

## Loss of Bullous Pemphigoid Antigen in Peritumoral Lacunas of Basal Cell Carcinomas

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Peritumoral lacunas of basal cell carcinoma (BCC) were for a long time misinterpreted as fixation artifacts. However, recent studies showed that they might be considered as a dynamic process related to degenerescence for the palisade cells. We studied three antigens of the epidermal basement membrane zone—type IV collagen, laminin, and bullous pemphigoid antigen—by indirect immunofluorescence in six cases of basal cell carcinoma. We could observe that type IV collagen as well as laminin were expressed as a linear continuous staining pattern surrounding each of the BCC buds. When there was a peritumoral lacuna, only the stroma side of the lacuna was stained. Bullous pemphigoid antigen showed either a linear continuous or discontinuous staining pattern along the basement membrane zone of the carcinomatous buds. At the site of a peritumoral lacuna bullous pemphigoid antigen abruptly disappeared. The loss of bullous pemphigoid antigen might be related to the lacuna formation. *Key words: Pemphigoid antigen; Basal cell carcinoma.* (Received July 21, 1983.)

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Peritumoral lacunas are frequently observed around buds of basal cell carcinomas (BCC) and appear as a clear halo on hematoxylin-eosin sections. Since the first report of Krompecher in 1903, they were considered as a technical artifact related to the fixation methods. This idea persists in some of the most important textbooks of dermatopathology (1, 2, 3). However, peritumoral lacunas can be observed on cryostat sections where there are no similar fixation artifacts.

Umiker & Director (4) first stated that the lacunas could be related to an enzymatic depolymerization process of peritumoral stroma. Pierard & Kint (5), Kint (6, 7) and more recently Franchimont et al. (8) eventually demonstrated that peritumoral lacuna must be interpreted as a dynamic process related to degenerescence of the most peripheral cells of the buds.

Basement membrane zone (BMZ) is a complex structure in which immunostaining procedures identify several components among which bullous pemphigoid antigen (BP Ag), laminin (Lam) and type IV collagen (Coll IV) are the most currently studied.

Bullous pemphigoid antigen is a high molecular weight (220 kd) glycoprotein synthesized by epidermal basal cells (9, 10, 11). Immunoelectronmicroscopic studies have demonstrated that it is localized in the lamina lucida of the basement membrane. BP Ag is also the first antigen appearing in the formation of the BMZ during embryonic life (12) or in the reepithelialization process of wound (13). BP Ag is distinct from laminin which is also localized in the lamina lucida (14, 15) and synthesized by epidermal cells (10). Type IV collagen is localized in the lamina densa of the basement membrane (14, 16) and also synthesized by epidermal basal cells (12, 14).

Since the study of the peritumoral lacunas of BCC may be considered as a good example of carcinoma-stroma interaction, we have studied the expression of the three antigens at this level.

## MATERIAL AND METHODS

### *Antisera*

Two sera from patients having bullous pemphigoid were used. One had a titer of 1/100 and the other 1/20 000. The latter has been demonstrated to react with an antigen localized in the lamina lucida by immunoelectronmicroscopy. Both were used diluted 1/10 and 1/100. Rabbit antisera to laminin and type IV collagen were kindly provided by Professor Fusenig, German Cancer Research Center, Heidelberg, FRG, and used at dilution 1/10 (lam.) and 1/100 (coll. IV).

### *Indirect immunofluorescence*

Six human basal cell carcinomas were obtained from excision specimens under local anesthesia with lidocaine 1%. Half of the specimen was proceeded for routine histology. The other half was immediately frozen in liquid nitrogen and stored at  $-20^{\circ}\text{C}$  within one or two weeks. They were serially sectioned with a cryostat microtome set at  $4\ \mu\text{m}$ .

The tissue sections were incubated with corresponding antiserum for 30 min at room temperature; after washing for 30 min in PBS pH 7.2 they were incubated with commercially prepared fluoresceine isothiocyanate (FITC) labelled sheep anti-rabbit IgG antiserum (Cappel Laboratories, Downing, PA, USA; specific antibody concentration: 0.8 mg/ml; F/P ratio 3.43 mg/g; working dilution in PBS 1/160 to obtain 50  $\mu\text{g}/\text{ml}$  of specific antibodies) for laminin and type IV collagen, or with commercially prepared FITC labelled goat anti-human IgG antiserum (Hyland, Deerfield, Illinois, USA; specific antibody concentration 1.8 mg/ml; F/P molar ratio 2.2; working dilution in PBS 1/36 to obtain 50  $\mu\text{g}/\text{ml}$  of specific antibodies) for bullous pemphigoid antigen. The slides were then washed for 30 min in PBS and mounted in Fluorep®. They were examined with a Zeiss fluorescent microscope equipped for incident illumination.

## RESULTS

The hematoxylin-eosin sections showed that the carcinomas were of superficial multicentric type, adenoid type, solid type or shared many of these features. We did not find any histologic evidence of keratinizing differentiation. Only four of the six carcinomas showed peritumoral lacunas.

*Laminin and type IV collagen.* Lam. and coll. IV immunoreactivities were similar in all BCC examined: both antigens were detected surrounding each aggregate of tumorous cells as an homogenous and continuous line. There was no staining within the carcinomatous buds. At the side of peritumoral lacunas, lam. and coll. IV immunoreactivities were detected only on the stromal side of the lacuna. We did not find any cytoplasmic staining.

*Bullous pemphigoid antigen.* BP Ag immunoreactivity showed several aspects. In the absence of peritumoral lacuna either a continuous or discontinuous linear pattern surrounding each carcinomatous buds or a granular pattern along the BMZ or an absence of the BP immunoreactivity (Fig. 1).

Moreover, some of the BCC cells showed a cytoplasmic staining pattern. This cytoplasmic BP Ag immunoreactivity could be seen either in peripheral cells which have close contacts with the basement membrane or in a central position. This pattern did not correlate with a particular histologic type of BCC. In a given specimen only a few buds showed this cytoplasmic BP Ag immunoreactivity.

At the level of the peritumoral lacuna, when the stroma separated from the carcinomatous epithelial proliferation an abrupt loss of the BP Ag immunoreactivity was always observed (Fig. 2). All along the lacunas we could observe BP Ag immunoreactivity neither along the epithelial side nor along the stromal side of the BCC buds, this contrasted with the expression of the lam. and coll. IV at the stromal side of the lacunas.

## DISCUSSION

Several workers had already studied the expression of BMZ antigens around BCC carcinomas but little attention was given to the peritumoral lacunas. Weber et al (17), Van

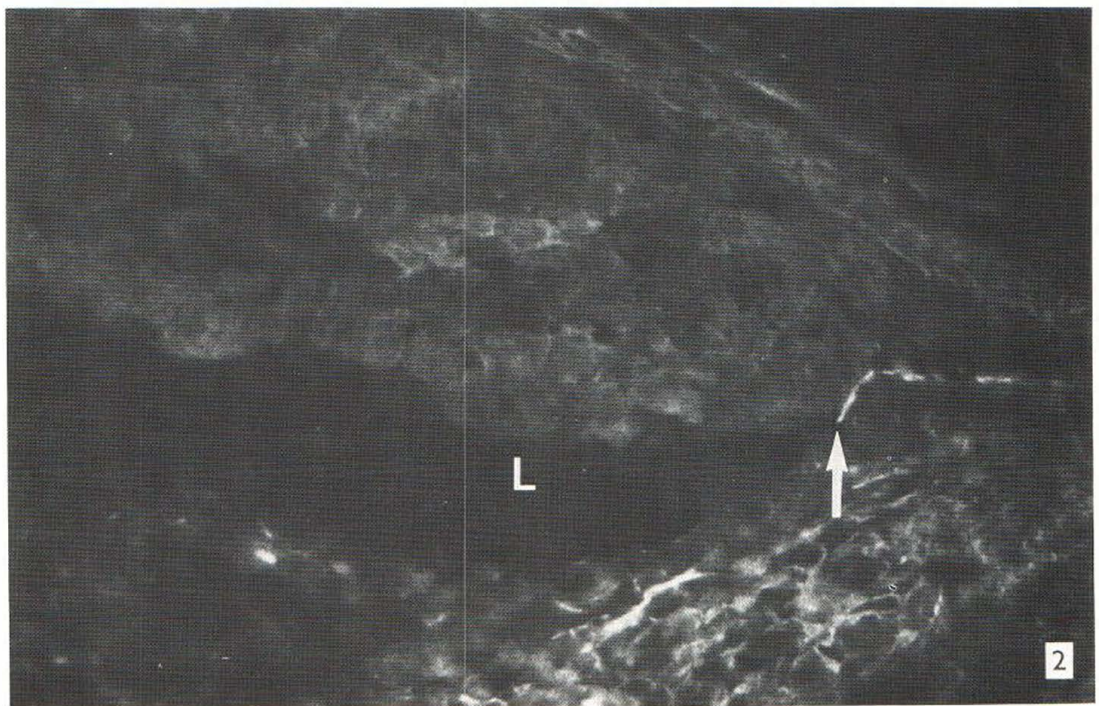
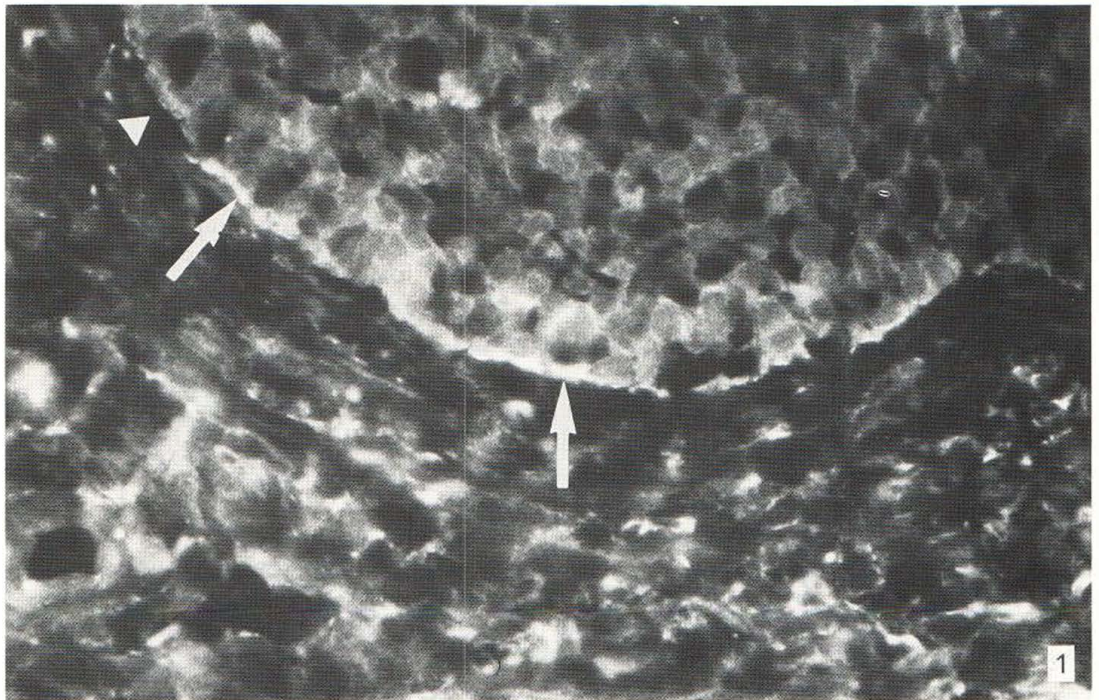


Fig. 1. Bullous pemphigoid antigen is present (→) or not (▲) along the carcinoma-stroma junction of BCC.  $\times 500$ .

Fig. 2. Bullous pemphigoid antigen: abrupt loss of the antigen immunoreactivity (→). L, lacuna.  $\times 300$ .

Cauwenberge et al. (18) reported that coll. IV and lam. were normally expressed in BCC, a fact that we could confirm in this study. As far as BP Ag is concerned the results of several groups were different. De Moragas, Winkelmann & Jordon (19), Tosca et al. (20) have found a persistence of BP Ag, although variable and faint. In contrast, Stanley et al. (21) reported a total loss of BP antigenicity in BCC.

Our study focused on the peritumoral lacuna and adds several points to this issue:

(i) A loss in BP immunoreactivity might be observed in several types of BCC: superficial multicentric, adenoid, solid or an admixture of these aspects, representing distinct types of BCC differentiation. This is of interest since Stanley et al. (13, 21) postulated that BP Ag might be required for differentiation of basal cells.

(ii) With one of the sera used in this study a cytoplasmic staining of BCC has been observed. This has been previously reported once by De Moragas, Winkelmann & Jordon (19), but not by others (22, 20). That a cytoplasmic staining was not reported in all the studies might be related to the recently recognized heterogeneity of BP Ag (23) and the BP sera used might not be directed to the same antigenic determinant. Moreover, a cytoplasmic expression might be related to a retention of BP Ag within the carcinomatous cells, illustrating therefore a defect in the excretion of the glycoprotein.

(iii) The immunomapping of BMZ antigens at the level of the lacuna showed that lam. and coll. IV were always detected around carcinomatous buds. Both were observed as a linear continuous pattern along the carcinoma stroma junction. This is in agreement with previous works of Weber et al. (17), Stanley et al. (21) and more recently Van Cauwenberge et al. (18). When the tumoral proliferation separated from the stroma these two antigens were still expressed along the stromal side of the lacunas. The immunostaining picture resembles that of the junctional type of congenital epidermolysis bullosa (24). Its constant character indicates that the lacunas are not processing artifacts but rather represent a specific defect in basal cell adhesion to the BCC.

(iv) BP Ag was constantly absent and/or abruptly lost at the level of the lacuna. Stanley et al. (21) did not indicate whether zones of loss in BP Ag immunoreactivity actually corresponded to lacunas. Therefore, the constant lack of BP Ag at the level of the lacunas might be regarded as a secondary event, the degenerated cells not being able to synthesize and excrete BP Ag. However, all the synthetic functions of the peripheral BCC cells bordering a lacuna do not seem to be lost since we recently demonstrated that a specific basal cell protein—the skin calcium binding protein—remained expressed within BCC peripheral cells at the level of the lacuna (22). This could also be due to a difference in the turnover rate of both proteins. A similar interpretation could apply to the persistence of lam. and col. IV which are also secreted by basal cells.

Whether the BP Ag loss is primarily responsible for the split is not established. However, Stanley et al. (25) have clearly demonstrated the role of BP Ag in the attachment process of the basal cell to the basement membrane. It is possible that this antigenic loss might imply impairment of the adhesion process and then the formation of the peritumoral lacunas. In that sense the peritumoral lacunas of BCC might represent a model for the study of the role of each biochemical component in the adhesion of basal cells to the BMZ structures.

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