Interleukin-8 Stimulates Calcium Transients and Promotes Epidermal Cell Proliferation

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The presence of large amounts of biologically active interleukin-8 (IL-8) in psoriatic involved skin suggests that it may contribute, in part, to the changes observed in psoriasis, including hyperproliferation of keratinocytes. To examine the effect of IL-8 on epidermal growth, we monitored cytosolic free Ca^{++} transients in human keratinocytes adult skin epidermis calcium reduced level, temperature elevated (HaCat) cells and normal keratinocytes loaded with the cell permeable, acetoxymethyl derivative, indo-1AM. Addition of IL-8 (0.06–47 nM) to the HaCat cells induced rapid rises in cytosolic free Ca^{++} from resting levels of 145 ± 38 to peak levels of 889 ± 10 nM. The induced rises in Ca^{++} were transient and concentration dependent. Half maximal effect was observed at 1.2 nM. Normal keratinocytes also responded to IL-8 (6 nM) by rises in cytosolic free Ca^{++} from a pre-stimulated level of 269 nM to transient peak value of 393 nM. In addition, IL-8 promoted epidermal cell proliferation. Polyclonal anti-IL-8 antibody blocked IL-8-induced calcium changes and proliferation. Under similar conditions, human neutrophils also responded to IL-8 in a similar dose range by a rapid and transient mobilization of Ca^{++}. The findings indicate that IL-8 has a wider range of responsive target cells than hitherto thought and acts as an autocrine growth factor.
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Psoriasis is a disease characterized by hyperproliferation of keratinocytes, and accumulation of activated T lymphocytes and neutrophils in the dermis and epidermis [1–3]. The molecular basis for the hyperplasia of keratinocytes or the increased trafficking of activated T cells and neutrophils into the skin appears to depend on locally produced growth factors and chemoattractants and the expression of adhesion molecules at the inflamed site (reviewed in [3]). Among potential proinflammatory cytokines that are detectable in psoriatic skin in biologically active form are members of a family of small chemotactic peptides, of which interleukin-8 (IL-8) and monocyte chemotactic factor (MCF) have been increasingly studied [4–6]. Although originally identified by its effects on neutrophils, IL-8 is now known to stimulate chemotaxis of T cells [7] and basophils [8], and to induce histamine release in basophils [9]. IL-8 is produced by various cell types including lymphocytes, monocytes, neutrophils, endothelial cells, dermal fibroblasts, and keratinocytes in response to inflammatory mediators such as interleukin-1 (IL-1), tumor necrosis factor alpha (TNF-α), interferon gamma (IFN-γ), or lipopolysaccharide (LPS) [10,11]. Intradermal or intracutaneous administration of IL-8 into experimental animals [7,11,12], or into human skin [13,14] causes an inflammation characterized by the accumulation of neutrophils and lymphocytes at the sites of injection.

In view of the indigenous cellular producers of IL-8 and their different responsiveness to proinflammatory cytokines [10,11], it is possible that IL-8 and its inducing mediators could be involved in the recruitment and proliferation of epithelial cells in cutaneous inflammation. The observation that IL-1α, TGF-α, and IL-6 are present in psoriatic skin [15–17] and that some cytokines can promote proliferation of keratinocytes in cultures [18,19] indicate that cytokines could potentially contribute to the profound keratinocyte hyperplasia seen in psoriasis. Indeed, recent studies have implicated IL-8 as both a potent chemoattractant [20] and a mitogen for epidermal keratinocytes [21,22].

One indicator of responsiveness of eukaryotic cells to certain growth factors is a transient rise in the intracellular concentration of free calcium ions ($[Ca^{++}]_i$) [23]. In this study, we examined whether or not HaCat cell line and normal human foreskin keratinocytes, like human neutrophils, respond to IL-8 by mobilization of cytosolic free calcium ($[Ca^{++}]_i$). We also investigated whether any IL-8–induced rise in $[Ca^{++}]_i$ was associated with stimulation of epidermal cell proliferation as measured by [^{3}H]thymidine incorporation and increase in cell numbers. The results show that under appropriate experimental conditions, IL-8 raises $[Ca^{++}]_i$ in HaCat cells and

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Abbreviations:
$[Ca^{++}]_i$: cytosolic free calcium
DMEM H21: Dulbecco's modified minimal essential medium
EGF: epidermal growth factor
EGTA: ethylene glycol tetraacetic acid
FCS: fetal calf serum
FMLP: formylmethionyl-leucyl-phenylalanine
HaCat: human keratinocytes adult skin epidermis calcium reduced level, temperature elevated
HBSS: Hanks' balanced salt solution
IFN-γ: interferon gamma
IgG: immunoglobulin G
IL-1: interleukin-1
IL-6: interleukin-6
IL-8: interleukin-8
KBM: keratinocyte basal medium
KGM: keratinocyte growth medium
LPS: lipopolysaccharide
MCF: monocyte chemotactic factor
PBS def.: phosphate-buffered saline without calcium and magnesium ion
SDS: sodium dodecyl sulphate
TCA: trichloroacetic acid
TGF-α: transforming growth factor alpha
TNF-α: tumor necrosis factor alpha

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human foreskin keratinocytes and promotes their proliferation, suggesting that this cytokine could contribute to the keratinocyte hyperplasia seen in psoriatic lesions.

MATERIALS AND METHODS

Source of IL-8 Recombinant human IL-8 was purified to homogeneity from *Escherichia coli* expressing the synthetic IL-8 gene as previously described [24]. Stock solutions of IL-8 were prepared in phosphate-buffered saline (PBS) containing 0.25% bovine serum albumin as a carrier protein and stored at −20°C. Specific polyclonal antibody to IL-8 was raised in rabbits and immunosorbent purified as previously described [25]. No endotoxin contamination (≤12.5 pg endotoxin activity/mg protein) was detected with the chromogenic Limulus amebocyte lysate reagent kit (Wittacker, MA, Bioproducts, Inc, Walkersville, MD) in either IL-8 or immunosorbent purified antibody preparations.

Cultivation of HaCat Cell Line The HaCat cells were maintained by subculturing in endotoxin-free modified Dulbecco's minimal essential medium (DMEM H21) enriched with 5% fetal calf serum (FCS) and 2.5% glutamine on plastic cover slips in Leighton tubes at 37°C in a humidified incubator [26]. At about 80% confluency, the DMEM H21 was replaced with endotoxin-free keratinocyte basal medium (KBM) (Clonetics) containing 0.06 mM Ca^{++}, and the cells were cultured for 2 d to induce quiescence as previously described [27].

Cultivation of Normal Human Epidermal Keratinocytes Normal human keratinocytes were obtained from fresh neonatal foreskin biopsies. Epidermal cells were disaggregated by trypsinization overnight at 4°C in Hanks' balanced salt solution (HBSS) without Ca^{++} and Mg^{++} containing 0.25% trypsin. The cells were plated out at 10^7 cells per 50 ml culture flask in endotoxin-free keratinocyte growth medium (KGM) containing 1.0 mM Ca^{++} and incubated at 37°C under 10% CO_2. Normal keratinocytes used in this study were derived from the second to fourth passages grown as a monolayer to 80% confluency on cover slips. The monolayers were then transferred to KBM containing 0.06 mM $CaCl_2$ and starved for 48 h at 37°C as described above for the HaCat cells.

Assay of Cytosolic Free Ca^{++} in Keratinocytes The KBM medium was replaced with a loading medium, i.e., fresh KBM containing 2 μM acetoxymethyl ester of indo-1 (indo-1/AM). The Leighton tubes were immersed in a 37°C bath and rotated so that the coverslips were flopped gently back and forth through the loading medium. After incubating for 30 min, coverslips were transferred into fresh KBM and then stored in the dark for 15 min at room temperature. The loaded HaCat cells were viable as judged by trypan blue exclusion (≥95% viability).

The coverslips were rinsed once in HBSS containing 0.06 mM Ca^{++}, placed in standard 1-cm square quartz cuvettes containing the same buffer, and transferred to a Perkin-Elmer fluorescence spectrophotometer (model LS-5B) maintained at 37°C with constant stirring. After equilibration for 5 min, IL-8 at various concentrations, or the PBS containing the carrier protein, was added and the resulting fluorescence recorded (excitation, 330 nm; emission, 400 nm). At the end of each assay, a calibration to determine F_{max} and F_{min} was performed by adding ionomycin (5 μM) and $MnCl_2$ (1 mM), respectively. The Ca^{++} levels were calculated according to the method of Grynkiewicz [28].

Keratinocyte Proliferation Assay Keratinocytes were plated at 5×10^3 cells per well of a 96-well microtiter plate and cultured in KGM or KBM for 3 d. The medium in each well was then replaced with KGM or KBM supplemented with epidermal growth factor (EGF) (0.01 μg/ml), IL-8 at various concentrations, or the PBS containing the carrier protein, and after 4 d replaced again with the same supplemented medium for a further 24 h. Proliferation was measured by labeling cultures with 0.5 μCi ml^{-1} [^{3}H]thymidine (specific activity 25 Ci $mmol^{-1}$, Amersham International) during the last 24 h of incubation. After labeling, the medium was removed, each well was washed 3 times with 200 μl of ice-cold PBS

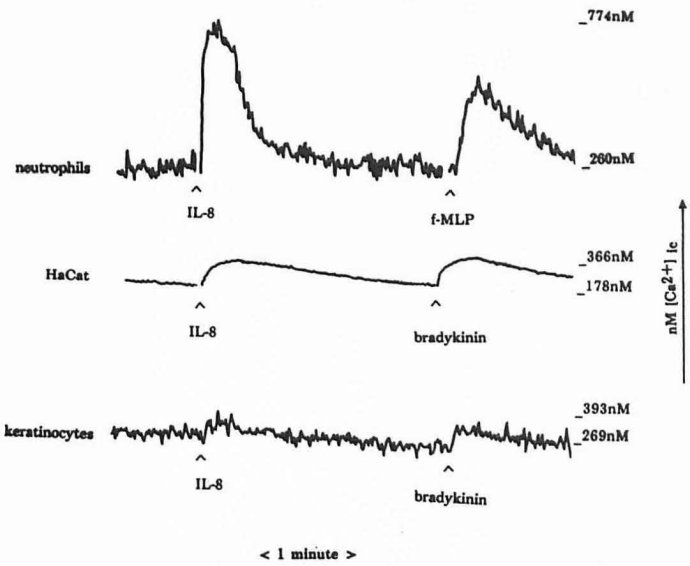

Figure 1. Stimulation of cytosolic free calcium transients in adherent HaCat cells, normal foreskin keratinocytes, and suspended human blood neutrophils by IL-8 (6 nM), bradykinin (2 μM), or FMLP (0.2 μM). Indo-1-loaded neutrophils and epidermal cells were exposed to the indicated stimuli, followed 2-3 min later by a second dose of FMLP or bradykinin, respectively. Cytosolic calcium was measured as described in *Materials and Methods*. *Arrows,* times of addition of the stimuli.

def. and 200 μl of ice-cold 10% trichloroacetic acid (TCA) was added, and the TCA-precipitated nucleic acid solubilized in 100 μl of 0.1 M sodium hydroxide, 1% sodium dodecyl sulphate (SDS) and 2% sodium carbonate for 30 min and counted in 2 ml Optifluor (Packard).

Isolation of Human Neutrophils Neutrophils were prepared from fresh blood obtained from healthy volunteers. The cells were purified by centrifugation through a layer of Ficoll-Paque and sedimentation through dextran followed by hypotonic lysis of residual erythrocytes [29]. The isolated neutrophil preparation contained >98% neutrophils, and cell viability was always ≥95% as determined by trypan blue exclusion.

For the measurement of Ca^{++}, the neutrophils were incubated with 2 μM acetoxymethyl ester of indo-1 (indo-1/AM) in HBSS without Ca^{++} and Mg^{++} for 30 min at 37°C. The loaded cells were washed once and suspended in HBSS containing 1 mM Ca^{++}. Cytosolic free Ca^{++} levels were measured as described above but in suspension cultures following addition of IL-8 at various concentrations, FMLP or the PBS containing bovine serum albumin. F_{max} and F_{min} were recorded after the addition of 0.05% Triton X-100 and ethylene glycol tetraacetic acid (EGTA) at pH 8.4, respectively.

Statistical Analysis Differences between groups were determined by a Student t test.

RESULTS

Stimulation of Calcium Transients in Keratinocyte-Like HaCat Cells and Human Blood Neutrophils by IL-8 As shown in Fig 1, the addition of IL-8 at a standard concentration of 24 nM induced a rapid and transient rise in cytosolic free Ca^{++} in adherent indo-1-loaded quiescent HaCat cells, from a resting level of 145 ± 38 nM to transient peak values in excess of 800 nM. The induced rises in $[Ca^{++}]_i$ were dose-dependent and peak values were observed at IL-8 concentrations of about 20 nM (Fig 2). Trypsinization of the HaCat monolayers for use as suspension cultures abolished the calcium transients stimulated by IL-8. It is not clear at present whether this effect is due to trypsin sensitivity of the IL-8 receptors on the HaCat cells. Evidence that the induced rises were

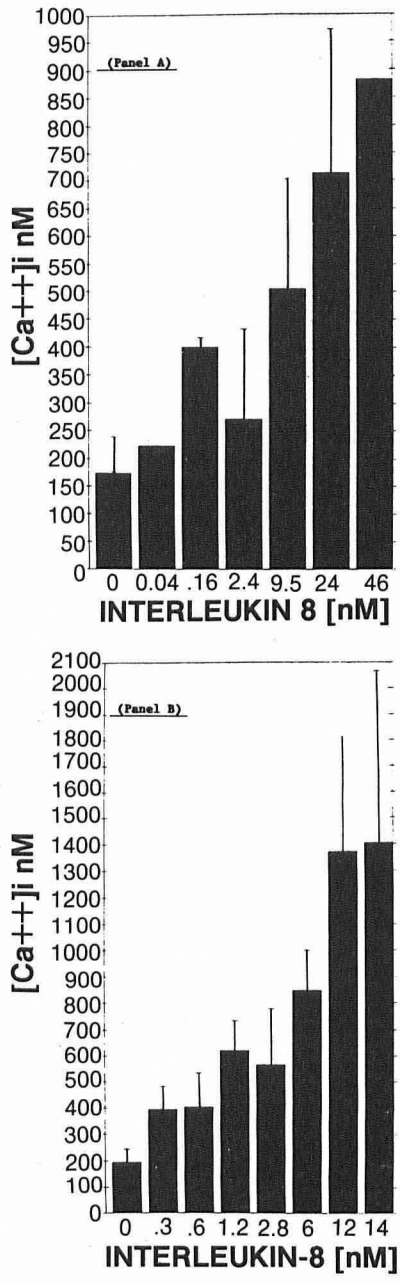

Figure 2. Comparative stimulation of cytosolic free Ca^{++} transients in indo-1-loaded HaCat cells *(A)* and blood neutrophils *(B)* by IL-8. Indo-1-loaded cells in duplicates were stimulated by IL-8 and cytosolic calcium was measured. Neutrophils were isolated from three different donors for every experiment. Results are the mean ± SD of more than six experiments.

an effect of IL-8 rather than contaminants was provided by the use of immunosorbent-purified anti-IL-8 polyclonal antibody. Whereas HaCat cells responded to IL-8 at 24 nM by raising resting calcium levels from 188 ± 23 nM to peak values in excess of 1050 nM, no calcium transients were observed when IL-8 at 24 nM was pre-incubated with excess neutralizing polyclonal anti-IL-8 antibody (2 μg/ml). For example, resting level of $[Ca^{++}]_i$ in HaCat cells measured in the presence of the antibody was 201 ± 12 and in cells stimulated with IL-8 pre-incubated with the antibody was 183 nM. Normal rabbit serum IgG preparation did not influence either the resting or stimulated level of $[Ca^{++}]_i$ in HaCat cells.

Under similar experimental conditions, IL-8 or FMLP stimulated rapid rises in cytosolic free Ca^{++} in neutrophils (Figs 1 and 2) to peak values of about 2000 nM. Also, whereas the fall of peak cytosolic free Ca^{++} to resting levels in HaCat cells was protracted, the fall of calcium to resting levels in the neutrophils was relatively rapid. Again, IL-8-induced Ca^{++} transients in neutrophils were dose-dependent (Fig 2) and could be inhibited by excess neutralizing rabbit polyclonal anti-IL-8 (data not presented).

Characteristics of the Response of HaCat Cells to IL-8 The response of the HaCat cells to IL-8 was transient, i.e., cytosolic free Ca^{++} rose to peak levels and gradually fell over the next few minutes (Fig 1). A second addition of IL-8 had no further effect on the levels of cytosolic free Ca^{++}, i.e., the first addition of IL-8 rendered the cells non-responsive to a second application. Incubation of the HaCat cells with pertussis toxin (1 μg/ml) for 1.25 h at 37°C abolished the responsiveness of the cells to IL-8. Whereas $[Ca^{++}]_i$ rose from resting levels of 211 ± 47 nM to peak levels of 439 ± 103 in control cells stimulated with IL-8 at 2.5 nM, pertussis toxin-treated cells stimulated with a similar concentration of IL-8 showed no calcium transients. Thus, the resting levels of $[Ca^{++}]_i$ of 227 ± 4 nM in pertussis toxin-treated cells remained more or less uninfluenced at 178 ± 93 nM following stimulation with IL-8 at 2.5 nM. These data indicate that guanosine triphosphate binding proteins (G-proteins) are involved in transducing the stimulatory signal of IL-8. Lastly, IL-8 did not cause any detectable rise in cytosolic free Ca^{++} in the absence of extracellular Ca^{++} (data not presented).

Responses of Normal Keratinocytes to IL-8 In Vitro The addition of nanogram quantities of IL-8 also stimulated calcium transients in adherent normal keratinocytes (Fig 1). However, compared to HaCat cells, normal keratinocytes responded weakly to IL-8 stimulatory effects. For example, in response to 6 nM IL-8, cytosolic calcium level increased from a resting level of 269 nM to transient peak value of only 393 nM in normal keratinocytes, whereas transient peak values in excess of 800 nM were recorded in HaCat cells stimulated with similar concentrations of IL-8. The normal keratinocytes also showed significant variability in their responses to the cytokine. Nevertheless, whenever the cells were responsive to IL-8, the addition of excess neutralizing rabbit anti-IL-8 polyclonal antibody blocked Ca^{++} transients. Normal rabbit serum immunoglobulin G (IgG) preparation used as a negative control did not influence calcium transients in keratinocytes (data not presented).

Mitogenic Effect of IL-8 for Normal Keratinocytes The mitogenic effect of IL-8 was assessed by measuring [^{3}H]thymidine incorporation into TCA-precipitable counts. The results in Table I show that normal keratinocytes did not proliferate significantly in KBM alone as assessed by the small amounts of [^{3}H]thymidine incorporated by control cultures. The addition of increasing concentrations of IL-8, however, resulted in three- to sevenfold increase in

Table I. Interleukin-8 Stimulates Proliferation of Normal Foreskin Keratinocytes[a]

Stimulus	Concentration (nM)	[^{3}H] Thymidine Incorporation (CPM ± SD) KBM	KGM
-	-	51.7 ± 40	13,827 ± 3,810
IL-8	0.012	181.3 ± 46	15,878 ± 737
	0.06	164.7 ± 44	17,106 ± 1,981
	0.12	149.3 ± 165[b]	19,549 ± 9,564[b]
	1.2	175.3 ± 34	15,798 ± 2,908
	6.0	163.3 ± 127	16,408 ± 1,685
	12.0	384.0 ± 360	16,545 ± 3,119
EGF	0.016	1273.0 ± 846	15,506 ± 3,888
	0.081	707.7 ± 442	10,139 ± 1,598

[a] Keratinocytes were cultured for 3 d, the indicated medium supplemented with either IL-8 or EGF added and cells were cultivated for a further 5 d. Proliferation was estimated by [^{3}H]thymidine incorporation during the last 24 h of culture. The results (CPM ± SD) are representative of three separate experiments that used cells from two different donors. CPM, counts per minute; SD, standard deviation.

[b] Addition of immunosorbent purified anti-IL-8 antibody reduced counts to control levels, whereas addition of normal rabbit serum IgG had no effect.

Table II. Effect of Interleukin-8 on the Proliferation of HaCat Cells In Vitro[a]

Stimulus	Concentration (nM)	[^{3}H]Thymidine Incorporation (CPM ± SD)[b] KBM	KGM
-	-	88.7 ± 16.8	12,845.3 ± 3,001
IL-8	0.012	117.3 ± 17.5	13,122.0 ± 728
	0.12	137.7 ± 35.5[c]	15,060.7 ± 1,101[c]
	1.2	129.7 ± 13.8	12,663.7 ± 2,589
	12.0	218.0 ± 19.5	11,965.0 ± 740
EGF	0.016	1,497.0 ± 207	11,903.0 ± 372

[a] Experiments were performed as in Table I using HaCat cells plated out at 5×10^3 per well. Representative data of three experiments are shown.
[b] CPM, counts per minute; SD, standard deviation.
[c] Immunosorbent purified anti-IL-8 reduced counts to control levels whereas normal rabbit IgG had no effect.

the amounts of [^{3}H]thymidine incorporated by the keratinocytes. Under similar conditions, EGF increased proliferation by up to 26 times. Evidence to support that IL-8-induced proliferation was due to the mitogenic effect of the cytokine rather than to contaminants in the preparation was provided by the use of immunosorbent purified neutralizing polyclonal anti-IL-8. Whereas normal rabbit serum IgG preparation had no effect on IL-8 stimulated proliferation, anti-IL-8 antibody reduced proliferation rate to background levels as evidenced by [^{3}H]thymidine incorporation. In KGM alone, the proliferation rate of keratinocytes was high. Nevertheless, IL-8 also enhanced the proliferation by 10% to 40%, levels comparable to that observed with EGF.

The mitogenic effects of IL-8 on HaCat cells were also examined (Table II). IL-8 increased [^{3}H]thymidine incorporation by HaCat cells cultivated in KBM dose dependently. Again, purified normal rabbit serum IgG had no effect on IL-8-stimulated [^{3}H]thymidine incorporation, whereas polyclonal anti-IL-8 antibody reduced the cytokine-mediated effects. A slight mitogenic effect was also observed when the cells were cultivated in KGM. The effect, however, was not dose dependent. Under similar conditions, EGF at 0.1 ng/ml also did not promote HaCat cell proliferation as was observed in KBM.

In several experiments, the mitogenic effect of IL-8 was also monitored by direct microscopic determination of cell numbers. IL-8 at 0.12 nM increased HaCat cell counts by 54.8% ± 23.2% over the counts obtained in control cultures. Again, polyclonal anti-IL-8 antibody reduced cell counts to control levels. Compared to the enhancement of [^{3}H]thymidine incorporation of 55.2% ± 40% at IL-8 of 0.12 nM, the effect of IL-8 was similar for both assays. These findings indicate that at the cell density used here catabolism of thymidine did not significantly result in underestimation of the mitogenic effect of IL-8.

DISCUSSION

IL-8 is now thought to play an important role in eliciting the neutrophil and the lymphocyte infiltrates that are characteristic features of early developing and established psoriatic lesions [5-7,30,31]. Support for the potential role of IL-8 in the pathogenesis of inflammatory skin diseases include its potent proinflammatory effects when injected in experimental animals [9,11,12] and volunteers [13,14], and the detection of considerable amounts of biologically active IL-8 and related peptides in psoriatic epidermis [5-7,30,31]. In addition, the presence of the cytokine in other inflammatory diseases, such as rheumatoid arthritis [25,32], and septic shock [10] at levels reflecting disease activity indicates a possible pathogenic role. Furthermore, cells indigenous to the inflamed sites, including fibroblasts, keratinocytes, and endothelial cells, can be stimulated in vitro by IL-1 or TNFα to produce significant amounts of IL-8 [10]. Although local production of the cytokine at inflamed sites is undoubtedly responsible, at least in part, for the cutaneous trafficking and activation of inflammatory cells, the role of IL-8 as an autocrine or paracrine growth factor regulating the characteristic keratinocyte hyperproliferation seen in skin diseases such as psoriasis is as yet unclear.

The results presented here demonstrate that IL-8 raises the $[Ca^{++}]_i$ of keratinocyte-like HaCat cells and normal human foreskin keratinocytes adhering to a substratum. It is not clear whether most of the Ca^{++} mobilized in HaCat cells in response to IL-8 came from an influx of exogenous Ca^{++}, and/or from intracellularly stored Ca^{++}. Whatever the source, the IL-8-stimulated rise in $[Ca^{++}]_i$ was transient, peaked within 30 seconds, and gradually declined to basal levels within 1 to 2 min. Once the $[Ca^{++}]_i$ returned to basal levels, a second addition of the cytokine had no effect (data not presented), but it did not interfere with a further rise in $[Ca^{++}]_i$ when an heterologous agonist such as bradykinin was added. The peak levels of the mobilized $[Ca^{++}]_i$ were dose dependent. The stimulation of human keratinocytes by IL-8 was lower and variable in samples obtained from different volunteers. This might be the reason why the effect of IL-8 on keratinocytes has been demonstrated only in some studies [20-22]. It is also possible that differences in the responsiveness of HaCat and normal keratinocytes were due to culture conditions to which the cell preparations were exposed 48 h prior to analysis. To avoid stratification and detachment from the slide, normal keratinocytes could be grown only in KGM, a medium with a lower calcium concentration than the recommended medium (DMEM) for cell growth [32]. Evidence that the calcium responses observed here were not due to any active contaminants was provided by neutralization of the cytokine with specific immunosorbent-purified anti-IL-8 antibody. Pre-incubation of the IL-8 with excess neutralizing polyclonal anti-IL-8 rendered the preparation ineffective in eliciting the calcium response in the HaCat cells, whereas pre-incubation of the cytokine with normal rabbit serum IgG did not interfere with the stimulatory effect of IL-8.

Two lines of evidence suggest that stimulation of the keratinocytes by IL-8 was induced through receptor-mediated events. Firstly, pre-treatment of the HaCat cells with *Bordotella pertussis* toxin for 1.25 h abrogated the calcium response, suggesting that IL-8 stimulated calcium transients through a pathway involving G proteins [33]. Secondly, trypsinization of the adherent cells for use in suspension cultures abolished the calcium transients. Under similar experimental conditions, specific receptors for IL-8 on the HaCat cells could not be detected (J. Besemer, personal communication). We cannot at present exclude the possibility that the lack of specific binding of IL-8 to epidermal keratinocytes in suspension cultures was due to the low numbers or to the sensitivity of the receptors to trypsin. Nevertheless, the observed desensitization of the cells to second applications of IL-8 could be explained by the presence of IL-8 receptors on keratinocytes.

Recent preliminary reports show that IL-8 is a chemoattractant for HaCat cells [20] and promotes the proliferation of normal keratinocytes in vitro [21,22]. The results presented here confirm the mitogenic effect of IL-8 for HaCat cells and extend these reports by showing that normal human foreskin keratinocytes also respond to IL-8 by mobilization of $[Ca^{++}]_i$ and by enhanced proliferation. The proliferation was best detected after 5 d incubation in KBM. As the level of free calcium in the skin is reported to be low [34], we speculate that locally produced IL-8 could promote, at least in part, the hyperproliferation of epidermal cells in certain cutaneous inflammatory diseases. Evidence supporting this contention come from reports showing that Ca^{++} regulates many aspects of epidermal differentiation [35-37]. Keratinocytes grown in a medium containing $\leq$ 0.1 mM Ca^{++} are phenotypically similar to basal epidermal cells, whereas cells grown in media containing high Ca^{++} ($\geq$ 0.1 mM) have many aspects of suprabasal phenotype in vivo. Taken together, the results suggest that IL-8 has a wider range of target cells than hitherto thought.

Although the precise mechanism(s) by which Ca^{++} can regulate keratinocyte terminal differentiation is unknown, increases in cytosolic free Ca^{++} may activate a series of regulatory enzyme systems that could induce keratinocytes to differentiate irreversibly. Over-

all, the findings reported here suggest that, in the microenvironment of an inflammatory site like a psoriatic lesion, locally produced IL-8 may be an important component in the trafficking of activated inflammatory cells and in maintaining the hyperproliferation of keratinocytes.

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