BP180 Autoantibodies Target Different Epitopes in Multiple Sclerosis or Alzheimer's Disease than in Bullous Pemphigoid

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BP180 AUTOANTIBODIES TARGET DIFFERENT EPITOPES IN MULTIPLE SCLEROSIS OR ALZHEIMER’S DISEASE THAN IN BULLOUS PEMPHIGOID

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Abbreviations: bullous pemphigoid (BP), Alzheimer’s disease (AD), multiple sclerosis (MS) immunofluorescence (IF), non-collagenous 16A (NC16A), enzyme-linked immunosorbent assay (ELISA), amino acids (AA), intracellular domain (ICD), extracellular domain (ECD)

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ABSTRACT

Neurological patients have an increased risk for bullous pemphigoid (BP) in which autoantibodies target BP180, a cutaneous basement membrane protein also expressed in the brain. Here we show that 53.6% sera of patients with multiple sclerosis (MS) (n=56) had IgG reactivity against full-length BP180 in immunoblotting, while in BP180-NC16A ELISA (n=143), only 7.7% MS samples studied were positive. Epitope mapping with 13 fusion proteins covering the entire BP180 polypeptide revealed that, in MS and Alzheimer’s disease (AD) patients, IgG autoantibodies target regions located in the intracellular and mid-extracellular parts of BP180, but not the well-known BP epitopes located in the NC16A domain and the distal part of extracellular domain. In indirect immunofluorescence analysis 8.1% MS sera recognized the cutaneous basement membrane and in full-length BP180 ELISA analysis 7.5% MS and AD sera were positive indicating that these autoantibodies rarely recognize BP180 in its native conformation. Thus, in MS and AD patients BP180 autoantibodies have a different epitope profile than in patients with BP and seldom bind to native BP180. This explains the inability of these autoantibodies to cause skin symptoms. Our results suggest that the autoantibodies against BP180 alone are not sufficient to induce BP in MS and AD patients.
INTRODUCTION

Bullous pemphigoid (BP) is a severe autoimmune blistering skin disease that usually first manifests when the patient is at an advanced age (Bagci et al., 2017; Nishie, 2014; Schmidt and Zillikens, 2013). Several epidemiological studies indicate that neurological and neurodegenerative diseases like multiple sclerosis (MS) and Alzheimer’s disease (AD) are common in BP patients and, more specifically, the presence of these neurological disorders increases an individual’s risk of developing BP (Försti et al., 2017; Lai et al., 2016). In BP the major autoantigen is the transmembrane hemidesmosomal protein BP180 (also known as collagen XVII or BPAG2) (Bagci et al., 2017). The autoantibodies against the extracellular non-collagenous (NC) 16A domain of BP180 are sufficient to induce hemidesmosomal breakdown and detachment of the epidermis and dermis and are believed to play a major role in BP pathogenesis (Ujiie et al., 2014). As well as being present in the skin BP180 is also expressed at low levels in the brain (Seppänen, 2013), but is not detectable in postmortem brain samples taken from patients with various neurodegenerative disorders (Barrick et al., 2016). The strong epidemiological association between neurological disorders and BP, alongside the neuronal expression of BP180 has led to the assumption that neurodegeneration or neuroinflammation could lead to the failure of self-tolerance against BP180 and thus the development of BP. This hypothesis was in some extent supported by our recent finding that about 20% of AD patients harbor anti-BP180-NC16A autoantibodies, although these autoantibodies neither recognize native cutaneous BP180 nor cause skin symptoms (Kokkonen et al., 2017). Autoantibodies against BP180 and BP230, another BP-associated autoantigen, have also been reported in small proportion of patients with Parkinson’s disease and non-specified dementia (Foureur et al., 2006; Messingham et al., 2016). To date circulating autoantibodies against BP autoantigens have not been detected in patients with MS (Recke et al., 2016), although MS is more strongly associated with BP than is any other neurological disease (Försti et al., 2017; Lai et al., 2016).
In the present study we investigated the prevalence of anti-BP180 IgG antibodies among patients with MS. Better to understand the differences in the skin and the brain in autoimmunization against BP180, we compared in detail the binding of autoantibodies of patients with BP, MS and AD to linear epitopes of BP180 and to natively folded BP180. Our results suggest alternative targets in the generation of autoantibodies against BP180 in cutaneous and neurological diseases.

**RESULTS**

**Patients with multiple sclerosis have autoantibodies against BP180**

Sera from patients with MS (n=143) and neurologically healthy control subjects (n=140) were tested using a well-established, commercially available ELISA analysis for BP180-NC16A and BP230 IgG autoantibodies (Table 1). Eight MS samples (5.6%) were positive for IgG against BP180-NC16A only, three (2.1%) were positive for IgG against BP230 only, and three (2.1%) were positive for both BP180 and BP230 (based on cut-off value 9 U/ml) (Table 1). Only two control samples (1.4%) were positive for BP180-NC16A IgG.

A subset of MS sera (n=56, including 14 that were positive for BP180-NC16A, BP230 or both, and 42 randomly selected negative samples) was further analyzed by immunoblotting against the full-length recombinant BP180. Thirty sera (53.6%) recognized a 180 kDa collagenase sensitive band corresponding to BP180 (Figure 1). There were no age or sex differences between patients from whom positive and negative MS sera were drawn. Interestingly, only seven of these sera were also found to be positive (9.1–27.0 U/ml) when the BP180-NC16A ELISA was used, suggesting that the
BP180 autoantibodies of MS patients may recognize epitopes other than NC16A in immunoblotting.

**Autoantibodies of patients with bullous pemphigoid, multiple sclerosis and Alzheimer’s disease target different epitopes in BP180**

Our finding that MS autoantibodies recognized the full-length BP180 in immunoblotting, but mainly not the NC16A domain in ELISA, prompted us to perform an epitope mapping analysis using 13 Glutathione-S-transferase (GST) fusion proteins (FP) covering most of the human BP180 molecule (Figure 2a). In addition to MS sera (n=35, including all 11 BP180-NC16A ELISA positive and 24 randomly selected ELISA negative sera), epitope mapping was also performed using previously characterized AD sera (n=32 including 22 randomly selected BP180-NC16A ELISA positive and 10 negative sera [Kokkonen et al., 2017]). The control samples were comprised of sera from BP patients (n=23); samples from randomly selected neurologically healthy subjects (n=24, including two BP180-NC16A ELISA positive and 12 negative samples from our previously characterized control group [Kokkonen et al., 2017]) and ten randomly selected BP180-NC16A ELISA negative samples from our current control group. The ability of each sera to recognize each FP (1-13) was classified in ordinal categories (0, 1, 2, 3) on the basis of densitometric quantitation of immunoblot signals (“0” being no band, “3” very strong band) (Figure 2c-g). The differences between the four groups (controls, BP, MS and AD) were analyzed using the Fisher’s test, which showed apparent inhomogeneity between the four groups (Supplementary Table S1 online). Most serum samples showed some immunoreactivity against at least one of the FPs, and only two control sera recognized none of them (Figure 2c-g and data not shown). In general, the pattern of recognized epitopes varied from sample to sample in all groups, but there was a clear tendency for
the healthy control samples to recognize fewer FPs and the intensities were weaker, suggesting lower autoantibody titers and specific affinities (Figure 2c-g, Supplementary Table S2 online).

Immunoblotting (Figure 2c-f) followed by densitometric quantitation (Figure 2g; Supplementary Figure S1 online) revealed that the epitope recognition pattern of the BP samples was clearly different from that of the others. The FP corresponding to the immunodominant NC16A domain (FP5; amino acids [AA] 489–567) was positive in 15 of the 23 BP sera, but in none of MS, AD or control sera (Figure 2c-g). FP5 remained negative independently of the phosphorylation of Ser544, which enhanced the recognition by BP sera (Supplemental results and Figure S2 online). The BP sera were also more frequently immunoreactive against FP1 (AA 2-168), FP10 (AA 936-1106) and FP12 (AA 1190-1399) (Figure 2c,g), all of which correspond with known BP autoepitopes (Di Zenzo et al., 2008). The reactivity against FP5, FP10 and FP12 was similar and the behavior of these epitopes in the BP group reflected that of the entire study sample (Figure 2g, Supplementary Table S4 online). The reactivity against the membrane proximal regions of the intracellular domain (ICD) (FP3 and FP4 AA 261-401) and the N-terminal regions of the extracellular domain (ECD) excluding NC16A domain (FP6-9 AA 558-994) showed a positive correlation across the whole study sample (Supplementary Table S4 online).

The strongest immunoreactivity for AD and MS samples was generally detected against those FPs that correspond to the ICD epitopes and the N-terminal half of the ECD, apart from the NC16A domain (Figure 2d,e,g). FP1 (AA 2-168) and FP3 (AA 261-401) were positive significantly more often in AD sera than in control samples (Figure 2d,f,g, Supplementary Table S3 online). Reactivity against FP4 (AA 377-455) was significantly more frequent in MS samples than in the other groups (Figure 2c-g, Supplementary Table S3 online). Finally, there was no difference between any of the groups in terms of recognition of three of the FPs: FP6, FP9 and FP11 (Supplemental Figure S1 online).
BP180 autoantibodies of few patients with multiple sclerosis or Alzheimer’s disease recognize natively folded BP180

Previous studies have indicated that BP180-IgG positive sera samples from only a small proportion of neurological patients with dementia, MS or Parkinson’s disease are able to bind to the skin basement membrane zone in indirect immunofluorescence (IF) analysis (Foureur et al, 2006, Messingham et al., 2016, Recke et al., 2016, Kokkonen et al., 2017). Therefore, indirect IF analysis was used to test all MS sera that were positive for BP180-NC16A (n=11) and BP230 (n=3) in the ELISA, and those that were positive for full-length BP180 in the immunoblotting (n=23). Three of the 37 samples (8.1%) reacted weakly against the epidermal side of the basement membrane zone on salt-split skin (Supplemental Figure S3 online). One of these samples was ELISA positive and the other two recognized the full-length BP180 in immunoblotting. A retrospective evaluation of the hospital records of the donors of these three samples revealed neither diagnosis nor clinical symptoms of BP.

We performed a further ELISA analysis on a full-length BP180 (Izumi et al., 2016) to investigate the ability of the IgG autoantibodies of neurological and BP patients and healthy controls to recognize BP180 in its native conformation. For the full-length ELISA we used the same MS (n=35) and BP (n=23) sera as in the epitope mapping, but included a larger sample of previously characterized AD (n=111) and control sera (n=40) due to the higher proportion of increased BP180-NC16A ELISA values (Kokkonen et al. 2017). We found that 18/23 (78.2%) of BP patient sera recognized the full-length BP180, whereas only 4/35 (11.4%) of MS, 7/111 (6.3%) of AD and 3/40 (7.5%) of healthy control samples were classified as positive (Table 2, Supplemental Figure S4 online). Comparison between the full-length BP180 and BP180-NC16A ELISA results showed that these assays correlated well among the samples from BP patients (Table 2). All four of the MS sera
that tested positive in the full-length BP180 ELISA and three of the seven AD sera were also positive in BP180-NC16A ELISA, suggesting that non-NC16A epitopes recognized in immunoblotting may not be commonly recognized by the cognate autoantibodies in their native folded conformation.

DISCUSSION

The significance of IgG autoantibodies against BP180 in the pathogenesis of BP is undisputable, while the factors and events leading to the breakage of immunotolerance against BP180 are still largely unknown. The concomitant occurrence of neurological diseases in BP patients has led to suggestions of neurodegeneration or neuroinflammation as triggering factors for BP development and to attempts to detect BP180 or BP230 autoantibodies in neurological patients as an initial sign of BP (Försti et al., 2017). Our current findings show that, like those with AD and Parkinson’s disease, patients with MS have IgG autoantibodies against BP180, but (again, as with those of AD and Parkinson’s patients) (Kokkonen et al., 2017; Messingham et al., 2016), these autoantibodies do not bind to the cutaneous basement membrane. Our most important finding is that autoantibodies in MS and AD patients target different epitopes of BP180 and/or have different conformation specificity to those in BP patients and healthy subjects, and are therefore not pathogenic.

The comprehensive epitope mapping we performed showed that anti-BP180 autoantibodies are relatively common both in healthy individuals and in patients with neurological disorders, but are more often detectable and tend to have greater immunoreactivity in patients with MS or AD. As expected, the autoantibodies of BP patients recognized the NC16A domain and distal C-terminal epitopes: the immunoreactivity against the NC16A domain both in immunoblotting and BP180-NC16A ELISA correlated strongly, and 65% of BP patients were positive both in the BP180-
NC16A and full-length BP180 ELISA assays. In contrast, the AD and MS sera were negative both for the phosphorylated and non-phosphorylated NC16A domain, but had some increased and characteristic reactivity against the ICD and mid-ECD epitopes in immunoblotting. Positive correlation, i.e. the co-presence or co-absence of these epitopes, was evident at the level of the whole study sample (Supplementary Table S4 online), which demonstrates that BP has its own distinct epitope profile. The presence of non-NC16A epitopes explains why the ELISA showed only a few MS patients with elevated levels of anti-BP180-NC16A, while more than half of the patient sera recognized the full-length BP180 in immunoblotting. This is in line what has been reported earlier for healthy elderly subjects (Desai et al., 2008). The ICD and mid-ECD regions of BP180 have been reported to be autoantigenic in a low proportion of BP cases (Di Zenzo et al., 2011), but to best of our knowledge, spreading from these regions to the NC16A domain has not been documented, even though epitope spreading from unspecified non-NC16A regions to NC16A has been recently described (Mai et al., 2018). This kind of epitope spreading would be expected to be seen in cases of MS or AD in which an autoimmune response against BP180 led to the clinical manifestation of BP.

Despite the fact that MS, of all neurological diseases, has the strongest association with BP (Lai et al., 2016) the existence of autoantibodies against BP180 in patients with MS has so far been analyzed by only one study, in which none of the MS sera tested (n=50) showed positivity in the BP180-NC16A ELISA nor in the immunoblotting against the full-length BP180 (Recke et al., 2016). We found that about half of MS sera recognize the full-length BP180 in immunoblotting, but the proportion that showed elevated anti-BP180-NC16A ELISA values was only slightly higher than in the control group and similar to that reported in general population (Wieland et al., 2010; Prussmann et al., 2015). The obvious explanation for why our results differed from those described above is the sensitivity of the immunoblotting assay, since we used recombinant BP180, while the
previous study employed a keratinocyte extracellular matrix. Furthermore, the patients in our study were notably older (mean age 50) than those in the previous study (mean age 33 years) and therefore more likely to be at higher risk of BP autoimmunity. Differences between the two populations in the duration and degree of activity of MS disease may also have contributed to the differing results of the two studies, as may differences in the immunomodulatory MS treatments administered.

Only a few sera from MS and AD patients were positive in the full-length BP180 ELISA and only three MS sera were able to bind to the cutaneous basement membrane in the indirect IF analysis. The AD samples used in the present study were the same as those investigated in our previous study (Kokkonen et al., 2017) and the current results confirm the previous finding that none of the AD sera were able to bind to the basement membrane. Also in line with the previous study’s result was our finding that none of the patients with AD whose samples showed any reactivity against BP180 had a BP diagnosis or any cutaneous symptoms related to BP. This was also true of the MS patients whose samples were positive in the present study. This suggests that most of the BP180 epitopes that are recognized by AD or MS sera in immunoblotting are cryptic and not exposed and/or not recognized in their natively folded trimeric protein form in vitro or in vivo. Interestingly, however, a few MS samples were positive in the BP180-NC16A-ELISA, as were several AD samples in our previous study (Kokkonen et al., 2017). We propose that autoantibodies in BP180-NC16A ELISA positive MS and AD sera likely recognize a folded conformer of an isolated NC16A domain, but not always when it is part of natively folded trimeric BP180 molecule. This hypothesis is supported by previous studies, which showed that a GST-FP corresponding to the NC16A domain adopts a secondary structure when expressed in E. coli (Laczko et al., 2000), and that as a part of the native trimeric BP180 molecule, the NC16A domain likely adapts to a coiled-coil structure (Nishie et al., 2012).
The exact mechanism of autoimmunization in BP is unknown, but it has been speculated that abnormal shedding and/or related processing events can create neo-epitopes that lead to the development of anti-BP180 autoantibodies (Izumi et al., 2016), as has been described for another anti-BP180 autoimmune disease, linear IgA dermatosis (Toyonaga et al., 2017). The epidemiological association between BP and neurological/neurodegenerative diseases alongside the presence of anti-BP180-NC16A autoantibodies in the sera of AD patients has raised a hypothesis that the autoimmunity against BP180 in AD and MS might precede the onset of BP. This could be explained by the exposure of neuronal BP180 neo-epitopes after neuronal damage, as BP180 is expressed in human brains (Seppänen, 2013). Our current results suggest that the picture is likely more complicated, because the autoantibodies in AD and MS sera frequently recognize only cryptic, non-NC16A epitopes and no epitope spreading from these specific regions to NC16A has been demonstrated so far (Di Zenzo et al., 2008). However, the current data do not exclude the possibility of epitope spreading from non-NC16A areas to the NC16A domain in patients with neurological diseases.

Bullous pemphigoid, MS and AD all have their own particular immunological mechanisms. While autoantibodies against BP180 lead the pathogenesis of BP, MS is an autoimmune disease mainly driven by T-cells, with autoantibodies against myelin components playing a contributing role (Dendrou et al., 2015). On the other hand, in AD the failure of the innate immune system to clear the amyloid load leads to neuroinflammation and astrogliosis (Heneka et al., 2015) and adaptive immunity may actually participate in restraining the disease’s pathogenesis (Butovsky et al., 2006; Schwartz and Baruch, 2014). This may reflect an example of how, in healthy individuals, transient autoimmunity and the presence of ‘naturally occurring autoantibodies’, instead of leading to a detrimental self-attack, can perform necessary physiological functions, including clearing apoptotic
cells, fighting malignant cells and maintaining sensitivity against viral pathogens (Avrameas and Selmi, 2013; Chen et al., 2009; Luo et al., 2016; Yatim et al., 2017). Autoantibodies against both major and minor neuronal proteins are actually relatively common among healthy subjects and undergo changes during diseases that affect neurons (Levin et al., 2010, Nagele et al., 2013). Disease-specific lymphocytes which produce moderate- or high- affinity antibodies or T-cell receptors against cryptic epitopes likely escape negative selection in the bone marrow or thymus, respectively, but are not re-activated as long as these epitopes stay invisible to the immune system. Therefore, it is possible that the elevated levels of anti-BP180 autoantibodies found in the sera of AD and MS patients merely reflect increased neuronal damage, altered proteolytic processing and antigen presentation to autoreactive lymphocytes. It remains to be examined whether the presence of autoantibodies against BP180 in MS, AD and the general population supports the concept of protective autoimmunity or if these antibodies participate in pathological processes, possibly via epitope spreading.

Taken together, our current data indicate that many MS and AD patients have IgG autoantibodies targeting linear BP180 epitopes. Although this reaction is not sufficient to cause skin symptoms, the recognition of BP180 may be linked to neuronal damage and trigger an epitope spreading phenomenon in some patients. Further studies of neurological patients with BP180 positivity are required to identify additional factors that may explain this predisposition.
MATERIALS AND METHODS

Patients and samples
The collection of human AD, MS and control sera and skin samples were approved by the research ethics committees of Kuopio University Hospital and the Northern Ostrobothnia Hospital District, Finland. Control samples were also obtained from Northern Finland Biobank Borealis, Oulu, Finland. The principles of the Declaration of Helsinki were followed and written informed consent was obtained for every sample. MS patients were diagnosed by according of the revised McDonald 2010 criteria (Polman et al., 2011). No clinical symptoms suggestive of BP were detected in any of the MS patients. The AD (Kokkonen et al., 2017) and BP patients were diagnosed as described previously (Försti et al., 2014). Two of the 23 BP patients had Parkinson’s disease and five had dementia. All patients and controls were of caucasian origin. The age and sex of patients and controls are indicated in Table 1.

DNA constructs, GST-fusion proteins and immunoblotting
Human BP180 cDNA (Franzke et al., 2004) was used for transfection and preparation of GST-fusion proteins. Techniques, including the immunoblotting procedure are described in Supplementary Materials and Methods.

ELISA assays and indirect immunofluorescence
The ELISA assays (BP180-NC16A, BP180-full-length and BP230) and indirect immunofluorescence analysis were done as described in Supplementary Materials and Methods.
Data analysis

Data were entered and statistical analyses were conducted using the IBM SPSS Statistics version 24.0 software for Windows (IBM, Chicago, IL). Differences between MS and control group means (BP180-NC16A ELISA, continuous non-normal variable) were analyzed using the Mann-Whitney U test. Mean and median values were reported, as appropriate. In epitope mapping antibody recognized bands were densitometrically analysed using the ImageJ software package and classified by ordinal scale: “0”= no band, “1”= weak, “2”=strong, “3”=very strong. Epitope mapping data were analyzed with Fisher’s exact test, the Chi² test and Spearman’s correlation analysis. A two-sided P-values of 0.05, 0.01 and 0.001 were considered as the limit of statistical significance *, ** and ***. In the full-length BP180 ELISA the cut-off value of 9 RU was determined by maximization of the Youden-index (YI = sensitivity+specificity-1), where sensitivity (0.783) and specificity (0.925) were calculated from BP and control patient data.

CONFLICT OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGEMENTS

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Table 1. Characteristics and bullous pemphigoid autoantibodies of patients with multiple sclerosis, Alzheimer’s disease, bullous pemphigoid and neurologically healthy controls

<table>
<thead>
<tr>
<th></th>
<th>MS patients</th>
<th>Controls</th>
<th>AD patients</th>
<th>Controls</th>
<th>BP patients</th>
</tr>
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<tbody>
<tr>
<td>n</td>
<td>143</td>
<td>140</td>
<td>111</td>
<td>40</td>
<td>23</td>
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<tr>
<td>Age</td>
<td>49.4±12.2</td>
<td>49.6±5.9</td>
<td>71.9±7.9</td>
<td>66.8±7.0</td>
<td>78.6±8.0</td>
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<tr>
<td>Sex (% female)</td>
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<td>70.7</td>
<td>64.0</td>
<td>65.0</td>
<td>52.2</td>
</tr>
<tr>
<td>BP180 (U/ml)³</td>
<td>2.85(1.4)±3.91</td>
<td>2.13 (1.81)±1.73</td>
<td>-</td>
<td>-</td>
<td>69.2(47)±60.2</td>
</tr>
<tr>
<td>BP180 +/- (%)⁴</td>
<td>11/132 (7.7%)</td>
<td>2/140 (1.4%)</td>
<td>-</td>
<td>-</td>
<td>18/23 (78.3%)</td>
</tr>
<tr>
<td>BP230 (U/ml)³</td>
<td>2.47 (1.5)±3.84</td>
<td>n.d.</td>
<td>-</td>
<td>-</td>
<td>n.d</td>
</tr>
<tr>
<td>BP230 +/- (%)⁴</td>
<td>6/132 (4.3%)</td>
<td>n.d.</td>
<td>-</td>
<td>-</td>
<td>n.d</td>
</tr>
</tbody>
</table>

Abbreviations: AD, Alzheimer’s disease; BP, bullous pemphigoid; MS, multiple sclerosis; n.d., not determined.

¹Data given as mean ± standard deviation. ²Mann-Whitney-U test. ³BP180-NC16A and BP230 autoantibodies were measured by ELISA [mean (median) ± SD]. A cut-off value of 9.0 U/ml was used. The NC16A-BP180 ELISA was performed on duplicate samples. ⁴+/−: Number of samples at or above / below cut-off, %: Share of positive samples. ⁵Chi-square test. ⁶AD patients, controls and their BP180-NC16A and BP230 values have been previously described by Kokkonen et al. (2017).
Table 2. Comparison of immunoreactivity of bullous pemphigoid, Alzheimer’s disease and multiple sclerosis samples in BP180-NC16A ELISA, immunoblotting against recombinant NC16A and full-length BP180 ELISA

<table>
<thead>
<tr>
<th>BP180-NC16A ELISA</th>
<th>NC16A immunoblotting</th>
<th>Full length-BP180 ELISA</th>
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</thead>
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<tr>
<td></td>
<td>pos.</td>
<td>neg.</td>
</tr>
<tr>
<td>BP NC16A pos</td>
<td>65.2</td>
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<tr>
<td>NC16A neg</td>
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<td>Σ</td>
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<td>NC16A neg</td>
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<td>NC16A neg</td>
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<td>68.6</td>
</tr>
<tr>
<td>Σ</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

All data are shown as %. Abbreviations: AD, Alzheimer’s disease; BP, Bullous pemphigoid; ELISA, enzyme-linked immunosorbent assay; MS, multiple sclerosis; NC, non-collagenous; neg., negative; pos, positive; Σ, sum.

1The criteria for classification (pos./neg.) in the immunoblotting against FP5 corresponding the NC16A domain is described in Materials and methods.

2In the full-length-BP180 ELISA the AD cohort (n=111) is the same as that described previously (Kokkonen et al., 2017) except that four samples were no longer available, while the BP (n=23) and MS (n=35) samples are the same ones used for the epitope mapping (Figure 2). The distribution of full-length BP180 values is shown in Supplementary Figure S4 online.
FIGURE LEGENDS

Figure 1. IgG autoantibodies present in the sera of patients with multiple sclerosis (MS) detect full-length BP180 in immunoblotting. Fifty-six MS samples including 14 sera which were positive for BP180-NC16A, BP230 or both, as well as 42 randomly selected ELISA negative samples were analyzed by immunoblotting using human recombinant full-length BP180 as a target antigen. Human BP180 specific NC16A antibody, sera taken from a patient with bullous pemphigoid (BP) and patients with MS recognized a 180-kDa protein (arrow) that was not detected in collagenase-treated cell extract. A high molecular weight band (asterisk, most likely an oligomeric form), and polypeptides with molecular mass of 97 kDa and ~85 kDa (arrowhead, likely processed ectodomains) were also detected in some MS sera. These all were collagenase sensitive.

Figure 2. Mapping of linear epitopes reveals differences between patients with bullous pemphigoid (BP), Alzheimer’s disease (AD), and multiple sclerosis (MS) and healthy controls. (a) Equal amounts of 13 GST-BP180 fusion proteins (FP) were used for immunoblotting with 35 MS, 32 AD, 23 BP and 24 control sera. (b)-(f) Representative examples from each group are shown. (g) Relative frequencies of densitometrically measured immunoblot signals are shown for indicated fusion proteins where the intensity of immunoreactivity (color code of bars indicated in the figure) was significantly different between at least two groups. A two-sided P-values of ≤0.05, ≤0.01 and ≤0.001 are shown as *, ** and ***, respectively. ICD, intracellular domain; TMD, transmembrane domain; ECD, extracellular domain. The grey areas in (a) corresponds to the collagenous domains.
Fig 1.
Fig 2.

a) Amino acid positions for ICD, TMD, and ECD regions.

b) Western blot analysis of Anti-GST antibodies against different peptides.

c) Western blot analysis of BP antibodies against different peptides.

d) Western blot analysis of AD antibodies against different peptides.

e) Western blot analysis of MS antibodies against different peptides.

f) Western blot analysis of Control antibodies against different peptides.

g) Graphs showing the percentage of bands for different peptides under various conditions.

Legend:
- : No band
- : Weak band
- : Strong band
- : Very strong band

Peptide identifiers:
- FP1: aa 2-168
- FP2: aa 147-305
- FP3: aa 261-401
- FP4: aa 377-455
- FP5: aa 480-567
- FP6: aa 558-672
- FP7: aa 661-825
- FP8: aa 802-997
- FP9: aa 847-994
- FP10: aa 936-1106
- FP11: aa 1116-1178
- FP12: aa 1190-1399
- FP13: aa 1278-1497