Describing a new animal model for bullous pemphigoid. In vitro experiments

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Introduction

Bullous pemphigoid (BP) is the most frequent autoimmune blistering disease, typical of the elderly. BP patients present with tense blisters on skin and occasionally on mucous membrane. Deposition of C3 and/or IgG is detected by direct immunofluorescence (DIF) on healthy perilesional skin. Circulating antibodies against the basement membrane zone (BMZ) can be detected by indirect immunofluorescence (IIF) in 90% of patients. Specific autoantibodies against BP180 (hemidesmosomal protein) and, less frequently, against BP230 (plakin member family protein) are detected by immunoblot with epidermal or cultured keratinocytes extracts. Most BP sera recognize the membrane-proximal non-collagen linker domain (termed NC16A)[1,2] of BP180 (Figure 1), which is considered the immunodominant epitope. Besides, the pathogenic role of the anti-NC16A autoantibodies has been demonstrated in several *in vitro* and *in vivo* experiments.

In vitro experiments [3,4]	In vivo experiments [5,6]
Dermal-epidermal separation on cryosections of human skin induced by BP patients' sera and purified anti-NC16A IgG	Passive transfer antibodies from BP patients or immunized animal against BP180 (NC16A). Active animal models inducing autoantibodies formation against NC16A.

To highlight, human NC16A epitope exhibits an unusually high degree of sequence divergence with its murine homologue (NC14A) (Figure 1), despite the high overall homology of human and murine BP180 (81.6%). For this reason, BP animal models needs to be more elaborated than in other autoimmune blistering skin disease.

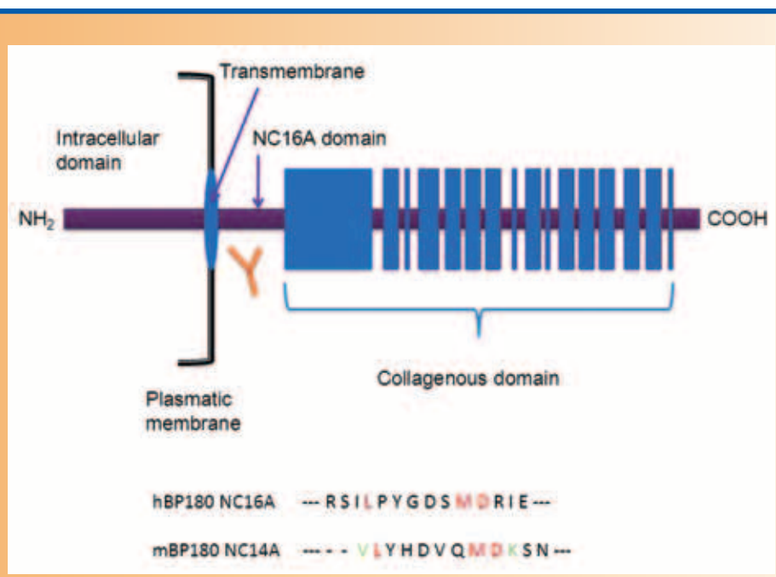

Figure 1. At the top, a schematic diagram of structural representation of the BP180 protein based on sequence analysis of the human cDNA. The bottom portion shows the amino acid sequence alignment of the human and murine forms of BP180 in the region of the epitope recognized by BP autoantibodies. Identical residues are marked with red colour, and conservative substitutions are marked with green colour.

Patients and methods

Patients' sera. Serum samples from 7 BP patients prior to the initiation therapy and 2 healthy donors were obtained. All BP patients were characterized by: a) blisters on the skin, b) subepidermal blisters on the skin biopsy, c) deposition C3 +/- IgG at the dermal-epidermal junction by direct immunofluorescence (DIF) of healthy perilesional skin. An enzyme-linked immunoabsorbent assay (ELISA) was performed for each sample to determine antibody levels against BP180.
Humanized skin on Swiss nude mice. Humanized skin was developed on the back of Swiss nude mice (athymic mice) by epidermal stem cells from the bulge of human hair follicle and cutaneous or mucous fibroblasts from C57BL/6 mice. A mixture of both cells were transplanted to and incubated for 1 week on a silicon chamber sticked on the mice back after performing an incisional wound, After 8 to 10 weeks, skin biopsies were obtained and embeded with optimum cutting temperature (OCT) compound. Humanized skin produced with mucous fibroblast was used, since it lacked hair follicles.
IIF microscopy studies were performed with patients' sera on salt split human skin, humanized murine skin and native murine skin. Two observers, subjectively, evaluated all the slides with a fluorescent microscope (Olympus BX51, San Diego, CA). The results were expressed from four pluses as maximum linear deposition on DE junction, to one plus as intermittent deposition.
Dermal-epidermal separation (DES) induced by BP patients sera and leukocytes. Human skin, humanized murine skin and native murine skin sections were co-incubated at 37ºC with patients' sera and leukocytes obtained from peripheral blood of healthy volunteers. The results of DES were expressed with four degrees, each defined by a gap percentatge of DE detachment along DE junction of skin section (e.g. grade IV means 75-100% of DE detachment from all skin section)[4,5].

Results and discussion

Patients	BP180 ELISA results	Sample	IIF	DE separation
BP1	159.03	HS	+++	IV
		HMS	++	IV
		NMS	-	-
BP2	13.00	HS	++	IV
		HMS	++	III
		NMS	+	NA
BP3	27.90	HS	-	IV
		HMS	-	III
		NMS	-	NA
BP4	2.00	HS	+++	IV
		HMS	++	II
		NMS	++	NA
BP5	45.90	HS	++	II
		HMS	++	II
		NMS	-	-
BP6	124.90	HS	++++	IV
		HMS	+++	III
		NMS	++	II
BP7	9.00	HS	+++	III
		HMS	++	III
		NMS	+	I

Table 2. IIF and DE splits induced on cryosections results. (BP180 ELISA values are positive if > 9 U/ml)

HS: human skin, HMS: humanized murine skin, NMS: native murine skin, NA: not available

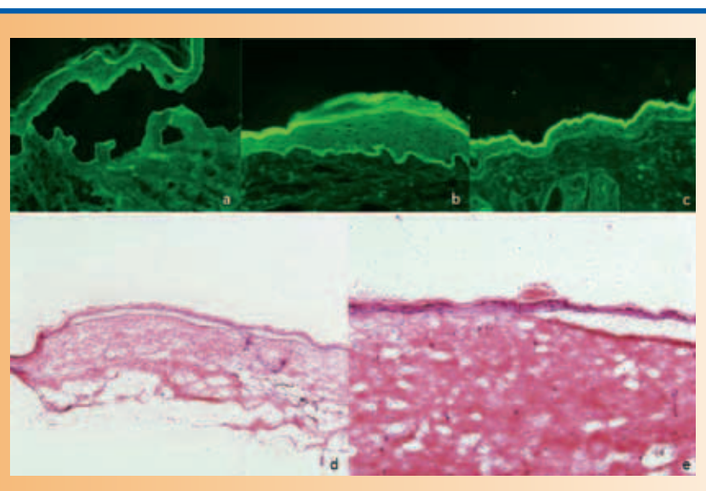

Figure 2. IIF from BP2 patient on the tree different substrates (a,b,c). a) 200x. Moderate continuous deposit of IgG in a mixed pattern (roof an floor) is observed on SSS human foreskin. b) 400x. Slight continuous deposit of IgG in BMZ on native murine skin. c) 200x. Intermittent deposition of IgG in BMZ on native murine skin. d) DES induced by BP6 sera. HE 100x. A whole length of DE separation is induced on humanized skin. e) DES induced by BP7 sera HE 200x. A close up view of blister formation.

The use of human skin grafts on mice represents an attempt to make animal models of inflammatory skin diseases closer to what really happens in human diseased skin. To our knowledge, only one study using engrafting human skin from healthy donors onto SCID immunodeficient mice has already been described for BP, unfortunately, with negative results[7], as this model failed to induce clinically evident subepidermal blisters or dermal-epidermal separation on histopathological study. Two other experimental studies using skin grafts of humanized murine skin have been reported. Both correspond to active models and human skin is not used: 1) Olasz at el, Grafts of murine skin expressing human type XVII collagen[8]; 2) Ujiie et al[9] engrafting murine skin expressing human type XVII collagen (from transgenic $COL17^{m-/-,h+}$ mice) on wild-type mice to induce an autoimmune response against human type XVII collagen. Splenocytes from the immunized wild-type mice were injected into $Rag\text{-}2^{-/-}$ / $COL17^{m-/-,h+}$ mice (expressing human type XVII collagen), which led to a continuous production of anti-human type XVII collagen IgG antibodies.
After comparing with the above-mentioned studies, and with the results obtained we can consider that our model is a probably novel model in which the recipient animals carry a skin graft obtained from human epidermal stem cells. In this regard, our grafts can be considered a chimeric skin formed by the interaction and differentiation of human epidermal stem cells and murine fibroblasts. Our grafts potentially express a variety of human epidermal and BMZ proteins. In contrast to other models using COL17-humanized transgenic mice, in which the only human protein expressed is type XVII collagen. This possibility would be of great interest, since BP and other related diseases are commonly associated to a heterogeneous response to more than one autoantigen, such as BP230, integrins, etc.

Conclusions

The results obtained, allow us to consider that it might be a new passive transfer animal model for BP, with the main feature that epidermal and BMZ proteins are of human origin. Based on this fact, this model would be usefull to study other autoimmune or inflammatory skin diseases.

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