

Report

Autoimmune subepidermal blistering diseases in Uganda: correlation of autoantibody class with age of patients

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Abstract

Background No data are available on the incidence and immunoreactivity of autoimmune subepidermal blistering skin diseases in East Africa.

Methods All patients with frank blisters/erosions on the skin and/or mucous membranes that attended the Department of Dermatology at Mbarara University, Uganda, from May 2000 to June 2002, were investigated. The diagnosis was based on the clinical presentation and on the presence of circulating autoantibodies detected by indirect immunofluorescence microscopy on 1 M NaCl-split human skin and by Western blotting of recombinant and cell-derived forms of BP180, BP230, and type VII collagen.

Results Twenty-two patients with autoimmune subepidermal blistering skin disorders were identified, including nine with bullous pemphigoid (41%), four with linear immunoglobulin A (IgA) disease (18%), three with mucous membrane pemphigoid (14%), two with linear IgG/IgA bullous dermatosis (9%), and one each with cicatricial pemphigoid and epidermolysis bullosa acquisita (5%). In addition, two patients with immunoreactivity to both the epidermal and dermal side of salt-split skin by indirect immunofluorescence microscopy, who were unreactive to type VII collagen, were provisionally diagnosed as "mixed pemphigoid" (9%). In patients with subepidermal blistering diseases, IgG reactivity correlated significantly with old age, whereas younger patients preferentially developed IgA autoantibodies ($P = 0.024$).

Conclusions The age of patients with autoimmune subepidermal blistering diseases appears to influence the immunoglobulin class of autoantibodies. The high frequency of IgA autoantibodies in Ugandan patients may be explained by the age distribution of the Ugandan population.

Introduction

Autoimmune subepidermal blistering disorders include bullous pemphigoid (BP), mucous membrane pemphigoid (MMP), cicatricial pemphigoid (CP), linear immunoglobulin A (IgA) disease (LAD), linear IgG/IgA bullous dermatosis, pemphigoid gestationis, anti-p200 pemphigoid, epidermolysis bullosa acquisita, lichen planus pemphigoides, and bullous systemic lupus erythematosus. In these diseases, autoantibodies are directed against proteins of the dermo-epidermal junction, such as BP180 and BP230 (BP, MMP, CP, LAD, linear IgG/IgA bullous dermatosis, pemphigoid gestationis, and lichen planus pemphigoides), laminin 5, $\alpha 6\beta 4$ integrin (MMP, CP), p200 protein (anti-p200 pemphigoid), and type VII collagen (epidermolysis bullosa acquisita, bullous systemic lupus erythematosus) (reviewed in Schmidt and Zillikens¹).

The relative frequency of autoimmune bullous skin diseases may vary between different geographic regions. In Germany, France, and Singapore, BP is the most common autoimmune

subepidermal blistering disorder with an incidence of about six new cases per 1 million inhabitants per year, followed by MMP, pemphigoid gestationis, and LAD.²⁻⁴ In contrast, in South Africa and Mali, LAD was reported to be more frequent, whereas in China and Malaysia, the incidence of all autoimmune subepidermal blistering diseases seems to be lower.⁵⁻⁸ So far, however, no data are available on the incidence of these disorders in East Africa.

In the present study, we determined the incidence and relative frequency of autoimmune subepidermal blistering disorders in south-western Uganda, and analyzed the specificities of both IgA and IgG autoantibodies against various recombinant and cell-derived forms of the relevant target antigens.

Materials and Methods

Patients

This study comprised 22 consecutive patients seen at the Department of Dermatology at Mbarara University Teaching



Figure 1 Papules, plaques, and tense blisters on the right arm and trunk of Patient 5 with bullous pemphigoid

Hospital, Mbarara, Uganda, between May 2000 and June 2002. All patients were included who: (i) presented with frank blisters and/or erosions on the skin and/or mucous membranes (Fig. 1); (ii) revealed reactivity by indirect immunofluorescence (IF) microscopy on 1 M NaCl-split human skin (Fig. 2); and (iii) had not yet been diagnosed with autoimmune blistering skin diseases. Sera from patients suspected to have pemphigoid gestationis were analyzed by indirect IF microscopy incubated with human complement.⁹ There were no exclusion criteria. No patient had been treated with systemic immunosuppressants or topical class III–IV corticosteroids prior to the first presentation when serum samples were obtained.

Immunoblotting and enzyme-linked immunosorbent assay

Sera were also analyzed by immunoblotting of a panel of recombinant fragments of the relevant target antigens, including the immunodominant 16th noncollagenous (NC) domain of BP180 (BP180 NC16A, amino acids 490–562), an immunodominant 55-kDa C-terminal stretch of BP230 (BP230-55 kDa, amino acids 1710–2189), and the 120-kDa soluble ectodomain of BP180 (LAD-1) from concentrated conditioned culture medium of HaCaT cells.^{10–12} In addition, sera with dermal binding by indirect IF were subjected to Western blotting with dermal extracts and the NC1 domain of type VII collagen (amino acids 1–125).^{13,14}

Skin biopsies for histopathology and direct IF microscopy were not taken because of the lack of liquid nitrogen as well as processing and storing facilities in Mbarara. The final diagnosis was therefore based on the clinical presentation and the results obtained by both indirect IF microscopy and Western blotting with the relevant target antigens as detailed in Table 1.

Demographic data

Data on the age and geographic distribution of the Ugandan population were retrieved from the *Annual Report of the Ugandan Bureau of Statistics (2002)*.¹⁵ Data for Singapore, China, and

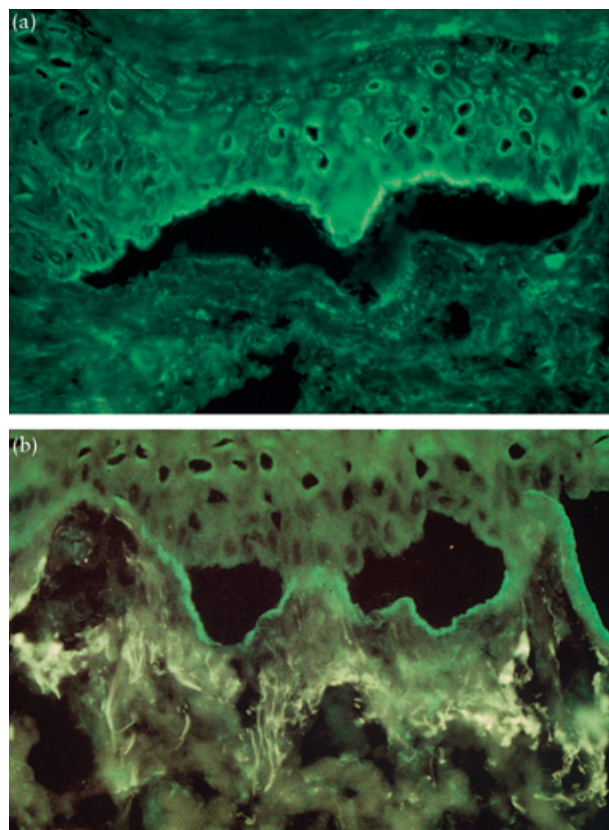


Figure 2 Indirect immunofluorescence microscopy on 1 M NaCl-split human skin, showing epidermal staining by immunoglobulin A (IgA) autoantibodies in Patient 18 with linear IgA disease (a) and dermal binding by IgG autoantibodies in Patient 22 with epidermolysis bullosa acquisita (b)

Germany came from the *US Census Bureau, International Data Base, October 2002 version*.¹⁶

Statistics

For statistical analyses, the Mann–Whitney *U*-test was used.

Results

All patients were of Ugandan origin; 11 were male and 11 were female. The age at disease onset ranged from 2 to 85 years (median, 34 years). All patients had skin lesions and, in five, oral lesions were also present that, in two, were accompanied by involvement of the genital mucosa. In six patients, skin lesions healed with scarring. The patients came from five districts in south-western Uganda with a total population of 2.058 million people. The patients' age, gender, and origin are detailed in Table 2. Based on these data, the annual incidence rate of autoimmune subepidermal blistering diseases in south-western Uganda was roughly estimated at 5.34 new cases per 1 million inhabitants.

Table 1 Diagnostic criteria for patients with subepidermal blistering diseases included in this study

Diagnosis	Criteria
Bullous pemphigoid*	Blisters/erosions <i>not</i> preferentially affecting mucous membranes; binding of predominant IgG antibodies to the epidermal side of salt-split skin by indirect IF microscopy; IgG antibodies against BP180
Linear IgA disease*	Blisters/erosions <i>not</i> preferentially affecting mucous membranes; binding of predominant IgA antibodies to the epidermal and/or dermal side of salt-split skin by indirect IF microscopy; IgA antibodies against LAD-1 and/or BP180
Mucous membrane pemphigoid†	Blisters/erosions preferentially affecting any or all mucous membranes, with or without scarring; binding of IgG and/or IgA antibodies to the epidermal and/or dermal side of salt-split skin by indirect IF microscopy
Cicatricial pemphigoid†	Blisters/erosions <i>not</i> preferentially affecting mucous membranes; skin lesions heal with scarring; binding of IgG and/or IgA antibodies to the epidermal and/or dermal side of salt-split skin by indirect IF microscopy
Linear IgG/IgA bullous dermatosis‡	Blisters/erosions <i>not</i> preferentially affecting mucous membranes; combined binding of IgG and IgA antibodies to the epidermal side of salt-split skin by indirect IF microscopy with titres not differing by more than 1 titre step; autoantibodies against BP180
Pemphigoid gestationis*	Like bullous pemphigoid; association with pregnancy
Anti-p200 pemphigoid*	Blisters/erosions; binding of IgG antibodies to the dermal side of salt-split skin by indirect IF microscopy; reactivity with the 200-kDa p200 antigen in dermal extracts by Western blotting
Epidermolysis bullosa acquisita*	Blisters/erosions; binding of antibodies to the dermal side of salt-split skin by indirect IF microscopy; reactivity with type VII collagen by Western blotting
Bullous systemic lupus erythematosus*	Blisters/erosions; fulfilling the American College of Rheumatology criteria for SLE; binding of antibodies to the epidermal and/or dermal side of salt-split skin by indirect IF
"Mixed pemphigoid"§	Blisters/erosions <i>not</i> preferentially affecting mucous membranes; combined binding of antibodies to the epidermal and dermal side of salt-split skin by indirect IF microscopy; reactivity with BP180 and/or BP230; absence of reactivity with type VII collagen and p200

IF, immunofluorescence; Ig, immunoglobulin; SLE, systemic lupus erythematosus.

*Reviewed in Schmidt and Zillikens.¹

†According to the 1st International Consensus on Mucous Membrane Pemphigoid.^{2,7}

‡As proposed in Zone *et al.*²³

§Provisional term, detailed in "Discussion" section.

The results obtained by indirect IF microscopy and the final diagnoses are presented in Table 2. Three patients with exclusive epidermal binding in whom some lesions, that were limited to the normal skin, healed with scarring were classified as BP or LAD depending on their IF reactivity. In these patients, scarring may be a result of the high incidence of secondary infection that was observed in our patients under the generally poor hygiene conditions. No patient with pemphigoid gestationis, lichen planus pemphigoides, anti-p200 pemphigoid, or bullous systemic lupus erythematosus was identified. In addition, in all sera with epidermal binding by indirect IF microscopy of human salt-split skin, reactivity with recombinant BP180 NC16A, BP230-55 kDa, and cell-derived LAD-1 was assayed; the results are summarized in Table 3.

The age of the patients with subepidermal blistering diseases was related to the immunoglobulin class of autoantibodies that bound to the epidermal side of artificially split skin by indirect IF microscopy (Table 2). When patients with exclusive generation of IgG autoantibodies were compared with those with exclusive IgA autoantibodies, a significantly different age was revealed ($P = 0.024$). Similarly, patients with predominant IgG autoantibodies were found to be statistically significantly older than patients who preferentially showed IgA autoantibodies ($P = 0.011$).

Discussion

In this study, the annual incidence of autoimmune subepidermal blistering diseases in Uganda was roughly estimated as 5.34 per 1 million people, which is lower than the values of 10.4 and 8.5 new cases per 1 million inhabitants in France and Germany, respectively.^{2,3} Data on incidence, however, were difficult to analyse in our patients as the Department of Dermatology at Mbarara serves a vast area of more than 500 km in diameter where transport is often difficult and not affordable to everybody. It is therefore likely that numerous cases were not seen.

Another particularity encountered in this study was that histopathology and direct IF microscopy could not be performed in Mbarara as a result of technical and storage problems. Thus, diagnoses were based on clinical presentation, indirect IF microscopy, and, in all patients, by the detection of circulating autoantibodies against cell-derived or recombinant forms of the relevant target antigens (Table 1). False positive detection of circulating autoantibodies is therefore unlikely. In contrast, patients who tested negative by indirect IF, but who may have been reactive by direct IF microscopy, were missed in our study. In patients with BP, LAD, and MMP/CP, which together account for more than 90% of autoimmune

Table 2 Demographics, indirect immunofluorescence reactivity, and diagnosis of patients included in this study

No.	Sex	Age (years)	District	Diagnosis	Mucosal involvement	Scar formation	Indirect IF*			
							Epidermal		Dermal	
							IgG	IgA	IgG	IgA
1	M	74	Mbarara	BP	–	No	640	–	–	–
2	F	85	Mbarara	MMP	Oral	Yes	20	–	–	–
3	M	26	Mbarara	BP	–	No	160	–	–	–
4	F	67	Bushenyi	BP	–	Yes†	160	–	–	–
5	M	45	Mbarara	BP	–	No	40	–	–	–
6	F	45	Mbarara	BP	–	No	640	–	–	–
7	M	2	Mbarara	LGAD	–	No	40	20	–	–
8	M	51	Bushenyi	MP	–	No	20	–	40	–
9	F	42	Mbarara	MMP	Oral, genital	No	40	20	80	–
10	M	15	Mbarara	MP	–	No	80	–	–	20
11	F	75	Ntungamo	BP	–	No	320	40	–	–
12	F	53	Rukungiri	BP	–	No	640	20	–	–
13	M	44	Mbarara	BP	–	No	320	10	–	–
14	F	7	Mbarara	BP	–	Yes†	40	–	–	–
15	F	85	Mbarara	MMP	Oral, genital	Yes	40	–	–	–
16	F	4	Bushenyi	LAD	–	No	–	160	–	–
17	M	10	Rukungiri	LAD	–	No	–	40	–	–
18	M	12	Mbarara	LAD	–	Yes†	40	320	–	–
19	F	17	Mbarara	LAD	–	No	–	320	–	–
20	F	3	Mbarara	LGAD	–	No	10	10	–	–
21	M	24	Bushenyi	CP	Oral	Yes	320	20	80	–
22	M	11	Mbarara	EBA	Oral	No	–	–	80	80

BP, bullous pemphigoid; CP, cicatricial pemphigoid; EBA, epidermolysis bullosa acquisita; F, female; LAD, linear IgA disease; LGAD, linear IgG/IgA bullous dermatosis; M, male; MMP, mucous membrane pemphigoid; MP, “mixed pemphigoid”.

*Indirect immunofluorescence (IF) microscopy was performed on 1 M NaCl-split human skin; titres of autoantibodies that bound to the epidermal and dermal side of the artificial split are presented.

†Scarring was only present in some lesions and was thought to be caused by secondary infection frequently observed in all patients.

subepidermal blistering diseases in Germany, France, and Singapore,^{2–4} circulating autoantibodies were reported by us and others to be detected in 90%, 50–90%, and 50–80%, respectively, by the use of indirect IF microscopy.^{17–22} Consequently, we under-estimated the incidence of autoimmune subepidermal blistering diseases by approximately 20%. Taking these data together, the incidence of these disorders in Uganda probably does not differ greatly from that in Europe.

In BP, pemphigoid gestationis, lichen planus pemphigoides, and epidermolysis bullosa acquisita, autoantibodies preferentially belong to the IgG class (reviewed in Schmidt and Zillikens¹), whereas, in LAD, IgA autoantibodies predominate.¹⁹ For patients with both IgG and IgA autoantibody reactivity that clinically presented as BP or LAD, the term linear IgG/IgA bullous dermatosis was proposed.²³ With further progress in the detection of circulating autoantibodies by the use of recombinant and cell-derived forms of BP180, it became clear, however, that, in the majority of BP patients, IgA autoantibodies were also present and, in most LAD patients, IgG autoantibodies could be found.¹¹

Table 3 Fine specificities of IgA and IgG autoantibodies in patients with autoimmune subepidermal blistering diseases analyzed by Western blotting of recombinant and cell-derived forms of BP180 and BP230 ($n = 21$ *)

BP180 NC16A†			LAD-1†			BP230†		
IgG	IgA	IgG + IgA	IgG	IgA	IgG + IgA	IgG	IgA	IgG + IgA
11	2	4	6	5	1	10	1	5
52%	10%	19%	29%	24%	5%	48%	5%	24%

*Excluding the patient with epidermolysis bullosa acquisita.

†For details, see “Materials and methods” section.

Interestingly, by indirect IF microscopy, 45% of the Ugandan patients with autoimmune subepidermal blistering diseases revealed IgA reactivity, compared to about 5% in European patients.¹¹ The high IgA reactivity by indirect IF microscopy is reflected by the large number of LAD (four patients, 18%) and linear IgG/IgA bullous dermatosis (two

patients, 9%) cases and the relatively low frequency of BP patients (nine patients, 41%) in our study. In contrast, in Europe and Singapore, about 80% of patients with autoimmune subepidermal bullous disorders are classified as BP and only about 3% as LAD.²⁻⁴

The exact cause of the relatively high rate of IgA reactivity amongst Ugandan patients (compared with European patients) remains elusive. It is remarkable, however, that the two populations have different age distributions. In Germany, about 16% of the population are 14 years and younger and about the same percentage are older than 65 years, compared with about 50% and 2% in Uganda, respectively.^{15,16} The different age distribution may thus explain the high percentage of IgA and low percentage of IgG reactivity in our study, and may also account for the relatively low incidence of BP in Uganda.

This idea is supported by the relatively high incidence of LAD in South Africa and Mali,^{5,6} whereas in China and Singapore, where the age distribution is similar to that in central Europe, BP is far more common than LAD.^{4,7,16} Further evidence for our hypothesis comes from correlation analyses between patient age and the immunoglobulin class of autoantibodies. Whereas older patients predominantly developed IgG autoantibodies, in younger patients, IgA autoantibodies were preferentially detected by indirect IF microscopy. In Europe, BP usually affects the elderly, with an average age of 75 years,²⁴ whereas children with BP are only rarely reported (reviewed in Nemeth *et al.*²⁵). In contrast, in LAD, two variants are known: chronic bullous dermatosis of childhood, with a peak incidence of about 4.5 years, and LAD of adulthood.¹⁹ We suggest that the correlation of autoantibody class and patient age is not unique for Uganda, but may not be detected in Europe because of the much smaller number of children in the population.

When the autoantibody reactivity in our patients was further dissected by the use of Western blotting of various recombinant and cell-derived forms of BP180 and BP230, no great difference was detected compared with data from European patients.^{11,12,26} Whereas, in previous studies, dual IgA/IgG autoantibody response was only observed by Western blotting, in the present study, it was also seen by indirect IF microscopy. This discrepancy may be explained by the somewhat stronger IgA reactivity in Ugandan patients that also allows the detection of autoantibodies by indirect IF microscopy.

In four of our patients, a combination of epidermal and dermal binding of autoantibodies by indirect IF microscopy of salt-split skin was observed. In these patients, Western blot reactivity with BP180 and/or BP230 was present explaining the epidermal staining. One of these patients with extensive oral lesions was diagnosed with MMP, and another, with mild oral involvement whose extensive skin lesions healed with scarring, was classified as CP.²⁷ As, in the two remaining patients, no reactivity with type VII collagen and the p200

antigen was detected, the target antigen(s) of autoantibodies labeling the dermal side of the artificial split remained elusive and the provisional diagnosis of "mixed pemphigoid" was made. Further studies are needed to identify the target antigen(s) and better classify these patients.

Because of the small number of patients and the use of indirect IF microscopy as the only screening method for autoantibodies, the incidence and relative frequency of MMP, CP, and epidermolysis bullosa acquisita (one patient with each diagnosis identified), as well as pemphigoid gestationis, lichen planus pemphigoides, anti-p200 pemphigoid, and bullous systemic lupus erythematosus (no patient identified), could not reliably be determined in this study.

In summary, we have reported the estimated incidence of autoimmune subepidermal blistering disorders in south-western Uganda. We observed a correlation of the autoantibody class with the age of the patients with autoimmune subepidermal blistering disorders. In addition, these patients revealed a high frequency of IgA autoantibodies, which may be explained by the different age distribution (compared with the European population) in the Ugandan population.

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