Lipocortin I (Annexin I) Is Preferentially Localized on the Plasma Membrane in Keratinocytes of Psoriatic Lesional Epidermis as Shown by Immunofluorescence Microscopy

Yasuo Kitajima, M. Koji Owada, Hiromi Mitsui, and Hideo Yaoita

Department of Dermatology, Jichi Medical School, Kawachigun, Tochigiken; Institute of Molecular and Cellular Biology for Pharmaceutical Sciences (MKO, HM), Kyoto Pharmaceutical University, Yamashina, Kyoto, Japan

Lipocortin I (LPC-I, also called annexin I) is a 35-kD protein that binds phospholipids and actin in a Ca^{++}-dependent manner. It is also a major substrate for EGF receptor/kinase and protein kinase C, and a putative inhibitor of phospholipase A_2, which produces chemical mediators to cause inflammation. Psoriasis (PS) is an inflammatory skin disease characterized by a rapid turnover of keratinocytes and a defect in keratinization with increased activities of phospholipase C and A_2, and EGF receptor. To understand the mechanism of the PS lesion formation and the function of LPC-I, its distribution was studied in the epidermis of PS, subacute eczema and normal skin, and in tumor cells of seborrheic keratosis and Bowen's disease. This study involved immunofluorescence and immunoblotting using affinity-purified polyclonal and monoclonal antibodies specific to LPC-I and to its Ca^{++}-bound form. In normal, nonlesional PS and subacute eczema epidermis, LPC-I was detected mainly in the cytoplasm of the suprabasal cells, although it was on the inner aspects of the plasma membrane in some parts of the granular layer. In lesional epidermis of PS, it was localized mainly on the inner aspects of the plasma membrane, but not in the cytoplasm of the whole suprabasal cells as the Ca^{++}-bound form, indicating a preferential localization on the plasma membrane. This membrane-binding of LPC-I was also observed in seborrheic keratosis, but not in Bowen's disease. These results suggest that the binding of LPC-I to the plasma membrane occurs actually in living cells, plays a role, not necessarily disease specific, in the PS lesion formation, and has some relevance to normal or abnormal differentiation of keratinocytes. *J Invest Dermatol 97:1032–1038, 1991*

Lipocortins are proteins that are known to have an inhibitory activity to phospholipase A_2 and to be involved in the regulation of inflammation [1]. Interest has been concentrated on two lipocortins: lipocortin I (also called annexin I), which has been found as a physiologic substrate for the epidermal growth factor (EGF) receptor/kinase [2,3], and lipocortin II (also called annexin II), a major substrate for $pp60^{v\text{-}src}$[4].

Lipocortin I, a putative glucocorticosteroid-induced inhibitor of phospholipase A_2 [1,5,6], is a protein with an apparent molecular weight of 35–36 kD [2,6,7], isolated first as a Ca^{++}-dependent substrate for EGF receptor/kinase from A-431 cells [7], and human placenta [8]. Recently, it has also been characterized as a substrate for protein kinase C [9–11]. This protein shows an ability to interact with phospholipids and actin in a Ca^{++}-dependent manner, so that it was also termed calpactin II [12].

The complete amino acid sequence of lipocortin I is now known [13]. This protein consists of two distinctive domains: a small amino (N-) terminal domain and a core domain that is formed by the fourfold repeat of a conserved 70-amino acid long segment. The N-terminal region is characteristic for each different lipocortin, but the core domain shows a 40–60% homology between lipocortins and contains the Ca^{++} and phospholipid binding sites [14,15].

Psoriasis vulgaris is an inflammatory skin disease characterized clinically by chronic relapsing erythematous plaques and papules with lamellated, fine, and silvery scales. Lesional epidermis shows a rapid keratinocyte turnover and a defect in keratinization. The hyperproliferation of psoriatic epidermis has been revealed to be associated with an increase in activities of phospholipase C [16,17] and phospholipase A_2 [18], which are thought to be involved in inflammatory processes. The lesional epidermis also shows an increase in activity of tyrosine kinase [19] and retaining of receptors for EGF in the suprabasal cells, where EGF receptors disappear in normal epidermis [20]. Furthermore, diacylglycerol (DG), one of the intracellular second messengers generated by phospholipase C, has been known to be increased in psoriatic epidermis as compared with normal controls [21], although the activity of protein kinase C, which is expected to be activated by DG [22], has been revealed to be decreased, possibly by downregulation [23].

These observations prompt us to study the involvement of lipocortin I in the mechanism of the formation of psoriatic lesions in epidermis, because lipocortin I is a major substrate for EGF receptor/tyrosine kinase and protein kinase C, both of which are altered in psoriatic epidermis, and is also suggested to have an inhibitory activity for phospholipase A_2, as mentioned above. On the other hand, the physiologic functions of lipocortin I are still unknown in the intact cells, although its biochemical characteristics in vitro have been elucidated. Therefore, the characterization of lipocortin I in

Manuscript received July 26, 1990; accepted for publication June 17, 1991.

This work was supported by a Grant-in-Aid for Science Research from the Ministry of Education (01480267), and a grant for 1989 from The Lydia O'Leary Memorial Foundation.

Reprint requests to: Dr. Yasuo Kitajima, Department of Dermatology, Jichi Medical School, Minamikawachimachi, Kawachigun Tochigiken, Japan 329-04.

0022-202X/91/$03.50

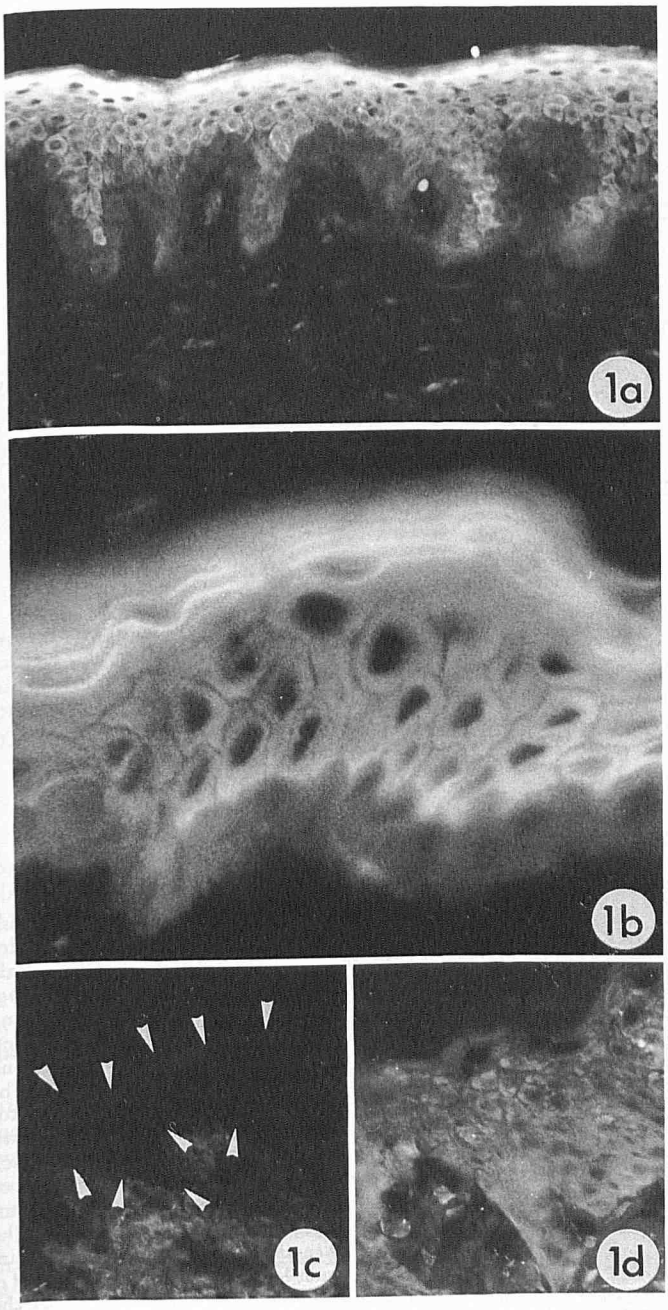

Figure 1. Immunofluorescence of normal human skin stained with monoclonal antibody, L1-MoAb (*a*), affinity-purified polyclonal antibody (*b*) specific to the whole molecule of lipocortin I, pre-absorbed monoclonal antibody, L1-MoAb (*c*), and non-absorbed L1-MoAb (*d*), which is the positive control for the pre-absorbed L1-MoAb (*c*). This positive control was prepared from the same culture medium of L1-MoAb without addition of purified lipocortin I and at the same time as the pre-absorbed antibody (see *Materials and Methods*). The whole suprabasal cells were stained in the cytoplasm, leaving unstained gaps between keratinocytes *(a,b)*. Some parts of skin showed a linear staining of the plasma membrane in the granular cells *(b)*. The basal layer generally reacted poorly with the antibody *(a,b)*, although in some cases it was stained as much as the surabasal cells *(d)*. The staining with pre-absorbed L1-MoAb showed a disappearance of fluorescence in the whole epidermis as shown between *arrowheads (c)*, suggesting that the fluorescence in the epidermis is specific to lipocortin I, including the weak fluorescence in the basal layer. Magnifications: *a*, ×300; *b*, ×1,300; *c* and *d*, ×500.

diseased cells seems to be one of the important approaches for understanding the function of this protein in vivo.

In the present study, affinity-purified polyclonal and monoclonal antibodies specific to lipocortin I, and a monoclonal antibody that binds specifically with the Ca^{++}-bound form of lipocortin I but not with the Ca^{++}-free form, were used to observe the distribution of this protein in epidermal cells of the skin of psoriasis vulgaris and controls—subacute eczema (an inflammatory skin disease), seborrheic keratosis (a benign epidermal tumor), Bowen's disease (a carcinoma in situ), and normal skin—by immunofluorescence microscopy and immunoblotting. We showed that most lipocortin I was localized as a Ca^{++}-bound form on the inner aspects of the plasma membrane, but not in the cytoplasm, in keratinocytes of psoriatic lesional skin, whereas this was not observed in normal and psoriatic non-lesional epidermis.

MATERIALS AND METHODS

Antibodies Polyclonal antibodies against lipocortin I, which were previously obtained by immunizing New Zealand white rabbits and affinity purified [24,25], were used over the range of 1/50 to 1/200 with positive results. Monoclonal antibodies against lipocortin I; L1-MoAb and L2-MoAb, which were also previously prepared [25–27], were used successfully at concentrations ranging from 10 μg/ml to 800 μg/ml in the present study. Monoclonal antibodies L1-MoAb, which reacts with lipocortin I, and L2-MoAb, which reacts specifically with a Ca^{++}-bound form, but not with the Ca^{++}-free form of lipocortin I, were prepared as described elsewhere [27]. The monoclonal antibody L2-MoAb requires at least 10 μM Ca^{++} in the reaction medium to bind lipocortin I, and does not bind lipocortin I in Ca^{++}-free conditions [27]. The specificity of these antibodies for lipocortin I was proved in previous studies [24–27]. The second antibodies, fluorescein isothiocyanate (FITC)-labeled goat anti-mouse IgG and alkaline phosphatase-labeled goat anti-rabbit IgG, were purchased from Cappel Laboratories (West Chester, PA). As negative controls, non-immunized rabbit serum, mouse IgG, and B27M2-ascites control (mouse IgG purified from ascites induced by SP2/0 myeloma cells in BALB/C mice) were purchased from Tago Inc. (Burlingame, CA), Zymed Laboratories, Inc. (San Francisco, CA), and Cappel Laboratories (West Chester, PA), respectively. They were used at the same range of concentrations as were used in the experiments with antibodies to lipocortin I. Preabsorbed control antibody solutions were prepared. Three hundred microliters of the culture medium of a hybridoma of L1-MoAb containing 3 μg of protein were mixed with 30 μl of a solution containing 45 μg of the same purified lipocortin I as that previously reported [8], and incubated for 1 h at 4°C. In addition, another 300 μl of this hybridoma culture medium was mixed with 30 μl of phosphate-buffered saline without lipocortin I and incubated for 1 h at 4°C. Both antibody solutions (culture medium) were used on serial sections of normal and psoriatic skin so as to check the binding specificity of the antibody to lipocortin I in the tissues.

Biopsy Specimens and Immunofluorescence Staining Procedures Biopsy specimens were taken from lesional skin including non-lesional sites of 13 patients with psoriasis vulgaris, three cases of subacute eczema, three cases of Bowen's disease, and three cases of seborrheic keratosis. Normal human skin was obtained from perilesional sites of benign skin tumors. The specimens were frozen in optimal cutting temperature compound, and used within 2 weeks. Four-micrometer sections were fixed with cold acetone (−20°C) or cold methanol (−20°C) or 10% formalin for 7–10 min and were stained for anti-lipocortin I antibodies. The sections were incubated with the first antibodies for 45 min at room temperature, followed by several washes in PBS, stained with the second antibodies labeled with FITC. The same procedures were employed for the negative controls (non-immunized rabbit serum, mouse IgG, B27M2-ascites control, and pre-absorbed antibody).

Immunoblotting The biopsied skin was cut into small pieces (5 × 5 mm) after fat tissues were removed, and incubated in a solu-

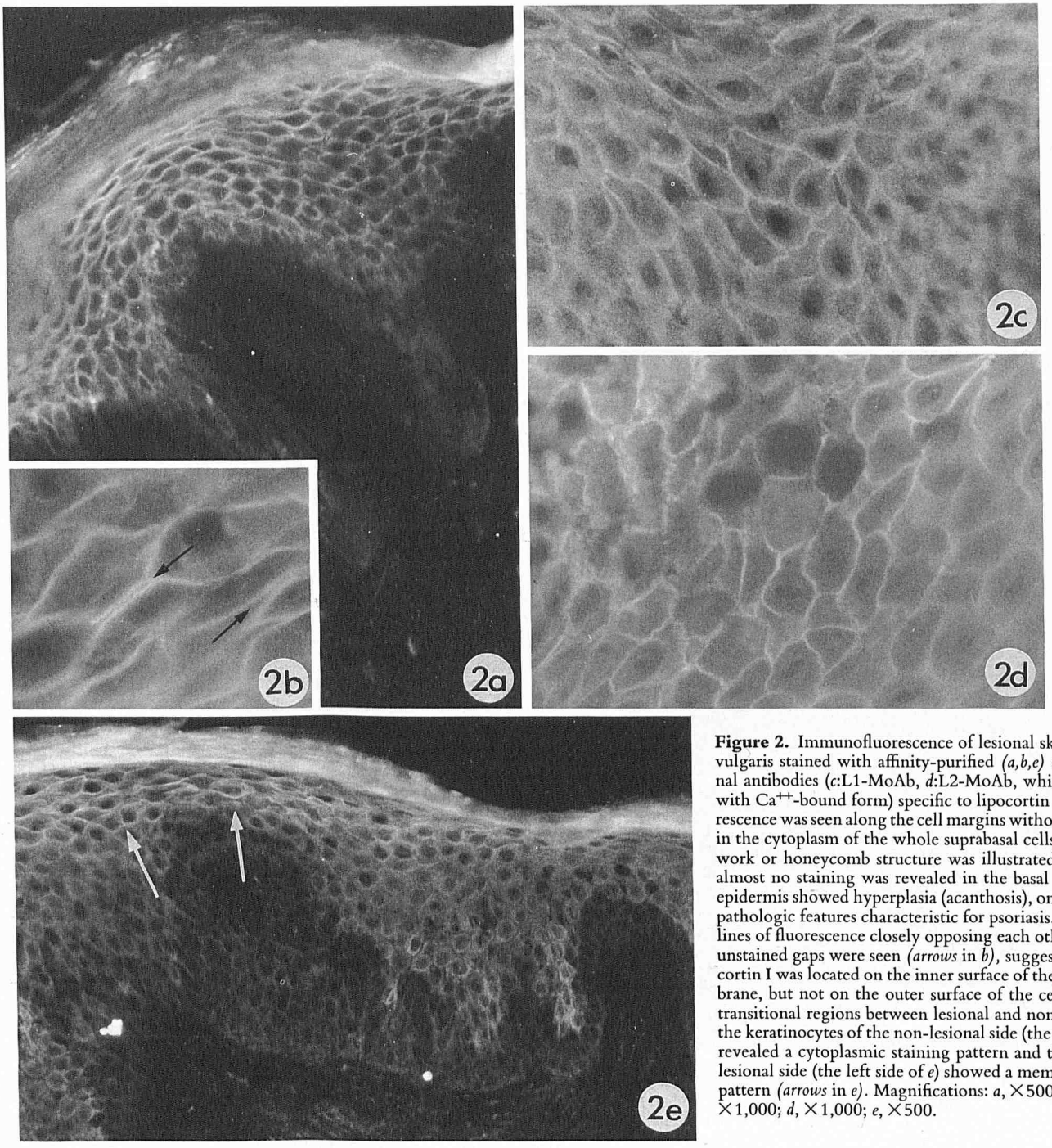

Figure 2. Immunofluorescence of lesional skin of psoriasis vulgaris stained with affinity-purified *(a,b,e)* and monoclonal antibodies (*c*:L1-MoAb, *d*:L2-MoAb, which reacts only with Ca^{++}-bound form) specific to lipocortin I. Linear fluorescence was seen along the cell margins without the staining in the cytoplasm of the whole suprabasal cells so that a network or honeycomb structure was illustrated *(a,b,c,d)*, and almost no staining was revealed in the basal cells *(a)*. The epidermis showed hyperplasia (acanthosis), one of the histopathologic features characteristic for psoriasis. Between two lines of fluorescence closely opposing each other, thin black unstained gaps were seen *(arrows* in *b)*, suggesting that lipocortin I was located on the inner surface of the plasma membrane, but not on the outer surface of the cells *(b)*. At the transitional regions between lesional and non-lesional skin, the keratinocytes of the non-lesional side (the right side of *e*) revealed a cytoplasmic staining pattern and the cells of the lesional side (the left side of *e*) showed a membrane staining pattern *(arrows* in *e)*. Magnifications: *a*, ×500; *b*, ×2,000; *c*, ×1,000; *d*, ×1,000; *e*, ×500.

tion of 5000 units/ml of dispase (Godo Shusei Co., Mastudo, Chiba, Japan) for 5 min at 37°C, so that the epidermis could be easily separated from the dermis. The epidermal sheets were treated with 0.25% trypsin containing 0.02% ethylenediamine tetraacetic acid sodium salt (EDTA) for 5–10 min, and free keratinocytes were obtained after removing cornified cells by filtering the suspension through a steel mesh. Cells (1×10^6) were suspended in 1 ml sample buffer (pH 6.8) containing 0.25 M Tris, 30% glycerol 10% 2 β -mercaptoethanol, and 2% sodium dodecyl sulfate (SDS) and solubilized by boiling at 98°C for 3 min. After 10 μl of this solution was subjected to SDS-polyacrylamide gel electrophoresis, the proteins were transfered to nitrocellulose paper. The paper was incubated with affinity-purified (1/200) and monoclonal (L1-MoAb, 50 μg/ml) antibodies to lipocortin I and control serum and IgG at appropriate concentrations, and visualized by antibodies conjugated with alkaline phosphatase.

RESULTS

Immunofluorescence Microscopy The fixation with cold acetone and cold methanol yielded positive results for the staining with both affinity-purified polyclonal and monoclonal (L1-MoAb and L2-MoAb) antibodies. However, after formalin fixation the antigenicity for monoclonal L2-MoAb antibody against the Ca^{++}-bound form of lipocortin I was not retained, although that for affinity-purified and monoclonal L1-MoAb antibodies was kept.

Normal and psoriatic non-lesional epidermis incubated with monoclonal (L1-MoAb, Fig 1*a*) and affinity-purified polyclonal (Fig 1*b*) antibodies to lipocortin I showed a strong positive staining

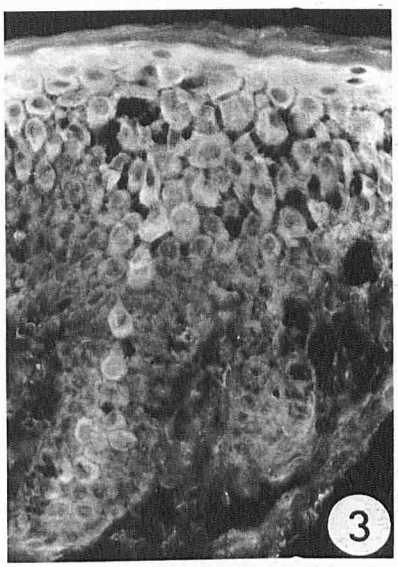

Figure 3. Immunofluorescence of the lesional skin of subacute eczema stained with an affinity-purified antibody specific to lipocortin I. A cytoplasmic staining pattern was seen, but not a membrane staining pattern, in the epidermal keratinocytes, which showed intercellular edema (spongiosis) and hyperplasia (acanthosis) characteristic of eczema. Magnification ×500.

in the cytoplasm of the suprabasal cells (Fig 1*a,b*), leaving unstained black thin gaps between the cells (Fig 1*b*). In some areas of epidermis, the linear staining on the plasma membranes was detected in the granular layers (Fig 1*b*). The basal cells in most cases were faintly positive or negligible (Fig 1*a,b*), but in some cases they were stained as much as suprabasal cells (Fig 1*d*). The same results were obtained by using the monoclonal antibody (L2-MoAb) to the Ca^{++}-bound form of lipocortin I (not shown).

In psoriatic lesional skin, the reactivity to antibodies against lipocortin I, including the Ca^{++}-binding form, was seen on the plasma membrane of the whole suprabasal cells, whereas almost no reactivity was detected in the cytoplasm, so that a staining pattern like a network or a honeycomb structure was generated, as seen in Fig 2. At a higher magnification, linear unstained gaps were also seen between two lines of fluorescence (Fig 2*b*), indicating that lipocortin I was located on the cytosolic face of the plasma membrane, but not in the extracellular space. The basal cells were almost negative for the antibodies in both the plasma membrane and the cytoplasm (Fig 2*a*). In the border areas between the lesional and non-lesional skin, there was a transitional staining; a membrane pattern in the cells in the lesional side and a cytoplasmic pattern in the cells in the non-lesional side were observed (Fig 2*e*).

In subacute eczema, the staining for lipocortin I was mainly localized in the cytoplasm of the suprabasal keratinocytes in the epidermis and was accompanied by intercellular edema (spongiosis) and hyperplasia (acanthosis), characteristic for eczema (Fig 3).

In the tumor cells of seborrheic keratosis, membrane staining was seen; this was especially prominent in the flattened keratinocytes just before changing to horny cells in the areas forming pseudohorn cysts (Fig 4). However, in Bowen's disease, the tumor cells showed a cytoplasmic, but not membranous, staining with anti-lipocortin I antibodies (Fig 5).

Incubation with each of the controls (non-immunized rabbit serum, mouse IgG, B27M2-ascites control, and pre-absorbed L1-MoAb) gave negative results on all normal and diseased skins used in the present study. Serial sections of normal and psoriatic skin that were stained with pre-absorbed L1-MoAb antibody showed negative results in whole epidermis, although a faint fluorescence was

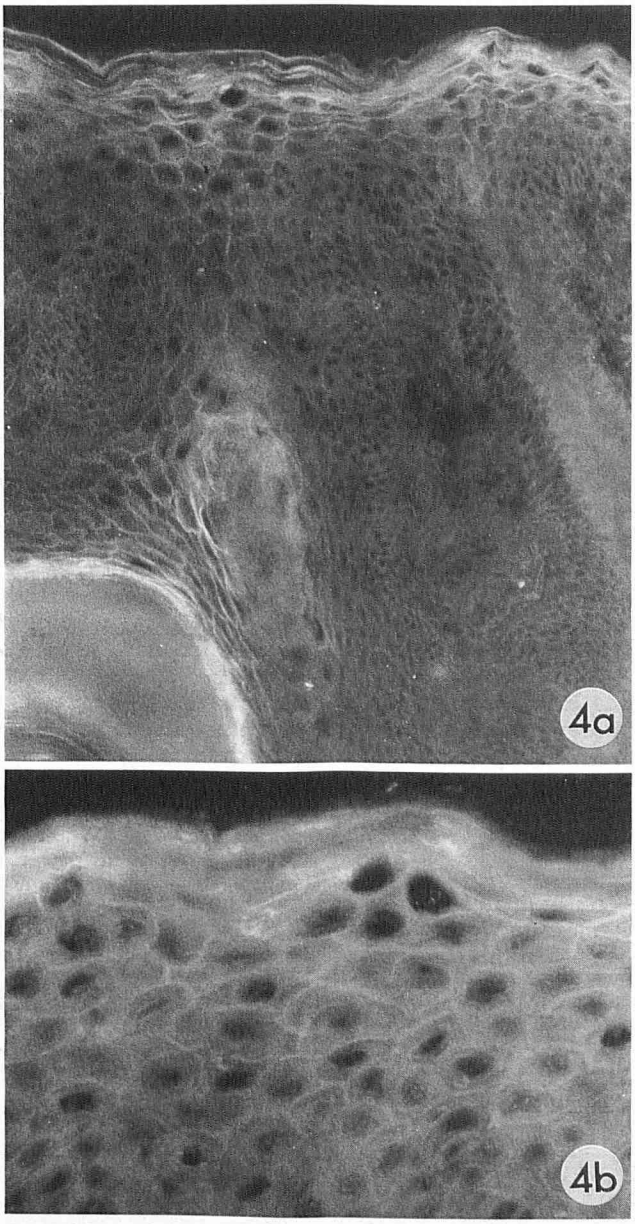

Figure 4. Immunofluorescence of seborrheic keratosis, a benign epidermal tumor, stained with an affinity-purified antibody *(a)* and monoclonal antibody, L1-MoAb *(b)*, specific to lipocortin I. A membranous staining was produced in the larger tumor cells located at closer positions to the tumor surface and pseudohorn cysts. The smaller cells located closely to the matrix (dermis) showed a poor or no staining. Magnifications: *a*, ×600; *b*, 1,200.

retained in the dermis (Figs 1*c*, 6*a*). However, non-absorbed L1-MoAb (positive control for pre-absorbed antibody) showed positive results, revealing a cytoplasmic pattern in normal epidermis (Fig 1*d*) and a membrane pattern in psoriatic epidermis (Fig 6*b*).

Immunoblotting Isolated keratinocytes were suspended in a sample buffer for SDS-PAGE (10^6 cell/ml) and solubilized by heating at 98°C for 3 min, then subjected to electrophoresis and immunoblotting. Both samples from normal and psoriatic lesional skins showed the same blotting pattern, featuring a single main peptide band with a molecular weight of 35 kD and a faint band at 32 kD, when blots were reacted with affinity-purified and monoclo-

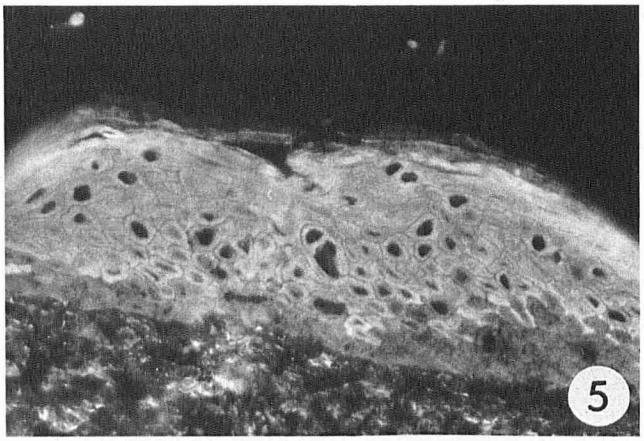

Figure 5. Immunofluorescence of Bowen's disease, an epidermal tumor of carcinoma in situ, stained with an affinity-purified antibody specific to lipocortin I. Tumor cells showed a cytoplasmic staining pattern without staining in the cells at one or two layers next to the dermis. Magnification ×600.

nal (L1-MoAb) anti-lipocortin I antibodies (Fig 7). A trace of another peptide band of 54 kD was detected by the affinity-purified antibody (Fig 7, column A,B), but not by the monoclonal antibody, L1-MoAb (Fig 7, column C).

DISCUSSION

The present immunofluorescence study using three different antibodies (an affinity-purified polyclonal and two monoclonal antibodies) to lipocortin I (annexin I) showed that lipocortin I was located on the plasma membrane of keratinocytes in psoriatic lesional epidermis but not in the cytoplasm, whereas it was located mainly in

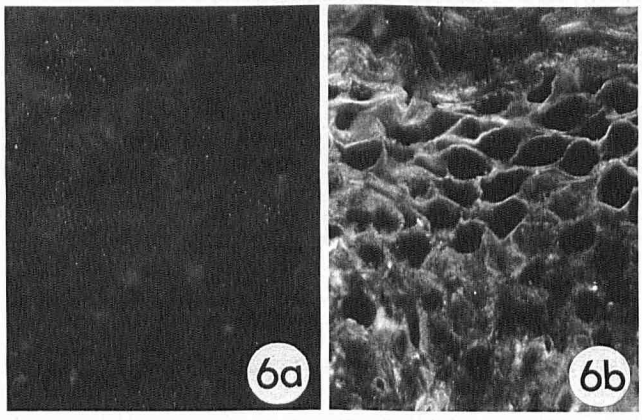

Figure 6. Psoriatic lesional epidermis (control experiments) stained with preabsorbed monoclonal antibody (L1-MoAb) *(a)* and non-absorbed L1-MoAb *(b)*. This is the positive control for the preabsorbed L1-MoAb *(a)*. This positive control was prepared from the same culture medium of L1-MoAb without addition of purified lipocortin I and at the same time as the preabsorbed antibody (see *Materials and Methods*). Preabsorbed antibody showed no reaction in psoriatic lesional epidermis *(a)*, but the positive control showed the membrane staining pattern *(b)* as shown in Fig 2, suggesting that this staining pattern is specific to lipocortin I. Magnifications: *a* and *b*, ×800.

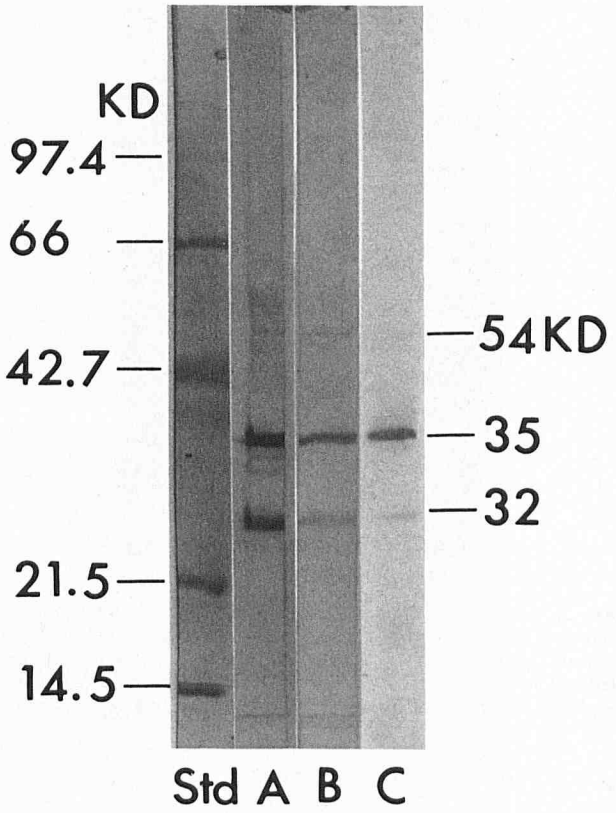

Figure 7. Western blotting of cellular extracts of normal and psoriatic epidermal keratinocytes with affinity-purified (columns *A, B*) and monoclonal antibody, L1-MoAb (column *C*), specific to lipocortin I. A main peptide with 35 kD of molecular weight was found in both of normal and psoriatic samples by affinity-purified (columns *A, B*) and monoclonal antibody (column *C*). A peptide of 32 kD was also detected by these antibodies (columns *A, B,* and *C*). Another trace peptide band of 54 kD was detected when reacted with affinity-purified antibody (columns *A, B*), but not with monoclonal antibody (column *C*). Std; molecular standard, A; specimen from normal skin; B and C, specimens from psoriatic lesional skin.

the cytoplasm of suprabasal keratinocytes in normal and psoriatic nonlesional skin. At the transitional regions between lesional and non-lesional skin, cells on the lesional side formed a membrane staining pattern and cells in the non-lesional side generated cytoplasmic staining. These observations suggest that lipocortin I was translocated from the cytoplasm to the plasma membrane in keratinocytes as the psoriatic lesions were formed.

Concerning the distribution of lipocortin I in normal skin, Fava et al reported recently that lipocortin I was readily detected in basal keratinocytes in rat, porcine, and human skin, and the ductal epithelium of apocrine sweat gland in porcine skin and in the eccrine sweat gland in human skin [28]. Our results in the present study that showed the localization of lipocortin I mainly in suprabasal cells in normal human skin are in conflict with their results showing its localization in basal cells in normal human skin. In this regard, our results, as shown in Fig 1*b*, demonstrated a faint positive staining, which upon first inspection appear to be negative because the staining of the suprabasal cells is very strong. The staining with preabsorbed antibody L1-MoAb showed a complete disappearance of fluorescence in whole epidermis, but showed some faint fluorescence in the dermis, indicating that the fluorescence in the epidermis, including that in the basal cells, was specific for lipocortin I, but that in the dermis was not. Actually, in some cases of normal skin, the basal cells showed positive fluorescence as positive as that in the

suprabasal cells by L1-MoAb staining. This suggests that the amount of lipocortin I in most cases is much smaller in the basal cells than in the suprabasal cells or that the epitopes for the antibody are masked in the basal cells but exposed in the suprabasal cells, possibly due to conformational changes. This observation may also explain the reason for the conflicting results on normal human epidermis between Fava's groups and ours. Alternatively, the epitopes of the antibodies used in the two laboratories may be different. Further studies are required to detemine whether the amount or the conformation of lipocortin I differs between the basal cells and the suprabasal cells.

Lipocortin I is one of several proteins known to undergo reversible Ca^{++}-dependent binding to phospholipid liposomes [29] and cellular membranes [30]. By using a monoclonal antibody (L2-MoAb), which specifically reacts with the Ca^{++}-bound form, but not with the Ca^{++}-free form of lipocortin I, it was shown that membrane-bound lipocortin I in the psoriatic lesional keratinocytes was the Ca^{++}-bound form. This suggests that Ca^{++} may play a key role in the translocation of lipocortin I from the cytoplasm to the plasma membrane. Ten micrometers Ca^{++} is required for lipocortin I to bind the L2-MoAb antibody, as described in *Materials and Methods*. This concentration of Ca^{++} is much higher than the basic Ca^{++} level of the cytoplasm [31]. In psoriasis, phospholipase C is activated, which may cause an increase in inositol trisphosphate and the Ca^{++} concentration in the cytoplasm [17], so that enough Ca^{++} to enable lipocortin I to be bound to the plasma membrane may be supplied.

Lipocortin I is a major substrate for EGF-receptor/tyrosine kinase and protein kinase C. It has been recently shown that the lipocortin I Ca^{++} requirement was reduced by five times when the protein was phosphorylated at Tyr-21 [15]. In this connection, it may be of interest also to note that psoriatic epidermis shows a retention of EGF receptors in suprabasal cells, whereas they disappear in normal skin [20].

The membrane-binding pattern of lipocortin I was also observed in some portions of the granular cells in normal skin and in tumor keratinocytes just before keratinizing to the horny cells at pseudohorn cysts in seborrheic keratosis, a benign epidermal tumor showing orthokeratosis. These findings indicate that the membrane binding of lipocortin I is not specific for keratinocytes in psoriasis, and may associate with some aspects of normal and abnormal keratinization. In this regard, lipocortin I has recently been shown to be a good substrate for tissue transglutaminase in human epidermoid carcinoma A431 cells [32]. Tissue transglutaminase is an intracellular, Ca^{++}-dependent enzyme contained in human keratinocytes and cross-links proteins via ϵ-(γ-glutamyl) lysine isopeptide bonds to form cornified envelope [33,34]. When lipocortin I was incubated with this enzyme, it formed cross-linked multimers, and this resulted in a remarkable enhancement of Ca^{++} sensitivity for phospholipid binding [32]. This suggests that this enhancement of Ca^{++} sensitivity may be involved in the formation of membrane-bound lipocortin I.

Lipocortin I has an inhibitory activity towards phospholipase A_2, which produces chemical mediators to cause inflammation. Psoriasis is an inflammatory disease associated with increased phospholipase A_2 activity and an abnormal keratinization featuring hyperplasia of the epidermis. Because phosphorylation of lipocortin I has been suggested to suppress its phospholipase A_2 inhibitory activity [35], it is feasible that the increased activity of phospholipase A_2 in psoriatic skin may be related with the formation of membrane-bound lipocortin I, which may have been phosphorylated by increased EGF receptor/tyrosine kinase.

As one of the disease controls, we checked the localization of lipocortin I in a typical inflammatory disease involving epidermis: subacute eczema, which is characterized by acanthosis (hyperplasia of epidermis) and intercellular edema with lymphocytic infiltration. In this disease, lipocortin I was retained in the cytoplasm of keratinocytes in epidermis. Taking the results on psoriasis into consideration, this suggests that there may be some substantial differences in the mode of involvement of lipocortin I in the formation of lesions between these two inflammatory diseases.

Bowen's disease is a squamous carcinoma in situ. Lipocortin I was localized in the cytoplasm, but not on the plasma membrane, of the tumor cells, although cells located at one or two layers from the basal layer showed an almost negative result, being like normal epidermis. This suggests that translocation of lipocortin I is not associated with the transformation of keratinocytes. More cases of Bowen's disease and metastatic squamous cell carcinoma should be examined to confirm and to understand the results.

By immunoblotting, it was shown that normal and psoriatic human epidermal keratinocytes contain lipocortin I and that there are no differences in the molecular size of lipocortin I between normal, nonlesional, and lesional epidermis. Beside the main 35-kD peptide of lipocortin I, two minor peptides were detected by immunoblotting. The peptide with a smaller molecular weight may be a breakdown product, as the detectable intensity of this peptide band was variable between specimens and periods of storage. Because the 54-kD peptide was detected, although faintly, with affinity-purified polyclonal antibody, it may be some undetermined protein related to lipocortin I or a new member of the lipocortin family. However, it may be some other substance rather than lipocortin I, as it was not detected with the monoclonal antibody L1-MoAb. It is still conceivable that the epitope for L1-MoAb may be masked or absent in this 54-kD peptide.

The peptides larger and smaller than 35 kD lipocortin I both appeared in normal and psoriatic lesional epidermal cells, indicating that they may not be related to the diseased conditions in psoriasis.

Although lipocortin I is well understood biochemically and structurally through in vitro study, almost nothing about the physiologic functions has been elucidated in vivo. Recently, however, it was suggested that lipocortin I may play a role in mediating the effects of glucocorticoids on epidermal differentiation [36]. The present study provides more new information concerning the function of lipocortin I in vivo, using epidermal cell and tissues of patients with skin diseases. It is suggested that this protein translocates from the cytosol to the inner face of the plasma membrane, possibly to bind phospholipids in living keratinocytes, probably via a Ca^{++}-dependent manner, as it does in vitro. This translocation may be one of the biologic events involved in some aspects of normal or abnormal differentiation of epidermal keratinocytes.

We thank Miss N. Ishizaki and Miss Y. Hamada for their technical assistance, and Professor Guy A. Thompson Jr., Department of Botany, University of Texas, for reviewing the manuscript.

REFERENCES

1. Flower RJ, Wood JN, Parente L: Macrocortin and the mechanism of action of the glucocorticoids. Ad Inflam Res 7:61–69, 1984
2. De BK, Misono KS, Lukas TJ, Mroczkowski B, Cohen S: A calcium-dependent 35 kilodalton substrate for epidermal growth factor receptor/kinase isolated from normal tissue. J Biol Chem 261:13784–13792, 1986
3. Pepinsky RB, Sinclair LK: Epidermal growth factor dependent phosphorylation of lipocortin. Nature (Lond) 321:81–84, 1986
4. Huang KS, Wallner BP, Mattaliano RJ, Tizard R, Burne C, Frey A, Hession C, McGray R, Sinclair K, Chow EP, Browing JL, Ramachandra KL, Tang J, Smart JE, Pepinsky RB: Two human 35kd inhibitors of phospholipase A_2 are related to substrates of $pp60^{v\text{-}src}$ and of the epidermal growth factor receptor/kinase. Cell 46:191–199, 1986
5. Hirata H: The regulation of lipomodulin, a phospholipase inhibitory protein, in rabbit neutrophils by phosphorylation. J Biol Chem 256:7730–7733, 1981
6. Sarias CJM, Tack BF, Kristensen T, Glenney JR, Hunter T: The cDNA sequence for the protein-tyrosine kinase substrate p36 (cal-

pactin I heavy chain) reveals a multidomain protein with internal repeats. Cell 46:201–212, 1986

7. Fava RA, Cohen S: Isolation of a calcium-dependent 35-kilodalton substrate for the epidermal growth factor receptor/kinase from A-431 cells. J Biol Chem 259:2636–2645, 1984
8. Hayashi H, Sonobe S, Owada MK, Kakunaga T: Isolation and characterization of three forms of 36-kDa Ca^{2+}-dependent actin- and phospholipid-binding proteins from human placenta membrane. Biochem Biophys Res Commun 146:912–919, 1987
9. Michener ML, Dawson WB, Creutz CE: Phosphorylation of a chromaffin granule-binding protein in stimulated chromaffin cells. J Biol Chem 261:6548–6555, 1986
10. Schlaepfer DD, Haigler HT: In vitro protein kinase C phosphorylation sites of placental lipocortin. Biochemistry 27:4253–4258, 1988
11. Campos-Gonzalez R, Kanemitsu M, Boynton AL: Tumor promoter-dependent phosphorlyation of a triton X-100 extractable form of lipocortin I in T51B rat liver cells. Exp Cell Res 184:287–296, 1989
12. Glenney JR Jr: Two related but distinct forms of the 36,000M_r tyrosine kinase substrate (calpactins) which interact with phospholipid and actin in a Ca^{2+}-dependent manner. Proc Natl Acad Sci USA 83:4258–4262, 1986
13. Wallner BP, Matterlino RJ, Hession C, Cate RA, Tizard R, Sinclair LK, Foeller C, Chow EP, Browning LJ, Ramachandran KL, Pepinsky RB: Cloning and expression of human lipocortin, a phospholipase A_2 inhibitor with potential anti-inflammatory activity. Nature (London) 320:77–81, 1986
14. Glenney JR: Phospholipid dependent Ca^{2+}-binding by the 36kd tyrosine kinase substrate (calpactin) and its 33kd core. J Biol Chem 261:7247–7252, 1986
15. Schlaepfer DD, Haigler HT: Characterization of Ca^{2+}-dependent phospholipid binding and phosphorylation of lipocortin I. J Biol Chem 262:6931–6937, 1987
16. Ziboh V, Casebolt TL, Marcelo CL, Duella EA, Anderson TF, Voorhees JJ: Biosynthesis of lipoxigenase products by enzyme preparations from normal and psoriatic skin. J Invest Dermatol 83:426–430, 1984
17. Bartel RA, Marcelo CL, Voorhees JJ: Partial characterization of phospholipase C activity in normal, psoriatic uninvolved and lesional epidermis. J Invest Dermatol 88:447–451, 1987
18. Foster S, Ilderton E, Norris JFB, Summerly R, Yardly HJ: Characterization and activity of phospholipase A_2 in normal human epidermis and in lesion-free epidermis of patients with psoriasis and eczema. Br J Dermatol 112:135–147, 1985
19. Gentleman S, Martensen TM, Digiovanna JJ, Chader GJ: Protein tyrosine kinase and protein phosphotyrosine phosphatase in normal and psoriatic skin. Biochim Biophys Acta 798:53–59, 1984
20. Nanney LB, Stocheck CM, Magid M, King LE: Altered [^{125}I] epidermal growth factor binding and receptor distribution in psoriasis. J Invest Dermatol 86:260–265, 1986
21. Talwar H, Fisher GJ, Voorhees JJ: $_{sn}$-1,2-diacyl glycerol is increased in psoriatic involved versus uninvolved and normal epidermis (abstr). J Invest Dermatol 90:612, 1988
22. Nishizuka Y: The role of protein kinase C in cell surface signal transduction and tumor promotion. Nature (Lond) 308:693–698, 1984
23. Horn F, Marks F, Fisher GJ, Marcelo CL, Voorhees JJ: Decreased protein kinase C activity in psoriatic versus normal epidermis. J Invest Dermatol 88:220–222, 1987
24. Hayashi H, Owada MK, Sonobe S, Kakunaga T, Kawakatsu H, Yano J: A 32-kDa protein associated with phospholipase A_2-inhibitory activity from human placenta. FEBS Letters 223:267–272, 1987
25. Hayashi H, Owada MK, Sonobe S, Kakunaga T: Characterization of two distinct Ca^{2+}-dependent phospholipid-binding proteins of 68-kDa isolated form human placenta. J Biol Chem 264:17222–17230, 1989
26. Ando Y, Imamura S, Hong Y-M, Owada MK, Kakunaga T, Kannagi R: Enhancement of calcium sensitivity of lipocortin I in phospholipid binding induced by limited proteolysis and phosphorylation at the amino terminus as analyzed by phospholipid affinity column chromatography. J Biol Chem 264:6948–6955, 1989
27. Hayashi H, Owada MK, Sonobe S, Doma K, Yamanouchi T, Kakunaga T, Kitajima Y, Yaoita H: Monoclonal antibodies specific to a Ca^{2+}-dependent phospholipid-binding ability from its ability to inhibit phospholipase A_2. Biochem J 269:709–715, 1990
28. Fava RA, McKanna J, Cohen S: Lipocortin I (p35) is abundant in a restricted number of differentiated cell types in adult organs. J Cell Physiol 141:284–293, 1989
29. Glenny JR Jr, Tack B, Powell MA: Calpactins: two distinct Ca^{2+}-regulated phospholipid- and actin-binding proteins isolated from lung and placenta. J Cell Biol 104:503–511, 1987
30. Krestsinger RH, Creutz CE: Consensus in exocytosis. Nature (Lond) 320:573, 1986
31. Sharpe GR, Gillespie JI, Greenwell JR: An increase in intracellular free calcium is an early event during differentiation of cultured human keratinocytes. FEBS Letters 254:25–28, 1989
32. Ando Y, Imamura S, Owada MK, Kakunaga T, Kannagi R: Cross-linking of lipocortin I and enhancement of its Ca^{2+} sensitivity by tissue transglutaminase. Biochem Biophys Res Commun 163:944–951, 1989
33. Thacker SM, Rice RH: Keratinocyte-specific transglutaminase of cultured human epidermal cells: relation to cross-liked envelope formation and terminal differentiation. Cell 40:685–695, 1984
34. Schmidt R, Michel S, Schroot B, Reichert U: Transglutaminases in normal and transformed human keratinocytes in culture. J Invest Dermatol 90:475–479, 1988
35. Hirata F, Matsuda K, Notsu Y, Hattori T, del Carmine R: Phosphorylation at a tyrosine residue of lipomodulin in mitogen-stimulated murine thymocytes. Proc Natl Acad Sci USA 81:4717–4721, 1984
36. Violette SM, King I, Browning JL, Pepinsky RB, Wallner BP, Sartorelli AC: Role of lipocortin I in the glucocorticoid induction of the terminal differentiation of a human squamous carcinoma. J Cell Physiol 142:70–77, 1990