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This supplementary material has been provided by the authors to give readers additional information about their work.
eMethods. Patients and Spiking Experiments

Patients

Of the 10 BP patients followed during the course of the disease 6 patients were treated with oral prednisolone 0.25-0.5 mg/kg/day, dapsone 75-125 mg/day, and topical class III or class IV corticosteroids. One patient was treated with oral prednisolone 0.5 mg/kg/day and topical class IV corticosteroids, one patient received doxycycline 200 mg/day plus topical class IV corticosteroids, and one patient topical steroids in combination with dapsone 100 mg/day that was changed to azathioprine 100 mg/day due to a dip in haemoglobin levels. In one patient, disease could be controlled with super potent topical corticosteroids alone. All patients on oral prednisolone followed a tapering scheme with reduction every second week after disease control was achieved.

Spiking experiments

For spiking experiments, increasing amounts of human IgE full length protein (ab65866, Abcam, Cambridge, UK), i.e. 100, 500, 1,000, and 5,000 IU were added to three BP sera (one of them with increased IgE anti-BP180 levels) and three sera from the elderly controls. Each of the 6 sera revealed total IgE serum levels <100 IU. After spiking, sera were assayed by the IgE BP180NC16A ELISA as described above.

Protein G affinity chromatography

Briefly, protein G agarose fast flow columns (Millipore, Temecula CA, USA) were incubated with 300 µl serum of 6 BP patients for 1h at 4°C, then serum flow through i.e. serum depleted of IgG was collected and columns were washed with PBS 5-8 times until the captured flow through OD was <0.05. Elution of IgG using 200 µl glycine buffer (0.1M, pH2.8) was performed until the OD of eluate reached values <0.01. Eluate was neutralized using Tris base (1M, pH9) to achieve a pH of 7.2. Subsequently, both serum depleted of IgG (concentrations of 1:101, 1:10 and pure) and purified IgG (concentrations of 1:101 and 1:20) were subjected to the newly developed IgE NC16A ELISA as described above. Secondary antibodies for IgG and IgE were used 1:10 and pure, respectively.
eResults. Verification of Specificity of the Novel IgE anti-BP180 NC16A ELISA

IgE spiking experiments

When different amounts of human IgE were added to 3 BP and 3 control sera IgE anti-BP180 NC16A ELISA values remained relatively constant in the BP serum with high anti-BP180 IgE levels (BP1) and in one control serum, whereas ELISA values increased in the 4 other sera (Fig. S2). These data were in line with our findings of the much higher sensitivity of the IgE anti-BP180 NC16A ELISA when age-matched controls were used compared to the use of total serum IgE-matched controls. These observations supported the hypothesis that high total serum IgE levels interfere with detecting anti-BP180 IgE and led us to the introduction of different cut-offs for sera with <100 IU, 100-500 IU, and >500 IU total serum IgE levels in the novel ELISA.

Elimination of IgG by Protein G did not yield significantly different extinctions

To control for interference of IgG in the detection of anti-BP180 IgE, serum depleted of IgG was subjected to the newly developed IgE NC16A ELISA. After eliminating IgG by protein G agarose columns the IgG-depleted sera showed about the same IgE anti-BP180 levels after compensating for the loss of IgE reactivity by the process of affinity chromatography compared to the naive BP sera (Table 1S). These data show a high specificity of the anti-IgE secondary antibody and speak against the hypothesis that anti-BP180 NC16A IgG may have blocked binding sites for the much rarer IgE anti-BP180 autoantibodies. Furthermore, cross reactivity between purified IgG and anti-IgE secondary antibody was excluded since purified IgG employed in the new IgE BP180 NC16A ELISA with anti-IgE detection antibody did only show an extinction slightly above the blank (Table 1S) while with anti-IgG secondary antibody extinction was in the same range compared to naïve BP sera (data not shown).

Total IgE levels influence measurements of BP180NC16A-specific IgE

ROC analysis of ELISA values of the cohorts of 65 BP patients and 49 elderly patients with non-inflammatory dermatoses resulted in a specificity of 100% and a sensitivity of 75% (cut off, 0.006; AUC, 0.921; 97.5 CI, 0.651-1; Fig. S1A). To validate these results and given that elevated total serum IgE levels were found in 59% of the BP cohort, but in only 16% of the elderly controls, the total serum IgE-matched cohort (n=127) was employed. Here, ROC analysis resulted in a sensitivity of 14 % when specificity was set to 99.2% for direct comparison with the cohort of elderly patients with non-inflammatory dermatoses (cut off, 0.312; AUC, 0.837; 97.5 CI, 0.64-0.964; Fig. S1B).

To account for these divergent findings cut off values were calculated by ROC analysis dependent on total serum IgE levels. Sera were classified in three groups, <100 IU, 100-500 IU, and >500 IU resulting in cut offs of 0.037, 0.113, and 0.173, respectively (Fig. S3). Using these cut offs 47 (40%) of the 117 BP sera contained anti-BP180 NC16A IgE including 13 (30%) of the 43 BP patients with total serum IgE of <100 IU, 15 (38%) of the 39 BP patients with total serum IgE of 100-500 IU, and 19 (54%) of the 35 BP patients with total serum IgE of >500 IU.
### eTable. Cross-reactivity of Purified IgG and IgE Secondary Antibodies

<table>
<thead>
<tr>
<th>Serum No.</th>
<th>OD value before depletion of IgG</th>
<th>OD value after depletion of IgG*</th>
<th>OD value of purified IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td>BP1</td>
<td>0.684</td>
<td>0.679</td>
<td>0.004</td>
</tr>
<tr>
<td>BP2</td>
<td>0.242</td>
<td>0.213</td>
<td>0.014</td>
</tr>
<tr>
<td>BP3</td>
<td>0.599</td>
<td>0.484</td>
<td>0.004</td>
</tr>
<tr>
<td>BP4</td>
<td>0.702</td>
<td>0.525</td>
<td>0.012</td>
</tr>
<tr>
<td>BP5</td>
<td>0.328</td>
<td>0.242</td>
<td>0.005</td>
</tr>
<tr>
<td>BP6</td>
<td>0.457</td>
<td>0.345</td>
<td>0.009</td>
</tr>
</tbody>
</table>

*Corrected for total IgE values to compensate for IgE loss during the process of affinity chromatography"
Receiver operating curves (ROC) are highly dependent on the selected control group. ROC analysis of BP patients (n=65) and age-matched controls (n=49) optimized for both sensitivity and specificity resulted in a specificity of 100% and sensitivity of 75% (cut off, 0.006) (A). ROC analysis of BP patients (n=65) and total serum IgE-matched controls (n=127) with a comparable specificity of 99.2% resulted in a sensitivity of only 14% (cut-off, 0.312) (B). Specificity is displayed as 1-specificity on the x-axis in both curves. Selected cutoff values are shown within the figures for various sensitivities and specificities.
Spiking of sera with human IgE did interfere with detection of anti-BP180 IgE serum levels. 100 IU, 500 IU, 1,000 IU, and 5,000 IU human IgE, respectively, was added to sera of BP patients (n=3) and age-matched controls (n=3) with total IgE serum levels below 100 IU. Subsequently, BP180 NC16A-specific IgE was detected by ELISA and only remained relatively unchanged in the BP serum with elevated anti-BP180 IgE levels (BP1) and one control serum (control 3).
eFigure 3. Detection of IgE anti-BP180 NC16A Reactivity

A

B

C
Receiver operating curves (ROC) based on three control groups with normal, medium, and high total serum IgE levels. ROC analyses were performed using BP and control sera matched for total serum IgE levels. Total serum IgE <100 IU: BP, n=27; aged-matched control, n=41; additional controls, n=54; cut off, 0.037; AUC, 0.791; specificity, 98.9%; sensitivity, 22.2% (A). Total serum IgE 100-500 IU: BP, n=22; aged-matched control, n=6; additional controls, n=41; cut off, 0.133; AUC, 0.915; specificity, 97.9%; sensitivity, 40.9% (B). Total serum IgE >500 IU: BP, n=16; aged-matched control, n=2; additional controls, n=31; cut off, 0.173; AUC, 0.922; specificity, 100.0%; sensitivity, 62.5% (C). Specificity is displayed as 1-specificity on x axis in all curves. Selected cut-off values are shown within the figures for various sensitivities and specificities.