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RESEARCH ARTICLE

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Reactivity against the BP180 ectodomain in patients with bullous pemphigoid, mucous membrane pemphigoid, multiple sclerosis and Parkinson disease

Jonathan Tegtmeyer[1,2] | Maurizio Romagnuolo[1,3] | Christoph M. Hammers[1,4] | Bianca Opelka[1] | Christian Probst[5] | Lars Komorowski[5] | Angelo V. Marzano[3,6] | Enno Schmidt[1,4] | Stephanie Goletz[1]

[1]Lübeck Institute of Experimental Dermatology (LIED), University of Lübeck, Lübeck, Germany

[2]Clinic and Polyclinic for Dermatology and Venereology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

[3]Dermatology Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy

[4]Department of Dermatology, Allergology and Venerology, University of Lübeck, Lübeck, Germany

[5]Institute of Experimental Immunology, EUROIMMUN AG, Lübeck, Germany

[6]Dermatology Unit, Department of Pathophysiology and Transplantation, Università degli Studi di Milano, Milan, Italy

Correspondence
Stephanie Goletz, Lübeck Institute of Experimental Dermatology, University of Lübeck, Ratzeburger Allee 160, Lübeck 23562, Germany.
Email: stephanie.goletz@uksh.de

Abstract

The 16th non-collagenous domain (NC16A) of BP180 is the main antigenic target of autoantibodies in bullous pemphigoid (BP) and mucous membrane pemphigoid (MMP). Commercially available assays detect serum autoantibodies against NC16A in the majority of BP (80%–90%) and in approximately 50% of MMP patients. However, a standardized test system for detecting antibodies against other regions of BP180 is still lacking. Moreover, anti-BP180 autoantibodies have been found in neurological conditions such as multiple sclerosis and Parkinson disease. This study aimed at identifying primary epitopes recognized by BP autoantibodies on the BP180 ectodomain. Serum samples of 51 BP and 30 MMP patients both without anti-NC16A reactivity were included along with 44 multiple sclerosis and 75 Parkinson disease sera. Four overlapping His-tagged proteins covering the entire BP180 ectodomain (BP180(ec)1–4) were cloned, expressed, purified and tested for reactivity by immunoblot. IgG antibodies to BP180(ec)3 were detected in 98% of BP, 77% of MMP and 2% of normal human sera. Only weak reactivity was detected for neurological diseases against BP180(ec)1, BP180(ec)2 and BP180(ec)4, in 3%, 11% and 7% of tested multiple sclerosis sera, respectively. 8% of Parkinson disease sera reacted with BP180(ec)2 and 9% with BP180(ec)4. In conclusion, this study successfully identified epitopes recognized by BP autoantibodies outside the NC16A domain in pemphigoid diseases. These findings contribute to a better understanding of the immune response in BP and MMP with potential implications for a future diagnostic assay for NC16A-negative pemphigoid patients.

KEYWORDS
BP180 ectodomain, bullous pemphigoid, mucous membrane pemphigoid, multiple sclerosis, Parkinson disease

Jonathan Tegtmeyer and Maurizio Romagnuolo contributed equally to this work.

1 | INTRODUCTION

Bullous pemphigoid (BP) antigen 180 (BP180, also called type XVII collagen or BPAG2) is a transmembrane protein mainly expressed in the dermoepidermal junction and in the brain, representing the major autoantigen in pemphigoid diseases, including BP and mucous membrane pemphigoid (MMP).[1] The BP180 ectodomain is divided into 16 non-collagenous (NC) domains, namely NC1 to NC16, and 15 collagenous domains.[2] The 16th non-collagenous domain (NC16A), located adjacent to the cellular membrane is the immunodominant region in BP, representing the target of the currently available commercial diagnostic tests—for example, enzyme-linked immunosorbent assay (ELISA) and indirect IF microscopy based on the BIOCHIP technology—used in clinical practice guiding the diagnostic work-up of bullous diseases.[3–7] The identification of various BP180 epitopes and their putative role in the pathogenesis of bullous diseases have recently raised interest, considering that approximately 15%–20% of BP patients and up to 50% of MMP patient sera do not recognize the NC16A domain used in the standardized serological assays.[8,9] Most of these sera react with the BP180 ectodomain also called linear IgA bullous dermatosis autoantigen (LAD-1), a cell-derived soluble protein, which is formed by shedding via different proteases of the disintegrin and metalloproteases (ADAM) family.[10]

The present study comprises an experimental validation of the reactivity of human IgG serum autoantibodies with recombinant fragments of the BP180 ectodomain in LAD-1-positive and NC16A-negative BP and MMP patients. In addition, recent evidence showed a significant epidemiological association between BP and neurological conditions, such as multiple sclerosis, Alzheimer disease and Parkinson disease.[11,12] Considering that BP180 regions may represent targets of IgG autoantibodies in neurological and neurodegenerative patients, verification of IgG reactivity against BP180 ectodomain fragments in a subset of multiple sclerosis and Parkinson disease patients' sera was performed.[13,14]

2 | MATERIALS AND METHODS

2.1 | Patients' sera

Inclusion criteria of patients used for the BP180 ectodomain immunoblot analysis were (i) clinical picture consistent with the diagnosis, (ii) no reactivity to the BP180-NC16 domain by ELISA (Euroimmun, Lübeck, Germany), and (iii) LAD-1 reactivity by immunoblot. In total, sera from 51 BP and 30 MMP patients were included. Two BP patients' sera and one MMP serum displayed additional reactivity with BP230 by ELISA. As controls, sera of healthy blood donors were used ($n = 54$, cohort A). Additionally, patients with Parkinson disease ($n = 75$), multiple sclerosis ($n = 44$) and neurological disease controls ($n = 75$) were included. Sera from these patients were not reactive against BP180-NC16A using BIOCHIP mosaic-aided indirect immunofluorescence microscopy as shown previously.[15] Sera from additional healthy blood donors ($n = 75$, cohort B) were included as negative controls. The study was approved by the ethics committee of the University of Lübeck (10-229, 20-421) and was conducted according to the Declaration of Helsinki Principles.

2.2 | Production of LAD-1 extract

LAD-1 was prepared as described previously.[16–18] In brief, HaCaT cells were cultured to 80% confluence at 37°C and 5% CO_2 in Keratinocyte Growth Medium 2 (PromoCell GmbH, Heidelberg, Germany) and then treated with 100 μg /mL vitamin C for 48 h. The cell supernatant was centrifuged for 10 min at 4°C and 1000× *g*. Afterwards, 0.5 M EDTA (pH 8, dilution 1:100) and 0.1 M PMSF (dilution 1:100) were added. After protein precipitation with ammonium sulphate (saturation of 30%), extracts were centrifuged at 4°C, 90 min, at 17000× *g* and precipitates were dissolved in PBS containing protease inhibitors (Protease Inhibitor Cocktail cOmplete mini™, Merck, Darmstadt, Germany) and concentrated using Amicon® Ultra Centrifugal Filters (MWCO 30 kD, Merck).

2.3 | Generation and expression of four different fragments of the BP180 ectodomain

For cloning of the four overlapping His-tagged fragments (BP(ec) 1–4, aa 563–807, 793–1039, 1024–1270, 1255–1497, Figure 1) of the BP180 ectodomain into pTriEx1, commercially available cDNA for BP180 (Acc-N. BC168368, imaGenes GmbH, Berlin, Germany) was used for generation of the 4 different fragments by PCR using the following primers sense BP180(ec)1, ATACGTCTCGCATGGGAAAT CTCCGAGGAAGCCCTGG, anti-sense BP180(ec)1, ATACGTCTC CTCGAGGATCTTG CCTGGAGCTCCTGGTTC, sense BP180(ec)2, ATACGTCTCGCATGGGCCGACC AGGAATAAAAGGTGAAC, anti-sense BP180(ec)2, ATACGTCTCCTCGAGTGGGGGA CCCTGAA CTCCGGATAG, sense BP180(ec)3, ATACGTCTCGCATGCAGAG TGACA GTATTAGATC, anti-sense BP180(ec)3, ATACGTCTCCTCG AGGGGGCCAACAAT GAAGCTGCGCAC, sense BP180(ec)4 ATAC GTCTCACATGAGCTACCTCACAA GTCCTGATGTG and anti-sense BP180(ec)4 TTAACGTCTCCTCGAGCGGCTTGACAGCAAT ACTTC TTCTCCTTCTCCG.

Transfection of HEK293 cells, cultured in DMEM (Capricorn Scientific GmbH, Ebsdorfergrund, Germany) with 10% fetal calf serum, with the different plasmids was done using 25 kDa linear Polyethylenimine (Polysciences, Hirschberg an der Bergstrasse, Germany). After 48 h, cells were lysed using a Tris–HCl buffer, pH 7.4, containing 10% sucrose, 5 mM EDTA and 1 mM PMSF and stored at −20°C. After buffer exchange, the His-tagged proteins were purified by immobilised metal affinity chromatography using Talon affinity matrix (Takara Bio Europe, Clontech, Saint-Germain-en-Laye, France) following the manufacturer's instructions. The protein concentrations were determined via SDS–PAGE using a BSA standard.

2.4 | Immunoprecipitation

To verify serum reactivity with BP180, immunoprecipitation was performed. As substrate LAD-1 extract and as antibodies a monoclonal rabbit anti-type XVII collagen antibody (10 μg, clone EPR18614, Abcam, Berlin, Germany), two different BP sera with LAD-1 IgG reactivity (no NC16A reactivity) and one serum of a healthy blood donor as control (15 μL of each serum) were used.

GammaBind Plus Sepharose (Cytiva, Marlborough, MA, USA) was equilibrated in PBS followed by coupling to the monoclonal BP180 antibody and to serum IgG for 3 h at RT on a rotator at 4°C. In the meantime, the extract was precleared at 4°C for 1 h using 100 μL of sepharose and 50 μL of normal human serum. After the 3 h incubation time, bound antibodies were washed twice with 1 mL of PBS and 200 μL of precleared extract was added to each sample and incubated over night at 4°C on a rotator. After four washing steps with PBS, beads were resuspended in 30 μL of Laemmli buffer and heated for 5 min at 95°C. Samples were separated via SDS–PAGE.

2.5 | SDS–PAGE and immunoblotting

LAD-1 extracts, precipitated proteins as well as recombinant proteins of BP180 (BP180(ec)1–4) were fractionated by SDS–PAGE, transferred to nitrocellulose membrane, and immunoblotted as described previously.[19] LAD-1 extracts and precipitated proteins were separated using 6% SDS–PAGE or precast gels (7.5%, Mini-PROTEAN TGX precast gels, BioRad) for LC–MS/MS. For the recombinant BP180 proteins with less molecular weight 8% SDS–PAGE gels were prepared. For immunoblotting, proteins were transferred to nitrocellulose (Amersham Protran NC, Cytiva) and blocked with 5%–8% skimmed milk powder in TBS-T (Tris-buffered saline pH 7.5 with 0.5% Tween20). Nitrocellulose stripes were incubated with monoclonal rabbit anti-BP180 antibody (dilution 1:3000) or sera (dilutions 1:50–1:800) in incubation buffer (5%–8% skimmed milk powder in TBS-T with 1% bovine serum albumin, BSA) over night at 4°C on a rocking platform. On the next day, blots were washed two times 12 min with TBS-T and incubated with the secondary antibodies (HRP-conjugated goat anti-rabbit IgG, 1:1000, Dako, Glostrup, Denmark and HRP-conjugated rabbit anti-human IgG, 1:500–1:4000, Dako) for 1 h at RT. After an additional washing step, binding of antibodies to the respective proteins were visualized using diaminobenzidine (Merck).

2.6 | Mass spectrometry

Liquid chromatography with tandem mass spectrometry (LC–MS/MS) analysis was performed by the Proteomics and Metabolomics Facility at the Wistar Institute in Philadelphia, PA, USA as described before.[20] The MS/MS spectra were compared against the UniProt human protein database and a database of common contaminants (Figure 1).

3 | RESULTS

3.1 | Optimization of the LAD-1 immunoblot

Serological diagnosis of BP and MMP requires the detection of circulating serum autoantibodies against various domains of the BP180 protein. In general, the commercially available Anti-BP180-NC16A-4X ELISA (IgG) (Euroimmun) is used, but there are patients with autoantibodies directed against epitopes outside the NC16A domain. For these patients, the LAD-1 immunoblot is used in the autoimmune laboratory of the Department of Dermatology in Lübeck, as well as for patient sera with linear IgA dermatosis. Therefore, this blot was considered as reference and serum reactivity with LAD-1 was a prerequisite for the immunoblots with BP180 ectodomain fragments 1–4 (BP180(ec) 1–4). To obtain a better separation of the

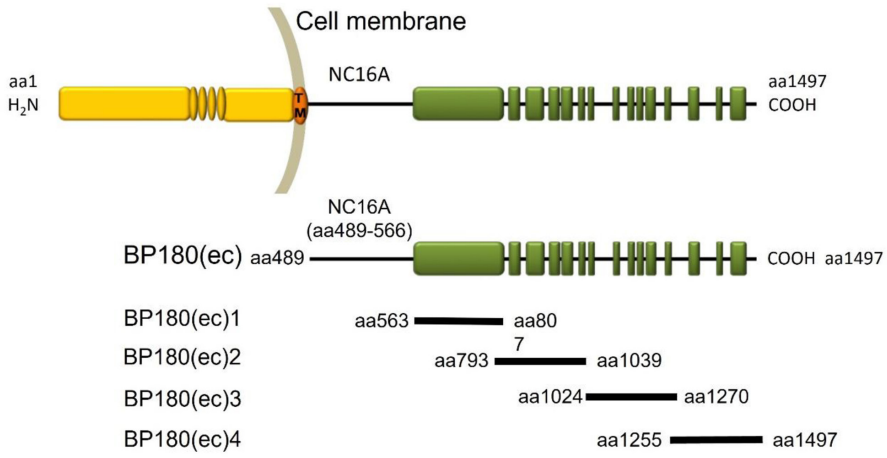

FIGURE 1 Schematic representation of BP180 structure and the location of the recombinant fragments used in this study. BP180, bullous pemphigoid antigen 180 kDa; aa, amino acids; H_2N, N-terminal domain; TM, transmembrane domain; NC16A, non-collagenous domain 16A; COOH, carboxy-terminal domain; BP180(ec), BP180 ectodomain.

protein bands in the LAD-1 extract, the SDS–PAGE was extended. This modification revealed the separation of the previous observed 120 kDa band into two bands (Figure 2) and in immunoblot only the 120 kDa band was considered as BP180 LAD-1, due to the fact, that the monoclonal rabbit anit-BP180 antibody showed reactivity with this protein band. All BP ($n = 51$) and MMP ($n = 30$) sera included in this study revealed reactivity with the newly identified BP180-LAD1 protein band. All tested sera of healthy blood donors were negative.

3.2 | Identification of anti-BP180 reactivity

To prove that the newly identified/separated protein band corresponds to BP180, immunoprecipitation using LAD-1-containing supernatant of HaCaT cells and the monoclonal BP180 antibody, two BP patient sera, and a serum of a healthy blood donor were used. Afterwards, a 120 kDa protein was detected by immunoblotting using again the anti-BP180 antibody (Figure 2). The corresponding bands in the Coomassie stained gel were analysed by LC–MS/MS, and as expected, BP180 was confidently identified by its peptides (Table S1).

3.3 | IgG reactivity in bullous pemphigoid and mucous membrane pemphigoid

Immunoblotting revealed that 50/51 (98%) of the BP sera recognized at least one fragment of the BP180 ectodomain; particularly, fragment 3 (BP180(ec)3), corresponding to amino acids (aa) 1024–1270, was recognized by almost all reactive BP sera (Figure 3, Table 1 and Figure S1). The other fragments, that is, BP180(ec)1, 2 and 4 were recognized by 22%, 41% and 71% of the BP sera, respectively (Table 1 and Figure S1).

Only one BP serum did not show any reactivity in immunoblot. Similar results were obtained testing MMP sera: most patients (77%) recognized BP180(ec)3, while BP180(ec)1, 2 and 4 were recognized by 13%, 53% and 63% of MMP sera, respectively (Table 1). Thirteen per cent (4/30) of the MMP sera were not reactive with any of the generated fragments. Multiple reactivities against different fragments were present in 50% (15/30) of the sera (Figure S1). In the control group, 81% of the sera showed no reactivity against the ectodomain fragments, while reactivity was detected in 10/54 sera (19%), mainly towards BP180(ec)2 (7%) and BP180(ec)4.

3.4 | IgG reactivity in multiple sclerosis, Parkinson disease and other neurological diseases

Immunoblot analyses of multiple sclerosis sera showed that the majority were not reactive against the generated fragments (81%), and none of the sera recognized BP180(ec)3. Reactivity to BP180(ec)1, BP180(ec)2 and BP180(ec)4 were found in 3%, 11% and 7% of the samples, respectively (Table 2). Similar results were obtained testing Parkinson disease sera: 80% (60/75) did not react with the fragments, while 8% (6/75) were positive for BP180(ec)2 and 7/75 (9%) for BP180(ec)4. BP180(ec)1 and BP180(ec)3 were recognized by 1/75 (1%) and 2/75 (3%) sera, respectively (Table 2). In the group of other neurological diseases, the results were partially overlapping as BP180(ec)3 was not recognized by any sera, and the positivity rate of BP180(ec)1, BP180(ec)2 and BP180(ec)4 was 3% (2/75), 7%

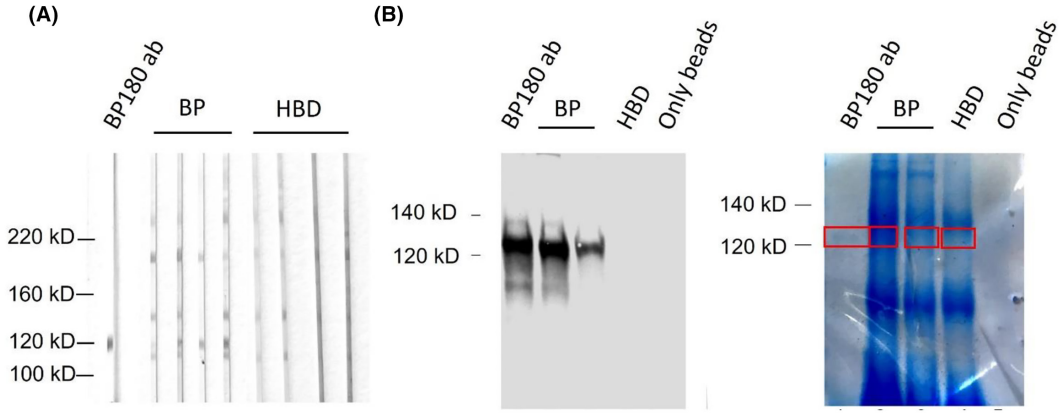

FIGURE 2 Confirmation of serum reactivity with BP180. (A) Representative LAD-1 immunoblot was performed using a monoclonal rabbit anti-human BP180 antibody (BP180 ab; dilution 1:3200) and human bullous pemphigoid (BP) sera (dilution 1:100). Sera of healthy blood donors (HBD) served as controls. After incubation with the corresponding secondary antibody-HRP conjugate two bands around 120 kDa were visable. The slightly lager band detected by the patients sera (arrow) is at the same height as the band detected by the rabbit anti-human BP180 antibody and corresponds to BP180/type XVII collagen. The position of the molecular weight markers is shown on the left. (B) Left: immunoblot analysis of precipitated proteins using a monoclonal anti-BP180 antibody (dilution 1:1000). For the immunoprecipitation of BP180, the monoclonal anti-human BP180 antibody (10 µg) and the sera of BP patients (each 15 µL) were used. Serum of a HBD and the GammaBind Plus Sepharose without any bound antibody served as controls (only beads). Right: Coomassie staining of a gel with the same samples as applied by immunoblotting. Red boxes marked the protein containing regions which were subjected to LC–MS/MS analysis. The positions of the molecular weight is given in kD.

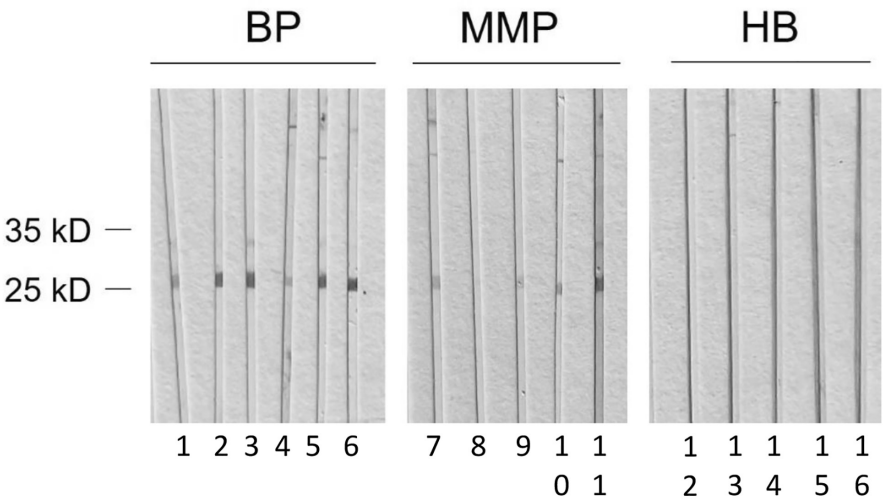

FIGURE 3 Sera of bullous pemphigoid and mucous membrane patients showed reactivity with a recombinant fragment of the BP180 ectodomain. Representative immunoblot analysis using recombinant BP180(ec)3 (amino acids 1024-1270). Strips were incubated with bullous pemphigoid (BP) sera (1:100, stripes 1–6), mucous membrane pemphigoid (MMP) sera (1:100, stripes 7–11) and sera of healthy blood (HB) donors (1:100, stripes 12–16). All BP sera, 4/5 MMP sera showed reactivity with BP180(ec)3, whereas all sera of HB donors were negative. The position of the molecular weight markers is shown on the left.

TABLE 1 Immunoblot reactivity of bullous pemphigoid and mucous membrane pemphigoid patients against the generated recombinant fragments of the BP180 ectodomain.

	BP180(ec)1 (aa563–807)	BP180(ec)2 (aa793–1039)	BP180(ec)3 (aa1024–1270)	BP180(ec)4 (aa1255–1497)	Reactivity	Multiple reactivity
BP sera ($n=51$)	11/51 (22%)	21/51 (41%)	50/51 (98%)	36/51 (71%)	50/51 (98%)	46/51[a] (90%)
MMP sera ($n=30$)	4/30 (13%)	16/30 (53%)	23/30 (77%)	19/30 (63%)	26/30 (87%)	15/30[a] (48%)
Healthy blood donors ($n=54$)	1/54 (2%)	4/54 (7%)	1/54 (2%)	5/54 (9%)	10/54 (19%)	1/54[a] (2%)

Abbreviations: aa, amino acids; BP, bullous pemphigoid; MMP, mucous membrane pemphigoid.
[a]Detailed reactivities are shown in supplementary materials.

(5/75) and 4% (3/75), respectively (Table 2). Most of the sera were not reactive with the generated fragments (65/75, 80%). Details of the group of other neurological diseases are described elsewhere.[15] Of the 75 sera of the healthy control group (cohort B), 70 (93%) were negative, while 5 showed reactivity against at least one fragment, which is in line with previous studies (Table 2).[21]

4 | DISCUSSION

Our results on the IgG immunoreactivity with the BP180 ectodomain, excluding the immunodominant NC16A region, implement and confirm the findings of other investigators who generated various recombinant fragments of BP180 for both pemphigoid diseases, BP and MMP,[8,22–27] as well as for patients with neurological disorders.[14,21,28]

It is known that autoantibodies in BP may recognize different antigenic sites of BP180 including the C-terminal and the N-terminal domains, probably due to epitope spreading phenomena starting from the immunodominant NC16A region.[24] However, limited data exist on the 10%–20% of NC16A-negative BP patients.

One of the first studies on the autoantibody reactivity against BP180 ectodomain fragments performed by Hoffmann and colleagues showed that 65% of NC16A-negative patients sera recognized a C-terminal portion of the protein corresponding to the aa 1351–1497.[23] Subsequently, Mariotti et al. showed that 50% of NC16A-negative BP sera recognized epitopes on the BP180 ectodomain comprising aa 1080–1107 and aa 1331–1404.[25] Similarly, Fairley et al. found that four NC16A-negative BP sera recognized one or more recombinant fragment of the BP180 ectodomain spanning from aa 1080 to aa 1413.[8] Of note, the recognition of the BP180 ectodomain seems to be a specific serological feature of dipeptidyl peptidase 4-inhibitors (DPP-4i, gliptins)-associated BP.[29,30] Interestingly, in our cohort, 98% (50/51) of the NC16A-negative BP sera recognized BP180(ec)3, corresponding to the aa 1024–1270, suggesting that in the majority of this patient subgroup, the main epitopes could be clustered in this region.

Our results show that also in the majority of BP180 positive/NC16A-negative MMP patients (23/30, 77%), BP180(ec)3 was preferentially targeted by IgG autoantibodies. These findings are in line with the work of other authors who assessed IgG reactivity generating different BP180 fusion proteins: Balding et al. demonstrated

TABLE 2 Immunoblot reactivity of patients with neurological diseases against the generated recombinant fragments of the BP180 ectodomain.

	BP180(ec)1 (aa563–807)	BP180(ec)2 (aa793–1039)	BP180(ec)3 (aa1024–1270)	BP180(ec)4 (aa1255–1497)	Reactivity	Multiple reactivity
MS sera ($n=44$)	1/44 (3%)	5/44 (11%)	0/44 (0%)	3/44 (7%)	9/44 (19%)	1/75[a] (1%)
PD sera ($n=75$)	1/75 (1%)	6/75 (8%)	2/75 (3%)	7/75 (9%)	15/75(20%)	1/75[a] (1%)
Other neurological diseases sera ($n=75$)	2/75 (3%)	5/75 (7%)	0/75 (0%)	3/75 (4%)	10/75 (13%)	0/75[a] (0%)
Healthy volunteer sera, cohort B ($n=75$)	1/75 (1%)	0/75 (0%)	1/75 (1%)	3/75 (4%)	5/75 (7%)	0/75[a] (0%)

Abbreviations: aa, amino acids; MS, multiple sclerosis; PD, Parkinson disease.

[a]Detailed reactivities are shown in supplementary materials.

that a major antigenic site in MMP patients was located in the C-terminal domain[26]; this observation was subsequently confirmed by Murakami and colleagues who showed that 40% and 30% of MMP sera reacted with two generated fusion proteins, respectively, corresponding to the BP180 C-terminal domain.[27] Finally, Lee and co-workers provided a more accurate analysis, generating three recombinant fusion protein covering the BP180 ectodomain (aa 1252- 1352, aa 1342–1442 and aa 1432–1532), and demonstrated that the majority of the MMP sera (38%) reacted with the most terminal portion of the domain.[31] In the control group, 81% of the sera exhibited no reactivity towards the ectodomain fragments. Reactivity was observed in 19% of the sera (10/54), primarily directed towards BP180(ec)2 (7%) and BP180(ec)4 (9%). This percentage was higher than anticipated based on previous studies, which reported anti-BP180 autoantibody rates in healthy subjects ranging from approximately 0% to 4%.[21]

Taken together, these findings indicate that, as for NC16A-negative BP, the main epitopes are localized in a specific portion of the C-terminal domain also in BP180-positive/NC16A-negative MMP patients. The development of a new serological detection system (e.g. ELISA or indirect immunofluorescence test) using recombinant BP180(ec)3 (aa 1024-1270), which display the highest sensitivity and specificity in immunoblotting, would increase the diagnostic accuracy of this subset of patients, facilitating the follow-up and clinical management of BP and MMP patients. The other fragments showed either non-specific signals or insufficient reactivity.

Finally, the study of the anti-BP180 ectodomain IgG reactivity in neurological diseases sera aimed to further elucidate if the strong epidemiological association between BP and neurological/neurodegenerative diseases could be linked to the autoantibody response affecting both the skin and the brain tissue.[2] It has been previously shown that some patients with Alzheimer disease, Parkinson disease and multiple sclerosis possess IgG against BP180, but they seem unable to bind the cutaneous basement membrane zone.[14] One of the first study by Messingham and colleagues on Parkinson disease showed that about one third of the analysed sera reacted with the BP180 ectodomain in immunoblot, and were able to bind neurons located in the substantia nigra of human brain tissue but not the cutaneous basement membrane zone, suggesting that the epitopes could differ in the two tissues (brain vs. skin).[28] Subsequently, Kokkonen et al. demonstrated in 115 Alzheimer disease patients that 20% of the tested sera were positive for BP180-NC16A using ELISA, and 18% reacted with the full-length protein in immunoblotting, with a positive correlation between high serum titres of anti-BP180 and the severity of dementia.[32] Similar results were obtained in 48 Alzheimer disease patients studied by Wang and co-workers.[2,13] Conversely, Recke et al., using a different panel of detection systems, found no significant increase of BP180-specific autoantibodies in 50 multiple sclerosis and 75 Parkinson disease sera compared to controls.[15] More recently, Tuusa and colleagues performed an epitope mapping in Alzheimer disease and multiple sclerosis sera, using glutathione-S-transferase generated fragments covering the full-length BP180, showing that different epitopes were targeted in those diseases: multiple sclerosis sera appeared to predominantly react with the BP180 intracellular (100%) and mid-extracellular domain (91%), while Alzheimer disease sera mainly recognized the intracellular (91%) and C-terminal domain (91%).[14]

The results of the present study show that 20% (15/75) of Parkinson disease and 19% (8/44) of multiple sclerosis sera react with at least one of the generated BP180 ectodomain fragments in immunoblotting, and mainly towards BP180(ec)2 (aa 793–1039) and BP180(ec)4 (1255 – 1497). Of interest, also 5/75 sera of the healthy control group showed reactivity with at least one fragment of the BP180 ectodomain, which is in line with previous studies.[21] However, no major differences can be seen between the control group and the neurological diseases regarding serum reactivity with BP180.

Of note, our Parkinson disease sera and multiple sclerosis cohorts were negative using BP180-NC16A-based ELISA. In addition, the Parkinson disease sera were previously also shown to be negative using BP180-transfected HEK293 cells (BIOCHIP technology) and human cell-derived extracellular matrix for the detection of full-length BP180 in immunoblot, confirming a higher sensitivity and specificity of the here used immunoblotting with the cell-derived BP180 fragments, although the autoantibodies were not able to bind human skin.[15] Taken together, these findings confirm that a minority of patients with neurological diseases classically associated to BP, such as Alzheimer disease, Parkinson disease, multiple sclerosis could present autoantibodies directed against BP180, mainly targeting epitopes outside the NC16A domain, preferentially in the intracellular domain and/or the protein ectodomain.

Little is known about the autoantibody's generation process in the brain: one hypothesis argues that chronic neuroinflammation

characterizing the neurological/neurodegenerative disease could expose brain antigens leading to the break of immune tolerance, and the subsequent production of autoantibodies.[33] However, these autoantibodies seem not to be pathogenetic for the skin: this could be explained by the generally low titres, the differences between skin and brain isoforms of BP180 and, probably, the multifactorial pathogenesis of these conditions in which autoantibodies could only play a relative role in disease onset, as suggested by the presence of these autoantibodies in otherwise healthy elderly patients.[21]

Moreover, the aim of our study was to assess the IgG reactivity against the BP180 ectodomain in different diseases. As such, IgA anti-BP180 reactivity, that has been described in 4%–37% of MMP sera, has not been studied.[9]

In conclusion, the present study describes the mid to C-terminal portion of BP180 represented here by recombinant BP180(ec)3 (aa 1024-1270) as immunodominant region of NC16A-negative BP and MMP patients. As such, BP180(ec)3 may be a promising candidate to develop a standardized detection system to further increase the diagnostic sensitivity for BP and MMP.

AUTHOR CONTRIBUTIONS

Jonathan Tegtmeyer: article drafting, acquisition and analysis of the data, visualization. Maurizio Romagnuolo: article drafting, acquisition and analysis of the data, visualization. Christoph M. Hammers: provided reagents, reviewing, editing, analysis of data; Bianca Opelka; Angelo V. Marzano: reviewing and editing; Christian Probst, Lars Komorowski: providing reagents and materials, reviewing and editing Enno Schmidt: concept and design, reviewing and editing; Stephanie Goletz: concept and design, writing—original draft, review and editing, acquisition and analysis of data. All the authors revised the manuscript and approved its final version.

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CONFLICT OF INTEREST STATEMENT

CP is employee, LK is member of the Board of EUROIMMUN. ES has a scientific cooperation with EUROIMMUN. The other authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Maurizio Romagnuolo https://orcid.org/0000-0002-7015-8319
Stephanie Goletz https://orcid.org/0000-0002-0355-4290

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Appendix S1: Supporting Information.

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