and full-length BP180 it has been postulated that LABD97 and LAD-1 are fragments of BP180 [38]. Subsequent independent studies confirmed that LABD97 and LAD-1 indeed represent portions of the extracellular domain of BP180 [8,9,39].

For the detection of autoantibodies against BP180, belonging to different immunoglobulin isotypes, several ELISA systems have been established. To detect IgG class autoantibodies from patients' sera has been the focus of immunoassays developed until now [19,20,40-43]. Two of these test systems are commercially available and widely used in diagnosis. Recently, ELISA systems using a recombinant form of BP180 NC16A domain were established for the detection of specific IgE in pemphigoid patients [31,44]. In contrast, an ELISA for the detection of IgA autoantibodies in pemphigoid diseases has not yet been developed. Both, (i) IgA deposition at the dermal-epidermal junction being a major diagnostic criterion and (ii) the demonstration of the pathogenic potential of IgA autoantibodies in patients with linear IgA disease emphasize the need of elaborating a sensitive and specific immunoassay to detect the IgA autoantibodies in this orphan disease.

Therefore, in the present work, we used a recombinant form of the ectodomain of human BP180 to establish an ELISA for the detection of IgA autoantibodies. Our results show that ELISA using the ectodomain of BP180 is a sensitive and most specific system for detection of circulating IgA autoantibodies in patients with pemphigoids.

Materials and methods

Human sera

Serum samples were obtained from patients with linear IgA disease (n = 30, mean age 56 years), bullous pemphigoid (n = 31, mean age 76.5 years), and dermatitis herpetiformis (n = 50, mean age 43 years) before initiation of treatment, as well as from healthy donors (n = 105, mean age 43 years). Our present study focuses on the IgA pemphigoid subgroup of the linear IgA disease patients, characterized by IgA binding to the epidermal side of the salt-split skin by indirect IF microscopy. Sera from patients with IgA epidermolysis bullosa acquisita or from patients with laminin autoimmunity were not included. The clinical diagnosis of IgA pemphigoid was confirmed by (i) subepidermal blisters, (ii) linear IgA deposition along the dermal-epidermal junction of perilesional skin by direct IF microscopy, and (iii) circulating IgA autoantibodies binding to the epidermal side of 1M salt-split skin by indirect IF microscopy. The clinical diagnosis of bullous pemphigoid was validated by (i) subepidermal blisters with inflammatory infiltrate, (iii) linear IgG deposition at the basement membrane zone revealed by direct IF microscopy, and (iii) circulating IgG autoantibodies to the epidermal side of 1M salt-split skin as shown by indirect IF microscopy and ELISA using a recombinant form of the 16^{th} non-collagenous region of BP180 expressed as a glutathione-S-transferase fusion protein. Patients with both IgG and IgA deposition at the dermal-epidermal junction were diagnosed as bullous pemphigoid. For the experiments conducted, we obtained approval from the Ethics Committee of the Medical Faculty of the University of Freiburg, Germany (Institutional Board Projects no 318/07 and 407/08). We obtained informed consent from patients whose material was used in the study, in adherence to the Helsinki Principles.

Cell culture

HaCaT human keratinocytes were cultured in serum free, low calcium KGM medium, supplemented with KGM Supplement Mix and $CaCl_2$ (all from PromoCell), as well as L-glutamine, penicillin and streptomycin (all from Biochrome). Transfected Flp-In HEK 293T cells (Flp-In™-293, Invitrogen) were cultured in DMEM medium, without phenol red (Gibco) supplemented with 10% FCS, L-glutamine, penicillin, streptomycin and hygromycin (all from Biochrome).

Production of recombinant BP180 ectodomain

The ectodomain of BP180, corresponding to amino acids 490-1497 was produced by stable expression in Flp-In HEK-293 cells (Invitrogen) as reported [45]. Briefly, the recombinant protein encoding for the ectodomain of human BP180 with an N-terminal Ig κ chain signal sequence and hexahistidine tag was affinity purified from culture medium by metallochelate affinity using Ni-NTA (Qiagen). Alternatively, the recombinant protein from harvested culture media was precipitated with 45% ammonium sulphate followed by dialysis against PBS. The purified protein's concentration was determined by spectrophotometry at 280 nm and this protein was subsequently used to determine the concentration of the specific protein in the precipitated protein by densitometric comparison of the specific bands of the Coomassie stained gel using the ImageJ software (http://rsb.info.nih.gov/ij).

Enzyme-linked immunosorbent assays

IgG pemphigoid autoantibodies were detected by ELISA utilizing a recombinant form of the 16^{th} non-collagenous region of BP180 expressed as a glutathione-S-transferase fusion protein following manufacturer's instructions (MBL Co, Nagoya, Japan). For detection of IgA autoantibodies, 96-well microtiter plates (Greiner Bio-One, Germany) were coated with 1.4 μg/well of recombinant BP180 ectodomain in 0.1 M bicarbonate buffer (pH 9.6), overnight at 4 °C. After washing with 0.05%Tween20-PBS (w/v) and subsequent 1 h blocking with 2% BSA-PBS (w/v) the plates were incubated for 1 h with 1:50 diluted sera in 1%

BSA-0.05% Tween20-PBS (w/v). Bound antibodies were detected using a 1000-fold dilution of a horseradish-peroxidase (HRP) conjugated rabbit anti-human IgA antibody (ab8510, Abcam) and orthophenylene diamine (Dako). All steps were carried out at room temperature. The optical density (OD) was read at 490 nm using an automated spectrophotometer (Sirius HT-TRF, MWG). Each serum was tested in duplicate. The cut-off for positivity was validated and optimized by receiver-operating characteristics (ROC) analysis as described below and was defined as 0.48 OD units at 490 nm.

In order to rule out the possibility of a nonspecific cross-reaction between the secondary HRP-labeled anti human IgA antibody and the IgG autoantibodies in bullous pemphigoid patients' sera we performed the ELISA described above with sera of known high IgA and IgG titers from IgA pemphigoid and bullous pemphigoid patients, respectively. Normal human serum was used as control. Bound IgG and IgA antibodies were detected in all samples with a 5000-fold diluted, HRP-labeled goat anti-human IgG antibody (ab6858, Abcam) and the HRP conjugated anti-human IgA antibody.

IgG autoantibodies against BP230 were detected by the MESACUP BP230 commercial ELISA kit (MBL Co, Nagoya, Japan) following the manufacturer's instructions [46]. For the detection of IgA autoantibodies against BP230, we have slightly modified the kit protocol. Briefly, BP230 microwell strips were incubated with 50 times diluted linear IgA disease patients' sera and appropriate control sera. After washing, the bound antibodies were detected using 1000-fold diluted HRP-conjugated anti-human IgA antibody (ab8510, Abcam) and the tetramethylbenzidine dihydrochloride (TMB)/ hydrogen peroxide substrate solution from the kit.

SDS-PAGE and immunoblot analysis

Immunoblotting with recombinant or native BP180 ectodomain proteins was performed as described with minor modification [18,47]. Briefly, preparations of recombinant BP180 ectodomain, native shed BP180 and precipitated supernatant of empty pcDNA5/FRT transfected HEK-293 cells were separated by SDS-PAGE on 6-8% preparative gels, under reducing conditions, followed by transfer onto nitrocellulose (Whatman/Protran BA85). Membrane strips were incubated overnight with 50-fold diluted serum or 800-fold diluted mouse mAb NC16A-3 directed against the 16th non-collagenous domain of BP180. Reactivity was visualized with secondary, HRP-conjugated rabbit anti-human IgA (Abcam) or anti-mouse IgG (BioRad) antibodies and diaminobenzidine (Merck).

Indirect immunofluorescence

Detection of circulating autoantibodies by indirect immunofluorescence followed published protocols [18,47]. Briefly, frozen sections of salt-split normal human skin were incubated in a first step with 10-fold diluted sera from patients with IgA pemphigoid, bullous pemphigoid and healthy donors and in a second step with 40-fold diluted, fluorescein isothiocyanate (FITC) conjugated polyclonal goat anti-human IgA antibody (Invitrogen).

Statistical analysis

Diagnostic parameters of our new ELISA were optimized by using the ROC analysis. Therefore, to determine the cut-off value for the ELISA using recombinant BP180 ectodomain, we performed a ROC analysis by plotting on the X-axis the 1 - specificity (the false positive rate) and on the Y-axis the sensitivity (the true positive rate). The diagnostic sensitivity and specificity are a function of the selected cut-off value. In the diagnostic context of pemphigoid diseases, which have a very low prevalence, it is advisable to choose a cut-off to maximise the specificity of the assay. In this regard, a specificity of 97.5% or higher may be considered an optimal target. A similar specificity would be aimed by applying an alternative method of calculating the cut-off based on the mean plus two standard deviations (2SD) of the negative reference sample [48]. Correlations were analyzed by the Spearman's rank correlation test. Proportion comparison was examined by Fisher's exact test. Data are considered significantly different if the $p < 0.05$. Statistical analyses were performed using the GraphPad Prism statistical package (v5; GraphPad Software, San Diego, CA).

Results

Generation of the recombinant autoantigen

When separated by SDS-PAGE without or with previous boiling, the recombinant protein migrated consistently with the calculated molecular masses of 360 kDa (Figure 1B, lane 2) and 120 kDa (Figure 1B, lane 3), for its trimeric and monomeric forms, respectively. This SDS-PAGE migration pattern matched the one observed for the keratinocyte-derived shed ectodomain of BP180 protein (data not shown) indicating that the recombinant protein forms a native-like collagenous trimerized structure. Monoclonal antibodies specific for the BP180NC16A domain of BP180, recognized the keratinocyte-derived shed ectodomain (Figure 1C, lane 1) and the recombinant protein by immunoblot analysis (Figure 1C, lane 2).

Immunoreactivity of recombinant BP180 with IgA autoantibodies

IgA autoantibodies from reference IgA pemphigoid patients' sera (n = 5) recognized the recombinant and the native BP180 ectodomain by immunoblotting. None of the normal human sera reacted with the autoantigens

and IgA antibodies from none of the tested sera bound to the precipitated proteins of the empty vector transfected HEK cell culture medium. Representative examples are shown in Figure 2.

Development of ELISA using recombinant BP180

The working conditions, including antigen amount/well, dilution of sera and secondary antibodies have been defined by an initial chessboard titration (data not shown). The secondary HRP-labeled anti-human IgA antibody was tested by ELISA (see Methods) in order to rule out nonspecific cross-reactivity with IgG autoantibodies. No cross-reactivity was found (data not shown). To determine the cut-off value of the newly established immunoassay, we performed a ROC analysis of the ELISA readings with sera from 30 IgA pemphigoid patients and 105 healthy donors as controls. Based on a calculated specificity of 100% and a sensitivity of 83.3% the cut-off was set at 0.48 OD reading units (Figure 3).

ELISA using recombinant BP180 is a sensitive and specific tool to detect IgA autoantibodies in pemphigoid patients

Applying the cut-off value of 0.48 defined by ROC analysis for the newly developed ELISA showed that 25 (83.3%; 95% CI: 65.2%-94.3%) of IgA pemphigoid and 8 (26.0%; 95% CI: 12.0%-45.0%) of bullous pemphigoid patients were positive, while 1 (2%; 95% CI: 0.05%-10.0%) of patients with dermatitis herpetiformis slightly topped the cut-off value (Figure 4). Serum from none of the healthy donors (0%; 95% CI: 0.0%-4.0%) showed IgA reactivity with the recombinant BP180 ectodomain (Table 1). Therefore, a sensitivity and a specificity of 83.3% (95% CI: 70-97%) and 100% (95% CI: 96-100%), respectively, were calculated for the ELISA detecting IgA autoantibodies against BP180 in patients with IgA pemphigoid. The area under the curve (AUC) was 0.993 (95% CI.: 98.4%-100%). IgA autoantibodies against BP180 ectodomain were primarily detected in bullous pemphigoid patients showing IgG reactivity against the BP180 NC16A region by ELISA (n = 8). IgA autoantibodies against BP180 ectodomain were found in only 1 of 16 bullous pemphigoid (6.25%, 95% CI.: 0%-28%) patients with negative BP180 NC16A.

Levels of BP230-specific IgA and IgG autoantibody in IgA pemphigoid patients' sera

While BP180 is considered the major autoantigen in IgA pemphigoid, BP230 has been also documented as target of these autoantibodies. Therefore, we have measured the IgA and IgG levels agains BP230 by ELISA in our 30 IgA pemphigoid patients' sera. In 5 patients, we have detected IgA autoantibodies against BP230, whereas none of the sera had detectable IgG against BP230 (Table 2).

IgA levels by BP180 ELISA correlate with the IgA reactivity against the dermal-epidermal junction by IF microscopy

The indirect IF microscopy on salt-split skin is a standard diagnostic tool in pemphigoid diseases. To further characterize the suitability of the newly developed ELISA for diagnosis of pemphigoid diseases, we correlated in IgA pemphigoid patients the IgA levels by ELISA with the semi-quantitative reactivity scores by IF microscopy on salt-split skin. When the IgA levels of BP180-specific IgA autoantibodies were plotted against the IgA indirect IF microscopy scores, a positive correlation ($r = 0.71$; 95% CI: 31-89%; $p < 0.005$) was obtained (Figure 5).

Analysis of IgA immunoreactivity against BP180 ectodomain in bullous pemphigoid patients

The reactivity of IgA autoantibodies from sera of bullous pemphigoid patients was analyzed by IF microscopy using frozen sections of human salt-split skin. Representative examples are shown in Additional file 1. Thirteen

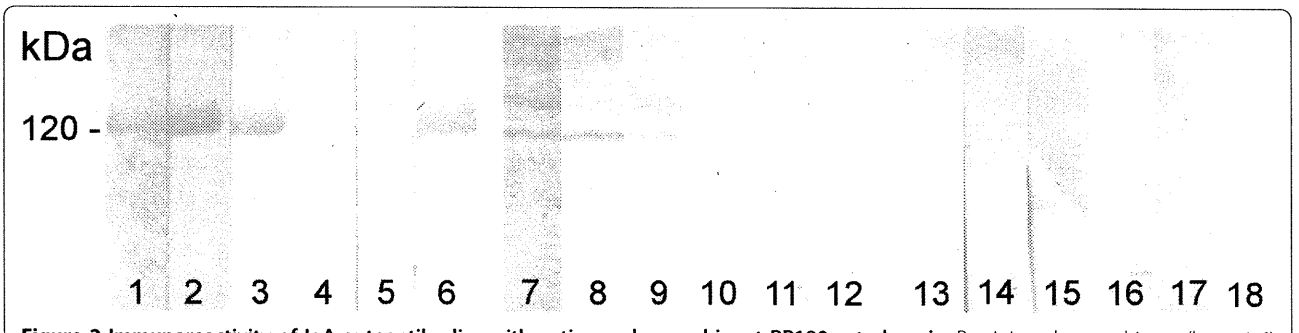

Figure 2 Immunoreactivity of IgA autoantibodies with native and recombinant BP180 ectodomain. Precipitated, recombinant (lanes 1-6) and native, keratinocyte-derived (lanes 7-12) BP180 ectodomain immunoblotted with IgA pemphigoid (LAD) patients' sera (lanes 1-3, 7-9) and control sera (NHS) (lanes 4, 5, 10 and 11). As substrate control (lanes 13-18), precipitated culture medium from cells transfected with empty vector was immunoblotted using the same LAD (lanes 13-15) and NHS (lane 16, 17) sera. Presence or absence of the 120 kDa ectodomain was visualized using a specific monoclonal Ab (lanes 6, 12 and 18).

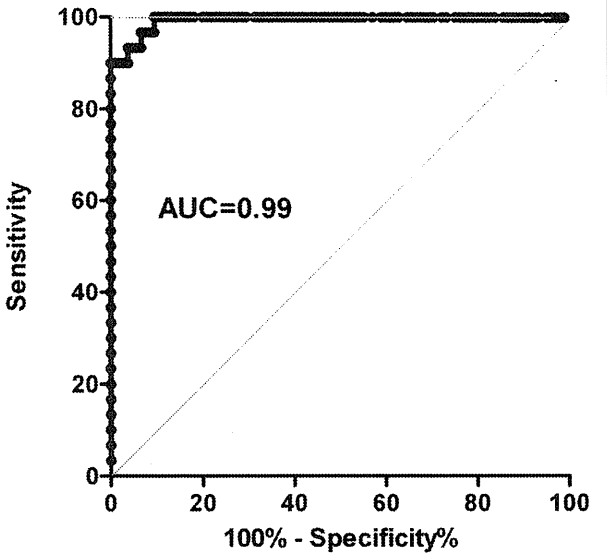

Figure 3 Receiver-operating-characteristic (ROC) curve. AUC, area under the curve. Test performed with sera from IgA pemphigoid patients (n = 30) and controls (n = 105).

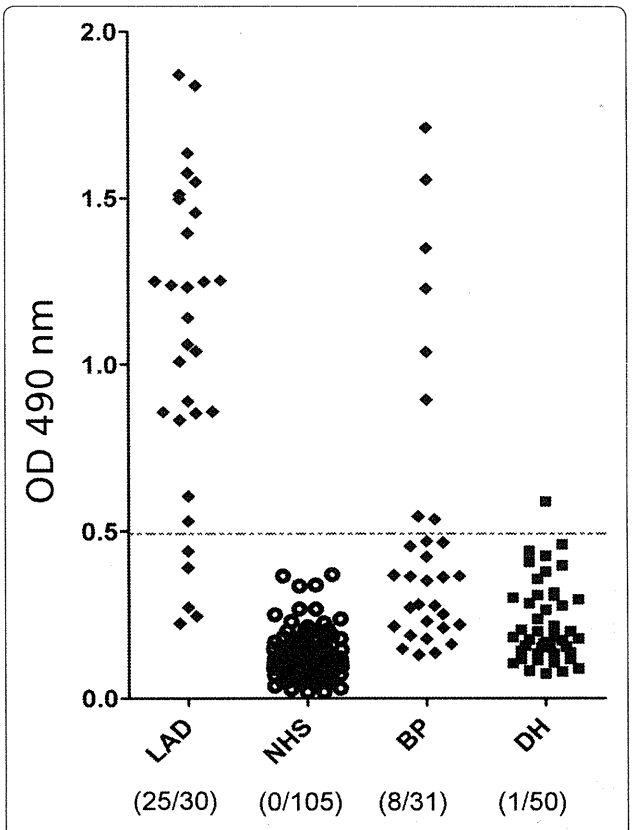

Figure 4 ELISA reactivity of linear IgA disease and control sera with the recombinant BP180 ectodomain. Scatter plots represent corrected optical density measurements of serum reactivity of IgA pemphigoid (LAD), dermatitis herpetiformis (DH), bullous pemphigoid (BP) patients and age matched, healthy donors (NHS) with the recombinant BP180 ectodomain. The cut-off of the assay is represented by a dashed line. Numbers in brackets are positive and total cases, respectively.

of 31 bullous pemphigoid sera had detectable autoreactive IgA antibodies binding to the epidermal side of the split skin. When immunoblot analysis was performed to identify IgA autoreactivity, we found that 13 and 19 of 31 patients recognized the native and the recombinant BP180 ectodomain, respectively. Characteristic examples are depicted in Additional file 1.

Comparison of different immunoassays for the detection of BP180-specific IgA autoantibodies in pemphigoid diseases

We compared using Fisher's exact test the findings in IgA pemphigoid patients obtained by ELISA using recombinant BP180 ectodomain and immunoblotting with native and recombinant LAD-1 antigen. Results are summarized in Table 2. The calculated p values of 0.703 and 0.502, respectively, show that the ELISA and immunoblotting results are not significantly different. Discrepancies were observed in only 4 (15%) and 5 (18%) patients having different results by ELISA compared with immunoblotting with the native LAD-1 and ELISA compared with immunoblotting with recombinant BP180 ectodomain, respectively.

Discussion

Several quantitative immunoassays for the detection of IgG and IgE autoantibodies against BP180 have already been established [19,20,31,40-44]. Using these tests, a plethora of clinical and experimental data have been generated, which decisively influenced our knowledge on disease pathogenesis and are firmly implemented in the diagnosis and monitoring of pemphigoid diseases [1,49]. In addition to IgG and IgE, IgA autoantibodies are present to different extent in pemphigoid diseases. While pemphigoid patients showing a predominant IgA autoimmune response directed against BP180 are diagnosed as linear IgA disease, IgA autoantibodies may complement a dominant IgG response in bullous pemphigoid or mucous membrane pemphigoid [1]. However, quantitative assays for measuring the levels of serum IgA autoantibodies in pemphigoid diseases have not been established yet. Therefore, in the present study, we

Table 1 Sensitivity and specificity of the IgA ELISA with recombinant BP180 ectodomain

Sera	Positive/Total	Sensitivity (95% CI)	Specificity (95%CI)
LAD	25/30 (83%)	83.3% (65.2%-94.3%)	100% (96.55%-100%)
BP	8/31 (26%)	26% (12.0%-45.0%)	100% (96.55%-100%)
DH	1/50 (2%)	2% (0.05%-10.0%)	100% (96.55%-100%)
NHS	0/105 (0%)	0% (0.0%-4.0%)	100% (96.55%-100%)

Table 2 Immunoreactivity profile of linear IgA disease patients

	Positive indirect immunofluorescence	Immunoblot with native shed BP180 ectodomain		Immunoblot with recombinant BP180 ectodomain		ELISA BP230-IgA		ELISA BP230-IgG	
		positive	*negative*	*positive*	*negative*	*positive*	*negative*	*positive*	*negative*
ELISA BP180 positive	25 (83%)	20(77%)	3 (11.5%)	20 (71.4%)	4 (14.3%)	4 (13.3%)	21 (70%)	0 (0%)	25 (83%)
ELISA BP180 negative	5 (17%)	1 (3.8%)	2 (7.7%)	1 (3.6%)	3 (10.7%)	1 (3.3%)	4 (13.3%)	0 (0%)	5 (17%)
	n = 30		n = 26		n = 28		n = 30		n = 30

n-total number of tested sera.

developed an ELISA using recombinant BP180 for the detection of IgA autoantibodies.

For detecting the IgA autoantibodies in pemphigoid diseases we have used a recently generated recombinant form of the ectodomain of BP180 [45]. The ectodomain of the autoantigen was expressed in a human cell line to ensure optimal posttranslational modifications of the protein, which were shown to influence the binding of pemphigoid autoantibodies of the IgG class [50]. IgA reactivity was found by ELISA using a recombinant form of the 16th non-collagenous region of BP180, which was expressed as a glutathione-S-transferase fusion protein in bacteria, in about 20% of IgA pemphigoid patients [51]. The expression of the antigen in mammalian cells and the fact that our recombinant form of BP180 contains its entire ectodomain significantly raised the sensitivity and specificity of the immunoassay and strongly support its use for the detection of IgA autoantibodies for the diagnosis of pemphigoid diseases.

The ELISA system developed in the present study was shown to be highly sensitive and specific for the detection of IgA autoantibodies in IgA pemphigoid. Since IgA reactivity with the epidermal side of the salt-split human skin by indirect IF microscopy was an inclusion criterion of the study patients, a direct comparison of the sensitivity of these 2 assays was not possible in the present work. The observation that IgA autoantibodies against BP180 were detected in bullous pemphigoid patients is in line with previous findings [7,52]. This intriguing finding prompted us to characterize the detection of IgA autoantibodies against BP180 by our ELISA in patients negative for IgG autoantibodies by the commercially available ELISA using the recombinant 16th non-collagenous region of BP180 (MBL Co, Nagoya, Japan). However, only 6.25% of patients negative for IgG BP180NC16A ELISA showed IgA reactivity against the BP180 ectodomain. Nevertheless, our finding, that a significant proportion of the bullous pemphigoid patients show IgA autoantibodies against the ectodomain of BP180 suggests that the newly developed IgA ELISA may be a useful ancillary diagnostic tool in patients with bullous pemphigoid. In our patient cohort, only a minority of sera (approximately 16%) showed IgA reactivity against BP230, confirming that BP180 is the major autoantigen in IgA pemphigoid.

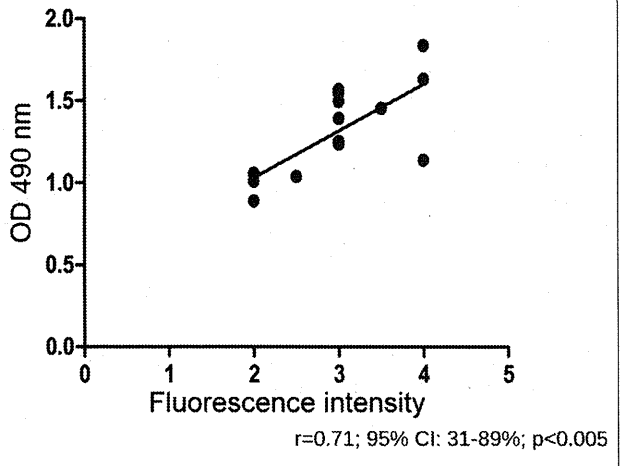

Figure 5 Correlation of BP180-specific IgA levels by ELISA with IgA reactivity by IF microscopy in IgA pemphigoid patients. Spearman rank coefficient (r) = 0.71, 95% confidence interval 31-89%, p < 0.005, n = 16.

In addition to facilitating translational research focusing on the IgA autoimmune response in pemphigoid diseases, the newly developed ELISA could complement or replace the traditional semi-quantitative, observer-dependent and time-consuming IF microscopy on salt-split skin and immunoblotting using concentrated conditioned supernatant of cultured keratinocytes. In contrast to IgG reactivity [20], the ELISA levels of BP180-specific IgA correlated well with the IgA reactivity by indirect IF microscopy. In a recent study, we have investigated the potential of neoepitope-specific rabbit IgG antibodies to induce dermal-epidermal separation in an *ex vivo* assay [45]. Our results showed as proof-of-principle that neoepitope-specific antibodies are pathogenic. However, the pathogenic potential of neoepitope-specific patient IgA autoantibodies has not been directly addressed yet. Using the newly developed ELISA with further recombinant forms of the BP180 ectodomain, which may better reproduce the neoepitopes of

the native shed ectodomain, may facilitate addressing the diagnostic relevance of measuring specifically the neoepitope-specific IgA autoantibodies.

In addition to healthy blood donors, our control group included patients with dermatitis herpetiformis. Dermatitis herpetiformis is a polymorphic autoimmune blistering disease with granular IgA and epidermal transglutaminase deposition in the papillary dermis, without circulating IgA autoantibodies binding to the basement membrane or hemidesmosomes. The disease is associated with a latent gluten-free enteropathy and the majority of patients have IgA type epidermal and tissue transglutaminase antibodies [53,54]. These diseases are associated with IgA autoantibodies staining the endomysium by IF microscopy and recognizing the tissue and epidermal transglutaminase [55,56]. Dermatitis herpetiformis shows overlapping clinical and histopathological features with the pemphigoids [1,53]. In 1979, linear IgA disease was defined as a new entity different from dermatitis herpetiformis on the basis of a linear IgA deposition at the dermal-epidermal junction, which still constitutes its golden diagnostic standard [33,34]. The characteristic diagnostic IF microscopy tests in dermatitis herpetiformis and linear IgA disease were complemented by the development of immunoassays for detecting IgA autoantibodies specific for tissue and epidermal transglutaminase [55-58]. The ELISA system developed in this study adds a new relevant tool, which further facilitates the positive and differential diagnosis of linear IgA disease and dermatitis herpetiformis.

In summary, our results establish an ELISA system for measuring IgA autoantibodies against BP180 in pemphigoid diseases. The newly developed ELISA is the first molecular quantitative immunoassay important for the diagnosis of linear IgA disease. In addition, this immunoassay should prove a useful tool for clinical research and could optimize the diagnosis and monitoring of pemphigoid diseases. Moreover, our findings strongly suggest that linear IgA disease and bullous pemphigoid represent two poles of the clinical spectrum of an immunological loss of tolerance against defined adhesion proteins of hemidesmosomes which is associated, with both IgG and IgA autoantibodies.

Additional material

Additional file 1: Characterization of IgA autoreactivity in bullous pemphigoid (BP) patients. A-F: Cryosections of human salt split skin were incubated with BP sera (A, B, D, E), serum from a patient with IgA pemphigoid (C), and a healthy donor (F). IgA autoantibodies were detected using a FITC-labeled goat anti-human IgA antibody (magnification, 200x). G: Keratinocyte-derived shed (lanes 1-5) and recombinant BP180 ectodomain (lanes 6-10) were separated by 6% SDS-PAGE and electrophoretically transferred to nitrocellulose. The membranes were immunoblotted with serum from BP patients (lanes 3-5 and 8-10), a healthy donor (lane 2 and 7) and a BP180-specific mouse monoclonal Ab (lane 1 and 6), as described in Methods.

Abbreviations

LAD: linear IgA disease; BP: bullous pemphigoid; DH: dermatitis herpetiformis; NHS: normal human sera.

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Authors' contributions

KC and CS designed and performed the ELISA, coordinated the data acquisition, analysed and interpreted the data and drafted the manuscript. SS produced the recombinant BP180 ectodomain and characterized its immunoreactivity. FF performed IF microscopy and immunoblot analysis of the major part of the sera in this study. WN provided us the transfected Flp-in HEK-293 cell line and made contributions to the conception of the study. NI, TH, MH, SK, and LBT provided serum samples used in the study and have participated in the experimental design and drafting of manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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IgA pemphigus

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Abstract Pemphigus is a life-threatening autoimmune blistering disease. Pemphigus is divided into 4 major types; pemphigus vulgaris, pemphigus foliaceus, paraneoplastic pemphigus, and IgA pemphigus. Among them, IgA pemphigus is characterized by tissue-bound and circulating IgA antibodies targeting desmosomal or nondesmosomal cell surface components in the epidermis. Histopathologically, slight epidermal acantholysis and extensive neutrophilic infiltration in either the upper part or all layers of the epidermis were observed. IgA pemphigus is subdivided into intraepidermal neutrophilic IgA dermatosis-type (IEN-type), whose target antigen is still unknown (probably nondesmosomal cell surface protein), and subcorneal pustular dermatosis-type (SPD-type), whose target antigen is desmocollin 1 (Dsc1). We summarize reported cases of IgA pemphigus and describe current knowledge including epidemiology, clinical manifestations, pathology, laboratory tests, pathophysiology, associated diseases, prognosis and treatment, and future perspectives of IgA pemphigus.

Introduction

Pemphigus is a life-threatening autoimmune blistering disease characterized by intraepidermal blisters owing to acantholysis and caused by circulating immunoglobulins, mainly IgG, directed against the cell-cell adhesive device, desmosomes.[1] Pemphigus is divided into 4 major types; pemphigus vulgaris (PV), pemphigus foliaceus (PF), paraneoplastic pemphigus, and IgA pemphigus[2] Although other pemphigus types show IgG autoantibodies, IgA pemphigus is characterized by tissue-bound and circulating IgA antibodies targeting desmosomal or nondesmosomal cell surface components in the epidermis.[3] Histopathological examination reveals slight epidermal acantholysis and extensive neutrophilic infiltration in either the upper part or all of the layers of the epidermis.[4,5] There are many synonyms for IgA pemphigus: intraepidermal neutrophilic IgA dermatosis, intercellular IgA dermatosis, intercellular IgA vesiculopustular dermatosis, intraepidermal IgA pustulosis, IgA pemphigus foliaceus, and IgA herpetiform pemphigus.[6-10] IgA pemphigus is subdivided into intraepidermal neutrophilic IgA dermatosis-type (IEN-type), whose target antigen is still

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unknown (probably nondesmosomal cell surface protein), and subcorneal pustular dermatosis-type (SPD-type), whose target antigen is desmocollin 1 (Dsc1).[5,11-13]

Epidemiology (incidence and prevalence)

IgA pemphigus is a relatively newly proposed disease entity, and about 60 cases of IgA pemphigus have been reported to date. Its frequency is currently not defined, and its race distribution is also unknown. Cases of IgA pemphigus have been reported from almost everywhere.[14] The sex distribution and the age distribution of IgA pemphigus, based on 28 cases from 1982 to 1997[14] reveals a male-to-female ratio of IgA pemphigus as 1:1.33.[14] The age distribution is 1 month to 85 years old (average: 53 years old). Thus far, there have been no reports of IgA pemphigus patients who have died directly from IgA pemphigus.[14] IgA pemphigus is considered to be less life-threatening than other types of pemphigus.

Clinical manifestations

IgA pemphigus is a vesiculopustular disease. The onset of IgA pemphigus is reported to be subacute.[14] Skin lesions initially appear as tense bullae but usually become translucent clear fluid-filled blisters (Figure 1).[14] They transform into pustules, owing to the accumulation of neutrophils.[14] IgA pemphigus patients usually develop erythematous plaques, but sometimes do not.[14] A herpetiform appearance has sometimes also been reported.[10] About half of IgA pemphigus patients suffer from pruritus.[14] Frequently, the affected sites for IgA pemphigus are the trunk and proximal extremities[14]; however, the scalp, postauricular areas, and intertriginous areas may be affected.[14] Yhr mucous membranes are usually spared in IgA pemphigus, although oral mucosal and perianal involvement has been reported in one patient with this condition.[15]

Pathology and laboratory tests

Histopathology

Histopathologically, hematoxylin and eosin staining of the blistered skin of IgA pemphigus shows slight acantholysis (often, no acantholysis is seen) and neutrophilic infiltration in the epidermis (Figure 2).[4] If present, acantholysis in IgA pemphigus is much milder than that seen in classic pemphigus.[4] Characteristically, the clefts and pustules localize in the subcorneal region in SPD-type IgA pemphigus, whereas they are present in the entire or mid epidermis in IEN-type IgA pemphigus.[14] Neutrophilic pustules in the epidermis are hallmarks for IgA pemphigus.[5]

Electron microscopy

The fine localization of the antigen molecule for IgA pemphigus was determined through postembedding immunoelectron microscopy. As a result, SPD-type IgA pemphigus reacted with extracellular space of the desmosomal area,

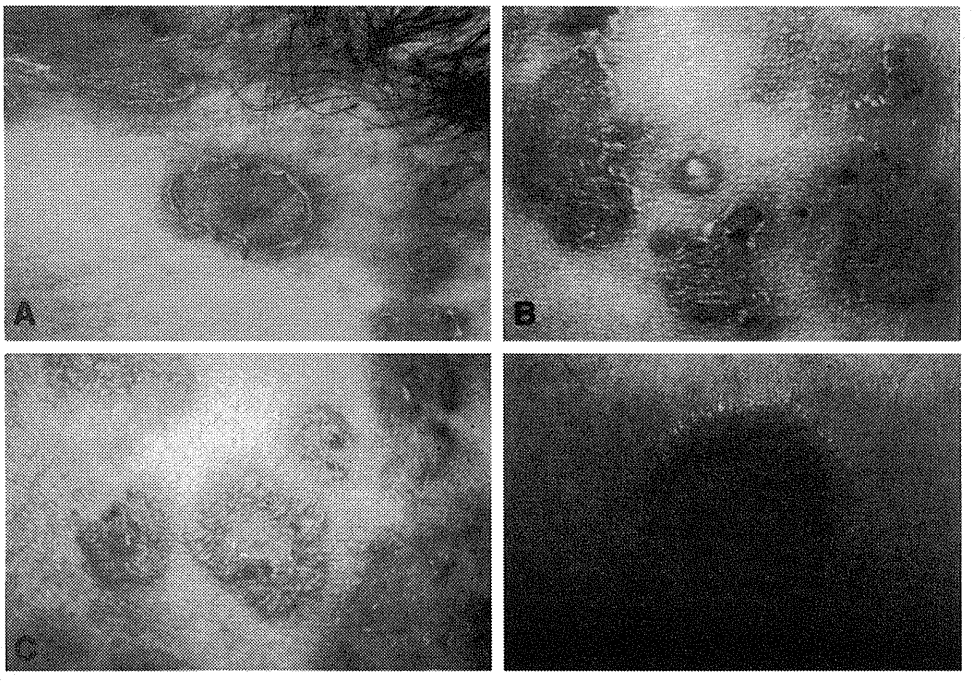

Fig. 1 Clinical appearance of IgA pemphigus. (A, B) SPD-type IgA pemphigus. (C, D) IEN-type IgA pemphigus. Note the yellowish fluid-filled blisters over the erythematous macule (A-D). Hypopyon is observed in (B).

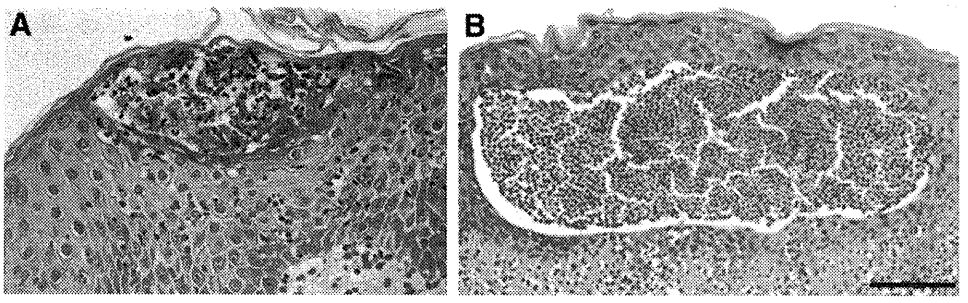

Fig. 2 Histopathology of IgA pemphigus. (A) SPD-type IgA pemphigus. (B) IEN-type IgA pemphigus. Note the subcorneal pustule in (A), and micropustule in the entire epidermis in (B) (hematoxylin and eosin, Bar = 100 μm).

which corresponds to the location of Dsc1, whereas IEN-type IgA pemphigus reacted with extracellular space outside the desmosomes (Figure 3).[16-18] These results suggested that the targets of IgA pemphigus autoantibodies are extracellular regions of cell-cell adhesive junction molecules; Dsc1 for SPD-type and nondesmosomal cell surface protein for IEN-type.

Immunopathology

Immunofluorescence

Direct immunofluorescence, using perilesional patient skin section and anti-human IgA secondary antibody, revealed that there is deposition of fluorescence in the cell-cell contact region[14] (Figure 4). IgG or complement component C3 is also sometimes deposited but is weaker than IgA.[14]

Indirect immunofluorescence using patient sera and various substrates, such as healthy human skin, monkey esophagus, or other epithelia shows the positive result in the cell-cell contact region.[14] The sensitivity of indirect immunofluorescence is reported to be about 50%.[14] The titers for autoantibodies are much lower than that in classic pemphigus.[14]

In addition, cultured COS-7 cells are also used for the detailed characterization of the target antigen for autoantibodies, particularly for Dsc1-Dsc3.[19-22] The vectors coding for Dsc1-Dsc3 are individually transfected to COS-7 cells. Then, patient sera are reacted with these transfected cells. Dotted fluorescent signals are obtained at the cell surfaces if patient sera reacts with such target antigens (Figure 5). This technique is only available in special facilities, including our laboratory.

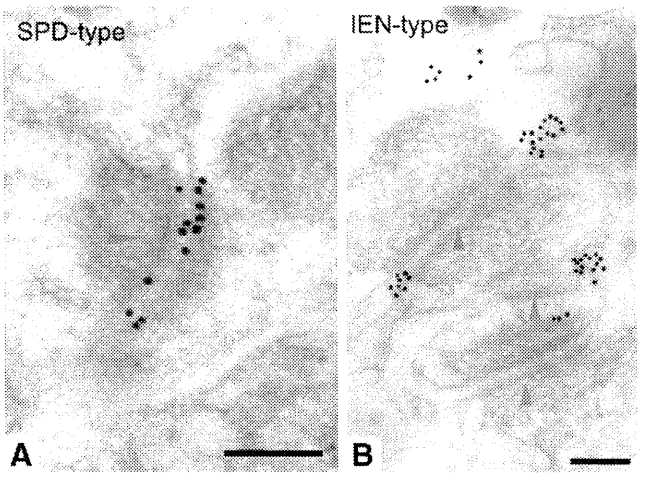

Fig. 3 Immunoelectron microscopy of IgA pemphigus. (A) SPD-type IgA pemphigus. (B) IEN-type IgA pemphigus. Note the deposition of immunogold at desmosome in SPD-type IgA pemphigus, whereas that at nondesmosomal cell-cell contact surface in IEN-type IgA pemphigus. Desmosome: arrowheads. Bar = 200 nm.

Enzyme-linked immunosorbent assay for immunodiagnosis

Enzyme-linked immunosorbent assay (ELISA) is used for the diagnosis of IgA pemphigus and for detection of autoantibodies in individual patients. We previously reported that ELISA detected IgA autoantibodies to either Dsg1 or Dsg3 in only very few patients of IgA pemphigus.[21] As for Dsc ELISA, the specificity and sensitivity is not very high, if compared with an immunofluorescence study using Dsc-transfected COS-7 cells.[11]

Immunoblotting

Immunoblotting of normal human epidermal extract is used to detect targets for autoantibodies in other types of pemphigus; however, such an attempt is not usually successful in IgA pemphigus, although immunoblotting of desmosome-enriched fraction obtained from bovine snout epidermis was successful for 10 of 17 IgA pemphigus cases.[5]

Pathophysiology

It is currently known that IgA autoantibodies bind to Dsc1 in SPD-type IgA pemphigus, as well as Dsg1 and Dsg3 in rare cases of IgA PF and IgA PV, respectively.[17,19,23-25] There is no clear explanation for the mechanism by which IgA autoantibodies produce characteristic skin lesions in IgA pemphigus. A few reports suggest mechanisms for the initiation of skin lesions in IgA pemphigus. Interleukin-5 (IL-5), Th2-type cytokine, which is known to produce IgA class antibodies in B cells, may be activated in patients with IgA pemphigus.[14] In addition, γδT cells are reported to be involved in IgA pemphigus process.[26] As IgA possesses

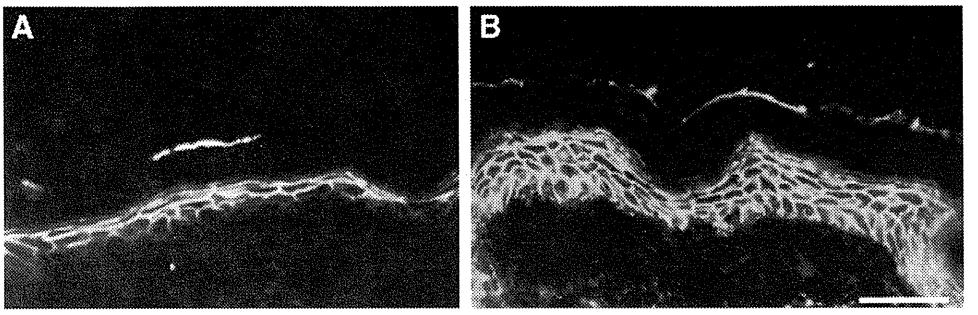

Fig. 4 Indirect immunofluorescence of IgA pemphigus. (A) SPD-type IgA pemphigus. (B) IEN-type IgA pemphigus. Note IgA cell-cell junction staining in the uppermost region in the epidermis in SPD-type IgA pemphigus, while in the entire epidermis in IEN-type IgA pemphigus. Bar =100 μm.

specific binding sites for the IgA-Fc receptor, CD89, in monocytes and granulocytes, accumulation of neutrophils in the epidermis is believed to occur via IgA autoantibodies; hence, proteolytic cleavage of the keratinocyte cell-cell junction may occur.[27] There have been no functional studies of the pathophysiology of acantholysis initiated and caused by IgA autoantibodies in IgA pemphigus.

The other issue to be considered is the possible epitope-spreading phenomenon, a newly proposed idea in which an inflammatory event releases new target antigens, exposes them to the immune system, and then induces subsequent autoimmunity to new related antigens.[28] In fact, in some IgA pemphigus patients, multiple target antigens have been identified: for example, single patients showed reactivity to all Dsc1-Dsc3, and some patients reacted with both Dsc and Dsg.[24,29]

Associated diseases

Malignancies are reported to be associated with IgA pemphigus. Chronic inflammation in IgA pemphigus may be a result of the development of cancers or drug-related immunosuppression. IgA gammopathy and lung cancer have been reported to be associated with IgA pemphigus.[30] There are some reports of cases with presence of both IgA and IgG antibodies, which raises the question of whether pemphigus with both IgG and IgA autoantibodies is a subset of IgA pemphigus or not.[31]

The other complications for IgA pemphigus are disease-related and treatment-related infections. Disease-related infections are caused by open wounds from the blister and erosion, whereas treatment-related infections are caused by systemic immunosuppressions.[14] Corticosteroids may cause growth retardation in childhood cases of IgA pemphigus.[32]

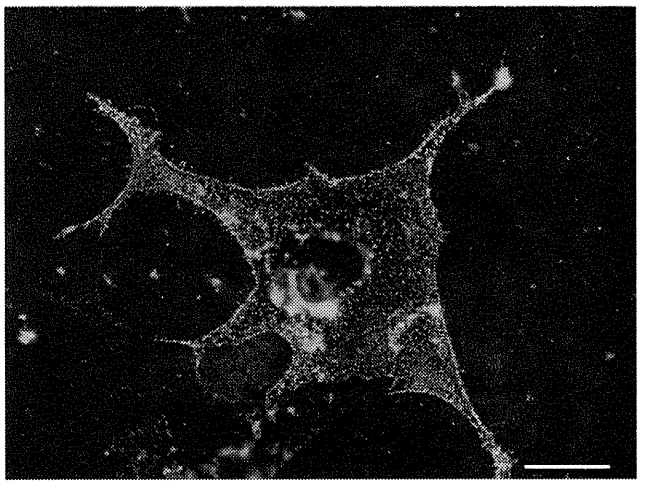

Fig. 5 Immunofluorescence by SPD-type IgA pemphigus serum using COS-7 cells transfected with Dsc1 encoding vector. Note the dotted positive signals on the cell surfaces of Dsc1-expressing cells. Bar = 10 μm.

Prognosis

The clinical phenotype of IgA pemphigus is much milder than classic pemphigus; however, as IgA pemphigus is a newly proposed entity, the clinical data for its prognosis are still limited. Histopathologically, because the cleft in IgA pemphigus occurs within the epidermis, almost no scarring is observed after healing.[14] Probably, the main issues that determine the prognosis are the side effects caused by immunosuppressive agents including corticosteroids.

Treatment

The limitation of reported cases of IgA pemphigus hinders the analyses of its effective treatments. Treatments of IgA pemphigus are performed, based on the disease pathomechanism and anecdotal reports. The mainstay for treatment of IgA pemphigus is oral and topical corticosteroids, owing to the inflammatory nature of IgA pemphigus.[33] Corticosteroid is considered to decrease inflammation by reversing increased permeability in capillaries and suppressing the activity of neutrophils[34,35]; moreover, corticosteroids stabilize lysosomal membranes and suppress lymphocytes and antibody production.[36] The usual side effects for oral corticosteroids, which need to be considered,

are gastric ulcer, osteoporosis, bone fracture, adrenal insufficiency, and diabetes.[37] In addition, dapsone may be useful in treating IgA pemphigus due to its effect in suppressing neutrophilic infiltration.[19] Isotretinoin and acitretin are also reported to be useful for the treatment of IgA pemphigus.[38,39] Recently, adalimumab and mycophenolate mofetil, which are known to be effective in classic pemphigus, are also reported to be useful in treating IgA pemphigus.[40]

Future perspectives

The classification and target antigens are not fully understood in IgA pemphigus; particularly, the autoantigen for IEN-type IgA pemphigus is really mysterious. This study should be the most important issue in future perspectives in IgA pemphigus. In terms of basic immunology, possible subclass switching between classic IgG pemphigus and IgA pemphigus should be an interesting aspect for future studies. In addition, the IgA-related autoimmune diseases are relatively rare, the study in IgA pemphigus should also be a paradigm for IgA autoimmunity. Finally, a guideline for the treatment of IgA pemphigus is to be established very shortly.

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CED
Clinical and Experimental Dermatology

Bullous pemphigoid associated with renal transplant rejection

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Bullous pemphigoid (BP) is a rare disease in childhood, characterized by subepidermal bullae and antibodies against the 180-kDa BP antigen. We report a male patient on renal dialysis who presented with BP after rejection of his donor kidney.

The patient was a 13-year-old boy, the fourth child of consanguineous parents, who had multiple congenital problems including cleft lip and palate, hypopituitarism and small genitalia. At the age of 9 years, a diagnosis of chronic renal failure due to congenital renal dysplasia was made. Almost immediately, the patient required haemodialysis treatment. He was transplanted with an unrelated donor kidney, and initially given immunosuppression with tacrolimus, mycophenolate mofetil and prednisolone. After approximately 26 months, because of severe rejection, tacrolimus was switched to sirolimus. After recurrent acute rejection and severe allograft nephropathy, the transplanted organ failed approximately 6 months later. The patient was started on peritoneal dialysis, and all renal immunosuppression was stopped after 3 months.

2 months following cessation of immunosuppression, the patient presented with multiple tense bullae over the trunk and limbs (Fig. 1). In addition, there were signs of skin fragility where dressings had been removed.

Histological examination of a skin biopsy confirmed a subepidermal blister containing fibrin and scanty eosinophils. Using direct immunofluorescence, linear C3 was seen at the dermoepidermal junction. Indirect immunofluorescence on salt-split skin found IgG anti-basement membrane zone antibodies binding to the epidermal surface (Fig. 2). IgG antibodies to the NC16A domain of BP180 were detected by ELISA at a titre of 133.62 (positive > 15). ELISA for BP230 was negative. Immunoblotting using epidermal extracts did not reveal any additional antigens.

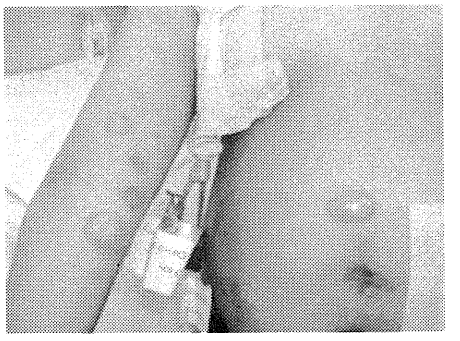

Figure 1 Tense bullae over the trunk and right arm.

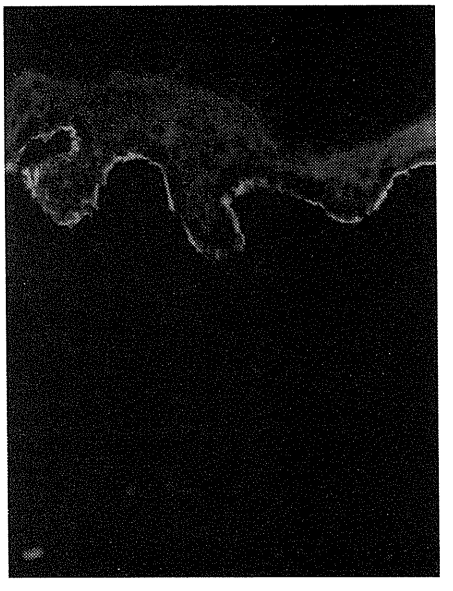

Figure 2 Indirect immunofluorescence on salt-split skin showing IgG anti-basement membrane zone antibodies at a titre of 1 : 160, binding to the epidermal surface.

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Table 1 Summary of published cases of BP in association with renal transplant rejection.

Reference	Age at presentation, years	IF Results	Type of transplant	Transplant status	Resolution
Yamazaki *et al.*[1]	9	IgG and C3 to BMZ on direct IF; antibodies to BP180 on immunoblotting	Related donor (mother)	Chronic rejection	Blistering resolved as the allograft shrunk and became atrophic
Feehally *et al.*[2]	15	IgG, C3 and C1q to BMZ on direct IF	Second cadaveric transplant	Chronic rejection	Transplant nephrectomy resulted in total resolution within 1 month
Tessari *et al.*[3]	47	Continuous C3 band on direct IF	Cadaveric	Chronic rejection	Excision of the allograft resulted in total remission
This study	13	IgG to BMZ on indirect IF; antibodies to BP180 on ELISA	Cadaveric	Chronic rejection	Excision of the allograft resulted in total remission

BMZ, basement membrane zone; IF, immunofluorescence.

Prednisolone 0.7 mg/kg successfully prevented blistering, but as the dose was reduced to 0.35 mg/kg, blistering relapsed. Owing to the possibility of an association between the patient's rejected but retained transplanted kidney and the immunobullous eruption, a decision was made to perform a transplant nephrectomy. Within 6 months, the patient was blister-free and off immunosuppressive therapy. At follow-up 4 years later, he was still on dialysis and had no recurrence of his BP.

Our case, like the three previous cases[1–3] (Table 1), have all been associated with failing renal transplant grafts. These cases raise the possibility of antibodies being present that are primarily directed at the renal allograft but that crossreact with the epidermal basement membrane, causing BP. The epidermal and glomerular basement membrane do share antigens, such as type IV collagen, and a subepidermal blistering disorder associated with glomerulonephritis, caused by antibodies to the NC1 domain on the α5 chain of type IV collagen, has been described.[4,5] However, shared antigens do not provide the explanation in our case, as anti-BP180 antibodies were present and BP180 is not expressed in the kidney[4,5] There was also no evidence of antibodies to type IV collagen, as there was no immunoreactivity with the dermal side of salt-split skin. It seems unlikely that the anti-BP180 antibodies were a secondary phenomenon as a result of epitope spreading, given that they were detected so early in the course of disease. Furthermore, there was no evidence of concurrent autoantibodies to other antigens.

An increased incidence of autoimmune disease has been seen in organ-transplant recipients on immunosuppression, and in children with primary immunodeficiency disease.[6] One hypothesis is that chronic infection and failure of pathogen eradication leads to an abnormal chronic inflammatory response and an intolerance to self antigens. However, in our case and the other cases documented, there was a temporal link between the onset of BP and the renal allograft rejection, and we suggest an indirect association as a result of immune upregulation in the context of renal allograft rejection.

In conclusion, BP is a rare disease in childhood, characterized by subepidermal bullae and antibodies against the 180-kDa BP antigen. In this case, subsequent removal of the rejected kidney resulted in total remission of the BP. Both renal physicians and dermatologists need to be aware of this probable link between BP and renal allograft rejection, as removal of the failed graft in our case and several others seems to be curative.

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It is important to note that Hoste *et al.* (2011) have not proposed that caspase-14 is the only protease that cleaves filaggrin. Rather, their report shows that, in the absence of caspase-14, other enzymes can initiate proteolysis of filaggrin. In addition, proteases such as calpain 1 and bleomycin hydrolase (Kamata *et al.*, 2009) are required to complete the degradation of filaggrin. Therefore, to understand the regulation of filaggrin degradation, it is necessary to determine the interplay of caspase-14 with these other proteases as well as the order in which the proteolytic cuts occur.

Concluding remarks

New evidence demonstrates that not only mutations in the filaggrin gene but also alterations in filaggrin processing may result in skin barrier defects and that caspase-14 takes part in this process. Hoste *et al.* (2011) provide a basis for improving strategies to diagnose filaggrin-associated skin disorders and to modulate caspase-14-dependent barrier function of the stratum corneum.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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See related article on pg 2271

Demonstration of Epitope Spreading in Bullous Pemphigoid: Results of a Prospective Multicenter Study

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Di Zenzo and colleagues have undertaken a multicenter prospective study to clarify the epitope profile for IgG anti-BP180 and BP230 antibodies in 35 patients with bullous pemphigoid (BP). Both intra- and intermolecular epitope spreading events were observed, in which epitopes shifted exclusively from extracellular to intracellular domains. The presence of IgG antibodies to the BP180 C-terminal domain and BP230, in addition to the BP180-NC16A domain, correlated with disease severity and activity, suggesting specific pathogenic relevance for anti-BP230 antibodies. Epitope spreading was found in both T- and B-cell recognition. IgA anti LAD-1 antibodies are frequently found in patients with BP; these antibodies appear to follow the development of IgG antibodies to BP180 and BP230 by epitope spreading. These observations provide direction for future studies of the pathogenesis of and treatments for BP.

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Epitope spreading plays an important role in the development of autoimmune diseases

Various autoimmune diseases develop and progress via epitope spreading (ES). In ES, inflammation induced by autoimmunity to an initial epitope damages target tissue, which subsequently induces antibodies to secondary epitopes on the same or different antigens (Chan *et al.*, 1998). Intra- or intermolecular ES is not simply an epiphenomenon, because it is important for the development of each disease.

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Clinical Implications

- Intra- and intermolecular epitope spreading (ES) is observed in bullous pemphigoid (BP); epitopes shift from extracellular to intracellular domains.
- The presence of IgG antibodies to the BP180 C-terminal domain and BP230, in addition to the BP180-NC16A domain, correlates with disease severity and activity. ES is found for both T- and B-cell recognition in BP.
- Analysis of ES may provide clues for developing novel therapies.

Previous studies in bullous pemphigoid (BP), using both human materials and mouse models, have clarified the progress and relevance of ES (Di Zenzo *et al.*, 2008, 2010). A retrospective study of BP sera by enzyme-linked immunosorbent assays (ELISA) that used recombinant proteins of BP180 and BP230 suggested the occurrence of ES in patients with BP (Yoshida *et al.*, 2006). However, the precise development and progress of ES and its correlation to BP disease activity have not been elucidated.

Di Zenzo *et al.* (2011, this issue) performed a multicenter prospective epitope profile study of IgG antibodies to both BP180 and BP230 during the clinical course of 35 patients with BP. ES occurred in about half of the patients. Intramolecular ES in BP180 occurred first in the extracellular domain and then spread to the intracellular domain. Intermolecular ES also occurred and spread from BP180 to BP230. Finally, reactivity, both with extracellular BP180 epitopes and with intracellular BP230 epitopes, correlated with disease severity and activity, suggesting pathogenic significance for the anti-BP230 autoantibodies.

ES occurred in patients with BP

Over a 12-month period, ES was found in 17 (49%) of 35 patients with BP, whereas 18 (51%) showed no epitope shifts. All 17 patients showing ES reacted with BP180 epitopes, and 12 reacted with BP230 epitopes at the time of diagnosis. Twenty-four different ES events were observed, and ES from extracellular BP180 epitopes to intracellular epitopes of BP180 and BP230 occurred most frequently. ES also occurred before the development of overt clinical manifestations of BP.

ES always spread from extracellular to intracellular domains

Three BP patients showed ES from extracellular BP180 epitopes to intracellular epitopes of BP180 and BP230, whereas the opposite was not observed. In addition, three patients showed intermolecular ES from BP180 epitopes to BP230 epitopes, whereas the opposite did not occur. This single direction of ES events should provide important insights into the mechanism of ES.

This study indicated clinical significance of ES events in BP

ES events occurred most frequently in the first 3 months after BP diagnosis, suggesting that ES is an early event in the course of the disease. Accordingly, ES was observed rarely during relapses of the disease. Perhaps immunosuppressive therapy impairs T- and B-cell activation, reducing subsequent ES events.

Severity and activity of disease were correlated with reactivity with both the NC16A and the C-terminal domains of BP180, but not with the N-terminal intracellular domain. Notably, two patients with active disease showed exclusive reactivity with the BP180 C-terminal domain. These results indicate that reactivity with the C-terminal domain of BP180 is pathogenic not only in mucous membrane pemphigoid but also in BP.

Importantly, ES was clearly related to disease severity and activity at the time of diagnosis. It is interesting that reactivity with epitopes occurred later via ES decreased rapidly. IgG reactivity with BP180 extracellular domains and with BP230 intracellular domains was clearly related to disease severity and activity, whereas reactivity with BP180 intracellular domains was not. These results suggest that not only antibodies to BP180 extracellular domains but also antibodies to BP230 are pathogenic, whereas reactivity with BP180 intracellular domains is an epiphenomenon.

Anti-BP230 autoantibodies were suggested to be pathogenic

Three patients without BP180-NC16A reactivity nonetheless reacted with other BP180 and BP230 epitopes. As mentioned above, IgG reactivity with BP230 was clearly related to disease severity and activity, suggesting that anti-BP230 antibodies are pathogenic. Although reactivity with BP230 has generally been considered a nonpathogenic epiphenomenon, previous evidence suggests a pathogenic role for anti-BP230 antibodies. Anti-BP230 antibodies induced skin lesions in a mouse model, and BP230 titers by immunoblotting and ELISA correlated with disease activity (Hamada *et al.*, 2001; Yoshida *et al.*, 2006). Recently, sera we collected from 35 patients with active BP showed only anti-BP230 antibodies by ELISA, reacted only with intracellular sites of hemidesmosomes by immunoelectron microscopy, and showed a unique ES profile (Fujihara *et al.*, 2011). This combination of evidence suggests pathogenic significance for BP230 antibodies in BP and would thus indicate that autoantibodies bind to intracellular antigens by unknown mechanisms.

Nonpathogenic IgA anti-LAD-1 antibodies were found frequently in BP

Di Zenzo *et al.* (2011) detected IgA reactivity with LAD-1 in 11 of their 35 patients. This result complements that of our study, in which a significant number of BP sera possessed IgA antibodies reactive with recombinant protein of LAD-1 by ELISA (Csorba *et al.*, 2011). In four patients in the present study, IgG reactivity with BP180 and BP230 spread to IgA reactivity with LAD-1. However, because IgA anti-LAD-1 antibodies showed no association with disease severity and activity, disease duration, mucosal involvement, or ES, they were considered to have a limited pathogenic role.

Nevertheless, because this finding may provide insight into development of pathogenic IgA anti-LAD-1 antibodies in linear IgA bullous dermatosis, further experiments should explore the mechanisms of class switching between IgG and IgA.

ES also occurred in T-cell recognition

ES in T-cell reactivity was examined in nine of the patients with BP, and a proliferative T-cell response to BP180 and/or BP230 was observed in five. Two of these five patients showed ES events in T-cell recognition. One showed a shift from the extracellular BP180 domain to the BP230 C-terminal domain, and another showed a shift from the BP180-NC16A domain to the BP180 C-terminal domain. In general, autoreactive B and T cells showed similar epitope profiles throughout the course of the disease.

Perspectives

In this report of their prospective study, Di Zenzo *et al.* reveal many novel and interesting results. In future studies, the pathogenic role in BP should be confirmed for IgG antibodies to the BP230 and the BP180 C-terminal domain, as well as for IgA anti-LAD-1 antibodies. Novel ES mechanisms may be discovered in the production of pathogenic IgG anti-BP230 antibodies and IgA anti-LAD-1 antibodies.

Di Zenzo and co-workers' results also give us insight into potential new treatments, including the use of decoy peptides, tolerance-inducing peptides, and antigen-specific immunoabsorption. In particular, the rapid decrease of autoantibodies against ES-induced late epitopes should yield clues for pursuing novel therapeutic approaches.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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IL-6 and IL-8 synthesis in tumor cells in our assay. To prove this assumption, MC supernatants were pre-incubated with a neutralising antibody against TNF-α or isotype control antibody before stimulation of the cancer cells. When TNF-α was neutralised, MC–mediated augmentation of IL-8 mRNA expression was almost completely lost and IL-8 concentration decreased to basal levels in Mel-1, SCL-1, SCC-12 and SCC-13 cells (Fig. 2a–d grey bars). Regarding IL-6 mRNA expression, blockade of TNF-α in MC supernatants also abrogated the increase in expression in SCL-1 and SCC-12 cells, but led to an only 50 percent inhibition in SCC-13 cells (Fig. 1d–f). Thus, regulation of IL-6 in SCC-13 cells seems not only processed through TNF-α but probably also through other factors like histamine or tryptase. Similar to our results obtained in SCC-13 cells, TNF-α seemed not responsible for IL-8 synthesis in Mel-4 cells. (Fig. 2e). However, stimulation of Mel-4 cells with histamine, another MC mediator, led to increased gene expression of IL-8 (Fig. 2f). Inhibition experiments with H1- or H2-receptor antagonists desloratadine or famotidine confirmed that up-regulation of IL-8 synthesis in Mel-4 cells was histamine dependent and mediated via the H2 receptor (Fig. 2f).

Conclusions

Using a novel model to study the communication between human MCs and human tumor cells derived from the same tissue site, we have been able to show that normal human MCs communicate with tumor cells and that TNF-α and histamine are key players in this process. Surprisingly, melanoma and squamous carcinoma cells differed in their response to MC-derived TNF-α: while IL-8 expression and release were up-regulated in melanoma and SCC cells, IL-6 was unaltered in melanoma cell lines, but up-regulated in SCC cell lines. In the SCC lines, MCs further increased constitutive IL-6 and IL-8 mRNA generation, whereas in the melanoma cell lines, Mel-1, Mel-2 and Mel-4, which exhibit an only marginal constitutive IL-8 production, IL-8 synthesis was enhanced by MC-derived TNF-α and/or histamine, far exceeding baseline levels.

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Conflict of interest

The authors have no conflicting interest.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Determination of TNF-α protein levels in co-culture supernatants.

Figure S2. Induction of IL-6 and IL-8 mRNA expression in mast cell/cancer cell co-cultures.

Appendix S1. Material and Methods.

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Letter to the Editor

Lesional Th17 cells and regulatory T cells in bullous pemphigoid

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Abstract: Th17 cells play crucial roles in the pathogenesis of autoimmune diseases. We previously reported that Th17 cells are recruited to the lesional skin in pemphigus vulgaris (PV) and pemphigus foliaceus (PF). The aim of this study was to evaluate

