

Human Melanocytic Naevi

III. Immunofluorescence and Immunoelectron Microscopy of the Basement Membrane Zone

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Serum pemphigoid antibody (BPA) was used to demonstrate the intraepidermal location of human pigmented naevi. Immunofluorescence was predominant along the epidermal-dermal junction and around the naevus nests seemingly located in the dermis. Serial sections showed that these naevus nests were intraepidermal. The immuno-gold labelling technique for electron microscopy, was improved by using uranyl acetate with no osmium tetroxide or lead citrate. Hemidesmosomes were absent from the epidermal-dermal junction apposed to naevus nests. Gold labelling occurred in patches of particles along the lamina densa. The number of gold particles per micron length of lamina densa were quantitated. Immunofluorescence microscopy and immuno-gold electron microscopy confirmed the intraepidermal location of pigmented naevi. *Key words: Immunology; Pemphigous; Basement membrane.*

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The intraepidermal location of pigmented naevi has been shown in serial section at the ultrastructural level by Lea & Pawlowski (1, 2, 3, 4).

Immunoelectron techniques were used to investigate the continuity of basement membrane from the level of the epidermal-dermal junction, with the basement membrane around pigmented naevus nests seemingly located in the dermis. The number of gold particles bound to the respective basement membranes were counted and quantitated.

In bullous pemphigoid, affected individuals have a serum antibody directed against basement membrane antigen (5, 6).

This antibody has been used at the light microscope level to confirm a clinical diagnosis of bullous pemphigoid. The location of immunoglobulins appeared to be limited to the basement membrane zone (7).

Serum pemphigoid antibodies have also been used to demonstrate various regions of the basement membrane zone (8, 9). High magnification in the light microscope, and subsequent immuno-electron microscopy, revealed that BPA seems to be preferentially associated with hemidesmosomes (10).

We observed that melanocytes and naevus cells in human melanocytic naevi lack hemidesmosomes (4), while still being apposed to basement membrane. To further investigate this finding, serum pemphigoid antibody was used.

MATERIAL AND METHODS

Human melanocytic naevi were surgically removed for medical or cosmetic reasons. Xylocaine (2%) was used as local anaesthetic. The biopsies were processed for light and electron microscopy (2).

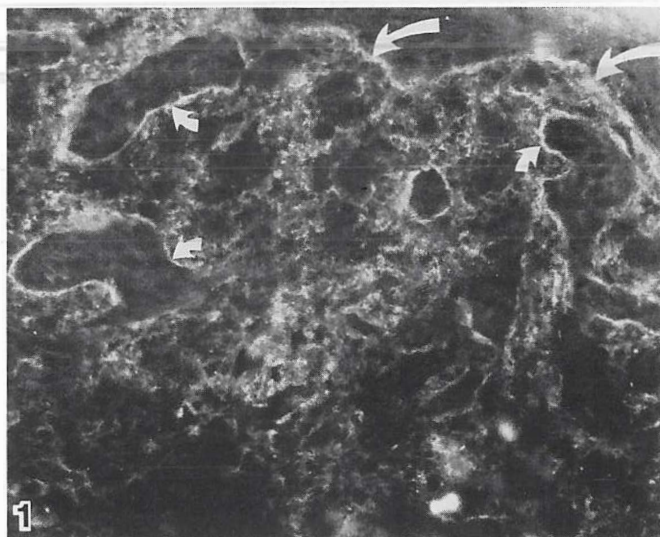


Fig. 1. Continuous indirect immunofluorescence along the epidermal-dermal junction (long arrows). Labelling at the junction between naevus nests and dermis (short arrows). $\times 1\,000$.

Immunofluorescence microscopy

The tissue samples were fixed in 0.2% glutaraldehyde in phosphate buffer (PB) pH 7.4 for 2 hours and frozen in O.C.T. on brass blocks. Serial sections, approximately 4 micrometers thick, were cut in a cryostat maintained at -30°C . The sections were placed on glass slides and labelled using Beutner's protocol (11). PB was used to rehydrate the sections. Incubation with pemphigoid serum, was carried out at room temperature for 30 min. The serum was titred at 320, and was used at a dilution of 1:5. Following a wash in PBS, the sections were incubated with rabbit anti-human IgG fluorescein conjugate at a concentration of 50 μg per ml for 30 min, washed with PBS and mounted in 50/50 PBS-glycerol. The sections were then examined under a fluorescence microscope.

Serial sections were photographed using both colour and black and white film.

Immunoelectron microscopy

Tissue samples were fixed in 0.2% glutaraldehyde in a 0.15 molar phosphate buffer, pH 7.4, for two hours, then embedded in Epon 812. The osmium tetroxide fixation step was purposely omitted. Ultrathin serial sections were cut with a glass knife, and placed on nickel grids.

The sections were treated for 5 min with 4% bovine serum albumin in PB to block nonspecific binding sites. Human BPA was allowed to react during a one hour incubation in pemphigoid serum titred at 640, diluted 1:8. This was followed by a buffer wash and then a one hour incubation in rabbit antihuman IgG diluted to 100 μg per ml. The final incubation with 5 nanometer gold particles coated with goat-anti-rabbit (Jensen Pharmaceuticals) diluted 1:50, lasted 30 min. In the controls, pemphigoid antibody was replaced by PB.

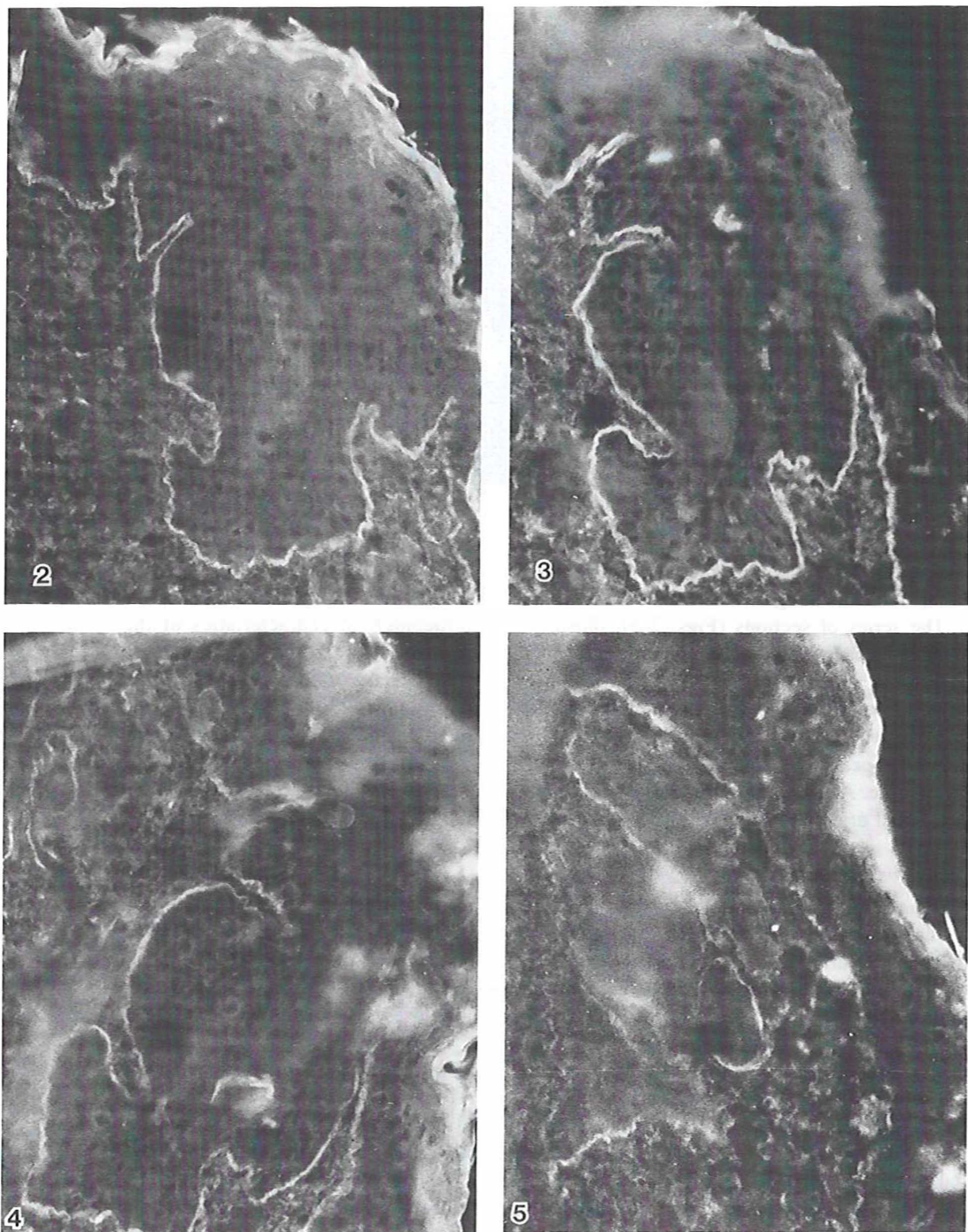
The sections were stained with uranyl acetate for 30 min. Lead staining was not used.

In order to quantitate the distribution of gold particles, lengths of basement membrane were measured with a non-dedicated image analysis system (12). The gold particles on the lamina densa were counted, and the number of gold particles per unit length, i.e. micrometer of basement membrane, calculated.

RESULTS

Immunofluorescence microscopy

Immunofluorescent staining in sections of human melanocytic naevi showed up in epidermis and in the dermis. Labelling was found in the basement membrane zone (BMZ). Staining of basement membrane along the epidermal-dermal junction was intense. It was not possible to differentiate where within the layers of the basement membrane labelling had occurred.



Figs. 2-5. Four consecutive sections. Fig. 2, naevus nest as an epidermal invagination into the dermis surrounded by a continuous epidermal basement membrane. Figs. 3 and 4, show lobate shape of the nest. Fig. 5, the naevus appears as islands in the dermis. $\times 1000$.

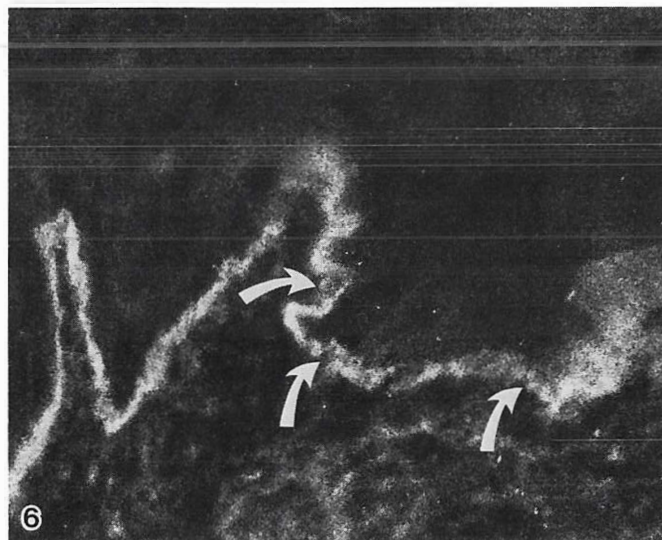


Fig. 6. Tangential section of epidermal-dermal junction showing intensely labelled circular areas, corresponding to hemidesmosomes. $\times 2880$.

Naevus cell nests, located entirely in the dermis at the plane of section, showed comparable labelling of the basement membrane around the nests (Fig. 1).

The series of sections (Figs. 2–5), illustrate the intraepidermal characteristics of the pigmented naevus. In Fig. 2, epidermal cells, limited by a labelled basement membrane are protruding into the dermis. This protrusion appears to be larger (Fig. 3), showing its lobular structure. The next consecutive section, (Fig. 4), shows the lower extent of the lobules, where the connections between them are thinner. In the last section (Fig. 5), the lobules appear to be separated from each other. They are surrounded by labelled basement membrane. At the level of the epidermal-dermal junction, where the sections were cut at a tangential orientation (Fig. 6), it was possible to see labelled spots.

Immunoelectron microscopy

Examination of gold particle distribution at the plane of the epidermal-dermal junction, including both the dermis and the epidermis, resulted in the following pattern. Gold labelling occurred in batches of particles along the lamina densa, on cytoskeletal elements of keratinocytes, in the vicinity of hemidesmosomes and on structural elements in the dermis (Fig. 7). There were very few gold particles not closely associated with structural components.

The control (Fig. 8), shows only the occasional random gold particle, i.e. demonstrating a low gold random background.

Sections of basement membrane in continuity with the epidermal-dermal junction, but located in the dermis due to the section plane (3), showed a distribution pattern of gold labelling (Fig. 9), very similar to that at the epidermal-dermal junction. The lamina lucida appeared to have no component that labelled specifically with antibody contained in the serum.

The control for this basement membrane (Fig. 10), shows a very low random background for gold particles.

The number of gold particles per micrometer of basement membrane, i.e. lamina densa, was calculated as 15 gold particles per micrometer at the level of the epidermal-dermal junction and 15 particles per micrometer for the intraepidermal nests. The control meas-

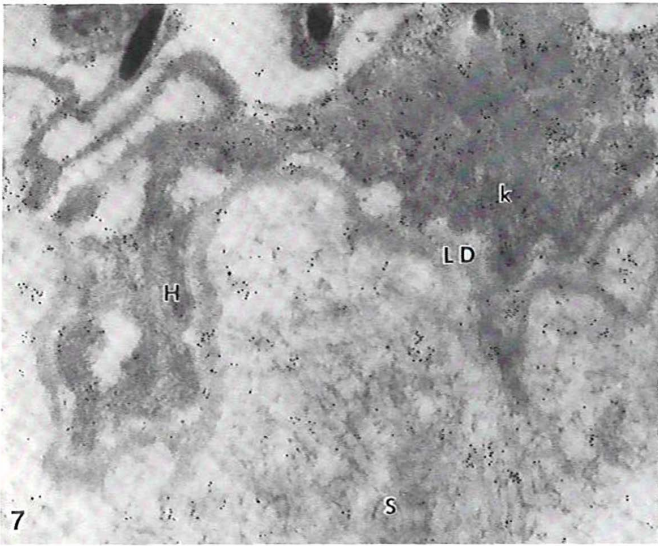


Fig. 7. Gold labelling along lamina densa (LD), in keratinocytes (K), in the vicinity of hemidesmosomes (H) and on structural elements in the dermis (S). $\times 32\,800$.

urements for each case resulted in 1 gold particle per micrometer. A minimum length of 100 micrometers of basement membrane was measured for each of the four experiments.

DISCUSSION

Bullous pemphigoid antigen is a normal component of the basement membrane zone (7, 10, 14). Indirect immunofluorescence labelled intensely and continuously along the border between the naevi and the dermis. This served to confirm the intraepidermal location of the naevi (3, 4).

This study performed on naevi obtained from human skin, showed that low level immunofluorescent labelling also took place in the epidermis and dermis. The interpretation of this labelling at the light microscope level is usually attributed to background (15).

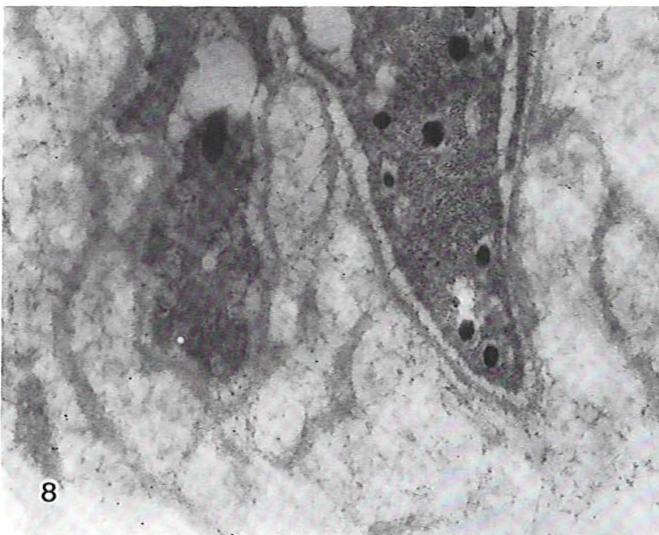


Fig. 8. Control for Fig. 7 showing background labelling. $\times 32\,800$.

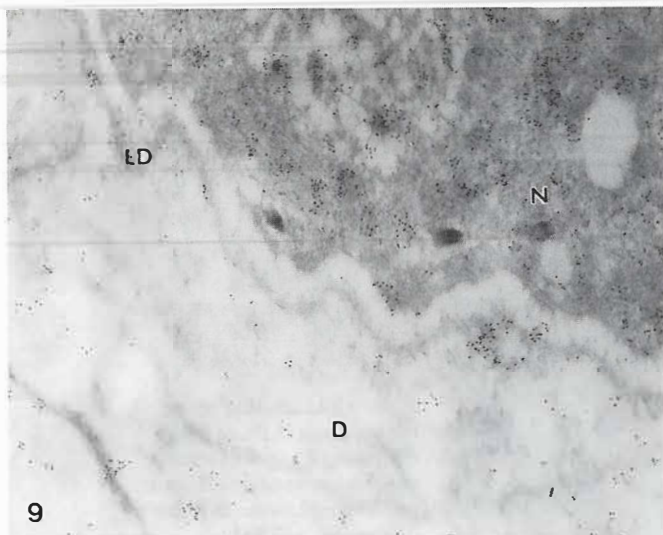


Fig. 9. Deeply protruding naevus cell showing a basement membrane with gold label distribution similar to Fig. 7. There are no regions corresponding to hemidesmosomes. *LD*, lamina densa; *N*, naevus cell; *D*, dermis. $\times 32\,800$.

Examination of immunogold labelling in the naevi showed that nearly all labelling found outside the BMZ, was in association with unidentifiable structural components.

When examining sections at the ultrastructural level, it was found that lead citrate caused dense staining, making it very difficult to see the 5 nanometer gold particles. By using only uranyl acetate, with no osmium tetroxide or lead citrate this was prevented and the gold particle distribution easily visible.

The distribution of gold particles in the basement membrane zone differed from the uniform pattern seen in the light microscope. The absence of structural hemidesmosomes did not appear to change the localization of gold.

It is generally accepted that melanocytes do not form hemidesmosomes or make basement membrane (16, 17, 18). No hemidesmosomes were found at the basement

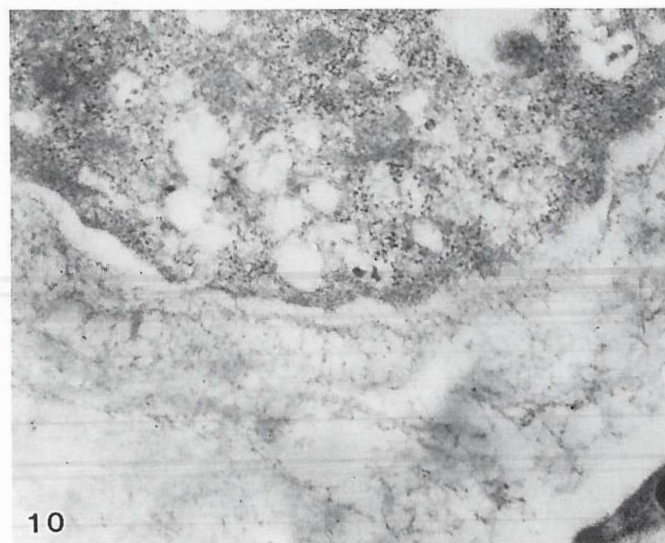


Fig. 10. Control for Fig. 9 showing background labelling. $\times 32\,800$.

membrane between naevi and dermis. Nevertheless, the distribution of gold particles in this zone was similar to the distribution of gold particles in keratinocytes and hemidesmosomes observed by Westgate et al. (10).

Quantitation of the density of gold particles per unit length of basement membrane gave similar concentrations of gold particles for basement membrane between naevus cells and dermis as at the epidermal-dermal junction. The gold distribution pattern, i.e. antigen location in the basement membrane, was therefore similar for both locations. The common distribution of bullous pemphigoid antigen supports the concept, that naevus nest basement membrane may stretch from the epidermal-dermal junction as the intraepidermal naevus nest protrudes into the dermis.

It is possible that melanocytes have an associated protein which binds with BPA. Since bullous pemphigoid antigen has been observed at the epidermal-dermal junction by indirect fluorescence, it appears likely that basal epidermal cells are capable of synthesizing this antigen. Some antigen may be deposited in the basement membrane. In vitro studies have also shown that basal epidermal cells synthesize bullous pemphigoid antigen in culture (19).

It has been suggested that the antigen may be necessary for cellular differentiation in the epidermis (20), and its close association to the basal cells may well reflect such a role for this BMZ component.

Pemphigoid antigen may also be needed for growth and differentiation of intraepidermal naevi. It may be possible, that as long as antigen derived from basal cells associated with the BMZ is present, the development and protrusion of the naevus into the dermis progresses (21). Once the basement membrane has stretched beyond the level of keratinocytes (3), farther proliferation of the naevus can be limited.

This study confirms the intraepidermal location of human pigmented naevi, and supports the concept that the basement membrane separating the naevus cells from the dermis, is similar to and continuous with the basement membrane at the epidermal-dermal junction.

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