0022-202X/83/8004-0297$02.00/0
THE JOURNAL OF INVESTIGATIVE DERMATOLOGY, 80:297–299, 1983

Vol. 80, No. 4
Printed in U.S.A.

RAPID COMMUNICATION

Plasminogen Is Present in the Basal Layer of the Epidermis*

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This study was undertaken to determine whether plasminogen was present within the epidermis. Cryostat sections of normal human skin were incubated with either whole rabbit antihuman plasminogen serum, the IgG fraction thereof, or the immune-specific IgG fraction thereof, purified by affinity chromatography on plasminogen-Sepharose. Standard indirect immunofluorescent techniques using rhodamine-conjugated goat antirabbit IgG were subsequently employed. Fluorescence was localized to the basal layer of the epidermis. Nonimmune serum and immune serum depleted of its specific antiplasminogen IgG by prior affinity chromatography yielded negative results, demonstrating the specificity of staining for plasminogen. Our findings indicate that plasminogen is present within the epidermis, localized to the peripheral intracytoplasmic area of basal keratinocytes. This finding supports the hypothesis that a proteolytic event mediated by plasminogen activator with the subsequent generation of plasmin may be involved in normal keratinocyte physiology.

A number of epidermal proteases have been the objects of recent investigations, and among them, the serine protease plasminogen activator (PA). It has been suggested that PA plays a physiologic role in the normal maturation of the epidermis, by either proteolytically assisting squame detachment [1] or by facilitating the terminal event of keratinocyte nuclear dissolution [2]. These suggestions have been supported by the demonstration that PA is indeed a normal keratinocyte cell product, whose intracellular levels increase with advanced differentiation [3]. There also have been suggestions that derangements in the control of levels of this enzyme may result in clinically recognizable disease states. For example, psoriatic lesions have been associated with local increases in PA [4], and, most recently, Lazarus and coworkers have suggested that PA and the subsequent generation of plasmin play a key role in the development of acantholysis in pemphigus [5].

Although both physiologic and pathologic roles have been postulated for the epidermal PA, there has not yet been any demonstration that the enzyme has access to its substrate, plasminogen (PLMG). Because PLMG is the only known natural protein that can serve as a substrate for PA [6], we felt that any postulated role for PA in epidermal biology would necessitate the presence of PLMG within the epidermis. This study was therefore undertaken to determine whether PLMG was indeed present within normal human epidermis.

MATERIALS AND METHODS

Sources of Skin

Clinically normal-appearing human skin was obtained from either reduction mammoplasties (×2), mastectomies for adenocarcinoma of the breast (×2), or adjacent to excised basal cell carcinoma (×1) or melanoma (×1). In all cases the pattern of staining observed was similar. Specimens were obtained within 4 h of surgery and were either cryostat-sectioned for immediate use or snap-frozen and stored in liquid nitrogen until time of use.

Sera and Antibody Preparation

For staining, 3 different commercially available preparations of rabbit antihuman PLMG (Behring, Lot 6505; Dako, Lot 099A; Calbiochem-Behring, Lot 009976) were used to assure that the observed staining pattern was not peculiar to any one antiserum. Either the diluted antisera, the IgG fractions thereof, or the affinity purified immune-specific IgG fractions thereof, were used in these studies. The IgG fraction of the rabbit antisera and normal rabbit serum were purified by Protein A Sepharose (Pharmacia) chromatography using standard procedures [7] and elution with 3.0 M potassium thiocyanate. Immune-specific, antihuman PLMG or antihuman albumin IgG fractions were prepared by affinity chromatography using CNBr-activated [8] Sepharose 4B (Sigma) linked to the appropriate ligand: either human albumin (Sigma) or human PLMG which had been prepared according to the technique of Deutsch and Mertz [9]. In each case, the immune-specific fraction was eluted from the column with 50 mM sodium citrate pH 3.8, immediately neutralized with 1 M KH_2PO_4, and dialyzed extensively against PBS. As a control, the non-immune-specific IgG fractions from the column flow-through were purified on Protein-A Sepharose as described above.

Immunofluorescence

Indirect immunofluorescence was carried out using standard procedures with the inclusion of 4% BSA in all rinsing and diluting buffers and the pretreatment of skin samples with normal goat serum diluted 1:5 with PBS/BSA to reduce nonspecific background fluorescence, as suggested by Stanley et al [10]. The first antiserum or antibody preparation was applied at the dilutions indicated in the figure legends. The second antibody, rhodamine-conjugated goat IgG, anti-rabbit-IgG (Cappel, Lot 14881) was used at a dilution of 1:60. The stained sections were examined with a Nikon Fluorofot microscope equipped with a 500–550 nm excitation filter and 570-nm absorption filters, and photographed on black and white Tri X Pan (Kodak) film.

RESULTS AND DISCUSSION

All samples examined, from either fresh or snap-frozen skin, revealed the same pattern when stained with antihuman PLMG antiserum: fluorescence of the basal layer of the epidermis (Fig 1*A*). However, when using whole serum for indirect immunofluorescence on human epidermis, much nonspecific staining (Fig 1*B*) occurs, presumably because of the propensity of the highly charged keratins to nonspecifically absorb serum pro-

Manuscript received September 13, 1982; accepted for publication October 19, 1982.

* A preliminary report of part of this work was presented at the 43rd Annual Meeting of The Society for Investigative Dermatology, Inc., May 1982, Washington, D.C. (J Invest Dermatol 78:359, 1982)

This work was supportd by a Dermatology Foundation Fellowship (RRI) and NIH grants CA 23753, AI 17365, and AM 07190 (DBR).

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Abbreviations:
BSA: bovine serum albumin
PA: plasminogen activator
PBS: phosphate-buffered saline
PLMG: plasminogen

teins. To obviate this problem, the IgG fraction purified from anti-PLMG serum was used; a similar staining of the basal layer was again noted (Fig 2).

To demonstrate that the observed staining pattern was indeed specific for PLMG, immune-specific anti-PLMG and antialbumin IgG fractions were prepared by affinity chromatography. Staining with the antialbumin IgG fraction demonstrated (Fig 3*A*) a brilliant fluorescence of the dermis and basement membrane zone; no fluorescence of the epidermis was seen. On the other hand, staining with an equal dilution (100 μg/ml) of anti-PLMG (Fig 3*B*) demonstrates bright fluorescence localized to the basal layer with some faint staining of occasional suprabasilar cells. No staining of the basement membrane zone was noted. In addition, brightly staining annular and/or linear areas were seen within the dermis (Fig 3*B*, *arrow*). These probably represent dermal vessels. When skin was stained with the control nonimmune IgG fraction, no staining of either epidermis or dermis was seen (Fig 3*C*), con-

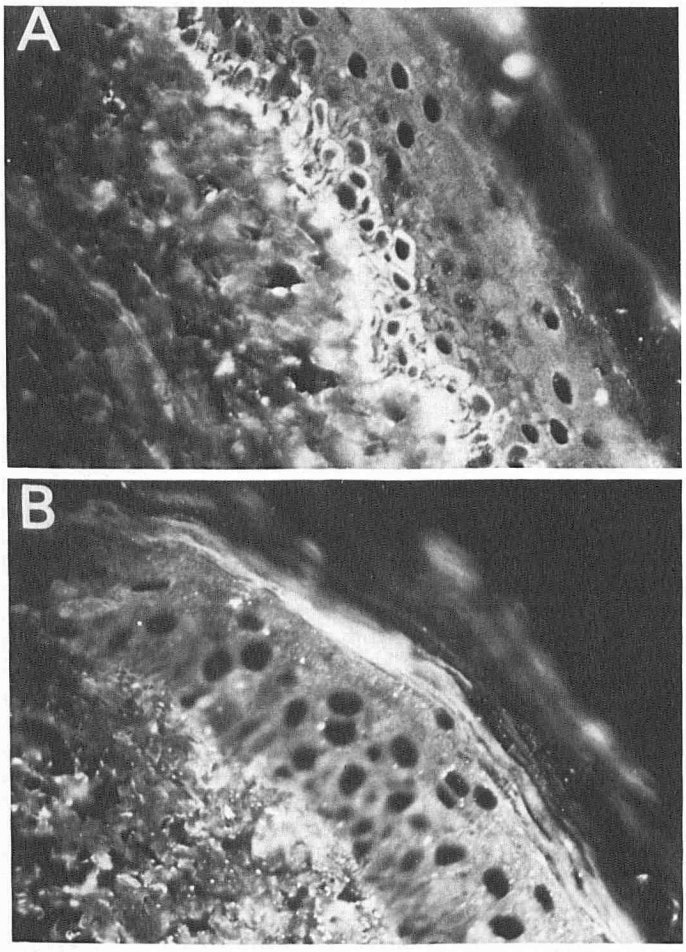

FIG 1. Indirect immunofluorescence of human skin. *A*, Using anti-PLMG rabbit serum diluted 1:100 with PBS/BSA as first antibody. *B*, Using nonimmune rabbit serum diluted 1:100 as first antibody. × 480.

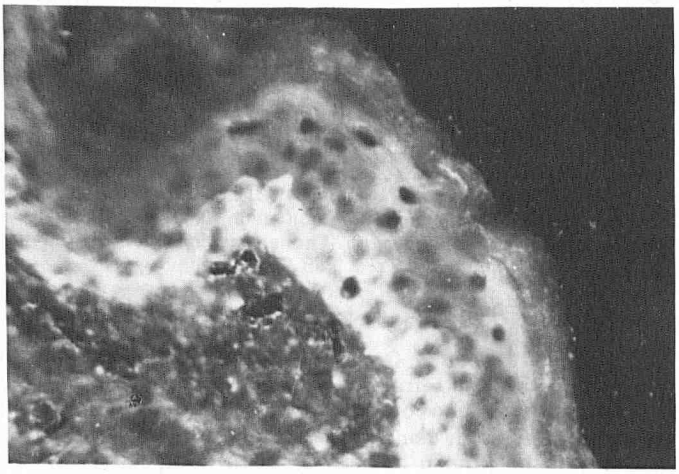

FIG 2. Indirect immunofluorescence of human skin using the IgG fraction of anti-PLMG serum diluted 1:100 with PBS/BSA. × 480.

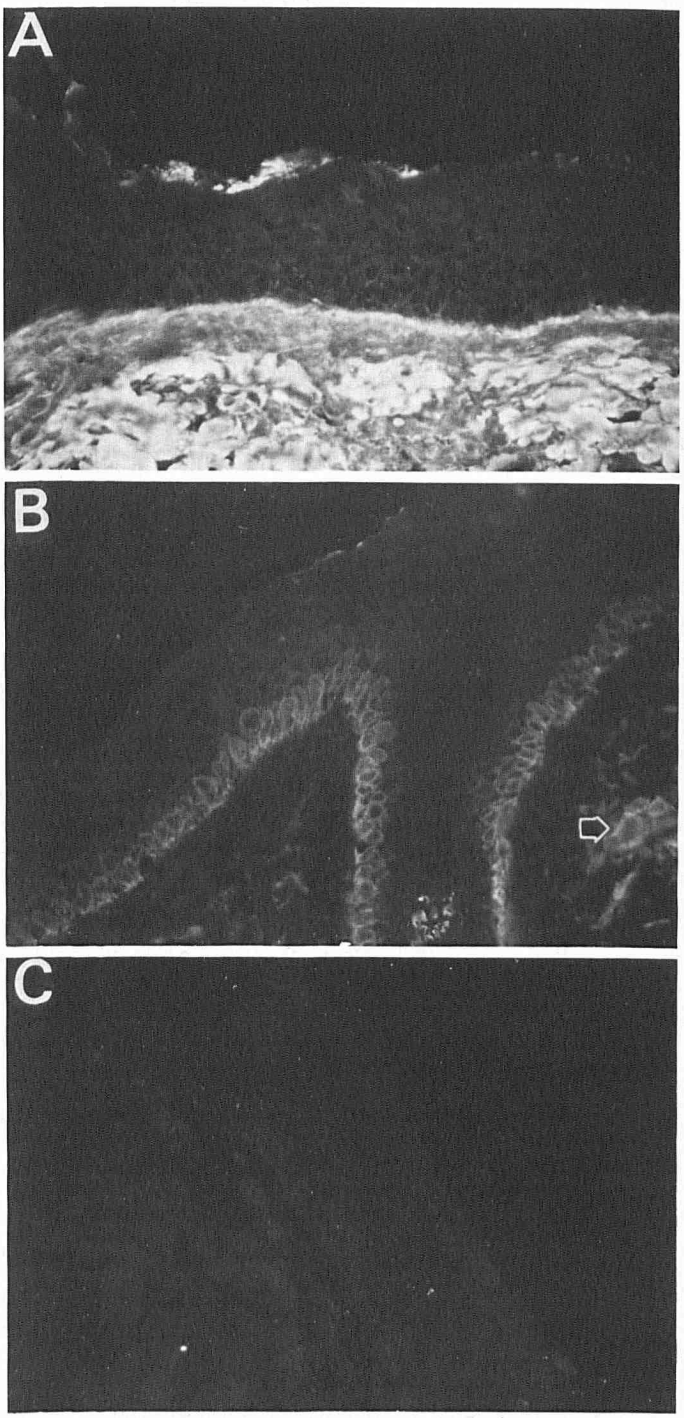

FIG 3. Indirect immunofluorescence demonstrating the different patterns observed when human skin was stained with: (*A*) immune-specific antialbumin IgG (100 μg/ml), (*B*) immune-specific anti-PLMG IgG (100 μg/ml), or (*C*) the control nonimmune IgG fraction (100 μg/ml) isolated from anti-PLMG serum as outlined in Materials and Methods. *Arrow* indicates bright annular area within dermis, representing dermal vessels. × 400.

firming the specificity of the previously observed pattern.

PLMG is localized to the peripheral intracytoplasmic area of the basal keratinocyte (Fig 4). This intracellular localization may suggest that PLMG is synthesized by the keratinocyte. Although it has been demonstrated that PLMG is synthesized by the liver [11], synthesis by other organs cannot be ruled out. Preliminary evidence, however, indicates that PLMG synthesis by human keratinocytes is unlikely, as no fluorescence can be demonstrated in vitro when cultured human keratinocytes are stained with anti-PLMG preparations (data not shown). Alternatively, and more likely, the observed intracytoplasmic staining may result from internalization of surface-bound PLMG. This may occur in vivo, or may be an artifact of freezing. These possibilities are currently being investigated.

The presence of PLMG within epidermal basal cells may be an important marker of the undifferentiated state. The bright basal fluorescence compared to the much fainter staining of the suprabasilar cells and total absence of staining within the upper layers of the epidermis suggest that PLMG is being consumed in the course of epidermal maturation. This finding supports the theories that plasmin, generated by the action of keratinocyte PA, plays a role in epidermal differentiation [1–3,12]. The exact nature of the ultimate epidermal substrate is not yet known and remains to be elucidated. Nevertheless, the demonstration of the zymogen PLMG within the epidermis is of key importance in any theory postulating a physiologic role for the keratinocyte protease PA. Additionally, the ability to identify basal cells with a commercially available and well-defined reagent, antihuman PLMG antiserum, may prove to be a valuable tool in other areas of epidermal research.

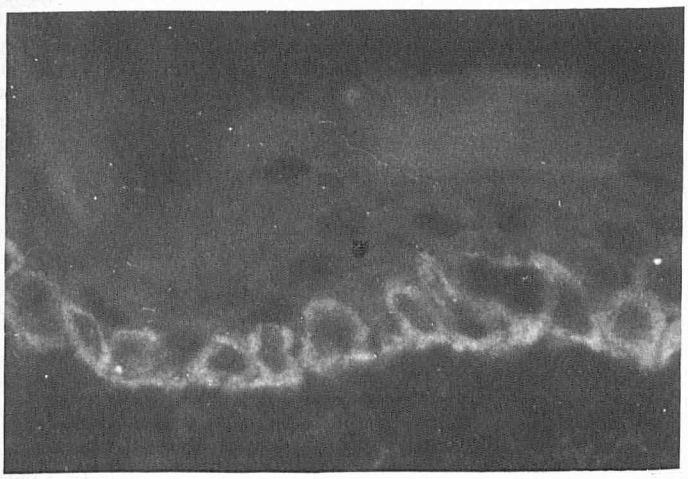

FIG 4. Photomicrograph demonstrating intracytoplasmic peripheral staining of basal keratinocytes when stained with immune-specific anti-PLMG IgG (70 μg/ml). × 756.

The careful secretarial skills of Carol Sweeten are gratefully acknowledged.

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