ANTIGEN MAPPING IN HEREDITARY EPIDERMOLYSIS BULLOSA

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Standard immunofluorescence tests are not positive in the various inherited epidermolysis bullosa (EB). Using antibodies to known antigens present in the basement membrane zone, antigen mapping can be done by immuno fluorescence, to determine the level of blistering and establish the diagnosis. We report three cases of junctional EB and one case of dystrophic EB in whom the diagnosis was confirmed by antigen mapping.

Key words: Hereditary epidermolysis bullosa, Antigen mapping, Immunofluorescence

Introduction

Standard direct and indirect immunofluorescence (IMF) are used to detect antibodies in various autoimmune vesicobullous disorders. Patients with inherited epidermolysis bullosa (EB) do not produce any autoantibodies to skin antigens and hence standard direct and indirect IMF tests are not useful. Based on the ultrastructural level of split, EB can be divided into simplex, junctional and dystrophic types.[1] Confirmation of the diagnosis often requires electron microscopy.[1,2] Using antibodies to known antigens like those of bullous pemphigoid (BP Ag) and EB acquisita antigen (EBA AG), the level of the split can be determined by an IMF technique. These antigens serve as markers in the basement membrane zone (BMZ), enabling us to establish the level of blistering. The site of splitting and therefore the localisation of BP Ag and EBA in the three common types of inherited EB is shown in figure 1 and table I.

Table 1 Localisation of antigens in EB by antigen mapping

Type of EB	Site of blister	BP Ag	EBA Ag
Simplex	Basal cell	Floor	Floor
Junctional	Lamina lucida	Roof	Floor
Dystrophic	Sublaminadensa	Roof	Roof

Fig.1. Level of blister and localisation of BP& EBA antigens in EB

Patients and Method:

Case 1 and 2 Eighteen and 12-year-old male siblings born of a non consanguinous marriage, with a history of generalised blistering since

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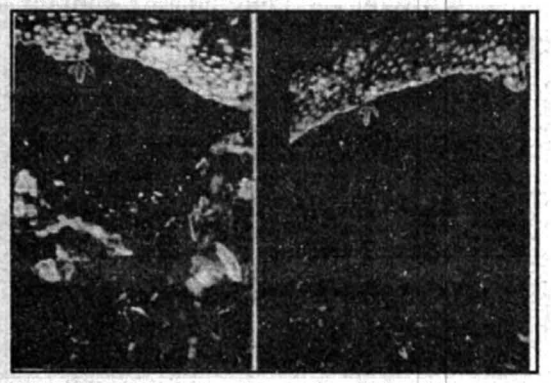
Fig.2. Scarring and hair loss over scalp.

infancy presented with multiple blisters and crusted lesions on trunk and limbs and post inflammatory hypopigmented macules, loss of eye brows and eye lashes and atrophic scarring over anterior aspect of legs. There were no milia. Scalp showed areas of atrophic scarring and hair was present only in central and lower occipital areas (Fig.2). Soles were hyperkeratotic with multiple pits. Nails were dystrophic or absent. The severity of blistering had been decreasing with age. Lesional biopsy for histopathology (H&E) showed subepidermal blister. Electron microscopy revealed defective hemidesmosomes, blistering through lamina lucida and normal anchoring fibrils (Fig.3).



3. BP Ag and EBA Ag localising in the roof of blister.

Case 3: A 5-year-old male born of a first degree consanguinous marriage had a history of generalised blistering since birth.

A few areas showed minimal scarring but no milia. There was no history of photosensitivity. H&E showed subepidermal blister.

Case 4: A 12-year-old male born of a first degree consanguinous marriage had a history of generalised blistering since birth. At presentation he had a few blisters, multiple post inflammatory hypopigmented and hyperpigmented lesions and atrophic scars with milia. Syndactyly with flexion deformities of fingers, dystrophic nails and ankyloglossia of tongue were also seen. There was no history of photosensitivity. H&E showed subepidermal blister.

Biopsy for antigen mapping was taken after mechanical friction (rubbing normal appearing skin with a pencil eraser for 5 minutes, and then the area biopsied after 15 minutes). The tissue was snap frozen with liquid nitrogen and stored in the deep freezer. Six micron thick sections were incubated with sera of BP and EBA patients respectively. This test was also done using monoclonal antibodies to 230 kd BP antigen and to type VII collagen (EBA Ag) at St. Thomas's hospital, London. Sections were then washed and made to react with fluoresceine labelled antihuman antibodies (FITC conjugate) to IgG and then observed through fluorescence microscope.

Results

Case 1,2 and 3 showed most of the BP antigen localising to the roof of the blister and EBA Ag to the floor of the blister. In case 4 both antigens localized in the roof of the blis-

ter (Fig.3). The results were similar even when monoclonal antibodies were used.

Discussion

In junctional EB the defect lies in the hemidesmosomes causing the split to occur through the lamina lucida, hence BP Ag goes to the roof and EBA Ag to the floor of the split. In cases 1, 2 and 3 localisation of BP antigen to the roof and EBA Ag/ Type VII collagen to the floor confirm a diagnosis of junctional EB (benign atrophic/non lethal type). In dystrophic EB the defect lies within the type VII collagen, causing blistering through sublamina densa, hence both BP Ag and EBA Ag localise in the roof of blister. In case 4 as both antigens localised to the roof,a diagnosis of dystrophic EB was confirmed.Hence with minor modifications IMF may be used for antigen mapping, and therefore help in the differentiation of the various inherited EB, especially when H&E is similar and in the absence of facilities for electron microscopy. In addition, antigen mapping may be useful in diagnosing the type of EB during early stages when other related signs and symptoms are yet to appear.

References

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