

IgE Autoantibodies Directed Against the Major Bullous Pemphigoid Antigen in Patients with a Severe Form of Pemphigoid¹

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Bullous pemphigoid (BP) is an autoimmune subepidermal blistering disease characterized in part by circulating and tissue-bound IgG autoantibodies directed against the basement membrane zone. In addition, most of the patients with BP have increased serum IgE levels which seem to be correlated with the disease activity, whereas the presence of circulating anti-basement membrane zone IgE Abs has been reported in some patients. To elucidate whether IgE-dependent mechanisms play a role in the physiopathology of BP, we looked for the presence of IgE Abs specifically directed against the major BP Ag (BPAg1) in sera of BP patients at the onset and after remission of the disease. A radioimmunoassay and a 55-kDa recombinant protein (rBP55) obtained from a cDNA sequence, encoding the C-terminal region of the BPAg1 and containing the BPAg1 immunodominant epitopes, were used. Anti-rBP55 IgE Abs were found in 12 of the 19 sera tested. When the patients were divided into two groups according to the disease severity, anti-rBP55 IgE Abs were found only in patients with a severe form of the disease. Cytophilic IgE was detected on ~20% of peripheral blood eosinophils purified from BP patients. Immunohistochemistry studies suggested that some of the IgE-bearing cells in the lesional skin of BP patients are eosinophils. Immunostaining experiments revealed the existence of FcεRI on both peripheral blood and tissue eosinophils. Taken together, these results suggest that IgE-dependent mechanisms could participate in the constitution of the lesions in BP. *The Journal of Immunology*, 1996, 157: 3642–3647.

Blood or tissue eosinophilia, together with increased serum IgE levels, is a prominent feature of diseases such as parasitic infections or allergic manifestations. In both cases, a preferential interaction between eosinophils and IgE Abs has been described (1, 2). Eosinophils purified from parasite-infected or allergic patients can be activated via surface IgE or with IgE immune complexes to release granule proteins such as eosinophil peroxidase or major basic protein (3–5). Various IgE-binding molecules, detected on eosinophil surfaces, have been incriminated in this triggering mechanism, including the low and high affinity IgE receptors, FcεRII/CD23 and FcεRI, respectively (6, 7). Bullous pemphigoid (BP)³ is the only autoimmune blistering disease in which blood eosinophils and IgE levels are increased in most cases (8–10). BP is characterized by circulating and tissue-bound autoantibodies directed against the basement membrane zone (BMZ) (11). These autoantibodies, mostly of the IgG class, react with components of hemidesmosomes, namely the major BP

Ag of 230 kDa (BPAg1) and another BP Ag of 180 kDa (BPAg2) (12–14). In some cases, IgE anti-BMZ autoantibodies have been reported in patients with BP (15, 16). Moreover, surface IgE-bearing cells have been detected in the lesional infiltrate (15, 17). BP patients frequently have eosinophilia, whereas eosinophils are the predominant cells of the lesional infiltrate (18). Eosinophils could play a crucial role in the dermal-epidermal separation through the release of their cytotoxic proteins and enzymatic mediators (19–22). However, the mechanism of cell activation that causes such mediators release in BP remains unknown. The weak correlation between anti-BMZ IgG titers and the disease activity (23), when both IgE levels and eosinophil counts seem to better correlate with the severity of the disease (8, 9, 24), led us to investigate whether IgE-dependent mechanisms could play some role in the physiopathology of BP.

In the present study, we could detect the presence of surface IgE and FcεRI on peripheral blood eosinophils, as well as on cells present in the lesional skin, identified as eosinophils. In addition, we have evaluated the presence, in the serum of BP patients, of specific IgE Abs directed against a recombinant Ag (rBP55) corresponding to the C-terminal domain of BPAg1 and containing the BPAg1 immunodominant epitopes. An increased frequency of specific IgE autoantibodies in severe patients was found. These results suggest that IgE-mediated cell activation could participate to the constitution of lesions in BP, and extend to an autoimmune disease, the findings obtained in parasitic infections and allergic diseases.

Materials and Methods

Patients

Nineteen patients (6 males, 13 females) with clinical, pathologic, and immunologic features typical of BP were studied. By direct immunofluorescence (IF), all patients showed IgG or C3 deposition along the BMZ of peribullous skin, and 12 of them had circulating autoantibodies to the

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³ Abbreviations used in this paper: BP, bullous pemphigoid; BMZ, basement membrane zone; IF, immunofluorescence; SPRIA, solid phase radioimmunoassay; rBP55, 55-kDa recombinant bullous pemphigoid protein; NHS, normal human serum; TBS, 10 mM Tris-HCl/140 mM NaCl, pH 7.4; SLE, systemic lupus erythematosus.

BMZ, as determined by indirect IF. All these patients were selected for the presence of autoantibodies reacting with BPAG1 on immunoblot assay with epidermal extracts. Sera from 7 patients also recognized BPAG2. These patients were all in an acute stage of the disease when the study was performed. Informed consent was obtained for all of them before inclusion in the study. Two groups of patients were defined according to the severity of the disease evaluated by the number of new blisters per day, counted for 3 days before treatment. The disease was considered as moderate if this number was ≤ 10 and as severe if ≥ 10 . All patients were treated with prednisone (1 mg/kg/day). Serum samples were taken before treatment for all patients and at 1 year follow-up for 6 of them. Control serum samples were obtained from patients with 2 other autoimmune blistering diseases, i.e., pemphigus vulgaris ($n = 3$) and linear IgA disease ($n = 3$), atopic dermatitis ($n = 4$), and from healthy donors [normal human serum (NHS); $n = 3$].

Total serum IgE assay

Total IgE levels were assayed with an enzyme-linked immunoassay kit (DPC, Los Angeles, CA) giving a normal rate inferior to 91 K international units (kIU)/L.

Indirect immunofluorescence assay

Indirect IF was performed on 1 M NaCl-split skin obtained by mammaplasty, as previously described (25) using FITC mAbs to human IgE (Cappel, Durham, NC). Skin sections were incubated with BP sera at a 1/20 dilution for 30 min at room temperature. After washing in PBS, the FITC-conjugated anti-human IgE was added at a final concentration of 1/20.

Immunohistochemistry

Immunohistochemistry was performed on lesional skin specimens from BP patients and from two pemphigus vulgaris patients, using polyclonal anti-human IgE Abs (Dako Corp., Carpinteria, CA) revealed by an avidin-biotin alkaline phosphatase method. The same experimental procedure was used with mAbs to Fc ϵ RI, mAb 15-1 (kindly provided by Dr. J. P. Kinet, Harvard Medical School), on 5 BP patients (BP4, -9, -11, -12, and -18). The paraffin-embedded tissue was deparaffinized using toluene and graded ethanol and incubated with rabbit polyclonal anti-IgE Abs and mouse monoclonal anti-Fc ϵ RI Abs at a 1/20 dilution for 1 h at room temperature. As the control, normal rabbit serum or a mAb of the same isotype but with a different specificity (anti-*Toxoplasma gondii*), were used, respectively, in the same conditions. After a washing in 10 mM Tris-HCl-140 mM NaCl, pH 7.4 (TBS)-BSA, biotinylated swine anti-rabbit or anti-mouse Abs were added for 30 min, followed by TBS washing and addition of streptavidin conjugated with alkaline phosphatase for another 30 min (Kit-LSAB 2, Dako Corp.). Specimens were developed with new fuchsin. The reaction was stopped with tap water and the specimens were counterstained with hemalum. Tandem slides were colored by May-Grünwald-Giemsa for cell identification.

Purification of peripheral blood eosinophils

Highly purified eosinophils (>90%) were obtained from five BP patients (BP4, -9, -11, -12, and -18) using a magnetic cell sorter (26). Briefly, after centrifugation of 15 ml of venous blood diluted 1/2 in HBSS on Ficoll-Paque and lysis of erythrocytes by osmotic shock, the granulocytes were obtained after 30 min of dextran sedimentation at room temperature. Eosinophils were then isolated from CD16-positive neutrophils by negative immunomagnetic selection, using anti-CD16 coated microbeads. The degree of purity and the morphologic integrity of cell population were estimated after staining of cytocentrifuged preparations with Giemsa.

Immunocytochemistry

Purified eosinophils were suspended in HBSS at a concentration of 10^6 cells/ml, and aliquots of 100 μ l were cytocentrifuged. The cytocentrifuged preparations were washed in TBS, and mouse monoclonal anti-human IgE Abs (Euromedex, Paris, France) or anti-Fc ϵ RI α -chain (15-1), were added at a final dilution of 1/20. After overnight incubation at 4°C, the slides were washed in TBS, and rabbit anti-mouse Abs were added at a 1/50 dilution for 45 min, followed by addition of mouse Abs coated with the enzymatic complex alkaline phosphatase-anti-alkaline phosphatase at 1/50 dilution. The development used new fuchsin. As the control, a monoclonal anti-*T. gondii* Ab was used in the same conditions.

BP recombinant protein

A 55-kDa M_r recombinant protein (rBP55) obtained from a cDNA sequence encoding the C-terminal region of the BPAG1 was used in this study. This recombinant protein has been shown to contain immunodom-

inant BPAG1 epitopes (27). Briefly, rBP55 was obtained by cloning a 1.5-kilobase BP cDNA (12) into the *Eco*RI site of a PGEX vector derived from PGEX-3X (Pharmacia, St. Quentin Les Yvelines, France). *Escherichia coli* DH5 α organisms transformed with this recombinant vector were cultured, harvested, and screened for expression of recombinant protein by SDS-PAGE analysis of total cell lysates. Purification of the recombinant protein was performed as previously described (27). The cultured cells were sonicated with 1% Triton X-100, and lysates were centrifuged for 5 min at 12,000 rpm and 4°C. Pellets were solubilized in 8 mmol/L urea in a lysis buffer containing 50 mmol/L Tris-HCl (pH 8), 1 mmol/L EDTA, 50 mmol/L NaCl, and 0.1 mmol/L PMSF. After vortexing for 1 h at room temperature, the solution was mixed with 9 volumes of KH_2PO_4 (pH 10.7), 1 mmol/L EDTA, and 50 mmol/L NaCl and left for 30 min. Finally the pH was adjusted to 8 using HCl, and the extract was left for 30 min at room temperature. Recombinant BP55 contains major B cell epitopes, since 60% of patients with BP have circulating autoantibodies to the recombinant protein (27). Bo, used as control protein, was obtained from the same but not recombinant PGEX vector.

Solid phase radioimmunoassay (SPRIA)

Radioiodination. Mouse monoclonal anti-IgE Abs (Euromedex) were radioiodinated by the chloramine-T procedure. Briefly, 10 μ g of purified anti-IgE Abs in 20 μ l of PBS were mixed with 3.7 MBq Na^{125}I (100 μ Ci) and 20 μ l of fresh chloramine-T (125 μ g/ml in 0.5 M sodium phosphate buffer, pH 7.4) were added. After gentle mixing for 1 min, 20 μ l of sodium metabisulfite (250 μ g/ml) in 0.5 M sodium phosphate buffer was added to stop the reaction. Separation of free iodine and the labeled fraction was conducted by gel filtration through a Sephadex G-25 column (PD10, Pharmacia, Uppsala, Sweden) equilibrated with 25 ml of 0.5% PBS-BSA. The iodination yield was estimated as the ratio of the activity in the protein fraction to the total radioactivity eluted, and the specific activity was calculated.

Solid phase radioimmunoassay procedure. rBP55-specific IgE Abs were detected by a SPRIA procedure, using the radiolabeled anti-human IgE Abs. For the immunoassay, polystyrene star tubes (Nunc, Intermed, Roskilde, Denmark) were coated for 24 h at room temperature with 1 μ g of rBP55 or Bo in 250 μ l of 0.015 M Na_2CO_3 buffer, pH 9.6. A 2-h saturation at room temperature was then conducted with 5% BSA-PBS. The tubes were then washed in PBS, and BP sera or control sera (pemphigus vulgaris, linear IgA disease and atopic dermatitis) were added at 1/2 dilution in PBS. Three control NHS were used at the same dilution. After 24 h of incubation at room temperature and three washes in 0.3% PBS-Tween, 18 ng of radiolabeled mouse mAbs to human IgE were added in each tube. After a 24-h incubation at room temperature, the tubes were washed three times and counted in a gamma counter (Clinigamma 1272, LKB, Sweden) with a counting efficiency of 99.9%. BP sera were considered as positive if the radioactivity (Rx) obtained was significantly superior to the mean radioactivity obtained with the NHS tested against rBP55 (Rn) and also significantly superior to the mean radioactivity obtained with the 19 BP sera tested against the control protein Bo (RBo), according to the formula: $Rx > Rn + tSD$ and $Rx > RBo + tSD$, where t was the Student t value with the considered degree of freedom, and the standard deviation SD was obtained according to the mean of the NHS results. Positive results obtained with rBP55 are shown as an index representing the increased (I) bound radioactivity calculated according to the formula: $I = (Rx - Rn)/Rn$.

Immunoblot analysis

Immunoblot reactivity of PB and control sera was performed using a protein extract of a normal human keratinocyte culture as previously described (25), or the purified recombinant BP protein. Briefly, the proteins were separated by SDS-PAGE electrophoresis using a 6% and a 10% acrylamide gel for the epidermal extract and the bacterial lysates, respectively. The proteins were electrophoretically transferred onto nitrocellulose membranes. The nitrocellulose strips were then sequentially incubated with: 1) 5% nonfat dry milk in TBS (TBS/milk) for 1 h at 37°C or overnight at 4°C; 2) 1/100 dilution of each serum sample in TBS/milk for 1 h at 37°C; 3) 1/250 dilution of a monoclonal mouse anti-human IgE (Advanced Immuno Chemical, Long Beach, CA), or biotinylated goat anti-human IgG (Amersham, Buckinghamshire, England) in TBS/milk for 40 min at room temperature; 4) 1/500 dilution of biotinylated rabbit anti-mouse Ig in case of using mouse anti-IgE; 5) 1/3000 dilution of streptavidin-alkaline phosphatase (Amersham). The reactions were revealed with 5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium-buffered substrate tablets (Sigma Chemical Co., St. Louis, MO). Reaction times for all lanes of individual blots were identical. Intervening washes were performed using 0.1% TBS.

Table I. Analysis of serum IgE levels, peripheral eosinophil blood count, IgE and total Ig BP Abs detected by immunoblotting performed with a normal human keratinocyte (NHK) culture extract and by SPRIA^a

Patients	Serum IgE (kIU/L)	Eosinophilia (cells/mm ³)	Indirect IF IgE Isotypes	Immunoblot (NHK extracts)		SPRIA : rBP55- Specific IgE (Index)
				IgG	IgE	
BP1	239	500	—	230, 180	—	—
BP2	406	1700	—	230	—	—
BP3	105	1100	—	230, 180	—	—
BP4	144	1300	—	230, 180	—	—
BP5	32	2400	—	230	—	0.85
BP6	759	900	—	230	—	0.83
BP7	4878	500	+	230, 180	230	11.06
BP8	233	1000	—	230, 180	—	—
BP9	4483	5100	—	230, 180	230	9.40
BP10	686	3200	+	230, 180	—	3.36
BP11	5000	6900	+	230	230	26.26
BP12	117	2400	+	230	—	1.77
BP13	352	2500	+	230	—	1.14
BP14	425	1100	+	230	—	5.57
BP15	130	2400	—	230	—	—
BP16	2056	1600	+	230	—	4.39
BP17	65	1500	+	230	—	—
BP18	5000	4600	+	230	230	16.20
BP19	2479	5300	+	230	—	5.90

^a Nineteen BP patients were distributed into 2 groups of disease severity: 4 patients had a moderate form (group 1: patients 1–4), and 15 others had a severe form (group 2: patients 5–19). Indirect IF was performed using normal human split skin as substrate and an anti-human IgE mAb. All BP sera were tested at 1/40 dilution. Anti-BMZ IgE Abs were detected in 10 patients, binding to the roof of the bullae in all cases. By immunoblotting, 4 BP sera were found to contain IgE autoantibodies directed against the BPAg1. No IgE Abs directed against the BPAg2 were detected. By SPRIA, specific IgE autoantibodies were detected in 12 of the 19 BP sera (60%), all of them having a severe form of BP. —, negative.

Statistical methods

Statistical analyses were conducted using the nonparametric Spearman's rank correlation test, the *r* coefficient of correlation, Student's *t* test, and the *K*² test with Yates' correction (Stat-View).

Results

Clinical and biologic data

Four patients (patients 1–4) were classified as having a moderate form of BP, while 15 patients (patients 5–19) had a severe disease. Seventeen patients (90%) had elevated IgE levels and all had blood eosinophilia >400/mm³. Total IgE levels were significantly higher in the patients with a severe form of BP (246 ± 72 vs 1780 ± 526; *p* < 0.01). The same observation was made with eosinophil counts (1150 ± 250 vs 2760 ± 490; *p* < 0.01). Circulating IgE autoantibodies to BMZ were detected by indirect IF on skin sections in 10 of the 15 BP sera from patients with the more severe form of the disease but in none of the 4 patients with the moderate form (Table I).

Presence of IgE- and FcεRI-bearing eosinophils in the skin and the blood of BP patients

IgE-bearing cells were detected in the lesional skin of patients under study (4–12 cells/high power field). They were found in the bullae and in the upper dermis, especially beneath the bullae (Fig. 1A). At high magnification, both mono- and polynucleated cells were stained, among which cells with a bilobed nucleus typical of eosinophils (Fig. 1B). On tandem slides, eosinophils constituted 25 to 80% of the infiltrating cells depending on the patients (10–160 cells/high power field). The distribution of the eosinophils was similar to those of IgE-bearing cells. In addition, there was a cor-

relation between the number of tissular eosinophils and IgE-expressing cells in lesional tissues (Spearman's test, *p* = 0.9; *p* < 0.01). No IgE-bearing cells were seen in lesional skin of the patient with pemphigus vulgaris, and no staining was detected with the normal rabbit serum. Moreover, peripheral blood eosinophils of five patients (BP4, -9, -11, -12, and -18) were purified and stained with anti-IgE Abs. A mean of 20% (5–35%) of circulating eosinophils were stained (Fig. 1D), whereas no staining was observed with the isotype control Ab (Fig. 1F). In addition, the presence of the high affinity receptor for IgE (FcεRI) was evaluated on skin and blood eosinophils of these same five BP patients by immunostaining with anti-FcεRI α-chain. FcεRI-bearing eosinophils were detected in lesional skin of the five patients (Fig. 1C) and in the blood (Fig. 1E) of the three patients (BP9, -11, and -18) with the highest IgE levels and blood IgE-bearing eosinophils. No labeling was obtained with the irrelevant Ab (Fig. 1F). On blood eosinophils, IgE seemed localized to cell surface, whereas staining of free FcεRI appeared rather intracytoplasmic (Fig. 1, D and E, respectively). The weak surface labeling with anti-FcεRI Abs might be the result of the receptor occupancy with IgE.

Detection of anti-BPAg1 IgE Abs in the serum of BP patients

The detection of IgE bound to eosinophil surface in BP patients led us to hypothesize that IgE could be directed against BPAg1 and/or BPAg2. The presence of specific IgE Abs was checked using an immunoblot assay on epidermal extracts. Four of the 15 severe patients had IgE Abs to BPAg1 (Table I). None of the patient sera contained IgE autoantibodies to BPAg2.

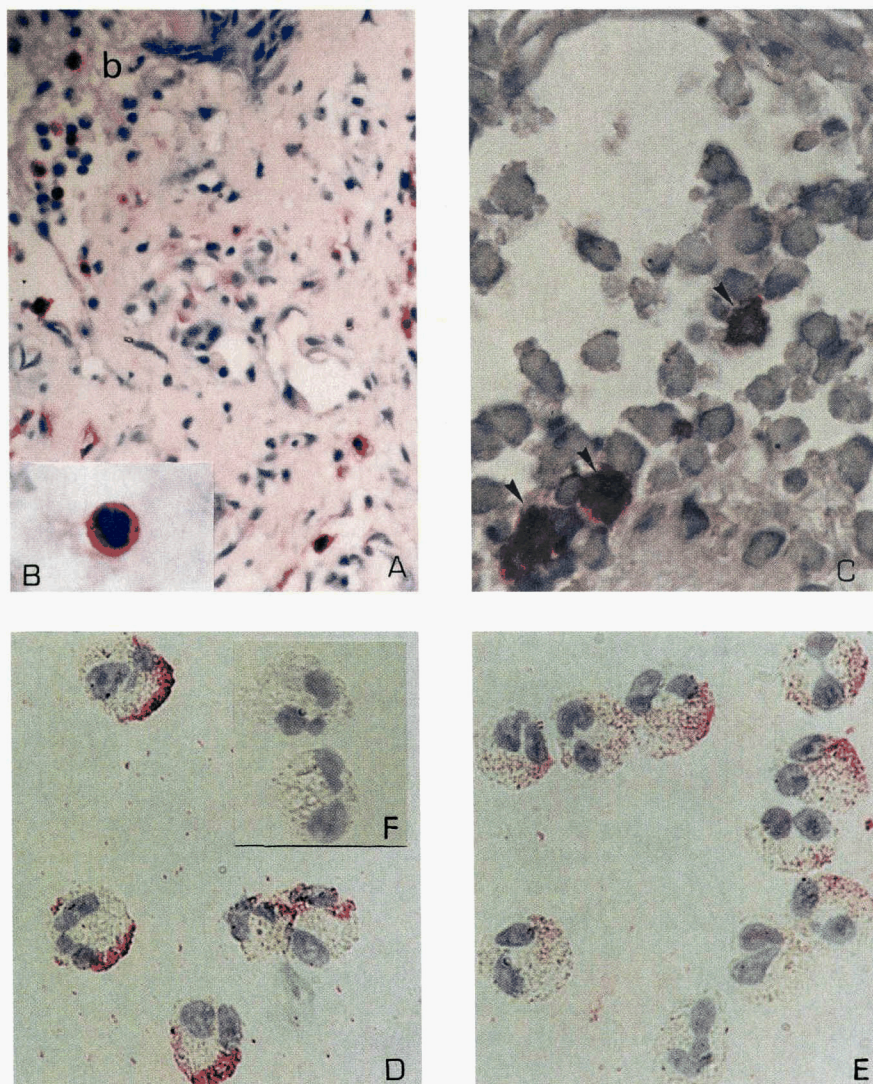
Because of the lack of sensitivity of the IgE immunoblot, a radioimmunoassay was next performed to obtain better insight into the presence of IgE directed against BPAg1. We used a recombinant protein of 55 kDa, encoding the C-terminal end of BPAg1 cDNA. Specific IgE autoantibodies were found only in patients with a severe form of BP (12 of 15) (Table I and Fig. 2). A highly significant correlation was observed between the titers of IgE autoantibodies and the total serum IgE levels on one hand (*r* = 0.886; *p* < 0.0001), and the peripheral eosinophil counts (*r* = 0.723; *p* < 0.0001), on the other hand. Moreover, the titers of IgE Abs to BPAg1 correlated with the numbers of IgE-bearing eosinophils in situ (Spearman's test, *p* = 0.68; *p* < 0.05), supporting the hypothesis that anti-BPAg1 IgE Abs could bind to eosinophils in BP lesional skin. No IgE reactivity was found in sera from patients with pemphigus vulgaris, linear IgA disease, or atopic dermatitis (Fig. 2).

To confirm the specificity of the SPRIA, sera from six BP patients (BP1, -5, -11, -14, -16, and -18) were submitted to immunoblotting on rBP55 (Fig. 3). Except for one serum (BP5), immunoblot and SPRIA gave concordant results. The discrepancy between these two tests, observed in one case, can be explained by the low titer of specific IgE (SPRIA index of 0.85) in this given patient.

Correlation between the presence of IgE Abs to BPAg1 and the severity of the disease

A correlation was found between the presence of specific IgE Abs and the severity of the disease (χ^2 , 8.502; *p* < 0.05) (Table I). Indeed, the 12 patients with IgE Abs to BPAg1 had a severe form of disease, as defined by the number of new bullous lesions occurring during the first 3 days before treatment. Moreover, in 5 of 6 patients followed up for 1 year, the clinical improvement observed under therapy was associated with the disappearance of specific IgE Abs. In the last patient, clinical improvement paralleled a dramatic decrease in the titer of these specific IgE (data not shown).

FIGURE 1. Detection of IgE and FcεRI on skin and blood eosinophils in BP patients. *A*, Positive IgE immunostaining of the inflammatory cells in the bullae (*b*) and beneath the bullae (×400). *B*, At a higher magnification, most of the positively labeled cells have a polylobulated nucleus (indirect immunophosphatase, ×1000). *C*, Indirect immunophosphatase staining with an Ab directed against FcεRI. Note the presence of labeled cells with bilobed nuclei (arrowheads) (×1000). *D* and *E*, Positive IgE (*D*) and FcεRI (*E*) immunostaining on cytopreparations of purified blood eosinophils from BP patients (×1000). *F*, No staining was observed with an irrelevant Ab on the same cytopreparations (×1000).



Discussion

The present study constitutes the first description of specific IgE autoantibodies directed against the BPAg1 in patients with a severe form of bullous pemphigoid. Anti-BMZ IgE Abs have been previously reported by direct IF in the lesional skin and sometimes by indirect IF in a limited number of patients (17, 28–31). In contrast, Bird et al. (32) and Hadi et al. (33) could not detect any anti-BMZ IgE Abs in their patients, using indirect and direct IF, respectively. More recently, anti-BMZ IgE Abs were detected by indirect IF in 6 of 9 patients by Parodi and Rebora (16) and in 9 of 17 patients by Soh et al. (15).

Thus far, the antigenic specificity of these IgE autoantibodies has been studied only twice, by two different techniques. Soh et al. found IgE directed against the BPAg1 in only 2 of 11 patients by immunoblotting (15), while Giudice et al., using an ELISA, failed to detect BPAg2-specific IgE Abs (34).

In the present study, anti-BPAg1-specific IgE Abs were detected in 12 of 19 patients (60%), using a 55-kDa recombinant protein and a radioimmunoassay. Recombinant BP55 has been shown to contain immunodominant epitopes of BPAg1; no sera from other autoimmune bullous diseases, i.e., epidermolysis bullosa acquisita and pemphigus vulgaris, reacted with rBP55 (27). Furthermore, in our study, anti-BPAg1 IgE Abs were detected only in BP patients

and not in control sera from pemphigus vulgaris and linear IgA disease, confirming their specificity. Like Soh et al. (15), using immunoblotting, we could only find IgE directed against the BPAg1. The four positive sera corresponded to the patients with the higher SPRIA index. This could be explained by the increased sensitivity of SPRIA vs immunoblotting for the detection of IgE Abs. IgE Abs directed against the BPAg2 were not detected by any of the techniques used. These data may indicate a preferential IgE response to the BPAg1.

The presence of these IgE Abs seemed correlated to the severity of the disease. Indeed, all the patients with specific IgE autoantibodies presented a more severe form of BP. Moreover, these IgE autoantibodies paralleled the disease activity, as shown by their disappearance with the clinical improvement under treatment. These data suggest the possible implication of IgE in the pathophysiology of BP. The detection of IgE-bearing cells in lesional tissues of BP patients and the correlation observed between the number of these cells and the levels of IgE Abs strongly support this hypothesis.

There is increasing evidence that eosinophils are the main effector cells in determining basement membrane injury in BP and pemphigoid gestationis, through the release of toxic granule proteins (18–21, 35) and proteases (22). Nevertheless, the mechanisms whereby

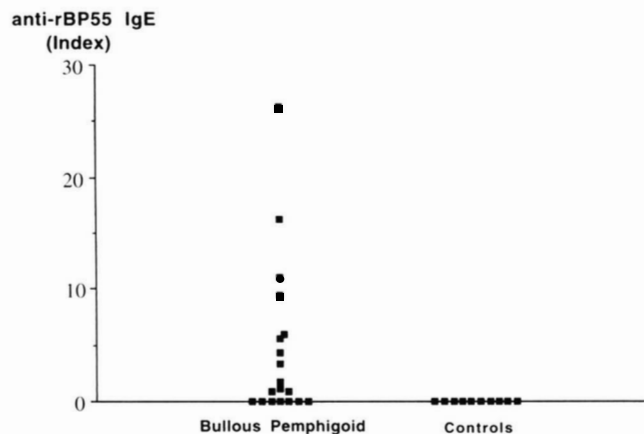


FIGURE 2. Scatterplot representation of SPRIA results. Patient sera and control sera were incubated in plates coated with a recombinant protein of 55 kDa corresponding to the C-terminal region of the BPAg1, and the bound Abs were detected with a murine radiolabeled Ab against human IgE. Each sample was counted in duplicate. The results are shown as an index representing the increase bound radioactivity of the BP sera compared with the mean radioactivity obtained with the NHS, according to the formula given in *Materials and Methods*.

eosinophils might degranulate are not known. In allergic inflammation (3) and parasitic diseases (2, 4, 7), interaction of eosinophils with IgE is one of the preferential mechanisms leading to activation of these cells. By analogy, we hypothesized that IgE-dependent degranulation of eosinophils could play a role in the physiopathology of BP.

Our results support such a hypothesis. Indeed, in this study, we found that in lesional tissues, numerous IgE-bearing cells are eosinophils. This is in agreement with the results of Soh et al. (15), who recently detected IgE-bearing cells in the lesional skin in 7 of 11 patients. These authors concluded that these cells could correspond in part to eosinophils. In our study, cytophilic IgE were also detected on peripheral eosinophils of BP patients. Moreover, the high affinity IgE receptor FcεRI was expressed on blood and skin eosinophils. Taken together, these data indicate that in BP, eosinophils could bind IgE and therefore be activated through IgE receptor engagement. These results are in agreement with our previous description of FcεRI on human blood and skin eosinophils (7), and with a recent study showing the expression of FcεRI on eosinophils in atopic dermatitis and BP lesions (36).

It has been shown that such an IgE-dependent activation of eosinophils could induce a selective release of eosinophil peroxidase and major basic protein (3–5), mediators present in lesional skin of BP (18, 20, 35) and able to exert toxic effects on epithelial cells. Cationic proteins, but also cytokines such as IL-5, could be released by eosinophils after stimulation by IgE (37). IL-5 is the main factor that promotes the terminal differentiation of eosinophil progenitors and enhances the effector capacity of mature eosinophils. It is of interest that the synthesis of this factor has been shown in BP (38, 39) and also in atopic dermatitis (40) in which IgE-dependent mechanisms are prominent events. In these two skin diseases, the synthesis of their own factors of activation by eosinophils suggests an autocrine pathway of activation that could explain the chronicity of the lesions (37).

In BP, blood eosinophilia on one hand and the predominance of IgG4 among the subclasses of Igs anti-BMZ (32, 41) together with the raised serum IgE levels on the other hand suggest an increased production of IL-5 and IL-4, respectively. These two cytokines are considered to be produced predominantly by activated T cells, especially of the Th2 subset. Th2-type immune response has been

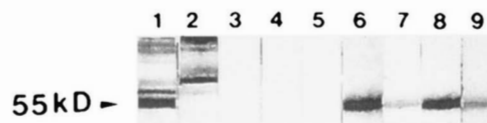


FIGURE 3. IgE immunoblot reactivity of patients with BP with rBP55. Lane 1, positive control: immunodetection with an anti-human IgG Ab of a serum containing anti-BPAg1 IgG Abs; lanes 2 to 3, negative control for rBP55: immunodetection with an anti-human IgG Ab (lane 2) and an anti-human IgE Ab (lane 3) of a pemphigus vulgaris serum; lanes 4–9, binding using serum from patients 1, 5, 11, 14, 16, and 18, respectively. A positive reaction with the rBP55 can be seen in lanes 6 to 9.

shown in allogeneic diseases and experimental models of systemic autoimmunity (42). In BP patients, it would be important to show a switch toward IL-4- and IL-5-producing Th2 cells. The results of Endo et al. (38), showing that PBMC of BP patients have increased in vitro IL-5 production compared with normal subjects, suggest an increased number of circulating T lymphocytes producing IL-5 in BP and argue in favor of this hypothesis.

Our results raised the question of the role of IgE in autoimmune diseases. Elevated serum IgE levels have been described in a few autoimmune diseases including systemic lupus erythematosus (SLE), rheumatoid arthritis, and dysthyroid orbitopathy (43–45), but not in other autoimmune bullous diseases. Moreover, antinuclear IgE Abs have been detected in SLE (43) and rheumatoid arthritis (44). In SLE, these IgE Abs could contribute to immediate-type hypersensitivity phenomena, including hives. In rheumatoid arthritis, IgE rheumatoid factor have been detected, especially in cases with extra-articular lesions, such as vasculitis, suggesting that IgE rheumatoid factor may be particularly relevant in the pathogenesis of systemic manifestations of rheumatoid arthritis (44). Furthermore, a correlation between the serum IgE levels and the clinical stages of dysthyroid orbitopathy has been recently reported (45). Taken together, these data indicate that in addition to IgG, IgE could play a role in some autoimmune diseases. Our results suggest that in BP, these IgE autoantibodies could take part in an effector mechanism involving IgE-mediated eosinophil activation contributing therefore to the dermal-epidermal damage.

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