Michel's medium: a potential alternative to cryoprotection for tissue transport in the investigation of genetic skin disease

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Summary

Michel's medium is now well established as a transport medium to maintain tissue-fixed immunoreactants prior to direct immunofluorescence and immunoelectron microscopy. This is a practical alternative to cryofixation prior to transportation when sending skin biopsies for the investigation of cutaneous immunobullous disease. In this study we have demonstrated preservation of the cutaneous basement membrane zone proteins in skin biopsies stored in Michel's medium for periods of up to 28 days – proving that Michel's medium can be used as a transport medium when sending skin biopsies for immunohistochemical studies to determine the structural and molecular deficiencies in genodermatoses such as inherited forms of epidermolysis bullosa.

Normal human skin, from a nonsun-exposed site, was obtained during a plastic surgical procedure. Excess dermis was removed and 4 mm punch biopsies were taken. These were divided into six groups of two biopsies each. The first of the six groups of samples (day 0) was immediately snap frozen and stored in liquid nitrogen. The remaining five groups were placed into Michel's medium: (1 M citrate buffer pH 7.4, 2.5 mL; 0.1 M magnesium sulphate, 5 mL; 0.1 M N-ethyl maleimide, 5 mL; ammonium sulphate 55 g; distilled water, 87.5 mL; total volume 100 mL adjusted to Ph 7.4 with 1 M sodium hydroxide),[1] and left at room temperature for 2, 5, 8, 14 and 28 days, washed in phosphate buffered saline, snap frozen and stored in liquid nitrogen.

The following monoclonal antibodies and serum from patients with acquired bullous disorders (and which had been characterized by immunoblotting) were used for epitope mapping.

HD4-233, IA8C. Monoclonal antibody 233 is specific to the extracellular part of BP 180 whereas 1A8C is specific to the intracellular part.[2,3] These monoclonal antibodies were provided by K. Owaribe (Nagoya University, Japan).

19DEJ-1. This antibody, also known as uncein, identifies a unique basement membrane zone (BMZ) protein localized in the upper lamina lucida region underneath the hemidesmosomes (supplied by J. D. Fine, University of North Carolina, USA).

GB3. This monoclonal antibody to laminin-5 (Harlan Seralab, U.K.) recognizes a 600 kDa glycoprotein which is localized to the interface between the lamina lucida and the lamina densa.[4]

Laminin-1 and Type IV collagen antibodies. These recognize proteins associated with the lamina densa region (from Dako, UK).

LH7:2. A monoclonal antibody that reacts with the NC-1 domain region of type VII collagen[5,6] (from Dako, Cambridgeshire, UK).

To preclude the existence of endogenous antibodies standard direct immunofluorescence was performed on tissue samples using fluorescein iso-thiocyanate (FITC) conjugated rabbit antihuman IgG and IgA antiserum (DAKO, Copenhagen, Denmark).[7] Standard indirect immunofluorescence was performed on each of the frozen samples.[7] Samples were first incubated with appropriately diluted monoclonal antibodies or the characterized sera (Table 1). Secondary labelling was

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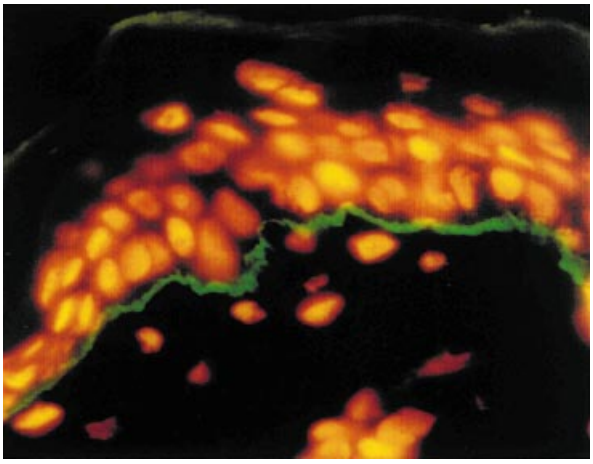

Figure 1 Monoclonal antibody GB3 showing positive staining on day 0 in Michel's medium.

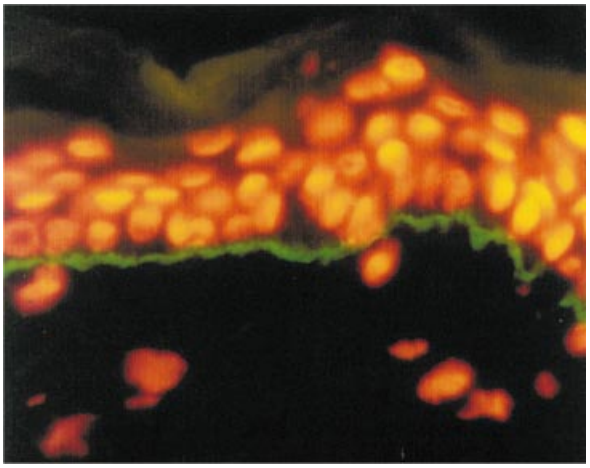

Figure 2 Undiminished staining intensity with GB3 after 28 days in Michel's medium.

performed using FITC conjugated rabbit antimouse and goat antihuman IgG and IgA antiserum, respectively (DAKO, Copenhagen, Denmark). Immunofluorescence was read by two independent observers.

Results

The monoclonal antibodies showed bright linear BMZ staining in all tissue specimens. Staining intensity was undiminished throughout the 28-day period of treatment with Michel's medium (Fig. 1). Bullous pemphigoid, pemphigus gestationis, epidermolysis bullosa aquisita and linear IgA disease (dermal and epidermal binding) patient's sera showed bright linear staining at the BMZ). No reduction in staining intensity was observed, even after 28 days in Michel's medium (Fig. 2).

The positive staining seen on skin maintained in Michel's medium with monoclonal antibodies and antibodies from patients with acquired subepidermal diseases suggests that the BMZ proteins (epitopes) are preserved. This is consistent with the ultrastructural preservation of the BMZ as described previously.[8,9]

Discussion

We have previously demonstrated preservation of cutaneous antigen–antibody complexes in tissues retained in Michel's medium for up to 6 months.[1] The immunohistochemical examination of patients' skin biopsies for any evidence of structural molecular deficiencies is essential when investigating genetic skin disorders such as epidermolysis bullosa (EB).[10–12] In spite of its value, the demonstration of a deficient cutaneous epitope, when diagnosing genetic skin diseases, has been impractical for routine use because frozen rather than fixed tissue is required. This has necessitated either the shipping of frozen specimens or patients travelling to attend clinics close to a specialist laboratory. These requirements have tended to discourage the routine use of immunohistochemical studies to determine aberrant protein expression in the dermatopathology laboratory.

In inherited EB there are selective abnormalities of individual proteins. The fact that these antigens are detectable in normal skin by immunofluorescence even after 28 days in Michel's medium means that any alteration in expression of these proteins in EB skin will provide clues as to the inherent gene/protein abnormalities in that case. Our findings indicate that tissues maintained in Michel's medium for periods up to 28 days have the potential to be used for antigen

Table 1 Control sera characterized by immunoblotting

Bullous disease	Cases	Target antigens as determined by Western blotting
Bullous pemphigoid	5	230 kDa protein
Pemphigus gestationis	5	180 kDa protein
Linear IgA disease – epidermal type	4	97 kDa protein
Linear IgA disease – dermal type	4	Unknown
Epidermolysis bullosa aquisita	5	290 kDa C terminal of type VII collagen dermal protein

mapping to determine the structural molecular deficiencies in EB. Further studies are necessary to confirm that Michel's medium is a practical alternative to cryoprotection when considering the transport of tissue for the investigation of genetic cutaneous disorders.

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