Gliptin-associated bullous pemphigoid shows peculiar features of anti-BP180 and -BP230 humoral response: Results of a multicenter study

ORIGINAL ARTICLE

Gliptin-associated bullous pemphigoid shows peculiar features of anti-BP180 and -BP230 humoral response: Results of a multicenter study

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Background: Recently, several case-control studies demonstrated an association between gliptins and bullous pemphigoid (BP) occurrence. However, data on the clinical and immunologic features of gliptin-associated bullous pemphigoid (GABP) are controversial.

Objective: This study aimed to clinically and immunologically characterize a large cohort of GABP patients to get an insight into the pathophysiology of this emerging drug-induced variant of BP.

Methods: Seventy-four GABP patients were prospectively enrolled and characterized from 9 different Italian dermatology units between 2013 and 2020.

Results: Our findings demonstrated the following in the GABP patients: (1) a noninflammatory phenotype, which is characterized by low amounts of circulating and skin-infiltrating eosinophils, is frequently found; (2) immunoglobulin (Ig)G, IgE, and IgA humoral responses to BP180 and BP230 antigens are reduced in frequency and titers compared with those in patients with idiopathic BP; (3) IgG reactivity targets multiple BP180 epitopes other than noncollagenous region 16A.

Limitations: A limitation of the study is that the control group did not comprise only type 2 diabetes mellitus patients with BP.

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Conclusion: GABP patients show peculiar features of anti-BP180 and -BP230 humoral responses, laying the foundation for diagnostic improvements and getting novel insights into understanding the mechanism of BP onset. (J Am Acad Dermatol https://doi.org/10.1016/j.jaad.2022.02.036.)

Key words: autoantibody; bullous pemphigoid; BP180; epitope; gliptin; humoral response.

INTRODUCTION

Bullous pemphigoid (BP) is an autoimmune bullous disease caused by autoantibodies (autoAbs) against the hemidesmosomal components BP180 and BP230. The diagnosis of BP relies on the clinical picture and the detection of linear immunoglobulin (Ig)G and/or C3 deposits along the dermal-epidermal junction using direct immunofluorescence.[1,2] Indirect immunofluorescence and enzyme-linked immunosorbent assays (ELISAs) based on the immunodominant portion of BP180, noncollagenous region 16A (NC16A), and the N- and C-terminal regions of BP230 also have diagnostic relevance.[3-5] BP, which predominantly affects elderly persons, is characterized by pruritic tense blisters and erosions over urticarial plaques on the trunk, extremities, and face.[1] Several factors, including drugs, can trigger BP.[6] Interestingly, recent studies showed an increasing prevalence of type 2 diabetes mellitus in BP patients close to the date of the approval of dipeptidyl peptidase 4 (DPP-4) inhibitors, a family of glucose-lowering agents also known as gliptins, by the European Medicines Agency (2006).[7-9] Recently, several case-control studies demonstrated an association between gliptins and BP occurrence.[10-13]

Clinical features peculiar to gliptin-associated bullous pemphigoid (GABP) have been reported in several Japanese studies, which showed the predominance of a noninflammatory phenotype (NIP).[14-16] On the contrary, no major clinical differences between GABP and idiopathic bullous pemphigoid (IBP) have been found in most European studies.[13,17,18]

Moreover, to date, immunologic data from the literature have been conflicting. Several studies have shown that GABP patients have a reduced reactivity to NC16A in comparison with IBP patients and tend to react to the midportion of the BP180 ectodomain.[14,15,19] In contrast, other studies have reported a similar reactivity profile between GABP and IBP patients.[13,20]

CAPSULE SUMMARY

- Data on the clinical and immunologic features of gliptin-associated bullous pemphigoid are controversial. Italian patients show a reduced humoral response to BP180 or BP230 and, frequently, a noninflammatory phenotype.
- In these patients, commercial assays often fail to detect autoantibodies. An assay based on a BP180 epitope is useful to support diagnosis.

The present study aimed to clinically and immunologically characterize a large cohort of GABP patients to get insights into the pathophysiology of this emerging drug-induced variant of BP.

MATERIALS AND METHODS

Study patients

In this study, 74 GABP patients were prospectively enrolled and characterized from 9 different Italian dermatology units between 2013 and 2020. In parallel, a cohort of 44 retrospectively collected IBP patients was also characterized. BP was diagnosed based on previously described criteria.[21] As for the association between gliptins and BP occurrence, the enrolled cases were classified, at least, as having "probable" adverse drug reactions, in accordance with the Naranjo score.[17,22,23] The clinical phenotype was determined as unblinded during clinical and anamnestic assessments. An inflammatory phenotype (IP) with erythema was distinguished from an NIP with scant or absent erythema.[14] Sera from 50 normal healthy individuals (NHIs) were used as controls. The study was conducted in accordance with the Declaration of Helsinki guidelines and approved by the local ethics committees of the participating institutions; all the patients gave written informed consent.

ELISA

BP IgG autoAbs against BP180-NC16A and BP230 were characterized using commercial ELISA (MBL Co), according to the manufacturer's protocol. For the detection of IgE and IgA autoAbs, the protocol was slightly modified. Briefly, BP180 and BP230 microwell strips were incubated with 5- and 50-times diluted BP and control sera for IgE and IgA detection, respectively. After washing, the bound antibodies were detected using 1000- and 10,000-fold diluted horseradish peroxidase-conjugated anti-human IgE (Southern Biotech) and IgA antibody (Sigma), respectively.[24,25] Instead, BP IgG autoAbs

Abbreviations used:

AutoAb: autoantibody
BP: bullous pemphigoid
ELISA: enzyme-linked immunosorbent assay
GABP: gliptin-associated bullous pemphigoid
IB: immunoblotting
IBP: idiopathic bullous pemphigoid
Ig: immunoglobulin
IP: inflammatory phenotype
LAD-1: linear IgA disease-1
NC16A: noncollagenous region 16A
NHI: normal healthy individuals
NIP: noninflammatory phenotype

against the epitopes were characterized using previously established ELISAs.[21] The cut-off value for IgG, IgE, and IgA ELISAs was determined as the mean of optical density measured in 50 NHI sera plus 3 SDs.

Immunoblotting (IB) analysis

IB studies were conducted using keratinocyte extracts and the 120-kDa soluble ectodomain of BP180 (linear IgA disease-1 [LAD-1]) as previously described.[21]

Statistical analyses

The Fisher's exact (probability) test and Student *t* test were used to compare the data of the GABP (NIP and IP) and IBP patients. A *P* value ≤.05 was considered significant. All the statistical analyses were performed using the SPSS statistical package.

RESULTS

An increased risk of BP occurrence is associated with linagliptin

The latency time between gliptin initiation and BP diagnosis ranged from 1 to 53 months, with a mean value of 9.5 months (8.5 months for IP and 12.9 months for NIP) (Table I). The mean latency time between gliptin initiation and pruritus development was 3.1 months (range, 0−12 months) (Table I). Although the most used gliptin in Italy is sitagliptin (44%), followed by vildagliptin (23%) and linagliptin (22%) (Table I),[26] in our GABP cohort, this gliptin's use showed a different distribution. In fact, an elevated risk of BP was significantly associated with the use of linagliptin (41/73 [56%]) (*P* < .001), whereas the use of vildagliptin (21/73 [29%]) was similar to that reported in type 2 diabetes mellitus patients without BP (Table I).

The NIP is frequent but does not affect the majority of GABP patients

In our GABP cohort, most patients (41/70, 59%) had an IP (Supplementary Fig 1, available via Mendeley at https://data.mendeley.com/datasets/sdcn39jrxm/1, Table I). However, the proportion of NIP cases was significantly higher among the GABP patients than among the IBP patients (29/70 [41%] vs 2/22 [9%], respectively; *P* = .002). Thus, the relative risk of NIP was 4.56 (95% confidence limits, 1.18-17.59) for the GABP patients compared with that for the IBP patients.

Table I. Demographic and clinical characteristics of 74 gliptin-associated bullous pemphigoid patients

Bullous pemphigoid patients	GABP	IBP
Sex, n (%)		
Male	46 (62)	27 (64)
Female	28 (38)	15 (36)
Mean age, y		
Male	77.1	76.2
Female	78.3	74.3
Clinical phenotype, n (%)		
Noninflammatory, NIP	29 (41)	2 (9)
Inflammatory, IP	41 (59)	20 (91)
Not detectable	4	22
Mucosal involvement	5/30 (17)	3/26 (12)
Mean latency time before BP diagnosis in 67 GABP patients, mo	9.5 (range, 1-53 mo)	UK
NIP (26 patients)	12.9 (range, 2-53 mo)	UK
IP (36 patients)	8.5 (range, 2-40 mo)	UK
Mean latency time before pruritus development in 36 GABP patients, mo	3.1 (range, 0-12 mo)	UK

Gliptin intake, n (%)	GABP	ARNO report[23]	*P* value
Linagliptin	41 (56)	13,788 (22)	**<.001**
Vildagliptin	21 (29)	14,659 (23)	.263
Sitagliptin	8 (11)	27,677 (44)	**<.001**
Alogliptin	3 (4)	5825 (9)	.216

In bold are *P* values that are statistically significant.
BP, Bullous pemphigoid; *GABP*, gliptin-associated bullous pemphigoid; *IBP*, idiopathic bullous pemphigoid; *IP*, inflammatory phenotype; *NIP*, noninflammatory phenotype; *UK*, unknown.

Circulating and infiltrating eosinophils are more abundant in IBP patients than in GABP patients

The mean circulating eosinophil count in 46 GABP patients was lower than that measured in 24 IBP patients (673.7 cells/μL vs 1090.4 cells/μL, respectively; *P* = .144) (Supplementary Fig 2, available via Mendeley at https://data.mendeley.com/datasets/sdcn39jrxm/1, and Methods). Of note,

Table II. IgG, IgE, and IgA humoral responses to BP180 and BP230 antigens in GABP and IBP patients

Autoantibody class	Target epitope/antigen	GABP, n/N (%)	IBP, n/N (%)	*P* value
IgG	BP180 NC16A	Total 37/60 (62)	Total 40/44 (91)	**<.001**
		NIP 10/22 (45)		.098
		IP 25/36 (69)		
	BP180 E-1080	Total 24/50 (48)	Total 15/44 (34)	.210
		NIP 7/18 (39)		.377
		IP 17/31 (55)		
	BP180 E-1331	Total 24/50 (48)	Total 18/44 (41)	.537
		NIP 10/18 (56)		.561
		IP 14/31 (45)		
	BP230	Total 12/57 (21)	Total 23/44 (52)	**.002**
		NIP 1/21 (5)		**.037**
		IP 10/34 (29)		
	BP180 and BP230 double negative	Total 22/60 (37)	Total 2/44 (5)	**.0001**
IgE	BP180 NC16A	Total 17/50 (34)	Total 35/44 (80)	**<.001**
		NIP 6/18 (33)		1.0
		IP 11/31 (35)		
	BP230	Total 12/50 (24)	Total 30/44 (68)	**<.001**
		NIP 1/18 (5)		.036
		IP 11/31 (35)		
IgA	BP180 NC16A	Total 14/50 (28)	Total 28/44 (64)	**.001**
		NIP 4/18 (22)		,527
		IP 10/31 (32)		
	BP230	Total 11/44 (22)	Total 30/44 (68)	**<.001**
		NIP 3/18 (17)		.724
		IP 8/31 (26)		

In bold are *P* values that are statistically significant. The first *P* value in each box was obtained by comparing GABP and IBP and the second one by comparing NIP and IP.
GABP, Gliptin-associated bullous pemphigoid; *IBP*, idiopathic bullous pemphigoid; *Ig*, immunoglobulin; *NIP*, noninflammatory phenotype; *IP*, inflammatory phenotype.

circulating eosinophils were lesser in GABP patients with an NIP than in GABP patients with an IP (295.6 cells/μL in 16 NIP patients vs 907.1 cells/μL in 28 IP patients, P = .035). Interestingly, peripheral eosinophilia (≥500 cells/μL) was also significantly associated with IP (50%, 14/28 IP patients vs 19%, 3/16 NIP patients; P = .040).

In parallel, the skin-infiltrating eosinophil density in periblister skin was assessed in 47 patients (20 with GABP and 27 with IBP). The mean eosinophil number in the GABP patients was lower than that in the IBP cases (2.3 cells/mm^2 vs 3.3 cells/mm^2, respectively; P = .096) (Supplementary Fig 2, *B* to *D*). In particular, the mean eosinophil number was lower in the NIP patients than in the IP patients (1.7 cells/mm^2 vs 2.7 cells/mm^2, respectively; P = .453).

GABP patients show reduced humoral immunity to BP180 and BP230 in comparison with IBP patients

IgG. The frequency of IgG reactivity to BP180-NC16A and BP230 was significantly lower in the GABP patients than that measured in 44 IBP cases (62% vs 91% and 21% vs 52%, respectively) (Table II). This reduction was more pronounced in GABP patients with an NIP, wherein only 45% reacted to BP180-NC16A, whereas 5% of the GABP patients had bound BP230. In contrast, the IP subgroup reacted to BP180-NC16A and BP230, with a frequency of 69% and 29%, respectively (Table II). In line with the reactivity data, in the IP patients, the mean titer of IgG for BP180 and BP230 was higher than that in the NIP patients (Supplementary Table I, available via Mendeley at https://data.mendeley.com/datasets/sdcn39jrxm/1).

IgE. Similarly, the IgE reactivity to either BP180 (34%) or BP230 (24%) in the GABP patients was significantly decreased in comparison with that measured in the IBP patients (Table II). In this case, a significant difference between NIP and IP reactivity was detected only for BP230. In line with the IgG data, the mean IgE titer was higher in the IP patients than in the NIP patients (Supplementary Table I).

IgA. As for circulating IgA, the reactivity to BP180 and BP230 in the GABP patients was reduced in

comparison with that in the IBP patients (Table II). In particular, in the NIP subgroup, 22% of the patients reacted to BP180-NC16A and 17% to BP230, whereas in the IP subgroup, 32% and 26% reacted to BP180-NC16A and BP230, respectively. However, the mean IgA titer was similar in the 2 subsets of patients (Supplementary Table I). Interestingly, although 35% (14/40) of the IBP patients showed IgA reactivity to LAD-1, as detected using IB, none of the 48 GABP patients had LAD-1 bound by IgA ($P < .001$).

IgG reactivity in GABP patients targets multiple BP180 epitopes

Similar to the 44 IBP patients (E-1080, 34%; and E-1331, 41%), the GABP patients frequently reacted to E-1080 and E-1331 epitopes (48% and 48%, respectively) (Supplementary Fig 3, available via Mendeley at https://data.mendeley.com/datasets/sdcn39jrxm/1, Table II). However, no significant difference in terms of IgG reactivity to the previously described BP180 regions between GABP patients with an NIP and those with an IP was found (Table II). In addition, most GABP patients (41/49, 84%) reacted to LAD-1, as detected using IB (Fig 1 and Supplementary Fig 3). Finally, as determined using IB of the human keratinocyte extracts, almost all patients analyzed (49/50, 98%) reacted to BP180, whereas only 16 of the 50 (32%) reacted to BP230 (Fig 1).

Of note, in the GABP cohort, the proportion of double-negative (BP180-NC16A and BP230) patients (37%) was remarkable in comparison with that of double-negative IBP patients (5%) ($P = .0001$) (Table II) and equally distributed between those with an NIP and those with an IP. Interestingly, double-negative GABP patients were positive for E-1080 (5/11 NIP and 7/10 IP; 57%) and E-1331 (38%), as detected using ELISA (Fig 2 and Supplementary Table II, available via Mendeley at https://data.mendeley.com/datasets/sdcn39jrxm/1). Moreover, 16 of 20 double-negative GABP patients reacted to LAD-1, and all reacted to BP180, as detected using IB of the human keratinocyte extracts.

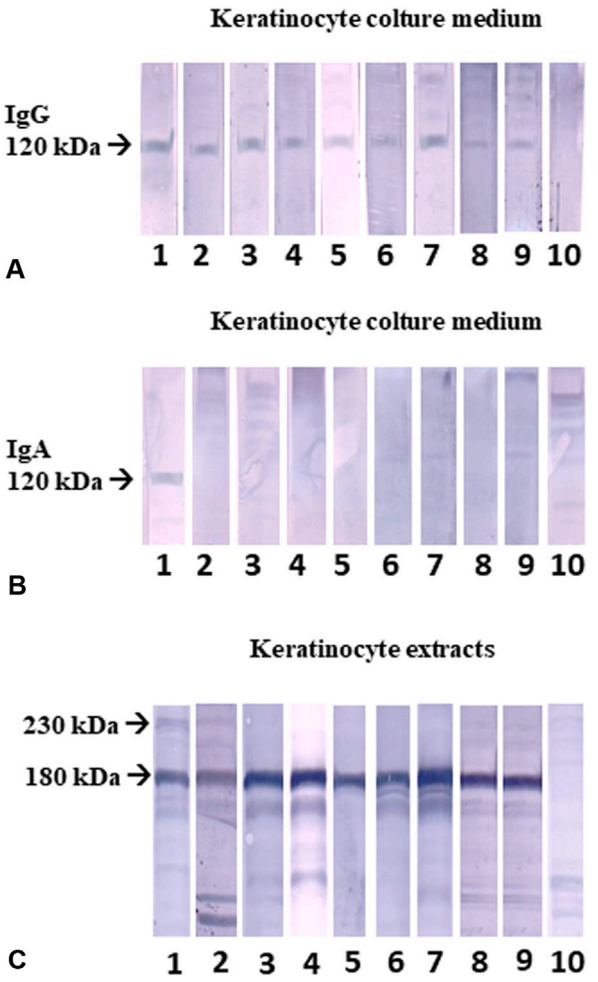

Fig 1. Humoral response of gliptin-associated bullous pemphigoid (GABP) patients against BP180 antigen. **A,** Immunoglobulin G reactivity to LAD-1 (120 kDa) by immunoblotting on a keratinocyte culture medium: lane 1, bullous pemphigoid-positive control serum; lane 2-9, GABP sera; lane 10, healthy volunteer serum. **B,** Immunoglobulin A reactivity to LAD-1 by immunoblotting on keratinocyte culture medium: lane 1, bullous pemphigoid-positive control serum; lane 2-9, GABP sera; lane 10, healthy volunteer serum. **C,** Immunoglobulin G reactivity to BP180 (180 kDa) and BP230 (230 kDa) by immunoblotting on keratinocyte extracts: lane 1, bullous pemphigoid-positive control serum; lane 2-9, GABP sera; lane 10, healthy volunteer serum.

DISCUSSION

There is a growing number of studies reporting the association between BP and gliptin intake.[10,27,28] Although distinct clinical and immunologic features have been reported in Japanese GABP patients,[14-16] according to other European studies, there are no major differences between GABP and IBP.[13,17,18] However, we observed that Italian GABP patients showed peculiar immunologic features and an increased frequency of the NIP. Of note, a clinical manifestation that is more evident and typical in IP patients than in NIP patients seems to expedite diagnosis, with a mean latency time before GABP diagnosis in IP patients shorter than that in NIP patients (8.5 vs 12.9 months, respectively). This finding can have clinical relevance in alerting providers that the diagnosis of NIP patients may be more challenging.

However, although the NIP was significantly more frequent in the GABP patients than in the IBP patients, the majority of our patients presented

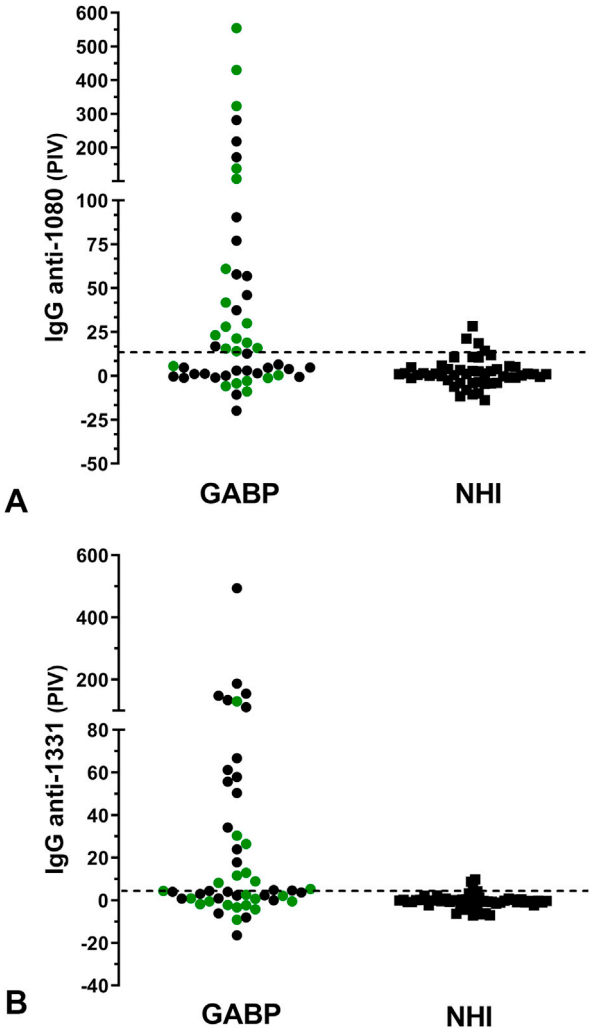

Fig 2. Immunoglobulin G reactivity to BP180 epitopes by E-1080 and E-1331 enzyme-linked immunosorbent assays. Scatter plot representation of **(A)** E-1080 and **(B)** E-1331 enzyme-linked immunosorbent assay results. Fifty gliptin-associated bullous pemphigoid sera and 50 normal healthy individuals' sera were analyzed. The *dashed lines* indicate the cutoff value, evaluated as described in the Materials and Methods section. The *green spots* are gliptin-associated bullous pemphigoid sera negative for BP180-NC16A and BP230, detected using enzyme-linked immunosorbent assays.

with an IP, in contrast to Japanese results. This may suggest that ethnic background plays a role in determining the phenotype of GABP.

Izumi et al[14] showed that eosinophil infiltration in periblister skin was related to the IP. In parallel, Kridin et al[29] reported that the mean of circulating eosinophil counts is lower in GABP patients than in IBP patients. In this study, we further investigated this issue, demonstrating that the lowering of eosinophil counts in the GABP patients may have been due to the large proportion of NIP patients in the GABP cohort in comparison with those in the IBP cohort. In fact, the number of circulating eosinophils was similar in GABP patients with IP and the IBP patients, whereas the lowering became relevant in the NIP patients. On the other hand, the number of skin-infiltrating eosinophils was not significantly different between the GABP and IBP patients or between the NIP and IP patients. Furthermore, the inhibition of DPP-4 has been postulated to enhance the activity of eotaxin 1, which can induce eosinophil recruitment in the skin and contribute to blister formation in patients with BP.[30,31] In this context, it can be speculated that the inhibition of DPP-4 by gliptins can induce eosinophil skin infiltration and blister formation even in the presence of normal ranges of circulating eosinophils.

In our cohort of GABP patients, we found significantly reduced IgG, IgE, and IgA humoral immune responses to BP180-NC16A and BP230 antigens. Of note, none of the 48 GABP sera analyzed had LAD-1 bound by IgA, whereas one-third of the IBP sera showed IgA reactivity to LAD-1, as detected using IB, highlighting the total absence of IgA reactivity to BP180 ectodomain epitopes as an additional peculiar feature of GABP patients. Because IgA represents the most prominent antibody isotype in the mucosal immune system and because IgA specific to BP180 has been found in mucous membrane pemphigoid patients, we evaluated the mucosal involvement of our IBP and GABP patients. Interestingly, mucosal involvement was found to be similarly represented in the 2 groups of patients. Thus, the absence of both IgA reactivity to LAD-1 and IgA deposition on the dermal-epidermal junction of the GABP patients (data not shown) suggest no or secondary role of IgA in GABP pathogenesis. A possible explanation for the absence of IgA bound to LAD-1 in the GABP patients could be selective isotype switching. Ellebrecht et al[32] reported that in pemphigus vulgaris patients, IgA clones to desmogleins were mainly generated by the isotype switching of IgG1 clones and not of IgG4 ones. In line with this finding, it can be hypothesized that reactivity to NC16A and BP230 could have been mainly due to an IgG1 response, whereas IgG4 mainly binds to LAD-1. Thus, similar to pemphigus patients, it can be speculated that IgG1 can induce IgA to NC16A and BP230 by isotype switching, whereas IgG4 cannot.

The reduction of circulating IgG, IgE, and IgA autoAbs in the GABP patients could be related to a milder disease and a good response to therapy, as reported in several studies,[11,14,18,20] further confirming the pathogenic role of circulating autoAbs.

This study investigated the largest cohort of GABP patients in which the humoral response has been

epitope-mapped so far. In Japanese GABP patients, the autoAbs mainly targeted the midportion of the extracellular domain of BP180 and did not react to the C-terminal domain of BP180.[14,19] On the contrary, in our study, the GABP patients frequently reacted not only to the epitope in the midportion of BP180 but also to the C-terminal portion. In addition, no significant difference in IgG reactivity to BP180 epitopes between the NIP and IP patients was found. The discrepancy between previously reported data and our findings may have been due to different ethnicities, which could have influenced the humoral response, and the size of the analyzed cohorts. In line with literature data, most GABP patients' IgG reacted to LAD-1, and almost all the patients showed reactivity to BP180, as detected using IB of the keratinocyte extracts. These findings suggest that the GABP patients reacted to multiple epitopes in the BP180 ectodomain in addition to NC16A and that this reactivity has a pathogenic role. In this context, it should be considered that DPP-4 is a cell-surface plasminogen receptor that, through the activation of plasminogen, leads to the formation of plasmin, a serine protease that cleaves the BP180 ectodomain. Therefore, it can be speculated that the inhibition of plasmin by gliptins can provoke alterations in the correct cleavage of BP180, inducing the preferential exposure of neoepitopes. In addition, the processing of BP180 may induce conformational changes on the molecule, which may also induce epitopes distinct from those on native BP180.[19] This phenomenon can induce the breakdown of the immune tolerance of BP180, with a change in the epitope profile recognized by BP autoAbs mainly targeting the antigen's midportion.

Epitope mapping unveiled a peculiar feature of GABP patients: the remarkable proportion of double-negative patients (37%) in comparison with our cohort of IBP patients (5%). As a consequence of this feature, the diagnostic performance of commercial ELISAs was reduced in this subset of patients. Of note, in previously published cohorts of BP, the percentage of double-negative patients (ranging from 9% to 34%)[33-38] seemed to increase with time. This could be due to the increasing use of gliptins for the treatment of type 2 diabetes mellitus patients and, in turn, an increasing proportion of GABP in recent cohorts of BP patients.

Of note, the double-negative sera were frequently positive (57%) for E-1080, as detected using ELISA. In light of these findings, an established ELISA based on E-1080[21] could be a sensitive tool to improve the diagnostic performance of commercial ELISAs in GABP patients.

CONCLUSION

Altogether these findings demonstrate that in GABP patients, the IgG, IgE, and IgA humoral responses to the BP180 and BP230 antigens is weakened and BP180 epitopes other than NC16A are targeted by circulating IgG. Specifically, the significant reactivity to E-1080 and E-1331 lays the foundation for diagnostic improvements and further studies aimed at understanding the mechanism of GABP onset.

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Conflicts of interest

None disclosed.

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