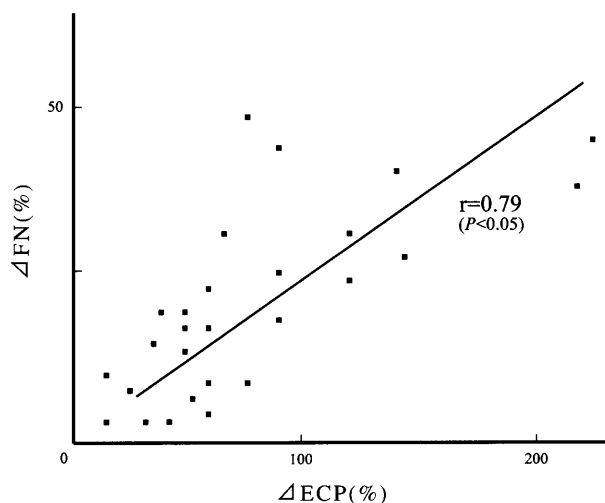


Fig. 6 The relative changes of serum ECP and plasma FN between the attack and non-attack stages were significantly correlated ($P < 0.05$). $\Delta ECP (\%) = (\text{serum ECP on attack} - \text{serum ECP in non-attack}) / \text{serum ECP in non-attack}$; $\Delta FN (\%) = (\text{plasma FN in non-attack} - \text{plasma FN on attack}) / \text{plasma FN in non-attack}$.

19]. Recently, FN was applied clinically to promote the repair of injured tissue [20, 21]. Therefore, FN might play roles in bronchial asthma, which is characterized by allergic and chronic airway inflammation. In this study, we examined the plasma FN levels of asthmatic patients both in the attack and non-attack stages. Plasma FN was significantly higher in the non-attack than in the attack stage, and the level in the non-attack stage was similar to that in healthy controls. In bronchial asthma, it is assumed that FN is maintained at higher levels due to its continuously accelerated production. Non-atopic asthmatics show more remarkable airway remodeling and fibrosis, and a thicker basement membrane of the bronchial mucosa than atopic asthmatics. FN is known to play a role in both airway remodeling and fibrosis. In this study, the fluctuation of FN between the attack and non-attack stages was not significant in atopic asthmatic patients, but was significant in non-atopic asthmatic patients. Therefore, the mechanism for allergic inflammation of the airways may be different in these 2 types of bronchial asthma. In chronic inflammatory disorders such as collagen diseases [22, 23], nephrotic syndrome [7], hyperthyroidism [24] and cancer [25], plasma FN levels are reported to be higher than in healthy controls. The abnormalities of plasma FN have tended to correlate with the disease activity in these inflammatory disorders. FN has been shown to be increased in the plasma of patients with collagen disease, and to show different molecular structures and antigenicity compared to FN in healthy subjects [22]; FN has a variety of structures after alternative splicing [26-29]. FN can be synthesized in culture by many cells, especially fibroblasts, hepatocytes, macrophages, and endothelial cells. Therefore, plasma FN might fluctuate not only quantitatively but also qualitatively in bronchial asthma. In relation to the severity of asthma attacks, a higher level of plasma FN was noted in the group of asthmatics who had more frequent and more severe attacks. Furthermore, the fluctuation of plasma FN between the attack and non-attack stages was more remarkable in asthmatic patients with severe attacks than in those with mild attacks. Because it has been reported that plasma FN is incorporated into the extracellular matrix [16], plasma FN could be mobilized during attacks to the extracellular matrix surrounding the airway in the lung in order to enhance and continue inflammation, while FN might be reduced transiently in plasma. The fluctuation of plasma FN in asthmatics should thus indicate the severity of

airway inflammation. Serum levels of ECP reflect the activation of eosinophils and the infiltration of eosinophils in the bronchial mucous membrane [30, 31]. In this study, the serum levels of ECP were high in asthmatic patients during attacks. The fluctuations of plasma FN and serum ECP between the non-attack and attack stages showed a positive correlation. This suggests that the decrease of plasma FN may reflect acute exacerbation of allergic inflammation of the bronchial wall, while the high level of plasma FN during the non-attack period may reflect the condition of chronic inflammation following the cessation of acute inflammation.

Although the exact roles of FN in bronchoalveolar lavage fluid remain obscure, FN could promote allergic inflammation during asthma attacks, and might contribute to down-regulation of allergic inflammation in order to decrease asthma attacks.

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