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CD151 is a novel autoantigen in patients with cicatricial pemphigoid

Dissertation

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Abbreviations

Ab	Antibody
Ag	Antigen
Auto-ab	Auto-antibody
BMZ	Basment membrane zone
BP	Bullous pemphigoid
BPAG1	Bullous pemphigoid antigen 1
BPAG2	Bullous pemphigoid antigen 2
cDNA	Complementary DNA
CP	Cicatricial pemphigoid
DEJ	Dermal epidermal junction
DIF	Direct immunoflourscence
dNTP	deoxyribonucleotide
g	gram
GST	Glutethione S transferase
h	Hour
HD	Hemodismosome
IgA	Immunoglobuline A
IgG	Immunoglobuline G
IIF	Indirect immunoflourscence
Kbp	Kilo base pair
kDa	Kilo dalton
l	Litre
M	Molar
mg	miligram
Minute	min
ml	mililitre
MMP	Mucous membrane pemphigoid
MW	Molecular weight
µg	microgram
µl	microlitre
µm	Micrometer

ng	nanogram
PAGE	Poly acryleamide gel electrophoresis
PCR	Polymerase chain reaction
RT-PCR	Reverse transcriptase -PCR
SDS	Sodium dodecylesulfate
SSS	Split salt skin
U	Unite

CD151 is a novel autoantigen in patients with cicatricial pemphigoid

CD151 ist ein neues Auto-Atigen in vernarbende Pemphigoid Patienten Seren

Zusammenfassung

Das vernarbende Pemphigoid ist ein autoimmune Blasenbilde Erkrankung, die vorwiegend Schleimhäute, wie orale, konjuktive, als auch genitele Mukose betrifft. Obwohl diese Erkrankung durch schon bekante Pathogene Auto-Antikörper der Klasse IgG charakterisiert ist, könnten noch weitere zusätzliche Krankheitspezifische Auto-Antikörpervorhanden sein, deren „Ziel-Antigen“ noch untersucht werden müssen.

Das transtransmembran protein CD151 wird kürzlich als Komponent der BMZ identifiziert, seine Rolle in autoimmune blasenbildende Erkrankungen ist noch unbekannt. In dieser Untersuchung, würde die Immun-Reaktivität von CP Patienten-Seren (n=22) mit CD151 durch Western Blot –Analyse nachgewiesen werden in den rekombinierten –und natives CD151 Proteine benutzt werden.

Erstmalig konnte gezeigt werden, dass CD151 als Auto-Antigen in 27% der Patienten Seren anzusehen ist.

Die durchgeführten Untersuchungen geben einen Hinweis darauf dass CD151 ein neues Auto-Antigen des vernarben Pemphigoid darstellt. Diese Ergebnisse geben noch weitere Hinweis für die immunologische Heterogenität dieser Krankheit.

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1. Introduction

Blistering diseases of the oral mucosa pose a major challenge in oral medicine, because they are chronic, painful, and interfere with the daily activities and quality of life of the patients, including disturbing eating, drinking, talking, and personal relationships. These diseases can be divided into an autoimmune variant, that is seen mainly in adults, and the inherited variant which is seen mainly in early childhood. Modern molecular biology has greatly increased our understanding of the mechanism of both autoimmune and inherited blistering disorders. Histologically these disorders could be divided into intraeipdermal and subepidermal blistering diseases (Megahed, 2004). Almost any blistering that occurs on the skin may manifest in the oral cavity (Cozzani *et al,* 2004). Cicatricial pemphigoid (CP) is predominantly a mucosal disease that affects the mucous membrane of the oral cavity, conjunctiva, nose, larynx, oesophagus, anus and genitalia (Yin *et al*, 2000). This disease generally affects old people with a peak incidence around 60 years of age, but cases involving children have been also reported (Wetter *et al*, 2005). Females are affected far more often than males, usually in a ratio of 2:1 or even 3:1 (Fine *et al*., 2004). Although this disease is characterised by the presence of auto-antibodies of IgG class binding to different antigens, however, its pathomechanism is not completely known. CP is a subepithelial autoimmune disease, which predominantly involves mucosal surfaces and results in mucosal blistering, ulceration, and subsequent scarring. The condition belongs to a group of mucocutaneous autoimmune blistering disorders collectively referred to as subepithelial bullous dermatoses (SEBDs). These disorders are characterised by blistering of the skin and/or mucosae (Cozzani *et al,* 2004). A final diagnosis may be reached by combining the clinical signs of the patient with the immunohistopathologic findings. CP involvement may include the eyes, oral cavity, and pharyngeal mucosa of patients usually over the age of 50 years. Although CP is a blistering disease

predominantly involving the mucosal surfaces, up to 30% of the patients may also have skin involvement (Cozzani et *al,* 2004). Autoantibodies produced by CP patients target one of several different auto antigens in the mucosal and cutaneous basement membrane zone (BMZ). The interaction between the autoantibodies and their target antigen supposed to cause cleaving of structural proteins in the basement membrane, as well as the activation of complements with the recruitment of neutrophils, which eventually results in subepithelial blistering (Ciarrocca *et al*, 1999): On a molecular level, each of autoimmune SEBDs has distinct autoantigen(s) to which the patient's circulating antibodies react. Over the past 20 years, several different epithelial basement membrane components have been identified.

1.1 Cicatricial pemphigoid

Cicatricial pemphigoid (CP) or Mucous membrane pemphigoid (MMP) is an autoimmune disease characterised by circulating antibodies to proteins of the BMZ of the skin and mucosae. Women are more commonly affected than men and the disease is usually diagnosed between the ages of 40-60 years. The typical lesion is small or large, clear-fluid blister which breaks fairly rapidly in the mouth to leave a flat white, somewhat tender ulcer with a thin red line around it. The gums are especially likely to be involved, resulting in sloughing during eating or tooth brushing ("desquamative gingivitis"). Usually patients with oral involvement will lack major skin involvement, but the eyelids and genital mucosa are quite susceptible to the blistering phenomenon. There is no cure for this pemphigus vulgaris look-alike disease, but lesions frequently respond well to topical or systemic corticosteroids. Death from this disease is extremely rare, but scar formation of the eyelids and eyeball surfaces may lead to blindness if an ophthalmologist does not closely monitor the eyes (Tauber *et al*, 1992).

CP is now recognised to be an immunologically heterogeneous group of diseases with different clinical phenotypes. Several subsets of patients with characteristic antibody systems have been identified. The majority of CP patients' sera recognizes the BPAG2 antigen, and several data indicate, in addition to the NC16A domain, a region nearer to the COOH-terminus as more frequently targeted by circulating IgG auto-antibodies (Bedaner *et al* 1997). Fewer patients have antibodies to the BPAG1 (Murakami *et al,* 1998). A small, about 5%, but well-characterized subset of patients have IgG antibodies directed against laminin 5, as shown by immunoprecipitation and immunoblot studies (Domloge-Hultsch *et al* 1992 and Lazarova *et al* 1998). These antibodies bind to the dermal side of salt split skin (SSS). In particular, the large

majority of anti-laminin 5 CP sera recognizes the α3 chain alone (Lazarova *et al* 1998). *In vitro* data showing that monoclonal anti-laminin 5 antibodies block keratinocyte adhesion and produce epithelial detachment in organotypic skin cultures have suggested a possible pathogenetic role of anti-laminin 5 antibodies in CP-affected patients (Rousselle *et al* 1991). An animal model has then been developed in which passive transfer of purified rabbit anti-laminin 5 IgG (human anti-laminin 5 antibodies do not bind murine DEJ) to neonatal mice induces non-inflammatory subepithelial blisters of skin and mucous membranes in a concentration-related fashion (Lazarova *et al* 1997). Moreover, development of the same lesions in C5- or mast cell-deficient mice, indicatied that anti-laminin 5 antibodies elicit blister formation in this experimental model directly and independently of an inflammatory cascade based on complement activation or mast cell degranulation (Lazarova *et al 1997).*

Among the remaining antigenic molecules possibly recognized by CP sera, the cytoplasmic domain of the ß4 integrin subunit has been reported to represent the target antigen in patients affected with ocular CP, a clinical subset of CP with prominent ocular involvement (Tayi *et al* 1996). A further putative CP mucosal antigen of 168 kDa (M168) has been identified using oral mucosa protein extracts in a subset of CP patients whose sera did not react with epidermal or dermal extracts (Ghohestani *et al* 1996).

Several studies showed that the existence of different immunological subsets of CP that are associated with distinct target molecules are involved in interactions between epithelial cells and extracellular matrix proteins of the basement membrane zone (Kawahara *et al,* 1998). In addition, the finding of CP antibodies not only directed against different epitopes of a single antigen but also recognizing more than one

target molecule at the same time in the same patient illustrates the complexity of the pathogenetic process in CP. However, the relevance of intermolecular epitope spreading of closely associated molecules in the production of these antibodies as well as the respective pathogenetic role of the different antibodies still remain to be established. Therefore to better understand this disease, basic knowledge of the component to the BMZ is needed.

1.2 Structure of basement membrane zone

The BMZ can be schematically divided into keratinocytes, lamina lucida, lamina densa, and sublamina densa (Garrod, 1993). Within the BMZ, hemidesmosomes anchor keratinocytes to the basement membrane. Hemidesmosomes (HD) are attachment structures that mediate adhesion of epithelium cells to the underlying extracellular matrix (Garrod, 1993). Together the HD-anchoring filament complex forms a continuous link between the basal keratinocyte intermediate keratin filaments and underlying BMZ and dermal component. Over the last 5 years, these structures have been shown to comprise of a variety of molecule components (Garrod, 1993).

1.2.1 Keratin intermediate filaments

In normal adult epidermis, the basal keratinocytes cytoskeleton comprises the intermediate filament cytokeratins 5 and 14 (McMillan, 2003). Intermediate filaments are small proteins that form semi-rigid intracellular cytoskeletal network throughout the epidermis and are involved in maintaining the cell shape and epithelial structural integrit (McMillan, 2003).

1.2.2 Plakin family

1.2.2.1 Plectin

There are two plakin family-associated molecules thought to be involved in providing link between the keratin intermediate filaments and the epidermal HD. Plectin and bullous pemphgoid antigen1 are high molecular weight molecules (Fontao *et al,* 2001). Plectin comprises a long rod domain flanked by two large globular domains and is able to self assemble into polymers (Wihe, 1998). Plectin has been identified in almost all cell types including dermal kerationcytes (Wihe, 1998). Plectin is a cytoskeltal cross-linker with its multidomin structure that binds and crosslinks the three major filament networks of actin, intermediate and microtubule filament systems (Fontao *et al,* 2001). Plectin also attaches the cytoskelton to components of certain membrane structures including the epidermal HD. Plectin and BPAG1 show tissue-specific isoform expression pattern and several different plectin alternative spliced isoforms have isolated from tissue including the epidermis (Fontao *et al,* 2001). The functional importance of plectin family is highlighted by plectin defects in the congenital disease epidermolysis bullosa simplex (EBS) associated with muscular dystrophy (McLean *et al, 1996*) and by being the target of an autoimmune disease in paraneoplastic pemphigus (Proby *et al,* 1999) and bullous pemphgoid (Niemi KM *et al*, 1988)

1.2.2.2 Bullous pemphgoid antigen1

The second plakin family member is the bullous pemphigoid antigen1 (BPAG1) that was originally identified as a large 230-KDa intercellular keratin intermediate filament and HD associated autoantigen (Stanle *et al,* 1981). BPAG1 comprises an amino terminal plaktin-like domain (Sawamura *et al,* 1991). BPAG1 is thought to be able to self assemble into dimers similar to other plektin family members (Sawamura *et al,* 1991). It is the most target antigen in bullous pemphigoid where greater than 90% of the patient with this disease have circulating antibodies against it (Yancey, 1995). It is

also reported as the autoantigen in some cases of lichen planus pemphigoid (Ogg *et al*, 1997) and herpes gestationis (Ghohestani *et al,* 1996).

1.2.3 Integrin family

Integrin family is a family of abundant cell surface extracellular matrix expressed on a variety of cell types (DiPersio *et al*, 2000). Integerins comprise covalently associated pairs of transmembrane α and β that act as receptors for specific extra cellular matrix components (Kikkawa *et al*, 2000). Many of the integrin α subunits require binding of divalent cations for stable ligand binding. The precise combination of α and β subunit gives these receptors a high level of specificity for their extra cellular ligands. Evidence from knockout murine model has recently demonstrated an important adhesion role for the α3, α6, β1 and β4 integrin subunits in normal epidermal basement membrane zone formation and adhesion (DiPersio *et al*, 2000). Among various integrins, the α6β4 integrin, expressed within the HD plaque complex, is the main receptor for the extra cellular glycoprotein laminin 5 (Porradori *et al,* 1999). The α6 and the β4 integrin subunits serve as autoantigens in CP. Evidence for the important comes from a severe blistering disease, pyloric atresia-junctional epidermolysis bullousa syndrome arising from defects in α6β4 integrin (Vidal *et al* 1995). Severe defects in the integrin α6 or β4 genes in this condition, lead to the assembly of hypoplastic HDs and poor sub-basal dense plate (Porradori *et al,* 1999).

1.2.4 Laminin family

Laminins are a family of large glycoprotein molecules that are abundant in basement membranes and comprise three distinct chains that together assemble into a cross-shaped molecule (Aumilly *et al,* 1999). Each laminin isoform comprises a unique

combination of three chains ($\alpha\beta\gamma$) with one or more cell adhesion domains. Laminins are part of crucial BMZ network that together with the collagen IV network forms the basic scaffolding for all basement membranes including the epidermal BMZ (Ghohestani *et al,* 2001). Multiple laminin isoforms are expressed in epidermal BMZ (Aumailly *et al,* 1999). Of these laminin 5, 6, and 10 are the main epidermal BMZ specific laminins (Aumilly *et al,* 1999). The structural diversity of laminin chain composition makes highly specialized functions possible (Aumilly *et al,* 1999). Laminin 5, one of the major epidermal laminins, is found at the upper lamina densa/lamina lucida border at the base of anchoring filaments (Masunaga *et al,* 1996). It is hypothesized that laminin 5 may be anchored to the BMZ by binding laminin 6.These dimers may in turn, be bound to other laminin isoforms to form large complexes (Cheng *et al,* 1997). Laminins 6 and 10 have multiple globular domains that enable them to bind nidogen and hence indirectly bind collagen IV within the epidermal BMZ (Aumilly *et al,* 1999). Previously the γ chain of laminin 5 has been implicated in the binding to the basement membrane components nidogen and fibulin 1/2 *in vitro* (Utani *et al*, 1997). These are bridging molecules that may link the laminin and collagen networks. In addition, direct laminin 5-collagen VII interactions have been reported providing multiple points of anchorage for lamina 5 (Rousselle *et al,* 1997). Thus, while many laminin molecules share some functional overlap, the individual chain combinations provide isoform specificity and enable the laminin 5, 6 and 10 to form large complexes. These large complexes enable multiple points of intermolecular binding that aid the incorporation of laminin into the BMZ network (Champliaud *et al*, 1996). The α3 chain serves as target autoantigen in subset of patients with CP (Chan *et al*, 1997).

1.2.5 Tetraspanin family

In the BMZ this family is represented by CD151. The tetraspanin family comprises a group of small, cell-surface proteins that span the membrane four times, forming two extra cellular loops (Maecker *et al,* 1997). CD151 is often codistributed with the laminin-binding integrins $\alpha3\beta1$, $\alpha6\beta1$ and $\alpha6\beta4$ at the keratinocyte basolateral surface where it is specifically enriched at the HD (Sincock *et al,* 1997). Other epidermal tetraspanins, such as CD9 and CD81, are more diffusely distributed around the basal keratinocyte cell surface, similar to the $\beta1$ integrins. It has a molecular weight of 29 kDa. This protein was first identified in cutaneous HDs (Sterk *et al,* 2000). Possible interaction partners for CD151, expressed in similar distribution to CD151, are the $\alpha3\beta1$ and $\alpha6\beta1$ integrins. CD151 like other tetraspanin family is thought to aid in clustering of sufficient numbers of integrin receptors to facilitate cell binding (Sincock *et al,* 1997). CD151 is implicated in cell signaling and morphogensis (Zhang *et a,l* 2002). In addition the CD151 tail is particular important in determining the outside-in functions of $\alpha6\beta1$ integrin that follow ligand engagement (Zhang *et al,* 2002).

1.2.6 Collagen family

Collagens are major connective tissue proteins that comprise a family of genetically distinct molecules all of which have a unique triple helix configuration of three polypeptide subunits with a gly-X-Y repeating amino acid sequence (Gohestani *et al,* 2001). To date, over 20 different collagen family members have been identified inculding collagen XVII, collagen IV and collagen VII (Gohestani *et al,* 2001).

1.2.6.1 Collagen XVII

Type XVII collagen (also known as BP180 or BPAG2), is a transmembrane molecule with type II molecular orientation (with the amino-terminus located in the cytoplasm), the extracellular domain contains interrupted collageous domains (gly-X-Y amino acid

repeats) (Giudice *et al,* 1991). Type XVII collagen molecules, which span the entire length of the lamina lucida (Masunaga *et al* 1997), may play a role in the structure or stability of anchoring filaments. It is a long-held hypothesis that the long extracellular and hook domain of collagen XVII functions as a cell-matrix adhesion molecule (Nonaka *et al,* 2000) which is supported by human disorder, non –Herlitz functional EB in which collagen XVII is genetically defective (McGrath *et al* 1995). In bullous pemphgoid, cicatricial pemphigoid, herpes pemphigoid gestationis and linear IgA disease autoantibodies are targeted against collagen XVII or its 97kDa extracellular domain cleavage product (Ishiko *et al,* 1996). The cytoplasmic domain of collagen XVII binds the $\alpha 6\beta 4$ integrin and BPAG1 (Hopkinson and Jones, 2000). The extracellular domain interacts with the $\alpha 6\beta 4$ integrin (Hopkinson et al, 1995).

1.2.6.2 Collagen IV

At least six forms of collagen IV have been identified in various tissues, the most abundant being the $\alpha 1$ (IV) and $\alpha 2$ (IV) chains present in all BMZ. The $\alpha 5$ (IV) and $\alpha 6$ (IV) chains are also expressed in the epidermal BMZ (Gohestani *et al,* 2001). The classical type IV collagen trimer $\alpha 1$ (IV) $2\alpha 2$(IV) comprises a C-terminal, NC-1 domain adjacent to a flexible 400 nm long interrputed triple helical domain. The heterotrimetric collagen IV molecule can self associate to form dimers via its NC-1 domain and tetramers via the N-terminal domains (Brown and Timpl, 1995). The collagen IV network is linked to the laminins 5/ 6 /10 complex by the small adhesion molecule, nidogen (Gohestani *et al*, 2001). In the Goodpasture's disease the antibodies are directed to the C-terminal non-collagenous domain of this collagen (Persson *et al*, 2004).

1.2.6.3 Collagen VII

Beneath the lamina densa lie anchoring fibrils, fan-like, cross-banded structures extending into the papillary dermis that form semi-circular loops, anchoring fibrils (Shimizu *et al,* 1997). Anchoring fibrils consist of antiparallel disulphide-bonded post-translationally modified collagen VII α1 (VII) 3 trimers. The large NC-1 globular domains are assembled from each of the three chains. The NC-1 domain, located exclusivly within the lamina densa, contains several motifs that are putatively involved in epidermal BMZ adhesion. Laminin 5 complexes may be directly bind to the collagen VII NC-1 domain via the short β and γ2 chain arms (Chen *et al,* 1997). Collagen VII is a 290 kDa (Burgeson, 1993). It is the auto-antigen in epidermolysis bullousa acquisita (Woodley *et al,* 1984), bullous systemic lupus erythematosus (Gammon *et al,* 1985) and a subset of linear IgA disease (Zambruno and kanitaki, 1996). Figure 1 show the protein constituents of the cutaneous BMZ.

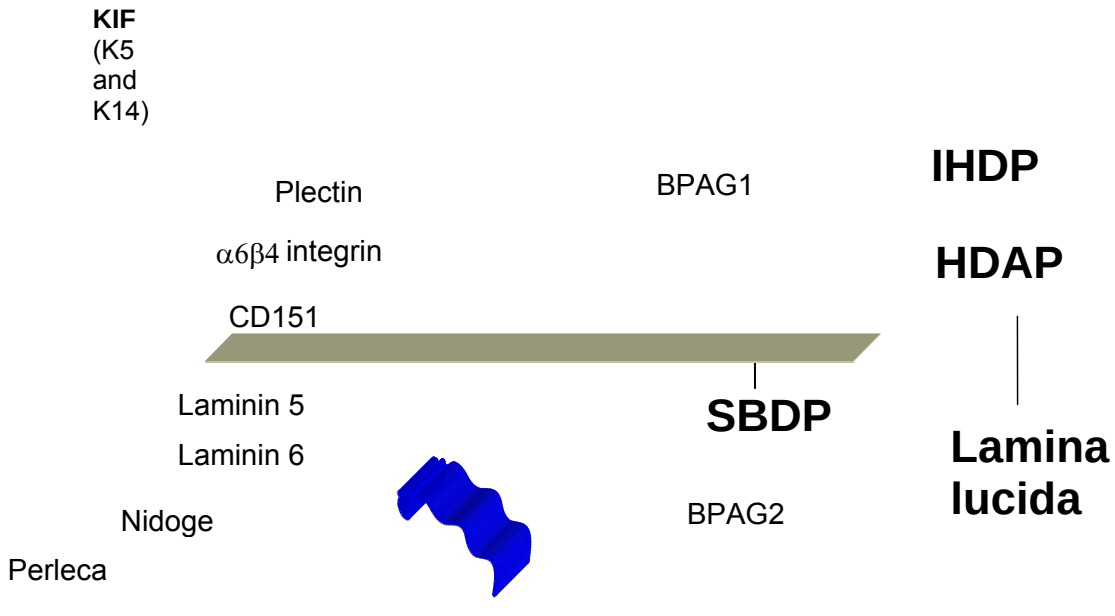

Figure. 1. Diagrammatic presentation of the protein constituents of cutaneous BM zone. (from Megahed 2004)

1.3 Epidemology of CP

The exact incidence of CP is unclear (Scully *et al*, 1999). Data in the medical literature suggest that CP is approximately seven times less common than BP (Bernard *et al*, 1995). Ocular CP may occur in 1 of every 15 000 to 40 000 individual seen by an ophthalmologist (Mondino *et al*, 1981). Retrospective immunoflurescent studies in oral mucosal diseases suggest that CP occurs up to three times more frequently than pemphigus (Daniels *et al,* 1981).

1.4 Clinical presentation of CP

The disease onset of CP is usually between 40 and 60 years of age, and oral lesions represent the first manifestation of the disease in two-third of cases. There is no racial or ethnic predilection, but the disease is much more common in women than in men in ratio 1:2 (Ahamed *et al*, 1991). Clinically, CP presents with erosive and/or vesiculobullous lesions that predominate on mucous membranes in the mouth, eye, throat, nose, esophagus, and/or anogenital regions (Yancey, 1999) Individual lesions develop slowly and are usually smaller and less frequent during the early months or years of the disease. Bullae may, however, become more than 3 cm in diameter and may remain intact long enough for the clinician to see them A ruptured blister leaves a shallow, mildly tender ulcer bed which heals in 7-10 days. Large or secondarily infected lesions may result in scar formation.

1.4.1 Oral manifistation

Oral lesions occur in more than 90% of patients with CP (Fine *et al*, 2004). Oral sites most often involved include the gingiva (90% of oral cases), palate and buccal mucosa. While individual blisters do not necessarily recur at the same exact site, new lesions do seem to remain localised within a limited anatomic region. When only the

gingiva or alveolar mucosa are involved, the disease has a special tendency to remain localized, and this has led to some authorities to prefer the more generic term, desquamative gingivitis. Oral manifestations of CP are variable. Patients often present with the complaint of bleeding, pain dysphasia and peeling of the mucosa (Mobini *et al,* 1998). Desquamative gingivitis is the most characteristic feature of CP (Sollecito and Parisi, 2005). Desquamation is likely resulted from frequent exposure of the oral mucosa to inflammation as well as trauma from mastication. It may present clinically as white areas of necrotic slough at the margins of the erythmatous zone and may be elicited by palpation with finger, mouth mirror or periodontal prob. Oral blisters or bulla may occasionally be seen, although they tend to rupture quickly as result of mechanical or traumatic force. Other regions of oral mucosa that may be involved are the tongue, palate, buccal mucosa, and floor of the mouth (Sacher *et al*, 2005). Patients with gingival involvement frequently have poor oral hygiene because of inability to clean the dentition effectively secondary to mucosal pain. Thus patients may often present with bleeding gums. Patients typically describe the inability to eat certain type of foods. Occasionally patients may complain of halitosis (Sollecito and Parisi, 2005) from lack of maintaining good oral hygiene. Other common clinical observations include delayed or incomplete healing following scaling and root planning or peeling of the gingival tissue with simple prophylaxis. Occasionally the signs and symptoms of CP may be subtle. Some patients may notice a superficial sloughing of the oral mucosa, whereas other patients may describe a transient fluid-filled blister that ulcerates and quickly heals. On the other hand, long standing lesions related to CP may be secondarily infected, sore, and slow to heal (Ahmed *et al*, 1991).

1.4.2 Extraoral manifestations

CP can involve the conjunctiva, genitalia, oesophagus, trachea, and larynx (Fleming *et al*, 2000). Eye involvement may be characterized by conjunctival injection; symblepharon formation may occur also, symblepalpebral formation results from the scarring and adhesion of the bulbar to the pleural conjunctiva. As a result, corneal damage is common; and progressive scarring can lead to blindness (Ahamed *et al*, 1991). Involvement of the oesophagus may results in dysphagia and odynophagia, whereas the tracheal involvement may lead to hoarseness (Warren and Lesher, 1993). Genital involvement may lead to sexual dysfunction resulting from pain. CP involves the skin in up to 30% of patients (Greenberg, 2003). Cutaneous lesion may be localised or disseminated (Ahmed *et al,* 1991). They consist of tense blisters, erosions and crusts on normal or erythematous skin and tend to heal with scar formation.

1.5 Aim of my study

CP is characterized by the presence of autoantibodies of IgG class binding to different antigens. However, there might be additional CP disease specific autoantibodies whose target antigens still remain to be determined. CD151 is recently identified as component of the BMZ, however its functional role in the autoimmune blistering diseases sofar is unkown.

The aim of this work was to identify a novel IgG autoantibody in cohort of CP sera that may be specific to tetraspan CD151.

The program which is proposed to carry out this study is as follow:

1- Production and purification of recombinant CD151 protein.
2- Evolution of the quality and the specifity of the recombinant CD151 using anti CD151 monoclonal antibody.

3- Carrying out immunoblotting and immunoprecipitation analysis using CP sera and recombinant as well as CD151 protein.

2. MATERIALS and METHODS

2.1 Materials

2.1.1 Chemicals

Chemicals used in this study were commercially purchased from the following companies:

Roche (Mannheim, Germany), Roth (Karlsruhe, Germany), Sigma (Munich, Germany) and Serva (Heidelberg, Germany). All chemicals were ordered at“ACS” or “p.A.” purity grade. All standard solutions, buffers, and media were prepared according to Sambrook *et al*. (1989), Ausubel *et al*. (1995) and Coligan *et al*, (1995). Compositions of non-standard solutions or buffers are listed at the end of the respective method section. Media for cultivating bacteria were sterilized by autoclaving (121oC/1-2 bar), all other solutions were sterile filtered (0.2 µm). Thermo labile components such as antibiotics were sterile filtered and added to the media after autoclaving and cooling to 50°C.

2.1.2 Buffers and Solutions

Buffer/solution	*Composition*
DNA loading buffer	20% Ficoll 400, 100mM EDTA, 0.25% Xylen-Cynol, 0.25% bromophenolblue, 0.25% OrangeG
1x PBS	140mM NaCl, 6.5mM Na_2HPO_4 2.5 mM KCL 1.5 mM KH_2PO_4 (pH 7.5)
1x TBE	100 mM Tris-HCl, 100 mM boric acid, 2.5 mM EDTA
1x TBS	20 mM Tris-HCl, 100 mM NaCl [pH 7.5]
1x TBS-T	1x TBS + 0,05% Tween 20
Stacking gel buffer (4x)	0.5 M Tris-HCl [pH 6.8]

Separating gel buffer (4x)	1.5 M Tris-HCl [pH 8.8]
Laemmli running buffer	25 mM Tris-HCl, 192 mM glycin, 0.1 % SDS [w/vol]
5 x SDS sample buffer	50 mM Tris-HCl [pH 6.8], 4% [w/vol] SDS, 12% glycerol, 0.01% [w/vol] Coomassie Brilliant Blue G-250, 4% [w/vol] ME
Cell lysis buffer (SDS-PAGE)	50 mM Tris-HCl [pH 7.5], 1% Triton, 1 mM Pefabloc
10 x blotting buffer	250 mM Tris-HCl [pH 8.3], 1.92 M glycin, 10% methanol, 0.01% SDS
Silver stain fixation solution	10% [vol/vol] acetic acid, 30% [vol/vol] ethanol
Silver stain sensitizer	30% [vol/vol] ethanol, 0.5M sodiumacetate, 0.5% [vol/vol] glutaraldehyde, 0.2% [w/vol] sodiumthiosulfate
Silver nitrate solution	0.1% AgNO3, 0.02% formaldehyde (37%)
Silver stain developer	2.5% [w/vol] Na2CO3, 0.01% [vol/vol] formaldehyde (37%)
Silver stain stop solution	1% [vol/vol] acetic acid
Western blot stripping buffer	5 mM NaPO4 [pH 7.5], 2 mM b-ME, 2% SDS
RIPA buffer	120 mM NaCl, 50 mM Tris-HCl [pH 7.5], 1% Triton X-100, 0,5% DOC, 0,1% SDS, 200 µM NaVO4, 20 mM NaF, 1 mM PMSF
IP wash buffer	50 mM Tris-HCl [pH 7.5]
Buffer (protein purification)	50 mM NaH2PO4 [pH 7.5], 300 mM NaCl

2.1.3 Chromatography column material

Glutathione sepharose 4B from Amersham Pharmacia Biotech was used for purification of GST fusion proteins

2.1.4 Antibodies

2.1.4.1 Primary antibodies

Mouse monoclonal anti CD151 antibody (serotech, Germany)

2.1.4.2 Secondary antibodies

Alkaline phosphatase-conjugated Goat Anti Mouse IgG (H+L) (Sigma, Germany)

Alkaline phosphatase-conjugated Goat Anti Human IgG (H+L) (Sigma, Germany)

Alkaline phosphatase-conjugated Goat Anti Mouse IgA (Sigma, Germany)

Alkaline phosphatase-conjugated Goat Anti Human IgA (Sigma, Germany)

FITC-conjugated goat anti-human IgG anti-human (Sigma, Germany)

FITC-conjugated goat anti-human IgA anti-human (Sigma, Germany)

2.1.5 Kits

Qiagen plasmid isolation kits (Qiagen, Germany)

QIAquick PCR purification/gel extraction/nucl. removal Kit (Qiagen, Germany)

Rneasy for RNA extraction (Qiagen, Germany)

Applied Biosystems Bigdye terminator sequencing ready reaction kit(Perken Elmer USA)

Bio-Rad protein Assay kit (Bio-Rad, Germany)

2.1.6 Enzymes

EcoR I enzyme (Genecraft,Germany)

T4-Ligase (Genecraft, Germany)

RNAse-inhibitor (Genecraft,Germany)

Alkaline phosphatase (Genecraft, Germany)

Taq DNA polymerase (Genecraft,Germany)

2.1.7 Media

Luria-Bertani broth and agar (Gibco BRL, Germany)

SOC medium was provided by (Invitrogen, Germany)

2.1.8 Antibiotics

Ampicillin: 50 mg/ml (Roth, Germany)

-Stock in H2O (aliquots stored at –20°C), used at 50-100 µg/mLfor the cultivation and selection of *E. coli* in liquid culture and on agar plates.

2.1.9 Bacterial strains

The *E. coli* strains used in this work are listed in the table1.

Table 1: *E. coli* strains used in this work.

Strain	**Genotype**	**Source**
One Shot Top 10 Chemically competent *E.coli*	F- mcrA Δ(mrr-hsdRMS-mcrBC) Δ80lacZΔM15 ΔlacX74 deoRrecA1 araD139 Δ(ara-leu)7697 galU galK rpsL(StrR) endA1nupG	Invitrogen
BL21(DE3)	F- *ompT hsdS*(rB- mB-) *dcm*+ Tetr *gal* λ(DE3) *endA* Hte (pLysS Camr)	Stratagene

2.1.10 Vectors and plasmids

Vector and plasmids used in this work work are listed in the table2.

Table 2: Plasmids that were used to express CD 151 in *E.coli*

Name	Description	Reference
PGEM-T easy vector	Cloning vector for cloning of Taq-amplified PCR products prior tosubcloning of these constructs into the final target vectors. Ampicillin and Kanamycin resistance	Invitrogen
PGEX-5X-3	*E.coli* medium copy number expression vector with ampicillin resistance gene. Expression is induced by IPTG. Proteins are expressed as N-terminal GST fusions. A Factor X cleavage site allows for the removal of the GST tag. Ampicillin resistance	Amersham Biosciences

2.1.11 Eukaryotic cell line

Human keratinocyte cell line (PromoCell, Germany)

2.1.12 Oligonucleotides

Primers used for PCR amplification of CD151 cDNA

Sense 5‘-CCGAATTCCGCGATGGGTAGTTCAACGAGAAG-3‘

Antisense 5‘-CCGAATTCCCGAGGGTCAGTAGTGCTCCAGCTT-3‘

2.1.13 Equipment and applications

Biochrom 20 amino acid analyser (Amersham Pharmacia Biotech) and EZchrom V6.7 data system software (Amersham Pharmacia Biotech).

Cameras: MP4 (Polaroid, Cambridge, MA, USA). E.A.S.Y 429K camera (Herolab, Wiesloch).

Centrifuges: AvantiTM 30 and AvantiTMJ-25 (Beckman, California, USA), Biofuge A (Heraeus, Hanau), Sigma 3-10 and Sigma 4-10 (Sigma, St. Louis, Missouri, USA), RC5C and RC5B plus (Sorval instruments, Du Pont, Bad Homburg). Rotors: F0650, F2402H, JLA 10.500 and JA 25.50 (Beckman), #1140 and #11222 (Sigma), RLA-300, SS-34 and GS-3 (Du Pont).

Chromatography equipment: ÄKTA explorer 10xT (Amersham Pharmacia Biotech), Gelfiltration XK26 column 26 mm inside diameter, 20 cm length (Amersham Pharmacia Biotech), 150 ml super-loop (Amersham Pharmacia Biotech) and UNICORN control, evaluation and documentation software (Amersham Pharmacia Biotech).

DNA gel electrophoresis apparatus: wide mini and mini cells for DNA agarose electrophoresis and power supplies (Bio-Rad).

DNA-sequencing machine: LI-COR IR2-4200 Sequencer (LI-COR, MWG-Biotech) and Base ImageIRTM 4.0 software (LI-COR).

Electroporation apparatus: “Gene pulserTM”, “Pulse controller” unit, Extender unit (BioRad) and 0.2 cm cuvettes (Bio-Rad).

InnovaTM 4340 incubator shaker (New Brunswick Scientific, Nürtingen).

PCR Thermocyclers: Primus and Primus 96 plus (MWG-Biotech).

Photometers: Spectrophotometer Uvikon 930 (Kontron, Neufahrn) and multi-channel spectrophotometer Spectromax 340 (Molecular Devices, Sunnyvale, Kalifornien).

Probe sonicator: (Braun Biotech, Melsungen).

Protein gel electrophoresis equipment: Mini PROTEAN IITM from BioRad. Gel Air Dryer (Bio-Rad).

Surface plasmon resonance: BIACORE® 2000 (BIACORE , Uppsala, Schweden) + PC, Windows NT 4.0 operating system (Microsoft) and Software (BIAControl 1.3 and BIAEvaluation 3.0).

UV-Transilluminators: wavelength 302 nm and UVT-20M (Herolab). UV-chamber (Bio-Rad).

Software: Windows NT 4.0 operating system (Microsoft); Microsoft Office 2000 (Microsoft); Adobe Photoshop 6.0 (Adobe); Chromas; Origin 6.0 (Data analysis and technical graphics, Microcal Software, Inc.); GCG (Wisconsin Package TM of Genetic Computer Group).

2.2 Methods

2.2.1 Selection of the patients

A survey of the archives of the departments of dermatology, oral surgery of Düsseldorf University between 1994-2005 revealed 70 patients with cicatricle pemphigoid .The selection of CP patients was based on the following criteria:

1-clinical features

2-histological findings

3-postive direct immunofluorescence DIF

4-postive indirect immunofluorescence IIF

2.2.1.1 Histological examination

To perform the histological examination, the specimens were fixed in 10% neutral buffered formalin embedded in paraffin. Serial sections were cut and stained with hematoxylin and eosin. The histological examination and evaluation of the specimens were performed according to the following qualitative and semiqualitative parameter:

1. Vacuolar degeneration of basal cell layer
2. Subepidemal split as well as blister formation
3. Infilteration distribution and muster
4. Cell infilterate
    - Eosinophil granulocytes
    - Neutrophil granulocytes
    - Lymphocytes
    - Plasma cell
5. Presence or absence of fibrosis

2.2.1.2 Direct immunofluorescence

For the direct immunofluorescence (DIF) the tissue samples (n=22) was cut (4µm) the section were put on the slide and allowed to air dry. The section were placed in ethanol/acetic acid fixative solution for 2-10 min, than rinsed three times in tap water 5 min each. The endogenous peroxidase and peroxidase-like activity were blocked by the incubation in 1.5 % H_2O_2 in PBS. The samples were then rinsed and incubated in FITC-conjugated goat anti-human IgG or IgA antibodies. After washing the slides were mounted with DAKO-Fluorescent mounting medium contain 15 mM NaN3.At the end, the immunoactivity of the antibodies were detected using a Zeiss fluorescence microscopy.

2.2.1.3 Indirect Immunofluorescence

Biopsies of normal human skin were mounted in OCT compound and immediately snap-frozen in liquid nitrogen. Cryostat sections (4µm) were mounted onto gelatin-coated slides and air-dried. Human sera and rabbit antisera diluted 1:50, 1:100 and 1:200 in PBS (150 mM NaCl, 16 mM Na_2HPO_4, 4 mM NaH_2PO_4, pH 7.3) were added and the slides were incubated at room temperature for 1 h. After washing in PBS for several times for 30 min, fluorescein conjugated secondary antibodies were incubated for 1 h at room temperature. The sections were then washed again in PBS and mounted in Vectorshield mounting medium (Vector Laboratories, Burlingame, CA) and viewed under a Zeiss fluorescence microscopy. The fluorescein conjugated secondary antibodies used here were: goat anti-human IgA and IgG (Sigma, Germany).

2.2.2 Microbiological methods

2.2.2.1 Cultivation of *E.coli*

E.coli was grown under aerobic conditions at 37°C on LB agar plates or in LB broth containing the appropriate antibiotics. Liquid cultures were incubated on a shaker at 220rpm. Glycerol stocks were prepared by mixing liquid cultures 1:1 with 40% glycerol [vol/vol], freezing the mixture in liquid nitrogen and keeping the stocks at -80°C for long-term storage.

2.2.2.2 Transformation of *E.coli*

For transformation 3-5 µl ligation reaction or 1 µl of prepared plasmid DNA were added to the chemically competent *E.coli* cells. The cells were incubated on ice for 5 to 10 min. Thereafter supplier's protocols for chemical transformation were followed. Finally, after addition of 250 µl SOC cells were grown at 37°C at 220 rpm for 60 min before the whole reaction was plated on selective agar plates.

2.2.2.3 Incubation of Keratinocytes cell culture

Normal human keratinocytes were cultured in154 CF keratinocyte medium (both obtained from PromoCell, Germany). Cell culture was maintained in a 37°C incubator in a moist atmosphere of 5% CO2.

2.2.2.4 Generation of cell lysates

After two washings with ice-cold PBS Keratinocyte cells were scraped off from 25 cmPP^2 cell culture flasks with a plastic scraper. Cells were resuspended in 200 µL/25 cm2 ice-cold cell lysis buffer and lysed by ultrasonication for 15 sec at setting two. Lysates were stored frozen at –20°C until analysis by SDS-PAGE and Western blotting.

2.2.3 Molecular biological methods

2.2.3.1 Isolation of RNA from keratinocytes cell culture

Total RNA was isolated from keratinocyte cell culture using the Qiagen RNEasy RNA extraction kit according to the manufacturer's suggestions. RNA was dissolved in H_2O and stored at -80°C.

2.2.3.2 Total RNA extraction

The total RNA was isolated from keratinocytes using Rneasy Mini Kit (Qiagen, Germany) as following, the cell were washed with PBS and then lysed with 500µl RTL Buffer. The cell lysate were applied into QIAshredder column and allowed to centrifuge at 10.000 rpm for 1min. After mixing with equal volume of 70% ethanol. The lysate were mixed well by pipting and then allowed to apply into Rneasy mini spin column and centrifuged at 10.000 rpm for 1min. The spin column was washed with 500 µl of RW1 buffer by centrifugation at 10.000 rpm for 1min. After washing with RPF buffer the spin column put in new collection tube. At the end, the spin column was allowed to centrifuge further for 2 min at 10.000 rpm. The total RNA was eluted with 40 µl of RNAase-free water, after the incubation at room temperature for 5 min the total RNA was collected by centrifugation for 2 min at 10.000 rpm. The concentration of total RNA was determined and stored at –80C until use.

2.2.3.3 Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)

In order to obtain cDNA coding for open reading frame (ORF) of the CD151, the following primers were used:

Sense 5'-CCGAATTCCGCGATGGGTAGTTCAACGAGAAG-3'

Antisense 5'-CCGAATTCCCGAGGGTCAGTAGTGCTCCAGCTT-3'

Primers were designed as to allow amplification of the 725 bp of the ORF coding for the CD151. In this work one step RT-PCR kit was used for amplified cDNA strand from a single stranded mRNA substrate. The cDNA was made by mixing the following in PCR soft tubes 0,2ml:

4 µl total RNA

1 µl sense primer

1µl antisense primer

2 µl dNTP

10 µl 5x QIAGEN One Step RT-PCR buffer

1 µl RNase

2 µl Enzyme mix

30µl RNase free water

The thermal cyler was programmed as follow:

Reverse transcription30 min 50 °C

Initial PCR activation 15 min 95 °C

Denaturation 1min 94 °C

Annealing 1min 60 °C

Extension 1min 72 °C

35 cycle

Final extension 10min 72 °C

The PCR products were then analyzed on a 2% agarose gel.

4.2.3.2 Standard PCR

Standard PCR reactions were carried out in 100 µl volumes using the GeneAmp PCR System 9700 from Applied Biosystems. The following reagents were mixed: Expand High Fidelity PCR System Polymerase with the supplied buffer 2, 100 ng

template DNA, 30 pmol of each primer, 10 mM dNTPs each. The cycling protocol was started with a denaturation step for 3 min at 94°C followed by 30 cycles with 30 sec at 94°C denaturation, 30 sec 50°C primer annealing and 3 min at 72°C polymerization, followed by a final polymerization step at 72°C for 7 min. For analysis PCR samples were mixed with SYBR Green according to the supplier's manual. Fluorescent PCR fragments were separated on 0.8-1.5% agarose gels prepared with 1x TBE and visualized with the GeneGenius Bio Imaging System from Syngene. Alternatively linear DNA could be analyzed with the Agilent 2100 Bioanalyzer according to the manufacturer's protocol using the DNA 1000, DNA 7500 or DNA12000 chips. For further use PCR fragments were purified using the Qiaquick PCR purification kit (Qiagen, Germany). Gel extraction was used if various products were generated.

2.2.3.5 PCR products purification

DNA was recovered from PCR reaction mixture according to the manufacturer's instructions .

2.2.3.6 Cloning procedures

PCR fragment and plasmid were digested with the appropriate restriction endonucleases using 10 U/µg DNA following the supplier's manual. After purification or gel extraction (QIAquick Gel extraction Kit) ligation of DNA fragments was performed using T4 DNA ligase *E. coli* Top 10 were used for plasmid transformation according to instructions. Plasmid isolation from 2 ml of an overnight culture was performed using Qiaprep Spin Kit and sequences were verified by restriction endonuclease cleavage, gel analysis and sequencing. Larger amounts of plasmid DNA were prepared with the Qiagen Plasmid Maxi Kit.

2.2.3.6 Cloning of PCR products in GST-expression vector

To clone the PCR products in PGEX-5X-3 vector, the PCR products and the expression vector were first treated with EcoR I restriction enzyme (according to the manufacture's instructions on the information sheets supplied with enzyme. Following the enzyme digestion, the expression vector was dephosphorylated in 1x dephosphorylation buffer and 1-2 U alkaline phosphatase at 37°C for 30 min to protect the vector from self-ligation. The dephosphorylated vector and the purified PCR products were mixed together in a reaction volume of 20 µl containing 50 ng vector, 150 ng PCR products, 2 µl of 10x ligase buffer and 5 U T4 ligase, then allowed to incubate over night at 4°C.

2.2.3.7 Restriction of PCR products with EcoR I enzyme

10 µl	PCR product
5 µl	10x EcoR restriction buffer (H)
1 µl	20 U EcoR I enzyme
44 µl	Dist. water

The reaction volume was then incubated at 37°C for 3 h

2.2.3.8 Restriction of Cloning vector (pGEX) with EcoR I enzyme

10 µl	pGEX vector
5 µl	10x EcoR restriction buffer (H)
1 µl	20 U EcoR I enzyme
44 µl	Dist. water

The react mixture was incubated at 37°C for 3 h

2.2.3.9 Dephosphoralation of the digested vector with alkaline phosphatase

30 µl	Digested vector
5 µl	alkaline phosphatase buffer
3 µl	alkaline phosphatase enzyme
12 µl	Dist. water

The react mixture was incubated at 37°C for 30 min.

2.2.3.10 Ligation of PCR products into pGEX vector

Blunt-ended PCR products generated by Pfu DNA polymerase were ligated into pGEX vector (Stratagene #211188), as following:

10 µl	DNA
5 µl	Vector (dephosphorelated)
5 µl	Ligation Buffer
2 µl	DNA ligase
8 µl	Dist. water

The reaction mixture was incubated at 4°C for 16 h. Ligation reaction were stored at 4°C until use.

2.2.3.11 Transformation of BL21 with Gst-CD151 plasmid

1 ml of the ligation mixture was added to 50 ml of competent BL121 cells in 1.5 ml Eppendorf tube. The tube was placed on ice for 30 min followed by heat shock for 90 sec in 42°C water bath. The sample was placed again on ice for 2 min and 200 ml LB was added. After incubation at 37°C with shaking (200 cycle/min) for 40 min, the cell culture was spread on LB ampicillin agar plates (50 ml/plate) and incubated overnight at 37°C. The growing colonies were transferred individually with a sterile toothpick to

10 ml LB supplemented with ampicillin at 50 mg/ml culture. The cells were grown to saturation at 37°C with shaking overnight. Plasmid DNA was then extracted using standard procedures. The DNA was then automatically sequenced and pGEX-5X-3 containing CD151 in the correct frame was used for fusion protein production**.**

2.2.3.12 DNA Sequencing

Sequencing of plasmid DNA was carried out with the Applied Biosystems Bigdye terminator sequencing ready reaction kit, basically as described in the manufacturer's manual. 2µL BigDye mix, 1 µL 5x buffer, 5 pmoles of sequencing primer and 250 ng plasmid DNA were brought to a total volume of 10 µL with HPLC grade H20 (Merck) and mixed in a PCR tube. The PCR protocol started with a denaturation step for 3 min at 96°C followed by 25 cycles with 10 sec at 96°C denaturation, 5 sec 50°C primer annealing and 4 min at 60°C polymerization, followed by a final polymerization step at 60°C for 1 min. The product DNA was purified with the Qiagen DyeEx spin kit 2.0 as described by the supplier, except that columns were additionally washed with 700 µL HPLC grade H20 prior to loading the PCR reaction onto the column. 10 µL of HPLC grade H_20 were added to the loaded columns prior to centrifugation. 10 µL of purified DNA were mixed with another 10 µL of HPLC grade H_20 in 0.5 mL sample tubes and were then ready for sequencing. DNA was sequenced in an ABI Prism 310 Genetic analyzer (Applied Biosystems) following the manufacturer's protocol. Sequences were analyzed with Chromas 1.45 and VectorNTI software.

2.2.4 Biochemical methods

2.2.4.1 Expression and purification of CD151 protein antigen

Protein expression was induced in transformed *E.coli* (strain BL21 (DE3), Stratagene) at an OD600 of 0.5 and a growth temperature of 21°C overnight or at

37°C for 4h with 1 mM IPTG. Cells were harvested by centrifugation. The pellet of GST-tagged protein was resuspended in 20 ml buffer I, ruptured in a French pressure cell at 16,000 p.s.i. and centrifuged for 30 min at 17.300 x g. In case of the protein being soluble, the supernatant was loaded onto a Ni-NTA superflow column from Qiagen (Hilden, Germany) equilibrated with buffer I. The column was washed with 20 mM imidazole and eluated with 250 mM imidazole, both in the same buffer. GST-tagged protein was loaded onto Glutathione-Sepharose columns (Amersham Biosciences) equilibrated in buffer I. After washing with buffer I, the protein was eluated with 10 mM glutathione. If the protein was insoluble in inclusion bodies, 6 M Gua was used to solubilize the protein. After an additional centrifugation step at 17.300 x g, the supernatant fraction was loaded onto the respective column. 8 M urea was added in this case to buffer I for all following purification steps. The eluates from either column type were run on preparative 20x14 cm SDS-PAGE gels, which were stained with Coomassie Brilliant blue in water. Following the destaining of the gels with water, the protein bands of interest were cut out. Elution of the protein was carried out, by placing the gel slice into a dialysis membrane bag in SDS running buffer and by applying an electric field in an SDS-PAGE chamber.

2.2.4.2 SDS-PAGE

10 to 15% polyacrylamide gels were prepared as described (Laemmli *et al*.1970). Full Range Rainbow molecular weight marker (Amersham Biosciences) was used as a protein standard. Samples were mixed with 5 x SDS sample buffer and boiled for 5 min at 95°C. After brief centrifugation samples were loaded onto SDS-PAGE gels. Minigels were run at 100 V to 200 V in a Mini-Protean II electrophoresis cell (Bio-Rad)

2.2.4.3 Silver staining

Silver staining of SDS-PAGE protein gels was performed according to a modified protocol of Heukeshoven. In brief, gels were fixed for 20 min in fixation solution, incubated for 30 min in sensitizer and then washed three times for 20 min in MQ. After incubating the sensitized gel in fresh silver nitrate solution for 30 min, the addition of developer led to visualization of protein bands/spots on the gel. Soaking the gel for 5 min in stop solution stopped this process. After four consecutive washing steps in MQ for 5 min the gels could be scanned using the FLA-5000 scanner (Raytest). For long term storage or for autoradiography gels were dried in between two sheets of cellophane in a GelAir Dryer (Bio-Rad) for 2h.

2.2.5 Immunological methods

2.2.5.1 Immunoprecipitation (IP)

In order to precipitate CD151 from a cell lysate with a specific monoclonal anti-CD151 antibody (Serotech), cell cultures in 10 cm tissue culture petri dishes were harvested in RIPA buffer. First, cells were washed twice with ice-cold PBS. Thereafter cells were scraped off with a plastic scraper, resuspended in 1 mL of ice-cold RIPA buffer containing protease inhibitors (1 mM Pefabloc SC (Serva) or PMSF (Roche) and optionally phosphatase inhibitors (20 mM NaF, 10 mM b-glycerophosphate, 1 mM sodium pyrophosphate, 200 µM NaVO4). Lysis was performed by ultrasonication with the Branson sonifier at level 3. Cell debris was removed by centrifugation at 12.000 x g for 20 min at 4°C. 50% slurry of Protein A Protein G (1:1) Sepharose beads with RIPA buffer was prepared with the IP Starter Pack (Amersham Biosciences) as described in the manual. Supernatants of the cell lysates were mixed 50:1 with antibody. In order to capture the antibody protein complexes 30µL slurry per mL lysate were added. The IP mixtures were incubated

overnight at 4°C in an overhead shaker. The next morning IP beads were pelleted by centrifugation with 12.000x g at 4°C for 30 sec. The supernatant was discarded and the beads were washed three times with 1 mL ice-cold RIPA buffer and once with IP wash buffer. Thereafter IP beads were ready for downstream kinase assays or SDS-PAGE analysis. For short term storage beads were frozen in 30% glycerol at –20°C.

2.2.5.2 Western blotting

Protein from SDS-PAGE gel was transferred to Protran Nitrocellulose transfer membranes (Schleicher & Schuell, Dassel, Germany) using a wet blotting system (TE series Transphor Electrophoresis unit by Hoefer) with 1 x blotting buffer at 950 mA for 45 min. Blots were blocked with 5% BSA in 1 x TBS by slight shaking for 2h at room temperature. Afterwards blots were incubated with antibodies, diluted in TBS + 0.05% BSA, overnight at 4°C. Antisera directed against CD151 protein was used at a 1:100 dilution, After three consecutive washing steps with TBS-T for 10 min, bound antibody was detected using a 1:5000 dilution of alklaine phosphtase conjugated secondary antibody (Sigma, Germany) for 2 h at room temperature. After three further washing steps with TBS-T, Western blots were developed with BCIP/NBT Alkaline phosphatase Western blotting substrate (Sigma, Germany).

2.2.5.3 Stripping of Western blots

Bound antibody was removed from Western blots by incubation for 30 min at 60°C in western blot stripping buffer. After washing the Western blots three times for 10 min with TBS-T, Western blots were ready for another immunodetection of protein.

2.2.5.4 Design and characterization of CD151 protein

For the construction of a CD151 fusion protein expression vector pGEX was used. Identity and size of the purified recombinant protein were investigated via SDS PAGE analysis followed by Western blotting. After gel filtration, a single band was visible in SDS-PAGE.

3. Resultes

3.1 Patient's data

During the past 8 years, 70 patients with CP were diagnosed and treated at the departments of dermatology/oral surgery university of dusseldorf. In all these patients the diagnosis of CP was confirmed by the histopathology and immunoflurscence examinations. Although DIF was positive in all 70 patients, IIF was only positive in 22 patients. The 22 CP patients with positive DIF and IIF were selected for this study. Of these 22 patients, 5 were males and 17 were females and their age ranged between 51-79 years. The clinical data of the patients and site of involvement are displayed in table 5.

Figures from 2 to 5 show representative picture of patients

Tablet 3. Clinical data of 22 CP patients including in the study

Patient No.	Age years	sex	duration of disease months	oral lesions		Extraoral lesions		
				Gingival	Buccal.	Cutaneous.	Ocular	Genital
1	67	F	12	+				
2	77	F	18	+			+	
3	61	M	48	+	+			
4	64	F	6		+	+		
5	55	F	48		+			+
6	66	M	120	+				
7	70	F	60	+	+	+		
8	68	F	72	+				+
9	53	M	28	+				
10	67	F	12	+				
11	66	F	7	+				
12	63	F	unknown	+				
13	78	F	12	+	+			
14	51	F	3	+				
15	58	F	6	+				
16	59	F	unknown	+				
17	63	F	48	+				
18	79	F	6	+				
19	62	F	unknown	+				
20	77	M	2	+	+	+		
21	70	M	6	+	+	+		
22	65	F	unknown	+	+			

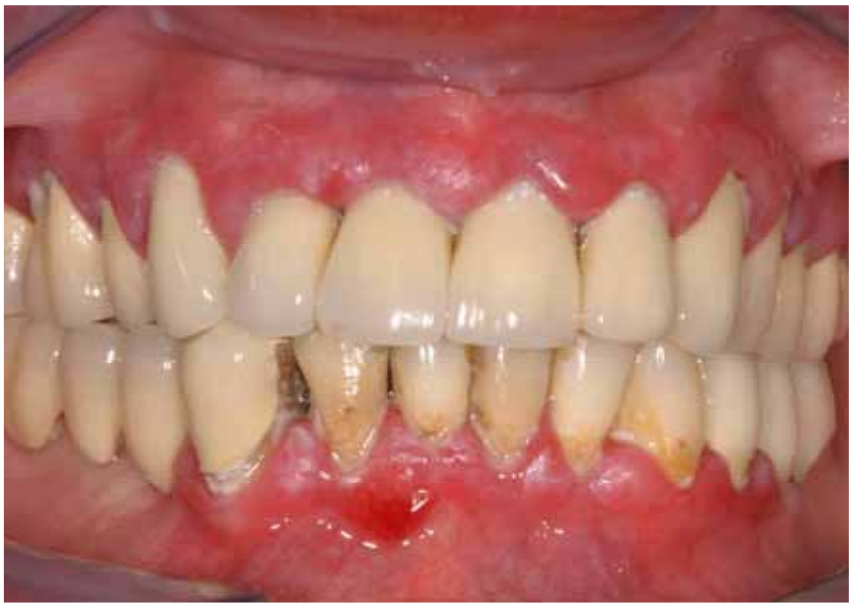

Fig.2 Patient no. 14 : Cicatricial pemphigoid affecting the gingiva and manifests as desqumative gingivitis**.**

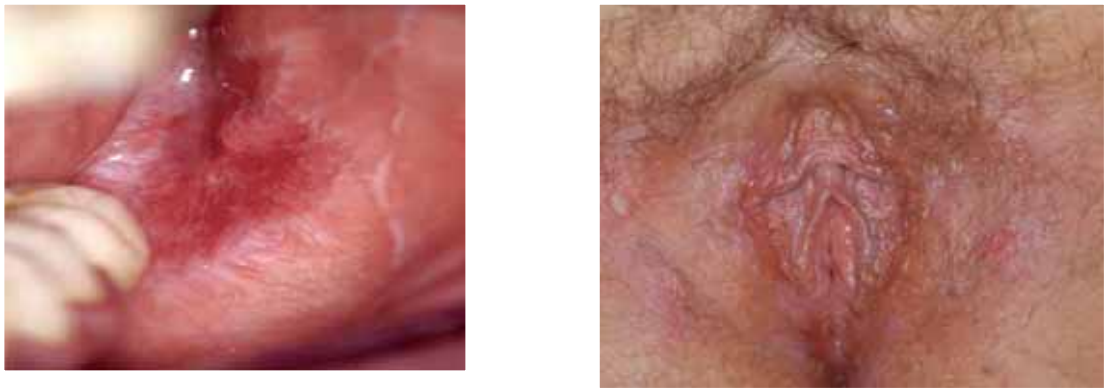

Fig.3 Patient no. 5: In this patient the CP affected the buccal mucosa as well as the vulva

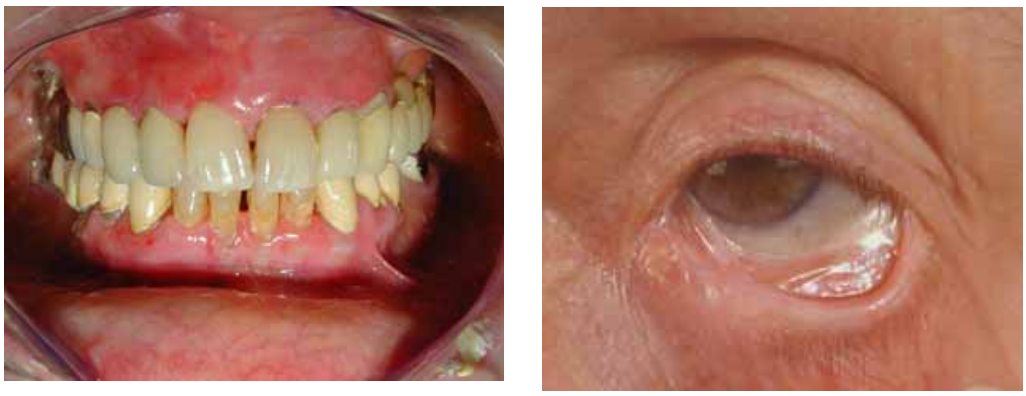

Fig. 4 Patient no. 2 : In this patient CO affected eye as well as the oral mucosa

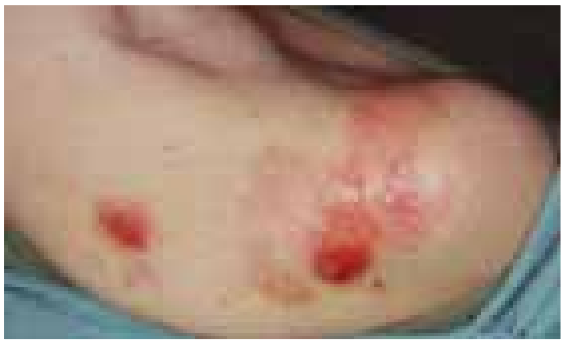

Fig. 5 Patient no.4 : In this patient CP affected the skin as well as the oral mucosa (picture of the oral mucosa is not shown)

3.2 Histological examination of patients' biopsies

On analyzing biopsies taken from cutaneous, oral and genital lesion of our 22 CP patients (formalin-fixed, hematoxylin-eosin stained) three patterns have been observed. In the first pattern (n=15) there is a subepidermal blister and inflammatory infiltrate. The infiltrate consists mainly of lymophcytes, esinophils and neutrophils (fig.6),

In the second pattern (n=5), there is a subepidremal blister and fibrosis. The degree of the fibrosis ranged from slight to moderate. There is also infiltrate of lymophcytes, esinophils and neutrophils.

In the third pattern (n=2), there are subepidermal blisters, fibrosis and band-like infiltrate of lymphocytes and esinophils. Occasionally neutrophils and plasma cells are present.

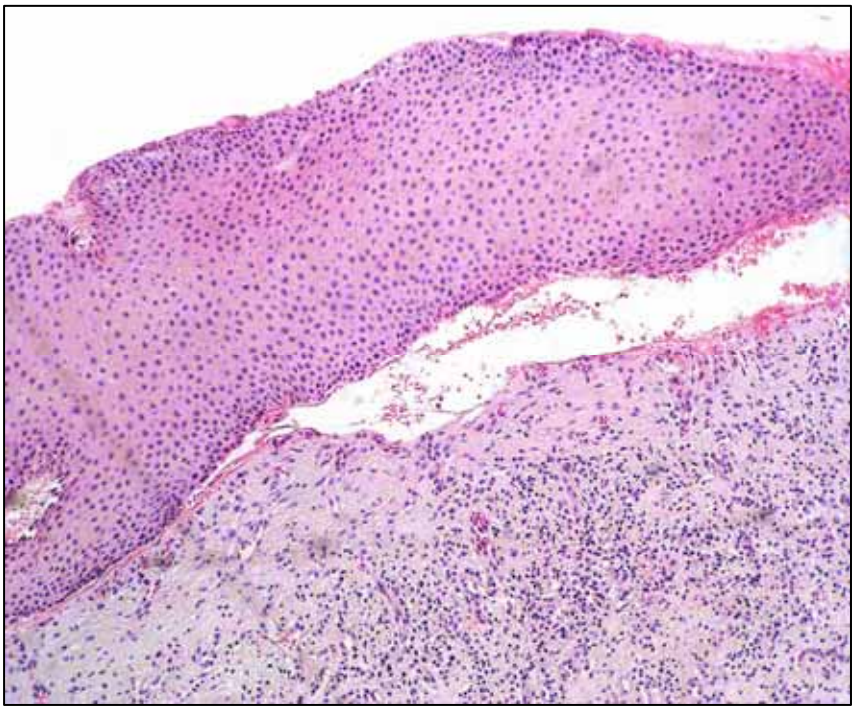

Fig. 6 Histopathology of a fully developed cicatricial pemphigoid lesion of oral mucosa with subepidermal blister, fibrosis and lymphocytic infiltration with few neutrophils and esionophils.

3.3 Characrization of patients' biopsies by direct immunoflulcent (DIF)

Data obtained from DIF examination using perilesional clinical normal appearing skin or mucosa in all 22 patients showed linear deposits of IgG along the basement membrane zone as showen in figure 7. No deposits of IgA were observed.

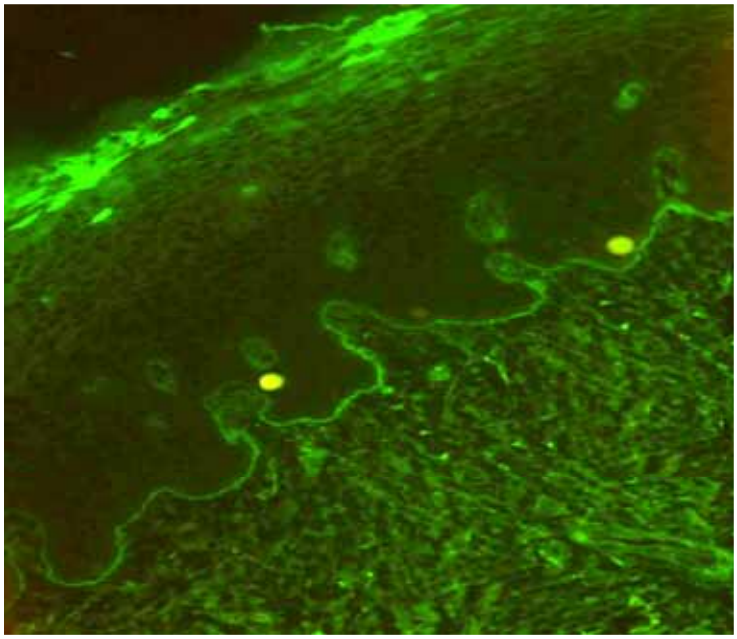

Fig. 7 DIF using periolesional normal appearing oral mucosa from a patein no. 14 with cicatrial pemphigoid. Linear IgG deposits can be seen along the BM zone.

3.4 Characterization of patient's sera by indirect immunofiulcent (IIF)

To examine whether CP patients sera have circulating autoantibodies react with BMZ pateints sera were tested for their immunoreactivity using salt split human skin. As positive control, a known positive patient serum was used, and as a negative control the dilution buffer was used for the treatment of the substrate. Of the 70 CP patients, only 22 patients showed positive IIF. These patients were selected for this study. Among the CP patients sera 1/22 (4.5%) showed a dermal, 16/22 (72%) an epidermal pattern, and 5/22 (22.7%) a combined epidermal/dermal as shown. All patients which reacted positive with CD151 reacted only with the epidermal side of the split in IIF (Fig. 8).

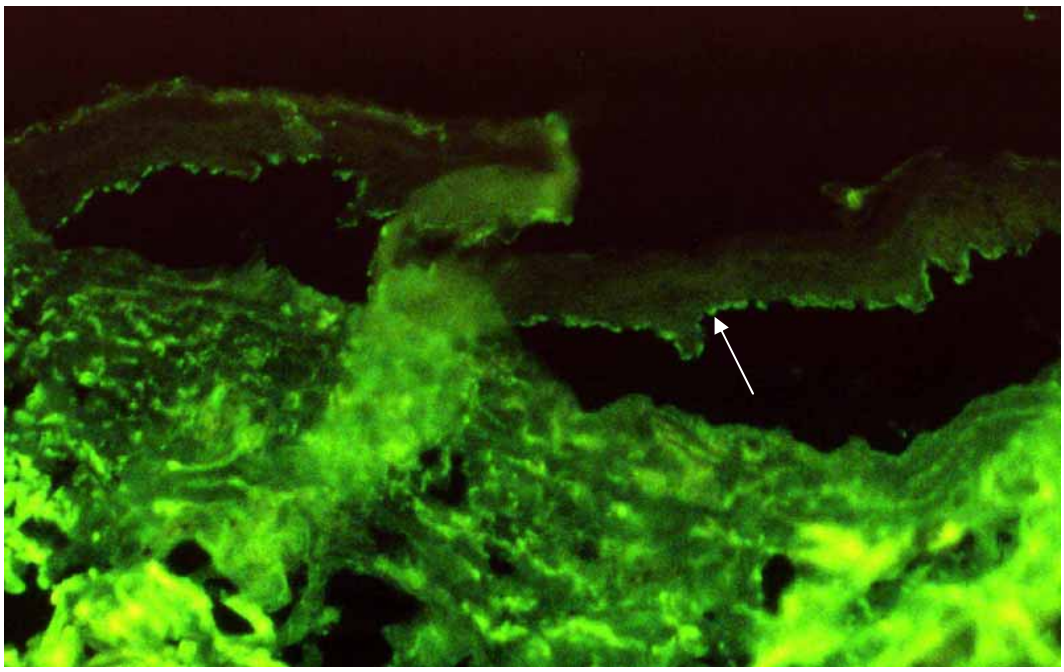

Fig. 8 IIF using salt split human skin. Linear IgG deposits can be seen along the epidermal side of the split (arrow)

3.5 Amplification of CD151 from total RNA

The amplification of encoding protein was performed using one step PCR as described under material and method. The total RNA was extracted from keratinocytes. The expected fragment of human CD151 was amplified from total RNA using RT-one step PCR. The PCR products were analyzed on 2% agrose gel electrophoresis, and the expected 762bp cDNA fragment was clearly seen (Figure 9). Results obtained from sequence analysis revealed that the cDNA fragment is 762bp and confirmed it as CD151-cDNA (fig, 10)

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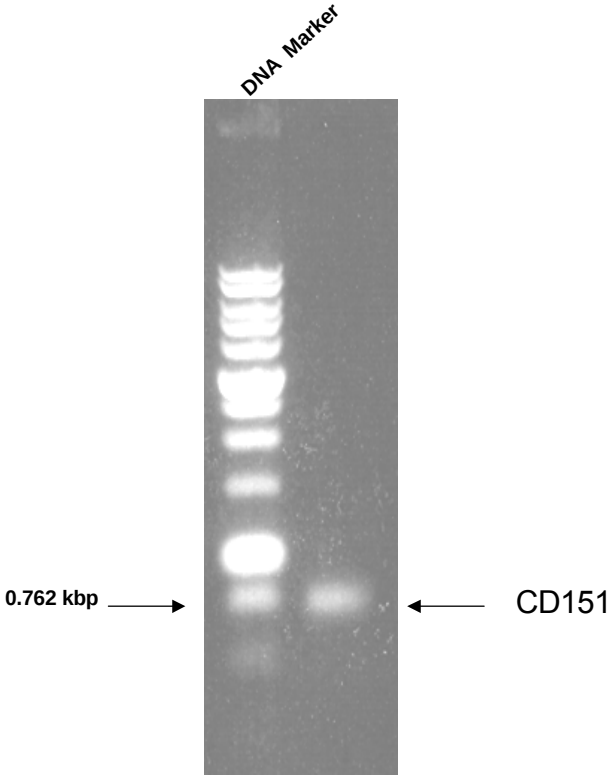


Fig. 9 RT-PCR amplification products obtained with keretinocytes total RNA. The expected sizes for PCR product is 725 bp.

```
Query: 378 gatcatcgctggtatcctcgcctacgcctactaccagcagctgaacacggagctcaagga 437
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 312 gatcatcgctggtatcctcgcctacgcctactaccagcagctgaacacggagctcaagga 371


Query: 438 gaacctgaaggacaccatgaccaagcgctaccaccagccgggccatgaggctgtgaccag 497
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 372 gaacctgaaggacaccatgaccaagcgctaccaccagccgggccatgaggctgtgaccag 431


Query: 498 cgctgtggaccagctgcagcaggagtcccactgctgtggcagcaacaactcacaggactg 557
           |||||||||||||||||||||||| ||||||||||||||||||||||||||||||||||
Sbjct: 432 cgctgtggaccagctgcagcaggagttccactgctgtggcagcaacaactcacaggactg 491


Query: 558 gcgagacagtgagtggatccgctcacaggaggccggtggccgtgtggtcccagacagctg 617
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 492 gcgagacagtgagtggatccgctcacaggaggccggtggccgtgtggtcccagacagctg 551


Query: 618 ctgtaagacggtggtggctctttgtggacagcgagaccatgcctccaacatctacaaggt 677
           ||| ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 552 ctgcaagacggtggtggctctttgtggacagcgagaccatgcctccaacatctacaaggt 611


Query: 678 ggagggcggctgcatcaccaagttggagaccttcatccaggagcacctgagggtcattgg 737
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 612 ggagggcggctgcatcaccaagttggagaccttcatccaggagcacctgagggtcattgg 671


Query: 738 ggctgtggggatcggcattgcctgtgtgcaggtctttggcatgatcttcacgtgctgcct 797
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 672 ggctgtggggatcggcattgcctgtgtgcaggtctttggcatgatcttcacgtgctgcct 731


Query: 798 g 798
           |
Sbjct: 732 g 732
```

Fig. 10 Nucleotide sequence of human CD151 cDNA. S15630611 Homo sapiens CD151 antigen mRNA, complete cds

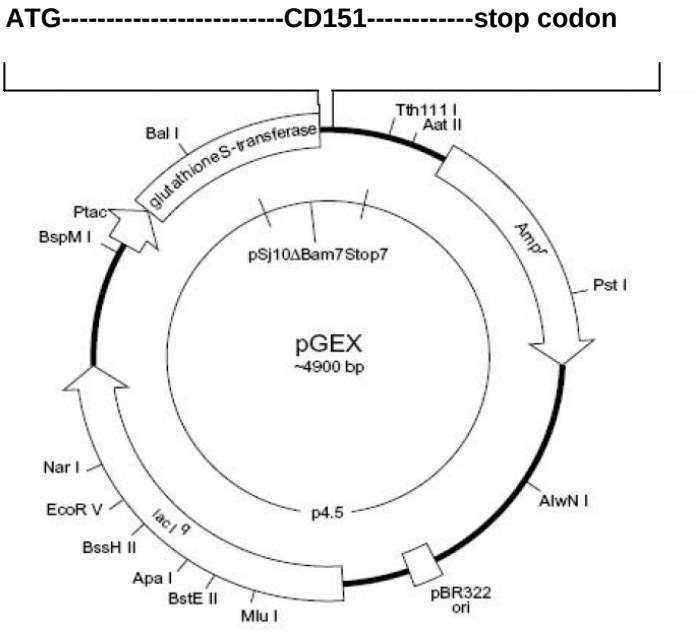


Fig. 11 Map of the glutathione S-transferase fusion PGEX-5x.3 expression vector showing the reading frames and main features with CD151 fragment

3.6 Cloning of CD151 open reading frame in expression vector pGEX-5X-3:

After purification of PCR product using PCR purification kit the DNA was subjected to digestion with EcoR I enzyme, the digasted fragment was extracted from gel using DNA extracted kit. The detected PCR product were cloned into pGEX vector to produce a plasmid containing cDNA encoding region of the human CD151. The transformed clones of BL21 were picked up manually and tested for presence of the cloned CD151 (Fig. 11). Only tested clones were used for the production of CD151 fusion protein. The digested CD151 encoding region was analysis on agars gel 2% to control the digation reaction (Fig. 12).

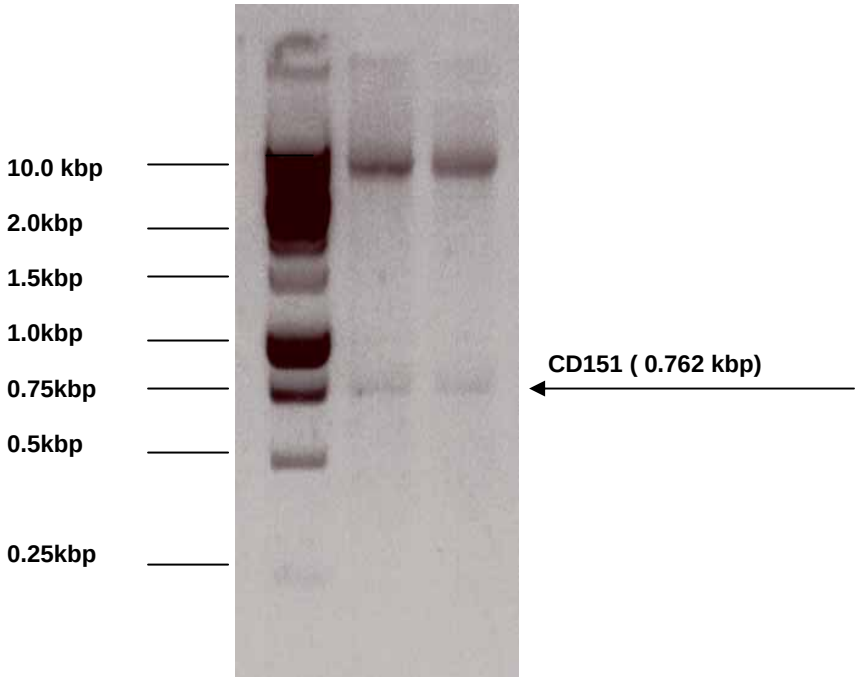
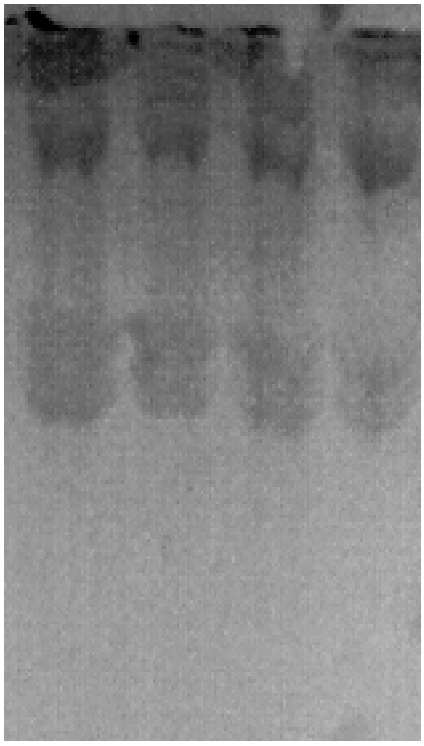


Fig. **12** Restriction of the pGEX-GST-CD151 plamid with the EcoR-I restriction enzyme.

3.7 Expression in *E.coli* and purification of CD151 protein

CD151 ORF were cloned as GST- fusion proteins into bacterial expression vectors as described. Plasmid was sequenced and inducible expression of the recombinent CD151 protein in *E.coli* was tested by SDS-PAGE analysis. The expression of CD151 protein was induced in *E.coli* strain BL21 (DE3) (Stratagene) after the addition of IPTG. The GST-CD151-fusion protein was purified by glutathione sepharose 4B. The expected molecular weight of the produced fusion protein (GST +CD151) 60 kDa and the pure CD151 (29kDa) following partial cleavage using factor X as shown (Fig. 13).



Fig, 13 SDS-PAGE analysis of CD151 fusion protein expressed in *Ecoli* after partial cleavage with factor X. A-induced CD151. Numbers on the right of the blot indicate the migration position of the molecular weight.

3.8 Examination of the Quality and immuneoreactivity of the produced CD151

To examine whether the produced CD151 fusion protein intact and immunoactive, CD151 fusion protein concentration were subjected to SDS-PGA gel electrophoresis and stained with commasie staining. The molecular weight of the produced CD151 detected to be 29kDa as expected. The immunoreactivity was tested by immunobloting using monoclonal antibody specific to the human CD151 protein as shown (Fig. 14). The monoclonal anti-CD151 antibody was found to detecte CD151 fusion protein. These data demonstrate that the purified CD151 fusion protein is intact and immunoactive.

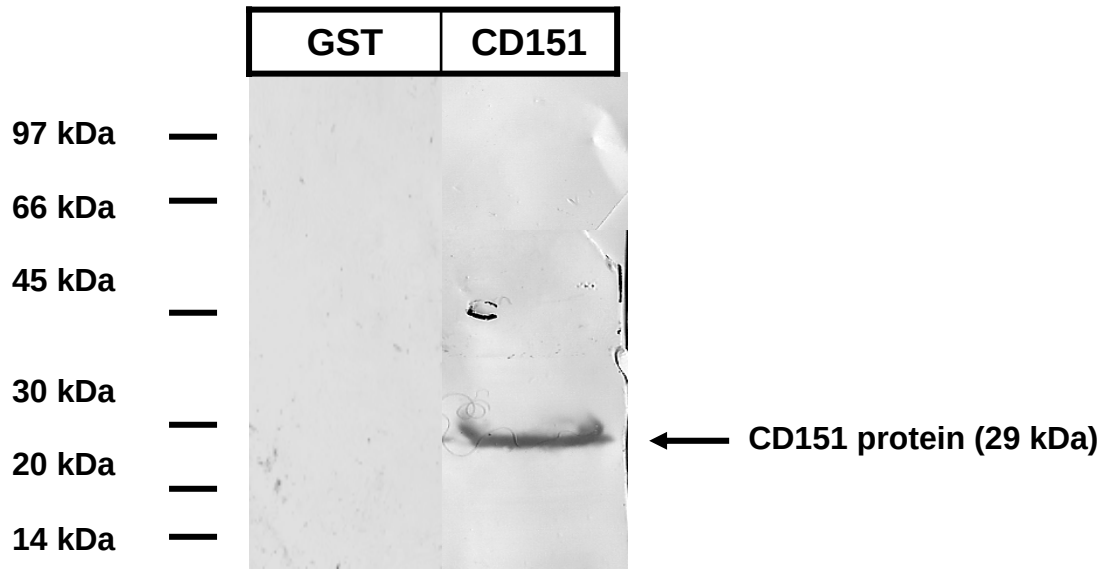


Fig. 14 Design, expression, purification and characterization of CD 151, CD151 was expressed in BL21 *E coli* and affinity-purified by 3-step chromatography., separated by SDS-PAGE and analyzed by Coomassie blue staining. CD151 protein of a molecular mass of approximately 29 kDa nitrocellulose filter CD151 was detected by the CD151specific monoclonal antibody and an AP-conjugated secondary goat-anti-mouse antibody. The position of molecular weight standards is shown on the left and their size given as kilo Dalton (kDa).

3.9 Detection of CD151-specific IgG in CP patients sera by western blot

To examine the immune reactivity of CD151 protein to CP patients sera, the purified CD151 protein was subjected to immunoblot analysis. A total 6/22 (27%) CP patients sera yielded positive results in western blot analysis using the recombinant CD151 (Fig. 15). Control sera of healty individual showed no reactivity with CD151 proteins. These results suggest that the circulating IgG autoantibodies in the 6 positive pateints are specific to CD151.

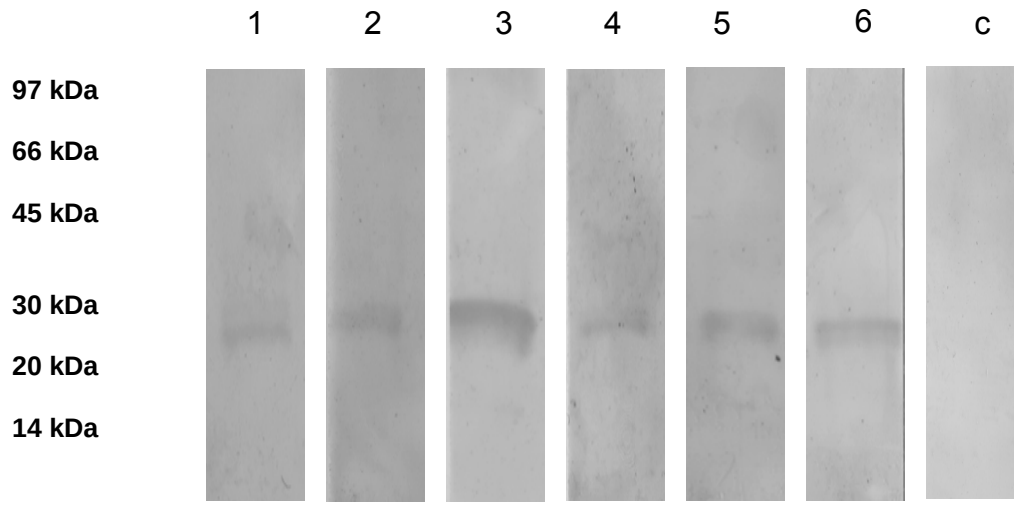


Fig. 15 Immunoblot assays using CD151 fusion protein.CD151 were separated by SDS-PAGE and transferred to nitrocellulose.lanes 1-6 crosbending to patients represented in table .lane c: negative control

3.10 Immunoprecipation/ western blot analysis

To confirm the immunoreactivity of CP patient sera to CD151 native protein, the total cell lysates prepared from human keratinocytes were immunoprecepitated using monoclonal anti-CD151 antibody. The immune complexes were analysed for CD151 IgG reactivity by Western blot using CP patients sera or control sera, or Abs against CD151 (Fig. 16). Specifically the 6 CP patients sera were who CD151-reactive by Western blot analysis were also positive by Immunoprecipation/Western blot analysis using recCD151(29 kDa). Control sera showed no reactivity with wild-type recCD151 .Immunoblotting with monoclonal Abs against the CD151 confirmed the expression of CD151 in keratinocytes. These data confirm the immune reactivity of recCD151 protein IgG produced by CP patients

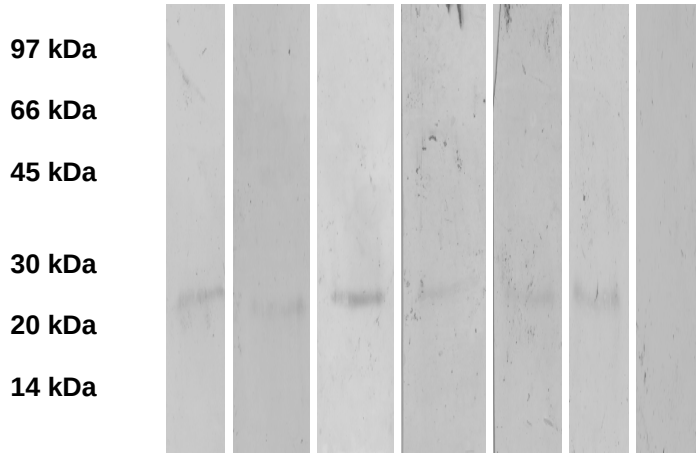


Fig. 16 Immunoprecipition/western blot analysis using keratinocyte cell lysate .Cell lysate were immunoprecipitated with CP sera. Immunoprecipites were separated by SDS-PAGE and transferred to nitrocellulose. Lanes 1-6 represented same positive patients with immunoblot, lane c: negative control

4. Discussion

This study provides evidences for the identification of CD151 specific autoantibodies in patients with CP. The specificity of these antibodies to CD151 was based on the fact that control sera from healthy individuals showed no immunoreactivity when analyzed in western blot using the recombinant CD151 as well as CD151 native protein.

In the present work the diagnosis of CP patients was confirmed by clinical presentation, routine histology, and immunopathological analysis of oral or cutaneous biopsies. Data presented in this work demonstrated that CP patients' sera contained CD151 specific IgG and suggesting furthermore the CD151 as a novel auto-antigen that may be specifically associated with CP disease. However, the inability of control sera to react with either recombinant or native CD151 protein supporting the specificity of CD151 antibodies to CP disease. Furthermore, the IIF examination on salt split skin revealed the presence of circulating IgG autoantibodies that are associated with CP disease. These antibodies were found to react with epidermal side, or epidermal and the dermal sides, or with the dermal side alone.

Based on the data obtained from IIF examination CP patients auto-antibodies that may be directed against one or more of the proteins present in BMZ namely BPAG1, BPAG2, α6ß4 integrin, laminin 5,6 or CD151.

$\alpha 6\beta 4$ integrin is heterodimer of integrin family of adhesion molecules and links, together with other transmembrane proteins, the intermediate filaments to the basement membrane of stratified epithelia of skin (Hertle *et al,* 1991). In addition, $\alpha 6\beta 4$ integrin plays a role in hemidesmosome assembly, and is important for cell proliferation, differentiation and migration. Null mutations of $\alpha 6\beta 4$ integrin lead to junctional epidermolysis bullosa (Inoue *et al*, 2000).

The different interactions between the various hemidesmosomal components were previously identified. The localization of BP180 in HDs containing a6b4 and plectin has previously been shown to depend on an interaction of BP180 with the cytoplasmic domain of β4 integrin (Borradori *et al*, 1997; Schaapveld *et al*, 1998). Interactions between the α6 subunit of integrin and the extracellular NC16a domain of BP180 are probably also implicated (Hopkinson *et al*, 1998). CP is a heterogeneous disease and both IgG and IgA antibodies against BMZ seem to be involved in the pathogenesis of this disease. Therefore, participation of additional autoantigens like CD151 is considered. CD151 has recently been characterized as a member of the tetraspan superfamily (Fitter *et al*, 1995). The tetraspan superfamily has 19 members each containing four highly conserved transmembrane domains (Maecker *et al*, 1997), a number of cysteine residues, and a major extracellular region between the third and fourth transmembrane domains. Although the biologic functions of the members of the TM4SF are poorly understood, several studies of their functions using specific mAbs have been undertaken, and the results obtained suggest a role for this superfamily in signal transduction pathways and the regulation of cell activation, development, proliferation, motility, and adhesion. Moreover, TM4SF members have been shown to form noncovalent associations with each other, integrins, and coreceptor molecules, such as those involved in adhesion and signal transduction. CD81, CD9, CD53, CD63, and CD82 have all been found in association with certain integrins in various types of human cells. All these molecules associate with the β1 integrins α3β1, α4β1, and α6β1. These associations appear to be important for cell-cell adhesion and migration. CD81 forms part of a signaling complex with CD21, CD19, and Leu 13 on B cells. CD81 also associates with CD82 as well as with CD4 and CD8 coreceptors on T cells. Like other tetraspan molecules CD151 contains one small and one large extra-cellular loop with short cytoplasmic

carboxy-and amino-terminal domains. The large extracellular loop is thought to be involved in the binding to other molecules, such as integrins. It has been implicated in variety of cell biological processes including cell adhesion and cell motility. CD151 is expressed by a variety of epithelial and mesenchymal cells, including basal keratinocytes. Endocytosis and subsequent recycling of integrins to the leading edge promotes cell migration (reviewed by Lauffenberger and Horwitz, 1996). Recycling of $\alpha5\beta1$, $\alpha5\beta3$ and $\alpha6\beta4$, which all associate with CD151, has been described (Bretscher, 1992; Sczekan and Juliano, 1990; Panetti and McKeown-Longo, 1993; Bretscher, 1989; Raub and Kuentzel, 1989). However, the mechanism by which integrins are internalised, sorted and recycled to the leading edge of migrating cells remains unclear. The similar intracellular localization of CD151 and integrins suggests that CD151 may enter the endocytic pathway complexed with these molecules. Interestingly, internalization of CD151/integrin complexes appears to be selective, as CD9, which is also present in integrin complexes on the plasma membrane, was not detected within the endocytic pathway. Therefore CD151 may play a specific role in the internalization and recycling of integrin complexes through the endocytic pathway. Furthermore, the functional effects of anti- CD151 antibodies may be due to disruption of integrin turnover. However the biological role CD151 is remains uncertain.

Therefore, the examination of these phenomena in the oral mucosa is of great interest. The characterization of CD151 expression in skin has been reported (Sincock *et al* 1996). Immunohistological studies showed that the expression of CD151 was found to express on the cell membrane of basal keratinocytes. CD151 was strongest on the membrane adjacent to the basal lamina. There are no data about the expression of CD151 in oral mucosa. The results show however that CD151 is expressed in the oral mucosa and it’s pattern of expression was similar to

that of the skin namely in the BMZ as well as the lateral surfaces of basal keratinocytes.

The analysis of the clinical data of the six CP patients whose were immunoreactive with CD151 protein showed that 3 patients have only oral lesions and one patient has oral and skin lesions. One patient has lesions of the oral mucosa as well as the conjunctiva and one patient has lesions of the oral mucosa and the vulva.

The diversity of the clinical pictures in relation to immunoreactivity of CD151 to patient's sera may duo to the presence of different epitopes that may be restricted to patients from which the sera are derived. Since CD151 is presumably the ligand of $\alpha 3\beta 1$ and $\alpha 6\beta 4$ integrins, CD151-specific autoantibodies may contribute to the pathogenesis of CP disease by interfering with CD151-integrins complex influencing hemidesmosome formation and other functions important for BMZ integrity. Mutations in the genes coding for laminin 5, $\alpha 6\beta 4$ integrin and BP180 were found to result in junctional epidermolysis bullosa, an autosomal recessively inherited blistering skin disorder associated with fragility at the BMZ. In analogy, one could speculate that mutations in the CD151 might also result in junctional epidermolysis bullosa.

In the present work, the quality and immunoreactivity of purified CD151 recombinant protein was evaluated by commasie saining and immunobloting, respectively. Thus, the production and purification led to substantial amounts of intact and fully immunoreactive CD151. Therefore, the detection of CD151 recombinant and native proteins by patients sera in dot- and western blot indicates that the immunoreactivity of CP associated autoantibodies is restricted to CD151 auto-antigen. Therefore, the identification of the CD151 as novel auto-antigen that is selectively recognized by an IgG autoantibody is considered.

Because CP sera showed much lower titers of anti-BMZ antibodies than BP sera the IIF was performed using 1M NaCL-split human skin. These techniques have been

reported to be much more sensitive than conventional IIF using non-split skin. These techniques actually raised the frequency of detection of anti-BMZ antibodies of both IgG and IgA classes in CP sera, confirming previous studies (Lazarova *et al* 2004).

Therefore to examine whether CD151 is favorite candidate among this suggested unknown auto-antigens, the immunoreactivity of patient's sera to recombinant CD151 was confirmed using immunopriciptated native CD151 obtained from keratinocytes. Interestingly, patient's sera, which showed immunreactivity to recombinant CD151, were also found to react with the native CD151 protein, when subjected for immunoprecipition/immunoblotting using keratinocytes lysate. The specificity of the detected band was further confirmed using anti-CD151 antibody that exactly detected CD151 band at same position. In contrast, no bands were found corresponding to BP 230 (230kDa), plectin (300 kDa), BP180 (180kDa), α6 (130 kDa) or ß4 (205 kDa) integrin subunits. These observations demonstrate that these patients sera contain antibodies that may be specific to CD151 antigen.

Based on the fact that there has been considerable interest to identifying various basement proteins that act as target antigen to autoantibodies in CP patients. The detection of autoantibodies in CP patients sera in addition to autoantibodies that have reported be specific for BPAG1 and BPAG2 in CP patients sera will improve the molecular diagnosis of CP disease.

BPAG1 has reported to be the major target antigen in CP patients, since 30%-78% of CP patients were found to react with BPAG1 (Megahed, 2004). In addition, a subset of CP patients was reported to be characterized with circulating IgG autoantibodies to β4 integrin subunit. Moreover, a subgroup of CP patients was reported with circulating IgG autoantibodies to the α3 chain of laminin 5 and laminin 6.

.Shawen *et al* demonstrated BPAG1 as target antigen for IgG autoantibodies produced by CP patients and showed that CP autoantibodies are directed against at

least two distinct antigenic sites of the extracellular domain of BPAG1. (Shawen *et al,* 1996.)

In summary my findings suggest that the CD151 is a noval candidate auto-antigen that may associated wirh CP, an event that crucial for the functional role of CD151 in the stability of BMZ. In addition, these data provide new evidance for the immunological heterogenecity of CP.

5. Summary

Cicatricial pemphigoid (CP) is an autoimmune blistering disease, which affects predominantly mucous membranes, such as the conjunctivae as well as oral and genital mucosae. Although this disease is characterized by the presence of pathogenic autoantibodies of IgG class binding to different antigens, however, there might be additional CP disease-specific auto-antibodies whose target antigens still remain to be determined. Transmembrane protein CD151 is recently identified as coponent of the basement membrane zone (BMZ), however, its functional role in autoimmune blistering diseases so far is unknown.

In this study, I demonstrated the immune reactivity of CP patients sera (n=22) to CD151 protein by western blot analysis using recombinant and native CD151 protein. I can show for the first time that the CD151 protein functions as autoantigen in 27% (6/22) of tested CP pateins sera. My finding suggest that the CD151 protein is a noval candidate autoantigen that may be associated with CP disease and provide new evidence for the immunological heterogeneity of the disease.

6. Zusammenfassung

Das vernarbende Pemphigoid ist eine autoimmun Blasenbilde Erkrankung, die vorwiegend Schleimhäute, wie konjuktive, orale und genitele Mukose betrifft. Obwohl diese Erkrankung durch schon bekante Pathogene Auto-Antikörper der Klasse IgG charakterisiert ist, könnten noch weitere zusätzliche Krankheitspezifische Auto-Antikörper vorhanden sein, deren „Ziel-Antigen“ noch untersucht werden sollte.

Das transtransmembran-protein CD151 wird kürzlich als komponent der Blasenmembranzone (BMZ) identifiziert. Seine Rolle in autoimmun blasenbildenden Erkrankungen ist noch unbekannt.

In dieser Untersuchung, wies ich mittls rekombinierten und nativen CD151 die Immun-Reaktivität der vernarbenden Pemphigoid Seren (n=22) mit CD151 durch Western Blot-Analyse nach. Meine Ergebnisse zeigten zum ersten Mal, dass das CD151 Protein als Auto-Antigen in 27% der vernarbenden Pemphigoid Patienten fungiert.

Die durchgeführten Untersuchungen geben einen Hinweis darauf dass CD151 ein neues Auto-Antigen des vernarben Pemphigoid darstellt. Darüber hinaus liefern diese Ergebnisse noch weitere Hinweis für die immunologische Heterogenität dieser Krankheit.

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