neutrophils stimulated by a chemotactic peptide (15). Thus, it is likely that, among CSFs, GM-CSF plays a more important role than G-CSF in the local immune or inflammatory responses in psoriasis. These data, however, do not exclude the need for further studies to elucidate the involvement of G-CSF in inflammatory skin diseases.

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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 CII 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 CII isozyme in Langerhans’ cells is a mouse-specific trait. Immunohistochemical studies revealed that protein kinase CII 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 CII isozyme in epidermal Langerhans’ cells in vivo is not a mouse-specific trait and that protein kinase CII 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.*
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 isoforms 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 isoforms 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 seborrhoeic keratosis. The skin pieces were embedded in O.C.T. compound (Miles, Naperville, Ill.) and quickly frozen at \(-80^\circ\text{C}\). Frozen sections (5 \(\mu\text{m}\)) 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, 1211-0881, 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 tetracetic acid (EDTA) in PBS at 37°C. Epidermal sheets were removed from the dermis and subjected to double staining.

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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
HTC fluorescence.

RESULTS AND DISCUSSION
Immunohistochemical staining of frozen skin sections
revealed non-specific staining with the control
McAb in the stratum lucidum 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 PKCII isozyme in vivo. Inohara et al.
(15) reported that PKCII and/or III isozyme is ex-
pressed 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 epider-
mis. Because we detected PKCII isozyme in LCs of
samples that had been treated by a process similar to,
though not identical with that, employed by In-
ohara et al. (15), it seems likely that the difference in
specificity of the anti-PKC isozyme McAbs is res-
ponsible 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. How-
ever, we can not rule out the possibility that PKC is
expressed in human keratinocytes in vivo in undetect-
able amounts or under specific conditions. Further
biochemical research should be done on the expres-
sion of PKC using a purified keratinocyte popula-
tion.
PKC is a key molecule controlling signal trans-
duction 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 epider-
mal LCs, as well as mouse Lcs (12), express
PKCII isozyme in vivo, further studies about ex-
pression and activation of PKCII isozyme in Lcs
should shed light on the mechanisms by which vari-
ous stimuli affect normal and diseased skin.

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Induction of Langerhans’ Cell Mitosis in vivo after Orchietomy on Mice
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We recently reported that male mice have fewer Langerhans’ cells (LCs) than females and that orchietomy resulted in an increased LC density. In this study we demonstrate that orchietomy induces a transient increase in the number of paired LCs (PLCs) before the increase in LC density occurs. The results of double staining for adenosine triphosphatase (ATPase) and 5-bromo-2’-deoxyuridine (BrdU) showed that all examined PLCs had incorporated the intraperitoneally injected BrdU, while only 0.7% of the unpaired LCs were BrdU-positive; that supported the notion of PLCs as being divided daughter LCs. Orchietomy appears to induce a transient increase in mitotic activity of LCs resulting in the increased LC density. Key words: Epidermis; Sex; ATPase; BrdU.

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Epidermal Langerhans’ cells (LCs) are bone-marrow-derived cells which play a pivotal role in cutaneous immune responses; they constitutively express Ia antigens and function as antigen-presenting cells (1, 2). Applying a foreign antigen, e.g., dimethylfluorobenzene, epicutaneously to the skin, usually induces immune reactions. When the same antigen is applied to skin whose LC density was greatly reduced, tolerance to the antigen occurs (3). LC density in the skin thus seems to be an important controlling factor in cutaneous immune responses.

We recently reported sex differences in the density of epidermal LCs: male mice had fewer LCs than females (4). Based on the observations that orchietomy resulted in an increased LC density and that treatment with testosterone propionate inhibited this increase (5), it has been assumed that testicular androgens are responsible for the sex differences in LC density. An increase in the density of epidermal LCs can be explained by (a) LC immigration exceeding emigration, and (b) LCs increasing their mitotic