BLOCKERS OF GSK3 FOR THE PREVENTION AND TREATMENT OF PEMPHIGUS VULGARIS

The invention relates to a method of preventing and treating pemphigus vulgaris comprising administering a GSK3\(\beta\) blocker, and the use of such blockers in said prevention and treatment and in the manufacture of medicaments for preventing and treating pemphigus vulgaris. In particular the invention relates to a method of preventing and treating pemphigus vulgaris comprising administering a GSK3\(\beta\) blocker, and the use of such blockers in said prevention and treatment and in the manufacture of medicaments for preventing and treating pemphigus vulgaris. Preferred is the use of GSK3\(\beta\) blockers which are selective for GSK3\(\beta\), i.e. which selectively reduce enzyme activity of GSK3\(\beta\) but not to a reasonable extent of GSK3\(\alpha\).
Blockers of GSK3 for the prevention and treatment of pemphigus vulgaris

Field of the invention

This invention relates to the prevention and treatment of pemphigus vulgaris using blockers of glycogen synthase kinase 3 (GSK3).

Background of the invention

Pemphigus vulgaris

Pemphigus vulgaris (PV) is an autoimmune blistering disease that affects skin and mucous membranes in man and domestic animals. It is characterized by auto-antibody binding to the desmosomal cadherin desmoglein (Dsg) 3 that results in suprabasal loss of intercellular adhesion in skin and mucous membranes (Amagai et al., Cell, 67, 869-877, 1991). Most PV patient's antibodies target the adhesive interface between juxtaposed Dsg3 molecules of adjacent cells, and are thought to disrupt their trans-adhesion by steric hindrance (Tsunoda et al., J Immunol, 170, 2170-2178, 2003). Initially, this leads to depletion of non-keratin anchored i.e. Triton X-100 soluble Dsg3, and ultimately to loss of Dsg3 from fully assembled desmosomes (Aoyama and Kitajima, 1999; Sato et al., 2000). Although the molecular mechanism of Dsg3 depletion and subsequent acantholysis so far was unknown, it had been previously shown that the armadillo protein plakoglobin (PG), that associates with the cytoplasmic tail of Dsg3, is crucially involved in this process (Caldelari et al., J. Cell Biol., 153, 823-834, 2001). This work suggested that secondary to antibody binding, modulation of available PG at the plasma membrane supports rapid degradation of Dsg3, and subsequently affects the ongoing differentiation process.

Glycogen synthase kinase 3 (GSK3)

GSK3 is a multifunctional serine/threonine kinase. It exists in two isoforms, GSK3α and GSK3β that are encoded by different genes. GSK3 is relatively unique among kinases because it is generally constitutively active in cells, and deactivation is responsible for propagation of intracellular signals. The two isoforms share high homology in their catalytic domain, and similar but not absolutely identical biological functions. For instance, both GSK3α and GSK3β are inhibited by protein kinase B (Toker and Yoeli-Lerner, Cancer Res, 66, 3963-3966, 2006) resulting in activation of proteins regulating cell cycle and apoptosis, while only GSK3β is inhibited by the canonical Wnt signaling pathway...
(Willert and Jones, Genes Dev, 20, 1394-1404, 2006) that promotes nuclear translocation of β-catenin and plakoglobin. Importantly, it was never shown before that this pathway is affected by PV antibodies.

Summary of the invention

The present invention relates to a method of preventing and treating pemphigus vulgaris comprising administering a GSK3 blocker, and the use of such blockers in said prevention and treatment and in the manufacture of medicaments for preventing and treating pemphigus vulgaris. Preferred as a GSK3 blocker is a GSK3β blocker, in particular a blocker which is selective for GSK3β, i.e. which selectively reduces enzyme activity of GSK3β but not to a reasonable extent of GSK3α.

The invention further relates to a method of screening for a compound effective in the prevention and treatment of pemphigus vulgaris comprising contacting a candidate compound with a GSK3, and choosing candidate compounds which selectively reduce activity of GSK3, preferably of GSK3β. The invention further relates to compounds selected by these methods of screening.

Brief description of the Figures

Fig. 1: Nuclear PG is reduced in PV antibody (PVIgG)-treated mouse keratinoocytes. Western blot analyses of (A) cytoplasmic/membrane and (B) nuclear fractions. Tubulin, E-cadherin, Dsg3, α-catenin and the nuclear envelope protein lamin B1 served as a loading or purity control, respectively. Graphs depict the relative change of indicated proteins with respect to control cells at 72h (set as 100). Axes: x: hours, y: relative pixel density. Insets indicate percentage PG reduction in PVIgG-treated cells at 72h as compared to control keratinocytes. Note that nuclear β-catenin was similar in control and PVIgG treated cells. Nuclear accumulation of armadillo proteins coincides with the phosphorylation of GSK3β on Ser9 (Calautti et al., J Biol Chem, 280, 32856-32865, 2005). PpM= plakophilin. **, p<0.009; n=5. Error bars are ± SD.

Fig. 2: PVIgG upregulate c-Myc in keratinocyte cultures and in PV patients. (A) Graph indicates the relative change (y axis) in c-Myc mRNA levels as compared to CS (calcium switch) in mouse keratinocyte cultures and was determined by Q-PCR. One representative result done in duplicates of three independent experiments is shown. Error
bars represent the range. (B) Western blot analyses for c-Myc was performed on total cell lysates obtained from mouse (upper panel) and human (middle panel) keratinocytes and on nuclear fractions from mouse keratinocytes (lower panel). (C) Consecutive sections of paraffin embedded biopsies from six PV patients (PV-1 to PV-6) and healthy donors (C and C-om (oral mucosa)) were stained for c-Myc, counterstained with Hoechst. For comparison, biopsies from healthy donors, patients with other autoimmune bullous diseases such as pemphigus foliaceous (PF) and bullous pemphigoid (BP) as well as chronic eczematous dermatitis were also investigated. C-Myc positive cells in the dermis (arrow heads) likely are leukocytes as judged from H&E stains (data not shown) which is consistent with their absence from non-lesional skin (PV-6). Arrows point to faint c-Myc staining in control skin. Scale bars, 200mm.

**Fig. 3: PG is a suppressor of c-Myc.**
(A) Graphs show reporter gene assays using the human c-Myc promoter transfected into mouse keratinocytes with indicated plasmids. The ratio of firefly over renilla luciferase activity (F/R) is indicated. One representative experiment of at least four independent experiments done in single measures per cell type is shown. PG ΔC, ΔN and ARM lack the C-terminal, N-terminal domain or both, respectively. (B) Top panel: scheme of the mouse c-Myc promoter and TCF/LEF binding site. Gray bars indicate positions of primer sets relative to the transcription initiation site used to amplify precipitated DNA by Q-PCR. Bottom panel: Chromatin immunoprecipitation (ChIP) using indicated antibodies for immunoprecipitation. Graphs depict relative amounts of amplification products (y axis: relative DNA amount) obtained by Q-PCR compared to control IgG with indicated primer sets. One of three independent experiments measured in duplicates is shown. Error bars indicate the range.

**Fig. 4: Blister formation in neonatal mice is prevented by c-Myc inhibitors as demonstrated by a passive transfer study**
Passive transfer study performed as described in Anhalt et al., N. Engl. J. Med., 306, 1189-1 196, 1982. (A) Top pictures show neonatal mice subcutaneously injected with vehicle (top panel) or 6μM c-Myc inhibitor 3 (MI-3, bottom panel) followed by PV1IgG, AK23 (pathogenic monoclonal Dsg3 antibody (Tsunoda et al., J Immunol, 170, 2170-2178, 2003)) or NAK3 (non-pathogenic Dsg3 antibody) together with half the minimal dose of a human PF serum. Discontinued lines indicate the area of injection. Micrographs underneath show lesions (H&E) and IgG binding in the peri-lesional area assessed by direct immunofluorescence (DIF). (B) Consecutive sections of the peri-lesional areas
shown in (A) were stained for c-Myc. c-Myc was mainly present in the cytoplasm of PVIgG and AK23 injected mice, and to some extent also of NAK3 injected mice. Arrows point to nuclei (insets) with slightly elevated c-Myc. V, vehicle.

Fig. 5: Blister formation in neonatal mice is prevented by GSK3 inhibitors as demonstrated by a passive transfer study showing that activated GSK3 accounts for loss of intercellular adhesion.


(A) As in Fig. 4, except that mice were pre-injected with inhibitors to GSK3. (B) Immunofluorescence analysis of c-Myc in consecutive sections of peri-lesional areas shown in (A). Scale bars, 1.6 mm for H&E, otherwise 200 mm.

Detailed description of the invention

The present invention relates to a method of preventing and treating pemphigus vulgaris comprising administering a GSK3 blocker, and the use of such blockers in said prevention and treatment and in the manufacture of medicaments for preventing and treating pemphigus vulgaris. In particular the invention relates to a method of preventing and treating pemphigus vulgaris comprising administering a GSK3β blocker, and the use of such blockers in said prevention and treatment and in the manufacture of medicaments for preventing and treating pemphigus vulgaris. Preferred is the use of GSK3β blockers which are selective for GSK3β, i.e. which selectively reduce enzyme activity of GSK3β but not to a reasonable extent of GSK3α.

GSK3 blockers are compounds which reduce the enzyme activity of a GSK3, or inhibit the production of GSK3 or the activation of GSK3 from its latent form, or inhibit the interaction between GSK3 and its activator axin (Willert and Jones, Genes Dev, 20, 1394-1404, 2006). Preferred GSK3 blockers are compounds which reduce the enzyme activity of GSK3β, or inhibit the production of GSK3β or the activation of GSK3β from its latent form, or inhibit the interaction between GSK3β and axin.

For example, compounds which inhibit GSK3 enzyme activity bind to a catalytic domain of GSK3 or bind to the ATP-binding pocket of GSK3. GSK3 production can be inhibited by anti-sense oligodeoxynucleotides or siRNAs to the GSK3 isoforms, particularly to GSK3β.
GSK3 blockers of the invention may belong to the class of inorganic compounds, e.g. lithium salts, or organic compounds, e.g. polypeptides or small organic compounds, preferably thiazolidinones, inositol-phosphate phosphatases, paullones (Indolo[3,2-d][1]benzazepines), 6-bromo-substituted indirubins (indigo isomer) or also maleimides.


Preferred GSK3 blockers according to the invention are:

- NP031 112 (Neuropharma S.A., Madrid, Spain; WO 2005/0971 17) and similar heterocyclic thiazolidinones
- lithium salts, such as LiCl, and lithium mimetics (such as mangan salts)
- antibodies and antibody fragments to GSK3, such as human or humanized antibodies or single chain antibodies to GSK3
- SB-216763 and SB-415286 (Coghlan et al., Chem. & Biol., 7:793-803, 2000)
- Indirubin (Leclerc et al., J Biol Chem, 276, 251-260, 2001)

Most preferred GSK3 blockers according to the invention are:
One aspect of the invention relates to a method of preventing and treating pemphigus vulgaris comprising administering GSK3 blockers as defined hereinbefore in a quantity effective against pemphigus vulgaris to a mammal in need thereof, for example to a human requiring such treatment. The treatment may be for prophylactic or therapeutic purposes. For the administration, the GSK3 blocker is preferably in the form of a pharmaceutical preparation comprising the GSK3 blocker in chemically pure form and optionally a pharmaceutically acceptable carrier and optionally adjuvants. The GSK3 blocker is used in an amount effective against pemphigus vulgaris. The dosage of the active ingredient depends upon the species, its age, weight, and individual condition, the individual pharmacokinetic data, the mode of administration, and whether the administration is for prophylactic or therapeutic purposes. In the case of an individual having a bodyweight of about 70 kg the daily dose administered is from approximately 1 mg to approximately 500 mg, preferably from approximately 10 mg to approximately 100 mg, of a GSK3 blocker and the daily dose of a GSK3 antibody administered is from approximately 0.1 mg to approximately 50 mg, preferably from approximately 1 mg to approximately 10 mg.

Pharmaceutical compositions for enteral administration, such as nasal, buccal, rectal or, especially, oral administration, for parenteral administration, such as subcutaneous, intravenous, or intramuscular or topical administration are considered. The pharmaceutical compositions comprise from approximately 1% to approximately 95% active ingredient, preferably from approximately 20% to approximately 90% active ingredient.

For parenteral administration preference is given to the use of solutions of the GSK3 blockers, and also suspensions or dispersions, especially isotonic aqueous solutions, dispersions or suspensions which, for example, can be made up shortly before use. The pharmaceutical compositions may be sterilized and/or may comprise excipients, for example preservatives, stabilizers, wetting agents and/or emulsifiers, solubilizers, viscosity-increasing agents, salts for regulating osmotic pressure and/or buffers and are
prepared in a manner known *per se*, for example by means of conventional dissolving and lyophilizing processes.

For oral pharmaceutical preparations suitable carriers are especially fillers, such as sugars, for example lactose, saccharose, mannitol or sorbitol, cellulose preparations and/or calcium phosphates, and also binders, such as starches, cellulose derivatives and/or polyvinylpyrrolidone, and/or, if desired, disintegrators, flow conditioners and lubricants, for example stearic acid or salts thereof and/or polyethylene glycol. Tablet cores can be provided with suitable, optionally enteric, coatings. Dyes or pigments may be added to the tablets or tablet coatings, for example for identification purposes or to indicate different doses of active ingredient. Pharmaceutical compositions for oral administration also include hard capsules consisting of gelatin, and also soft, sealed capsules consisting of gelatin and a plasticizer, such as glycerol or sorbitol. The capsules may contain the active ingredient in the form of granules, or dissolved or suspended in suitable liquid excipients, such as in oils.

Transdermal application is also considered, for example using a transdermal patch, which allows administration over an extended period of time, e.g. from one to twenty days.

Especially preferred are pharmaceutical compositions for topical administration, e.g. epicutaneou administration, for which preference is given to the use of solutions of the GSK3 blockers, and also suspensions or dispersions, ointments, creams and the like. A suitable solution is a GSK3 blocker in 25% DMSO and 75% 1,2-propanediol.

Another aspect of the invention relates to the use of GSK3 blockers as described hereinbefore in the treatment of pemphigus vulgaris and in the manufacture of medicaments for treating these diseases. Such medicaments are manufactured by methods known in the art, especially by conventional mixing, coating, granulating, dissolving or lyophilizing.

The GSK3 blocker can be administered alone or in combination with one or more other therapeutic agents, possible combination therapy taking the form of fixed combinations of a GSK3 blocker and one or more other therapeutic agents known in the treatment of pemphigus vulgaris, the administration being staggered or given independently of one another, or being in the form of a fixed combination.
Possible combination partners considered are glucocorticosteroids and/or immunesuppressiva such as Azathioprine and/or Myc inhibitors, such as the Myc inhibitors described by Yin et al., Oncogene, 22, 6151-6159, 2003.

The invention further relates to a method of screening for a compound effective in the treatment of pemphigus vulgaris comprising contacting a candidate compound with GSK3 and choosing candidate compounds which selectively reduce the activity of GSK3, preferably the activity of GSK3β but not to a reasonable extent GSK3α.

The invention further relates to compounds selected by these methods of screening.

Inhibitors of GSK3 activity are identified by contacting a GSK3 with a candidate compound. A control assay with the corresponding GSK3 — in the absence of the candidate compound - is run in parallel. A decrease in activity in the presence of the candidate compound compared to the level in the absence of the compound indicates that the compound is a GSK3 inhibitor. Kinase activity is measured using methods known in the art. For example recombinant active GSK3α or β are incubated with inhibitors to be tested, and GSK3 activity measured using a peptide-based kinase assay (Cross, D.A. et al., Biochem. J. 303, 21-26, 1999). Preferred candidate compounds cause marked decrease of GSK3β enzyme activity while having no or only marginal influence on GSK3α enzyme activity.

**Concepts and Evidence behind the Invention**

It was found for the first time that pemphigus vulgaris antibodies induce constitutive and pathogenic expression of c-Myc that results from transient degradation of Dsg3 followed by reduced plakoglobin levels at the plasma membrane, in the cytoplasm and nucleus. As a consequence, plakoglobin-mediated c-Myc suppression is abrogated and it was shown that this is central for PV pathogenesis. In normally terminal differentiating keratinocytes, PG functions as the main docking partner for phosphatidylinositol-3-kinase (PI3K) in intercellular junctions (Calautti et al., J Biol Chem, 280, 32856-32865, 2005). The signal of activated PI3K is then propagated via inhibition of one of its down-stream target glycogen-synthase-kinase (GSK) 3. GSK3β in turn regulates the nuclear trafficking of PG. The proof that GSK3 inhibition prevents blister formation or in other words PV antibody binding to Dsg3 induce blister formation via lack of GSK3 inhibition was provided herein by a passive transfer study (Anhalt et al., N. Engl. J. Med., 306, 1189-1196, 1982) in presence or absence of GSK3 inhibitors SB216763 (Coghlan et
al., Chem Biol, 7, 793-803, 2000) and LiCl (Beaulieu et al., Proc Natl Acad Sci U S A., 101, 5099-5104, 2004). The importance of Dsg3 as trigger of the signaling cascade leading to GSK3 inactivation was demonstrated herein by the mouse monoclonal Dsg3-specific antibody AK23 which has the same pathogenic activity as patients antibodies (Tsunoda et al., J Immunol, 170, 2170-2178, 2003). AK23 is a pathogenic PV antibody which targets the adhesive interface between juxtaposed Dsg3 molecules. The mouse monoclonal antibody NAK3 was used as a negative control (Amagai et al., unpublished). NAK3 also targets Dsg3, is unable to induce blisters on its own, but has weak pathogenic activity in combination with other blister-inducing antibodies.

Specifically, AK23 and NAK3 were subcutaneously injected into the lumbar area of neonatal mice that had been pre-injected at the same site with GSK3 inhibitors or vehicle. After 24 hours blisters were observed in PVIgG/vehicle and AK23/vehicle injected mice but not in their siblings pre-treated with GSK3 inhibitors. Despite binding of NAK3 to the surface of keratinocytes, no blisters developed under any condition, similarly as seen in nhlgG injected mice. This indicates that GSK3 blockers are effective therapeutic agents for the treatment of pemphigus vulgaris.

Examples

Experiment demonstrating that nuclear PG is reduced in PV antibody (PVIgG)-treated mouse keratinocytes (see Fig. 1).

Methodology: Isolation and characterization of mouse C57BL/6 keratinocytes was described previously (Caldelari et al., J. Cell Biol., 153, 823-834, 2001). Conventional submerged cultures and stimulation conditions were according to a previously established protocol for studies on PV (Caldelari et al., J. Cell Biol., 153, 823-834, 2001) except that the medium was changed to CnT-02 (CELLnTEC, Switzerland) or Cnt-02 supplemented with 1.2 mM calcium (referred to as "high calcium medium"). PV antibodies (PVIgG) and normal human IgG (nhlgG) were obtained by Protein A-Sepharose affinity purification (Caldelari et al., J. Cell Biol., 153, 823-834, 2001). Normal human IgG (nhlgG) were derived from >1'000 healthy donors (Sandoglobulin). Cytoplasmic/low-detergent soluble membrane and nuclear extracts were prepared as described for keratinocytes by Corsini et al with some modifications (Corsini et al., J. Invest. Dermatol., 107, 720-725, 1996). Briefly, cells were lysed with hypotonic lysis buffer containing 0.58% NP-40, scraped and centrifuged. The supernatant (referred to as cytoplasm/membrane fraction) was removed. Pelleted nuclei were resuspended with the aid of a 23 gauge needle and additionally
centrifuged through a 0.85 M sucrose cushion for 15 min at 11700g to remove nucleus-associated endoplasmic reticulum and cellular debris.

*Experiment demonstrating that PVIgG upregulates c-Myc in keratinocyte cultures and in PV patients* (see Fig. 2).

Mouse keratinocytes were cultured as described in above. Human keratinocytes from foreskin were a kind gift form CELLnTc Advanced Cell Systems AG, Switzerland and were cultivated in CnT-07 (CELLnTc, Switzerland). For quantitative PCR (Q-PCR) total RNA was extracted and analyzed using TaqManTM quantitative RT-PCR technology and primers designed and validated as previously described (Kolly et al., J Invest Dermatol, 124, 1014-1025, 2005). All samples were normalized against cyclophilin S1 mRNA and results are reported as n-fold change relative to the sample at calcium switch. Analyses were done in duplicates of at least three independent experiments. Total protein lysates were obtained by scraping keratinocytes into SDS loading buffer and nuclear extracts as described in Fig. 1. Lesional and peri-lesional biopsies of skin, scalp and oral mucosa performed for clinical purposes were used according to the rules of the Medical Faculty. All biopsies were fixed with 4% formaldehyde and paraffin embedded. For immunofluorescence analysis, Biopsies were deparaffinized and antigens retrieved by microwaving three times 5 min at 720 watt in 0.01 M sodium citrate buffer pH 6.0. Sections were stained with c-Myc antibody (Upstate Biotechnology) and nuclei counterstained with Hoechst 33258 (H-1398, Molecular Probes).

*Experiment to demonstrate that PG is a suppressor of c-Myc* (see Fig. 3)

A reporter gene assay was done using the DualLuciferase™ Reporter Assay System (Promega, Wallisellen, Switzerland). One day after seeding, mouse keratinocytes were transfected with Polyethyleneimine (PEI, linear, MW-25,000; Polysciences, Inc Warrington, PA). Briefly, 50 ml medium was mixed with 2 mg of DNA per 15 ml of PEI (1 mg/ml), incubated for eight min, mixed with 450 ml medium/10% FCS and added onto cells in six well plates 24 h post seeding. Two hours later cells were washed with PBS, and incubated with fresh medium for 24 h to 28 h prior to lysis. In each case 1.25 µg for reporter genes (kind gifts of E. Fearon, Ann Arbor, MI (Kolligs et al., Genes Dev., 14, 1319-1331, 2000) and, unless stated otherwise, 0.4 µg of plasmids encoding Lef-1, Tcf-4, DLeM, and PG (all kind gifts R. Klemmer, Freiburg, Germany (Huber et al., Mech. Dev., 59, 3-10, 1996)), respectively, and deletion mutants of PG (kind gift A. Ben-Ze'ev, Rehovot, Israel (Zhurinsky et al., Mol Cell Biol, 20, 4238-4252, 2000)) were transfected. In each case, vector was added to obtain a total of 0.8 µg plasmid encoding exogenous factors. Seven
ng Renilla Luciferase-encoding plasmid was co-transfected as normalizer for transfection efficiency. The ChIP assay was done according to Frank et al. (Frank et al., Genes Dev, 15, 2069-2082, 2001) Briefly, cells were fixed with formaldehyde to cross-link DNA and associated proteins. Samples were sonicated to obtain DNA fragments between 500 and 1000 bp in length as confirmed by gel electrophoresis (data not shown). Prior to immunoprecipitation, samples were adjusted for DNA input as measured by photospectrometry and confirmed by Q-PCR. Each sample was split and immunoprecipitated with LeM, PG or b-catenin antibodies, or rabbit IgG as negative control. 30% of the precipitated material was controlled by western blotting, the remaining reverse cross-linked, and the DNA subjected to Q-PCR using three sets of primers to the mouse c-Myc promoter. The MatInspector program was used to define the TCF/LEF binding site in the mouse c-Myc promoter and primers to areas of interest were designed as described (Kolly et al., J Invest Dermatol, 124, 1014-1025, 2005). The primer express program (Applied Biosystems) did only allow to design Q-PCR primers adjacent to the TCF/LEF binding site. Results are presented as relative change compared to the IgG control. The experiment was done three times.

Experiment to demonstrate that blister formation in neonatal mice is prevented by c-Myc inhibitors as demonstrated by a passive transfer study (see Fig. 4)

Around 16 h after birth, Albino DDY mice where subcutaneously injected in the lumbar area with 6mM c-Myc inhibitors (5521700 (MI-1) or 540471 1 (MI-3); Chembridge) or vehicle (DMSO/PBS). Two hours later, 15mg PVIgG or nhlgG, or alternatively 75µg mouse monoclonal AK23 (Tsunoda et al., J Immunol, 170, 2170-2178, 2003) or NAK3 antibody (M. Amagai, unpublished) were injected in the same area in combination with half the minimal dose of a human PF serum inducing blister formation (Mahoney et al., J. Clin. Invest., 103, 461-468, 1999). Vehicle or c-Myc inhibitors alone were used as negative controls. After 24 h the animals were evaluated for macroscopic blistering. Biopsies were taken from lesional areas, fixed in 4% buffered formaldehyde over night at 4°C and paraffin embedded. Biopsies were deparaffinized and stained with H&E or processed for direct immunofluorescence as described under immunofluorescence analyses. Four animals per treatment (PVIgG, AK23, NAK3, nhlgG, no treatment) each with vehicle or c-Myc inhibitors were analyzed in two independent experiments which showed consistent results (in total 20 animals). Immunofluorescence analysis were as described above.
Experiment demonstrating that blister formation in neonatal mice is prevented by GSK3 inhibitors as demonstrated by a passive transfer study (see Fig. 5)

A passive transfer study (Anhalt et al., N. Engl. J. Med., 306, 1189-1196, 1982) in presence or absence of GSK3 inhibitors SB216763 (Coghlan et al., Chem Biol, 7, 793-803, 2000) and LiCl (Beaulieu et al., Proc Natl Acad Sci U S A, 101, 5099-5104, 2004) was performed. Pemphigus vulgaris antibodies were subcutaneously injected into the lumbar area of neonatal mice that had been pre-injected at the same site with GSK3 inhibitors or vehicle. After 24 hours blisters were observed in pemphigus vulgaris antibody/vehicle injected mice but not in their siblings pre-treated with GSK3 inhibitors or any control conditions. This indicates that these inhibitors are effective therapeutic agents.

Around 16 h after birth, Albino DDY mice where subcutaneously injected in the lumbar area with chemical GSK3 inhibitors, SB216763 (100 mM; 2 mg/g body weight; Calbiochem) and LiCl (200 mg/g body weight) (Beaulieu et al., Proc Natl Acad Sci U S A, 101, 5099-5104, 2004) or vehicle (DMSO/PBS). Two hours later, 15mg pemphigus vulgaris antibodies or the same amount of control antibodies or alternatively 75µg mouse monoclonal AK23 (Tsunoda et al., J Immunol, 170, 2170-2178, 2003) or NAK3 antibody (M. Amagai, Department of Dermatology, Keio University School of Medicine, Tokyo Japan) were injected in the same area in combination with half the minimal dose of a human PF serum inducing blister formation (Mahoney et al., J. Clin. Invest., 103, 461-468, 1999). Vehicle or GSK inhibitors alone were used as negative controls. After 24 h the animals were evaluated for macroscopic blistering. Biopsies were taken from lesional areas, fixed in 4% buffered formaldehyde over night at 4°C and paraffin embedded. Biopsies were deparaffinized and stained with H&E or processed for direct immunofluorescence as described under immunofluorescence analyses. Six animals were pre-injected with vehicle followed by pemphigus antibody or control antibody or vehicle alone. Results obtained were identical per treatment group. Immunofluorescence analysis were as described in Fig. 2.
Claims

1. Use of a glycogen synthase kinase 3 (GSK3) blocker in the manufacture of medicaments for treating pemphigus vulgaris.

2. Use according to claim 1 wherein the GSK3 blocker is a compound which reduces the enzyme activity of a GSK3, inhibits the production of GSK3 or the activation of GSK3 from its latent form, or inhibits the interaction between GSK3 and axin.

3. Use according to claim 1 wherein the GSK3 blocker is a compound which reduces the enzyme activity of a GSK3β, inhibits the production of GSK3β or the activation of GSK3β from its latent form, or inhibits the interaction between GSK3β and axin.

4. Use according to claim 1 wherein the GSK3 blocker is selected from the group consisting of lithium salts, GSK3 antibodies and antibody fragments, thia diazolidinones, inositol-phosphate phosphatases, paullones, 6-bromo-substituted indirubins and maleimides.

5. Use according to claim 1 wherein the GSK3 blocker is selected from the group consisting of NP031 112, lithium salts, LiCl, lithium mimetics, antibodies and antibody fragments to GSK3, SB-216763, SB-415286, indirubin and flavopiridol.

6. Use according to claim 1 wherein the GSK3 blocker is selected from the group consisting of NP031 112, antibodies and antibody fragments to GSK3β, indirubin and flavopiridol.

7. A method of treating pemphigus vulgaris comprising administering a GSK3 blocker in an effective amount to a patient in need thereof.

8. A method of screening for a compound effective in the treatment of pemphigus vulgaris comprising contacting a candidate compound with a GSK3, and choosing candidate compounds which selectively reduce activity of GSK3.
9. A method of screening for a compound effective in the treatment of pemphigus vulgaris comprising contacting a candidate compound with a GSK3, and choosing candidate compounds which selectively reduce activity of GSK3β but not to a reasonable extent GSK3α.

10. A compound selected by the method of claim 8 or claim 9.