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# Perip7lakin is a target for autoimmunity in asthma

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## Abstract

The role of autoimmunity targeting epithelial antigens in asthma has been suggested, in particular in non-atopic and severe asthma. Periplakin, a desmosomal component, is involved in epithelial cohesion and intracellular signaling. We detected anti-periplakin IgG antibodies in 47/260 (18 %) patients with asthma, with no association with severity or atopy. In addition, anti-periplakin IgE antibodies were detected in 12 of 138 tested patients (8.7 %) and were more frequently observed in patients with than without nasal polyposis. This study identifies a new autoimmune epithelial target in asthma. Whether periplakin autoimmunity (both IgG and IgE auto-antibodies) is involved in asthma pathogenesis remains to be studied during the disease course of these patients.

**Keywords:** Airway epithelium, Asthma, Autoimmunity, Desmosome, Nasal polyposis

To the Editor,

Autoimmunity targeting the bronchial epithelium has been consistently observed in patients with asthma, and various auto-antigens have been identified, including alpha enolase, [1] cytokeratine 8 or 9, [2] epidermal growth factor receptor (EGFR), activin A type 1 receptor, collagen V or α-catenin [3]. Autoantibodies (Abs) are observed in 30 % to 40 % of non-atopic patients and 0 to 10 % of atopic ones [3]. Autoimmunity may result from the exposure of self-antigens due to airway epithelial damage induced by respiratory viruses or allergens. However, the demonstration that epithelial-targeted autoimmunity contributes to asthma pathogenesis and severity is still tenuous and poorly documented: in non-atopic patients, asthma severity has been found associated with anti-collagen V Abs [3] and with the presence of IgG Abs, with a cytotoxic effect on airway epithelial cells [4].

Periplakin (PPL), a component of desmosomes, is expressed in proximal and distal airway epithelia [5].

PPL acts as a cytolinker between intermediate filament scaffolding and the desmosomal plaque and is involved in epithelial cohesion, intracellular signal transduction and antigen presentation [5]. We previously described PPL as a target for autoimmunity in idiopathic pulmonary fibrosis and that anti-PPL IgG Abs reduced alveolar cell migration in vitro [6]. Here, we hypothesized that PPL may be a target for autoimmunity in asthma and that anti-PPL IgG frequency might be different in severe compared with mild-to-moderate patients and/or in atopic compared with non-atopic patients.

We prospectively evaluated the prevalence of anti-PPL IgG Abs in the French asthma cohort COBRA (Cohorte Obstruction Bronchique et Asthme). All patients gave written informed consent for follow-up in the COBRA cohort according to the procedure validated by the local Ethics Committee (Comité de Protection des Personnes d'Ile de France 1), which approved the study.

We analysed serum obtained at enrolment in 260 patients (188 with severe and 72 mild to moderate asthma according to GINA guidelines); 40 healthy donors were used as controls. Western blot assay was used to test the presence of anti-PPL IgG Abs as previously described [6]. In 138 patients and 18 healthy donors, we also tested for the presence of anti-PPL IgE Abs. Results were considered positive when a 195-kDa band was detected with both a human placental extract and the recombinant PPL as

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antigenic targets. Western blot results from 6 selected patients are in Fig. 1.

Statistical analysis involved use of SAS 9.2 (SAS Inst. Inc., Cary, NC). Continuous variables were compared by Student $t$ test or Wilcoxon rank-sum test and categorical variables by chi-square test or Fisher exact test, as appropriate. We tested for a differential association between anti-PPL IgG and severity or atopy with an interaction term in a multivariate logistic regression analysis explaining asthma severity. We did not adjust for multiple comparisons as it is not required in exploratory analysis [7].

Anti-PPL IgG Abs were detected in 47/260 patients (18 %) and in no control subjects. The characteristics of anti-PPL IgG–positive and –negative patients are in Table 1.

The proportion of patients with anti-PPL IgG was similar in mild-to-moderate asthma (19.4 % [95 % CI 10.3–28.6 %]) and in severe asthma (17.6 % [95 % CI 12.6–23 %]) ($p = 0.72$). However, the association between anti-PPL IgG positivity and asthma severity differed by atopy ($p_{interaction} = 0.04$): anti-PPL IgG–positive non-atopic patients tended to have less severe asthma, although not significantly (odds ratio [OR] 0.26 [95 % CI 0.07–1.05], $p = 0.059$), while anti-PPL IgG-positivity was not associated with asthma severity in atopic patients (OR 1.42 [95 % CI 0.59–3.42], $p = 0.42$).



**Detection of anti PPL Abs of IgE and/or IgG isotypes in 6 selected patients by immunoblot**
Panel I and II illustrate the serum reactivities on recombinant PPL protein using, respectively anti-IgE or anti-IgG revelation (Sigma)
lane 1 : molecular weight
lane 2-4 : three patients with mild to moderate asthma
lane 5-7 : three patients with severe asthma
lane 8 : healthy control
lane 9 : polyclonal anti PPL Ab (sc-16754, Santa Cruz)

**Fig. 1** Western blot analysis of anti-periplakin (PPL) antibodies (Abs) of IgE isotype (I) and IgG isotype (II) in 6 patients. Lane 1, molecular weight; lanes 2–4, 3 patients with mild to moderate asthma; lanes 5–7, 3 patients with severe asthma; lane 8, healthy control; lane 9, polyclonal anti-PPL Ab (sc-16754, Santa Cruz Biotechnology)

Moreover, anti-PPL IgG–positive and –negative patients only differ for the mean daily inhaled corticosteroids dose, which was lower in the positive group. Non-significant trends to a greater proportion of males, frequency of occupational asthma and nasal polyposis were observed in the anti-PPL IgG–positive group, together with a trend to a lower number of asthma exacerbations and a longer median duration of asthma.

Anti-PPL IgE Abs were detected in none of the healthy donors and in 12/138 patients (8.7 %) (Table 1); 7 were negative for anti-PPL IgG Abs. Anti-PPL IgE–positive and –negative patients did not differ in clinical characteristics, particularly atopy, except for the proportion of nasal polyposis, which was higher for anti-PPL IgE–positive than –negative patients (50 % vs 16.7 %, $p < 0.01$).

This study identifies a new epithelial target for autoimmunity in asthma. Regardless of atopic status and degree of asthma severity, about 20 % of our asthmatic patients exhibited circulating auto-Abs (IgG and/or IgE) targeting PPL. Anti-PPL IgG-positive patients received lower doses of inhaled corticosteroids, suggesting a less active disease, which is in line with a trend for a lower proportion of patients presenting an asthma exacerbation, but could also suggest a potential modulation of the anti-PPL Abs production by corticosteroids.

We consider anti-PPL Abs a marker of airway epithelium damage. Because the presence of anti-PPL IgG or IgE Abs was tested only once during the asthma time course, we cannot determine how anti-PPL autoimmunity developed during the disease course nor how it could relate to earlier periods of uncontrolled disease, beyond the 1-year period preceding inclusion. A close prospective follow-up of anti-PPL Ab–negative patients could help assess the history of anti-PPL autoimmunity, as well as the role of asthma exacerbations and inhaled/oral steroids in the development of anti-PPL Abs. A longer follow-up could also help determine whether anti-PPL–positive patients may have a different prognosis than –negative patients, for example, show a more pronounced obstructive pattern or not. The titration of Abs could also give additional information because anti-collagen V Ab levels have been found to be increased in severe asthma [3].

Our results emphasize the potential role of autoreactive IgE (alone or associated with IgG) in asthma. Of note, anti-PPL IgE Abs were more frequent in patients with than without nasal polyposis. This is an intriguing observation because nasal polyposis is characterized by altered upper airway epithelium (loss of epithelial barrier function and loss of desmosomes) [8], as is observed in asthma. Autoreactive IgE have been described in auto immune diseases, where they can act in synergy with IgG auto-Abs to potentiate the activation of inflammatory components [9]. Most importantly, IgE auto-Abs have been also reported in atopic dermatitis [10] and in

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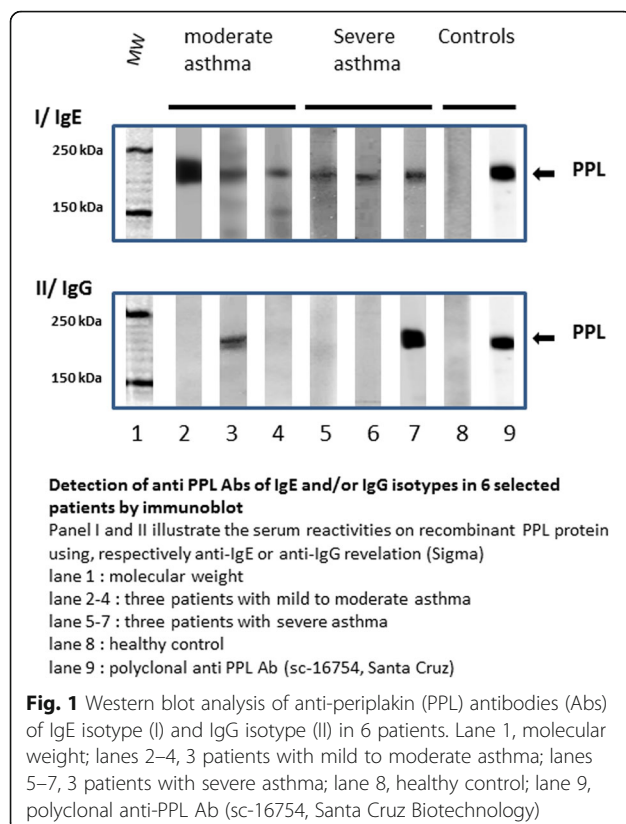
**Table 1** Characteristics of anti-periplakin IgG and IgE –positive and –negative patients

| | Total (n = 260) | IgG PPL + (n = 47) | IgG PPL– (n = 213) | P value | Total (n = 138) | IgE PPL + (n = 12) | IgE PPL– (n = 126) | P value |
|---|---|---|---|---|---|---|---|---|
| Male | 80 (30.8 %) | 20 (42.6 %) | 60 (28.2 %) | 0.053 | 44 (31.9 %) | 6 (50.0 %) | 38 (30.2 %) | 0.19 |
| Age (yr) | 48.5 (14.2) | 47.5 (13.4) | 48.7 (14.4) | 0.59 | 49.5 (15.1) | 47.1 (16.3) | 49.7 (15.0) | 0.57 |
| Asthma severity | | | | 0.89 | | | | 0.67 |
|   Mild | 35 (13.4 %) | 6 (12.7 %) | 29 (13.6 %) | | 16 (11.6 %) | 2 (16.6 %) | 14 (11.1 %) | |
|   Moderate | 37 (14.2 %) | 8 (17.0 %) | 29 (13.6 %) | | 16 (11.6 %) | 1 (8.3 %) | 15 (11.9 %) | |
|   Severe | 188 (72.3 %) | 33 (70.2 %) | 155 (72.8 %) | | 106 (76.8 %) | 9 (75.0 %) | 97 (77.0 %) | |
| Atopy | 162 (69.8 %) | 34 (77.3 %) | 128 (68.1 %) | 0.23 | 95 (72.0 %) | 10 (83.3 %) | 85 (70.8 %) | 0.5 |
| BMI | 26.9 (5.6) | 26.9 (5.7) | 27.0 (5.6) | 0.91 | 27.5 (5.3) | 25.4 (3.0) | 27.7 (5.4) | 0.14 |
| Asthma duration (yr) | 23.2 [9.1–35.9] | 28.4 [14.5–37.7] | 21.1 [8.0–35.7] | 0.28 | 21.5 [8.2–36.4] | 13.6 [8.0–31.7] | 23.0 [8.8–36.4] | 0.31 |
| FEV1 (% predicted) | 81.0 (23.4) | 84.1 (20.8) | 80.4 (23.9) | 0.32 | 78.7 (24.8) | 87.8 (28.2) | 77.8 (24.4) | 0.18 |
| FEV1/FVC (%) | 68.6 (14.4) | 68.3 (15.6) | 68.7 (14.2) | 0.88 | 66.5 (15.3) | 65.3 (15.0) | 66.7 (15.4) | 0.77 |
| Total IgE (kUI/l) | 424.0 (643.5) | 446.6 (826.7) | 418.6 (593.9) | 0.45 | 425.0 (665.5) | 318.2 (377.9) | 436.1 (688.8) | 0.82 |
| Blood eosinophils (/mm3) | 305.4 (278.8) | 322.3 (185.1) | 301.4 (297.1) | 0.06 | 316.5 (251.7) | 378.9 (248.0) | 310.7 (252.2) | 0.32 |
| Nasal polyposis | 51 (19.9 %) | 13 (27.7 %) | 38 (18.2 %) | 0.14 | 27 (19.6 %) | 6 (50.0 %) | 21 (16.7 %) | 0.01 |
| Smoker or former smoker | 100 (38.6 %) | 22 (46.8 %) | 78 (36.8 %) | 0.26 | 56 (38.6 %) | 4 (33.3 %) | 52 (41.2 %) | 0.8 |
| Occupational asthma | 32 (12.4 %) | 9 (19.1 %) | 23 (10.9 %) | 0.12 | 18 (13.1 %) | 1 (8.3 %) | 17 (13.6 %) | 1 |
| GERD | 103 (39.6 %) | 19 (40.4 %) | 84 (39.4 %) | 0.9 | 58 (42.0 %) | 4 (33.3 %) | 54 (42.9 %) | 0.52 |
| Asthma control | | | | | | | | |
|   well-controlled | 78 (30.2 %) | 17 (36.2 %) | 61 (28.9 %) | 0.61 | 38 (27.7 %) | 4 (33.3 %) | 34 (27.2 %) | 0.92 |
|   partly controlled | 76 (29.5 %) | 13 (27.7 %) | 63 (29.9 %) | | 36 (26.3 %) | 3 (25.0 %) | 33 (26.4 %) | |
|   uncontrolled | 104 (40.3 %) | 17 (36.2 %) | 87 (41.2 %) | | 63 (46.0 %) | 5 (41.7 %) | 58 (46.4 %) | |
| JUNIPER score | 10.2 (7.3) | 9.1 (8.1) | 10.5 (7.0) | 0.29 | 10.4 (7.3) | 6.1 (8.4) | 10.8 (7.1) | 0.08 |
| At least 1 exacerbation in the previous year | 147 (58.8 %) | 22 (48.9 %) | 125 (61.0 %) | 0.13 | 81 (60.4 %) | 8 (72.7 %) | 73 (59.3 %) | 0.52 |
| No. of oral steroid bursts in the previous year | 4.0 [2.0–8.0] | 3.0 [2.0–4.5] | 4.0 [2.0–8.0] | 0.39 | 4.0 [2.0–8.0] | 3.5 [2.0–4.0] | 5.0 [2.0–8.0] | 0.38 |
| Inhaled steroids | 223 (85.8 %) | 42 (89.4 %) | 181 (85.0 %) | 0.43 | 123 (89.1 %) | 10 (83.3 %) | 113 (89.7 %) | 0.62 |
| Daily dose of inhaled steroids (µg/d) | 1781.1 (1154.1) | 1463.5 (1113.6) | 1854.8 (1153.8) | 0.01 | 1801.8 (1092.8) | 1700.0 (1032.8) | 1810.8 (1101.9) | 0.86 |
| Daily oral steroids | 47 (18.1 %) | 7 (14.9 %) | 40 (18.9 %) | 0.52 | 25 (18.2 %) | 2 (16.7 %) | 23 (18.4 %) | 1 |
| Omalizumab | 15 (5.8 %) | 2 (4.3 %) | 13 (6.2 %) | 1 | 8 (5.8 %) | 0 (0.0 %) | 8 (6.3 %) | 0.36 |

Data are mean (SD), n (%) or median (interquartile range)
*PPL* periplakin, *GERD* gastroesophageal reflux disease, *FEV* forced expiratory volume in 1 s, *FCV* forced vital capacity, *BMI* body mass index Atopy was defined by a positive skin prick test and/or specific IgE level > 0.15 kU/l for at least one aeroallergen

chronic urticaria [11], and are considered as potential targets for omalizumab treatment in this setting [11].

The present data do not allow for determining whether anti-PPL autoimmunity is really involved in the pathogenesis of asthma. However, since we previously showed that anti-PPL Abs inhibit epithelial repair in vitro [6], we suggest anti-PPL Abs may contribute to a vicious cycle leading to chronic airway epithelial injury, a central feature of asthma.

To conclude, in this exploratory analysis, we observed autoimmunity features (IgG and/or IgE Abs) directed against PPL, an important airway epithelial protein, in 20 % of a large cohort of patients with asthma. These results add to the controversial hypothesis about the role of autoimmunity in the pathogenesis of asthma and autoimmunity as a target for asthma treatment but also emphasize the potential role of IgE auto-Abs in asthma and nasal polyposis.

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