Acquired Fas ligand (FasL)-mediated cytolytic activity of human keratinocytes causes the massive keratinocyte cell death that occurs during toxic epidermal necrolysis, a deadly adverse drug eruption. Under normal conditions keratinocyte apoptosis is a rare event in the epidermis although keratinocytes express the death receptor Fas and its ligand. Here we have investigated why this is so. We show that Fas, FasL, Fas-associated death domain, and caspase-8 mRNA are detectable in the epidermis, primary keratinocyte cultures, and keratinocyte cell line and that Fas protein is expressed in keratinocytes of all subcorneal layers of the epidermis, whereas FasL is only expressed in the basal and first suprabasal layers. Coexpression of Fas and FasL therefore occurs in basal and suprabasal keratinocytes. In vitro, keratinocytes are killed by recombinant FasL in a dose-dependent manner, but they are unable to kill Fas-sensitive target cells despite FasL expression. Analysis of keratinocyte culture supernatants and treatment of keratinocytes with metalloproteinase inhibitors excluded cell surface expression of FasL and rapid metalloproteinase-mediated cleavage of cell surface FasL. Fluorescence-activated cell sorter, confocal microscopic, and electron microscopical analysis revealed that keratinocyte FasL is localized intracellularly predominantly associated to intermediate filaments. These data suggest that the observed inability of keratinocyte FasL to induce apoptosis under physiological conditions is due to its cellular localization and also indicate that intermediate filaments may be involved in regulating the subcellular localization of FasL.

FasL is a member of the tumor necrosis factor protein superfamily. It is a type II membrane protein lacking a signal sequence, but having an internal hydrophobic domain that allows membrane anchorage of the ligand (1). Upon contact with FasL, cells expressing Fas rapidly undergo apoptosis (2, 3) by way of activation of an intracellular signaling pathway. This pathway involves a distinct cytoplasmic motif called the “death domain” (4, 5) that interacts with a protein called FADD (6, 7). The recruitment of FADD allows the connection of the Fas receptor to the cysteine protease caspase 8 (ICE-like cysteine proteases) (FLICE/MACH) (8, 9).

The Fas system has proven to be essential in contributing to the functional integrity of the epidermis. Recent evidence has shown that Fas-induced keratinocyte apoptosis in response to UV prevents the accumulation of procarcinogenic p53 mutations by deleting UV-mutated keratinocytes (10). Furthermore, strong evidence exists that dysregulation of Fas expression and/or signaling contributes to the pathogenesis of diseases such as toxic epidermal necrolysis (11) and acute cutaneous graft versus host disease (12, 13).

Immunohistochemical studies of normal human skin have reported the expression of Fas on keratinocytes (14–17) and shown that agonistic anti-Fas antibody can mediate apoptosis of interferon-γ pretreated cultured keratinocytes (15–17). To date, only two studies have reported FasL protein expression in the epidermis in vivo (11, 18). Several studies have however shown FasL protein in cultured keratinocytes by Western blot analysis (18–21). The exact cellular localization and the cytolytic potential of keratinocyte FasL are not yet known under normal conditions. Here we investigated this in the aim of better understanding how the cytolytic activity of keratinocyte FasL is regulated.
verse transcription and amplification were performed from total RNA using the iQ One-step RT-PCR kit (Qiagen AG, Basel, Switzerland). RT-PCR for FasL was carried out using the following forward and reverse primers 5′-CTC TGG AAT GGG AAG ACA CC-3′ and 5′-ACC AGA GAG AGC TCA GAT ACG-3′. For β-actin 5′-TGA TGG ACT CCG GTG ACG G-3′ and 5′-TGG TAC CCA GCA TTT CCC GC-3′. PCR products (40 cycles) of FasL (381 bp, intron spanning) and β-actin (179 bp) were analyzed by 1.5% agarose gel electrophoresis.

**Immunohistochemistry and Immunofluorescence—Immunohistochemistry** was performed on cryosection as described (23) using UB2 anti-Fas antibody (Immunotech, Marseille, France) and isotype-matched control antibody (mouse IgG1).

For immunofluorescence, 6-μm-thick cryostat sections were air-dried for 30 min with ice-cold methanol (1:1) and then rehydrated with PBS. Sections were then incubated at room temperature for 60 min with anti-Fas rabbit IgM antibody A-11 (Alexis, San Diego, CA) and with anti-β1-integrin mouse IgG antibody AA2 (kindly provided by C. E. Klein, University of Würzburg, Würzburg, Germany) (24), diluted 1:60 and 1:40 in PBS supplemented with 1% bovine serum albumin, respectively. For negative control, sections were incubated with rat IgM and mouse IgG, respectively. Sections were then revealed with fluorescein isothiocyanate-conjugated mouse anti rat IgM diluted 1:200 or with Texas red-conjugated anti mouse IgG diluted 1:200 (Jackson ImmunoResearch, West Grove, PA). Results were routinely analyzed under fluorescence microscope. Some experiments were analyzed using a confocal laser-scanning microscope Zeiss LSM 410 invert, equipped with argon (488 nm) and HeNe (543 nm) lasers.

**FACS Staining—**FACS staining was performed on HaCaT cells permeabilized or not by 1% formaldehyde in PBS. Cells were then incubated at 4 °C for 1 h in FACS buffer (PBS, 5% FCS, plus 1% saponin for permeabilized cells) containing 1 μg of mouse anti-FasL G247-4 antibody (PharMingen) or 1 μg of the isotype-matched control MOPC-21 (PharMingen). After two washings in FACS buffer, cells were stained at 4 °C for 45 min with 1 μg of Alexa 488-conjugated anti-mouse (Molecular Probes Inc., Eugene, OR). After two washings, cells were directly analyzed on a FACScan™ (BD Biosciences) using Cellquest 3.0 software. For FACS analysis in non-permeabilized cells, PI exclusion was used to eliminate dead cells.

**Fas and FasL Functionality Assays—**For analysis of Fas death signaling function, cells were seeded in 96-well microtiter plates at a density of 104 cells per well. After adhesion, serial concentrations of recombinant soluble FLAG-tagged FasL (Alexis, San Diego, CA) plus enhancer (anti-FLAG Ab, Alexis) at 1 μg/ml concentration were added. Viability of cells was determined 24 h later by using the Boehringer cell proliferation reagent WST-1 according to the manufacturer’s instructions (Boehringer GmbH, Mannheim, Germany). Percentage of cell viability was determined against control untreated cells.

**Cytolytic FasL function of cultured keratinocytes was determined using a 2-deoxyuridine release assay.** A20 and A20R target cells were labeled with 1×103 of [125I]-deoxyuridine (Amersham Biosciences) for 2 h at 37 °C in a humidified 5% CO2 incubator and then washed three times in medium. Labeled target cells were seeded at 104 cells per well with keratinocytes at the indicated effector/target ratios in flat bottom 96-well microtiter plates. After incubation for 20 h at 37 °C, 2-deoxyuridine-labeled fragmented DNA released in culture medium was pooled with cytoplasmic 125I-deoxyuridine-labeled fragmented DNA recovered by cell lysis. Pooled supernatants were assayed for radioactivity using a γ-counter. Percentage of apoptosis was calculated as: 100 × (sample 125I release – spontaneous 125I release)/maximum 125I release – spontaneous 125I release).

**Cytolytic function of FasL in human epidermis was determined as described (11). Briefly, 600,000 Jurkat cells (in 100 μl of medium) were overlaid on human skin cryosections and incubated for 6 h at 37 °C. Jurkat cell apoptosis was subsequently assessed on 200,000 recovered cells by FACS analysis using annexin-V assay (PharMingen). Jurkat cells overlaid on 293 and 293-FasL (previously cultured on coverslips) for 6 h were used as negative and positive controls, respectively.

**Immunoprecipitation—**To identify soluble FasL in the supernatant of 293-WT, 293-FasL, and HEK cells, cells were cultured in appropriate medium containing 230 μCi/ml of 35S-Cyst/Met (Amersham Biosciences) at 37 °C for 24 h, and supernatants were collected. After preclearing for 5 h with protein A, supernatants were incubated with 10 μl of protein A-agarose beads for 2 h at 4 °C. Following incubation, the incubation of supernatants was continued overnight at 4 °C. After three washings with buffer (0.5%-Triton X-100, 20 mM Tris-HCl, pH 7.6, 150 mM-NaCl), material bound to protein A was eluted with electrophoresis sample buffer containing 50 μM diethiothreitol and subjected to SDS-PAGE. For fluorography, the gel was incubated with Amplify (Amersham Biosciences) for 30 min, dried, and exposed to x-ray film at -80 °C for 3 days.

**Electron Microscopy—**After informed consent of the patients and authorization of the institutional ethical committee for clinical investigation, keratome samples of control human skin were obtained from specimens of abdominal reduction surgery.

Immediately after sampling, fragments were fixed at room temperature in a 0.1% glutaraldehyde, 4% paraformaldehyde solution for 5 min and then exposed for 55 min to 4% paraformaldehyde. The fragments were then embedded at low temperature in Lowicryl K4M resin and thin-sectioned using a Reichert OM10 ultramicrotome. Sections were first incubated 10 min in PBS containing 0.5% bovine serum albumin (1:1) and then exposed at room temperature for 2 h to unlabeled anti-FasL mouse IgG1 monoclonal antibody NOK-1 (PharMingen International). After rinsing, the sections were exposed 1 h at room temperature to a preparation of 15-nm gold particles, coated with protein A, and diluted 1:200. Sections were stained 10 min with 2% uranyl acetate and examined in a Phillips CM10 electron microscope.

Negative controls included 1) skin sections incubated with undiluted isotype matched control antibody MOPC-21 (PharMingen International), followed by protein A-coated gold particles; 2) skin sections incubated with protein A-gold particles only; 3) wild type 293 cells (not expressing human FasL) incubated with the NOK-1 monoclonal antibody followed by protein A-coated gold particles. None of these controls resulted in a positive staining (not shown). Positive controls were provided by incubations of 293 cells that were stably transfected for human Fas ligand, with the NOK-1 monoclonal antibodies, followed by protein A-coated gold particles. These incubations resulted in a clear cytoplasmic labeling of the transfected cells (not shown).

Evaluation of labeling distribution was performed by taking photographs of about 40 keratinocytes, in the suprabasal layers of epidermis of three skin samples, at a constant 60,000-fold magnification. Gold particles were scored in each photograph (representing each a surface of 7.93 μm2) and attributed to one of the following compartments: junctional membrane, non-junctional membrane (desmosomes), cytosol, lysosomes, and other vesicles (Golgi apparatus and endoplasmic reticulum). The membranes and the cytosol of the keratinocytes and the membranes of the other vesicles, whose membranes are unsatisfactorily preserved after K4M embedding, were not scored separately and were included in the “vesicle” counts, intermediate filaments, mitochondria, and nucleus. Values were calculated as number of protein A-coated gold particles per 100 μm2 of cytoplasm and compared between groups by analysis of variance and Student’s unpaired t tests, using the Statistical Package for Social Sciences (SPSS Inc., Chicago).

**RESULTS**

The Fas Death-signaling Pathway Is Functional in Human Keratinocytes Cultured under Basal Conditions—RIPase protection was performed for Fas, FADD, and caspase 8 using total RNA extracted from whole skin, split dermis and epidermis, primary keratinocyte cultures (HEK, interfollicular epidermal keratinocytes and ORS, outer root sheath keratinocytes). As FasL was not detectable by RIPase protection (data not shown), we used the more sensitive RT-PCR technique to analyze FasL mRNA expression as indicated in the next result paragraph. As shown in Fig. 1a, specific protected fragments for Fas, FADD, and caspase 8 were detected in each RNA sample, demonstrating that key initial effectors of the Fas signaling pathway are expressed by human keratinocytes, both in vitro and in vivo. Immunohistochemical staining revealed, as reported previously, a membrane pattern of Fas staining in all subcorneal layers of the epidermis (Fig. 1b). To investigate whether Fas signaling pathway is functional in human keratinocytes, we tested the ability of recombinant soluble FasL (rFasL) to induce apoptosis of immortalized HaCaT and primary HEK cells under basal culture conditions. As shown in Fig. 1c, under these conditions, recombinant human FasL (rFasL) induced dose-dependent death of Fas-sensitive Jurkat cells and HaCaT and HEK cells, HaCaT and HEK cells being, however, less sensitive (IC50 = 89 and 232 ng/ml, respectively) to rFasL than Jurkat cells (IC50 = 25 ng/ml).

**FasL Is Expressed in the Basal and Suprabasal Layers of Human Epidermis—**FasL mRNA expression was investigated
by RT-PCR in the same total RNA samples previously used to analyze Fas expression by RNase protection assay. Indeed, RNase protection that is less sensitive than RT-PCR technique did not allow detection of FasL mRNA. As shown in Fig. 2a, a 381-bp FasL fragment was amplified in all samples, demonstrating mRNA FasL expression in keratinocytes of normal human skin. To investigate whether FasL mRNA is translated into protein in human skin, we analyzed FasL distribution by immunofluorescence in tissue sections of normal human skin.

Keratinocyte FasL Is Not Capable of Inducing Cell Death in Vitro and ex Vivo—The above experiments demonstrate that Fas and FasL are coexpressed in the lower epidermis and that keratinocyte Fas death signaling pathway is functional under basal culture conditions. To determine whether keratinocyte FasL is cytolytically active and able to induce apoptosis in Fas-expressing keratinocytes, we analyzed the capacity of primary HEK to induce Fas-mediated cell death. When keratinocytes were incubated with radioactively labeled A20 (Fas-sensitive) or A20R (Fas-resistant) cells at various effector:target cell ratios, no significant level of apoptotic cell death could be detected in A20 cells after coincubation with HEK. Thus under tested conditions keratinocyte FasL is non-cytolytic. This result was confirmed by the inability of human skin samples to induce apoptosis of Fas sensitive Jurkat cells (Fig. 3b). The low percentage of apoptotic cells detected (5–10%) in both experiments could not be decreased by incubation with Fas-Fc fusion protein, demonstrating that keratinocyte FasL is indeed non-lytic in vitro and ex vivo.

Absence of Surface FasL in Human Keratinocytes in Vitro—To investigate whether the lack of keratinocyte cytolytic activity was due to the absence of FasL at keratinocyte membrane, we analyzed FasL expression at the surface of HEK cells by FACS. As shown in Fig. 4a, HEK cells were clearly negative for surface FasL.
Intracellular Localization of FasL

Fig. 4. Analysis of the localization of FasL in cultured keratinocytes. a, FACS analysis of cell surface (non-permeabilized cells, upper left) or intracellular (permeabilized cells, upper right) FasL expression in HEK cells transiently transfected with a FasL antibody (black line) or an isotype-matched control antibody (gray histogram). 293-WT and 293-FasL, transfected cells were used as negative and positive controls, respectively. b, analysis of HEK culture supernatants for the presence of soluble FasL by immunoprecipitation using Fas-Fc fusion protein. 293-WT and 293-FasL culture supernatants were used as negative and positive controls, respectively, and CD44-Fc as a control Fc-fusion molecule.

Intracellular Localization of FasL

for membrane FasL (upper left). To investigate whether the absence of membrane FasL on keratinocytes could be due to proteolytic cleavage and release of surface FasL into the culture medium, we performed an immunoprecipitation of conditioned culture medium with a Fas-Fc fusion protein. As shown in Fig. 4b, immunoprecipitation of cell culture supernatant from 293-FasL-transfected cells revealed significant levels of soluble FasL at 25 kDa, whereas culture supernatants from wild type 293 and primary cultures of HEK did not. These data suggest that FasL protein may not be translocated to the membrane of keratinocytes and may be retained within their cytoplasm. This hypothesis was confirmed by FACS analysis performed on permeabilized HEK cells (Fig. 4a, upper right), showing a marked shift in fluorescence intensity (35% of cells) in comparison to non-permeabilized cells and permeabilized cells incubated with an isotype-matched control antibody. The marked shift between both antibodies correspond to specific binding as it was cancelled by incubation of anti-FasL G247-4 antibody by a 5-fold excess of recombinant FasL protein (data not shown). These data point out that FasL is localized intracellularly in human HEK cells.

Keratinocyte FasL Is Cytoplasmic in Vivo—To establish the in vivo localization of FasL in keratinocytes, normal human skin was immunostained both with anti-FasL and anti-integrin mAbs and analyzed by confocal microscopy (Fig. 5a). This double labeling revealed that cellular localizations of keratinocyte FasL (green staining) and membrane specific integrin (red staining) were distinct although some little colocalization occurred in suprabasal epidermal layers (Fig. 5a, yellow). The same experiment performed in cultured human keratinocytes HEK (Fig. 5, b and d) confirmed the predominant cytoplasmic localization of FasL. These data show that keratinocyte FasL is predominantly located inside the cytoplasm both in vitro and in vivo.

FasL Is Associated with Intermediate Filaments in Keratinocytes—Using anti-FasL antibody (NOK-1) and protein A-coated gold particles for immunoelectron microscopical analysis of human skin, FasL labeling was detected in most living keratinocytes of human interfollicular epidermis in agreement with the previously shown molecular, biochemical, and immunohistochemical data. In the plastic- and low temperature-embedded material, which we used, this labeling was almost exclusively restricted to the cytoplasm of keratinocytes (Fig. 6, a and b). The labeling of keratinocytes was considered specific in view of the various controls (see “Experimental Procedures,” including the observation that labeling was absent when using the isotypic irrelevant antibody MOPC-21) (Fig. 6c). Quantitative evaluation further showed that different organelles of keratinocytes were not similarly labeled under the experimental conditions that elicited a sizeable staining of these cells for FasL. FasL was predominantly observed in the cytosol and intermediate filament compartments (total number of gold particles per 100 μm² of these compartments was 113.1 ± 15.8, n = 39 and 105.4 ± 14.2, n = 39, respectively) (Table 1), irrespective of whether these organelles were located close to (Fig. 6a) or at distance from the cell membrane (Fig. 6b) and also to some extent in lysosomes and vesicles (22.9 ± 6.8, n = 39). In contrast, several other organelles, including desmosomal and non-junctional regions of the cell membrane, mitochondria (Fig. 6b), and nucleus failed to show any specific labeling above background levels (Table 1).

DISCUSSION

Several studies have shown that Fas-mediated apoptosis is important for the maintenance of epidermal homeostasis (for review, see Ref. 25). This is best illustrated by the fact that mice with mutant non-functional FasL are significantly more resistant to UVB-induced keratinocyte apoptosis (“sunburn cells”) and, as a consequence, accumulates UV-induced p53 mutations significantly more rapidly than control mice (10). It is therefore very likely that keratinocyte FasL expression...
and intact Fas signaling pathway in keratinocytes are crucial for the prevention of UV induced skin cancer. Although Fas is known to be expressed in human keratinocytes, and capable of signaling death when triggered in the presence of interferon-γ (17), little is known about the expression, cellular localization, and cytolyl function of FasL in resting human keratinocytes in vitro and in vivo. The present report analyzes for the first time the exact cellular localization of keratinocyte FasL, and provides evidence that FasL in resting keratinocytes has no cytolyl activity because of its intracellular localization.

Previous studies on human keratinocytes have shown FasL expression at the mRNA and protein levels in vitro (18, 20, 21). Our data confirm these observations as they show constitutive keratinocyte FasL expression in vitro and additionally show constitutive in vivo expression of FasL at the protein level in lower human epidermis. FasL protein expression therefore colocalizes with Fas expression at this location. In addition, our data demonstrate that the Fas death signaling pathway is functional in resting keratinocytes, even without interferon-γ pretreatment, contrary to what was suggested by a previous report (17). Coexpression of functional Fas and FasL set the conditions for spontaneous Fas-mediated cell death of keratinocytes in the basal and first suprabasal layers of the epidermis. However, spontaneous keratinocyte apoptosis in these layers is very seldom observed under physiological conditions (26). We therefore investigated the cytolyl capacity of FasL expressed by normal human keratinocytes in vitro and ex vivo. Our data clearly show that keratinocyte FasL does not induce apoptosis of either adjacent keratinocytes in culture or Fas-sensitive cells overlaid on sections of normal human skin. This lacking of cytolyl activity is probably due to the absence of FasL at the surface of keratinocyte cells, as demonstrated by FACS analysis. We cannot exclude that lack of FasL surface expression could be due to the weak expression of FasL. Indeed, we have unpublished data that showed cell surface expression of FasL in cells treated to increase FasL mRNA transcription level. However, previous reports in other cell types, including activated T cells, have shown that the levels of FasL expressed at the cell surface can be regulated by rapid metalloproteinase cleavage of cell surface FasL, despite stable FasL gene expression (27, 28). In these cell types, treatment with inhibitors of matrix metalloproteinases specifically led to the accumulation of cell surface FasL and to a decrease in soluble FasL in the culture medium. Thus, we investigated whether the lack of membrane FasL in normal human keratinocytes was due to excessive metalloprotease cleavage. This is most likely not the case, since soluble FasL could not be detected in culture supernatants of keratinocytes and furthermore, since treatment of keratinocytes with metalloproteinase inhibitors did not cause

![Intracellular Localization of FasL](image)

**Fig. 6.** Electron microscopical analysis of the subcellular localization of keratinocyte FasL. **a,** Fas ligand immunolabeling is detected with NOK-1 antibody in several cytoplasmic compartments of human interfollicular epidermal keratinocytes. Specific labeling was mostly found over cytosol (gold particles *circled in black*) and intermediate filaments (gold particles *circled in white*), but was not above background level in other organelles, including desmosomal regions of the cell membrane (portions of two adjacent keratinocytes are seen in this large view). **b,** the cytosolic and intermediate filament-associated labeling is also observed in regions of the cytoplasm of spinous keratinocytes, which were distant from the cell membrane and contained unstained organelles, such as mitochondria. **c,** incubation of adjacent sections from the very same skin sample with an isotypic but irrelevant antibody (MOPC-21) did not result in a significant labeling of human keratinocytes (the single gold particle visible in this field is *circled*). The bar represents 300 nm in **a** and **c** and 335 nm in **b**.

| Type of keratinocyte | Organelle                  | FasL immunoreactivity (Nok-1 antibody) | Background immunoreactivity (MOPC-21 antibody) |
|---------------------|---------------------------|----------------------------------------|-----------------------------------------------|
|                     |                           | 11.6 ± 3.4, n = 39                     | 3.9 ± 3.9, n = 13                              |
|                     | Junctional membrane       | 2.9 ± 1.6, n = 39                      | 0, n = 13                                     |
|                     | Non-junctional membrane   | 115.1 ± 15.8, n = 39                   | 17.4 ± 4.7, n = 13                            |
|                     | Cytosol                   | 105.4 ± 14.2, n = 39                   | 25.2 ± 5.5, n = 13                            |
|                     | Intermediate filaments    | 1.0 ± 0.5, n = 39                      | 0, n = 13                                     |
|                     | Mitochondria              | 2.2 ± 0.8, n = 39                      | 1.0 ± 1.0, n = 13                             |
|                     | Lysosomes and vesicles     | 8.7 ± 2.2, n = 39                      | 0, n = 13                                     |
|                     | Nucleus                   | 37.8 ± 23.2, n = 5                     | 25.2 ± 12.0, n = 5                            |

*p < 0.001.*

*p < 0.002.*

*p < 0.07.*
an increase in cell surface FasL staining, as evaluated by FACS (data not shown).

We provide here first evidence for an intracellular localization of keratinocyte FasL. Indeed, FasL was clearly detected by FACS in permeabilized but not in non-permeabilized keratinocytes and was shown to be essentially localized in the cytoplasm of keratinocytes in vitro and in vivo, using confocal and electron microscopical analysis. Maintenance of keratinocyte FasL in an intracellular localization may therefore be a physiological means by which to guarantee a rapid availability of cell surface FasL if required, while keeping this protein out of reach of cell surface Fas that is constitutively expressed and functional in keratinocytes. In this way, keratinocytes can protect themselves from spontaneous Fas-mediated apoptosis under physiological situations despite harboring all the proteins necessary to rapidly trigger it. In this way, keratinocytes can also suicide themselves under stress situations. Indeed, one can hypothesize that in a situation such a harmful UVB exposition of skin, intracellular FasL can be translocated to the keratinocyte cell surface where Fas receptor is present and then lead to apoptotic destruction of UVB-damaged keratinocytes and then avoid accumulation of mutated cells in skin. Such an exclusive intracellular localization of FasL has also been shown for cytotoxic T lymphocytes, in which FasL is stored in secretory lysosomes and exocytosed following T cell receptor engagement (29).

Using immunoelectron microscopy we have further analyzed the subcellular localization of FasL and shown that this protein is predominantly associated with intermediate filaments, the remaining being localized to lysosomes and cytoplasmic vesicles. Our observations suggest that FasL may be docking to intermediate filaments, even though the molecular interaction between FasL and intermediate filaments remain to be elucidated. Thus it is possible that intermediate filaments of human keratinocytes are somehow involved in regulating the transport of FasL to the plasma membrane. Intermediate filaments have already been involved in the translocation of proteins to the cell surface in response to certain stimuli. Indeed, in 3T3-L1 adipocytes disruption of intermediate filaments has been shown to induce marked disruption of the perinuclear GLUT4 sugar transporter to peripheral regions of the cells (30). Further investigation of the association between FasL and keratins may help explain how keratinocyte FasL can become cytolitic under selected circumstances and contribute to the pathogenesis of skin diseases such as toxic epidermal necrolysis (11).

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Intracellular Localization of Keratinocyte Fas Ligand Explains Lack of Cytolytic Activity under Physiological Conditions
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