

A COMPARATIVE STUDY OF SEBORRHEIC KERATOSES AND BASAL CELL EPITHELIOMAS BY COMPLEMENT IMMUNOFLUORESCENCE AND ELECTRON MICROSCOPY

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Abstract. Eight cases of seborrheic keratosis (SK) and 7 cases of basal cell epithelioma (BCE) were studied comparatively by complement immunofluorescence using pemphigoid serum which was reactive not only to the basement membrane zone (BMZ) but also to the cell membrane or the intercellular space (CMICS) of basal cells in normal human epidermis. Positive immunofluorescence both at the BMZ and the CMICS of proliferating basaloid cells was observed in all cases of SK, whereas in BCE, immunofluorescence weaker than that in SK was observed only at the BMZ of tumor cell nests. These tumors were also examined by light and electron microscopy, and differences already described in the literature between SK and BCE were confirmed. These findings suggest that antigenic properties of the BMZ of the skin are affected by proliferating epithelial cells and that epithelial cells are involved in the formation of the BMZ.

Key words: Complement immunofluorescence; Seborrheic keratosis; Basal cell epithelioma; Basement membrane zone

Antigenic properties of epidermal tumor cells in relation to the underlying basement membrane zone have never been investigated by complement immunofluorescence (CIF) using pemphigoid sera, except for one preliminary study (5), although a few investigations using ordinary IgG indirect IF (IIF) have been performed to date (3, 4, 10, 11, 15). In the present study, seborrheic keratoses (SK) and basal cell epitheliomas (BCE), both of which consist of proliferating basaloid cells, were investigated comparatively by CIF using pemphigoid serum which is reactive not only to the basement membrane zone (BMZ) but also to the cell membrane or the intercellular space (CMICS) of basal cells of normal human epidermis (13), and by light and electron microscopy. The details are described and antigenic properties of proliferating cells are discussed.

MATERIALS AND METHODS

Materials

Eight cases of SK and 7 cases of BCE recently experienced at the Department of Dermatology, Keio University School of Medicine, were examined. SK showed a variety of histologic patterns, such as solid, reticulated, and adenoid. BCE also showed various histologic types, including solid, adenoid, cystic and keratotic. Biopsy specimens were cut into three pieces and each piece was subjected to the following investigations.

CIF

Specimens were immediately frozen and cut into 4-6 μ m thick sections at -20°C . These sections were then subjected to CIF tests according to Nishikawa et al. (12). Normal human fresh sera were used as a source of complement. Pemphigoid serum TH (1:640) which was reactive not only to the BMZ but also to the CMICS of basal cells of normal human epidermis (13) was used at 1:20 dilution. Phosphate-buffered saline with Ca^{++} and Mg^{++} was used throughout the experiment. For staining, fluorescein isothiocyanate-labelled anti-human C3 goat antiserum (Hyland Division, Travenol Lab., U.S.A., Lot 2210T004A1) was used at 1:32 dilution. Photomicrographs were taken with a Tiyoda fluorescence microscope equipped with Osram HBO 200 and darkfield condenser. As a control, pemphigoid serum CS (1:640) which was reactive only to the BMZ of normal human epidermis (8) was used for CIF, and ordinary IgG IIF testing was also performed using pemphigoid serum TH and CS at the same dilution.

Light microscopic examination

Formalin-fixed and paraffin-embedded sections were stained with hematoxylin and eosin, periodic acid-Schiff (PAS) with and without diastase digestion, and colloidal iron with and without bovine testicular hyaluronidase digestion. Frozen sections used for CIF and IIF were also stained with hematoxylin and eosin.

Electron microscopic examination

Specimens were cut into further small pieces and immediately fixed with 2.5% glutaraldehyde in Millonig's phosphate buffer (pH 7.3) for 2 h at 4°C and rinsed overnight in the same buffer. These pieces were post-fixed

Table 1. Summary of the results

CMICS = cell membrane or intercellular space, BC = basaloid cells, BMZ = basement membrane zone, ICS = intercellular space

| Methods | Observations | Tumors | |
|-------------------------------|--|----------------------|-------------------------|
| | | Seborrheic keratoses | Basal cell epitheliomas |
| Complement immunofluorescence | Immunofluorescence in the CMICS of BC | + | ~+~ |
| | Immunofluorescence in the CMICS of squamoid cells | - | - |
| | Immunofluorescence at the BMZ | + | Weakly + |
| Light microscopy | PAS-positive substance in the ICS of BC | ± | Partly + |
| | Colloidal iron-positive substance in the ICS of BC | ± | Partly + |
| | Continuity of the basement membrane | + | ~+~ |
| Electron microscopy | Widening of the ICS of BC | Slight | Moderate |
| | Amorphous substance in the ICS of BC | Normal | Increased |
| | Flocculent substance on the surface of BC | Normal | Decreased |
| | Continuity of the basal lamina | + | +~+ |
| | Width of the basal lamina | Normal | Partly increased |
| | Width of the lamina lucida | Normal | Mostly narrowed |

with 1% osmium tetroxide in the same buffer for 2 h at 4°C. These were dehydrated in graded ethanols, substituted by QY-1, and embedded in epoxy resin. After polymerization of the resin at 54°C for 4 days, these blocks were cut into ultrathin sections and stained with uranyl acetate and lead citrate. Photomicrographs were taken with a Hitachi HU-12AS electron microscope.

RESULTS

Results are summarized in Table 1. The most remarkable differences between SK and BCE were revealed by CIF and electron microscopy.

CIF

By CIF using pemphigoid serum TH, SK showed similar reactions to those of normal human epidermis. Positive immunofluorescence in the CMICS was observed in accordance with the areas occupied by proliferating basaloid cells (Fig. 1a) regardless of their histologic patterns. No immunofluorescence was observed in the CMICS of squamoid cells. Squamous eddies showed in part non-specific fluorescence as seen in the CMICS of thickened horny layer and pseudo-horn cysts. Some basaloid cells showed intracellular immunofluores-

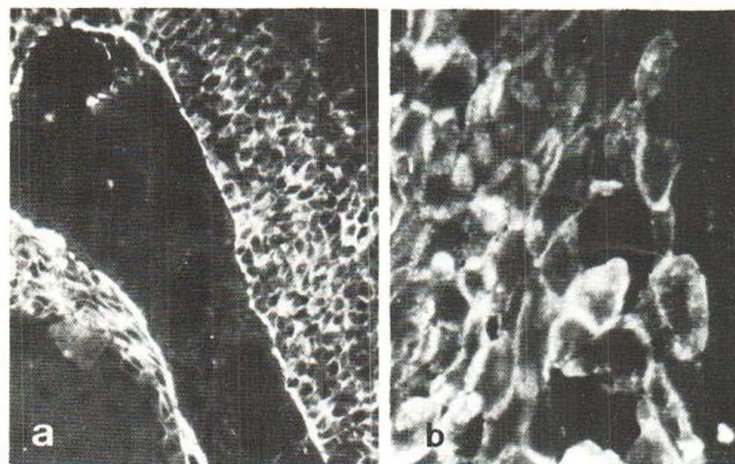


Fig. 1. (a) Complement immunofluorescence (IF) staining of seborrheic keratoses (SK) showing positive IF in accordance with the cell membrane or intercellular space (CMICS) of basaloid cells and at the basement membrane zone (BMZ). $\times 400$. (b) Complement IF staining of SK showing IF not only in the CMICS but also in the cytoplasm of basaloid cells. $\times 800$.



Fig. 2. Complement IF staining of basal cell epitheliomas (BCE) showing positive IF only at the BMZ. $\times 800$.

cence in their cytoplasm (Fig. 1*b*). Immunofluorescence in the CMICS was not observed, either when using pemphigoid serum CS or by ordinary IgG IIF using any pemphigoid serum. Immunofluorescence at the BMZ of SK was almost indistinguishable from that of normal human epidermis by CIF using any pemphigoid serum (Fig. 1*a*). Immunofluorescence at the BMZ by CIF was stronger than that by ordinary IgG IIF.

On the other hand, in BCE, although immunofluorescence at the BMZ was almost continuously

observed by CIF using any pemphigoid serum (Fig. 2), it was extremely weaker compared with that seen in SK and in normal human epidermis, and it was in part discontinuous. Only a quantitative difference was observed in BCE between CIF and ordinary IgG IIF. No distinct immunofluorescence in the CMICS and in the cytoplasm of BCE was observed by CIF as well as ordinary IgG IIF using any pemphigoid serum.

Light microscopy

In SK light microscopic basement membrane of proliferating cell nests, visualized by PAS staining, was indistinguishable from that of normal human epidermis with regard to continuity and thickness. In the CMICS of basaloid cells only a trace of PAS-positive and colloidal iron-positive substance, similar to that in normal human epidermis, was observed.

In BCE, PAS stain revealed partial discontinuity and, in part, thinning and thickening of the light microscopic basement membrane. In the CMICS of basaloid cells, moderate amounts of PAS-positive and colloidal iron-positive substance were observed in part. This substance was diastase-resistant but was almost completely digested by testicular hyaluronidase.

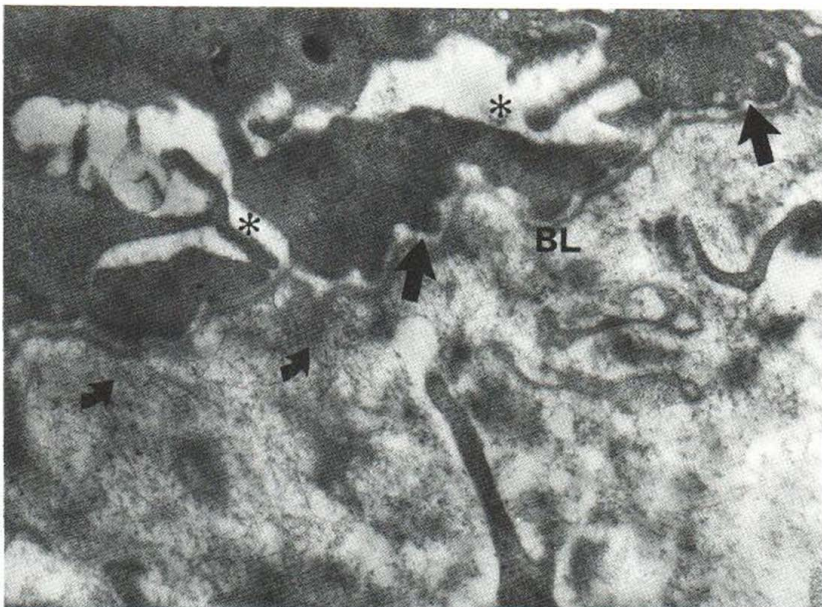


Fig. 3. Ultrastructure of the BMZ and basaloid cells of SK showing almost normal structure. Arrows, Lamina lucida; BL, basal lamina; curved arrows, anchoring fibrils; asterisk, flocculent substance on the surface of basaloid cells. $\times 17\ 600$.

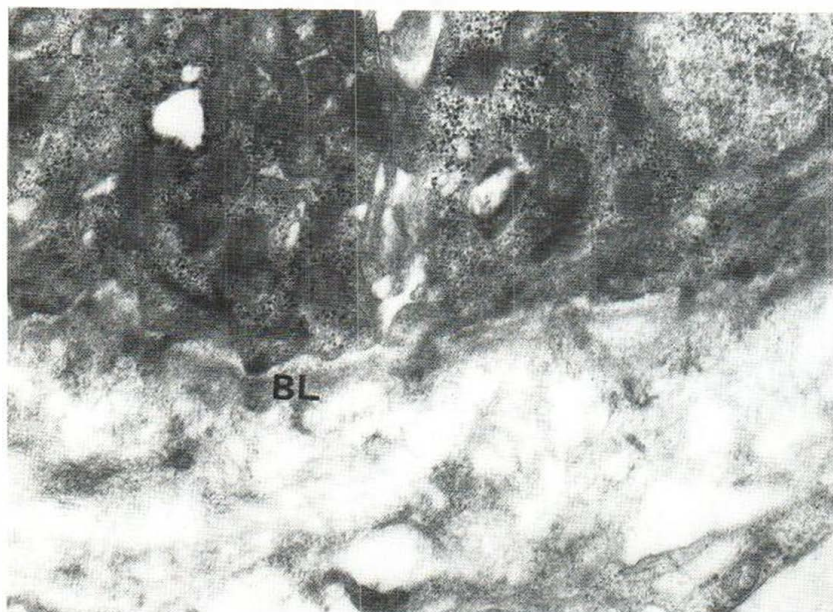


Fig. 4. Ultrastructure of the BMZ and basaloid cells of BCE showing thickening of basal lamina (BL) or basal lamina-like substance. $\times 17600$.

Electron microscopy

SK showed slight and moderate widening of the intercellular space (ICS) and a slight increase in microvilli formation of basaloid cells. No distinct change was observed in the amount of amorphous or flocculent substance on the surface of basaloid cells. Ultrastructure of the BMZ, including lamina lucida, basal lamina and anchoring fibrils, was also indistinguishable from that of normal human epidermis (Fig. 3).

In BCE, moderate and prominent widening of the ICS of basaloid cells was observed, with a moderate increase in the amount of amorphous substance in the ICS. There was less amorphous or flocculent substance on the surface of basaloid cells than that in SK and in normal human epidermis. Ultrastructure of the BMZ varied from tumor to tumor and from part to part in the same tumor. Although discontinuity and disappearance were observed in part, the basal lamina generally showed slight and moderate thickening with irregular narrowing of the lamina lucida (Fig. 4). These findings are consistent with those reported by Kobayashi (9) and Ono (14).

DISCUSSION

Complement immunofluorescence was first described for the detection of complement-fixing autoantibodies (CFA) in bullous pemphigoid by Jor-

don et al. (8). Recently, Harada et al. (5) found that some pemphigoid sera reacted not only to the BMZ but also to the ICS of proliferating basaloid cells in a case of SK, by using CIF. Using the same technique, Nishikawa et al. (13) also reported the binding capacity of those pemphigoid sera to normal human epidermal basal cells. In the present study, CIF was applied to learn about the antigenic properties of tumor cells and BMZ underlying epidermal tumors.

Complement IF on SK using pemphigoid serum TH revealed remarkable immunofluorescence, in accordance with the CMICS of proliferating basaloid cells. This is the main difference of staining attitude between CIF and ordinary IgG IIF. Previous studies using ordinary IgG IIF with pemphigoid sera revealed positive immunofluorescence at the BMZ (3, 4, 10, 11, 15). It can be said that CIF detects epidermal antigens more sensitively than does ordinary IgG IIF, as reported by Hertz et al. (6).

What, then, gives rise to the differences in staining attitude between SK and BCE in CIF findings? These differences may be attributed to the degree of differentiation, to the differences in their origin, or to differences in their antigenic properties, as suggested by Muller et al. (10, 11). The difference in antigenic properties may in part be correlated to their structural differences both in the BMZ and in the CMICS of basaloid cells of these two tumors.

In SK, immunofluorescence in the CMICS was observed only in the areas of basaloid cells. Squamoid cells did not show any positive immunofluorescence in their CMICS. These findings suggest that reactive substances in the CMICS of basaloid cells have the same antigenic properties as those at the BMZ, that reactive substances both in the CMICS and at the BMZ of basaloid cells are produced by basaloid cells, and that basaloid cells play a part in the formation of the components of the BMZ which have the antigenic properties of the BMZ.

This last concept may be supported by the finding that immunofluorescence by CIF as well as ordinary IgG IIF at the BMZ is obviously influenced by proliferating epithelial cells. This theory conforms with the hypothesis that epidermal basal lamina is of epidermal origin (1, 2). Interrelationships between CIF findings and electron microscopic ones should, however, be interpreted separately, since PAS-positive light microscopic basement membrane of the epidermis lies beneath the electron microscopic basal lamina (2), and IgG deposition of pemphigoid skin has been immunoelectron microscopically observed to react mostly to lamina lucida (7, 16). Although narrowing and in part disappearance of lamina lucida in BCE may correspond to weak or negative staining in CIF on BCE, immunoelectron microscopy must be performed to reveal precise ultrastructural localization of the reaction of CFA.

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