Variation of the epidermal expression of glucocorticoid receptor-beta as predictive marker of bullous pemphigoid outcome

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INTRODUCTION

BACKGROUND

Systemic and local corticosteroid (CS) treatment are considered as the gold standard for treatment of bullous pemphigoid (BP). While most of patients with BP are clinically controlled upon treatment with high dose of CS within 1 or 2 weeks, about 30% of patients with BP show a relapse during the first year of treatment. In many inflammatory diseases glucocorticoid insensitivity has been associated with the up-regulation of the glucocorticoid receptor- β (GR β), one of the GR isoforms. GR α and GR β isoforms were shown to be expressed in skin of patients with BP. We hypothesized that GR isoform expression could differ in BP patients with on-going remission and those who relapsed during the first year of treatment.

OBJECTIVE

To investigate at baseline the expression of the total GR and GR β isoform in keratinocytes at the peri-lesional site in order to provide a potential tool to predict disease outcome

METHODS

METHODS

All peri-lesional skin biopsy specimen were obtained from the department of pathology at Reims University Hospital and performed in patients with BP before the introduction of any CS treatment.

The expression of GC receptors by keratinocytes was investigated *in situ* by immunohistochemistry using one primary antibody directed against total GR (including GR α and GR β isoforms) (sc-8992; Santa Cruz Biotechnology) and one primary specifically directed against GR β isoform (ab130227; Abcam).

Immunostaining intensity of total GR was evaluated in the keratinocytes either in the whole cell or more precisely by discriminating between the cytoplasmic and the nuclear compartments. Values were attributed with respect to a negative control without primary antibody (value = 0) up to 4 for intense labelling. The quantification was performed twice by two independent evaluators in double blind analysis.

STATISTICAL ANALYSES

Descriptive statistics such as means and SDs were performed for all quantitative measures. As the number of included BP patients in each group was smaller than 30, the studied populations could not be assumed to be normal, and then nonparametric testing, specifically Mann Whitney test, was used to compare them. The results were considered significant if *P* values were 0.05 or less.

PATIENTS

26 consecutive patients with BP (according to Vaillant's well established criteria), 13 with on-going remission and 13 with relapse within the first year of treatment, were included in this ancillary observational study conducted by the French dermatology department of Reims University Hospital. Among them, 3 patients were excluded from the study due to poor quality of the biopsies. Clinical data were recorded at baseline (Table 1). Control biopsy specimen were obtained from 14 control patients without inflammatory and autoimmune diseases. All subjects gave their informed and written consent before participating to the study in accordance with the Helsinki Declaration.

	Patients without relapse	Patients with relapse	<i>P</i> value
Patients No	11	12	
Mean age $\pm$ SD (range), years	81.1 $\pm$ 6.3	75.4 $\pm$ 20.9	0.83
Female/Male	5/6	6/6	
Extensive disease ¹ , n (%)	7 (64)	10 (83)	0.28
Baseline treatment			
TCS	11	12	
SCS	0	1	
MTX	1	3	
IS	0	0	
Doxycyclin	2	1	
Baseline comorbidities			
Cardiovascular disorders	10	8	
Kidney disorders	3	3	
Diabetes mellitus	5	3	
Neurologic diseases	3	3	

Abbreviations: TCS: topical corticosteroids; SCS: systemic corticosteroids; MTX: methotrexate; IS: immunosuppressive drugs

¹ i.e. more than 10 daily blisters at diagnosis

Table 1: Baseline clinical characteristics of patients with BP

RESULTS (1)

Total GR expression in bullous pemphigoid

Figure 1: Total GR expression in healthy and BP skin epidermis. Representative high magnification pictures of total GR expression in keratinocytes after immunohistochemistry on skin biopsy specimen from control patients (B) and patients with BP with on-going remission (C) or who relapsed during the first year of follow-up (D). Negative control without primary antibody was shown in (A). Inserts in (A-D) showed low magnification picture of the area from which high magnification pictures have been taken. Staining intensity reflecting total GR expression was quantified in keratinocytes of control patients and patients with BP at whole cell level (E, H) or by discriminating cytoplasmic (F, I) and nuclear (G, J) compartments. Comparison was performed either between control and total BP population (E-G) or between control patients and patients with BP according to disease outcome (H-J) (*, *P*<0.05; **, *P*<0.01; ***, *P*<0.001)

RESULTS (2)

GR β expression in bullous pemphigoid

Figure 2: Increased GR β expression in BP skin epidermis. Representative high magnification photomicrographs of GR β expression in keratinocytes after immunohistochemistry on skin biopsy specimen from control patients (A) and patients with BP with on-going remission (B) or who relapsed during the first year of follow-up (C). Inserts in (A-C) showed low magnification picture of the area from which high magnification pictures have been taken. Bars indicate 10 μ m in high magnification pictures and 50 μ m in low magnification pictures. Staining intensity reflecting GR β expression was quantified in keratinocytes of control patients and patients with BP at whole cell level (D, G) or by discriminating cytoplasmic (E, H) and nuclear (F, I) compartments. Comparison was performed either between control and total BP population (D-F) or between control patients and patients with BP according to disease outcome (G-I) (*, *P*<0.05; **, *P*<0.01; ***, *P*<0.001)

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CONCLUSION

Overall, the present study demonstrates an increased expression of GR β in the keratinocytes of BP patient who later relapsed compared with patients with on-going remission. These results suggest that increased expression of GR β in the skin epithelial cells could be predictive of reduced CS treatment efficacy, and therefore of increased risk of disease relapse in the population of patients with BP.