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

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Neurologic 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% of sera from patients with multiple sclerosis (MS) (n = 56) had IgG reactivity against full-length BP180 in immunoblotting, while in BP180 non-collagenous 16A ELISA (n = 143), only 7.7% of 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 non-collagenous 16A domain and the distal part of extracellular domain. In indirect immunofluorescence analysis, 8.1% of 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 epidemiologic studies indicate that neurologic and neurodegenerative diseases like multiple sclerosis (MS) and Alzheimer's disease (AD) are common in BP patients and, more specifically, the presence of these neurologic 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

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Abbreviations: AA, amino acid; AD, Alzheimer's disease; BP, bullous pemphigoid; FP, fusion protein; MS, multiple sclerosis; NC16A, non-collagenous 16A

known as collagen XVII or BPAG2) (Bagci et al., 2017). The autoantibodies against the extracellular non-collagenous 16A (NC16A) 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 epidemiologic association between neurologic disorders and BP, alongside 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 development of BP. This hypothesis was, to 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 nonspecified 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 neurologic 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. To better 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 auto-antibodies against BP180 in cutaneous and neurologic diseases.

RESULTS

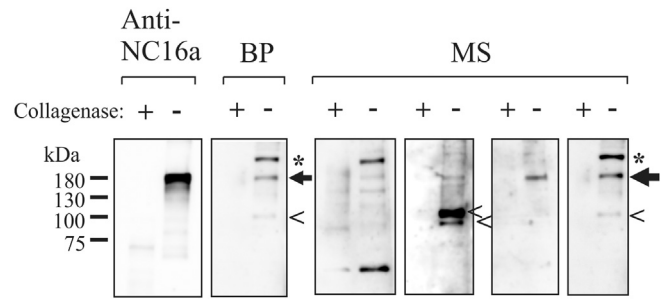### Patients with multiple sclerosis have autoantibodies against BP180

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 cutoff value of 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 (53.6%) sera 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 fusion proteins (FPs) covering most of the human BP180 molecule

Figure 1. IgG autoantibodies present in the sera of patients with multiple sclerosis detect full-length BP180 in immunoblotting. Fifty-six multiple sclerosis samples including 14 sera that were positive for BP180 non-collagenous 16A, 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 non-collagenous 16A antibody, sera taken from a patient with bullous pemphigoid and patients with multiple sclerosis 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 multiple sclerosis sera. These all were collagenase-sensitive. BP, bullous pemphigoid; MS, multiple sclerosis; NC16A, non-collagenous 16A.

(Figure 2a, 2b). 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 10 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–2g).

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

Characteristics	MS Patients	Controls	P-Value	AD Patients[1]	Control[1]	BP Patients
n	143	140	—	111	40	23
Age, y, mean ± SD	49.4 ± 12.2	49.6 ± 5.9	0.797[2]	71.9 ± 7.9	66.8 ± 7.0	78.6 ± 8.0
Sex (% female)	75.5	70.7	—	64.0	65.0	52.2
BP180, U/ml,[3] mean (median) ± SD	2.85 (1.4) ± 3.91	2.13 (1.81) ± 1.73	0.21[2]	—	—	69.2 (47) ± 60.2
BP180 +/− (%)[4]	11/132 (7.7)	2/140 (1.4)	0.02[5]	—	—	18/23 (78.3)
BP230, U/ml,[3] mean (median) ± SD	2.47 (1.5) ± 3.84	ND	—	—	—	ND
BP230 +/− (%)[4]	6/132 (4.3)	ND	—	—	—	ND

Abbreviations: AD, Alzheimer's disease; BP, bullous pemphigoid; MS, multiple sclerosis; ND, not determined; SD, standard deviation.

[1]AD patients, controls, and their BP180 non-collagenous 16A and BP230 values have been described previously by Kokkonen et al. (2017).

[2]Mann-Whitney-U test.

[3]BP180 non-collagenous 16A and BP230 autoantibodies were measured by ELISA. A cutoff value of 9.0 U/ml was used. The non-collagenous 16A BP180 ELISA was performed on duplicate samples.

[4]+/−, Number of samples at or above/below cutoff; %, share of positive samples.

[5]χ^2 Test.
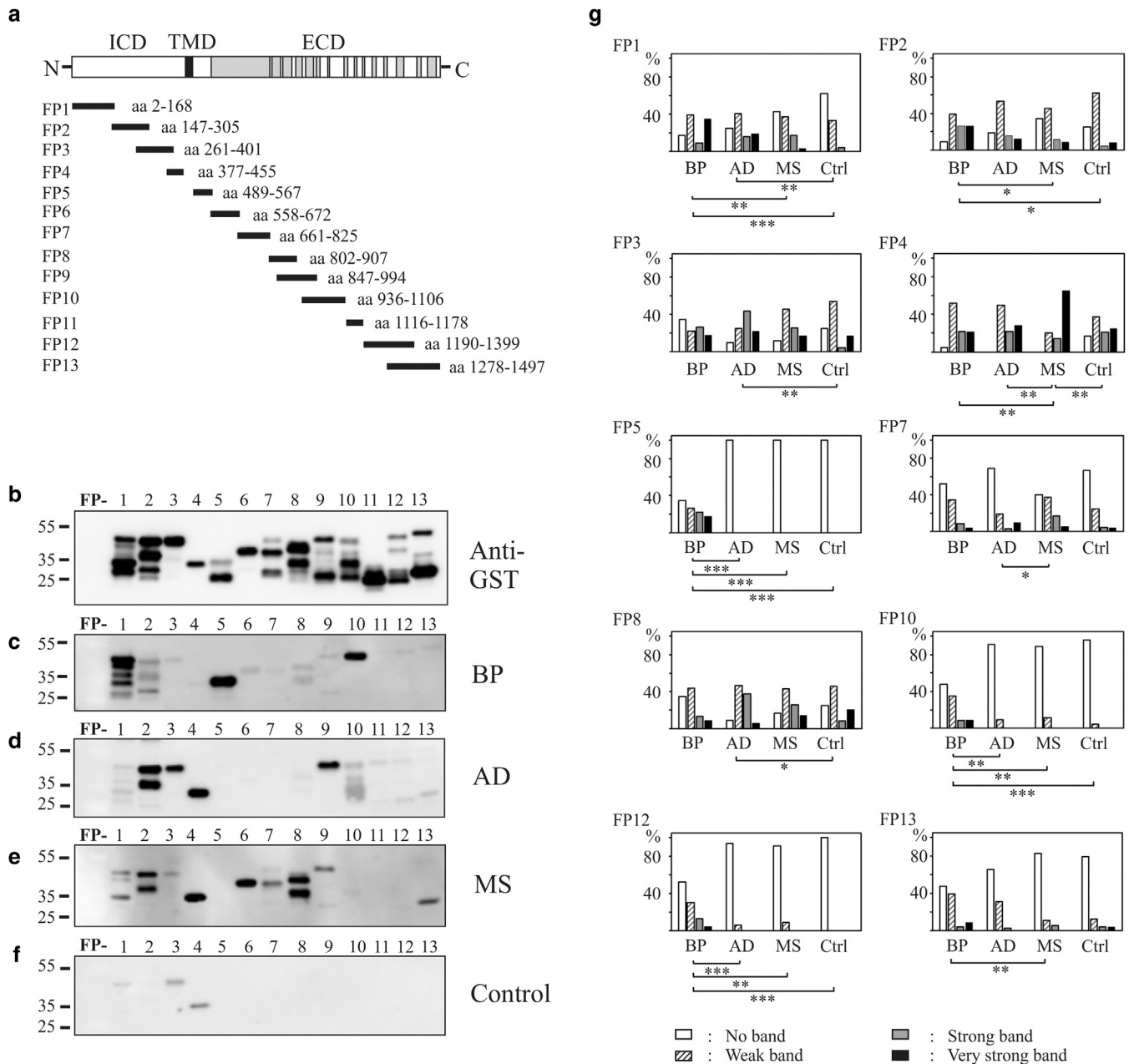
Figure 2. **Mapping of linear epitopes reveals differences between patients with bullous pemphigoid, Alzheimer's disease, and multiple sclerosis, and healthy controls.** (**a**) Equal amounts of 13 glutathione-S-transferase-BP180 fusion proteins were used for immunoblotting with 35 multiple sclerosis, 32 Alzheimer's disease, 23 with bullous pemphigoid, 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. Two-sided *P*-values: *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$. The gray areas in (**a**) correspond to the collagenous domains. BP, bullous pemphigoid; ECD, extracellular domain; GST, glutathione-S-transferase; ICD, intracellular domain; MS, multiple sclerosis; TMD, transmembrane domain.

The differences between the four groups (controls, BP, MS, and AD) were analyzed using Fisher's exact 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–2g 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–2g, Supplementary Table S2 online).

Immunoblotting (Figure 2c–2f) 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–2g). FP5 remained negative independently of the phosphorylation

of Ser544, which enhanced the recognition by BP sera (Supplementary Results and Supplementary 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, 2g), 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 (FP3 and FP4 AA 261−455) and the N-terminal regions of the extracellular domain, excluding NC16A domain (FP6-9 AA 558−994) showed a positive correlation across the whole study sample (Supplementary Table S4).

The strongest immunoreactivity for AD and MS samples was generally detected against those FPs that correspond to the intracellular domain epitopes and the N-terminal half of the extracellular domain, apart from the NC16A domain (Figure 2d, 2e, and 2g). FP1 (AA 2−168) and FP3 (AA 261−401) were positive significantly more often in AD sera than in control samples (Figure 2d, 2f, and 2g, Supplementary Table S3 online). Reactivity against FP4 (AA 377−455) was significantly more frequent in MS samples than in the other groups (Figure 2c−2g, Supplementary Table S3). Finally, there was no difference between any of the groups in terms of recognition of three of the FPs: FP6, FP9, and FP11 (Supplementary Figure S1).

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 neurologic patients with dementia, MS, or Parkinson's disease are able to bind to the skin basement membrane zone in indirect immunofluorescence analysis (Foureur et al., 2006; Kokkonen et al., 2017; Messingham et al., 2016; Recke et al., 2016). Therefore, indirect immunofluorescence 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 (Supplementary 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 of 23 (78.2%) BP patients' sera recognized the full-length BP180, whereas only 4 of 35 (11.4%) of MS, 7 of 111 (6.3%) of AD, and 3 of 40 (7.5%) of

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

BP180-NC16A ELISA	NC16A Immunoblotting, %[1]			Full-Length BP180 ELISA, %[2]		
	Positive	Negative	Sum	Positive	Negative	Sum
BP						
NC16A-positive	65.2	13.0	78.3	65.2	13.0	78.2
NC16A-negative	0	21.7	21.7	13.0	8.7	21.7
Sum	65.2	34.8	100	78.2	21.7	100
AD						
NC16A-positive	0	68.8	68.8	2.7	17.1	19.8
NC16A-negative	0	31.3	31.3	3.6	76.6	80.2
Sum	0	100	100	6.3	93.7	100
MS						
NC16A-positive	0	31.4	31.4	11.4	20	31.4
NC16A-negative	0	68.6	68.6	0	68.6	68.6
Sum	0	100	100	11.4	88.6	100

Abbreviations: AD, Alzheimer's disease; BP, bullous pemphigoid; MS, multiple sclerosis; NC16A, non-collagenous 16A.

[1]The criteria for classification (positive/negative) in the immunoblotting against FP5 corresponding the NC16A domain is described in the Materials and Methods.

[2]In 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.

healthy control samples were classified as positive (Table 2, Supplementary 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 neurologic 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 neurologic 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 from 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 neurologic 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 in both the BP180-NC16A and full-length BP180 ELISA assays. In contrast, the AD and MS sera were negative for both the phosphorylated and non-phosphorylated NC16A domain, but had some increased and characteristic reactivity against the intracellular domain and mid-extracellular domain epitopes in immunoblotting. Positive correlation, that is, the co-presence or co-absence of these epitopes, was evident at the level of the whole study sample (Supplementary Table S4), 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 intracellular domain and mid-extracellular domain regions of BP180 have been reported to be auto-antigenic 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 described recently (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 neurologic 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 or 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 (Prussmann et al., 2015; Wieland et al., 2010). The obvious explanation for why our results differed from those described is the sensitivity of the immunoblotting assay, because 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 years) 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 immunofluorescence 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 glutathione-S-transferase 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 epidemiologic association between BP and neurologic/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 neurologic diseases.

BP, MS, and AD all have their own particular immunologic mechanisms. While autoantibodies against BP180 lead the pathogenesis of BP, MS is an autoimmune disease driven mainly 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 physiologic 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., 2011). Disease-specific lymphocytes that 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 reactivated 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 neurologic 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, glutathione-S-transferase fusion proteins, and immunoblotting

Human BP180 cDNA (Franzke et al., 2004) was used for transfection and preparation of glutathione-S-transferase FPs. Techniques, including the immunoblotting procedure, are described in Supplementary Materials and Methods online.

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 online.

Data analysis

Data were entered and statistical analyses were conducted using the IBM SPSS software for Windows, version 24.0 (IBM, Armonk, NY). 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 analyzed using the ImageJ software package (NIH, Bethesda, MD) 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, χ^2 test, and Spearman's correlation analysis. Two-sided P-values of 0.05, 0.01, and 0.001 were considered as the limit of statistical significance. In the full-length BP180 ELISA, the cutoff value of 9 relative units was determined by maximization of the Youden Index (sensitivity + specificity − 1), where sensitivity (0.783) and specificity (0.925) were calculated from BP and control patient data.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2018.09.010.

REFERENCES

Avrameas S, Selmi C. Natural autoantibodies in the physiology and pathophysiology of the immune system. J Autoimmun 2013;41:46−9.

Bagci IS, Horvath ON, Ruzicka T, Sardy M. Bullous pemphigoid. Autoimmun Rev 2017;16:445−55.

Barrick BJ, Ida CM, Laniosz V, Jentoft ME, Sominidi-Damodaran S, Wieland CN, et al. Bullous pemphigoid, neurodegenerative disease, and hippocampal BP180 expression: a retrospective postmortem neuropathologic study. J Invest Dermatol 2016;136:2090−2.

Butovsky O, Koronyo-Hamaoui M, Kunis G, Ophir E, Landa G, Cohen H, et al. Glatiramer acetate fights against Alzheimer's disease by inducing dendritic-like microglia expressing insulin-like growth factor 1. Proc Natl Acad Sci USA 2006;103:11784−9.

Chen Y, Khanna S, Goodyear CS, Park YB, Raz E, Thiel S, et al. Regulation of dendritic cells and macrophages by an anti-apoptotic cell natural antibody that suppresses TLR responses and inhibits inflammatory arthritis. J Immunol 2009;183:1346−59.

Dendrou CA, Fugger L, Friese MA. Immunopathology of multiple sclerosis. Nat Rev Immunol 2015;15:545−58.

Desai N, Allen J, Ali I, Venning V, Wojnarowska F. Autoantibodies to basement membrane proteins BP180 and BP230 are commonly detected in normal subjects by immunoblotting. Australas J Dermatol 2008;49:137−41.

Di Zenzo G, Thoma-Uszynski S, Calabresi V, Fontao L, Hofmann SC, Lacour JP, et al. Demonstration of epitope-spreading phenomena in bullous pemphigoid: results of a prospective multicenter study. J Invest Dermatol 2011;131:2271−80.

Di Zenzo G, Thoma-Uszynski S, Fontao L, Calabresi V, Hofmann SC, Hellmark T, et al. Multicenter prospective study of the humoral

autoimmune response in bullous pemphigoid. Clin Immunol 2008;128:415−26.

Försti AK, Huilaja L, Schmidt E, Tasanen K. Neurological and psychiatric associations in bullous pemphigoid-more than skin deep? Exp Dermatol 2017;26:1228−34.

Försti AK, Jokelainen J, Timonen M, Tasanen K. Increasing incidence of bullous pemphigoid in northern Finland: a retrospective database study in Oulu University Hospital. Br J Dermatol 2014;171:1223−6.

Foureur N, Mignot S, Senet P, Verpillat P, Picard-Dahan C, Crickx B, et al. Correlation between the presence of type-2 anti-pemphigoid antibodies and dementia in elderly subjects with no clinical signs of pemphigoid. Ann Dermatol Venereol 2006;133:439−43.

Franzke CW, Tasanen K, Borradori L, Huotari V, Bruckner-Tuderman L. Shedding of collagen XVII/BP180: structural motifs influence cleavage from cell surface. J Biol Chem 2004;279:24521−9.

Heneka MT, Carson MJ, El Khoury J, Landreth GE, Brosseron F, Feinstein DL, et al. Neuroinflammation in Alzheimer's disease. Lancet Neurol 2015;14:388−405.

Izumi K, Nishie W, Mai Y, Wada M, Natsuga K, Ujiie H, et al. Autoantibody profile differentiates between inflammatory and noninflammatory bullous pemphigoid. J Invest Dermatol 2016;136:2201−10.

Kokkonen N, Herukka SK, Huilaja L, Kokki M, Koivisto AM, Hartikainen P, et al. Increased levels of the bullous pemphigoid BP180 autoantibody are associated with more severe dementia in Alzheimer's disease. J Invest Dermatol 2017;137:71−6.

Laczko I, Vass E, Toth GK, Marczinovits I, Kiss M, Husz S, et al. Conformational consequences of coupling bullous pemphigoid antigenic peptides to glutathione-S-transferase and their diagnostic significance. J Pept Sci 2000;6:378−86.

Lai YC, Yew YW, Lambert WC. Bullous pemphigoid and its association with neurological diseases: a systematic review and meta-analysis. J Eur Acad Dermatol Venereol 2016;30:2007−15.

Levin EC, Acharya NK, Han M, Zavareh SB, Sedeyn JC, Venkataraman V, et al. Brain-reactive autoantibodies are nearly ubiquitous in human sera and may be linked to pathology in the context of blood-brain barrier breakdown. Brain Res 2010;1345:221−32.

Luo CT, Liao W, Dadi S, Toure A, Li MO. Graded Foxo1 activity in treg cells differentiates tumour immunity from spontaneous autoimmunity. Nature 2016;529:532−6.

Mai Y, Nishie W, Izumi K, Yoshimoto N, Morita Y, Watanabe M, et al. Detection of anti-BP180 NC16A autoantibodies after the onset of dipeptidyl peptidase-IV inhibitor-associated bullous pemphigoid: a report of three cases. Br J Dermatol 2018;179:790−1.

Messingham KA, Aust S, Helfenberger J, Parker KL, Schultz S, McKillip J, et al. Autoantibodies to collagen XVII are present in Parkinson's disease and localize to tyrosine-hydroxylase positive neurons. J Invest Dermatol 2016;136:721−3.

Nagele E, Han M, Demarshall C, Belinka B, Nagele R. Diagnosis of Alzheimer's disease based on disease-specific autoantibody profiles in human sera. PLoS One 2011;6:e23112.

Nishie W. Update on the pathogenesis of bullous pemphigoid: an autoantibody-mediated blistering disease targeting collagen XVII. J Dermatol Sci 2014;73:179−86.

Nishie W, Jackow J, Hofmann SC, Franzke CW, Bruckner-Tuderman L. Coiled coils ensure the physiological ectodomain shedding of collagen XVII. J Biol Chem 2012;287:29940−8.

Polman CH, Reingold SC, Banwell B, Clanet M, Cohen JA, Filippi M, et al. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. Ann Neurol 2011;69:292−302.

Prussmann W, Prussmann J, Koga H, Recke A, Iwata H, Juhl D, et al. Prevalence of pemphigus and pemphigoid autoantibodies in the general population. Orphanet J Rare Dis 2015;10:63.

Recke A, Oei A, Hubner F, Fechner K, Graf J, Hagenah J, et al. Parkinson disease and multiple sclerosis are not associated with autoantibodies against structural proteins of the dermal-epidermal junction. Br J Dermatol 2016;175:407−9.

Schmidt E, Zillikens D. Pemphigoid diseases. Lancet 2013;381:320−32.

Schwartz M, Baruch K. Breaking peripheral immune tolerance to CNS antigens in neurodegenerative diseases: boosting autoimmunity to fight-off chronic neuroinflammation. J Autoimmun 2014;54:8−14.

Seppänen A. Collagen XVII: A shared antigen in neurodermatological interactions? Clin Dev Immunol 2013;2013:240570.

Toyonaga E, Nishie W, Izumi K, Natsuga K, Ujiie H, Iwata H, et al. C-terminal processing of collagen XVII induces neoepitopes for linear IgA dermatosis autoantibodies. J Invest Dermatol 2017;137:2552−9.

Ujiie H, Sasaoka T, Izumi K, Nishie W, Shinkuma S, Natsuga K, et al. Bullous pemphigoid autoantibodies directly induce blister formation without complement activation. J Immunol 2014;193:4415−28.

Wieland CN, Comfere NI, Gibson LE, Weaver AL, Krause PK, Murray JA. Anti-bullous pemphigoid 180 and 230 antibodies in a sample of unaffected subjects. Arch Dermatol 2010;146:21−5.

Yatim N, Cullen S, Albert ML. Dying cells actively regulate adaptive immune responses. Nat Rev Immunol 2017;17:262−75.