

# Platelet-derived growth factor in asthma

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Platelet-derived growth factor (PDGF) controls cellular growth, migration, and differentiation. It is secreted by various cell types, including macrophages, and participates in tissue repair and epithelial regeneration. PDGF may therefore be involved in airway remodeling in asthma. This study compared the immunoreactivity of PDGF and its receptors ( $R\alpha$  and  $R\beta$ ) in bronchial biopsies and the levels of PDGF in bronchoalveolar lavage (BAL) fluid of asthmatics and control subjects. Bronchial biopsies were done in a subsegmental bronchus of 11 asthmatics and 11 control subjects by flexible bronchoscope. PDGF AA and BB, and PDGF receptors  $R\alpha$  and  $R\beta$  were studied with monoclonal antibodies and revealed by immunoperoxidase staining. The percentage of subjects presenting positive staining with PDGFs and its receptors was studied in the epithelium and submucosa. PDGF AA, AB, and BB were measured in BAL fluid of 18 asthmatics and 10 controls by specific ELISA. In biopsies, there was no significant difference between asthmatics and controls for PDGF AA, BB, PDGF- $R\alpha$  and  $R\beta$  (Fisher's exact test and Bonferroni's correction). Moreover, the levels of PDGF, AA, AB, and BB were similar in asthmatics and controls. This study does not support a role for PDGF in the repair processes of asthma.

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Asthma is a chronic inflammatory disease of the airways (1–3) associated with variable airways remodeling (4). Among the numerous growth factors that are active in tissue repair, platelet-derived growth factor (PDGF) may have a prominent role since most inflammatory cell types of the airways inflammation are capable of synthesizing and secreting PDGF. Many of the postulated roles of PDGF are probably involved in tissue repair of chronic inflammatory diseases of the airways including repair damage via migration and proliferation of connective-tissue cells and proliferation of smooth muscle (5, 6). Although initial studies have suggested that PDGF is primarily a mitogen for connective-tissue cells, recent findings have shown that it also induces growth of epithelial cells (7–9). In lung inflammation, attention has been focused on the increased expression of PDGF  $\beta$  chain expression in alveolar macrophages of patients suffering from interstitial pulmonary fibrosis (10–13). PDGF consists of two peptide chains, denoted A and B, which can be dimerized into various active isoforms (PDGF AA, BB, and AB). The three isoforms act on two types of receptors,  $\alpha$  and  $\beta$  (PDGF- $R\alpha$  and PDGF- $R\beta$ ), and differ in their biologic and functional properties and their secretory behavior.

Since macrophages and other cells types able to release PDGF are increased in number and activated in the airways of asthmatics (14, 15), a study was undertaken to investigate the presence of PDGF AA and BB, and PDGF- $R\alpha$  and PDGF- $R\beta$  in bronchial biopsies of 11 control subjects and 11 asthmatics. PDGFs and PDGF-Rs were revealed by immunohistochemistry using highly specific monoclonal antibodies. The study was completed by the measurement of PDGF AA and BB in the bronchoalveolar lavage (BAL) fluid of control subjects and asthmatics.

## Material and methods

### Subjects

Eighteen asthmatics (18–62 years of age,  $35.0 \pm 15.2$  years) were studied. Asthma was defined, as previously (2), according to the criteria of the American Thoracic Society (16), and the severity was assessed by the clinical score of Aas (17). Immediate-type hypersensitivity was confirmed by positive skin prick tests with common aeroallergens and serum allergen-specific IgE (Phadebas RAST®, Pharmacia Diagnostics AB, Uppsala, Sweden). No subject had respiratory tract infection or acute exacerbation during

the month preceding the test. None of the patients had been treated with oral or inhaled corticosteroids for at least 3 months. Other treatments were stopped either 48 h (theophylline) or 8 h ( $\beta_2$ -agonists) prior to the study.

Eleven healthy subjects (25–59 years of age,  $38.3 \pm 12.7$  years) were used as a control group. None of them were smokers. Their pulmonary function was within normal range, they were nonallergic, and they had never suffered from asthma or chronic bronchitis. None of them were receiving therapy. No subject had suffered from any respiratory tract infection during the month preceding the test.

The study was performed after informed consent was obtained and approval of the ethics committee of the hospital was given.

#### *Pathologic examination of bronchial mucosa*

In 11 asthmatics and all controls, biopsies were recovered by fiberoptic bronchoscopy performed as previously described (2) using alligator forceps in subsegmental bronchi. Specimens were fixed in 3.7% weight/volume formaldehyde and embedded in paraffin blocks. Four-micrometer sections were deparaffined and rehydrated before immunohistochemistry. After blocking endogenous peroxidase with 3% hydrogen peroxide followed by incubation with non-immune goat serum, slides were incubated with the mouse monoclonal antibodies against PDGF AA, PDGF BB, PDGF-R $\alpha$ , or PDGF-R $\beta$  (gift of Dr Pech, Hoffman-La Roche, Basel, Switzerland), at a dilution of 1:25. The antibodies are highly specific and their description has been previously reported (18–20). The reaction was revealed using the streptavidin biotin immunoperoxidase method (Dako LSAB<sup>®</sup>, Glostrup, Denmark), according to the package insert. Control slides were prepared by using an irrelevant mouse antibody of the same isotype at the same concentration as the specific primary monoclonal antibody (Dako). Two different investigators, both unaware of the clinical findings, examined the slides.

#### *Measurement of PDGF in BAL fluid*

The BAL was carried out identically in all patients and controls and performed in one of the subsegmental bronchi of the middle lobe using the injection of several aliquots of sterile saline (total volume of 200 ml) reaspirated by gentle syringe suction. Immediately after lavage, the BALF was centrifuged at 4°C and 400 g for 5 min and the supernatant stored at –20°C until assay of the mediators. PDGF AA and BB were measured with an isoform-specific ELISA using specific monoclonal antibodies (19), as previously described (21). The assay was kindly car-

ried out by Dr H. Gallati (Pharma Division, Pre-clinical Research, F. Hoffman-La Roche Ltd, Basel).

#### *Statistical analysis*

Fisher's exact test was used for statistical comparisons of immunoreactivity between the groups. The Mann-Whitney U test was used for comparisons of demographic characteristics of the patients and for the comparisons of BAL fluid results. For multiple correlations, Bonferroni's correction was used. Results are given as medians and 95% confidence intervals.

## Results

#### *Patient characteristics*

Ten patients had mild asthma (Aas score 1–2), four moderate asthma (Aas score 3), and four severe asthma (Aas score 4). None of the patients were receiving corticosteroid treatment in any form. FEV<sub>1</sub> ranged from 75% to 116% of predicted values. None of the patients were suffering from nocturnal asthma. However, two patients with severe asthma had unstable asthma. Thirteen patients were allergic. There was no significant difference between asthmatics and normal subjects in age and sex.

#### *Pathologic examination of the biopsies*

The mucosa of asthmatic subjects was characteristic of the disease; in particular, basement membrane thickness was increased in asthmatics in comparison with normal subjects.

Immunoreactivity with PDGF AA and BB mAb was observed for epithelial cells and submucosal

Table 1. Immunohistochemistry of PDGF and its receptors

|          | Epithelium |        | Submucosa |        |
|----------|------------|--------|-----------|--------|
|          | Controls   | Asthma | Controls  | Asthma |
| PDGF AA  |            |        |           |        |
| Positive | 6          | 2      | 3         | 1      |
| Negative | 4          | 7      | 4         | 6      |
| ND       | 1          | 2      | 4         | 4      |
| PDGF BB  |            |        |           |        |
| Positive | 0          | 3      | 0         | 2      |
| Negative | 10         | 6      | 5         | 5      |
| ND       | 1          | 2      | 6         | 4      |
| PDGF-rA  |            |        |           |        |
| Positive | 10         | 10     | 3         | 5      |
| Negative | 0          | 0      | 5         | 2      |
| ND       | 1          | 2      | 3         | 4      |
| PDGF-rB  |            |        |           |        |
| Positive | 10         | 8      | 2         | 2      |
| Negative | 0          | 0      | 6         | 4      |
| ND       | 1          | 3      | 3         | 5      |

cells in some but not all subjects (Figs. 1 and 2, and Table 1). There was no staining with irrelevant antibodies. In epithelial cells, ciliated and basal cells were stained, but in the former cells, the reactivity

was mainly restricted to the apical pole. When a subject had PDGF-immunoreactivity among epithelial cells, most, if not all, cells were stained. PDGF BB immunoreactivity was only found in a few asth-

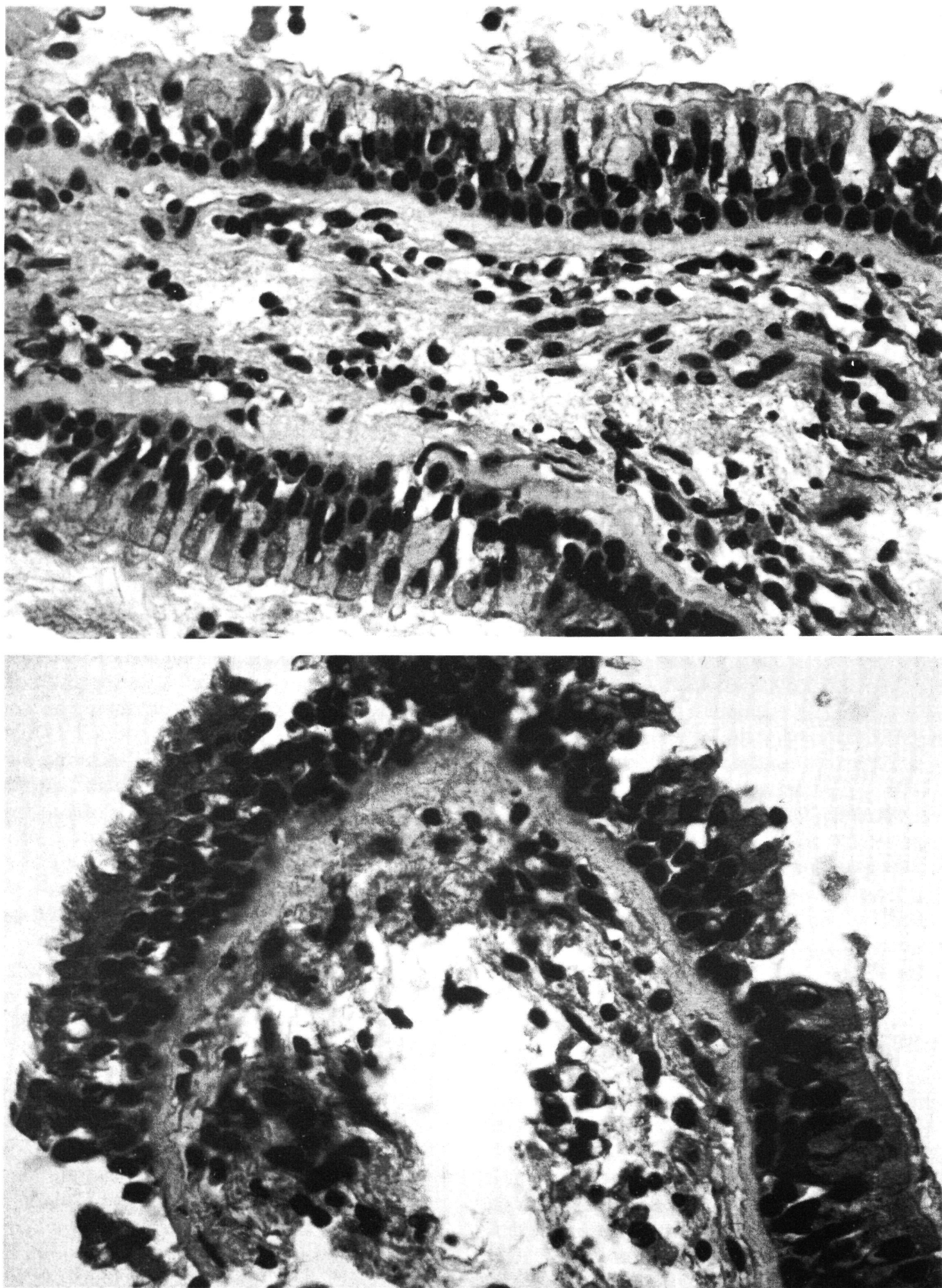


*Fig. 1.* PDGF AA immunoreactivity in asthmatic patients. Upper panel: slide stained with irrelevant antibody; lower panel: slide stained with anti-PDGF AA monoclonal antibody.

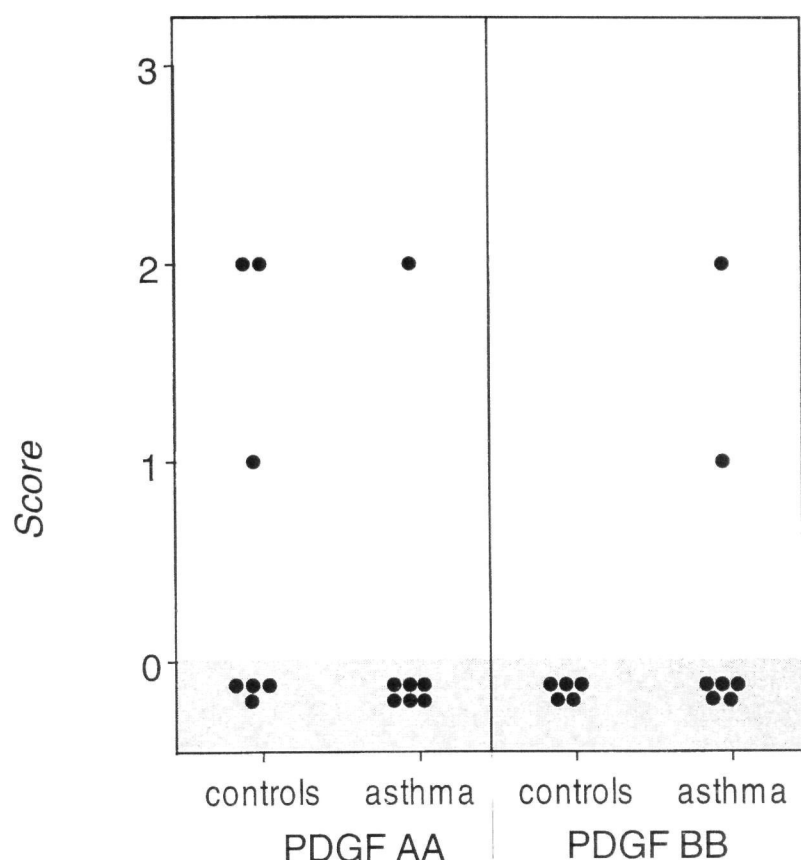


Fig. 2. PDGF immunoreactivity in submucosa: arbitrary score from 0 (no reactivity) to 3 (strong reactivity).

matics. PDGF AA immunoreactivity was more rarely found in asthmatic in comparison with normal subjects, but the difference did not reach significance when Bonferroni's correction was used. In the submucosa, PDGF AA was found in a greater number of control subjects than asthmatics. On the other hand, PDGF BB was found in none of the control subjects and in 2/7 asthmatic patients. Since we did not use double staining, it was not possible to determine which cells were positive in the submucosa, but, in asthmatic subjects, granulocytes and mononuclear cells were positive and in normal subjects

Table 2. Levels of PDGF in BAL fluid (results expressed in pg/ml)

|         |        | Controls  | Asthma    |
|---------|--------|-----------|-----------|
| PDGF AA | Median | 17.8      | 11.2      |
|         | 95% CI | 13.9–23.9 | 10.5–18.6 |
| PDGF AB | Median | 23.3      | 23.4      |
|         | 95% CI | 17.2–22.0 | 20.6–28.3 |
| PDGF BB | Median | 19.9      | 35.6      |
|         | 95% CI | 13.9–31.6 | 28.1–40.0 |

only mononuclear cells were positive. Smooth-muscle cells were seen in only about one-third of the biopsies and there was positive staining in some subjects.

The median size of the basement membrane was 11.5  $\mu$ m in asthma and 7  $\mu$ m in normal subjects. There was no correlation between basement membrane size and PDGF immunoreactivity.

Immunoreactivity of PDGF receptors was found to be present in epithelial and submucosal cells of the two groups of subjects without any apparent difference.

#### Measurement of PDGF in BAL fluid

The levels of PDGF AA, BB, and AB were similar and nonsignificantly different in the two groups of subjects (Table 2 and Fig. 3). However, five patients were clinically indistinguishable from the others that had an increased PDGF BB level.

#### Discussion

The present study does not favor a role for PDGF in the generation of the airways remodeling in asthma.

This study used previously validated methods. Although some of the patients had moderate to severe

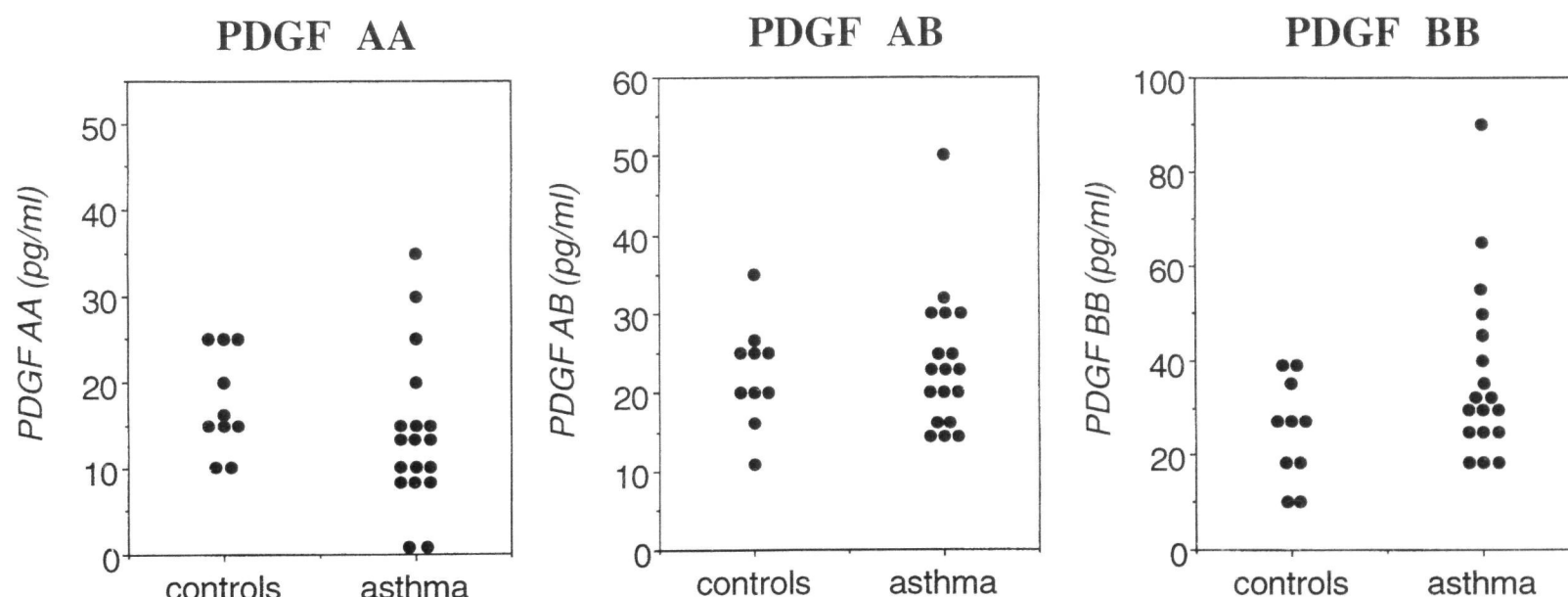


Fig. 3. Levels of PDGF AA, BB, and AB in BAL fluid of asthmatic and control subjects.

asthma, none of them had received inhaled corticosteroids for at least 3 months, since guidelines (22) are not always optimally followed. The study was not quantitative for the epithelium since when a subject had positive staining, most, if not all, cells were stained. The specificity of the antibodies used is critical in any immunohistochemical or ELISA study and is well documented in the present study. We used monoclonal antibodies whereas Aubert et al. (23) used a polyclonal antibody against the three forms of PDGF.

Asthma is a chronic inflammatory disease of the airways and some pathologic features of asthma indicate the presence of airways remodeling. There is pseudothickening of the basement membrane due to collagen deposition (24) and myofibroblast activation (25). In the submucosa, there is an increase in muscle mass (26–30). The clinical consequences of remodeling are not fully understood. For decades, asthma has been considered a completely reversible obstructive disease, but many asthmatics, both children and adults, show evidence of residual airway obstruction (31). This irreversible component of the airways obstruction is more prominent in severely ill patients and even persists after aggressive anti-inflammatory treatment. During adult life, clinical asthma is associated with an increase in the rate of decline in FEV<sub>1</sub> (32). CT-scan findings confirm airways remodeling in asthma, irreversible abnormalities being observed in many asthmatics (33).

We studied PDGF for the following reasons:

- 1) PDGF is a growth factor released by macrophages in fibrosis (12, 13), and PDGF mRNA was found to be present in macrophages from normal subjects and asthmatics (34), these cells being upregulated in asthma (14, 15).
- 2) In asthma there is an increase in smooth-muscle mass (27–30) and PDGF is one of the smooth-muscle growth factors (5, 35).
- 3) In asthma, epithelial cells are activated and possibly present an increased regeneration rate because of the excessive shedding, and PDGF is implicated in the regeneration of cells in general and of epithelial cells in particular, as shown in fetal lung and corneal and glomerular epithelium (7–9, 36–38).
- 4) PDGF is involved in lung fibrosis (10–12) and fibroblasts are activated in asthma (25).

The expression of PDGF AA and its receptors on bronchial epithelial cells was investigated in the present study, but there was no significant difference between asthmatic and control subjects. For PDGF BB, immunohistochemistry with PDGF was most often negative among epithelial cells, but PDGF-R $\beta$  was revealed on most cells of asthmatics and controls. This study differs from that of Aubert et al. (39), who found little PDGF immunoreactivity

among epithelial cells, whereas submucosal cells (mainly macrophages) were positive for PDGF. However, in the latter study, biopsy specimens were obtained at autopsy; most asthmatics in whom treatment was known had received corticosteroids, and the antibodies used differed in both studies. Since corticosteroids modify significantly the phenotype of epithelial cells of asthmatic patients (40), differences in PDGF immunoreactivity of epithelial cells in both studies may be explained. Macrophages express PDGF in smokers, chronic obstructive pulmonary disease (COPD) patients, asthmatics (39), and patients suffering from fibrosis (10, 12), whereas in normal subjects 0–25% of lung macrophages express PDGF (12). Thus, in the present study, the lack of immunoreactivity of the submucosa in many normal subjects and asthmatic patients does not contradict previous studies. BAL fluid levels have not been measured in asthmatic patients in any previous study, and the data of this study did not indicate any significant difference between control subjects and asthmatics.

Overall, the present study was unable to confirm the involvement of PDGF AA or BB in asthma by using immunohistochemistry on bronchial biopsies and measurements of PDGF in BAL fluid. However, it is possible that due to the large heterogeneity of asthma and the number of subjects investigated, some asthmatic patients, particularly those with severe disease, present increased PDGF expression in the airways, but we studied patients of variable severity and both allergic and nonallergic asthmatics. These results in asthmatics possibly explain, at least partly, why the remodeling of the airways in asthma is a slow process, whereas fibrotic diseases of the lung in which PDGF appears to be involved sometimes develop rapidly toward severe fibrosis (41).

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