
Diagnosis of blistering autoimmune diseases

The present invention relates to a polypeptide comprising laminin beta-4, to a carrier comprising the polypeptide, to an antibody, preferably autoantibody, against laminin beta-4, to use of the polypeptide, carrier or autoantibody for the diagnosis of a disease and to a method comprising the step of detecting an autoantibody against laminin beta-4 in a sample.

Autoimmune diseases are understood to mean diseases in which the immune system is directed against intact structures endogenous to the body. In many autoimmune diseases, the body produces detectable autoantibodies which can bind against proteins endogenous to the body and thus cause disorders. In other cases, the presence of autoantibodies does not appear to be causally responsible for the outbreak of disease.

Blistering autoimmune diseases are distinguished by autoantibodies attacking target structures in the skin. Immune mechanisms bring about the detachment of the upper parts of the skin, and this leads to the formation of blisters.

On the basis of the target antigens and of the localization of split formation, a distinction is made between 4 main groups: pemphigus and pemphigoid diseases, epidermolysis bullosa acquisita (EBA) and dermatitis herpetiformis. Blistering is intraepithelial in the case of pemphigus diseases and subepithelial in the case of the other bullous autoimmune dermatoses.

Pemphigus vulgaris (PV) is the most common pemphigus disease, followed by pemphigus foliaceus, whereas other forms of pemphigus diseases such as paraneoplastic pemphigus occur very rarely. Pemphigus vulgaris (PV) is distinguished by blisters which burst relatively rapidly and which leave behind redness, erosions and crusts on the skin, due to dissolution of associated cells in the epidermis. Infections commonly occur at the affected sites. In many cases and at the start of the disease, the oral mucous membranes or the genital mucous membranes are affected by the blisters with severe soreness. If the disease is not identified and therapied in time, severe and, in some cases, fatal courses of the disease can be expected. PV occurs rarely with about 1 to 2 new cases per one million people.

In the case of the autoimmune diseases which form blisters subepidermally, the autoantibodies are directed against structural proteins in the region of the dermal-epidermal junction zone (DEJ). Said proteins are of great importance for the adhesion of the basal keratinocytes on the underlying dermis. In the case of this group of diseases, a distinction is made between bullous pemphigoid, anti-p200 pemphigoid, pemphigoid gestationis, mucous membrane pemphigoid, linear IgA dermatosis, lichen ruber pemphigoides, epidermolysis bullosa acquisita (EBA) and dermatitis herpetiformis.

Bullous pemphigoid (BP) is the most common subepidermally blistering autoimmune disease. It is characterized by bulging blisters on healthy or reddened skin that are formed because of detachment of the epidermis and that may be accompanied by severe itching as a preliminary sign. The blisters commonly occur on the trunk and on the thighs and arms. In 20 percent of patients, the mucous membranes also become involved. If the blisters burst, the wounds usually heal without scarring. BP is a relatively common autoimmune disease, with about 15 to 20 new cases per one million people.

After bullous pemphigoid, mucous membrane pemphigoid (MMP, a variant thereof is anti-laminin 332 MMP) is the second most common bullous autoimmune dermatosis. Clinically, MMP is a heterogeneous syndrome in which, as per the definition, the mucous membranes are predominantly affected. The oral and ocular mucous membranes are most commonly affected. Blistering lesions sometimes heal with scarring. If the conjunctivas are affected, this may lead to blindness.

The clinical picture of anti-p200 pemphigoid is variable, with the BP-similar type occurring most commonly. This disease is rarer than BP, and this may also be due to the fact that many of these patients are misdiagnosed as EBA or anti-laminin 332 mucous membrane pemphigoid because of their similar immunofluorescence pattern.

Epidermolysis bullosa acquisita (EBA) is a very rare subepidermal bullous autoimmune disease which is characterized by autoantibodies against type VII collagen. Clinically, a distinction can be made between the mechanobullous form and inflammatory variants (similar to BP or to mucous membrane pemphigoid).

It is difficult to distinguish between the diseases clinically or histologically. However, in the prior art, a range of serological assays which allow a diagnosis in many cases have been described. In the case of PV, autoantibodies against the proteins desmoglein 1 and/or 3

occur. In the case of BP, it is possible to detect autoantibodies against the proteins BP180 and/or BP230 in almost all cases and even against laminin 332 in some cases.

Anti-p200 pemphigoid is characterized by autoantibodies against a protein of the dermal-epidermal junction zone that has an apparent molecular mass of about 200 kDa. Said protein was described in 2009 (Dainichi, T., Kurono, S., Ohyama, B., Ishii, N., Sanzen, N., Hayashi, M., Shimono, C., Taniguchi, Y., Koga, H., Karashima, T., Yasumoto, S., Zillikens, D., Sekiguchi, K., Hashimoto, T. (2009). Anti-laminin gamma-1 pemphigoid. 106(8): 2800-5) as laminin gamma-1. In addition, as part of epitope spreading, it is also possible to find antibodies against type VII collagen, BP180 or else against laminin 332.

What are used in this connection are especially ELISA and indirect immunofluorescence, but other immunobiochemical methods are also usable in principle. For example, the dermatology profile of EUROIMMUN Medizinische Labordiagnostika AG (EA 1490-1208-1 G), which encompasses BP180, NC16A-4X, BP230-CF, desmoglein 1, desmoglein 3, envoplakin and collagen type VII, is suitable.

The prior art discloses diagnostic procedures, in particular indirect immunofluorescence with esophagus tissue sections from primates or ELISA with the recombinant autoantigens as substrate (cf. van Beek, N., Zillikens, D., and Schmidt, E. (2018): Diagnosis of autoimmune bullous diseases, *J. Dtsch. Dermatol. Ges.* 16(9), pages 1077-1091). A particularly reliable assay, in terms of diagnostics, for the detection of anti-laminin 332 mucous membrane pemphigoid can be carried out using immunofluorescence and recombinant laminin 332 (Goletz, S., Probst, C., Komorowski, L., Schlumberger, W., Fechner, K., van Beek, N., Holtsche, M. M., Recke, A., Yancey, K. B., Hashimoto, T., Antonicelli, F., Di Zenzo, G., Zillikens, D., Stöcker W., and Schmidt, E. (2018): Sensitive and specific assay for the serological diagnosis of anti-laminin 332 mucous membrane pemphigoid, *Br. J. Dermatol.*, DOI 10-1111/bjd.17202). The prior art does not describe autoantibodies against laminin beta-4.

However, what becomes apparent is that the detection via laminin gamma-1 is not positive in all patients. In a western blot with recombinant forms of laminin gamma-1 and in an ELISA with the C-terminal domain of laminin gamma-1, it was possible to detect, respectively, 90% and 69% of the tested anti-p200 pemphigoid patients (Dainichi et al., 2009; Groth, S., Recke, A., Vafia, K., Ludwig, R.J., Hashimoto, T., Zillikens, D., Schmidt, E. (2011). Development of a simple enzyme-linked immunosorbent assay for the detection of autoantibodies in anti-p200 pemphigoid. *Br J Dermatol.* 164(1): 76-82).

The clinical course of anti-p200 pemphigoid is variable. In contrast to epidermolysis bullosa acquisita and bullous pemphigoid, what is concerned here is a relatively benign disease with a good therapeutic response. Therefore, it is of great clinical importance to differentiate
5 between these diseases.

US6,682,911 discloses laminin 12 and preparations of isolated laminin 12 and of subunits thereof.

10 Against this background, there is a need for a detection procedure for anti-p200 pemphigoid that has higher diagnostic reliability, especially sensitivity. At the same time, there is the additional aim of improving the differentiability between the various blistering autoimmune diseases of the skin.

15 In a first aspect, the object underlying the invention is achieved by a polypeptide comprising laminin beta-4 or a variant thereof, preferably in recombinant and/or isolated form.

In a preferred embodiment, the polypeptide is immobilized.

20 In a second aspect, the object is achieved by a carrier comprising the polypeptide according to the invention, and further comprising at least one antigen, preferably all the antigens, from the group comprising laminin gamma-1, BP180, BP230, desmoglein 1, desmoglein 3, envoplakin, deamidated gliadin, laminin 332 and collagen type VII or variants thereof.

25 In a preferred embodiment, the carrier is selected from the group comprising a glass slide, preferably slide for microscopy, a biochip, a microtiter plate, a lateral flow device, a test strip, a membrane, preferably a blot, more preferably a line blot, a chromatography column material and a bead, preferably a magnetic or fluorescent bead.

30 In a third aspect, the object is achieved by a therapeutically useful carrier comprising the polypeptide according to the invention, wherein the carrier allows the contacting of human blood with the polypeptide followed by the recirculation of the blood into the body of a patient, the carrier preferably being an apheresis apparatus.

35 In a fourth aspect, the object is achieved by an antibody, preferably autoantibody, against laminin beta-4, preferably in isolated form.

In a fifth aspect, the object is achieved by the use of the polypeptide, carrier or autoantibody according to the invention for the diagnosis of a disease.

- 5 In a sixth aspect, the object is achieved by a method for producing a diagnostically or therapeutically useful carrier, comprising the step of coating the carrier with the polypeptide according to the invention.

10 In a seventh aspect, the object is achieved by a method for purifying an autoantibody, comprising the steps of a) contacting a liquid comprising the autoantibody with the polypeptide according to the invention under conditions which allow the formation of a complex comprising the autoantibody and the polypeptide, b) isolating the complex from step a), and c) optionally detecting the complex from step a) or dissociating the complex isolated in step b) followed by removal of the autoantibody from the polypeptide.

15 In an eighth aspect, the object is achieved by a method comprising the step of detecting an autoantibody against laminin beta-4 in a sample.

20 In a ninth aspect, the object is achieved by a pharmaceutical composition comprising the polypeptide according to the invention or a variant thereof, preferably together with a pharmaceutically acceptable carrier.

25 In a tenth aspect, the object is achieved by a kit comprising the polypeptide according to the invention or the carrier according to the invention, wherein the kit contains one reagent or more than one reagent, preferably all the reagents, from the group comprising a wash solution, a calibrator solution, an antibody against laminin beta-4, and an agent for the detection of an autoantibody against laminin beta-4, preferably a secondary antibody.

30 In an eleventh aspect, the object is achieved by the use of the polypeptide according to the invention, of the carrier according to the invention or of an antibody, preferably autoantibody, against laminin beta-4 for the production of a kit or diagnostically or therapeutically useful carrier.

35 In a preferred embodiment, an autoantibody against laminin beta-4 is detected using a method selected from the group comprising immunodiffusion, immunoelectrophoresis, light scattering, agglutination, immunoassay with labeling such as that from the group comprising immunoassay with radioactive labeling, with enzymatic labeling, more preferably ELISA, with

chemiluminescence labeling, more preferably electrochemiluminescence labeling, and with immunofluorescence labeling, preferably indirect immunofluorescence.

In a twelfth aspect, the object is achieved by the use of the polypeptide according to the invention or of the antibody according to the invention for the determination of the capability, preferably capacity, of a diagnostic or therapeutic device or of a diagnostic or therapeutic reagent to bind an antibody against laminin beta-4.

The present invention is based on the surprising finding by the inventors that an autoantibody against laminin beta-4 exists and can be specifically detected in patients who suffer from anti-p200 pemphigoid.

The inventors further found that the detection of said autoantibody increases the sensitivity of the serological diagnostic assay for anti-p200 pemphigoid. It can be detected in some patients in whom it is not possible to detect the autoantibody against laminin gamma-1, which autoantibody was hitherto considered to be of sole importance.

Laminins are trimeric basal membrane proteins which consist of alpha, beta and gamma chains. Laminin beta-4 is a beta chain. Laminins are involved in many physiological functions such as, for example, cell adhesion, migration, differentiation, neuronal development and in the regulation of gene expression.

Choi *et al.* (2015) observed a reduced expression of laminin beta-4 in cancer tissue of the stomach and intestine. They identified somatic laminin beta-4 mutations in cancer tissue that possibly contributed to a reduced expression of laminin beta-4 (Choi, M. R., An, C. H., Yoo, N. J., Lee, S. H. Laminin gene LAMB4 is somatically mutated and expressionally altered in gastric and colorectal cancers. *APMIS* 123: 65-71, 2015). Moreover, reduced levels of laminin beta-4 might also play a role in diverticulitis (Coble JL, Sheldon KE, Yue F, Salameh TJ, Harris LR III, Deiling S, Ruggiero FM, Eshelman MA, Yochum GS, Koltun WA, Gerhard GS, Broach JR. Identification of a rare LAMB4 variant associated with familial diverticulitis through exome sequencing. *Hum Mol Genet.* 2017; 26(16): 3212-3220).

The present invention provides a polypeptide comprising a mammalian laminin beta-4, preferably from humans, simians, cattle, sheep or pigs and particularly preferably from humans.

In a preferred embodiment, laminin beta-4 is the polypeptide which is encoded by the database codes (BC140804), preferably SEQ ID NO27, or a variant thereof. In this application, all the cited database codes represent the Uniprot database or other databases, more precisely the version thereof on the filing date of said application or its earliest priority application. In a further preferred embodiment, the further antigens have the sequences respectively indicated between parentheses: laminin gamma-1 (LAMC1 P11047), laminin 332 selected from the group comprising LAMA3 (Q16787), LAMB3 (Q13751) and LAMC2 (Q13753), BP180 (Q9UMD9), BP230 (SEQ ID NO8 from EP3260864, listed here as SEQ ID NO 28), desmoglein 1 (Q02413), desmoglein 3 (P32926), envoplakin (Q92817), gliadin, preferably selected from the group comprising LGQQQPFPPQQPYQPQPFPSQQPY (SEQ ID NO 29), QLQPFQPELPYPQPQS (SEQ ID NO 30) and QQLPQPEQPQQSFPEQERPF (SEQ ID NO 31), and collagen type VII (Q02388) or variants thereof.

However, it is possible to carry out the teaching according to the invention not only by using polypeptides or nucleic acids, more precisely a polypeptide comprising the wild-type sequence of laminin beta-4 or a nucleic acid coding therefor, which have the exact sequences stated expressly or implicitly in this application, but also by using variants of such polypeptides or nucleic acids.

In a preferred embodiment, the term “variant”, as used herein, can mean at least one fragment or one full-length sequence, more precisely one or more than one amino acid sequence or nucleic acid sequence, which is truncated at one end or both ends by at least one or more than one amino acid relative to the full-length sequence. Such a fragment comprises or encodes a peptide containing at least 6, 7, 8, 10, 12, 15, 20, 25, 50, 75, 100, 300, 400, 600, 800, 1000 or 1200 successive amino acids of the original sequence or a variant thereof. The total length of the variant can be at least 6, 7, 8, 9, 10, 11, 12, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 800, 1000, 1200, 1500, 1800 or more amino acids. A particularly preferred fragment is selected from the group comprising SEQ ID NO 8, SEQ ID NO 9, SEQ ID NO 10, SEQ ID NO 11, SEQ ID NO 12, SEQ ID NO 13 and SEQ ID NO 14 and variants thereof.

In a further preferred embodiment, the term “variant” means not only at least one fragment, but also a polypeptide or fragment thereof which comprises amino acid sequences which are at least 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 92%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence or the fragment thereof to which express reference has been made, with deletion or substitution of amino acids other than those essential for biological activity, for example the capabilities of an antigen of binding to the

(auto)antibody or the folding or structure of the polypeptide, and/or conservative substitution of one or more than one such essential amino acid and/or addition of amino acids such that the biological activity of the polypeptide is maintained. The prior art comprises various methods which can be used for aligning two given nucleic acid or amino acid sequences and for calculating the degree to which they are identical; see, for example, Arthur Lesk (2008), Introduction to bioinformatics, Oxford University Press, 2008, 3rd edition. In a preferred embodiment, the ClustalW software is used with use of the basic settings (Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P. A., McWilliam, H., Valentin, F., Wallace, I. M., Wilm, A., Lopez, R., Thompson, J. D., Gibson, T. J., Higgins, D. G. (2007). Clustal W and Clustal X version 2.0. Bioinformatics, 23, 2947-2948).

In a preferred embodiment, the variant is a linear, nonfolded polypeptide which is optionally denatured.

In a preferred embodiment, the polypeptide and variants thereof can additionally contain chemical modifications, for example isotopic labels, or covalent modifications such as a glycosylation, phosphorylation, acetylation, decarboxylation, citrullination, methylation, hydroxylation and the like. A person skilled in the art is familiar with methods for modifying polypeptides. Any modification is designed such that it does not cancel the biological activity of the variant.

Furthermore, variants can also be generated by N-terminal and/or C-terminal fusion with other polypeptides, peptides or amino acids or variants thereof and can comprise active parts or domains, for example having a sequence identity of at least 70%, 75%, 80%, 85%, 90%, 92%, 94%, 95%, 96%, 97%, 98% or 99% when they are aligned with the active part of the wild-type sequence, the term "active part", as used herein, meaning the amino acid sequence which is shorter than the full-length amino acid sequence, or in the case of a nucleic acid sequence encodes less than the full-length amino acid sequence, and/or is a variant of the natural sequence, but contains at least some of the biological activity. Fused-on sequences are configured such that they do not interfere with the binding of the autoantibody to the laminin beta-4 or the variant thereof, especially not to the epitopes which are comprised by the sequences SEQ ID NO 8, SEQ ID NO 9, SEQ ID NO 10, SEQ ID NO 11, SEQ ID NO 12, SEQ ID NO 13 and SEQ ID NO 14 and variants thereof. A tag, for example from the group comprising GST, His, MBP and FLAG, which is fused on directly or via a flexible linker at the N-terminus and/or C-terminus is suitable for example.

In a preferred embodiment, the term “variant” encompasses nucleic acids of the complementary strand which hybridizes to the reference nucleic acid or wild-type nucleic acid under stringent conditions. The stringency of hybridization reactions can be easily determined by a person skilled in the art and is generally an empirical calculation which depends on the probe length, the wash temperatures and the salt concentration. It is generally the case that longer probes requires higher temperatures for specific annealing, whereas shorter probes require a less high temperature. Hybridization generally depends on the capability of a denatured DNA to hybridize once again to the complementary strand in an environment below its melting temperature. The higher the degree of the required homology between the probe and the sequence capable of hybridization, the higher the relative temperature that can be used. As a result, higher relative temperatures would tend to shape the reaction conditions more stringently, whereas a lower temperature brings this about to a lesser degree. For additional details and explanations in relation to stringency and hybridization reaction, see Ausubel, F. M. (1995), *Current Protocols in Molecular Biology*. John Wiley & Sons, Inc. Furthermore, a person skilled in the art can follow the instructions found in the manual from Boehringer Mannheim GmbH (1993) (*The DIG System Users Guide for Filter Hybridization*, Boehringer Mannheim GmbH, Mannheim, Germany) and in Liebl, W., Ehrmann, M., Ludwig, W., and Schleifer, K. H. (1991) *International Journal of Systematic Bacteriology* 41: 255-260, in relation to the question of how DNA sequences can be identified by means of hybridization. In a preferred embodiment, stringent conditions are applied to every hybridization, i.e. hybridization occurs when the probe is 70% identical or higher to the target sequence. Probes having a lower degree of identity with respect to the target sequence can hybridize, but such hybrids are unstable and are removed in a wash step under stringent conditions, for example by lowering the salt concentration to 2 x SSC or optionally and then subsequently 0.5 x SSC, while the temperature is, with increasing preference, about 50°C – 68°C, about 52°C – 68°C, about 54°C – 68°C, about 56°C – 68°C, about 58°C – 68°C, about 60°C – 68°C, about 62°C – 68°C, about 64°C – 68°C, about 66°C – 68°C. In a particularly preferred embodiment, the temperature is about 64°C – 68°C or preferably 66°C – 68°C. It is possible to adjust the salt concentration to 0.2 x SSC or even 0.1 x SSC. Nucleic acid sequences having a degree of identity with respect to the reference sequence or wild-type sequence of at least 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% can be isolated. In a preferred embodiment, the term “variant of a nucleic acid sequence”, as used herein, means any nucleic acid sequence encoding the same nucleic acid sequence and variants thereof as the reference nucleic acid sequence in line with the degeneracy of the genetic code.

The variant of the polypeptide exhibits biological activity. In a preferred embodiment, this is the capability of binding to an autoantibody against laminin beta-4, as is found in a patient suffering from an autoimmune disease associated with such an autoantibody, preferably anti-p200 pemphigoid. It is possible to clarify whether, for example, a variant of laminin beta-4 has such biological activity by detecting whether said variant binds to an autoantibody from a patient sample, preferably by western blotting and/or indirect immunofluorescence, preferably indirect immunofluorescence, using recombinant proteins as described in the examples. Moreover, the inventors have identified the sequences SEQ ID NO 8, SEQ ID NO 9, SEQ ID NO 10, SEQ ID NO 11, SEQ ID NO 12, SEQ ID NO 13 and SEQ ID NO 14, which are reactive toward an autoantibody against laminin beta-4. Variants of laminin beta-4 to which such an autoantibody binds can thus be easily generated by the generation of an epitope from the laminin beta-4 sequence that comprises at least one of these sequences, it being optionally possible to generate additional variants by conservative substitutions of amino acid residues.

The polypeptide according to the invention can be provided in any form and any purification stage, from liquid samples, tissues or cells comprising the polypeptide in an endogenous form, more preferably cells overexpressing the polypeptide, crude or enriched lysates of such cells, up to purified and/or isolated polypeptide which is optionally substantially pure. In a preferred embodiment, the polypeptide is a native polypeptide, the term "native polypeptide", as used herein, describing a folded polypeptide, more preferably a folded polypeptide purified from tissues or cells, more preferably from mammalian cells or tissues, optionally from nonrecombinant tissues or cells. In a further preferred embodiment, the polypeptide is a recombinant protein, the term "recombinant", as used herein, meaning a polypeptide which was produced in any phase of the production process using gene-technology methods, for example by fusion of a nucleic acid encoding the polypeptide to a strong promoter for overexpression in cells or tissues or by modification of the polypeptide sequence itself. A person skilled in the art is familiar with methods for modifying nucleic acids and polypeptides encoded thereby by means of gene technology (e.g., described in Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989), Molecular Cloning, CSH or in Brown T. A. (1986), Gene Cloning – an introduction, Chapman & Hall), and also with methods for producing and purifying native or recombinant proteins (e.g., the handbooks "Strategies for Protein Purification", "Antibody Purification", "Purifying Challenging Proteins" (2009/2010), published by GE Healthcare Life Sciences, and in Burgess, R. R., Deutscher, M. P. (2009), Guide to Protein Purification). In a preferred embodiment, a polypeptide is pure when at least 60%, 70%, 80%, 90%, 95% or 99% of the total polypeptide in the relevant sample consists of the polypeptide, as assessed by means of SDS-polyacrylamide gel

electrophoresis followed by Coomassie Blue staining and estimation with the naked eye. If the polypeptide according to the invention is provided in the form of a tissue, it is preferred that the tissue is a mammalian tissue, for example tissue from humans, from primates, from donkeys, from goats, from horses, from sheep, from pigs or from cows, preferably skin tissue. If the polypeptide is provided in the form of a recombinant cell, it is preferred that the recombinant cell is a eukaryotic cell such as a yeast cell, more preferably a cell from a multicellular eukaryote such as a plant, a mammal, frog or insect, most preferably from a mammal, for example rat, human, primate, donkey, mouse, goat, horse, sheep, pig or cow.

The polypeptide for carrying out the teaching according to the invention is preferably of such a nature that it comprises at least one epitope which is recognized by the autoantibody which binds to laminin beta-4, or that it binds specifically to said autoantibody. The epitope is preferably an epitope which is only recognized by such an autoantibody and not by other antibodies, such as autoantibodies against laminin gamma-1, BP180, BP230, desmoglein 1, desmoglein 3, envoplakin, gliadin, laminin 332 or collagen type VII. In such an embodiment, such an epitope comprises a segment of 6, 7, 8, 9, 10, 11, 12, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100 or more, preferably at least 9, but not more than 16, successive amino acids from laminin beta-4. A person skilled in the art is familiar with guidelines for designing peptides having sufficient immunogenicity, for example as have been described in Jackson *et al.* (Jackson, D. C., Fitzmaurice, C. J., Brown, L. E., Zeng, W. (1999), Preparation and properties of totally synthetic immunogenes, Vaccine, volume 18, issues 3-4, September 1999, pages 355-361; and Black, M., Trent, A., Tirrell, M. and Olive, C. (2010), Advances in the design and delivery of peptide subunit vaccines with a focus on Toll-like receptor agonists, Expert Rev Vaccines, 2010 February; 9(2): 157-173).

The polypeptide according to the invention, which encompasses laminin beta-4 or a variant thereof, can be provided in any conformation to carry out the present invention. For example, the polypeptide can be a substantially unfolded or partially or completely folded polypeptide. In a preferred embodiment, the polypeptide is folded in the sense that the epitope(s) required for the binding to the autoantibody according to the invention assume(s), in its or their entirety, the folding which is assumed by the native protein in its natural environment. A person skilled in the art in the field is familiar with methods which can be used to determine whether the polypeptide is folded, and if it is folded, what structure it assumes, for example limited proteolysis, NMR spectroscopy, CD spectroscopy or X-ray crystallography (see, for example, Banaszak L. J. (2008), Foundations of Structural Biology, Academic Press, or Teng Q. (2013), Structural Biology: Practical Applications, Springer), preference being given to using CD spectroscopy.

The polypeptide according to the invention can be a fusion protein which comprises, at the C-terminus and/or N-terminus, amino acids and/or amino acid sequences other than those from laminin beta-4, especially a C-terminal or N-terminal tag, preferably a C-terminal tag, which, in a preferred embodiment as used herein, comprises an additional sequence motif or a polypeptide that has some biological or physical function and can, for example, be realized for the purification, immobilization, precipitation or identification of the polypeptide according to the invention. In a more preferred embodiment, the tag is a sequence or domain which is capable of binding specifically to a ligand, for example a tag selected from the group comprising His-tags, thioredoxin, maltose-binding protein, glutathione S-transferase, a fluorescence tag, for example from the group comprising green fluorescent protein (GFP).

The polypeptide according to the invention can be an immobilized polypeptide. In a preferred embodiment, the term “immobilized”, as used herein, means a molecule which is bound to a solid carrier which is insoluble in an aqueous solution, more preferably via a covalent bond, electrostatic interaction, encapsulation or inclusion, for example by denaturation of a globular polypeptide in a gel, or via hydrophobic interaction, most preferably via one or more than one covalent bond. Various suitable carriers, for example paper, polystyrene, metal, silicone or glass surfaces, microfluidic channels, membranes, beads such as magnetic beads, chromatography column media, biochips, polyacrylamide gels and the like, are described in the literature, for example in Kim, D., and Herr, A. E. (2013), Protein immobilization techniques for microfluidic assays, *Biomicrofluidics* 7(4), 041501. In this way, the immobilized molecule together with the insoluble carrier can be easily separated from an aqueous solution in a simple manner, for example by filtration, centrifugation or decanting. An immobilized molecule can be reversibly or irreversibly immobilized. For example, the immobilization is reversible when the molecule interacts with the carrier via an ionic interaction which can be masked by addition of a high concentration of a salt or when the molecule is bound via a cleavable covalent bond such as a disulfide bridge which can be cleaved by addition of thiol-containing reagents. In contrast, the immobilization is irreversible when the molecule is attached to the carrier via a covalent bond which cannot be cleaved in aqueous solution, for example a bond which is formed by reaction of an epoxide group and an amino group, as is commonly used for binding lysine side chains to affinity columns. The protein can be indirectly immobilized, for example by immobilization of an antibody or some other unit having affinity for the molecule, followed by formation of a complex, with the effect that the molecule–antibody complex is immobilized. Various options for immobilizing molecules are described in the literature, for example in Kim, D., and Herr, A. E. (2013), Protein immobilization techniques for microfluidic assays, *Biomicrofluidics* 7(4), 041501. In

addition, various reagents and kits for immobilization reactions are commercially available, for example from Pierce Biotechnology.

It is necessary that the sample used for diagnosis by means of the detection of autoantibodies according to the present invention comprises antibodies, which are also referred to as immunoglobulins. Typically, the sample of a body fluid comprises a representative selection of the entirety of immunoglobulins of the patient. However, after provision, the sample can be subjected to further processing steps, which can encompass fractionation, centrifugation, enrichment or isolation of the entirety of immunoglobulins or of a certain immunoglobulin class of the patient and can influence the relative distribution of the immunoglobulins of the various classes.

The reagents, devices, methods and uses that are described in this application can be used for the diagnosis of a disease. In a preferred embodiment, the disease is an autoimmune disease, even more preferably an autoimmune disease of the skin and/or of the eye. Such diseases are distinguished by an overlapping spectrum of symptoms, including blisters, redness and bleedings and also secondary diseases such as an infection of the affected sites. Accordingly, what is also possible is a diagnosis, diagnostic differentiation or indirect diagnosis of an infection of the skin, of the eye or of an allergy. The autoimmune disease is particularly preferably one from the group comprising anti-p200 pemphigoid, anti-laminin 332 mucous membrane pemphigoid, epidermolysis bullosa acquisita, BP and PV.

In a preferred embodiment, the term "diagnosis", as used herein, means any procedure which is aimed at obtaining information which is used to estimate whether a patient suffers from a certain disease or disorder in the past, at the time of diagnosis or in the future and/or has a higher probability of suffering than the average comparative person, preferably with similar symptoms, in order to find out how the disease is progressing or is likely to progress in the future or in order to evaluate the response of a patient to a certain treatment, for example the administration of immunosuppressants. In other words, the term "diagnosis" encompasses not only the making of a diagnosis, but also the prognostication and/or the monitoring of the course of a disease or disorder.

In many cases, mere detection, in other words determining whether detectable concentrations of the antibody are present in the sample or not, is sufficient for diagnosis. If the autoantibody can be detected, this is a piece of information which is important for the diagnosis by the physician and indicates that there is an increased probability of the patient suffering from the disease. Preferably, said piece of information, or a signal containing said

piece of information, can be communicated to a patient or the physician treating said patient in written form, by telephone or by electronic means, for example via the Internet, for example as an e-mail. The piece of information enables the physician in a particularly preferred embodiment to draw a diagnostically useful conclusion and optionally to take it into consideration in the treatment of the patient. In a preferred embodiment, the autoantibody is considered detectable if it can be detected using one or more than one method selected from the group comprising immunoprecipitation, indirect immunofluorescence, ELISA or blot, preferably blot. The experimental details are as described in the experimental section of this application or as described in textbooks or practical protocols as were available on the earliest priority date of this application. In a preferred embodiment, the relative concentration of the antibody in serum is determined in comparison with the concentration found in an average normal person. While it is sufficient in many cases to establish whether the autoantibodies are present or detectable in the sample, the method for obtaining useful information for diagnosis can comprise determination of whether the concentration is at least 0.1, preferably 0.2, 0.5, 1, 2, 5, 10, 20, 25, 50, 100, 200, 500, 1000, 10 000 or 100 000, times higher than the concentration found in the normal healthy person. In a preferred embodiment, the relative concentration of the autoantibody is determined using one or more than one method selected from the group comprising semiquantitative immunoprecipitation, semiquantitative indirect immunofluorescence, ELISA or line blot, preferably ELISA.

The present invention provides a complex comprising an antibody, preferably autoantibody, which binds against the polypeptide according to the invention. It is possible for such a complex to be used or to be detected as part of a method for diagnosing a disease. A liquid sample comprising antibodies from a patient can be used for carrying out the method in which an autoantibody against laminin beta-4 is to be detected. Such a liquid sample can encompass any body fluid comprising a representative selection of antibodies from the person, preferably a sample comprising antibodies of immunoglobulin class IgG from the person. For example, a sample can be cerebrospinal fluid, blood or blood serum, lymph, interstitial fluid and is preferably serum or cerebrospinal fluid, more preferably serum.

The step of contacting a liquid sample comprising antibodies with the polypeptide according to the invention can be carried out by incubation of an immobilized form of the polypeptide in the presence of the sample, of the antibody-comprising sample, under conditions which are compatible with the formation of a complex comprising the polypeptide and an antibody, preferably autoantibody, which binds to the polypeptide according to the invention. The liquid sample in which antibodies which bind against the polypeptide(s) according to the invention are depleted can subsequently be removed, followed by one or more than one wash step.

Lastly, the complex comprising the antibody or the antibodies and the polypeptide or the polypeptides can be detected. In a preferred embodiment, the term “conditions which are compatible with the formation of the complex” means conditions which allow the formation of the specific antigen–antibody interactions for the assembly of the complex comprising the polypeptide and the antibody. In a preferred embodiment, such conditions can comprise the incubation of the polypeptide in a sample diluted 1:100 in PBS buffer at 25°C for 30 minutes. In a preferred embodiment, the term “autoantibody”, as used herein, means an antibody which binds specifically to an endogenous molecule of the animal, preferably mammal, which produces the autoantibody itself, with the concentration of the autoantibody being elevated preferably compared to the average of any antibodies which bind against such an endogenous molecule, preferably compared to a healthy person. In a most preferred embodiment, the autoantibody is an autoantibody against laminin beta-4.

The method according to the invention is preferably an *in vitro* method.

In a preferred embodiment, the detection of the complex for the prognosis, diagnosis, methods or assay kit according to the present invention comprises the use of a method selected from the group comprising immunodiffusion assays, immunoelectrophoresis assays, light-scattering immunoassays, agglutination assays, immunoassays with labels such as those from the group comprising radiolabeled immunoassays, enzyme immunoassays, preferably ELISA, chemiluminescence immunoassays and immunofluorescence assays, preferably indirect immunofluorescence assays. A person skilled in the art is familiar with such methods, and they are described in the prior art, for example in Zane, H. D. (2001), Immunology – Theoretical & Practical Concepts in Laboratory Medicine, W. B. Saunders Company, particularly in chapter 14.

Alternatively, a tissue sample, i.e., preferably a sample comprising tissue comprising the polypeptide according to the invention, can be used instead of a liquid sample, especially split skin, which can be prepared as described in the prior art (Zillikens, D., Kawahara, Y., Ishiko, A., Shimizu, H., Mayer, J., Rank, C. V., Liu, Z., Giudice, G. J., Tran, H. H., Marinkovich, M. P., Brocker, E. B., and Hashimoto, T. A novel subepidermal blistering disease with autoantibodies to a 200-kDa antigen of the basement membrane zone. J Invest Dermatol, 1996. 106(6): pp. 1333-8). The tissue sample preferably originates from a tissue comprising endogenous laminin beta-4, preferably expressing the polypeptide to an increased extent compared to the average tissue in the relevant organism, preferably the human body. Such a sample, which can then be present in the form of a tissue section fixed on a carrier, for example a slide for microscopic analyses, can then be contacted with the

antibody according to the invention, preferably autoantibody which binds against the polypeptide according to the invention. The antibody is preferably labeled in order to allow distinguishing of endogenous autoantibody which binds against the polypeptide according to the invention, and so it is possible to detect and optionally quantify newly formed complexes with autoantibodies from a subsequently contacted sample that compete with the first antibody. If the number of the newly formed complexes is higher than the number found with a sample from a healthy person, it is likely that the person from whom the investigated sample was obtained is suffering from the disease.

Any data showing the presence or absence of the complex comprising the antibody and the polypeptide according to the invention can be compared with reference data. For example, the detection of the complex indicates that the patient from whom the analyzed sample was obtained suffered from a disease, is suffering from it or is likely to suffer from the disease in the future. If a patient has been previously diagnosed and the method is carried out again to obtain diagnostically relevant information, the amount of the complex obtained in both passes can be correlated in order to find out the progression of the disease and/or the success of a treatment. If, for example, it is found that the amount of the complex has increased, this suggests that the disease is progressing, is likely to manifest itself in the future and/or that an attempted treatment is unsuccessful and vice versa. The first detection of the complex indicates that the patient from whom the sample originates is suffering from a high or increased risk of developing the disease in the future.

In a preferred embodiment, a microtiter plate, a membrane, a blot such as dot blot or line blot is used in order to carry out the diagnostic method according to the invention. A person skilled in the art is familiar with the experimental setup, and said setup is described in the prior art (Raoult, D., and Dasch, G. A. (1989), The line blot: an immunoassay for monoclonal and other antibodies. Its application to the serotyping of gram-negative bacteria. J. Immunol. Methods, 125 (1-2), 57-65; WO2013041540).

In a further preferred embodiment, what is envisaged for the prognosis, diagnosis, methods or kit as per the teaching according to the invention is the use of indirect immunofluorescence. A person skilled in the art is familiar with such techniques and the preparation of suitable samples, and these are described in the prior art (US4647543; Voigt, J., Krause, C., Rohwäder, E., Saschenbrecker, S., Hahn, M., Danckwardt, M., Feirer, C., Ens, K., Fechner, K., Barth, E., Martinetz, T., and Stöcker, W. (2012), Automated Indirect Immunofluorescence Evaluation of Antinuclear Autoantibodies on HEp-2 Cells, Clinical and Developmental Immunology, vol. 2012, doi:10.1155/2012/65105; Bonilla, E., Francis, L.,

Allam, F., *et al.*, Immuno-fluorescence microscopy is superior to fluorescent beads for detection of antinuclear antibody reactivity in systemic lupus erythematosus patients, *Clinical Immunology*, vol. 124, no. 1, pp. 18-21, 2007). Suitable reagents, devices and software packages are commercially available, for example from EUROIMMUN, Lübeck, Germany.

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As per the teaching according to the invention, what is provided is an antibody, preferably an autoantibody, which binds against the inventive polypeptide used for the diagnosis of a disease. A person skilled in the art in the field is familiar with methods for purifying antibodies, for example those described in Hermanson, G. T., Mallia, A. K., and Smith, P. K. (1992), *Immobilized Affinity Ligand Techniques*, San Diego: Academic Press. In brief, an antigen which binds specifically against the autoantibody of interest, the antigen being the polypeptide according to the invention, is immobilized and used in order to purify the autoantibody of interest from a suitable source via affinity chromatography. A liquid sample comprising antibodies from a patient suffering from a neurological disorder or disease which is associated with the occurrence of the autoantibody can be used as the source.

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According to the invention, what is provided is an antibody, for example an autoantibody, which is capable of binding specifically to the polypeptide according to the invention. In a preferred embodiment, the term "antibody", as used herein, means any immunoglobulin-based binding units, preferably a unit comprising at least one heavy chain and one light chain of an immunoglobulin, encompassing, if not limited to, monoclonal and polyclonal antibodies and also variants of an antibody, especially fragments, with the binding units being capable of binding against the corresponding antigen, more preferably of binding specifically thereto. In a preferred embodiment, the term "bind specifically", as used herein, means that the bond is stronger than a binding reaction characterized by a dissociation constant which is 1×10^{-5} M, more preferably 1×10^{-7} M, more preferably 1×10^{-8} M, more preferably 1×10^{-9} M, more preferably 1×10^{-10} M, more preferably 1×10^{-11} M, more preferably 1×10^{-12} M, as determined by surface plasmon resonance using Biacore equipment at 25°C in PBS buffer at pH 7. The antibody is preferably recombinant and/or isolated. The antibody detected or provided according to the invention preferably binds specifically to an epitope from the sequence SEQ ID NO 27 comprising one or more than one sequence from the group comprising the sequences SEQ ID NO 8, SEQ ID NO 9, SEQ ID NO 10, SEQ ID NO 11, SEQ ID NO 12, SEQ ID NO 13 and SEQ ID NO 14. The autoantibody is preferably an IgG antibody.

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The antibody can be part of an autoantibody preparation which is heterogeneous or can be a homogeneous autoantibody, a heterogeneous preparation comprising a multiplicity of

different autoantibody species, as can be obtained by preparation from the sera from human donors, for example by affinity chromatography using the immobilized antigen, for the purification of any autoantibody which is capable of binding to the antigen. The antibody can be glycosylated or nonglycosylated. A person skilled in the art in the field is familiar with methods which can be used for the identification, production and purification of antibodies and variants thereof, for example those described in EP 2 423 226 A2 and references therein. The antibody can be used by itself or in combination as diagnostic agent, for example in a complex with the polypeptide according to the invention.

The present invention provides a method for isolating an antibody, preferably an autoantibody, which binds against the polypeptide according to the invention, comprising the steps of a) contacting a sample comprising the antibody with the polypeptide according to the invention such that a complex is formed, b) isolating the complex which is formed in a), c) dissociating the complex which was isolated in step b), and d) removing the antibody from the polypeptide according to the invention. A sample from a patient suffering from anti-p200 pemphigoid can be used as the source of the autoantibody. Suitable methods are described in the prior art, for example in the handbooks "*Affinitätschromatografie*" [Affinity Chromatography], "Strategies for Protein Purification" and "Antibody Purification" (2009/2010), published by GE Healthcare Life Sciences, and in Philips, Terry, M., Analytical techniques in immunochemistry, 1992, Marcel Dekker, Inc.

The invention provides a pharmaceutical composition comprising the polypeptide according to the invention, the composition preferably being suitable for administration to a subject, preferably a mammalian subject, more preferably a human. Such a pharmaceutical composition can comprise a pharmaceutically acceptable carrier. The pharmaceutical composition can, for example, be administered orally, parenterally, via an inhalation spray, topically, via eye drops, rectally, nasally, buccally, vaginally or via an implanted reservoir, the term "parenterally", as used herein, encompassing subcutaneous, intracutaneous, intravenous, intramuscular, intraarticular, intrasynovial, intrasternal, intrathecal, intralesional and intracranial injection or infusion techniques. The pharmaceutical composition can be provided in suitable dosage forms, for example capsules, tablets and aqueous suspensions and solutions, preferably in sterile form. It can be used in a treatment method, the method comprising the administration of an effective amount of the polypeptide according to the invention to a subject.

Within the scope of protection of the present invention, what is provided is a medical or diagnostic device comprising, preferably coated with, a reagent for the detection of the

(auto)antibody according to the invention and/or the polypeptide according to the invention. Preferably, such a medical or diagnostic device comprises the polypeptide according to the invention in a form which allows the contacting of the polypeptide with an aqueous solution, more preferably the liquid human sample, in a simple manner. In particular, the polypeptide according to the invention can be immobilized on the surface of a carrier, preferably selected from the group comprising glass plates or glass slides, microscope slides, biochips, microtiter plates, beads, for example magnetic beads, apheresis devices, chromatography columns, membranes or the like. Exemplary medical devices encompass line blots, microtiter plates, glass slides for microscopy, beads, preferably magnetic beads, and biochips. In addition to the polypeptide according to the invention, the diagnostic device can comprise further polypeptides, for example positive or negative controls such as samples which contain or do not contain an antibody against the polypeptide of interest, or other known antigens which bind against autoantibodies of diagnostic value, especially with regard to other diseases which are associated with one or more than one identical or similar symptom.

Within the scope of protection of the present invention, what is also provided is a therapeutic device comprising, preferably coated with, laminin beta-4 or a variant thereof, the nature of which is such that the autoantibody against laminin beta-4 can be removed from the blood of a patient with minimal change to the blood composition and especially with removal of as few essential components as possible. This can be an apheresis device, preferably for plasmapheresis. Suitable reagents and methods are described in EP17001759.4 and in WO2007/085240, which are incorporated herein in their entirety by reference. In the prior art, it is already described that specific immunoabsorption for the treatment of bullous pemphigoid is promising (Mersmann *et al.*, Arch Dermatol Res 2015: "Immunoabsorber for specific apheresis of autoantibodies in the treatment of bullous pemphigoid"). Preferably, the device is sterilized in the sense that incoming and outgoing blood only comes into contact with sterilized components.

As per the teaching according to the invention, what is provided is a kit, preferably for the diagnosis of a disease. Such a kit can comprise instructions describing in detail how the kit is to be used and also the carrier according to the invention as means for the contacting of the polypeptide according to the invention with a body fluid sample from a subject, preferably a human subject. Furthermore, the kit can comprise a positive control, for example a batch of autoantibody or recombinant antibody that is known to bind against the peptide according to the present invention, or a diluted sample from a patient comprising the autoantibody. The kit can contain a negative control, for example a protein having no detectable affinity for the

polypeptide according to the invention, such as bovine serum albumin, or a sample from a healthy individual. Such a kit can comprise one or more than one standard solution of the antibody for the generation of a calibration curve, also referred to as calibrator, it being possible for the antibody to be a recombinant and/or monoclonal antibody or the diluted antibody from a sample from a patient, preferably against an epitope from the group comprising SEQ ID NO 8, SEQ ID NO 9, SEQ ID NO 10, SEQ ID NO 11, SEQ ID NO 12, SEQ ID NO 13 and SEQ ID NO 14. The kit can furthermore comprise a secondary antibody, preferably having a detectable label, which recognizes an autoantibody against laminin beta-4, preferably a secondary antibody which recognizes IgG antibodies.

In a preferred embodiment, the kit comprises a means for the detection of an autoantibody which binds against the polypeptide according to the invention, preferably by detection of a complex comprising the polypeptide according to the invention and an antibody which binds against the polypeptide according to the invention. Such a means is preferably an agent which binds against the complex and modifies the complex or bears a label which makes the complex detectable. For example, such a means can be a labeled antibody which binds against the polypeptide at a binding site differing from the binding site to which the primary autoantibody binds or which binds against a constant region of the primary antibody. Alternatively, the agent can be a secondary antibody against laminin beta-4 that binds against the constant region of the autoantibody, preferably a secondary antibody which is specific for mammalian antibodies of the IgG class. A multiplicity of methods and means for the detection of such a complex is described in the prior art, for example in Philips, Terry, M., *Analytical techniques in immunochemistry*, 1992, Marcel Dekker, Inc.

The polypeptide comprising laminin beta-4 or a variant thereof can be produced or provided in the form of a cell which comprises and/or expresses a nucleic acid encoding the polypeptide. If a nucleic acid comprising a sequence encoding the polypeptide according to the invention or a variant thereof is used, such a nucleic acid can be an unmodified nucleic acid. In a preferred embodiment, the nucleic acid is a nucleic acid which does not occur as such in nature and comprises, compared with a natural nucleic acid, at least one modification, for example contains an isotope or a chemical modification, for example a methylation, a sequence modification, a label or the like, which indicates the synthetic origin. In a preferred embodiment, the nucleic acid is a recombinant nucleic acid or, in a more preferred embodiment, part of a vector in which it is functionally connected to a promoter allowing the expression, preferably overexpression, of the nucleic acid. A person skilled in the art is familiar with a multiplicity of suitable vectors which are commercially available, for example from *Origene*. For example, a vector encoding fusion constructs having a

C-terminal GFP can be used. The vector can comprise a bacterial origin of replication and a promoter for expression in eukaryotic, preferably mammalian, cells, for example an SV40 promoter or chicken beta-actin promoter. The cell can be a eukaryotic or prokaryotic cell, preferably a eukaryotic cell, such as, for example, a yeast cell, and is more preferably a mammalian cell, even more preferably a human cell such as a HEK293 cell. Examples of a mammalian cell encompass HEK293, CHO or COS-7 cells. The cell comprising the nucleic acid encoding the polypeptide according to the invention can be a recombinant cell or an isolated cell, the term "isolated" meaning that the cell is enriched such that the environment thereof contains, in comparison with the wild-type of the cell or the environment thereof, fewer cells of different differentiation or species or in fact no other such cells. The cell or the vector can be used for the expression of the polypeptide, followed by the use thereof for the production of a composition or of a kit for the diagnosis of an autoimmune disease. The cell can be a live cell or a fixed cell which is no longer metabolically active. Fixation of cells is possible by means of treatment with acetone or formalin, as described in Probst *et al.* (Probst, C. Saschenbrecker, S., Stoecker, W., and Komorowski, L. (2014) Anti-neuronal autoantibodies: Current diagnostic challenges, Multiple Sclerosis and Related Disorders 3, 303-320).

The teaching according to the invention can be used not only for a diagnosis, but also for the prevention or for the treatment of a disease, more precisely for a method for preventing or treating a disease, comprising the steps of a) reducing the concentration of autoantibodies which bind against the polypeptide according to the invention in the blood of a subject and/or b) administering one or more than one immunosuppressive pharmaceutical substance, preferably from the group comprising rituximab, prednisone, methylprednisolone, cyclophosphamide, mycophenolate mofetil, intravenous immunoglobulins, FK506, cyclosporin, methotrexate, clobetasol propionate, dapsone, tetracycline, nicotinamide, colchicine and azathioprine.

In a preferred embodiment, the present invention provides for use of a means or reagent for the detection of an antibody against laminin beta-4 or of a nucleic acid comprising a sequence encoding laminin beta-4 or a variant thereof or of a vector comprising said sequence or of a cell comprising the vector or said nucleic acid for the production of a kit for the diagnosis of an autoimmune disease.

In a preferred embodiment, any method or any use according to the present invention can be intended for a nondiagnostic purpose, especially determination of the presence of an autoantibody against laminin beta-4 for some use other than diagnosing a patient. For

example, the method or the use can be intended for the determination by *in vitro* testing of the efficiency of a medical device which is configured to remove an autoantibody from the blood of a patient, the testing being carried out with a liquid which is not patient blood. In a preferred embodiment, it is possible to use any method and any use according to the present invention with the intention of generating an autoantibody profile, preferably for the detection of a disease in a mammal, preferably a human. In a preferred embodiment, it is possible to use a method or use for the detection of disease-associated markers in a sample from patients suffering from a skin disease.

In a preferred embodiment, the method according to the present invention is a method for calibrating a diagnostic assay system or for confirming the reliability and/or sufficient capacity of such an assay system or of a therapeutic system for the removal of autoantibodies from the blood of a patient. In the case of a diagnostic assay system, autoantibodies are not detected in the sample from a patient who is to be diagnosed, but in an artificial solution having a known composition, especially having a defined, known concentration of the antibody or of a recombinant antibody of known concentration that binds against the autoantibody. The diagnostic system can be any system which allows the detection of autoantibodies in a sample, for example a medical diagnostic device according to the present invention.

In the case of a therapeutic system, for example an apparatus for apheresis, the method can be used for developing such a system and for testing its reliability and/or efficiency and/or capacity. For example, it is possible after an apheresis run or therebefore for a solution comprising a known concentration of an antibody which binds against the polypeptide according to the invention to be contacted with the system, and the method according to the present invention can be used for confirming that the system is capable of removing the antibody from the solution.

In a preferred embodiment, the invention provides an apparatus for the analysis of a sample from a patient in order to detect one or more than one autoantibody against laminin beta-4, the autoantibody or autoantibodies indicating an increased probability of the patient suffering from an autoimmune disease, the apparatus comprising the following:

- a. a carrier which comprises a means for the capture of at least one autoantibody in the sample when the sample is contacted with the carrier,

- b. a detectable means which is capable of binding to the captured antibody when the detectable means is contacted with the carrier, the detectable means preferably being a labeled secondary antibody which is capable of binding to the antibody captured on the carrier,

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- c. optionally a means for the removal of a sample from the carrier and for the removal of the detectable means, preferably by washing;

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- d. a detector for the detection of the presence of the detectable means and for the conversion of the results into an electrical signal, and

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- e. optionally a means for the receiving of the electrical signal from the detector and determination of whether the intensity of the signal indicates an increased probability of an autoimmune disease, by comparison of the intensity of the signal with the intensity of a background signal or with an inputted reference value obtained using samples from healthy persons.

The present application comprises a series of sequences of novel nucleic acids or polypeptides:

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SEQ ID NO 1: Primer sense LAMB4:

ATAGGTCTCACATGCAATTTCAACTGACCCTTTTTTTGCACCTTG

SEQ ID NO 2: Primer anti-sense LAMB4:

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ATAGGTCTCGTCGAGGCTATAGCACCTAGCATATTTTTTTTCTTG

SEQ ID NO 3: Primer anti-sense LAMB4-Stop:

ATAGGTCTCCTCGAGCTAGCTATAGCACCTAGCATATTTTTTTTCTTG

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SEQ ID NO 4_pTriEx-1 (standard vector)

GGGGAATTGTGAGCGGATAACAATTCCCCGGAGTTAATCCGGGACCTTTAATTCAACCC
AACACAATATATTATAGTTAAATAAGAATTATTATCAAATCATTTGTATATTAATTAATA
CTATACTGTAAATTACATTTTATTTACAATCAAAGGAGATATACCATGGCGATATCCCGG
GAGCTCGTGATCCGAATTCTCAGATCTCGGCGCGCCTGCAGGTCGACGGTACCGGTT
CGAAGCTTGCGGCCGCACAGCTGTATACACGTGCAAGCCAGCCAGAACTCGCCCCGG
AAGACCCCGAGGATCTCGAGCACCACCATCACCATCACTAAGTGATTAACCTC

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AGGTGCAGGCTGCCTATCAGAAGGTGGTGGCTGGTGTGGCCAATGCCCTGGCTCACA
 AATACCACTGAGATCGATCTTTTTCCCTCTGCCAAAAATTATGGGGACATCATGAAGCC
 CCTTGAGCATCTGACTTCTGGCTAATAAAGGAAATTTATTTTCATTGCAATAGTGTGTTG
 GAATTTTTTGTGTCTCTCACTCGGAAGGACATATGGGAGGGCAAATCATTTAAAACATC
 5 AGAATGAGTATTTGGTTTtagagTTTGGCAACATATGCCCATATGTAAGTAgCATAACCCC
 TTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTGCTGAAAGCATGCGGAGGAAATTC
 TCCTTGAAGTTTCCCTGGTGTTCAAAGTAAAGGAGTTTGCACCAGACGCACCTCTGTTC
 ACTGGTCCGGCGTATTA AAAACACGATACATTGTTATTAGTACATTTATTAAGCGCTAGAT
 TCTGTGCGTTGTTGATTTACAGACAATTGTTGTACGTATTTTAATAATTCATTAATTTAT
 10 AATCTTTAGGGTGGTATGTTAGAGCGAAAATCAAATGATTTTCAGCGTCTTTATATCTGA
 ATTTAAATATTAATCCTCAATAGATTTGTAAAATAGGTTTCGATTAGTTTCAAACAAGGG
 TTGTTTTTCCGAACCGATGGCTGGACTATCTAATGGATTTTCGCTCAACGCCACAAAAC
 TGCCAAATCTTGTAGCAGCAATCTAGCTTTGTGCGATATTCGTTTGTGTTTTGTTTTGTAA
 TAAAGGTTGACGTCGTTCAAATATTATGCGCTTTTGTATTTCTTTCATCACTGTCGTT
 15 AGTGTACAATTGACTCGACGTAAACACGTTAAATAGAGCTTGGACATATTTAACATCGG
 GCGTGTTAGCTTTATTAGGCCGATTATCGTCGTCGTCCCAACCCTCGTCGTTAGAAGTT
 GCTTCCGAAGACGATTTTGCCATAGCCACACGACGCCTATTAATTGTGTGCGGCTAACAC
 GTCCGCGATCAAATTTGTAGTTGAGCTTTTTGGAATTATTTCTGATTGCGGGCGTTTTTG
 GGCGGGTTTCAATCTAACTGTGCCCCGATTTTAATTCAGACAACACGTTAGAAAGCGATG
 20 GTGCAGGCGGTGGTAACATTTcagacGGCAAATCTACTAATGGCGGCGGTGGTGGAG
 CTGATGATAAATCTACCATCGGTGGAGGCGCAGGCGGGGCTGGCGGCGGAGGCGGA
 GGCGGAGGTGGTGGCGGTGATGCAGACGGCGGTTTAGGCTCAAATGTCTCTTTAGGC
 AACACAGTCGGCACCTCAACTATTGTACTGGTTTCGGGCGCCGTTTTTGGTTTGACCGG
 TCTGAGACGAGTGCGATTTTTTTTCGTTTCTAATAGCTTCCAACAATTGTTGTCTGTGCTC
 25 TAAAGGTGCAGCGGGTTGAGGTTCCGTCGGCATTGGTGGAGCGGGCGGCAATTCAGA
 CATCGATGGTGGTGGTGGTGGTGGAGGCGCTGGAATGTTAGGCACGGGAGAAGGTGG
 TGGCGGCGGTGCCGCCGGTATAATTTGTTCTGGTTTtagTTTGTTCGCGCACGATTGTG
 GGCACCGGCGCAGGCGCCGCTGGCTGCACAACGGAAGGTCGTCTGCTTCGAGGCAG
 CGCTTGGGGTGGTGGCAATTCAATATTATAATTGGAATACAAATCGTAAAAATCTGCTAT
 30 AAGCATTGTAATTTcGCTATCGTTTACCGTGCCGATATTTAACAACCGCTCAATGTAAGC
 AATTGTATTGTAAAGAGATTGTCTCAAGCTCGGAACGCTGCGCTCGGTCTGTTCCGGCTG
 CGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGG
 ATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAA
 GGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAT
 35 CGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTC
 CCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCT
 GTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCAATGCTCACGCTGTAGGTAT

CTCAGTTCGGTGTAGGTCGTTGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTC
 AGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACA
 CGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTA
 GGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAG
 5 TATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCT
 TGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTGCAAGCAGCAGAT
 TACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGTTACCAATGCTTAATCAGTGAG
 GCACCTATCTCAGCGATCTGTCTATTTGTTTCATCCATAGTTGCCTGACTCCCCGTCGT
 GTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCG
 10 CGAGACCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGG
 CCGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGC
 CGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGC
 TACAGGCATCGTGGTGTACGCTCGTCGTTTGGTATGGCTTCATTCAGCTCCGGTTCC
 CAACGATCAAGGCGAGTTACATGATCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTT
 15 CGGTCCTCCGATCGTTGTCAGAAGTAAGTTGGCCGCGAGTGTTATCACTCATGGTTATGG
 CAGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGT
 GAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCC
 GGCGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAGTGCTCATCATTG
 GAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTTCG
 20 ATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTACTTTACCAGCGTTTCT
 GGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGG
 AAATGTTGAATACTCATACTCTTCCTTTTTCAATATTATTGAAGCATTATCAGGGTTATT
 GTCTCATGTCCGCGCGTTTTCTGTCATCTTTAATCAAATCCCAAGATGTGTATAAACCAC
 CAAACTGCCAAAAAATGAAAAGTGTGACAAGCTCTGTCCGTTTGCTGGCAACTGCAAG
 25 GGTCTCAATCCTATTTGTAATTATTGAATAATAAAACAATTATAAATGTCAAATTTGTTTTT
 TATTAACGATACAAACCAACGCAACAAGAACATTTGTAGTATTATCTATAATTGAAAAC
 GCGTAGTTATAATCGCTGAGGTAATATTTAAATCATTTTCAAATGATTCACAGTTAATTT
 GCGACAATATAATTTTATTTTACATAAACTAGACGCCTTGTCGTCTTCTTCTTCGTATTC
 CTTCTCTTTTTCATTTTTCTCTTCATAAAAATTAACATAGTTATTATCGTATCCATATATGT
 30 ATCTATCGTATAGAGTAAATTTTTGTTGTCATAAATATATATGTCTTTTTTAATGGGGTG
 TATAGTACCGCTGCGCATAGTTTTTCTGTAATTTACAACAGTGCTATTTTCTGGTAGTTC
 TTCGGAGTGTGTTGCTTTAATTATTAAATTTATATAATCAATGAATTTGGGATCGTCGGT
 TTTGTACAATATGTTGCCGGCATAGTACGCAGCTTCTTCTAGTTCAATTACACCATTTTT
 TAGCAGCACCGGATTAACATAACTTTCCAAAATGTTGTACGAACCGTTAAACAAAAACAG
 35 TTCACCTCCCTTTTCTATACTATTGTCTGCGAGCAGTTGTTTGTGTTAAAAATAACAGC
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SEQ ID NO 5_pTriEx-1-LAMB4(IF1)[human] – encodes – human laminin beta-4 without His-tag

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SEQ ID NO 6_pTriEx-1-LAMB4(IF1)[human](dHis) – encodes – human laminin beta-4 with His-tag

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 35 ACGAATGCCCTCCAAAAAGCCCAGGAAGCAAAATCCATTATTCGTAATTTGGACAAACA
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 5 TGAAGGCCAAAGCAGCTGAGAAAGCAGCAAATATTCTATTAAATCTTGACAAAACATTGA
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 10 CTGAATCTGCCCAACACCAGGCTGGGAGTCTTGAGAAGGAATTTGTTGAGCTGAAAAAA
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 15 GAACAAGAAAAAAAATATGCTAGGTGCTATAGCTAGCTCGAGCACCACCATCACCATCA
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 15 CCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTC
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 30 GCAAAAAAGCGGTTAGCTCCTTCGGTCCTCCGATCGTTGTCAGAAGTAAGTTGGCCGC
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 5 GTAGTATTATCTATAATTGAAAACGCGTAGTTATAATCGCTGAGGTAATATTTAAAATCAT
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 10 AACAGTGCTATTTTCTGGTAGTTCTTCGGAGTGTGTTGCTTTAATTATTAAATTTATATAA
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 15 GAAATGTCTATCAATATATAGTTGCTCTAGTTATTAATAGTAATCAATTACGGGGTCATTA
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 CGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGACTATTTACGGTAACTGCCAC
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 20 TAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGC
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 TTAATTATTTTGTGCAGCGATGGGGGCGGGGGGGGGGGGGGGGGGGCGCGCGCCAGGCG
 GGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTGCGGCGGCAG
 25 CCAATCAGAGCGGCGCGCTCCGAAAGTTTCCTTTTATGGCGAGGCGGCGGCGGCGGC
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SEQ ID NO7:>LAMB4-His – human laminin beta-4 with His-tag

MQFQLTLFLHLGWLSYSKAQDDCNRGACHPTTGDLLVGRNTQLMASSTCGLSRAQKYCIL
 30 SYLEGEQKCFICDSRFPYDPYDQPNSTIENVIVSFEPDREKKWWQSENGLDHVSIRLDLEA
 LFRFSLILTFKTRPAAMLVERSTDYGHNWKVFKYFAKDCATSFPNITSGQAQGVGDIVCD
 SKYSDIEPSTGGEVVLKVLDPSTFEIENPYSPIQDLVTLNLRINF TKLHTLGDALLGRRQND
 LDKYYYALYEMIVRGSCFCNGHASECRPMQKMRGDVFSPPGMVHGQCVCQHNTDGPNC
 RCKDFFQDAPWRPAADLQDNACRSCSCNSHSSRCHFDMTTYLASGGLSGGVCEDCQHNT
 35 EGQHCDRCRPLFYRDPLKTISDPYACIPCECDPDGTISGGICVSHSDPALGSVAGQCLCKEN
 VEGAKCDQCKPNHYGLSATDPLGCQPCDCNPLGSLPFLTCDVDTGQCLCLSYVTGAHCEE
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LNFYLYEAAEATTQGLAPLGSETFGQSPAVHVVLGEPVPGNPVTWTGPGFARVLPAGL
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 LLPTPICLEPDVQYSIDVYFSQPLQGESHASHVLDLGLIPQINSLENFCQDLDEYQLH
 NCVEIASAMGPQVLPGACERLIISMSAKLHDGAVACKCHPQGSVGSSECSRLGGQCQCKPLV
 5 VGRCCDRCTGSYDLGHHGCHPCHPQGSKDTVCDQVTGQCPCHGEVSGRRCDRCLA
 GYFGFPSCHPCPCNRFAELCDPETGSCFNCGGFTTGRNCERCIDGYGNPSSGQPCRPC
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 CNNNIDVTDPESCSRVTGECLRCLHNTQGANCLCKPGHYGSALNQTCRRCSCHASGVSP
 MECPGGGACLCDPVTGACPCLPNVTGLACDRCADGYWNLPGRGCQSCDCDPRTSQS
 10 SHCDQLTGQCPCKLGYGGKRCSECQENYYGDPGRCIPCDNRAQTQKPCDPDTGMCR
 CREGVSGQRCDRCARGHSQEFPTCLQCHLCFDQWDHTISSLSKAVQGLMRLAANMEDKR
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 SANTRNDLLTILDTLTSKGNLSLERLKQIKIPDIQILNEKVCGDPGNVPCVPLPCGGALCTGRK
 15 GHRKCRGPGCHGSLTLSTNALQKAQEAksiRNLDKQVRGLKNQIESISEQAEVSKNNALQL
 REKLGNIERNQSDSEENINLFIKKVKNFLLEENVPPEDIEKVANGVLDIHLPIPSQNLDELVKI
 QKHMQLCEDYRTDENRLNEEADGAQKLLVKAKAAEKAANILLNLDKTLNQLQQAQITQGRA
 NSTITQLTANITKIKKNVLQAENQTREMKSELELAKQRSGLEDGLSLLQTKLQRHQDHAVNA
 KVQAESAQHQAGSLEKEFVELKKQYAILQRKTSTTGLTKETLGKVKQLKDAAEKLADTEAK
 20 IRRITDLERKIQDLNLSRQAKADQLRILEDQVVAIKNEIVEQEKKYARCYSLEHHHHHHHH

SEQ ID NO 8: Autoantibody epitope 1

EFQDL

25 **SEQ ID NO 9: Autoantibody epitope 2**

ADLLLEDLQE

SEQ ID NO 10: Autoantibody epitope 3

ADLLLEDLQEEIDLQS

30

SEQ ID NO 11: Autoantibody epitope 4

DLLTILDTLTSK

SEQ ID NO 12: Autoantibody epitope 5

35 QIKIPDIQILNEK

SEQ ID NO 13: Autoantibody epitope 6

VRGLKNQIESISE

SEQ ID NO 14: Autoantibody epitope 7

5 LLEENVPPEDI

SEQ ID NO 15: Subfragment 1

MQDDCNRGACHPTTGDLLVGRNTQLMASSTCGLSRAQKYCILSYLEGEQKCFICDSRFPY
DPYDQPNSTIENVIVSFEPDREKK**WW**QSENGLDHVSIRLDLEALFRFSHLILTFKTFRPAAM
10 LVERSTDYGHN**W**KVFKYFAKDCATSFPNITSGQAQGVGDIVCDISKYSIEPSTGGEVVLKV
LDPSFEIENPYSPYIQDLVTLTNLRINF TKLHTLGDALLGRRQNDSLDKYYYYALYEMIVRGSCF
CNGHASECRPMQKMRGDVFSPPGMVHGQCVCQHNTDGPNCERCKDFFQDAP**W**

SEQ ID NO 16: Subfragment 2

15 MPNCERCKDFFQDAP**W**RPAADLQDNACRSCSCNSHSSRCHFDMTTYLASGGLSGGVCE
CQHNTGQHCDCRPLFYRDPLKTISDPYACIPCECDPDGTISGGICVSHSDPALGSVAGQ
CLCKENVEGAKCDQCKPNHYGLSATDPLGCQPCDCNPLGSLPFLTCDVDTGQCLCLSYVT
GAHCEECTVGY**W**GLGNHLHGCSPCDIDIGGAYSNVCSPKNGQCECRPHVTGRSCSEPAP
GYFFAPLNFYLYEAEATT LQGLAPLGSETFGQSPAVHVVLGEPVPGNPVT**WT**GPGFARVL
20 **W**

SEQ ID NO 17: Subfragment 3

MGNPVT**WT**GPGFARVLPGAGLRFAVNNIPFPVDFTIAIH YETQSAAD**W**TVQIVVNPPGGSE
HCIPKTLQSKPQS FALPAATRIMLLPTPICLEPDVQYSIDVYFSQPLQGESHASHVLVDSL
25 LIPQINSLENFCSKQDLDEYQLHNCVEIASAMGPQVLPGACERLIISMSAKLHDGAVACKCHP
QGSVGSSCSRLGGQCQCKPLVVGRCDCRSTGSYDLGHHGCHPCHHPQGSKDTVCDQ
VTGQCPCHGEVSGRRCDRCLAGYFGFPSCHPCPCNRFAELCDPETGSCFNCGGFTTGRN
CERCIDGYYGN**WGW**

SEQ ID NO 18: Subfragment 4

30 MTGRNCERCIDGYYGNPSSGQPCRPCLPDDPSSNQYFAHSCYQNL**W**SSDVICNCLQGY
TGTQCGECSTGFYGNPRISGAPCQPCACNNNIDVTDPECSRVTGECLRCLHNTQGANCO
LCKPGHYGSALNQT CRRCSCHASGVSPMECPPGGGACLCDPVTGACPCLPNVTGLACDR
CADGY**W**NLVPGRGCQSCDCDPRTSQSSHCDQLTGQCPCKLGYGGKRCSECQENYYGDP
35 PGRICIPCDCNRAFTQKPICDPDTGMCRCREGVSGQRCDRCARGHSQE**WGW**

SEQ ID NO 19: Subfragment 5

MSGQRCDRCARGHSQEFPTCLQCHLCFDQWDHTISSLSKAVQGLMRLAANMEDKRETL
VCEADFKDLRGNVSEIERILKHPVFPSPGKFLVKVDYHDSVRRQIMQLNEQLKAVYEFQDLKD
TIERAKNEADLLLEDLQEEIDLQSSVLNASIADSSSENIKKYYHISSSAEKKINETSSSTINTSANT
5 RNDLLTILDTLTSKGNLSLERLKQIKIPDIQILNEKVCGBPNCVPLPCGGALCTGRKGHR
KCRGPGCHGSLTLSTNALQKAQEAISIIRNLDKQVRGLKNQIESISEQAEVSKNNALQLREK
LGNIWGWGW

SEQ ID NO 20: Subfragment 6

10 MSKNNALQLREKLGNIIRNQSDSEENINLFIKKVKNFLLEENVPPEDIEKVANGVLDIHLPIPS
QNLTDELVKIQKHMQLCEDYRTDENRLNEEADGAQKLLVKAKAAEKAANILLNLDKTLNQLQ
QAQITQGRANSTITQLTANITKIKKNVLQAENQTREMKSELELAKQRSGLDGLSLLQTKLQR
HQDHAVNAKVQAESAQHQAGSLEKEFVELKKQYAILQRKTSTTGLTKETLGKVKQLKDAE
KLAGDTEAKIRRIDLERKIQDLNLSRQAKADQLRILEDQVVAIKNEIVEQEKKYARCYSWGW
15 GWGW

SEQ ID NO 21: Subfragment 1, with four tryptophans and C-terminal His-tag (AMB4-TF1-4W-His)

MQDDCNRGACHPTTGDLLVGRNTQLMASSTCGLSRAQKYCILSYLEGEQKCFICDSRFPY
20 DPYDQPNSTIENVIVSFEPDREKKWWQSENGLDHVSIRLDLEALFRFSHLITFKTFRPAAM
LVERSTDYGHNWKVFKYFAKDCATSFNITSGQAQGVGDIVCDSKYSDIEPSTGGEVVLKV
LDPSFEIENPYSPYIQDLVTLTNLRINF TKLHTLGDALLGRRQNDSLDKYYYALYEMIVRGSCF
CNGHASECRPMQKMRGDVFSPPGMVHGQCVCQHNTDGPNCERCKDFFQDAPWLEHHH
HHH

SEQ ID NO 22: Subfragment 2, with four tryptophans and C-terminal His-tag (LAMB4-TF2-4W-His)

MPNCERCKDFFQDAPWRPAADLQDNACRSCSCNSHSSRCHFDMTTYLASGGLSGGVCE
CQHNTGQHCDCRPLFYRDPLKTISDPYACIPCECDPDGTISGGICVSHSDPALGSVAGQ
30 CLCKENVEGAKCDQCKPNHYGLSATDPLGCQPCDCNPLGSLPFLTCDVDTGQCLCLSYVT
GAHCEEECTVGYWGLGNHLHGCSPDCDIDGAYSNVCSKNGQCECRPHVTGRSCSEPAP
GYFFAPLNFYLYEAEATTQGLAPLGSETFGQSPAVHVVLGEPVPGNPVTWTGPGFARVL
WLEHHHHHHH

SEQ ID NO 23: Subfragment 3, with four tryptophans and C-terminal His-tag (LAMB4-TF3-4W-His)

MGNPVTWTGPGFARVLPGAGLRFVNNIPFPVDFTIAIHYETQSAADWTVQIVVNPPGGSE
HCIPKTLQSKPQS FALPAATRIMLLPTICLEPDVQYSIDVYFSQPLQGESHASHVLVDSL
LIPQINSLENFCSKQDLDEYQLHNCVEIASAMGPQVLPGACERLIISMSAKLHDGAVACKCHP
QGSVGS SCSRLGGQCQCKPLVVGRCCDR CSTGSYDLGHHGCHPCHHPQGSKDTVCDQ
5 VTGQCPCHGEVSGRRCDRCLAGYFGFPSCHPCPCNRFAELCDPETGSCFNCGGFTTGRN
CERCIDGYYGNWGWLEHHHHHH

**SEQ ID NO 24: Subfragment 4, with four tryptophans and C-terminal His-tag
(LAMB4-TF4-4W-His)**

10 MTGRNCERCIDGYYGNPSSGQPCRPCLPDDPSSNQYFAHSCYQNLWSSDVICNCLQGY
TGTQCGECSTGFYGNPRISGAPCQPCACNNNIDVTDPESCSRVTGECLRLHNTQGANCQ
LCKPGHYGSALNQTCRRCSCHASGVSPMECPPGGGACLCDPVTGACPCLPNVTGLACDR
CADGYWNLVPGRGCQSCDCDPRTSQSSHCDQLTGQCPCKLGYGGKRCSECQENYYGDP
PGRICIPCDCNRAGTQKPICDPDTGMCRCREGVSGQRCDRCARGHSQEWGWLEHHHHHH

**SEQ ID NO 25: Subfragment 5, with four tryptophans and C-terminal His-tag
(LAMB4-TF5-4W-His)**

15 MSGQRCDRCARGHSQEFPTCLQCHLCFDQWDHTISSLSKAVQGLMRLAANMEDKRETL
VCEADFKDLRGNVSEIERILKHPVFPSPGKFLVKDYHDSVRRQIMQLNEQLKAVYEFQDLKD
20 TIERAKNEADLLLEDLQEEIDLQSSVLNASIADSSSENIKKYYHISSSAEKKINETSSTINTSANT
RNDLLTILDTLTSKGNLSLERLKQIKIPDIQILNEKVC GDPGNVPCVPLPCGGALCTGRKGHR
KCRGPGCHGSLTLSTNALQKAQEAksiIRNLDKQVRGLKNQIESISEQAEVSKNNALQLREK
LGNIWGWGWLEHHHHHH

**SEQ ID NO 26: Subfragment 6, with four tryptophans and C-terminal His-tag
(LAMB4-TF6-4W-His)**

25 MSKNNALQLREKLGNI RNQSDSEENINLFIKKVKNFLLEENVPPEDIEKVANGVLDIHLPIPS
QNLTDELVKIQKHMQLCEDYRTDENRLNEEADGAQKLLVKAKAAEKAANILLNLDKTLNQLQ
QAQITQGRANSTITQLTANITKIKKNVLQAENQTREM KSELELAKQRSGLEDGLSLLQTKLQR
30 HQDHAVNAKVQAESAQHQAQSLEKEFVELKKQYAILQRKTSTTGLTKETLGKVKQLKDAAE
KLAGDTEAKIRRIDLERKIQDLNLSRQAKADQLRILEDQVVAIKNEIVEQEKKYARCYSWGW
GWGWLEHHHHHH

SEQ ID NO 27: Laminin beta-4

35 MQFQLTLFLHLGWLSYSKAQDDCNRGACHPTTGDLLVGRNTQLMASSTCGLSRAQKYCIL
SYLEGEQKCFICDSRFPYDPYDQPN SHTIENVIVSFEPDREKKWWQSENGLDHVSIRLDLEA

LFRFSHLILTFKTRPAAMLVERSTDYGHNWKVFKYFAKDCATSPFNITSGQAQGVGDIVCD
 SKYSDIEPSTGGEVVLKVLDPSEFIENPYSPYIQDLVTLTNLRINF TKLHTLGDALLGRRQNDS
 LDKYYYALYEMIVRGSCFCNGHASECRPMQKMRGDVFSPPGMVHGQCVCQHNTDGPNC
 RCKDFFQDAPWRPAADLQDNACRSCSCNSHSSRCHFDMTTYLASGGLSGGVCEDCQHNT
 5 EGQHCDRCRPLFYRDPLKTISDPYACIPCECDPDGTISGGICVSHSDPALGVSAGQCLCKEN
 VEGAKCDQCKPNHYGLSATDPLGCQPCDCNPLGSLPFLTCDVDTGQCLCLS YVTGAHCEE
 CTVGYWGLGNHLHGCSPCDCDIGGAYSNVCSPKNGQCECRPHVTGRSCSEPAPGYFFAP
 LNFYLYEAAEATTLQGLAPLGSETFGQSPAVHVVLGEPVPGNPVTWTGPGFARVLPAGL
 RFAVNNIPFPVDFTIAIH YETQSAADWTVQIVVNPPGGSEHCIPKTLQSKPQS FALPAATRIM
 10 LLPTPICLEPDVQYSIDVYFSQPLQGESHASHVLDLGLIPQINSLENFC SKQDLDEYQLH
 NCVEIASAMGPQVLPGACERLIISMSAKLHDGAVACKCHPQGSVGS SCSRLGGQCCKPLV
 VGRCCDR CSTGSYDLGHHGCHPCHHPQGSKDTVCDQVTGQCPCHGEVSGRRCDRCLA
 GYFGFPSCHPCPCNRFAELCDPETGSCFNCGGFTTGRNCERCIDGY YGNPSSGQPCRPC
 LCPDDPSSNQYFAHSCYQNLWSSDVICNCLQGYTGTQC GECSTGFYGNPRISGAPCQPCA
 15 CNNNIDVTDPESCSRVTGECLRCLHNTQGANCQLCKPGHYGSALNQT CRRCSCHASGVSP
 MECPGGGACLCDPVTGACPCLPNVTGLACDRCADGYWNLVPGRGCQSCDCDPRTS QS
 SHCDQLTGQCPCKLGYGGKRCSECQENYYGDPPGRCIPDCN RAGTQKPIDPDTGMCR
 CREGVSGQRCDRCARGHSQEFPTCLQCHLCFDQWDHTISSLSKAVQGLMRLAANMEDKR
 ETLPVCEADFKDLRGNVSEIERILKHPVFPSGKFLKV KDYHDSVRRQIMQLNEQLKAVYEFQ
 20 DLKDTIERAKNEADLLLEDLQEEIDLQSSVLNASIADSS ENIKKYHISSSAEKKINETSSTINT
 SANTRNDLLTILDTLTSKGNLSLERLKQIKIPDIQILNEKVC GDPGNVPCVPLPCGGALCTGRK
 GHRKCRGPGCHGSLTLSTNALQKAQEAksiRNLDKQVRGLKNQIESISEQA EVSKNNALQL
 REKLGNI RNQSDSEENINLFIKKVKNFLLEENVPPEDIEKVANGVLDIHLPIPSQNLTD ELVKI
 QKHMQLCEDYRTDENRLNEEADGAQKLLVKAKAAEKAANILLNLDKTLNQLQQAQITQGRA
 25 NSTITQLTANITKIKKNVLQAENQTREMKSELELAKQRSGLEDGLSLLQTKLQRHQDHAVNA
 KVQAESAQHQAGSLEKEFVELKKQYAILQRKTSTTGLTKETLGKV KQLKDAAEKL AGDTEAK
 IRRITDLERKIQDLNLSRQAKADQLRILEDQVVAIKNEIVEQEKKYARCYS

SEQ ID NO 28: SEQ ID NO 8 from EP3260864

30 MDCTFKPDFEMTVKECQHSGELSSRNTGHLHPTPRSPLLRWTQEPQPLEEKWQHRVVEQI
 PKEVQFQPPGAPLEKEKSQQCYSEYFSQTSTELQITFDETNPITRLSEIEKIRDQALNNSRPP
 VRYQDNACEMELVKVLTPL EIAKNKQYDMHTEVTTLKQEKNPVPSAE EWMLEGCRASGGL
 KKGDFLKKGLEPETFQNF DGDHACSVRDDEFKFQGLRHTVTARQLVEAKLLDMRTIEQLRL
 GLKTVEEVQKTLNKFLT KATSIAGLYLESTKEKISFASAAERIIIDKMVALAFLEAQAATGFIIDP
 35 ISGQTYSVEDAVLKGVDPEFRIRLLEAEKAAVGYSYSSKTL SVFQAMENRMLDRQKGKHL
 EAQIASGGVIDPVRGIRVPPEIALQQGLLNAILQFLHEPSSNTRVFPNPNNKQALYYSELLR
 MCVFDVESQCFLFPFGERNISNLNVK KTHRISVVDTKTGSELTVYEA FQRNLIKSIYLELSG

QQYQWKEAMFFESYGHSSHMLTDTKTGLHFNINEAIEQGTIDKALVKKYQEGLITLTELADS
LLSRLVPKKDLHSPVAGYWLTASGERISVLKASRRNLVDRITALRCLEAQVSTGGIIDPLTGK
KYRVAEALHRGLVDEGFAQQLRQCELVITGIGHPITNKMMSVVEAVNANIINKEMGIRCLEFQ
YLTGGLIEPQVHSRLSIEEALQVGIIIDVLIATKLKDQKSYVRNIICPQTKRKLTKEALEKADFD
5 FHTGLKLLEVSEPLMTGISSLYYSSLLE

SEQ ID NO 29: Gliadin peptide

LGQQQPFPFPQQPYQPQPFPSQQPY

SEQ ID NO 30: Gliadin peptide

QLQPFPQPELPYPQPQS

SEQ ID NO 31: Gliadin peptide

QQLPQPEQPQQSFPEQERPF

The invention is elucidated below on the basis of exemplary embodiments with reference to the figures. The embodiments described are merely exemplary in every respect and not to be understood as limiting, and various combinations of the features cited are encompassed by the scope of the invention.

Fig. 1 shows an immunoprecipitation with anti-p200 pemphigoid patient IgG or serum without LAMC1 reactivity and extract of human dermis. A: Western blot analysis: detection of the immunoprecipitated proteins by incubation with an anti-p200 pemphigoid serum as primary antibody (dilution 1:50). Lane 1: anti-p200 IgG (purified) without LAMC1-cterm reactivity; lane 2: anti-p200 pemphigoid serum without LAMC1-cterm reactivity; lane 3: normal human IgG (purified); lane 4: normal human serum; lane 5: Gammabind G Sepharose on its own. B: Silver blue (Coomassie) staining of a gel containing the same samples as in A. Bands 1, 2 and 3 were cut out and subjected to an LC-MS/MS analysis. Laminin beta-4 was identified as possible autoantigen (bands 1 and 2).

Fig. 2 shows the detection of laminin beta-4 in the skin. A, B: Indirect immunofluorescence microscopy: staining of laminin beta-4 on the dermal side of the artificial split in salt-split human skin with the aid of a commercial polyclonal antibody (Cloud-Clone; PAC079Hu01). B: Negative control. C: Testing of the laminin beta-4 antibody in urea extracts of human dermis by means of western blot analysis. Strips 1 and 2: anti-200 pemphigoid sera; strip 3: laminin gamma 1 antibody; strip 4: laminin beta-4 antibody; strips 5 and 6: normal human

sera. D: Immunoprecipitation with laminin beta-4 antibody and extract of human dermis followed by western blot analysis (staining with a pool of anti-p200 pemphigoid patient IgG without laminin gamma 1 reactivity). Lanes 1 and 2: anti-laminin beta-4 antibody (5 µg and 10 µg, respectively); lane 3: anti-NC16A IgG (control IgG, 10 µg); lane 4: normal rabbit IgG (10 µg); lane 5: Gammabind G Sepharose on its own.

Fig. 3 shows a representative western blot analysis with laminin beta-4-expressing HEK293 cells and anti-p200 pemphigoid sera (n=29). HEK293 cells were transfected with LAMB4-pTriEx1 (jetPRIME; Polyplus) and lysed in RIPA buffer after 48 h. The protein extract was resolved by means of SDS-PAGE, the proteins were transferred to a nitrocellulose membrane, and the membrane was blocked and was incubated overnight with the laminin beta-4 antibody (commercial polyclonal antibody from Cloud-Clone; 1:2000), the anti-p200 pemphigoid sera (1:50) and the normal human sera (NHS, 1:50) from healthy blood donors. An anti-human IgG4 HRP conjugate (1:2000) was used as secondary antibody. The blot was developed with DAB for 1 min. All the anti-p200 pemphigoid sera shown here reacted with laminin beta-4, while the controls exhibited no reaction.

Fig. 4 shows the results of the preadsorption study. Anti-p200 pemphigoid sera were preadsorbed with the HEK293-laminin beta-4 extract (overnight) and then tested for their reactivity in a western blot. A: Left: Three anti-p200 pemphigoid sera were preadsorbed overnight with HEK293-laminin beta-4 extract and no longer exhibited a signal (strips 2, 4, 6) in comparison with the non-preadsorbed sera (1, 3, 5) in the subsequent western blot with laminin beta-4; strip 7: normal human serum; strip 8: preadsorbed normal human serum. Right: The same samples were also incubated with nitrocellulose strips of dermal extract and exhibited the same result. Only serum No. 2 reacted, despite preadsorption, in the western blot with dermal extract. B: In a western blot with recombinant hLAMC1-cterm, it was possible to show that serum No. 2 also reacted with laminin gamma-1. C: Renewed preadsorption of serum No. 2 with hLAMC1-cterm and the cell extract with expression of laminin beta-4. It was possible to cancel the autoantibody reactivity with both extracts. Strip 1: anti-p200 pemphigoid serum (No. 2); strip 2: preadsorbed anti-p200 pemphigoid serum (No. 2); strip 3: normal human serum; strip 4: preadsorbed normal human serum.

Fig. 5 shows exemplary primary data from precise epitope mapping. What can be seen is the signal obtained from a microarray after the incubation of four patient sera and a sample from a blood donor (each 1:10 diluted) and IgG secondary antibody.

Example 1: Identification of laminin beta-4 as diagnostically relevant autoantigen

Preparation of dermal extract

- 5 A further autoantigen of anti-p200 pemphigoid was identified by first carrying out an immunoprecipitation with extract of human dermis and purified patient antibody.

To this end, the dermal extract was first prepared (Zillikens D, Kawahara Y, Ishiko A, Shimizu H, Mayer J, Rank C V, Liu Z, Giudice G J, Tran H H, Marinkovich M P, Bocker E B, and Hashimoto T, A novel subepidermal blistering disease with autoantibodies to a 200-kDa antigen of the basement membrane zone. J Invest Dermatol, 1996. 106(6): pp. 1333-8). Human skin pieces were incubated at 4°C for 48 h in PBS (phosphate-buffered saline) containing 1 M NaCl, 5 mM EDTA, 1 mM PMSF and protease inhibitor cocktail (Set III, Merck). After 48 h, it was then possible to separate the dermis and the epidermis from one another. The proteins were extracted from the dermis by first overlaying the dermis with a 4 M urea buffer (4 M urea, 12.5 mM Tris-HCl, pH 6.8, 5 mM EDTA, 1 mM PMSF) for 10 min, then removing the buffer and subsequently carrying out an incubation with a 9 M urea buffer (9 M urea, 12.5 mM Tris-HCl, pH 6.8, 2% SDS, 100 mM DTT, 5 mM EDTA and 1 mM PMSF) for 1 hour. The resulting extract was rebuffed in RIPA buffer (radioimmunoprecipitation assay buffer: 0.1% SDS, 1% NP-40, 0.5% Na deoxycholate, protease inhibitors (1 tablet of SIGMAFAST™ Protease Inhibitor Cocktail Tablets, EDTA-Free, Sigma Aldrich)) using desalting columns (Zeba™ Spin Desalting Columns, Thermo Scientific) and stored at -20°C until use.

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Affinity purification of IgG antibodies

Anti-p200 pemphigoid IgG was purified using Protein G Sepharose (Genscript) according to the instructions from the manufacturer. Thereafter, the hLAMC1-cterm (C-terminus of human laminin gamma-1, amino acids 1363-1609) specific autoantibodies were isolated with the aid of an affinity purification procedure (Vafia K, Groth S, Beckmann T, Hirose M, Dworschak J, Recke A, Ludwig R J, Hashimoto T, Zillikens D, and Schmidt E, Pathogenicity of autoantibodies in anti-p200 pemphigoid. PLoS One, 2012. 7(7): p. e41769). For the specific purification of the patient antibodies, hLAMC1-cterm-pQE40 was first transformed into *E. coli* Rosetta 2 DE3 (Novagen/Merck, Darmstadt) and the protein was expressed and purified (by means of IMAC). Lastly, the recombinant hLAMC1-cterm protein was covalently coupled to Affigel 15 (BIO RAD) according to the instructions from the manufacturer (Groth S, Recke A,

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Vafia K, Ludwig R J, Hashimoto T, Zillikens D, and Schmidt E, Development of a simple enzyme-linked immunosorbent assay for the detection of autoantibodies in anti-p200 pemphigoid. *Br J Dermatol*, 2011. 164(1): pp. 76-82). With the aid of this specific affinity chromatography, it was possible to remove the hLAMC1-cterm-specific autoantibodies from
5 the IgG preparations of the anti-p200 pemphigoid patient sera.

Immunoprecipitation

What were used in the immunoprecipitation were the rebuffered dermal extract as protein source and the purified patient IgG without hLAMC1-cterm reactivity, an anti-p200 pemphigoid serum without hLAMC1-cterm antibodies, and the corresponding controls from healthy blood donors.

First, the Protein G Sepharose (GammaBind G Sepharose, GE Healthcare) was washed with RIPA buffer and preadsorbed with serum from a healthy blood donor. After the one-hour preadsorption and centrifugation, the Sepharose was incubated with in each case:

1. 30 µg of patient IgG (pool) without hLAMC1-cterm reactivity
2. 20 µl of patient serum
3. 30 mg of NH IgG (normal human IgG from healthy blood donors)
4. 20 µl of NHS (normal human serum from a healthy blood donor)
5. only RIPA buffer

in RIPA buffer at room temperature for 1 h with rotation and subsequently washed with RIPA buffer. 200 µl of the rebuffered dermal extract were then pipetted into each reaction tube and the reaction preparations were then incubated overnight at 4°C on a rotator. A wash step with RIPA buffer was followed by a wash step with sucrose buffer (1 M sucrose, 150 mM NaCl, 10 mM EDTA, 10 mM HNaPO₄). Finally, a wash step with RIPA buffer was carried out once again. The samples were each heated at 95°C for 5 min with 20 µl of Laemmli buffer and centrifuged and the samples were loaded on 7.5% PROTEAN TGX protein gels (BIO RAD).

One half of the gel was Coomassie (silver blue) stained and the other half was, after transfer of the proteins to a nitrocellulose membrane, analyzed in a western blot with an anti-p200 pemphigoid serum (dilution 1:50, incubation overnight at 4°C). The buffers used were TBS-T containing 5% milk powder for saturating free binding sites and TBST containing 5% milk powder and 1% BSA as incubation buffer. The secondary antibody used was an anti-human IgG4 HRP antibody (1:2000, clone HP6025, Southern Biotech). The blot was developed with DAB (3,3'-diaminobenzidine). It was possible to observe a distinct signal at approx. 200 kD (**Fig. 1**). The corresponding bands from the Coomassie-stained gel were subjected to an LC-MS/MS analysis (Wistar Institute, Philadelphia) and it was possible to identify them as laminin beta-4 (LAMB4).

Example 2: Checking of the localization of laminin beta-4

The localization of laminin beta-4 in the skin was checked by first using a commercially available anti-laminin beta-4 antibody (polyclonal, Cloud-Clone; PAC079Hu01) in indirect immunofluorescence. In contrast to the negative control, the anti-laminin beta-4 antibody, just like the anti-p200 pemphigoid sera (not shown), binds in the base of the artificial blister in human salt-split skin. In addition, the anti-laminin beta-4 antibody was also tested in a western blot with dermal extract (see above for the procedure). The western blot with dermal human extract is the current diagnostic standard for diagnosing anti-p200 pemphigoid. As shown by **Fig. 2**, the anti-laminin beta-4 antibody (strip 4) reacts with a protein band situated on the same level with the signal of the anti-p200 pemphigoid sera (strips 1 and 2). The signal of the commercial monoclonal laminin gamma-1 antibody (clone B-4, Santa Cruz) is shifted slightly upward.

In addition, the commercial anti-laminin beta-4 antibody was used for an immunoprecipitation. Here too, the laminin beta-4 source used was again the dermal extract. 5 µg and 10 µg of anti-laminin beta-4 antibody were used for the immunoprecipitation (see above for the procedure). The controls used were anti-NC16A rabbit IgG (10 µg), normal rabbit IgG (10 µg) and GammaBind G Sepharose on its own. In the subsequent western blot analysis, laminin beta-4 was detected using a pool of anti-p200 pemphigoid IgG without laminin gamma-1 reactivity. On **Fig. 2**, it can be clearly seen that the anti-p200 pemphigoid patients have autoantibodies against laminin beta-4.

Example 3: Cloning

Laminin beta-4 (LAMB4) was recombinantly produced as a variant with C-terminally fused polyhistidine-tag and as an authentic variant. The cDNA clone IRCBp5005F2212Q, encoding isoform 1 of human laminin beta-4, was purchased from Source BioScience (United Kingdom) and used as template for PCR.

For the amplification of the coding sequence of laminin beta-4, the sense primer LAMB4 (SEQ ID NO 1) and the anti-sense primer LAMB4 (SEQ ID NO 2) were used for the fragment without translation stop codon at the end of the sequence encoding laminin beta-4. The amplification of the laminin beta-4 cDNA with translation stop codon at the end of the

coding sequence was carried out with the sense primer LAMB4 (SEQ ID NO 1) and the anti-sense primer LAMB4-Stop (SEQ ID NO 3).

At the ends, both PCR products contained restriction sites for the restriction enzyme BsaI.

After BsaI digestion, the laminin beta-4 amplicons were ligated with the NcoI/XhoI-linearized plasmid vector pTriEx-1 (Merck, Darmstadt, Germany, SEQ ID NO 4), yielding the constructs as per SEQ ID NO 5 and SEQ ID NO 6. The ligation preparations were transformed into *E. coli* NEB 5-alpha (New England Biolabs GmbH) and transformants were selected by means of 100 µg/ml ampicillin. The isolated plasmids were checked for correctness by means of restriction analyses and DNA sequencing and used for the transfection of HEK293 cells.

Example 4: Transfection of HEK cells and western blot analysis

To check that laminin beta-4 is actually a target antigen of anti-p200 pemphigoid, HEK293 cells were transfected with LAMB4-pTriEx1 (with His-tag, SEQ ID NO 7) or the empty vector by means of jetPRIME (Polyplus transfection) and expression was checked after 48 h in a western blot by means of a commercial anti-laminin beta-4 antibody (see above). Altogether, 49 anti-p200 pemphigoid sera (dilution 1:50) and 20 sera from healthy blood donors (NHS, normal human sera, dilution 1:50) were tested for their reactivity with laminin beta-4 in a western blot with the laminin beta-4-HEK293 extract (RIPA buffer). To this end, the finished nitrocellulose membrane was cut into strips. The commercial anti-laminin beta-4 antibody (dilution 1:2000) was used as positive control. The strips were incubated with the primary antibodies overnight at 4°C. Here too, as described above, an anti-human IgG4 HRP antibody and a mouse anti-rabbit HRP antibody were used as secondary antibody. The blot was developed with DAB for 1 min. Altogether, 47 of the 49 anti-p200 pemphigoid sera reacted with laminin beta-4, whereas the negative controls exhibited no reactions.

Example 5: Preadsorption study

The correct identification of laminin beta-4 was confirmed by a preadsorption study. To this end, three anti-p200 pemphigoid sera (without laminin gamma-1 reactivity) were preadsorbed with the laminin beta-4-HEK293 extract overnight at 4°C and then tested for their remaining reactivity in a western blot with dermal extract and laminin beta-4-HEK293

extract (**Fig. 4**). The western blot analysis was carried out as described above. Compared to the non-preadsorbed sera (**Fig. 4**, strips 1, 3 and 5), the preadsorbed sera no longer exhibited reactivity with the laminin beta-4-HEK293 extract, and 2 of the 3 preadsorbed sera also no longer reacted with the 200 kDa sized protein in the dermal extract. The reaction could thus be neutralized. Only serum No. 2 still exhibited an attenuated reaction at about 200 kDa. This anti-p200 pemphigoid serum should actually exhibit no reactivity with laminin gamma-1, but testing was nevertheless carried out again using an hLAMC1-cterm western blot. It emerged that serum No. 2 actually has laminin gamma-1 autoantibodies, and this can thereby explain the reaction in the dermal extract.

By means of a preadsorption of serum No. 2 with hLAMC1-cterm and the laminin beta-4-HEK293 extract, it was possible to cancel the autoantibody reactivity with both extracts. The negative control used in this study was the serum from a healthy blood donor (NHS).

Example 6: Rough epitope mapping

To identify rough epitopes, six subfragments of laminin beta-4 (SEQ ID NO 15, SEQ ID NO 16, SEQ ID NO 17, SEQ ID NO 18, SEQ ID NO 19 and SEQ ID NO 20) were cloned and expressed in a pET24d vector with C-terminal His-tag using standard protocols (SEQ ID NO 21, SEQ ID NO 22, SEQ ID NO 23, SEQ ID NO 24, SEQ ID NO 25 and SEQ ID NO 26). Where the constructs did not already have four tryptophan residues, they were artificially fused on in a number for each construct to have four tryptophans for concentration measurement.

The constructs were subjected to a western blot analysis, as described in Example 1.

The results are combined in Table 1. Essentially, what became apparent was considerable activity against subfragment (SF) 5 and SF 6. By contrast, the sera from blood donors (BD) showed practically no reactivity.

	Sera	SF 1	SF 2	SF 3	SF 4	SF 5	SF 6
p200	1	+	weak +	+	weak +	+	+++
	2	-	-	-	-	weak +	+++
	3	+	+	+	-	-	+++
	4	-	-	-	-	-	+++
	5	-	+	-	+	++	+++
	6	-	-	-	-	++	+++
	7	+	+	-	-	++	+++

	8	+	+	weak +	+	++	+++
	9	-	-	-	-	++	+++
	10	-	-	+	-	+	+++
	11	+	-	weak +	-	++	+++
	12	+	+	weak +	-	++	+++
	13	-	-	-	-	+	+++
	14	-	-	-	-	++	++
	15	-	-	-	-	+	++
	16	-	-	-	-	-	++
	17	-	-	-	-	+	+++
	18	-	-	-	+	-	+++
	19	-	-	-	-		+++
	20	-	-	-	+		+++
		6/20	6/20	6/20	5/20	14/20	20/20
NHS	BD1		-	-	-	-	-
	BD2	-	-	-	-	-	-
	BD3	-	-	-	-	-	-
	BD4	-	-	-	-	-	-
	BD5	-	-	-	-	-	-
	BD6	-	-	-	-	-	-
	BD7	-	-	-	-	-	-
	BD8	-	-	-	-	-	-
	BD9	-	-	-	-	-	-
	BD10	-	-	-	-	-	-
	BD11	-	-	-	-	-	-
	BD12	-	-	-	-	-	-
	BD13	-	-	-	-	-	-
	BD14	unspecific	unspecific	unspecific	unspecific	unspecific	unspecific
	BD15	-	-	-	-	-	-
	BD16	-	-	-	-	-	-
	BD17	-	-	-	-	-	-
	BD18	-	-	-	-	-	weak?
	BD19	unspecific	unspecific	unspecific	unspecific	unspecific	unspecific
	BD20	-	-	-	-	-	-
							poss. 1/20

Example 7: Precise epitope mapping

5 Four patient sera comprising autoantibodies against laminin beta-4 were used for epitope mapping. The serum from a blood donor was additionally incubated in order to be able to identify unspecific reactions. Use was made of linear 15mer peptides with an overlap of 14 amino acids, which map the C-terminal portion, more precisely subfragments 5 (SEQ ID NO 19) and 6 (SEQ ID NO 20). The peptides were contacted with the samples at a dilution of

1:100 in incubation buffer (PBS, pH 7.4 containing 0.05% Tween 20 with 10% Rockland blocking buffer MB-070) and incubated in incubation buffer for 16 h at 4°C and with shaking at 140 rpm and in dilutions of 1:500, 1:100 and 1:10, followed by 45 min of staining with secondary (mouse anti-human IgG (Fc) DyLight680 (0.5 µg/ml)) and control (mouse monoclonal anti-HA (12CA5) DyLight680 (0.2 µg/ml)) antibodies. Reading was carried out using a LI-COR Odyssey Imaging System. Quantification of the spot intensities and peptide annotation were carried out using a PepSlide Analyzer. Exemplary primary data can be seen in **Fig. 5**.

Result: What were determined were low to moderate specific antibody reactions with peptides, meaning that it was possible to identify the epitopes having the sequences SEQ ID NO 8, SEQ ID NO 9, SEQ ID NO 10, SEQ ID NO 11, SEQ ID NO 12, SEQ ID NO 13 and SEQ ID NO 14.

Claims

- 5 1. A polypeptide comprising laminin beta-4 or a variant thereof, preferably in recombinant and/or isolated form.
2. The polypeptide as claimed in claim 1, wherein the polypeptide is immobilized.
- 10 3. The carrier as claimed in claim 2, further comprising at least one antigen, preferably all the antigens, from the group comprising laminin gamma-1, laminin 332, BP180, BP230, desmoglein 1, desmoglein 3, envoplakin, gliadin and collagen type VII or variants thereof and split skin.
- 15 4. The carrier as claimed in claim 3, wherein the carrier is selected from the group comprising a glass slide, preferably slide for microscopy, even more preferably with a eukaryotic cell overexpressing laminin beta-4 or a variant thereof, a biochip, a microtiter plate, a lateral flow device, a test strip, a membrane, preferably a blot, more preferably a line blot, a chromatography column material and a bead, preferably a magnetic or fluorescent bead.
- 20 5. A therapeutically useful carrier comprising the polypeptide as claimed in either of claims 1 and 2, wherein the carrier allows the contacting of human blood with the polypeptide followed by the recirculation of the blood into the body of a patient, the carrier preferably being an apheresis apparatus.
- 25 6. An antibody, preferably autoantibody, against laminin beta-4, preferably in isolated form.
- 30

7. The use of the polypeptide, carrier or autoantibody as claimed in any of claims 1 to 6 for the diagnosis of a disease.

8. A method for producing a diagnostically or therapeutically useful carrier, comprising the step of coating the carrier with the polypeptide as claimed in claim 1.

9. A method for purifying an autoantibody, comprising the steps of

a) contacting a liquid comprising the autoantibody with the polypeptide as claimed in claim 1 under conditions which allow the formation of a complex comprising the autoantibody and the polypeptide,

b) isolating the complex from step a), and

c) optionally detecting the complex from step a) or dissociating the complex isolated in step b) followed by removal of the autoantibody from the polypeptide.

10. A method comprising the step of detecting an autoantibody against laminin beta-4 in a sample.

11. A pharmaceutical composition comprising the polypeptide as claimed in claim 1 or a variant thereof, preferably together with a pharmaceutically acceptable carrier.

12. A kit comprising the polypeptide as claimed in either of claims 1 and 2 or the carrier as claimed in either of claims 3 and 4, wherein the kit contains one reagent or more than one reagent from the group comprising a wash solution, a calibrator solution, an antibody against laminin beta-4, and an agent for the detection of an autoantibody against laminin beta-4, preferably a secondary antibody.

13. The use of the polypeptide as claimed in claim 1, of the carrier as claimed in either of claims 3 and 4 or of an antibody, preferably autoantibody, against laminin beta-4 for the production of a kit or diagnostically or therapeutically useful carrier.

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14. The method, use or kit as claimed in any of claims 7 to 10 and 12 to 13, wherein an autoantibody against laminin beta-4 is detected using a method selected from the group comprising immunodiffusion, immunoelectrophoresis, light scattering, agglutination, immunoassay with labeling such as that from the group comprising
10 immunoassay with radioactive labeling, with enzymatic labeling, more preferably ELISA, with chemiluminescence labeling, more preferably electrochemiluminescence labeling, and with immunofluorescence labeling, preferably indirect immunofluorescence.

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15. The use of the polypeptide as claimed in claim 1 or of the antibody as claimed in claim 6 for the determination of the capability, preferably capacity, of a diagnostic or therapeutic device or of a diagnostic or therapeutic reagent to bind an antibody against laminin beta-4.

20

16. The method as claimed in claim 10, wherein the autoantibody is detected in a tissue sample from a patient or with a carrier comprising laminin beta-4 or a variant thereof, preferably as claimed in claim 3 or 4.