Abstract  Linear IgA disease (LAD) is an autoimmune subepidermal blistering skin disease characterized by the linear deposition of IgA at the dermoepidermal junction. Serum from patients with LAD most commonly contains autoantibodies that are directed against the hemidesmosomal transmembrane glycoprotein BP180 (type XVII collagen). Various antigenic sites on the extracellular domain of this anchoring filament protein have been shown to be targeted by autoantibodies in different autoimmune bullous skin diseases, including bullous pemphigoid and cicatricial pemphigoid (CP). However, little is known about epitopes on BP180 recognized by autoantibodies in LAD. In this study, we used three recombinant GST fusion proteins, together roughly covering the entire BP180 ectodomain, to characterize the autoimmune response in serum from patients with LAD. Interestingly, we found both IgA and IgG reactivity to all three portions of the BP180 ectodomain. The strongest reactivity was observed with the C-terminal portion of BP180. This is also the major region recognized by autoantibodies in patients with CP. This finding correlates with the observation that there may be significant overlap of the clinical and immunopathological findings in LAD and CP.

Keywords  Autoantibodies · Dermoepidermal junction · Linear IgA disease · Cicatricial pemphigoid

Introduction

Linear IgA disease (LAD) of adulthood and childhood, the latter also called chronic bullous disease of childhood, are autoimmune blistering skin diseases characterized by linear deposits of IgA autoantibodies at the dermoepidermal junction (DEJ) [1]. In addition, autoantibodies of the IgG subclass may also be detected in LAD [2, 3, 4, 5, 6], and the term linear IgA/IgG bullous dermatosis has been proposed for this condition [2]. Immunoelectron microscopic studies have shown that serum from some LAD patients binds within the lamina lucida, serum from others labels the sublamina densa region, and serum from still others reacts with both locations [7, 8]. Indirect immunofluorescence (IF) microscopic studies using salt-split human skin have shown that serum from most LAD patients labels the epidermal side of the split, serum from some patients shows dermal binding, and serum from still others stains both sides [9, 10]. LAD serum showing dermal binding has been reported to react with a 255 kDa dermal antigen [10], and with type VII collagen, the autoantigen in epidermolysis bullosa acquisita [11]. LAD serum showing epidermal binding has been found to react with various antigens, including a 285 kDa protein in dermal extracts [12], a 97 kDa antigen (LABD97) mainly extracted from epidermis [13], a 120 kDa protein (LAD-1), detected in conditioned medium of cultured human keratinocytes [14], and the two major bullous pemphigoid (BP) antigens BP180 and BP230 [2, 15].

BP180 is a hemidesmosomal transmembrane glycoprotein with a type II orientation, i.e. its aminoterminal portion localizes to the cytoplasm and the carboxyterminus projects into the extracellular space, spanning the lamina lucida of the DEJ (Fig. 1). The protein forms a homotrimer and the ectodomain consists of 15 interrupted collagenous domains [16]. LABD97 has been shown to share sequence homology with the ectodomain of BP180 and appears to be a proteolytic product of LAD-1 [14, 17], which itself represents a portion of the extracellular domain of BP180 [18, 19].
of the fragments GST moiety. Amino acid residue numbers are shown to the right in this study. Each of the fusion proteins contains an N-terminal GST-BP915) represent the recombinant GST fusion proteins used in this study. The three fragments (GST-BP1050, GST-BP963, and GST-BP915) represent the recombinant GST fusion proteins used in this study. Each of the fusion proteins contains an N-terminal GST moiety. Amino acid residue numbers are shown to the right of the fragments.

Several other acquired blistering diseases have been shown to be associated with an autoimmune response to BP180. Autoantibodies in the serum from the majority of patients with BP and pemphigoid gestationis show reactivity with four epitopes clustered within the aminoterminal 45 amino acids of the extracellular 16th noncollagenous domain (NC16A) [20, 21]. In contrast, antibodies in patients with cicatricial pemphigoid (CP) preferentially target the C-terminus of BP180 localizing to the lamina lucida/lamina densa interface [22, 23, 24]. Autoantibodies in serum from patients with lichen planus pemphigoides mainly react with an epitope in the C-terminal portion of the NC16A domain [25]. In addition, we have recently demonstrated that the NC16A domain may also be targeted by IgA autoantibodies in a subgroup of LAD patients, although this region does not appear to represent the major antigenic site on the BP180 ectodomain recognized by IgA autoantibodies [26].

The aim of the present study was to further characterize IgA and IgG immunoblot reactivity in serum from patients with lamina lucida-type LAD using three recombinant fusion proteins which together roughly cover the entire ectodomain of human BP180.

### Materials and methods

#### Patient and rabbit serum

The study included serum from 26 patients with LAD of adulthood and from one male patient with LAD of childhood (Table 1). All patients had blisters on their skin located subepidermally as shown by histopathology of a lesional skin biopsy. In addition, six patients showed cicatricizing lesions on mucous membranes. The mean age of the patients with LAD of adulthood was 61 years, with a range from 17 to 89 years; the child patient was 22 months old. Direct IF microscopy of perilesional skin biopsies showed that all LAD patients had linear deposits of IgA at the DEJ, and seven patients also showed weaker IgG deposits. Indirect IF analysis of 1 M NaCl-separated skin was performed as described previously [27]. The serum from 74% and 26% of patients contained IgA and IgG antibodies, respectively, that bound to the epidermal side of the split (lamina lucida-type LAD). Six patients showed some degree of scarring conjunctivitis. However, in three of these six patients direct IF microscopy revealed only IgA deposits. In the remaining three patients, IgA deposits were stronger than IgG deposits. In addition, the titres of circulating IgG antibodies were much lower than the titres of IgA antibodies (n = 2) or were completely negative (n = 1). These six patients were therefore designated as LAD rather than CP. As negative controls, we used serum from 20 healthy individuals. Positive controls for immunoblotting of LAD-1 included well-characterized serum from five BP patients. Rabbit anti-GST serum (Sigma, Deisenhofen, Germany) was used as a positive control for immunoblot studies with recombinant glutathione-S-transferase (GST) fusion proteins.

### Keratinocyte-derived LAD-1

The 120 kDa soluble ectodomain of BP180 (LAD-1) was produced from the medium of cultured human keratinocytes as described previously [26].

### Human BP180 recombinant proteins

Cloning procedures for GST-BP1050, GST-BP963 and GST-BP915 were as reported previously [24]. GST-BP1050 comprises 350 amino acids and localizes to the N-terminal portion of the BP180 ectodomain (amino acids 542–892) according to the BP180 sequence data of Giudice et al. [28], GST-BP963 comprises 321 amino acids on the central portion of the BP180 ectodomain (amino acids 885–1206), and GST-BP915 comprises 305 amino acids and localizes to the C-terminus (amino acids 1227–1532; Fig. 1). Recombinant GST and GST fusion proteins were expressed in *E. coli* strains DH5α (GST), XL-Blue (GST-BP1050), TG1 (GST-BP963), and BL21 (GST-BP915) as described previously [20, 24] with the following modifications to the culture conditions that resulted in an increased rate of intact protein: *E. coli* XL-Blue were cultured at 30°C overnight and induced with isopropyl-β-D-thiogalactopyranoside also at 30°C for 2 h; *E. coli* TG1 were cultured at 20°C overnight and induced at 20°C for 5 h; and *E. coli* BL21 were cultured at 20°C overnight and induced at 14°C for 7 h. GST fusion proteins were purified by glutathione agarose affinity chromatography as described previously [20].

### Immunoblotting

Immunoblotting was performed as reported previously [26]. Briefly, affinity-purified fusion proteins and concentrated keratinocyte culture medium were fractionated by 15% and 8% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), respectively, and electrophoretically transferred to nitrocellulose. Blots were blocked for 45 min with 3% skimmed milk in Tris-buffered saline which comprised 0.02 M Tris-(hydromethyl)-aminomethane.