Archives of Dermatological Research (2019) 311:93–97
https://doi.org/10.1007/s00403-018-1884-y

ORIGINAL PAPER

Downregulation of aquaporin 3 in bullous pemphigoid patients

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Received: 16 August 2018 / Revised: 24 November 2018 / Accepted: 30 November 2018 / Published online: 4 December 2018

Abstract
Bullous pemphigoid (BP) is a chronic autoimmune skin disease. Aquaporin 3 (AQP 3) has a possible role in the pathogenesis of many dermatological diseases. In this work, we aimed to evaluate the expression of AQP 3 in BP. Perilesional skin biopsies were taken from 24 BP patients and 13 controls. The biopsies were stained by direct immunofluorescence using rabbit anti-human AQP 3 FITC antibody. The expression of AQP 3 was weak in 5 patients (20.8%), moderate in 18 patients (75%), strong in 1 patient (4.2%) in the suprabasal layers. It was negative in 4 patients (16.7%), weak in 18 patients (75%), moderate in 2 patients (8.3%) and no strong fluorescence was seen in the basal layers. In the controls, the expression was strong in ten controls (76.9%), moderate in three controls (23.1%) and no controls showed weak fluorescence in the suprabasal layer. The basal layer showed strong fluorescence in 11 controls (84.6%), moderate in 2 controls (15.4%) and no controls showed mild or no fluorescence. There was a statistically significant difference in the expression of AQP 3 between basal and suprabasal layers of BP patients but not of the controls. There was statistically significant difference in the expression of AQP 3 between patients and controls in both the basal (P value < 0.001) and the suprabasal layers (P value < 0.001). In conclusion, AQP 3 was downregulated in BP patients especially in the basal cell layer. This suggests that AQP 3 plays a role in the pathogenesis of BP.

Keywords Aquaporin 3 · Basal · Bullous pemphigoid · Epidermis · Immunofluorescence · Suprabasal

Introduction

Bullous pemphigoid (BP) is a chronic autoimmune skin disease characterized by subepidermal blisters. BP is the most common autoimmune bullous disease in elderly [5, 11]. The incidence of BP in UK and France is 43 [10] and 21.7 cases [8] per million per year, respectively. Initially the disease presents with urticarial lesions, subsequently tense blisters develop. However, pruritus is the most frequent symptom of the disease [11, 18]. The disease is caused by pathogenic autoantibodies targeting the bullous pemphigoid 1 (230 kDa) and 2 (180 kDa) components of the hemidesmosome. Complement activation is crucial for neutrophil and eosinophil chemotaxis and mast cell degranulation which induce the inflammatory cascade of BP [13].

Aquaporin 3 (AQP3) is a member of a family of aquaporins which are transmembrane proteins that are responsible for skin hydration through the transport of water and glycerol [2]. It is also involved in wound healing through its effect on keratinocyte migration and proliferation. It is expressed in the keratinocytes of the basal layer and stratum spinosum of the epidermis. Its expression in the basal layer is more than the stratum spinosum [2, 7].

The expression of AQP 3 was found to be altered in several dermatological diseases. For example, AQP 3 was reduced in basal cell carcinoma, it was patchy in squamous cell carcinoma [19], it was reduced in vitiligo [8] and it increased in atopic dermatitis [15]. Moreover, the expression of AQP 3 was cytoplasmic instead of membranous in psoriatic skin [19].

In this work, we aimed to evaluate the expression of AQP 3 in bullous pemphigoid disease.

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Materials and methods

Twenty-four patients fulfilling the clinical, pathological and immunofluorescence criteria of BP were included in this study. Thirteen normal biopsies were collected from the remains of abdominoplasty operations. We tried to match the ages and sexes of the cases and controls as much as possible. All individuals were subjected to history taking and clinical examination. The disease severity of the patients was measured by the bullous pemphigoid disease area index (BPDAI) score [11]. Moreover, 4 mm skin biopsy was taken from the perilesional skin. The lesional skin was avoided as the immune deposits might be degraded in lesional skin leading to false-negative results.

The skin biopsies were cut into 5-µm sections and stained by rabbit anti-human AQP 3 antibody (Sigma, Aldrich, St Louis, MO, USA). The immunofluorescence pattern and intensity were evaluated using a scoring system were 0 = no staining, 1 = weak, 2 = moderate and 3 = strong [4, 12]. The images were also analyzed objectively using Image J software for windows version 1.52 h, Maryland, USA. Immunofluorescence pattern and intensity were compared between the different layers in the epidermis in the patients and controls and between patients and controls.

Comparisons between basal and suprabasal layers in patients and controls and between patients and controls were done using Chi-square (χ^2) test. Comparison between AQP 3 immunofluorescence in patients and controls regarding age and sex and comparison between patients on therapy and patient who did not receive any treatment were done using Mann–Whitney test. Correlations were done using Spearman correlation coefficient. *P* values less than 0.05 were considered as statistically significant.

All the subjects signed an informed written consent. The study was approved by the Ethical committee and was conducted according to the Declaration of Helsinki principles.

Results

This study included 24 BP patients and 13 controls, with 7 males (29.2%) and 17 females (70.8%) in the BP group and 4 males (30.7%) and 9 females (69.2%) in the control group. The age of the patients ranged from 46 to 78 years (mean = 62.38 ± 8.56). The age of the control group ranged from 47 to 74 years (mean = 59.7 ± 7.4).

Thirteen patients (54.2%) were on therapy in the form of systemic steroids (either daily or pulse steroids) and immunosuppressive therapy (azathioprine, mycophenolate mofetil or cyclophosphamide) and 11 patients (45.8%) did not receive any therapy.

BPDAI scoring for erosions ranged from 1 to 61 (mean = 17.6 ± 16.8) and BPDAI scoring for urticaria ranged from 0 to 43 (mean = 7.29 ± 9.54).

AQP 3 immunofluorescence was positive in the epidermis in all patients and controls and it showed mainly membranous pattern. In the dermis AQP 3 was positive in 10 patients (41.7%) and in 7 controls (46%) and it mainly stained sweat glands and blood vessels.

In BP patients, the expression of AQP 3 was weak (+1) in 5 patients (20.8%), moderate (+2) in 18 patients (75%) and strong (+3) in 1 patient (4.2%) in the suprabasal layers. In the basal layer, negative immunofluorescence was found in 4 patients (16.7%), while 18 patients (75%) showed weak immunofluorescence, 2 (8.3%) patients showed moderate fluorescence and no strong fluorescence was seen (Fig. 1). There was a statistically significant difference in the expression of AQP 3 between basal and suprabasal layers of BP patients (*P* value < 0.001) (Table 1).

In the controls, the suprabasal layers showed strong AQP 3 fluorescence in ten controls (76.9%), moderate in three controls (23.1%) and no controls showed weak or no fluorescence. The basal layer showed strong fluorescence in 11 controls (84.6%), moderate fluorescence in 2 controls

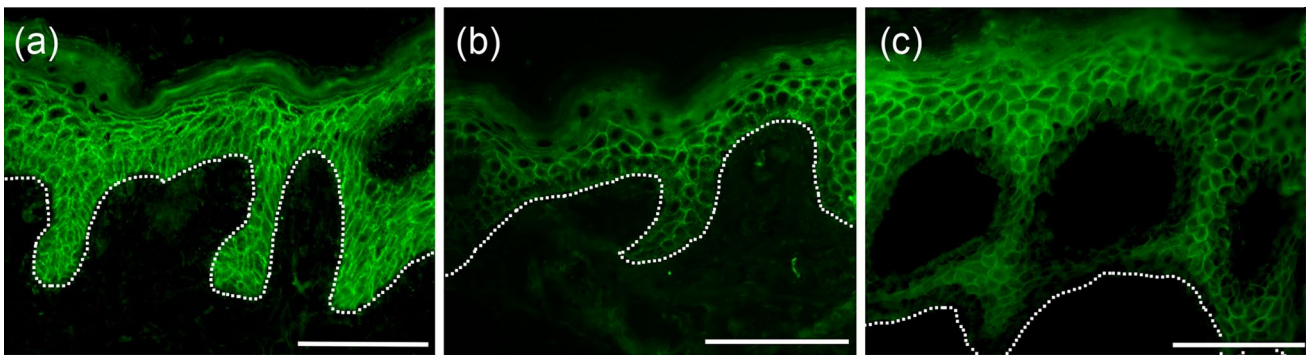

Fig. 1 AQP 3 expression in the epidermis of bullous pemphigoid patients and control. Skin biopsies were stained with rabbit anti-human AQP 3 antibody, scale bar = 50 µm. Strong expression of AQP 3 in a normal control (**a**), AQP 3 was weak in the basal layer of patient 1 (**b**), it was absent in the basal layer of patient 2 (**c**)

Table 1 AQP 3 staining of the basal and suprabasal layers of the perilesional skin biopsies of BP patients

AQP 3	Basal		Suprabasal		*p* value
	Count	%	Count	%	
No	4	16.7	0	0	< 0.001
Weak	18	75	5	20.8	
Moderate	2	8.3	18	75	
Strong	0	0.0	1	4.20	

Table 2 AQP 3 staining of the basal and suprabasal layers of the skin biopsies of the controls

AQP 3	Basal		Suprabasal		*p* value
	Count	%	Count	%	
No	0	0	0	0	1
Weak	0	0.00	0	0.00	
Moderate	2	15.40	3	23.10	
Strong	11	84.60	10	76.90	

Table 3 Comparison between AQP 3 staining of the skin biopsies of the BP patients and controls

AQP 3	Cases		Controls		*p* value
	Count	%	Count	%	
Suprabasal layer					
No	0	0	0	0	< 0.001
Weak	5	16.70	0	0.00	
Moderate	18	79.20	3	23.10	
Strong	1	4.20	10	76.90	
Basal layer					
No	4	16.70	0	0.00	< 0.001
Weak	18	75.00	0	0.00	
Moderate	2	8.30	2	15.40	
Strong	0	0.00	11	84.60	

(15.4%) and no controls showed weak or no fluorescence. There was no statistically significant difference in the expression of AQP 3 between basal and suprabasal layers of the controls (P value = 1) (Table 2).

There was statistically significant difference in the expression of AQP 3 between patients and controls as regards both the basal layer (P value < 0.001) and the suprabasal layers (P value < 0.001) (Table 3).

There was no statistical significance in the expression of AQP 3 between patients and controls regarding age and sex. There was no statistical significance in the expression of AQP 3 between patients on therapy and patient who did not receive any treatment (Table 4).

Table 4 Comparing AQP 3 of BP patients on therapy and those who did not receive any treatment

AQP 3	Therapy				*p* value
	Yes		No		
	Count	%	Count	%	
Suprabasal layer					
No	0	0	0	0	1
Weak	2	15.40	2	18.20	
Moderate	10	76.90	9	81.80	
Strong	1	7.70	0	0.00	
Basal layer					
No	4	30.80	0	0.00	0.128
Weak	8	61.50	10	90.90	
Moderate	1	7.70	1	9.10	
Strong	0	0.00	0	0.00	

There was significant negative moderate correlation between the BPDAI score of the erosions and the expression of AQP 3 in the suprabasal layer, $r = -0.587$, P value = 0.003. There was no significant correlation between the BPDAI score of the urticaria and the expression of AQP 3 in the suprabasal layer, $r = -0.320$, P value = 0.127. There was no significant correlation between the BPDAI score of the erosions and the expression of AQP 3 in the basal layer, $r = 0.063$, P value = 0.771. There was no significant correlation between the BPDAI score of the urticaria and the expression of AQP 3 in the basal layer, $r = 0.204$, P value = 0.338.

Discussion

Studies have shown that AQP 3 was not only involved in water transport and hydration of the human epidermis, but also in the regulation of keratinocyte proliferation, differentiation, cell migration, and tumorigenesis [2, 7, 19]. Thus AQP 3 was found to have a possible role in the pathogenesis of many dermatological diseases as psoriasis, atopic dermatitis and vitiligo together with the aging process where its pattern and expression were altered in these conditions.

The current study aimed at examination of the expression AQP 3 in the perilesional skin of BP patients and comparing it to controls. There was a statistically significant decrease in AQP 3 in BP patients compared to controls. Furthermore, in BP patients, AQP 3 was significantly reduced in the basal layer compared to suprabasal layers.

In normal skin, AQP 3 is expressed in the basal layer and stratum spinosum of the epidermis, in which the expression is more in the basal layer than the stratum spinosum [14]. On the other hand, decreased AQP 3 was associated with intercellular edema due to defective water homeostasis [2]. Early

bullous pemphigoid lesions appear clinically as urticarial lesions and histopathologically as dermal edema, perivascular lymphohistocytic infiltrate with eosinophils. Eosinophilic spongiosis is sometimes present. Established bullous pemphigoid lesions appear clinically as intact blisters and histopathologically as subepidermal blister with coagulated serum, fibrin and eosinophils and sometimes neutrophils [3]. In this work, we showed that in BP patients the expression of AQP 3 was weak or absent in the basal layer of 91.7% of the patients which might contribute to the accumulation of fluids and formation of dermal edema in early lesions or subepidermal blisters in established lesions of BP.

E-cadherin, catenins and integrins are essential components for the dynamic inter-keratinocyte and keratinocyte–matrix adhesion [6]. A functional link exists between E-cadherin and AQP 3. Both E-cadherin and AQP 3 colocalize in normal skin and cultured keratinocytes [9]. It was suggested that AQP 3 is an upstream signaling molecule for E-cadherin and catenin which are involved in cell survival. AQP 3 knockout mice showed decreased keratinocyte survival and decreased E-cadherin. In patients with vitiligo, downregulation of AQP 3 and its downstream molecules (E-cadherin and catenins) was followed by defective keratinocyte adhesion [1]. Autoimmune bullous diseases are caused by autoantibodies that inhibit the inter-keratinocyte adhesion or the keratinocyte–matrix adhesion. Autoantibodies of the autoimmune bullous diseases activate a cascade of signaling molecules which result in keratinocyte death [16]. E-cadherin was downregulated in several autoimmune bullous diseases [6, 17]. Since our results showed deceased expression of AQP 3 in BP patients compared to controls, therefore, AQP 3 may be involved in the pathogenesis of BP. The downregulation of AQP 3 resulted in downregulation of E-cadherin and caused defective keratinocyte adhesion, resulting in decreased keratinocyte survival which facilitated the acantholytic process and the development of the BP disease.

We propose that AQP 3 shared in keratinocyte adhesion. Interestingly, our results showed negative correlation between the suprabasal expression of AQP 3 and the BPDAI score of the erosions. This suggests that low AQP 3 leads to less keratinocyte adhesion and more skin erosions. Therapies targeting AQP 3 expression might improve the clinical picture of BP disease with fewer side effects.

Although systemic steroids upregulate several adhesion molecules [14], our results showed that there was no significant difference in the expression of AQP 3 between treated and untreated patients. Therefore, we think that the systemic steroids and immunosuppressive treatment does not affect the expression of AQP 3 in BP patients. However, further research is needed to prove this.

In conclusion, AQP 3 was downregulated in BP patients especially in the basal cell layer. This suggests that AQP 3 plays a role in the pathogenesis of BP disease. Therapies targeting AQP 3 expression might improve the clinical picture of BP disease with fewer side effects.

Funding None.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Informed consent Informed consent was obtained from all individual participants included in the study.

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