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 between these diseases.

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

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.

Summary

In one example, the object underlying the disclosure is achieved by a polypeptide comprising laminin beta-4 or a variant thereof, preferably in recombinant and/or isolated form.

In a preferred example, the polypeptide is immobilized.

In a second example, the object is achieved by a carrier comprising the polypeptide according to the disclosure, 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.

In a preferred example, 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.

In one aspect, the present disclosure refers to a kit for the detection of anti-p200 pemphigoid or an autoimmune disease of the skin comprising a polypeptide consisting of laminin beta-4 according to SEQ ID NO 27 or a variant thereof, preferably in recombinant and/or isolated form, and an agent for the detection of an autoantibody against laminin beta-4 according to SEQ ID NO 27, wherein the polypeptide binds to an autoantibody against laminin beta-4 according to SEQ ID NO 27, wherein the variant a) comprises at least 90% sequence identity to SEQ ID NO 27 or b) 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 wherein the agent is a secondary antibody that binds to the autoantibody bound to the polypeptide.

In another aspect, the present disclosure refers to a therapeutically useful carrier comprising laminin beta-4 according to SEQ ID NO 27 or a variant thereof, wherein the therapeutically useful carrier allows the *ex vivo* contacting of human blood with laminin beta-4 according to SEQ ID NO 27 or the variant thereof followed by the recirculation of the blood into the body of a patient, wherein laminin beta-4 according to SEQ ID NO 27 or the variant has the capability of binding to an autoantibody against laminin beta-4 according to SEQ ID NO 27, as is found in

a patient suffering from an autoimmune disease associated with such an autoantibody, wherein the variant a) comprises at least 90% sequence identity to SEQ ID NO 27 or b) 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.

In another example, the object is achieved by a therapeutically useful carrier comprising the polypeptide according to the disclosure, 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.

In another aspect, the present disclosure refers to an *in vitro* use of the kit as described herein for the diagnosis of anti-p200 pemphigoid or an autoimmune disease of the skin.

In another aspect, the present disclosure refers to an *in vitro* method for purifying an autoantibody against laminin beta-4 according to SEQ ID NO 27, comprising the steps of: a) contacting a liquid human sample comprising autoantibodies from a patient suffering from anti-p200 pemphigoid or an autoimmune disease of the skin, wherein the autoantibodies are associated with the occurrence of anti-p200 pemphigoid with a polypeptide consisting of laminin beta-4 according to SEQ ID NO 27 or a variant thereof under conditions which allow the formation of a complex comprising the autoantibody and the polypeptide, wherein the polypeptide binds to an autoantibody against laminin beta-4 according to SEQ ID NO 27, wherein the variant a) comprises at least 90% sequence identity to SEQ ID NO 27 or b) 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; b) isolating the complex from step a); and c)detecting the complex from step a) and dissociating the complex isolated in step b) followed by removal of the autoantibody from the polypeptide.

In another aspect, the present disclosure refers to an *in vitro* method for the detection of anti-p200 pemphigoid or an autoimmune disease of the skin comprising the step of detecting an autoantibody specifically binding to laminin beta-4 according to SEQ ID NO 27 in a sample.

Claims

1. A kit for the detection of anti-p200 pemphigoid or an autoimmune disease of the skin comprising a polypeptide consisting of laminin beta-4 according to SEQ ID NO 27 or a variant thereof, preferably in recombinant and/or isolated form, and an agent for the detection of an autoantibody against laminin beta-4 according to SEQ ID NO 27, wherein the polypeptide binds to an autoantibody against laminin beta-4 according to SEQ ID NO 27, wherein the variant a) comprises at least 90% sequence identity to SEQ ID NO 27 or b) 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 wherein the agent is a secondary antibody that binds to the autoantibody bound to the polypeptide.

2. The kit as claimed in claim 1, wherein the polypeptide is immobilized on a carrier.

3. The kit as claimed in claim 2, wherein the carrier further comprises 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.

4. The kit as claimed in claim 2 or 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 according to SEQ ID NO 27 or a variant thereof, wherein the variant a) comprises at least 90% sequence identity to SEQ ID NO 27 or b) 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, 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.

5. A therapeutically useful carrier comprising laminin beta-4 according to SEQ ID NO 27 or a variant thereof, wherein the therapeutically useful carrier allows the *ex vivo* contacting of human blood with laminin beta-4 according to SEQ ID NO 27 or the variant thereof followed by the recirculation of the blood into the body of a patient, wherein laminin beta-4 according to SEQ ID NO 27 or the variant has the capability of binding to an autoantibody against laminin beta-4 according to SEQ ID NO 27, as is found in a patient suffering from an autoimmune disease associated with such an autoantibody, wherein the variant a) comprises at least 90% sequence identity to SEQ ID NO 27 or b) 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.

6. *In vitro* use of the kit as claimed in any of claims 1 to 4 for the diagnosis of anti-p200 pemphigoid or an autoimmune disease of the skin.

7. An *in vitro* method for purifying an autoantibody against laminin beta-4 according to SEQ ID NO 27, comprising the steps of

a) contacting a liquid human sample comprising autoantibodies from a patient suffering from anti-p200 pemphigoid or an autoimmune disease of the skin, wherein the autoantibodies are associated with the occurrence of anti-p200 pemphigoid or an autoimmune disease of the skin, with a polypeptide consisting of laminin beta-4 according to SEQ ID NO 27 or a variant thereof under conditions which allow the formation of a complex comprising the autoantibody and the polypeptide, wherein the polypeptide binds to an autoantibody against laminin beta-4 according to SEQ ID NO 27, wherein the variant a) comprises at least 90% sequence identity to SEQ ID NO 27 or b) 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,

b) isolating the complex from step a), and

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

8. *An in vitro* method for the detection of anti-p200 pemphigoid or an autoimmune disease of the skin comprising the step of detecting an autoantibody specifically binding to laminin beta-4 according to SEQ ID NO 27 in a sample.

9. The kit as claimed in any of claims 1 to 4, wherein the kit further contains one reagent or more than one reagent from the group comprising a wash solution, a calibrator solution and an antibody against laminin beta-4 according to SEQ ID NO 27.

10. The method, use or kit as claimed in any of claims 6 to 9 wherein the autoantibody against laminin beta-4 according to SEQ ID NO 27 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.

11. The method as claimed in any of claims 7, 8 or 10, wherein the autoantibody is detected in a tissue sample from a patient or with a carrier consisting of laminin beta-4 according to SEQ ID NO 27 or a variant thereof, wherein laminin beta-4 or the variant binds to an autoantibody against laminin beta-4 according to SEQ ID NO 27, as is found in a patient suffering from an autoimmune disease associated with such an autoantibody, wherein the variant a) comprises at least 90% sequence identity to SEQ ID NO 27 or b) 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.

12. The method as claimed in any one of claims 8, 10 or 11, wherein the sample is cerebrospinal fluid, blood, blood serum, lymph, interstitial fluid or split skin.