

# Specific Epidermal Protein Markers Are Modulated during Calcium-induced Terminal Differentiation

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**ABSTRACT** Extracellular calcium concentration has been shown to be an important determinant of proliferation rate in a number of cell culture models. Recently, the role of calcium as a regulator of cellular differentiation has also become apparent. This effect of calcium was exemplified by the discovery that keratinocytes of mouse or human origin grew as a proliferating monolayer in medium with a calcium concentration of 0.02–0.09 mM but that proliferation ceased and cells stratified and cornified when calcium was increased  $>0.1$  mM. While the morphological and biological effects of changes in calcium concentration are dramatic in keratinocyte cultures, it has been difficult to identify specific protein changes associated with the modulation of maturation. *In vivo*, however, several proteins that are markers for stratified squamous epithelia have been identified by specific autoimmune sera. Pemphigoid antigen is a 220-kdalton protein found in the basement membrane and closely associated with the plasma membrane of the basal cell. Pemphigus antigen is a 130-kdalton glycoprotein found on the cell surface of stratifying epithelial cells. Immunofluorescence staining of cells cultured in low  $\text{Ca}^{2+}$  or cells switched to high  $\text{Ca}^{2+}$  for 48 h before staining demonstrated that pemphigoid antigen was detected in low  $\text{Ca}^{2+}$  cultures but was diminished or absent in high  $\text{Ca}^{2+}$  cultures and that pemphigus antigen was seen only in high  $\text{Ca}^{2+}$  cultures. The synthesis of each antigen was studied in immunoprecipitates of cell lysates radiolabeled with  $^{14}\text{C}$ -amino acids or D-[ $^{14}\text{C}$ ]glucosamine. Pemphigoid antigen was synthesized mainly by proliferating cells in low  $\text{Ca}^{2+}$  medium and its synthesis was decreased by  $>90\%$  in cells switched to high  $\text{Ca}^{2+}$  medium. In contrast, synthesis of pemphigus antigen was detected only in stratifying cells cultured in high  $\text{Ca}^{2+}$  medium. These studies indicate that extracellular calcium concentrations which modulate the transition between proliferating and stratifying epidermal cells also modulate, in parallel, the synthesis of specific marker proteins for these cell types.

The control of cellular proliferation by ions has been studied extensively since the introduction of cell culture methodology into biological research (1, 2). In particular, calcium ions have been identified as regulators of proliferation in many model systems (1–3). The regulation of cell growth by calcium is demonstrated directly by modulating extracellular calcium concentration or indirectly in experiments in which calcium fluxes across the plasma membrane or intracellular membranes regulate the response to other trophic stimuli. Calcium has also been implicated in the control of cellular differentiation in several model systems (4, 5), but these effects have been less extensively studied than effects on proliferation.

Mouse keratinocyte cultures are a model system in which extracellular calcium regulates growth and differentiation (5). In medium containing 0.05–0.1 mM calcium (low  $\text{Ca}^{2+}$ ), mouse keratinocytes proliferate rapidly with a high growth fraction,

do not stratify, and are separated by widened intercellular spaces. When the calcium concentration in culture medium is switched to 1.2 mM (high  $\text{Ca}^{2+}$ ), cell-to-cell contact occurs, cell stratification and cornification take place, and DNA synthesis is inhibited (5, 6). These changes mimic, in many ways, the orderly transition of keratinocytes *in vivo* from the proliferative, basement membrane-anchored, basal cell layer to stratified, nonproliferating, differentiating cells. It is also notable that just as the program of differentiation of neoplastic epidermal cells is altered *in vivo*, this program of differentiation in response to calcium *in vitro* is altered for transformed or carcinogen-treated keratinocytes (7–9).

In an effort to elucidate the molecular mechanisms involved in this calcium regulation of normal epidermal cells, we investigated whether the synthesis of specific proteins was associated with the maintenance of proliferation or the induction of

stratification by calcium. Here we show that the synthesis of pemphigoid and pemphigus antigens, two specific proteins that are found in epithelia which stratify but, in general, not in other tissues (10), is profoundly modulated by extracellular calcium concentrations that regulate the proliferation or stratification of epidermal cells. The diseases bullous pemphigoid and pemphigus are both autoimmune bullous diseases in which the antigen-antibody interaction is thought to be important in the pathophysiology of blister formation (11). Pemphigoid antigen, defined by antibodies in the sera of patients with bullous pemphigoid, is a normal basement membrane protein of ~220 kdalton (12). It is located ultrastructurally in the lamina lucida (13, 14) and is in close association with the epidermal basal cell plasma membrane (15-17). Pemphigus antigen is defined by antibodies in the sera of patients with pemphigus, and is a normal cell surface antigen (or antigens) of stratified squamous epithelial cells (10). It has been postulated to be part of the "intercellular cement" of stratifying cells (18). Most, but not all, pemphigus sera bind a cell surface glycoprotein of ~130 kdalton when reduced (19). (In this paper we are using "pemphigus antigen" to refer to this particular glycoprotein.)

We show here that proliferating keratinocytes in culture in low  $\text{Ca}^{2+}$  medium synthesize pemphigoid antigen, but not detectable pemphigus antigen, and that when the cells are induced to stratify by high  $\text{Ca}^{2+}$  medium, pemphigoid antigen synthesis is shut off or markedly decreased while pemphigus antigen synthesis is initiated.

## MATERIALS AND METHODS

**Cell Culture:** Epidermis from newborn BALB/c mice was used to establish cultures (20). As previously described (5), these cultures were grown in Medium 199 plus 2% fetal bovine serum, but with the medium calcium concentration adjusted to 0.07 mM (low  $\text{Ca}^{2+}$  cells). After 6 d of culture, some cultures were switched to the same medium containing 1.2 mM calcium (high  $\text{Ca}^{2+}$ ), and the remaining cultures were re-fed with low  $\text{Ca}^{2+}$  medium.

**Antibodies and Sera:** The sera used in this study to identify pemphigoid antigen were from bullous pemphigoid patients who had circulating anti-basement membrane zone antibodies (as detected by indirect immunofluorescence on normal skin). These sera have been used previously to characterize the pemphigoid antigen (12). Antibodies to laminin, another basement membrane protein found in the lamina lucida, were used to determine whether changes seen in pemphigoid antigen synthesis were a reflection of changes in overall basement membrane synthesis. These antibodies were affinity purified as previously described (21). The sera used in this study to identify pemphigus antigen were from two pemphigus vulgaris patients who had circulating antibodies which bound normal human epidermis in a typical cell surface pattern as determined by indirect immunofluorescence. These two sera have been used previously to characterize the 130-kdalton pemphigus antigen (19).

**Indirect Immunofluorescence:** Cells, grown on glass or plastic coverslips, were cultured in low  $\text{Ca}^{2+}$  or switched to high  $\text{Ca}^{2+}$  for 48 h prior to staining, and then indirect immunofluorescence was performed to detect pemphigoid or pemphigus antigens. The methods used have been previously described in detail (7, 19). To detect pemphigoid antigen, we first permeabilized the cells with methanol and acetone if they were grown on glass coverslips (7) or with methanol alone at  $-20^{\circ}\text{C}$  for 10 min if they were grown on plastic coverslips. To detect pemphigus antigen (a cell surface antigen), we stained living cells which were not fixed or permeabilized (19). In these indirect immunofluorescence studies, normal human sera were used as controls. Binding of human antibodies was detected by using fluorescein-conjugated goat anti-human IgG (Cappel Laboratories, Cochranville, PA).

**Radiolabeling and Extraction of Cell Cultures:** To demonstrate cell biosynthesis of antigens, we cultured confluent cells in low  $\text{Ca}^{2+}$ , or switched them to high  $\text{Ca}^{2+}$  for 6 to 24 h prior to labeling, and then radiolabeled them with a mixture of  $^{14}\text{C}$ -amino acids (specific activity 55 mCi/matom carbon, New England Nuclear, Boston, MA) at 4-8  $\mu\text{Ci}/\text{ml}$  or with D-[ $^{14}\text{C}$ ]glucosamine (specific activity 55 mCi/mmol, Amersham Corp., Arlington Heights, IL) at 12.5  $\mu\text{Ci}/\text{ml}$ . After a 24-48-h labeling period, cell culture medium was removed and dialyzed and cells were extracted in situ with nonionic detergent as previously described (12, 19).

**Immunoprecipitation:** To identify newly synthesized antigens, we used previously described immunoprecipitation techniques (12, 19). Radiolabeled medium or cell extracts were incubated with antibodies or serum, and immune complexes were precipitated using either protein A-bearing staphylococci (12) or an anti-IgG antibody (19).

We used SDS PAGE (22) and fluorography (23, 24) to identify specifically precipitated radiolabeled antigens. In each gel, immunoprecipitates shown in each lane were performed starting with equal amounts of radioactivity. The relative amounts of precipitated antigens were determined by densitometric scanning of fluorographs (Quick Scan, Helen Laboratories Corp., Beaumont, TX). Densitometric scanning of the specifically precipitated antigen bands is more accurate than determining counts precipitated because there is a certain (and variable) amount of nonspecific counts precipitated by each serum.

To demonstrate that immunoprecipitations of antigens were complete, we rereacted supernatants with an aliquot of the same immune serum or antibodies used in the initial immunoprecipitation. In general, there was no detectable antigen left after the first reaction with the immune serum or antibodies.

## RESULTS

### Cell Culture

As previously shown (5), mouse epidermal cells cultured in low  $\text{Ca}^{2+}$  medium grew as monolayer with widened intercellular spaces. When the cells were then switched to high  $\text{Ca}^{2+}$  medium, cell-to-cell contacts were established within 4-6 h and stratification took place within 24-48 h.

### Indirect Immunofluorescence

Pemphigoid antigen was detected in low  $\text{Ca}^{2+}$  cultures of mouse epidermal cells as a perinuclear granular fluorescence seen in >90% of cells (Fig. 1a). This is the same immunofluorescence pattern which we have previously described for pemphigoid antigen found in human epidermal cells (12, 25) and Pam cells, spontaneously transformed mouse keratinocyte cell lines (7). This pattern indicates antigen within or under the cells. In cultures of mouse epidermal cells switched to high  $\text{Ca}^{2+}$  for 48 h, the results of immunofluorescence for detection of pemphigoid antigen were variable. In some cultures a faint perinuclear granular fluorescence was detected to be associated only with cells on the substrate (Fig. 1b), and in some cultures the antigen could not be detected at all. These results suggested that there might be a decrease in pemphigoid antigen in the cell layer of mouse epidermal cells switched to high  $\text{Ca}^{2+}$  medium compared to the cell layer of low  $\text{Ca}^{2+}$  cultures. To determine whether this decrease in staining for pemphigoid antigen was simply due to a decrease in antibody access to antigen in stratified cells, we stained the cells by indirect immunofluorescence for laminin. Laminin is a basement membrane glycoprotein that is distinct from pemphigoid antigen (26) and, unlike pemphigoid antigen, is found in all basement membranes (not just stratified squamous epithelial basement membrane) (27). However, like pemphigoid antigen, laminin is found in the lamina lucida of epidermal basement membrane (27). The results of immunofluorescent staining for laminin demonstrated that laminin was detected in or under cells on the substrate in both low  $\text{Ca}^{2+}$  cultures and high  $\text{Ca}^{2+}$  cultures (Figs. 1c and d) and, thus, that the decrease or absence of pemphigoid antigen in stratifying cells was not simply due to poor access of antibody to antigen.

Pemphigus antigen could not be detected by immunofluorescence on keratinocytes grown in low  $\text{Ca}^{2+}$  (Fig. 1e). However, when the cells were switched to high  $\text{Ca}^{2+}$  for 48 h before staining, pemphigus antigen was visualized on the cell surfaces of the stratified cells (Fig. 1f). These findings suggested that cells induced to stratify by high  $\text{Ca}^{2+}$  medium synthesize pemphigus antigen but that proliferating cells in low  $\text{Ca}^{2+}$  medium may not.

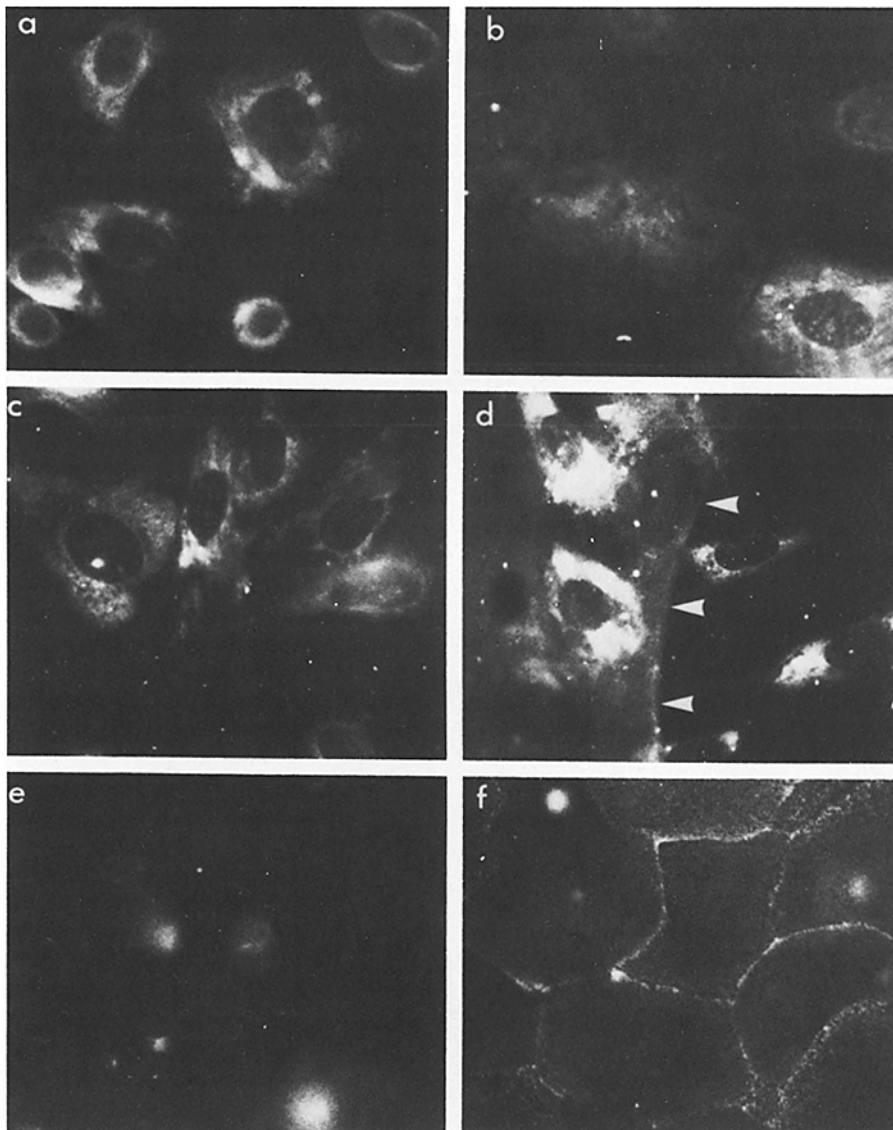


FIGURE 1 Immunofluorescent staining for pemphigoid antigen, laminin or pemphigus antigen in mouse epidermal cells cultured in low  $\text{Ca}^{2+}$  medium (low  $\text{Ca}^{2+}$  cells) or switched to high  $\text{Ca}^{2+}$  medium (high  $\text{Ca}^{2+}$  cells) for 48 h before staining. (a) Pemphigoid antigen, low  $\text{Ca}^{2+}$  cells. Cells display a bright perinuclear granular fluorescence. (b) Pemphigoid antigen, high  $\text{Ca}^{2+}$  cells. Some cells on the substrate display a dull perinuclear granular fluorescence. In some cultures of high  $\text{Ca}^{2+}$  cells, no fluorescence was detectable. (c) Laminin, low  $\text{Ca}^{2+}$  cells. Cells display a bright perinuclear granular fluorescence. (d) Laminin, high  $\text{Ca}^{2+}$  cells. Small cells on the substrate display a bright perinuclear granular fluorescence. The edge of a colony is marked by arrowheads, and cells can be seen both isolated on the substrate (to the right of the colony) and under the colony. (e) Pemphigus antigen, low  $\text{Ca}^{2+}$  cells. No specific fluorescence is seen. (f) Pemphigus antigen, high  $\text{Ca}^{2+}$  cells. There is a cell surface fluorescence. (a-d)  $\times 420$ . (e and f)  $\times 260$ .

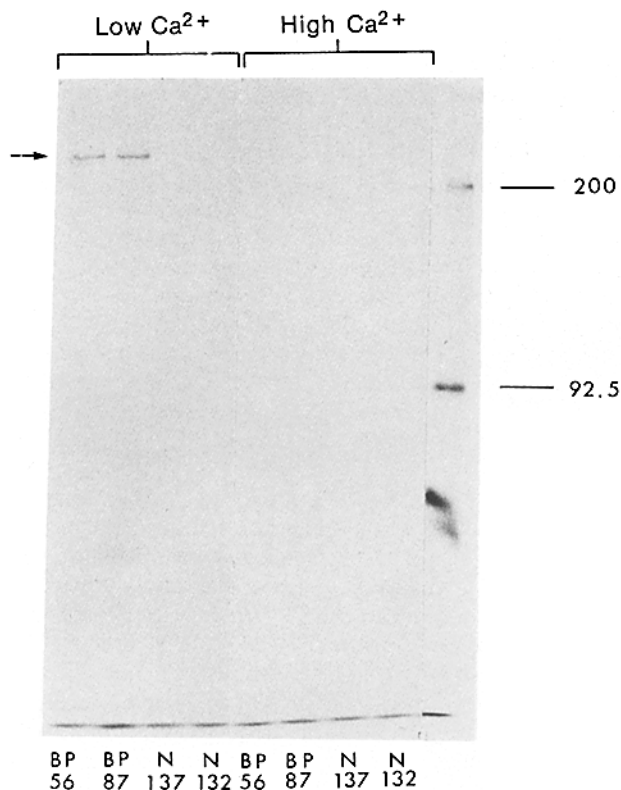
### Synthesis of Pemphigoid Antigen

To determine whether  $\text{Ca}^{2+}$ -induced changes in epidermal morphology correlated with changes in the synthesis of pemphigoid antigen, we cultured mouse epidermal cells for 6 d in low  $\text{Ca}^{2+}$  medium, then either maintained them in fresh low  $\text{Ca}^{2+}$  medium or switched them to high  $\text{Ca}^{2+}$  medium. 24 h later,  $^{14}\text{C}$ -amino acids were added for a 24-h labeling period. Newly synthesized, radiolabeled pemphigoid antigen was detected by immunoprecipitation followed by SDS PAGE and fluorography. Under reducing conditions, pemphigoid antigen appears as a band at  $\sim 220$  kdalton (12).

Newly synthesized pemphigoid antigen was precipitated from detergent extracts of monolayer epidermal cells grown in low  $\text{Ca}^{2+}$  but was not seen, or was barely detected, in gels of immunoprecipitates from extracts of stratifying epidermal cells cultured in high  $\text{Ca}^{2+}$  medium (Fig. 2). There was at least a 90% reduction in newly synthesized pemphigoid antigen extracted from cells switched to high  $\text{Ca}^{2+}$ , as determined by densitometric scanning of the fluorographs. Cells in which radiolabeled amino acids were added only 6 h after the switch to high  $\text{Ca}^{2+}$  medium also showed a  $>90\%$  reduction in newly synthesized pemphigoid antigen compared to cells maintained in low  $\text{Ca}^{2+}$ . To determine whether these differences reflected

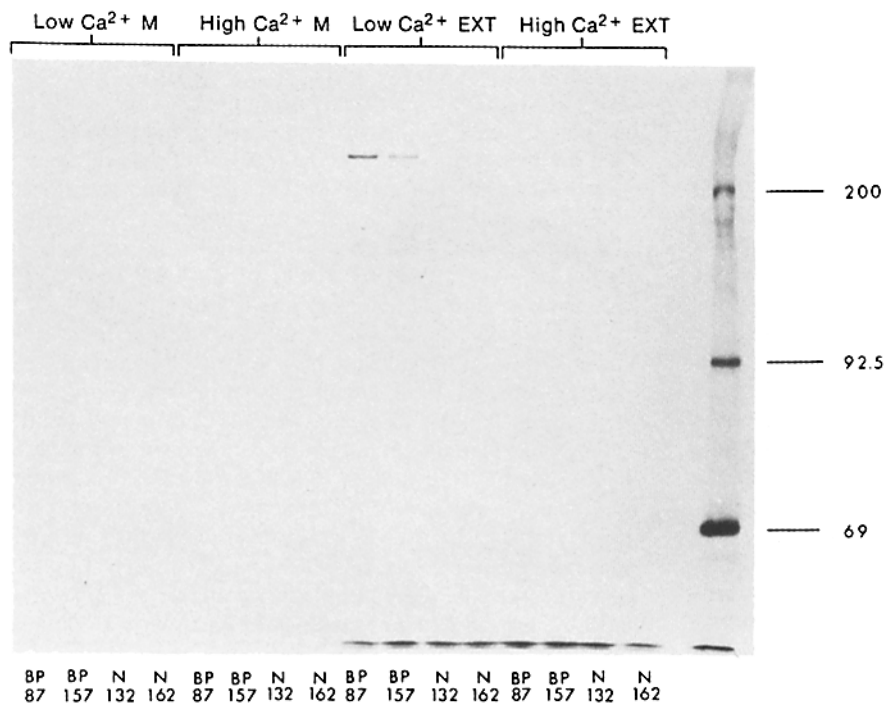
a real change in synthesis or whether the newly synthesized antigen was lost from the cell layer in high  $\text{Ca}^{2+}$  cultures, we attempted to immunoprecipitate pemphigoid antigen from the medium of these cells. Newly synthesized pemphigoid antigen could not be precipitated from the medium of cells cultured in either high or low  $\text{Ca}^{2+}$  (Fig. 3). Thus, the inability to detect pemphigoid antigen in the cell layer of high  $\text{Ca}^{2+}$  epidermal cells is not simply because it is shed into the medium. In addition, it is very unlikely that the failure to detect newly synthesized pemphigoid antigen in cells grown in high  $\text{Ca}^{2+}$  is due to increased degradation, because there was no increase in smaller molecular weight proteins precipitated by pemphigoid sera from extracts of those cells or from their medium. Thus, these results indicate that extracellular  $\text{Ca}^{2+}$  modulates the synthesis of pemphigoid antigen in parallel with the state of differentiation of the epidermal cells. At low  $\text{Ca}^{2+}$  concentrations, in which epidermal cells proliferate as a monolayer, pemphigoid antigen is synthesized. However, when the  $\text{Ca}^{2+}$  concentration is raised to a level which induces stratification and cessation of proliferation, pemphigoid antigen synthesis is dramatically decreased. These results are consistent with the immunofluorescence findings.

To learn whether this decrease in synthesis of pemphigoid



BP BP N N BP BP N N  
56 87 137 132 56 87 137 132

FIGURE 2 Newly synthesized pemphigoid antigen (arrow identifies 220-kdalton band) is immunoprecipitated from radiolabeled extracts of mouse epidermal cells cultured in low  $\text{Ca}^{2+}$  medium, but not from extracts of cells switched to high  $\text{Ca}^{2+}$  medium for 24 h before exposure to the radiolabel ( $^{14}\text{C}$ -amino acids). Immunoprecipitation was performed with pemphigoid sera (BP) or, as controls, normal human sera (N). (The different numbers indicate sera from different patients.) Radiolabeled immunoprecipitated antigen was electrophoresed, under reducing conditions, on SDS PAGE gels and identified by fluorography.  $^{14}\text{C}$ -Labeled molecular weight standards are shown in the far right lane. In this figure (and in all subsequent figures) immunoprecipitates shown in each lane were performed starting with equal amounts of radioactivity. Measurements in kilodaltons.



BP BP N N BP BP N N BP BP N N BP BP N N  
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FIGURE 3 Newly synthesized pemphigoid antigen is not immunoprecipitated from the medium of mouse epidermal cell cultures. The medium (M) and extracts (EXT) of cells, cultured in low  $\text{Ca}^{2+}$  or switched to high  $\text{Ca}^{2+}$  for 24 h before radiolabeling, were used to immunoprecipitate pemphigoid antigen. See legend for Fig. 2 for abbreviations and details. Pemphigoid antigen is detected in the extract of cells cultured in low  $\text{Ca}^{2+}$  and is just barely detectable in the extract of cells switched to high  $\text{Ca}^{2+}$ ; however, it is not detectable in the medium of either cell type. Measurements in kilodaltons.

antigen by stratifying epidermal cells in high  $\text{Ca}^{2+}$  medium was a reflection of a general overall decrease in the synthesis of basement membrane components, we determined whether laminin synthesis was also decreased by high  $\text{Ca}^{2+}$  medium. Newly synthesized laminin was immunoprecipitated from extracts of epidermal cells grown in low  $\text{Ca}^{2+}$  medium or switched to high  $\text{Ca}^{2+}$  medium. In contrast to the profound decrease in the synthesis of pemphigoid antigen induced by high  $\text{Ca}^{2+}$  medium, laminin synthesis was unchanged (or showed a slight decrease only) compared to synthesis in low  $\text{Ca}^{2+}$  medium (Fig. 4). These findings are consistent with the immunofluorescence results.

### Synthesis of Pemphigus Antigen

Mouse epidermal cells were grown in low  $\text{Ca}^{2+}$  medium for 6 d prior to switching some plates to high  $\text{Ca}^{2+}$  medium for 24 h, after which cultures were radiolabeled with D-[1- $^{14}\text{C}$ ]glucosamine. Newly synthesized pemphigus antigen was immunoprecipitated from extracts of these cells or from their medium. Pemphigus antigen, when reduced, can be identified by SDS PAGE as a 130-kdalton band (19). Newly synthesized pemphigus antigen was detected only in extracts of epidermal cells cultured in high  $\text{Ca}^{2+}$  but not in extracts of cells grown in low  $\text{Ca}^{2+}$  (Fig. 5). The antigen was not found in the medium of either high or low  $\text{Ca}^{2+}$  epidermal cells. This finding demonstrated that pemphigus antigen is not shed from the cell surface. The absence of smaller molecular weight molecules from immunoprecipitates of extracts or the medium of cells grown in low  $\text{Ca}^{2+}$  suggests that increased degradation does not account for the absence of pemphigus antigen in these cell cultures. It is very unlikely that failure to detect pemphigus antigen by immunoprecipitation of extracts of cells grown in low  $\text{Ca}^{2+}$  is due to decreased glycosylation resulting in poor radiolabeling of the antigen, because the immunofluorescence findings confirm the fact that there is no detectable immunoreactive antigen on these cells. Thus, taken together, these results demonstrate a profound decrease in the synthesis of a normal immunoreactive cell surface pemphigus antigen by proliferating monolayer epidermal cells cultured in low  $\text{Ca}^{2+}$  medium. We have not

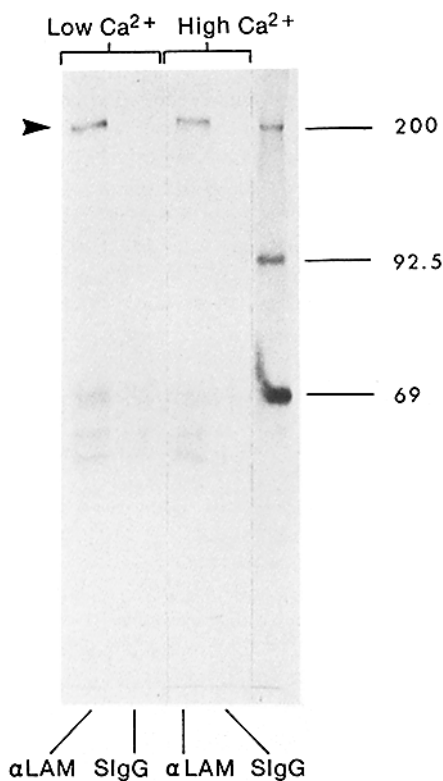


FIGURE 4 Newly synthesized laminin (arrowhead) is immunoprecipitated from radiolabeled extracts of mouse epidermal cells grown in low  $\text{Ca}^{2+}$  medium or switched to high  $\text{Ca}^{2+}$  medium for 24 h before radiolabeling. Affinity-purified antibodies against laminin ( $\alpha\text{LAM}$ ) immunoprecipitate approximately equal amounts of radiolabeled laminin from both cell extracts. Sheep IgG (*SIgG*) was used as a control. Only the 200-kdalton band of laminin is detected in these cell extracts, as has been reported previously (26). Measurements in kilodaltons.

ruled out that some form of aberrant synthesis may take place under these culture conditions. In contrast, immunoreactive pemphigus antigen is synthesized by, and found on the cell surface of, differentiating epidermal cells cultured in high  $\text{Ca}^{2+}$  medium.

## DISCUSSION

The transition between proliferating monolayer cells and stratifying nonproliferating cells induced by  $\text{Ca}^{2+}$  in vitro is associated with a marked decrease in pemphigoid antigen synthesis and an initiation of, or marked increase in, pemphigus antigen synthesis.

The finding that high medium  $\text{Ca}^{2+}$  concentration induces stratification and inhibits proliferation and, at the same time, affects basement membrane protein synthesis is interesting in light of the possible functions ascribed to basement membrane components. It has been demonstrated, for example, that epidermal cells with pemphigoid antigen on their surface, compared to other epidermal cells, preferentially attach to substrates (15). If pemphigoid antigen was involved in the attachment of epidermal cells to their substrate, then a decrease in its synthesis could result in facilitation of stratification. However, laminin is also thought to be involved in epidermal cell attachment to substrates (28), and there is little or no change in newly synthesized laminin in the cell layer of cells switched from low to high  $\text{Ca}^{2+}$  medium. Another possible function of pemphigus antigen might be maintenance of the basal cell phenotype. This

function has been shown to require direct basal cell contact with a substrate (29–32). In normal skin the basal cell contacts its substrate at the lamina lucida of the basement membrane, and the lamina lucida component most closely associated with the basal cell surface is pemphigoid antigen (15–17). Thus, if pemphigoid antigen is important in maintaining the basal cell phenotype, a decrease in newly synthesized pemphigoid antigen in the cell layer of epidermal cells switched from low to high  $\text{Ca}^{2+}$  medium could be involved in a causative way in the transition between proliferating basal cells and stratifying nonproliferating cells. A full understanding of the actual role that these basement membrane components play in this transition will depend on the elucidation of their biological functions.

The initiation of the synthesis of pemphigus antigen in stratifying epidermal cells is consistent with its tissue localization as well as a postulated function of the antigen. Antibodies in the sera of patients with pemphigus define normal cell surface antigens found only in stratified squamous epithelia (10). It has been thought that pemphigus antibodies bind a molecule in the “intercellular cement” or glycocalyx of the epidermis (18). The results of the present study are consistent with the hypothesis that pemphigus antigen is involved in holding stratified squamous cells together. Although this study demonstrates an association between synthesis of pemphigus antigen and stratification, an understanding of the role that pemphigus antigen actually plays in stratification awaits the elucidation of its biological function.

Regardless of the biological functions of each of the antigens studied here, the modulation of pemphigoid and pemphigus antigen synthesis by extracellular  $\text{Ca}^{2+}$  concentrations which regulate differentiation is not just a reflection of a general

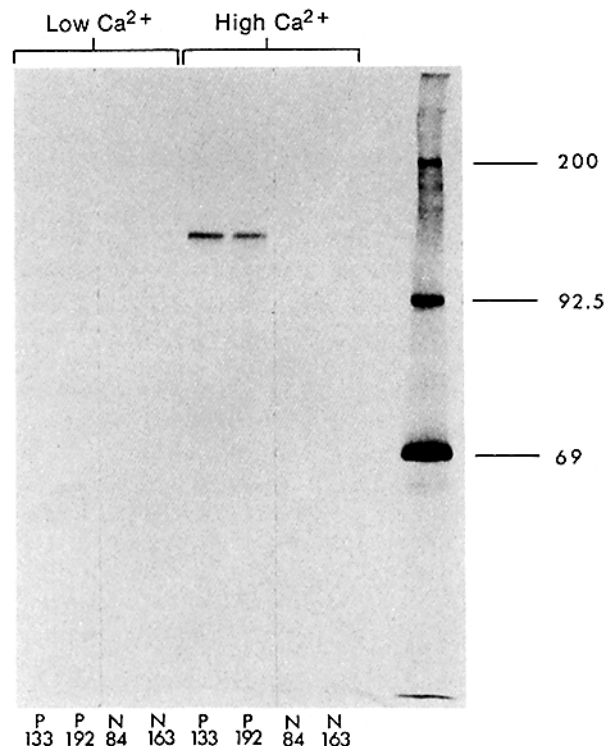


FIGURE 5 Pemphigus antigen is synthesized by mouse epidermal cells cultured in high  $\text{Ca}^{2+}$  medium but is not detectable in cells cultured in low  $\text{Ca}^{2+}$  medium. Cells were radiolabeled with  $\text{D-[1-}^{14}\text{C]glucosamine}$ . *P* indicates pemphigus sera. See legend for Fig. 2 for other abbreviations and details. Pemphigus antigen is seen as a 130-kdalton band. Measurements in kilodaltons.

modulation of the synthesis of most of the cellular proteins. The electrophoretic profiles of the newly synthesized major proteins of epidermal cells grown in low and high  $\text{Ca}^{2+}$  medium are similar (5), showing that there are no major changes in the levels of most of the cellular proteins. In particular, extracellular  $\text{Ca}^{2+}$  does not seem to regulate synthesis of the major differentiation-associated keratin proteins (5) or, as shown here, the basement membrane protein laminin. Thus, there is some specificity to the regulation of pemphigoid and pemphigus antigens by extracellular  $\text{Ca}^{2+}$ . We anticipate, however, that other specific proteins, whose synthesis is regulated in parallel with  $\text{Ca}^{2+}$ -induced differentiation, will be identified and that ultimately we will be able to identify a sequence of changes which regulate and define the program of epidermal differentiation. For example, previous studies have shown that extracellular  $\text{Ca}^{2+}$  also modulates the activity of epidermal transglutaminase (6), although synthesis of the enzyme protein was not directly determined.

The changes described here in the synthesis of pemphigoid and pemphigus antigens may be a result of the differentiation induced by extracellular  $\text{Ca}^{2+}$ . On the other hand, these antigens may prove to be important molecular regulators of the transition between proliferating monolayer cells (basal cells) and stratifying, nonproliferating differentiating epidermal cells. This epidermal cell model system should be useful in studying the biological and molecular functions of these two proteins. Such information is also crucial for understanding the disease states associated with autoantibodies to these antigens.

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