

Bullous pemphigoid antigen 1 isoforms: potential new target autoantigens in multiple sclerosis?

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Summary

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None declared.

Background The simultaneous occurrence of bullous pemphigoid (BP) and multiple sclerosis (MS), two autoimmune diseases involving the skin and the central nervous system (CNS), respectively, has been described.

Objectives As the BPAG1 gene encodes the epithelial isoform of BP antigen 1 (BPAG1-e), a major autoantigen of BP, as well as additional variants expressed in the neurones of the CNS and peripheral nervous system and in Schwann cells, we tested the hypothesis that products of the BPAG1 gene act as shared autoantigens in both BP and MS.

Methods The reactivity of cerebrospinal fluid (CSF) obtained from 18 patients with MS, 10 patients with other inflammatory CNS diseases and 20 normal controls was assayed by immunoblotting against two recombinant fragments of BPAG1-e encompassing regions that are also found in the neuronal variants BPAG1-n and BPAG1-a.

Results The recombinant protein glutathione-S-transferase (GST)-BPAG1-e¹⁻⁸⁸⁷ was recognized by five of 18 (27%) CSF samples obtained from patients with MS, two of 10 (20%) samples from patients with other inflammatory neurological diseases and five of 20 (25%) samples from normal controls. Furthermore, two of 18 (11%) CSF samples from patients with MS bound to GST-BPAG1-e¹⁸⁸⁰⁻²⁶⁴⁹, whereas none of the samples obtained from patients with other inflammatory neurological diseases or from control subjects showed reactivity.

Conclusions These results raise the possibility that a subset of patients with MS develops an autoantibody response to the neuronal variants of BPAG1. These findings potentially open the avenue of neuronal BPAG1 variants being novel targets of autoantibodies in neurological diseases.

Bullous pemphigoid (BP) is an autoimmune bullous disorder of the skin associated with an antibody response to the BP antigen 1 (BPAG1) and the BP antigen 2, two components of junctional adhesion complexes in stratified and certain complex epithelia.^{1,2} The disease typically affects the elderly and has an estimated incidence of seven new cases per one million individuals per year. In anecdotal observations, BP has been reported to occur in some patients concomitantly with multiple sclerosis (MS),^{3,4} usually at a younger age, raising the possibility of a common pathway underlying both conditions. MS is an autoimmune demyelinating disease of the central nervous system (CNS) characterized by an abnormal cellular and humoral response to myelin epitopes produced by oligodendrocytes including myelin basic protein, cyclic nucleotide phosphodiesterase and

myelin oligodendrocyte glycoprotein.⁵⁻⁸ Its prevalence is estimated to be between 80 and 130 per 100 000 inhabitants in the U.K.⁹⁻¹¹ The potential co-occurrence of BP and MS is particularly intriguing in light of recent findings indicating that the BPAG1 gene encodes not only the epithelial isoform BPAG1-e, but also additional variants expressed in neurones of the CNS and peripheral nervous system as well as in Schwann cells.⁸ These variants appear to contribute critically to the maintenance of the cytoarchitecture of neurones and Schwann cells by cross-linking the microfilament, intermediate filament and microtubule networks together.¹²⁻¹⁴ This idea is supported by the observation that BPAG1 null-mutant mice develop a dystonia musculorum-like phenotype characterized by dystonia and ataxia,¹²⁻¹⁴ severe neurodegeneration and profound myelin

abnormalities within the CNS and peripheral nervous system.^{7,15}

While BPAG1-e consists of an NH₂-terminal plakin domain, a coiled-coil rod domain and a COOH-terminal intermediate filament-binding domain (IFBD), the neuronal variants are peculiar in that they contain an actin-binding domain (ABD), spectrin repeats and/or a microtubule-binding domain (MTBD) (Fig. 1).^{8,16,17} Specifically, the BPAG1-a isoform differs from BPAG1-e by the presence of an ABD at the extreme NH₂-terminus of the protein. Instead of having a coiled-coil rod domain, this variant contains a spectrin repeat domain followed by a distinct COOH-terminus containing an MTBD. BPAG1-a was detected in the brain, the spinal cord and during development in pituitary primordium, cranial and dorsal root ganglia.⁸ The BPAG1-b isoform resembles BPAG1-a but has an insertion between the plakin domain and the spectrin domain containing a putative IFBD. BPAG1-b expression is more restricted to striated muscles and bone cartilage in developing mouse embryos.⁸ Finally, BPAG1-n (also referred to as dystonin) is also identical to BPAG1-e except for the insertion of an ABD at the extreme NH₂-terminus.^{6,12,17} The exact expression profile of BPAG1-n is still unclear. Based on *in situ* hybridization and immunohistochemistry studies, Yang *et al.*^{16,17} reported that postnatally this variant is predominantly expressed in primary sensory neurones and some secondary neurones, whereas little if any expression of BPAG1-n is detectable in motor neurones, satellite cells, oligodendrocytes or Schwann cells. However, Bernier *et al.*¹⁴ found BPAG1-n expression not only in peripheral sensory neurones but also in Schwann cells and neurones of the CNS (extrapyramidal motor system, cerebellum, motoneurones). In the latter cells, BPAG1-n is thought to play a critical role in cytoskeletal organization. This idea is supported by the observation that *dt*-mice, which carry mutations in the BPAG1-n/dystonin gene, exhibit abnormal Schwann cells with a disorganized cytoskeleton and defective myelination.⁷ While these previous expression studies of

BPAG1-n were obtained utilizing probes against the ABD and plakin domains common to various isoforms of the BPAG1 gene, Leung *et al.*⁸ found only small amounts of BPAG1-n (or BPAG1-e) in the brain by polymerase chain reaction (PCR) using a specific set of primers.

In this study, we tested the hypothesis that products of the BPAG1 gene could act as shared autoantigens in both MS and BP. Therefore, we assayed by immunoblotting the reactivity of cerebrospinal fluid (CSF) obtained from patients with MS, other inflammatory CNS diseases and normal controls against two recombinant fragments of BPAG1-e encompassing the NH₂- and COOH-terminal regions of BPAG1-e, that are usually recognized by at least 30% and 80% of BP sera, respectively.^{18–21} The NH₂-terminal plakin domain of BPAG1-e is also found in the neuronal variants BPAG1-a and BPAG1-n, while its COOH terminal region is also possibly found in BPAG1-n.⁸

Materials and methods

Patients

CSF was obtained from 18 patients with MS (according to the criteria of Poser *et al.*¹⁸) prior to treatment initiation, 10 patients with other, non-MS inflammatory CNS diseases (two with neuroborreliosis, three with human immunodeficiency virus encephalitis, two with neurosyphilis, two with neuroleptus and one with vasculitis) and 20 subjects with idiopathic peripheral facial nerve palsy as controls.

Recombinant proteins

The first recombinant protein, glutathione-S-transferase (GST)-BPAG1-e^{1–887}, consisted of a large portion (approximately 80%) of the plakin domain of BPAG1-e (residues 1–887), which is homologous to that found in BPAG1-a and BPAG1-n, fused to GST, whereas the second recombinant protein, GST-BPAG1-e^{1880–2649}, contained the IFBD of BPAG1-e (residues 1880–2649), homologous to that present in BPAG1-n, fused to GST (Fig. 1). They were expressed in the *Escherichia coli* and the *Saccharomyces cerevisiae* expression systems, respectively, as previously described.^{19,20}

Immunoblotting

One hundred micrograms of total yeast extracts containing GST-BPAG1-e^{1880–2649}, 250 µg of human keratinocyte extracts, 1 µg of purified GST-BPAG1-e^{1–887} or GST were loaded per well on sodium dodecyl sulphate-polyacrylamide gels, separated, and electrophoretically transferred to polyvinylidene fluoride membranes (Immobilon-P; Millipore, Bedford, MA, U.S.A.). The membranes were incubated with undiluted CSF overnight at 4 °C. After washing, the blots were incubated with peroxidase-conjugated antihuman IgG antibodies for 1 h at room temperature, washed, and developed using enhanced chemiluminescence (Amersham, Little Chalfont, U.K.).

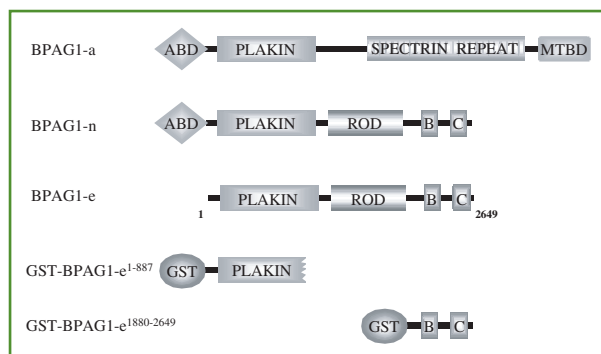


Fig 1. Schematic representation of two putative neuronal variants of bullous pemphigoid antigen 1 (BPAG1), BPAG1-n and BPAG1-a, of the epithelial isoform BPAG1-e.^{6,12,17} The various protein subdomains are depicted. The region of BPAG1-e with the amino acid range covered by the two recombinants expressed as glutathione-S-transferase (GST) fusion proteins is indicated. ABD, actin-binding domain; MTBD, microtubule-binding domain.

Table 1 Reactivity of cerebrospinal fluid (CSF) samples with recombinant NH₂-terminal and COOH-terminal fragments of the epithelial isoform of bullous pemphigoid antigen 1 (BPAG1-e) expressed as glutathione-S-transferase fusion proteins as detected by immunoblotting

	Multiple sclerosis (n = 18)	Other neurological diseases (n = 10)	Normal CSF (n = 20)
BPAG1-e ¹⁻⁸⁸⁷	5/18 (27%)	2/10 (20%)	5/20 (25%)
BPAG1-e ¹⁸⁸⁰⁻²⁶⁴⁹	2/18 (11%)	0/10 (0%)	0/20 (0%)
Keratinocyte extracts	0/18 (0%)	0/10 (0%)	0/20 (0%)

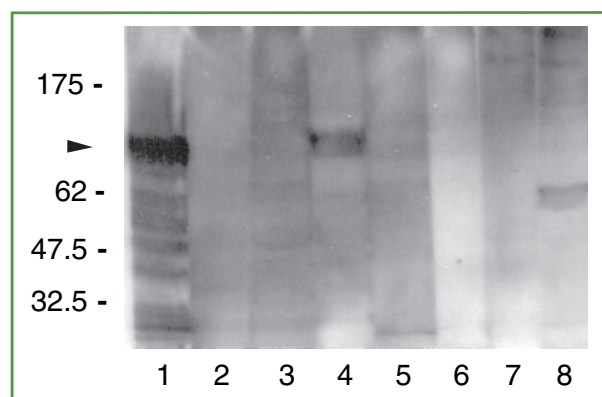


Fig 2. Reactivity of cerebrospinal fluid (CSF) obtained from patients with multiple sclerosis (MS) and normal controls with the epithelial isoform of bullous pemphigoid antigen 1 (BPAG1-e¹⁸⁸⁰⁻²⁶⁴⁹). Extracts of transformed *Saccharomyces cerevisiae*-producing glutathione-S-transferase (GST)-BPAG1-e¹⁸⁸⁰⁻²⁶⁴⁹ were assayed by immunoblotting with monoclonal antibody 5E against BPAG1-e (lane 1), CSF from patients with MS (lanes 2–5) and from subjects with idiopathic peripheral facial nerve palsy as controls (lanes 6–8). The arrowhead indicates the estimated 85-kDa band corresponding to GST-BPAG1-e¹⁸⁸⁰⁻²⁶⁴⁹. Reactivities are observed in lanes 1 and 4. Molecular weight standards are indicated by bars in kDa.

Results

Five of the 18 (27%) CSF samples obtained from patients with MS immunoblotted the recombinant protein GST-BPAG1-e¹⁻⁸⁸⁷ (Table 1). The latter was also recognized by two of 10 (20%) samples from patients with other inflammatory neurological diseases and five of 20 (25%) samples obtained from normal controls. None of the CSF samples which were reactive with GST-BPAG1-e¹⁻⁸⁸⁷ immunoblotted purified GST (not shown). Furthermore, two of the 18 (11%) CSF samples from patients with MS bound, although in one case marginally, to the GST-BPAG1-e¹⁸⁸⁰⁻²⁶⁴⁹ from yeast extracts, whereas none of the samples obtained from patients with other neurological diseases or from normal controls bound to this recombinant (Fig. 2).

Discussion

These results suggest that the reactivity profile of MS CSF does not significantly differ from that of either non-MS inflammatory or normal CSF with respect to BPAG1 variants. Binding activity to the NH₂-terminal plakin domain was observed in all

three study groups, most probably reflecting a nonspecific binding to the fusion protein. However, 11% of the CSF samples from MS patients, but none of the non-MS inflammatory or control CSF samples, reacted with the IFBD of BPAG1-e. The latter is also present in the neuronal BPAG1-n variant, small amounts of which appear to be present in the brain by PCR using a specific set of primers.²¹ Because BPAG1-e is not expected to be naturally present in the CSF,²¹ these observations raise the possibility that a subset of patients with MS can develop antibodies directed against the neuronal variants of BPAG1, most probably in the context of an intermolecular epitope-spreading phenomenon.²¹ As the CSF samples obtained from the non-MS inflammatory population, in which intrathecal synthesis of immunoglobulins was also present in the form of oligoclonal bands (not shown), did not exhibit such a reactivity, this abnormal immune response against GST-BPAG1-e¹⁸⁸⁰⁻²⁶⁴⁹ may be specific for MS. The low frequency of reactivity of MS patients may be only apparent, and testing using either (i) eukaryotic recombinant proteins under native nondenaturing conditions different from those of immunoblotting or (ii) concentrated CSF may prove more sensitive. Nevertheless, a better knowledge of the expression profile and distribution of these BPAG1 variants at protein level in the CNS is mandatory for better delineation of their role in maintenance of tissue homeostasis and disease development. Although these preliminary findings do not enable any conclusion to be drawn, the possibility that neuronal BPAG1 variants are potential novel targets of CNS diseases should be further investigated.

Acknowledgments

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