Anti-laminin β4 – a new autoantibody marker in pemphigoid disease

by Dr Jacqueline Gosink

Laminin β4 (lamβ4) has been identified as a novel target antigen in anti-p200 pemphigoid, a rare autoimmune disease manifesting with subepidermal blisters. Anti-lamβ4 antibodies bind to the region of the dermal–epidermal junction and demonstrate pathogenic effects. A recombinant-cell indirect immunofluorescence assay has been developed for specific detection of anti-lamβ4 antibodies.

Autoimmune bullous skin diseases

Autoimmune bullous dermatoses (AIBD) are a heterogeneous group of immune-mediated diseases of the skin and mucous membranes [1]. They are characterized by autoantibodies against structural components of desmosomes and hemidesmosomes. Desmosomes are involved in cell-to-cell adhesion within the epidermis, while hemidesmosomes anchor the epithelial cells to the underlying basement membrane (Fig. 1). The pathogenic autoantibodies disrupt the adhesive functions leading to splitting. Blisters form when tissue fluid flows into the splits.

In pemphigus diseases the autoantibodies target desmosomal proteins such as desmoglein 1 (Dsg1), desmoglein 3 (Dsg3) and plakins. This leads to formation of intraepidermal blisters, which are typically fragile and easily ruptured. Pemphigus vulgaris (PV), pemphigus foliaceus (PF) and paraneoplastic pemphigus (PNP) are the major disease forms.

In pemphigoid diseases, the autoantibodies are directed against hemidesmosomal proteins, including BP180, BP230 and laminins. These diseases manifest with subepidermal blisters that are tense and fluid-filled. Different disease forms include bullous pemphigoid (BP), pemphigoid gestationis (PG), mucous membrane pemphigoid (MMP), linear IgA dermatosis and anti-p200 pemphigoid.

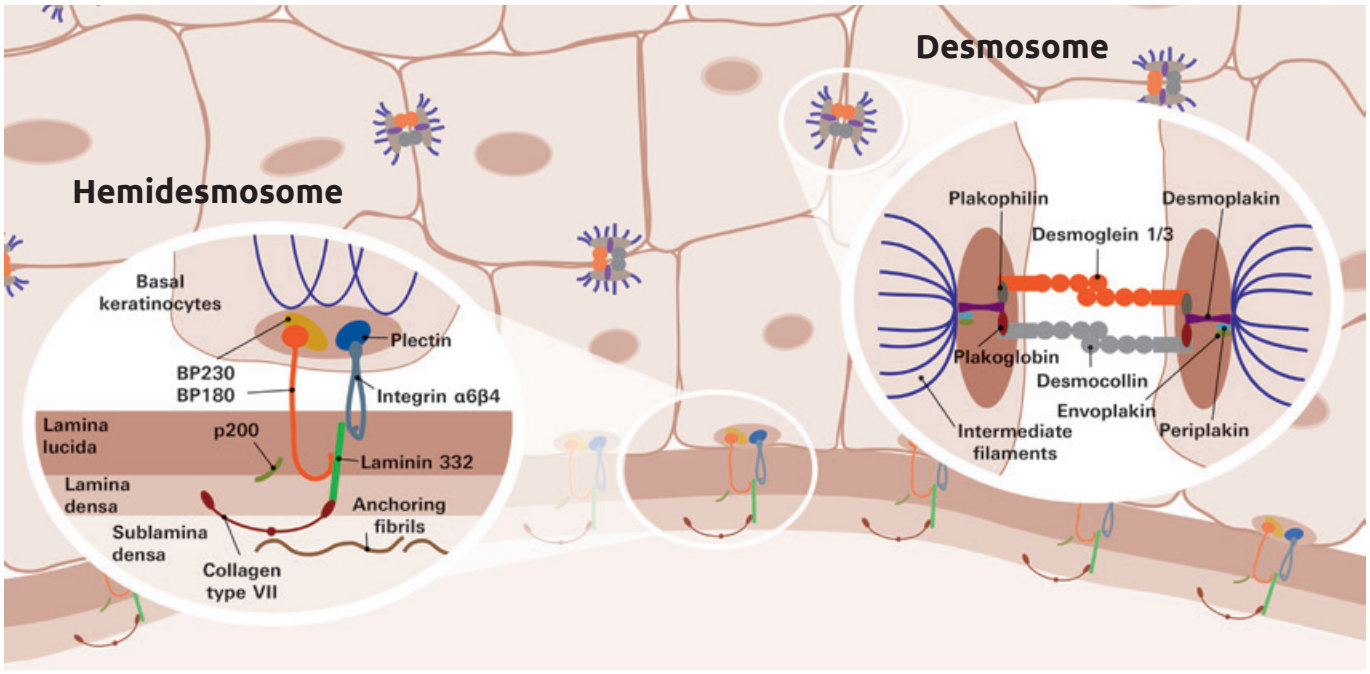

Figure 1. Antigens of desmosomes and hemidesmosomes

Epidermolysis bullosa acquisita (EBA) is a further subepidermal blistering disease, which is characterized by autoantibodies against collagen type VII, a key component of anchoring fibrils that stabilize the dermal–epidermal junction.

Autoantibody detection methods

Analysis of autoantibodies facilitates categorization of the type of AIBD. Direct immunofluorescence is used to detect tissue-bound autoantibodies in vivo on skin biopsy samples, while serological methods allow detection of circulating autoantibodies in serum [1,2].

Indirect immunofluorescence assay (IFA) is the most important serological method. IFA using tissue sections of esophagus enables detection of autoantibodies against desmosomes (pemphigus) and the basal lamina (pemphigoid). Salt-split skin is a technique that involves creating an artificial split in a skin sample to separate the epidermis from the dermis. This 'split' is achieved by soaking normal human skin in a salt solution, which causes the skin to separate at the lamina lucida. This substrate then allows differentiation of autoantibodies against antigens of the epidermal (BP180, BP230) and dermal (collagen type VII, laminins) sides of the basement membrane (Fig. 2). These substrates do not, however, allow identification of the precise target antigen.

Autoantibodies can be characterized monospecifically using recombinant-cell IFA (RC-IFA). This innovative method utilizes transfected human cells expressing defined antigens or optimized fragments thereof as the antigenic substrate. The technique offers high sensitivity and reproducibility due to the controlled high-level expression of the target antigens, and results are easy to interpret. Further monospecific detection methods include IFA based on purified antigen dots and multiparameter ELISA profiling. Ready-to-use BIOCHIP Mosaic slides, which are composed of miniature sections of different cell and tissue substrates, are advantageous for analysing multiple autoantibodies in parallel or for antibody screening and confirmation in one test.

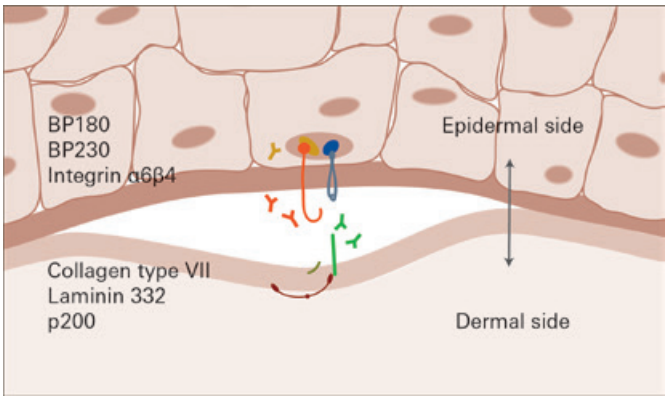

Figure 2. Localization of antigens on salt-split skin

Dermal binder autoantibodies

Of the relevant autoantibodies in AIBD, three main types bind to the dermal side of artificially (salt) split skin in IFA. This is referred to as the 'floor' pattern, in contrast to the 'roof' pattern seen with autoantibodies in BP. Two of these floor-binding antibodies, anti-collagen type VII and anti-laminin 332 (anti-lam332), are associated with severe forms of AIBD, namely EBA and a subform of MMP (anti-lam332 MMP), respectively. The third dermal binder autoantibody occurs in anti-p200 pemphigoid, which until recently was not well characterized.

Anti-p200 pemphigoid

Anti-p200 pemphigoid is a rare form of AIBD, with an estimated annual incidence of 0.7 per million inhabitants in Germany [3]. Since many patients are presumed to be misclassified as BP or EBA, this is most likely an underestimate. Anti-p200 pemphigoid appears to be more frequent than anti-lam332 MMP and EBA, making it the most common pemphigoid disease associated with floor-binding autoantibodies [4,5].

The disease manifests with erosions and blisters on the skin and/or mucosal membranes. It is characterized by tissue-bound IgG and/or complement C3 along the cutaneous basement membrane zone (BMZ) and serum autoantibodies against a 200-kDa protein in extracts of dermis [3].

Laminin subunit gamma 1 (lamγ1) was initially described as a target antigen recognized by 70–90% of patient sera. However, anti-lamγ1 failed to show pathogenic effects *in vivo*, raising doubts as to whether lamγ1 was the sole antigen in anti-p200 pemphigoid [3].

Identification of lamβ4

Laminin subunit beta 4 (lamβ4) was recently identified as an additional target antigen in anti-p200 pemphigoid [3]. Reactivity to lamβ4 was detected by immunoblotting in all sera from patients with anti-p200 pemphigoid (*n*=60), with results additionally corroborated by preclearance assays. No reactivity was detected in control sera from patients with other AIBD (*n*=33), including BP, anti-lam332 MMP and EBA, and from healthy individuals (*n*=29), indicating that anti-lamβ4 is specific for anti-p200 pemphigoid. Epitope mapping revealed that the C-terminal stretch of lamβ4 is the immunodominant region of the protein.

To localize the antigen, antibodies against a lamβ4 fragment were isolated from patient serum. In IFA, they showed a clear binding to the dermal side of salt-split skin, as typically seen with sera of anti-p200 pemphigoid patients. In direct immunofluorescence assays, anti-lamβ4 antibodies localized to the BMZ of skin samples [3].

Lamβ4 was shown to be expressed predominantly in epidermal keratinocytes at the RNA and protein level. In contrast, no protein and considerably less mRNA was detected in fibroblasts. It is, therefore, assumed that lamβ4 is produced in keratinocytes and subsequently secreted and incorporated into the BMZ [3].

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Pathogenic role of anti-lamβ4

The precise biological function of lamβ4 is currently unknown. Laminins are a family of structural proteins that perform crucial functions in the skin and other epithelial tissue. They typically consist of heterotrimer structures composed of an alpha chain, a beta chain and a gamma chain. For example, laminin 332 is made up of the subunits α3, β3 and γ2. So far, no heterotrimer containing the β4 subunit has been reported. Recent evidence suggests that lamβ4 interacts with α3 and potentially γ1/2 [6].

Anti-lamβ4 immune complexes appear to have a pathogenic effect. They were able to activate leukocytes, leading to the dose-dependent release of tissue-damaging molecules [6]. Moreover, anti-lamβ4 antibodies from patient serum caused dermal–epidermal splitting of skin sections in the presence of leukocytes, while anti-lamγ1 antibodies did not. This splitting is also observed with other pemphigoid auto-antibodies such as anti-BP180 and anti-collagen type VII.

Anti-lamβ4 assays

A new RC-IFA was developed for monospecific detection of anti-lamβ4 antibodies [Euroimmun Anti-Laminin Subunit Beta 4 (LAMB4) IIFT; for research use only]. The assay utilizes lamβ4-expressing HEK293 cells as the detection substrate together with control cells [7].

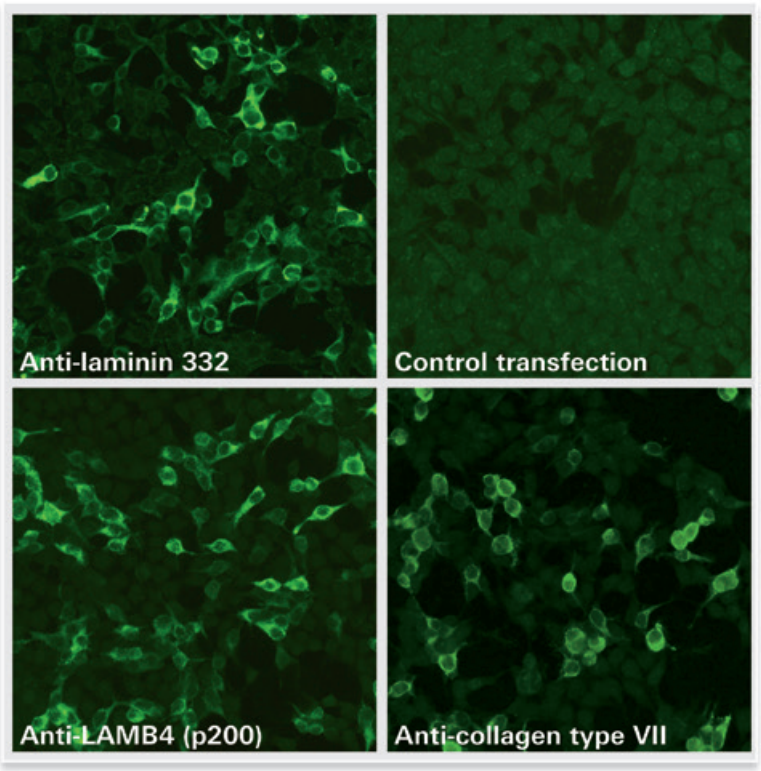

Figure 3. BIOCHIP Mosaic for detection of antibodies against dermal binder autoantibodies

A further RC-IFA for simultaneous detection of anti-lamβ4, anti-lam332 and anti-collagen type VII autoantibodies was also developed (Euroimmun Dermal Binder Mosaic 1; for research use only). Since these dermal-binding autoantibodies are difficult to tell apart in tissue-based IFA, monospecific detection is a useful additional analysis. The new test comprises a BIOCHIP Mosaic of transfected-cell substrates expressing each of the respective antigens, together with control cells (Fig. 3). The multiparameter analysis with a derived laboratory-developed test (LDT) can aid in the delimitation of the disease forms anti-p200 pemphigoid, anti-lam332 MMP and EBA.

Clinical evaluation: anti-lamβ4 IFA

The anti-lamβ4 assay was validated using serum samples from patients with anti-p200 pemphigoid (*n*=293) and control sera from patients with PV (*n*=20), BP (*n*=100), anti-lam332 MMP (*n*=19) or EBA (*n*=35) and healthy blood donors (*n*=300) [7].

Reactivity with lamβ4 was seen in 99.2% (237/239) of the anti-p200 pemphigoid sera. Notably, reactivity was detected in four sera that were unreactive on salt-split skin, indicating that the new test might help to increase the serological detection rate for anti-p200 pemphigoid. The two anti-lamβ4-negative sera were reactive with p200 antigen and lamγ1 in immunoblotting.

None of the PV, BP or anti-lam332 sera exhibited anti-lamβ4 antibodies, confirming the antibody specificity for anti-p200 pemphigoid. Three patients with EBA showed reactivity to lamβ4 as well as to lamγ1 and collagen type VII, likely as a result of epitope spreading. Two of the blood donors were anti-lamβ4 positive. The overall specificity of the assay, therefore, amounted to 99.3%. The high sensitivity and specificity of the new IFA make it a useful tool for standardized detection of anti-lamβ4 autoantibodies.

Prospective study: Dermal Binder Mosaic 1

In a prospective international multicentre study, the Dermal Binder Mosaic 1 was used to investigate 41 samples from AIBD patients with dermal IgG binding on salt-split skin [8]. All sera showed reactivity with at least one of the target antigens: 27 with lamβ4, two with lam332 and 15 with collagen type VII. No sera from patients with PV (*n*=50) or from healthy blood donors (*n*=50) showed a reaction. From a panel of sera with epidermal IgG binding on salt-split skin (*n*=51), three reacted with lamβ4-expressing cells. Upon retesting, these samples exhibited faint dermal staining in addition to the epidermal staining, suggesting dual reactivity. These findings underscore the high sensitivity and specificity of the RC-IFA and highlight the benefits of composite testing.

Outlook

The identification of lamβ4 as the target antigen in anti-p200 pemphigoid, along with evidence of its pathogenic role, has advanced the understanding of this rare disease. An in-depth characterization of the disease is essential, as it is frequently misdiagnosed yet appears to be more prevalent than other AIBD with dermal-binding autoantibodies. Further research will focus on determining the physiological binding partners of lamβ4 both within and beyond the laminin family, exploring the immunogenic epitopes of the protein, and investigating the pathogenic relevance of anti-lamβ4 autoantibodies *in vivo*. The standardized anti-lamβ4 IFA and BIOCHIP Dermal Binder Mosaic 1 are relatively simple, highly sensitive and specific tools that will facilitate ongoing research into anti-p200 pemphigoid.

Regulatory status, precise intended use and product availability must be verified for the user's individual jurisdiction.

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For further information see:
www.euroimmun.com

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