CD69, An Early Activation Antigen on Lymphocytes, Is Constitutively Expressed by Human Epidermal Langerhans Cells

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When screening skin cryosections with a panel of monoclonal antibodies (MoAb), we found that the anti-CD69 MoAb Leu-23 reacted with a subpopulation of epidermal dendritic cells, presumably Langerhans cells (LC). The staining intensity was enhanced by gentle trypsin pretreatment of the sections. Flow cytometric analysis of LC-enriched epidermal cells (EC) revealed that nearly all CD1a-bearing LC display anti-CD69 reactivity when tested briefly after termination of the enrichment procedure. Immunoprecipitation experiments showed that isolated LC specifically express a disulphide-linked dimer composed of 26/30-kDa subunits that therefore slightly differs from the 28/32-kDa CD69 complex described on activated T or natural killer (NK) cells. This difference is probably due to a different post-translational glycosylation pattern as evidenced by Endoglycosidase-F treatment of the immunoprecipitate disclosing the 24-kDa core protein of CD69. When freshly isolated LC-enriched EC were kept in culture, anti-CD69 reactivity gradually decreased but the addition of IFN-γ to the culture medium sustained the CD69 expression on LC in vitro. These results strongly suggest that resident but not LC recovered from EC cultures bear CD69 moieties. It remains to be seen whether the expression of this antigen can be linked to (a) particular functional property (ies) of intraepidermal LC. J Invest Dermatol 98:771–776, 1992

A few hours after polyclonal activation, T cells, B cells, and natural killer (NK) cells will react with the MoAb Leu-23 [1], MLR3 [2-4], EA 1 [5], and anti-ATMIP [6]. Phenotypic and biochemical analyses revealed that the surface moieties recognized by these antibodies are localized on the very same cell surface structure, which, at the occasion of the IVth Leucocyte Typing Workshop, has been classified as CD69 [7]. Current evidence indicates that this antigen represents the first cell-surface glycoprotein detected after lymphocyte activation [3,6]. Biochemically, this molecule represents a disulfide-linked phosphorylated dimer consisting of two homologous 24-kDa polypeptide chains that are differently glycosylated in 28- and 32-kDa species [1,2,5,6]. Although CD69 is not detectable on unstimulated peripheral blood leukocytes and activated monocytes, this molecule is constitutively expressed by mature and, to a lesser extent, by immature CD1a-positive thymocytes [8] as well as by platelets [9]. Although the exact function of CD69 has yet to be clarified, it has been suggested that this molecule plays a role in either cellular interaction, interaction with a yet-to-be-defined growth factor, or interleukin 1-dependent functions of T cells [3].

When investigating the phenotypic features of T cells in inflammatory infiltrates of various skin diseases, we found that CD69 expression was not confined to CD3-positive T cells but was also seen on some epidermal dendritic cells. We therefore undertook a study to characterize the anti-CD69-reactive cells within the human epidermis and to define the molecular configuration of the anti-CD69-reactive moieties.

**MATERIALS AND METHODS**

**Reagents** The first step MoAb included anti-CD1a (JOT6a, IgG1, Immunotech, Marseille, France; T6/RD1, IgG1, Coultertronics, Krefeld, Germany; OKT6, IgG1, Orthopharmaceuticals, Raritan, NJ), anti-CD3 (Leu-4, IgG1, Becton Dickinson [BD] and Co., Sunnyvale, CA), anti-CD25 (anti-Tac, IgG1, BD) and anti-CD69 (Leu-23, IgG1, BD) reagents. Isotype controls were obtained from BD. All these antibodies were used either unlabeled or as fluorescein-isothiocyanate (FITC) and/or phycoerythrin (PE) conjugates. For immunohistologic purposes, we additionally used rabbit-anti-mouse antibody (RAM/Ig) and alkaline-phosphatase

**Abbreviations:**

APAAP: alkaline-phosphatase mouse anti–alkaline phosphatase
cLC: cultured LC
EC: epidermal cell
Endo-F: endoglycosidase F
FCS: fetal calf serum
fILC: freshly isolated LC
LC: Langerhans cells
MoAb: monoclonal antibodies
NK: natural killer
PBS: phosphate-buffered saline
PE: phycoerythrin
PKC: protein kinase C
RAM/Ig: rabbit-anti-mouse antibody
rLC: resident LC
TBS: Tris-buffered saline

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mouse anti-alkaline -phosphatase complexes (APAAP) (Dakopatts, Hamburg, FRG).

In Situ Immunolabeling on Cryosections Split-thickness specimens from cadaver skin (buttocks) were obtained by using a hand dermome (Weck, NJ). For the preparation of vertical skin sections, 6-mm punch biopsies were taken from these specimens, snap-frozen in liquid nitrogen, and stored at −70 °C. Six-micrometer cryosections were then prepared, air-dried, fixed for 10 min in pure acetone, and processed for immunohistochemistry using the alkaline-phosphatase mouse anti-alkaline phosphatase (APAAP) technique as described previously in detail [10]. Briefly, the sections were first incubated in Tris-buffered saline (TBS), followed by an incubation with either IOT6a, Leu-4, Leu-23, anti-Tac, or the relevant isotype control (all at 10 μg/ml) for 1 h. In some experiments, cryosections were exposed to 2.5 μg/ml trypsin (Trypsin XI, Sigma Chemical Co, St. Louis, MO) for 5 min at 37 °C, washed in TBS, and then incubated with Leu-23. Then, the sections were washed twice in TBS and incubated for 1 h with a RAM/Ig antibody diluted 1:10. After further washes in TBS, the sections were incubated for 1 h with the APAAP complexes at a final dilution of 1:10. The second and third steps were repeated once. Then the sections were washed twice in TBS and the substrate was added. For the preparation of the substrate, 125 mg N-AS-B1-phosphate (Sigma) were first dissolved in 1.5 ml dimethylformamide. This mixture was then added to a 0.04 M TBS solution containing 90 mg levamisole. Immediately before the staining procedure, 50 mg sodium nitrate and 0.5 ml newfuchsin was diluted in the substrate solution, which was then filtered and added for 10 min to the sections. Color reaction was controlled under the light microscope. Finally, the sections were washed in distilled water and mounted in Kaiser's gelatine (Merek, Darmstadt, FRG).

LC-Enriched Epidermal Cell Suspensions Epidermal cell (EC) suspensions were obtained by trypsinization of split-thickness cadaver skin specimens. After incubation for 2 h at 37 °C in 0.5% trypsin (Trypsin XI, Sigma) in phosphate-buffered saline (PBS, pH 7.2), the loosened epidermis was peeled off and vigorously agitated in fetal calf serum (FCS) (Gibco, Berlin, FRG) — supplemented RPMI 1640 (Flow Laboratories, Meckenheim, FRG) containing 0.01% DNAse (Sigma). Cell suspensions thus obtained were then filtered through sterile gauze, washed in supplemented RPMI 1640, resuspended in Dulbecco's modification of Eagle's medium (DMEM) (Flow Laboratories, Irvin, Scotland), and finally incubated for 45 min at 37 °C on Collagen G (a mixture of 90% collagen type I and 10% collagen type II extracted from calf skin) (Biochrom, Berlin, FRG) — coated culture Flasks, which resulted in a preferential attachment of basal keratinocytes. Based on our previous observation that differentiating keratinocytes are more susceptible to osmotic shock treatment than other EC (Yokozeki et al, in preparation), non-adherent cells were then collected by vigorous pipetting and subjected to a hypotonic mixture of eight volumes PBS and two volumes distilled water for 1 min under mechanical agitation. Resulting cells (viability ~ 20%) were then subjected to Lymphoprep (Nyegaard, Oslo) density gradient centrifugation. The interface cell layer (viability > 90% as determined by trypan blue exclusion) was then collected and the enrichment for LC was controlled by OKT6/FITC immunolabeling. These cells will now be referred to as LC-enriched EC.

Culture of LC-Enriched Epidermal Cells 5 × 10⁶ LC-enriched EC were cultured for 15 to 18 h in 24-well plates (Costar) in keratinocyte-conditioned medium (KCM). For this purpose, collagen-adenherent keratinocytes were cultured for 38 h in DMEM supplemented with 10% FCS, 1% antibiotics/antimycotics (Gibco), 2 mM L-glutamine (Gibco), 25 mM Hepes buffer (Seromed, Berlin, FRG), 5 mM sodium-pyruvate (Gibco), and 1% non-essential amino acids (Gibco). The culture supernatants were then collected, filtered through 0.2-μm Millipore filters, and stored frozen at −20 °C. This KCM was found to be active in supporting the viability of LC-enriched EC (see Results). In certain experiments, either recombinant human interferon gamma (rhIFN-γ, 100 U/ml) or recombinant α-defensin (fuscin, Genzyme Corporation, Boston, MA), phorbol 12-myristate 13-acetate (PMA, 5 ng/ml, Sigma), or the calcium ionophore A23187 (0.5 mM FmC, Sigma) were added to the cultures.

Human Cell Line Jurkat The cell-line Jurkat cells were obtained from the ATCC (American Tissue Culture Collection, Rockville, MD). These cell lines were continuously maintained in culture with RPMI 1640 supplemented with 10% FCS, 1% antibiotics/antimycotics (Gibco) and 2 mM L-glutamine (Gibco). Jurkat cells constitutively express CD69, which is upregulated [5] upon stimulation with phorbol-myristate acetate (PMA). Consequently, Jurkat cells stimulated with PMA (Sigma; 5 ng/ml) for 24 h were used as control for immunoprecipitation and immunolabeling experiments.

Flow Cytometric Analysis 1 × 10⁸ freshly prepared or cultured LC-enriched EC were incubated for 30 min with heat-inactivated AB serum. After several washes in PBS supplemented with 1% FCS + 0.1% sodium azide, double-staining experiments were performed by simultaneously incubating the cells with i) Leu-23/FITC (5 μg/ml) and T6/RD1/PE (1 μg/ml), ii) Leu-23/FITC and anti-Tac/PE (5 μg/ml), iii) anti-Tac/PE and OKT6/FITC (2 μg/ml), or iii) Leu-4/FITC (5 μg/ml) and T6/RD1/PE for 40 min on ice. Isotype controls were performed with non-relevant FITC- and PE-conjugated mouse IgG1 (BD). The cells were then washed twice in supplemented PBS at 4 °C and were analyzed by flow cytometry using a FACScan (BD). Fluorescence parameters were collected using a built-in logarithmic amplifier after gating on the combination of forward light scatter and OKT6/FITC labeling. Viable cultured cells were excluded by ethidium bromide labeling. Fluorescence data of 10,000 cells obtained with Consort 30 software were analyzed with the Lysis-I program (BD) and expressed as a percentage of fluorescent cells tested with the appropriate MoAb compared to the isotype control.

Immunoprecipitation and Electrophoresis LC-enriched EC or PMA-activated Jurkat cells (5 × 10⁶) were washed twice in PBS (pH 7.4) and resuspended in 50 μl cold PBS (4 °C). For surface iodination, cell suspensions were incubated under gentle agitation with 500 μCi [125I]-NaI (Amersham, Little Chalfont, England), 15 μl lactoperoxidase at 2 mg/ml (Sigma), and increasing amounts of H₂O₂ (5 μl/5 min, 7.5 μl/10 min, 10 μl/15 min). The enzymatic reaction was stopped by washing the cells in 1 ml PBS, followed by two washes in 20 mM sodium iodide (Sigma). The cells were then resuspended in 500 μl of lysis buffer (Tris 50 mM, NaCl 150 mM, EDTA 5 mM, 0.5% NP-40) containing the following protease inhibitors: 10 mM iodoacetamide (Serva, Westbury, NY), 0.1 mM phenylmethylsulfonyl fluoride (PMSF) (Sigma), 50 mM e-amino-caproic acid (Sigma), and 10 mM benzamidin (Sigma). After a 2-h incubation at 4 °C, lysates were first freed of insoluble material by centrifugation and then subjected to sequential preclarifying using 50 μl normal rabbit serum/100 μl protein A sepharose beads (Boehringer Mannheim Biochemicals, Mannheim, FRG), 50 μl normal goat serum/100 μl protein G sepharose beads (Pharmacia LKB, Uppsala, Sweden), and, finally, 100 μl protein A sepharose beads alone (Boehringer). For specific immunoprecipitation, pre-cleared lysates were incubated with 20 μl of either MoAb OKT6, Leu-23, Leu-4 or an isotype control, respectively, in the presence of 20 μl protein-G sepharose beads (Pharmacia LKB) overnight at 4 °C. Subsequently, immunoprecipitates were washed five times with lysis buffer and complexes were eluted by boiling the samples in a sample buffer (125 mM Tris-HCl, pH 6.8) containing 3% SDS, 15% glycerol, 0.01% bromophenol blue in the presence or absence of 10% D-Mercaptoethanol. Eluted proteins were subjected to a sodium dodecyl sulfate PAGE (Sigma) 10% polyacrylamide gel electrophoresis (PAGE) with 40 mA constant current. Gels were then dried and autoradiographed using Hyperfilm-MP (Amersham) at −70 °C for 7 d.

For endoglycosidase-F (Endo-F) treatment, immunoprecipitates were resuspended in 10 μl 0.5% SDS and boiled for 2 min. Then
RESULTS

Leu-23 Antigen Is Expressed by Resident Human Langerhans Cells  Incubation of cryostat skin sections with the MoAb Leu-23 in an APAAP technique resulted not only in the marked labeling of certain perivascular and/or peri-appendageal mononuclear cells in the upper dermis, but also in the visualization of a few weakly stained dendritic cells and/or dendrites within the basal and suprabasal layers of the epidermis (Fig 1A) and, even more pronounced, of the follicular epithelium (not shown). In contrast, staining with anti-CD3 mAb Leu-4 revealed only very few labeled cells in the epidermis (less than 1 cell/microscope field at ×250 magnification) which exhibited a round rather than a dendritic morphology. Immunolabeling with anti-CD25 MoAb or with IgG1 isotype control reagents consistently yielded negative results. Gentle proteolytic pretreatment of sections followed by the Leu-23 incubation resulted in a clearcut staining of numerous cells with dendritic morphology (Fig 1B) in the same localization as described above. Similar results were obtained using other anti-CD69 MoAb as anti-MLR-3 and anti-AIM (data not shown). Typical dendritic pattern was seen after anti-CD1a-labeling of untreated serial cryosections. As opposed to its effect on Leu-23 staining, trypsin pretreatment of skin cryosections did not influence the anti-CD3 or anti-CD25 staining pattern. These data suggest but do not prove that epidermal LC may express Leu-23 antigen in situ.

In order to address this issue, we performed Leu-23/CD1a double-labeling experiments on LC-enriched EC suspensions. Depending on the number of LC in the starting EC suspension (0.3–2% CD1a positive cells), the procedure described in Materials and Methods resulted in EC suspensions that contain CD1a-bearing cells (i.e., LC) in the range from 20% to 80%. Analysis of these CD1a-enriched EC suspensions by double-labeling and flow cytometry showed that the vast majority (>80%) of these CD1a-positive EC exhibits Leu-23 reactivity (Fig 2A) but fail to react with the anti-CD25 reagent (Fig 2B). CD3-bearing T cells were only detected in minute quantities (<0.1% of non-gated cells) and were consistently Leu-23-negative. These experiments strongly suggest that Leu-23 expression by EC is confined to the LC population.

Biochemical Analysis of the Leu-23 Antigen on Human Langerhans Cells  In order to ascertain that the Leu-23 reactivity of LC reflects actual CD69 expression, we performed immunoprecipitation experiments with Leu-23 on lysates of surface-iodinated LC-enriched EC and, for control purposes, on PMA-stimulated Jurkat cells. While under non-reducing conditions, the latter cells gave a broad band in the 50–60 kDa range ([5]; Fig 3A, lane 6), Leu-23

40 μl of a 0.1 M sodium phosphate buffer containing 50 mM EDTA and 0.5% octylglycoside (pH 5.5) were added and again boiled for 2 min. After removal of sepharose beads by centrifugation, immunoprecipitates were incubated for 18 h at 37°C in the presence of 0.5 U endoglycosidase F/N-glycosidase F (Boehringer) in a final volume of 60 μl. Enzyme-treated proteins were then subjected to SDS-PAGE under reducing conditions as described above.

Figure 1. Immunohistochemical reactivity of Leu-23 on normal human skin. Routine (A) or trypsin-pretreated (2.5 μg/ml, 5 min at 37°C) (B) cryosections of biopsy specimens from human skin were incubated with MoAb Leu-23 and stained with the highly sensitive APAAP technique as described. Note the increased reactivity of epidermal dendritic cells (arrow) after enzyme digestion on Fig 1B.

Figure 2. CD69 expression on freshly isolated Langerhans cells. LC-enriched EC were subjected to immunofluorescence double-labeling using either PE-conjugated anti-CD1a MoAb T6/RD1 and Leu-23/FTTC (A) or anti-CD1a MoAb OKT6/FTTC and PE-conjugated anti-CD25 MoAb (B). Using a FACScan (BD), LC (as defined by anti-CD1a reactivity and forward light scatter pattern) were gated and analyzed for CD69 (A) and CD25 (B) expression, respectively. Markers defining quadrants were positioned to include >98% of the control MoAb stained cells in the lower left quadrant.
Figure 3. Biochemical analysis of Leu-23 reactive molecules on Langerhans cells. (A) Freshly isolated LC-enriched EC (lanes 2, 3, and 4) or PMA (10 ng/ml, 24 h)–activated Jurkat cells (lanes 5, 6, and 7) were radiolabeled with ¹²⁵I and detergent solubilized. Lysates were immunoprecipitated with control IgG/MoAb (anti-CD1a MoAb OKT6, lanes 2 and 5), or with the anti-CD69 Leu-23 MoAb (lanes 3 and 4) and then electrophoresed under either reduced (lanes 2, 4, 5, and 7) or non-reduced (lanes 3 and 6) conditions. Size markers are included in lane 1. (B) Effect of deglycosylation on Leu-23 reactive molecules. Leu-23-immunoprecipitates derived from lysates of surface-iodinated freshly isolated LC-enriched EC and PMA-activated Jurkat cells (as described above) were exposed to Endo-F (0.5 U for 18 h) and then subjected to SDS-PAGE under reduced conditions (lane 1, LC-enriched EC; lane 2, Jurkat cells).

immunoprecipitation of LC-enriched EC yielded a specific band with a Mr ranging from 46 to 55 kDa (Fig 3A, lane 3). Under reducing conditions, the Leu-23 immunoprecipitated material resolved into a 26/30 kDa dimer on LC-enriched EC (Fig 3A, lane 4) and into a 28/32 kDa dimer on PMA-stimulated Jurkat cells (Fig 3A, lane 7). Deglycosylation experiments of the reduced immunoprecipitates with Endo-F revealed a single core protein of 24 kDa on both LC-enriched EC (Fig 3B, lane 1) and PMA-activated Jurkat cells (Fig 3B, lane 2). These results confirm the specificity of the Leu-23 reactivity of LC and strongly imply that LC express a 26/30 kDa variant of the CD69 antigen.

Langerhans Cells Rapidly Lose Their Surface-Bound CD69 Antigens in Culture Upon mitogen stimulation of lymphocytes, CD69 expression can be detected during the first hour, peaks at 18–24 h, and then gradually decreases [5,11]. This suggests that, on lymphocytes, CD69 expression is linked to a functional property of these cells in their early activation phase. In EC cultures, LC undergo profound phenotypic and functional changes [12–14], and this process is dependent upon the presence of keratinocyte-derived cytokines [15–17]. It was therefore of interest to follow the fate of CD69 expression on LC-enriched EC in culture, and to compare it with the emergence kinetics of CD25 present on cultured, but not on resident, LC [13,14]. For this purpose, LC-enriched EC were cultured for either 15 or 38 h in KCM [this was done in order to provide cytokines (GM-CSF, IL-1, TNF-α) in quantities needed to secure LC viability and differentiation (15–17)] and then subjected to phenotypic analysis. After 15 h of culture, the intensity of both anti-CD1a and anti-CD69 labeling of viable LC-enriched EC was clearly less than that recorded on freshly prepared LC-enriched EC and this phenomenon was even more pronounced after a culture period of 38 h (Fig 4). Gentle tryptic digestion of cultured LC did not lead to an increase of CD69 expression excluding a masking phenomenon by structures recovered in vitro. In reverse fashion, CD25 was not detectable on freshly prepared LC-enriched EC, but was readily discernible after culture (Fig 4).

Effect of IFN-γ on CD69 Expression by Cultured Langerhans Cells Because it has been shown that i) PMA or the calcium ionophore A23187 are able to induce/potentiate CD69 expression on thymocytes or T cells [8,18] and that ii) IFN-γ induces its appearance on monocytes ([19], Bieber et al, unpublished data), we investigated the effect of these factors on the CD69 expression by LC-enriched EC in culture. Therefore, LC-enriched EC were cultured in KCM as described above in either the presence or absence of PMA (5 ng/ml), calcium ionophore (0.5 mM), or rhIFN-γ (100 U/ml). After 38 h of culture, the cells were harvested, double stained with either anti-CD1a and Leu-23 or anti-CD1a and anti-CD25, and finally analyzed by flow cytometry.

As shown above, CD69 expression was rapidly lost by LC in KCM without exogenous factors. The addition of PMA or Ca ionophore had no effect on this phenomenon (Fig 5) although Jurkat cells cultured under the same conditions increased their CD69 expression (data not shown). In contrast, we found that CD69 expres-
CD69 expression by Langerhans cells

Figure 4. Flow cytometric analysis of cultured LC. LC-enriched EC were cultured in KCM for either 15 h (viability >80%) or 38 h (viability >60%) and then subjected to immunofluorescence double-labeling using i) FITC-anti-CD1a (for gating LC) versus either PE-anti-CD25 (B); or ii) PE-anti-CD1a (for gating LC) versus FITC-anti-CD69 (C). In order to allow a simultaneous demonstration of culture time-dependent shifts in the various antibody reactivities, results are presented as histograms. Dotted lines, cells stained with irrelevant isotype-matched control antibodies.

Figure 5. Effect of rIFN-γ, PMA, and Ca ionophore on the expression of CD69 (right column) and CD25 (left column) by LC. LC-enriched EC were cultured in either KCM or in KCM supplemented with PMA (5 ng/ml), Ca ionophore (0.5 mM), or rIFN-γ (100 U/ml). After a culture period of 38 h, LC (gated on the base of their anti-CD1a reactivity and their forward light scatter pattern) were analyzed for CD25 and CD69 expression. Results are shown by means of histograms. Dotted lines, cells stained with irrelevant isotype-matched control antibodies.

Discussion

In this study, we have shown that i) the anti-CD69 MoAb Leu-23 reacts with epidermal dendritic cells in situ; ii) Leu-23-reactive EC belong to the LC population as evidenced by anti-CD1a/Leu-23 double-labeling experiments on LC-enriched EC; iii) Leu-23 recognizes a disulphide-linked 26/30 kDa CD69 variant on LC; and iv) CD69 expression by LC is rapidly lost in EC cultures but can, at least partly, be sustained by the addition of IFN-γ to the culture medium. With regard to the Leu-23 antigen expression in situ, this molecule could be readily visualized on dermal mononuclear cells, but was barely detectable on epidermal dendritic cells unless a gentle protease pretreatment was performed prior to immunolabeling. This observation indicates that, on resident LC, the Leu-23-reactive epitope of CD69 is a hidden determinant that can be unmasked by trypsin pretreatment. A similar phenomenon has been described for other LC-bound antigens such as D47 and MHCl class I [20]. Notwithstanding the caveat that the definitive (immuno-electronmicroscopic) proof for the LC nature of the Leu-23-reactive epidermal dendritic cells has yet to be provided, we conclude from our in situ studies that CD69 is constitutively expressed on resident LC. This contention is strongly supported by our immunolabeling studies on LC-enriched EC documenting the presence of Leu-23 on the vast majority of CD1a-bearing EC (i.e., LC) and, conversely, revealing the Leu-23 non-reactivity of CD1a-negative EC (i.e., keratinocytes).

Concerning the molecular configuration of Leu-23-reactive determinant, immunoprecipitation experiments revealed the presence of a disulphide-linked 26/30 kDa dimer on LC that is slightly different from the 28/32 kDa dimer found on PMA-induced Jurkat cells ([5], this study), on activated T cells [2–5], on activated NK cells [1], and on thymocytes [8]. Our assumption that the Leu-23-reactive moiety on LC is indeed the CD69 antigen is based on our further observation that Endo-F treatment of the Leu-23-immunoprecipitates from both LC-enriched EC and PMA-stimulated Jurkat cells yielded a protein backbone of 24 kDa in either cell population. It thus appears that post-translational glycosylation mechanisms of CD69 in LC are different from those in most other cell types.

At this moment, very little is known about the functional role of CD69 on lymphoid cells, which makes it difficult to speculate about the significance of CD69 expression by resident LC. Using a PKC-dependent pathway, CD69 is rapidly induced on lymphocytes, but is then progressively lost [5,11]. These findings suggest that the functional role of CD69 on lymphocytes is linked to events occurring during the early steps of activation. The further observation that anti-CD69 antibodies can provide a comitogenic signal for preactivated lymphocytes [4,21] supports this assumption and will
Although upon stimulation keratinocytes can produce a variety of cytokines [23], their capacity to produce IFN-γ has yet to be demonstrated. Thus, our finding that IFN-γ-supplemented KCM, but not KCM alone, leads to a prolonged expression of CD69 by LC may seemingly argue against the above hypothesis. On the other hand, the demonstration of a favorable effect of IFN-γ on LC CD69 expression does by no means argue against a similar capacity of other substances. Furthermore, one should not forget that the supply of KCM cannot be regarded as an equivalent for an intact epidermis.

Ample evidence now exists that certain phenotypic and functional features of resident/freshly isolated LC differ from those of LC recovered from EC cultures ("cultured LC") [12,13]. In this regard, it has been observed that freshly isolated LC are capable of antigen uptake/processing of protein antigens but are only poor stimulators of resting T cells; conversely, cultured LC are extremely potent stimulators of resting T cells but are deficient in the capacity of processing certain protein antigens [24]. The demonstration in this study of CD69 expression on resident rather than on cultured LC may therefore suggest that this molecule is involved in functional duties typical for resident LC. If this should prove to be correct, the interference with CD69 expression by LC may be a useful strategy to influence immune response originating in the epidermis.

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