Expression of Protein Kinase C Isozyme in Human Langerhans' Cells

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Protein kinase C is a key molecule controlling signal transduction into the cell. We recently reported that protein kinase C II isozyme, but neither I nor III isozyme, was expressed in epidermal Langerhans' cells of the adult mouse, and that none of these isozymes was detected in keratinocytes. In this study, we examined the expression of protein kinase C isozymes in human Langerhans' cells in vivo to see whether the expression of protein kinase C II isozyme in Langerhans' cells is a mouse-specific trait. Immunohistochemical studies revealed that protein kinase C II isozyme, but neither I nor III isozyme, was expressed in epidermal Langerhans' cells. None of these isozymes was detected in keratinocytes. These results suggest that the expression of protein kinase C II isozyme in epidermal Langerhans' cells in vivo is not a mouse-specific trait and that protein kinase C II isozyme is a novel phenotypic marker for epidermal Langerhans' cells in human as well as mouse skin.
Key words: Epidermis; Signal transduction; Monoclonal antibody; Immunohistochemistry.

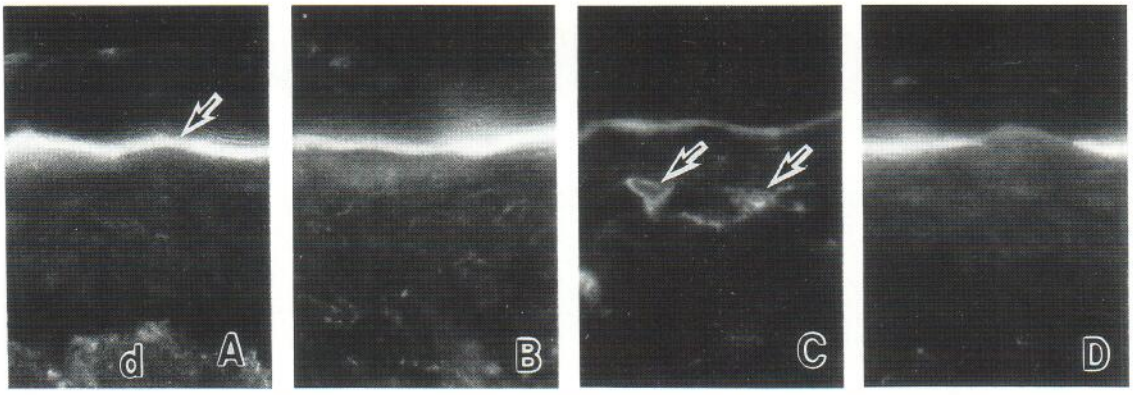

Fig. 1. Immunofluorescence staining of PKC isozymes in normal human skin. Frozen sections were stained using control McAb (A), MC-1a (B), MC-2a (C), and MC-3a (D) as the first antibody. Non-specific reactions were observed in the stratum licidum (Fig. 1A, *arrow*) and in the dermis (Fig. 1A, *d*). In the epidermis, MC-2a-positive dendritic cells were observed (Fig. 1C, *arrows*). None of the isozyme was detected in keratinocytes (×640).

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Protein kinase C (PKC) is a Ca^{2+}- and phospholipid-dependent protein kinase, which transduces various extracellular signals into the cell (1). PKC is encoded by a gene family, and its multiple isozymes are expressed in various mammalian tissues (2–5). PKC activity was detected in established lines of keratinocytes (6, 7), primarily cultivated keratinocytes (7), and whole epidermal cells (8–11). However, these observations do not prove that PKC is expressed in keratinocytes in vivo, since (*a*) cultivation in vitro may have led to the expression of PKC in keratinocytes; and (*b*) the epidermis contains not only keratinocytes but also several other types of cells such as melanocytes, Merkel cells, Langerhans cells (LCs), and Thy-1-positive dendritic epidermal cells, some of which potentially express PKC too.

We recently reported that in keratinocytes of adult mouse in vivo neither PKC I, II, nor III isozyme was detected by immunohistochemical and immunoblot analyses using isozyme-specific monoclonal antibodies (McAbs), but that PKC II isozyme was highly expressed in epidermal LCs (12). These observations point to PKC II isozyme playing an important role in the functioning of mouse LCs. The expression of PKC in human LCs, however, has not been reported on so far. In this study we immunohistochemically investigated the expression of PKC isozymes in human LCs in vivo to see whether or not the expression of PKC II isozyme in LCs is a mouse-specific trait.

MATERIALS AND METHODS

Monoclonal antibodies

Preparation and properties of the McAbs used in this study were described previously (13). All three clones, MC-1a (anti-PKC I), MC-2a (anti-PKC II), and MC-3a (anti-PKC III), produced immunoglobulin G (IgG).

Histochemical and immunohistochemical staining

Skin biopsy specimens were obtained from 11 persons of both sexes aged 18–80.

(a) *Frozen section:* Normal skin samples were taken near benign skin tumours such as epidermal cysts, naevus or seborrheic keratosis. The skin pieces were embedded in O.C.T. compound (Miles, Naperville, Ill.) and quickly frozen at −80°C. Frozen sections (5 μm) were cut and air-dried on egg albumin-coated glass plates. After fixation in ethanol for 20 min at 4°C, the samples were washed in phosphate-buffered saline (PBS). Non-specific binding of antibodies was blocked by incubation at room temperature for 30 min in PBS containing 1% bovine serum albumin and 0.05% sodium azide (P/B). The samples were then incubated for 30 min at room temperature in a culture supernatant of either MC-1a, MC-2a, or MC-3a. Another McAb of the IgG class was used as negative control. After washing, the samples were incubated for 30 min at room temperature in fluorescein isothiocyanate (FITC)-conjugated anti-mouse IgG goat IgG (Cappel, 1211-0081, Malvern, Pa) diluted to 1 : 100 with P/B.

(b) *Epidermal sheet:* During plastic surgery involving skin grafts, normal skin was obtained with a dermatome and treated for 1.5 h in 20 mM ethylenediamine tetraacetic acid (EDTA) in PBS at 37°C. Epidermal sheets were removed from the dermis and subjected to double staining

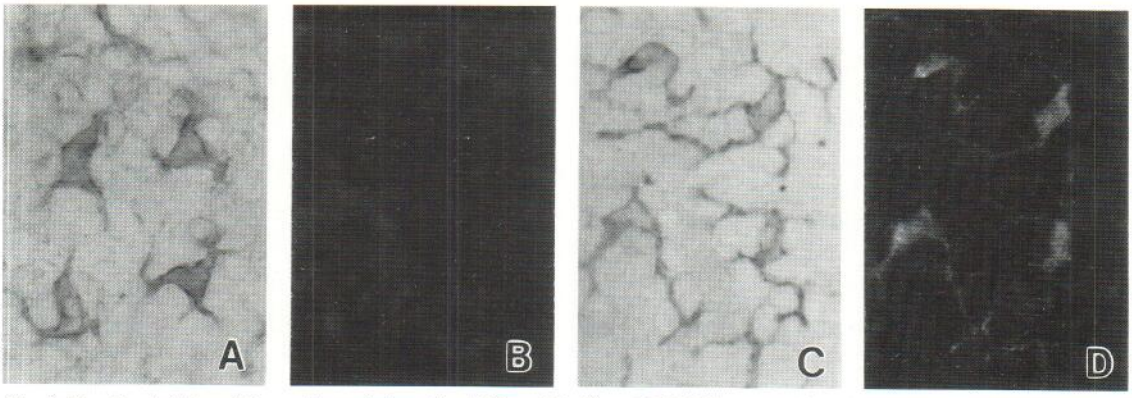

Fig. 2. Double staining of the epidermal sheet for ATPase (A, C) and PKC II isozyme (B, D). Control McAb did not stain ATPase-positive cells (A, B). On the contrary, all ATPase-positive cells are positive for MC-2a (C, D), indicating that the PKC II isozyme-positive epidermal cells are LCs (×500).

for ATPase and PKC isozymes as described previously (12).

Specimens were mounted in PBS containing 1 mg/ml *p*-phenylenediamine and were examined under a Zeiss microscope (Axiphot) with epi-illumination and filters for FITC fluorescence.

RESULTS AND DISCUSSION

Immunohistochemical staining of frozen skin sections revealed non-specific staining with the control McAb in the stratum licidum and in the dermis (Fig. 1A). MC-1a and MC-3a McAb did not cause any positive staining of the epidermis (Fig. 1B, D). On the contrary, MC-2a-positive cells were observed in the epidermis of all samples examined (Fig. 1C). In order to examine the identity of these MC-2a-positive cells, epidermal sheets were double stained for PKC isozymes and ATPase, a reliable marker for epidermal LCs (14). The control McAb did not stain ATPase-positive cells (Fig. 2A, B). On the contrary, ATPase-positive cells showed positive reaction to MC-2a McAb (Fig. 2C, D) indicating that MC-2a-positive epidermal cells are LCs. MC-1a and MC-3a did not produce any positive staining. These results suggest that human epidermal LCs as well as mouse LCs express PKC II isozyme in vivo. Inohara et al. (15) reported that PKC II and/or III isozyme is expressed in vivo in keratinocytes of the granular layer of the human skin, but they did not demonstrate positive staining in any other cell types of the epidermis. Because we detected PKC II isozyme in LCs of samples that had been treated by a process similar to, though not identical with that, employed by Inohara et al. (15), it seems likely that the difference in specificity of the anti-PKC isozyme McAbs is responsible for the discrepancy in the expression of PKC isozymes in human LCs.

In this study we did not find any positive reaction in keratinocytes to anti-PKC isozyme McAbs. However, we can not rule out the possibility that PKC is expressed in human keratinocytes in vivo in undetectable amounts or under specific conditions. Further biochemical research should be done on the expression of PKC using a purified keratinocyte population.

PKC is a key molecule controlling signal transduction into the cell (1). At present, little is known about extracellular signals that control functions of LCs in vivo. Since we showed here that human epidermal LCs, as well as mouse LCs (12), express PKC II isozyme in vivo, further studies about expression and activation of PKC II isozyme in LCs should shed light on the mechanisms by which various stimuli affect normal and diseased skin.

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REFERENCES

1. Nishizuka Y. Studies and perspectives of protein kinase C. Science 1986; 233: 305–312.
2. Knopf JL, Lee MH, Sultzman LA, Kriz RW, Loomis CR, Hewick RM, Bell RM. Cloning and expression of multiple protein kinase C cDNAs. Cell 1986; 46: 491–502.

3. Parker PJ, Coussens L, Totty N, et al. The complete primary structure of protein kinase C – the major phorbol ester receptor. Science 1986; 233: 853–859.
4. Coussens L, Parker PJ, Rhee L, et al. Multiple, distinct forms of bovine and human protein kinase C suggest diversity in cellular signaling pathways. Science 1986; 233: 859–866.
5. Ohno S, Kawasaki H, Imajoh S, et al. Tissue-specific expression of three distinct types of rabbit protein kinase C. Nature 1987; 325: 161–166.
6. Chida K, Kato N, Kuroki T. Down regulation of phorbol diester receptors by proteolytic degradation of protein kinase C in a cultured cell line of fetal rat skin keratinocytes. J Biol Chem 1986; 261: 13013–13018.
7. Chida K, Kato N, Yamada S, Kuroki T. Protein kinase C activities and binding of a phorbol ester tumor promoter in 41 cell lines. Biochem Biophys Res Commun 1988; 157: 1–8.
8. Gschwendt M, Horn F, Kittstein W, Furstenberger G, Besemfelder E, Marks F. Calcium and phospholipid-dependent protein kinase activity in mouse epidermis cytosol. Stimulation by complete and incomplete tumor promoters and inhibition by various compounds. Biochem Biophys Res Commun 1984; 124: 63–68.
9. Koizumi H, Adachi K, Tsutsui M, Itami S, Katayama H, Halprin KM. Calcium-activated, phospholipid-dependent protein kinase in pig epidermis. J Invest Dermatol 1984; 83: 261–264.
10. Fournier A, Murray AW. Application of phorbol ester to mouse skin causes a rapid and sustained loss of protein kinase C. Nature 1987; 330: 767–769.
11. Fisher GJ, Harris VA, Voorhees JJ. Purification and characterization of calcium/phospholipid-dependent kinase from adult human epidermis. J Invest Dermatol 1987; 89: 484–488.
12. Koyama Y, Hachiya T, Hagiwara M, et al. Expression of protein kinase C isozyme in epidermal Langerhans cells of the mouse. J Invest Dermatol 1990; 94: 677–680.
13. Hidaka H, Tanaka T, Onoda K, et al. Cell type-specific expression of protein kinase C isozymes in the rabbit cerebellum. J Biol Chem 1988; 263: 4523–4526.
14. Wolff K, Stingl G. The Langerhans cell. J Invest Dermatol 1985; 80 (Suppl) 96–98.
15. Inohara S, Tatsumi Y, Tanaka Y, Tateishi H, Sagami S. Immunohistological identification of protein kinase C isozymes in normal and psoriatic epidermis. Arch Dermatol Res 1988; 280: 454–455.