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Evidence for Multiple Bone Resorption-Stimulating Factors Produced by Normal Human Keratinocytes in Culture*

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ABSTRACT. Conditioned medium from cultured normal human foreskin keratinocytes enhanced the release of calcium from neonatal mouse calvaria in organ culture. Unfractionated keratinocyte-conditioned medium (KCM) stimulated bone resorption in a dose-dependent manner, but it did not increase the concentration of prostaglandin E₂ (PGE₂) in the bone culture medium until a maximal dose of KCM for resorption was used. Furthermore, inhibitors of PGE₂ synthesis, indomethacin, ibuprofen, and piroxicam, did not inhibit KCM-induced calcium release. High concentrations of KCM increased cAMP production by calvaria in the presence of isobutylmethylxanthine, but the increase was small compared with that produced by a dose of bovine PTH that caused a similar level of bone resorption. The bone resorption-stimulating activity of KCM was lost after 56 C for 60 min, but it was lost after heating at 100 C for 10 min. Fractionation of KCM by gel filtration chromatography revealed two distinct peaks of bone resorption-stimulating activity. One peak, KCM₁, caused a significant increase in bone resorption at 2 µg protein/ml. KCM₁ did not increase medium PGE₂, and inhibition of PGE₂ synthesis in bone had no effect on KCM₁-induced bone resorption. KCM₂ failed to increase cAMP production by human osteosarcoma SaOS-2 cells. Another peak, KCM₃, caused a dose-dependent increase in bone resorption, and a significant increase in medium calcium was noted at a 20-fold lower concentration (0.1 µg protein/ml) than with KCM₁. In contrast to KCM₂, the increase in bone resorption stimulated by KCM₃ was accompanied by a parallel increase in the production of PGE₂, and inhibition of PGE₂ synthesis completely inhibited the bone resorption-stimulating activity of KCM₃. KCM₄ also caused an increase in cAMP production by SaOS-2 cells. We conclude that KCM contains at least two distinct bone resorption-stimulating factors, one of which acts via a PG-mediated mechanism and the other by a PG-independent pathway. (Endocrinology 122: 2497–2475, 1988)

KNOWLEDGE of the multiple immune and endocrine functions of epidermal keratinocytes has been evolving rapidly. Keratinocytes secrete various cytokines that may play a role in local immune responses. Among the first of these cytokines from keratinocytes to be studied was an interleukin-1-like (IL-1-like) molecule termed epidermal thymocyte-activating factor (ETAF) (1, 2). Etaf is pyrogenic and has chemotactic activity for neutrophils (3, 4). Keratinocytes also express mRNAs that are homologous or identical to monocyte-derived IL-1α and IL-1β (5, 6). In addition, keratinocytes have been shown to produce a natural killer cell activity-augmenting factor (7), a T cell growth factor (8), and an IL-3-like factor (9), suggesting a complex role for these cells in immunological responses. Although IL-1 has been shown to cause bone resorption in vitro (10, 11), Etaf-induced bone resorption has not been documented.

Recently, normal human epidermal keratinocytes in culture have been found to produce a PTH-like protein (12) that has been characterized by its ability to increase cAMP in PTH target tissues and to be inhibited by the PTH antagonist PTH(3–34). An essential characteristic of any suspected PTH-like protein would be its ability to enhance bone resorption; however, keratinocyte-conditioned medium (KCM) has not been evaluated for bone resorption-stimulating activity. It is possible that factors released by keratinocytes may have a profound effect on local and systemic bone metabolism. Therefore, we have characterized the action of KCM on bone resorption. We have found that conditioned medium from normal human epidermal keratinocytes in culture markedly stimulates bone resorption in vitro. Furthermore, we have partially separated two distinct factors released by keratinocytes that cause bone resorption. One of these factors induces bone resorption via a prostaglandin (PG)-
dependent mechanism, while the other mediates resorption by a PG-independent pathway.

**Materials and Methods**

**Cell culture**

Normal human foreskin keratinocytes were grown by standard procedures (13). Briefly, the cells were cultivated on an irradiated 3T3 feeder layer in Dulbecco-Vogt Eagle’s Medium supplemented with hydrocortisone (0.4 μg/ml), epidermal growth factor (10 ng/ml), insulin (5 μg/ml), transferrin (5 μg/ml), adenine (0.1 mM), T3 (20 pm), and fetal bovine serum (5%). Four-day-old medium was collected from the cell cultures that were near or at confluence. This KCM was pooled and frozen at -20° C for later experimentation. Serum-free KCM (without fetal bovine serum or epidermal growth factor) as well as serum-containing KCM without epidermal growth factor were collected as described above.

The human osteosarcoma cell line SaOS was grown in monolayer culture, as described previously (14, 15).

**Organ culture of bone**

Neonatal mouse calvariess were placed in organ culture as free-floating bones, as previously described (16, 17). The medium was Dulbecco’s Modified Eagle’s Medium supplemented with 15% heat-inactivated (60 C for 1 h) horse serum. Incubation of half-calvaria (by sagittal sectioning into two symmetrical portions) was performed at 37 C in a roller drum apparatus under an atmosphere of 90% O2, 5% CO2, and 5% N2. Calvaria were preincubated for 24 h before experimental treatment. The medium was changed at this time, and fresh control medium or medium containing specific treatments was then added. Bone resorption was determined by measuring the release of calcium (45Ca2+) from the calvaria into the culture medium 72 h after addition of the test agents.

**Measurement of calcium**

The concentration of total calcium in the bone culture medium was measured in 400-μl samples by a calcium-selective electrode using a NOVA 7 + 7 automatic calcium analyzer (NOVA Biomedical, Waltham, MA).

**Measurement of PGE2**

PGE2 produced by the bones and released into the culture medium was measured by RIA, as described previously in detail (18).

**Measurement of cAMP**

Calvaria were prepared and cultured as described above. After incubation for 24 h, the medium was removed, and calvaria were rinsed in serum-free Dulbecco’s Medium. Washes were discarded. Serum-free control medium or medium containing specific test agents was added, and incubations were continued for 1 h (37 C; 50% O2, 5% CO2, and 45% N2). Serum-free medium used for the 1-h incubation contained 3 μM isobutylmethylxanthine (IBMX). After the 1-h incubation, medium samples were collected, placed in a boiling water bath for 5 min, and stored at -20° C. The cAMP concentration was measured by RIA (14, 19).

SaOS-2 cells were grown to confluence in 16-mm culture wells. Growth medium was aspirated, and the cells were washed twice with Hank’s Balanced Salt Solution (HBSS). HBSS with 400 μM IBMX in the absence or presence of various treatment agents was then added to the cells. The cells were incubated for 20 min (37 C; 95% air and 5% CO2). After incubation, the medium was collected, boiled for 5 min, and stored at -20° C until measurement of cAMP by RIA (14, 19).

**Gel chromatography**

KCM was concentrated 10- to 15-fold by lyophilization and then chromatographed at 4°C on a siliconized Bio-Gel P-60 (2.5 × 110 cm; Bio-Rad Laboratories, Richmond, CA) column. The column was eluted using a 0.15-μM NaCl solution containing 0.02 M HEPES, pH 7.4. The eluate was collected in 5-ml fractions, and the absorbance at 280 nm was determined for each fraction. The fractions were stored at -20° C until assay.

**Measurement of protein**

The protein concentration in all samples was determined using a bicinchoninic acid (Pierce Chemical Co., Rockford, IL) assay (20).

**Statistical analysis**

Results of each experiment were subjected to an analysis of variance, and the SE values were calculated from the residual error term of that analysis.

**Materials**

Swiss albino mice (CD-1 strain) were obtained from the Harvard Animal Resource Center. Media and sera were obtained from Gibco (Grand Island, NY) with the exception of Dulbecco’s Modified Eagle’s Medium which was from M. A. Bioproducts (Walkersville, MD). Indomethacin, ibuprofen, and IBMX were obtained from Sigma Chemical Co. (St. Louis, MO). Bovine PTH (bPTH; 190 U/mg) was used as a partially purified material from Wilson Laboratories (Chicago, IL). Piroxicam was a gift from Pfizer Pharmaceuticals (Groton, CT). Radioactive tracer for cAMP, adenosine 3’,5’-cyclic phosphoric acid [125I]2’-O-succinyl (>150 Ci/mmol) was purchased from ICN (Irvine, CA). Anti-cAMP was obtained from Miles (Elkhart, IN).

**Results**

**Actions of KCM on bone in organ culture**

KCM from confluent cultures of normal human foreskin keratinocytes stimulated bone resorption in neonatal mouse calvaria in organ culture. Unfractionated KCM elicited a large increase in calcium release from calvaria, but there was no associated increase in PGE2 accumu-
lation in the bone culture medium (Fig. 1A). To control for the effects of EGF and other factors present in the keratinocyte growth medium, conditioned medium from keratinocytes maintained at confluence without EGF or without serum was tested for bone resorption-stimulating activity. The activity of KCM without added EGF did not differ quantitatively from that prepared in the presence of EGF (Fig. 1A). The results in Fig. 1B show that serum-free KCM lacking EGF also contained bone resorption-stimulating activity. Thus, the activity in KCM is not derived from EGF or serum, and these medium supplements are not essential for the production and release of bone resorption-stimulating factors from keratinocytes. KCM diluted in bone culture medium is not acidic; therefore, the effect of KCM on calcium release from bone is not due merely to solubilization of mineral at low pH.

KCM, KCM without EGF, and serum-free KCM did not enhance the release of PGE₂ by mouse calvaria (Fig. 1), suggesting that KCM-induced bone resorption does not proceed via a PG-mediated mechanism. However, it was possible that local production of PGE₂ by certain bone cells that could not be detected by measurement of PGE₂ accumulation in bulk medium might still be the mechanism by which KCM stimulates bone resorption. Such a mechanism has indeed been described recently in this culture system for tumor necrosis factor by Tashjian, Jr., et al. (21). To test this possibility the following experiments were performed. Indomethacin, a well characterized inhibitor of PGE₂ synthesis in mouse calvaria, was added to the bone culture medium before treatment with KCM. Indomethacin had no inhibitory effect on the bone resorption-stimulating activity of KCM (Fig. 2).

Furthermore, two other inhibitors of PG synthesis (ibuprofen and piroxicam) also failed to decrease KCM-induced bone resorption despite the fact that they all prevented the small increase in PGE₂ production induced by KCM in this experiment (Table 1).

Several stimulators of bone resorption, such as PTH, vasoactive intestinal peptide, and exogenous PGE₂, act via a cAMP-mediated mechanism. Therefore, KCM was tested for its ability to acutely increase cAMP accumulation in the medium and mouse calvaria. KCM at near-maximal concentrations for bone resorption-stimulating activity did cause a small but significant (P < 0.01) increase in cAMP production by mouse calvariae (Fig. 3). Bones treated with PTH, at a dose that caused comparable bone resorption, induced a large increase in medium cAMP. At a lower concentration (150 μg protein/ml) of KCM no increase in cAMP accumulation above the control value was noted. This lower concentration, however, caused significant bone resorption (Fig. 4). In addition, KCM did not contain a factor that inhibited the production of cAMP induced by PTH (Fig. 3, last column).

Dose response of KCM

Crude KCM increased bone resorption in a dose-dependent manner, reaching a maximum at 500 μg protein/ml (Fig. 4). KCM caused a significant increase in bone resorption at 75 μg protein/ml. An increase in medium PGE₂ induced by KCM was not noted until bones were incubated with 500 μg protein/ml KCM, a concentration that caused maximal stimulation of bone resorption. However, lower concentrations of KCM in-

![Graph](image-url)
BONE-RESORBING FACTORS FROM KERATINOYCTES

Fig. 2. KCM-induced bone resorption is not inhibited by indomethacin (INDO). KCM at a concentration of 300 µg protein/ml bone culture medium caused a significant increase in the medium Ca²⁺ concentration (□) compared with the control value in a 3-day incubation period. Addition of 400 ng/ml indomethacin did not inhibit the KCM-induced increase in medium Ca²⁺. The medium PGE₂ concentration (●) did not vary from the control value in any of the treatment groups. Each bar gives the mean value, and the brackets give the SE for four bones per group. **, Significantly different from control ($P < 0.01$).

Table 1. Actions of inhibitors of PG synthesis on KCM-induced bone resorption

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Medium Ca²⁺ (mg/dl)</th>
<th>Medium PGE₂ (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>7.5 ± 0.26</td>
<td>0.34 ± 0.11</td>
</tr>
<tr>
<td>KCM</td>
<td>12.9 ± 1.2*</td>
<td>1.04 ± 0.14*</td>
</tr>
<tr>
<td>KCM + indomethacin</td>
<td>12.7 ± 1.5*</td>
<td>0.07 ± 0.01*</td>
</tr>
<tr>
<td>KCM + ibuprofen</td>
<td>12.5 ± 1.8*</td>
<td>0.59 ± 0.27*</td>
</tr>
<tr>
<td>KCM + piroxicam</td>
<td>12.4 ± 2.0*</td>
<td>0.10 ± 0.01*</td>
</tr>
</tbody>
</table>

Values are the mean ± SE (three or four bones per group; 72-h treatment). Neonatal mouse calvariae were incubated with crude KCM at 300 µg protein/ml. Indomethacin, ibuprofen, and piroxicam were used at 200 ng/ml.

* Significantly greater than no treatment control ($P < 0.01$).
* Significantly less than control ($P < 0.05$).
* Significantly less than KCM alone ($P < 0.05$).

Fig. 3. Effects of KCM on cAMP release by mouse calvariae. After a 24-h preincubation, calvariae were washed twice in serum-free medium containing 3 µM IBMX. Test and control media with IBMX were then added to the bones and incubated for 60 min at 37°C. Medium was then removed, boiled for 5 min, and assayed for cAMP by RIA. KCM at 300 µg protein/ml increased cAMP significantly, but the increase was small compared with the large increase in cAMP induced by 2 U/ml PTH. KCM (150 µg protein/ml) did not increase medium cAMP. Addition of both KCM (300 µg protein/ml) and PTH (2 U/ml) to mouse calvariae did not alter medium cAMP levels from those observed with PTH alone. Each bar gives the mean value, and the brackets give the SE for three bones per group. **, Significantly different from control ($P < 0.01$).

Crude KCM was not due to a nonspecific physicochemical influence on calcium mobilization.

Partial purification of KCM

KCM was fractioned by gel filtration using Bio-Gel P-60. Fractions (5 ml each) were collected and pooled; protein concentrations were determined and then assayed for bone resorption-stimulating activity at a standard protein concentration. In addition, these fractions were assayed for their ability to stimulate PGE₂ production by bone. The biological activity did not elute with the majority of the protein present in KCM. In fact, the active fractions contained less than 1% of the total protein. Two distinct peaks of bone resorption-stimulating activity were obtained (Fig. 5). Fractions 51–60 (termed KCM₁), with an approximate mol wt of 10,000–20,000, contained substantial activity. The bone resorption stimulated by KCM₁ was not associated with an increase in medium PGE₂ (Figs. 5 and 6). Fractions 81–90 (KCM₁₁), of lower mol wt range (estimated 3,000–6,000), also increased bone resorption. In contrast to the activity of KCM₁, KCM₁₁ markedly increased the production of PGE₂ (Figs. 5 and 7). KCM₁ elicited bone resorption in a dose-dependent manner, showing significant activity at 2 µg protein/ml. No increase in medium PGE₂ was noted at any concentration of KCM₁ used (Fig.

Stability of bone resorption-stimulating activity

The bone resorption-stimulating activity in crude KCM was not lost after repeated freezing and thawing or after heating at 56°C for 60 min (data not shown). However, the activity was decreased markedly when KCM was heated for 10 min at 100°C (Table 2).

Maximal concentrations of KCM had no effect on medium calcium in devitalized bones, while the same concentration of KCM caused a significant increase in medium calcium in vital bones (Table 3). These results indicate that the bone resorption-stimulating activity in

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FIG. 4. Dose response for KCM on bone resorption and PGE_2 production. KCM was concentrated by lyophilization and then dialyzed (3500 mol wt cut-off) against 0.02 M HEPES buffer before addition to the bone culture medium. KCM increased bone resorption significantly above control at 75 μg protein (P)/ml, while a significant increase in PGE_2 was first observed only at 500 μg protein/ml KCM. Each point gives the mean ± SE for five bones per group. SE bars for the control point in the lower panel is contained within the symbol. **, Significantly greater than control (P < 0.01).

TABLE 2. Heat stability of the bone resorption-stimulating activity of KCM

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Medium Ca^{2+} (mg/dl)</th>
<th>Medium PGE_2 (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>7.5 ± 0.29</td>
<td>&lt;0.12</td>
</tr>
<tr>
<td>KCM</td>
<td>11.2 ± 1.0*</td>
<td>0.29 ± 0.09</td>
</tr>
<tr>
<td>KCM (100 C; 10 min)</td>
<td>7.7 ± 0.08</td>
<td>0.15 ± 0.01</td>
</tr>
</tbody>
</table>

Values are the mean ± SE (four bones per group; 72-h treatment). Neonatal mouse calvaria were incubated with crude KCM at 300 μg protein/ml either fresh or heated to 100 C for 10 min.

*Significantly greater than no treatment control (P < 0.01).

6). KCMII elicited a dose-dependent increase in bone resorption, with significant activity at a concentration as low as 100 ng protein/ml. Thus, KCMII is 10- to 20-fold more potent than KCMI at this stage of partial purification. In addition, KCMII caused a dose-dependent increase in medium PGE_2 (Fig. 7). A slight increase in bone resorption-stimulating activity was noted in fractions 10–24, but the increase was not significant (Fig. 5).

To determine if the bone resorption-stimulating activity in KCMII and KCMIII was mediated by PGE_2, indomethacin was used to inhibit the synthesis of PGs. When bones were treated with KCMII plus indomethacin, the activity was unaffected (Fig. 8). In contrast, the bone resorption-stimulating activity of KCMII was inhibited completely by indomethacin (Fig. 8). These results suggest that the activity of KCMII is mediated via a PG-

TABLE 3. KCM does not cause calcium release from devitalized bone

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Medium Ca^{2+} (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VITAL BONES</td>
<td>Devitalized bones</td>
</tr>
<tr>
<td>None</td>
<td>7.7 ± 0.32</td>
</tr>
<tr>
<td>KCM</td>
<td>9.8 ± 0.47*</td>
</tr>
<tr>
<td>PTH</td>
<td>11.0 ± 0.93*</td>
</tr>
</tbody>
</table>

Values are the mean ± SE (five bones per group; 72-h treatment). Neonatal mouse calvaria were devitalized by repeated freezing and thawing and then treated in the same manner as the vital bones. The calvaria were incubated with crude KCM (600 μg protein/ml) or PTH (2 U/ml).

*Significantly greater than no treatment control (P < 0.01).
dependent pathway, while KCMI acts via a PG-independent mechanism. Moreover, these findings indicate that KCMI and KCMII represent two different bone resorption-stimulating factors. Neither KCMI nor KCMII contains immunoreactive epidermal growth factor (assay generously performed by Dr. David Orth).

**Actions of KCM on the production of cAMP**

Both KCMI and KCMII were studied for their ability to enhance cAMP production by the human osteosarcoma cell line SaOS-2. These osteoblast-like cells increase cAMP in response to agents such as PTH and vasoactive intestinal peptide (14, 22). At concentrations that caused maximal bone resorption, KCMI did not increase cAMP release by SaOS-2 cells (Fig. 9A). In contrast, KCMII (0.5 μg protein/ml) elicited a 5-fold increase in medium cAMP. KCMII, at concentrations as low as 0.1 μg protein/ml, caused a significant increase in cAMP release from SaOS-2 cells (Fig. 9B). Addition of indomethacin to KCMII completely inhibited the stimulated release of cAMP from the cells (Fig. 9B). This finding suggests that KCMII-induced stimulation of cAMP production was mediated indirectly via the production of PGs. Indomethacin does not inhibit PTH-stimulated production of cAMP.

**Discussion**

Keratinocytes, the predominant proliferating cell type in the epidermis, have been shown to produce several cytokines that may play an important role in the immunological function of the skin. The results of the present study have shown that cultured normal human keratinocytes also produce at least two distinct factors that cause bone resorption. These bone resorption-stimulating factors are produced by keratinocytes even in the absence of serum. The bone resorption-stimulating activity in the crude KCM was not dependent on PG production and only increased cAMP in bones to a small degree compared with PTH. Fractionation of KCM revealed two distinct peaks of biological activity, one at 10,000–20,000 daltons called KCMI and one at 3,000–6,000 daltons called KCMII. Although both KCMI and KCMII cause bone resorption, they do so by different mechanisms. These findings suggest the presence of two separate factors, rather than KCMII being a degradation product of KCMI.

KCMI stimulates bone resorption in neonatal mouse calvariae via a PGE2-independent pathway. KCMI does not increase cAMP production in SaOS-2 cells, unlike certain other PGE2-independent bone resorption-stimulating agents, such as PTH and vasoactive intestinal...
peptide. In contrast, KCMII stimulates an increase in the production of PGE2 in mouse calvaria, and the bone resorption-stimulating activity of KCMII is inhibited completely by doses of indomethacin that are not non-specific inhibitors of resorption (22). These results indicate that KCMII induces bone resorption via a PGE2 mediated mechanism. In addition, these data suggest that the majority of the bone resorption-stimulating activity measured in crude KCM is derived from KCMII. At high concentrations of crude KCM, bone cell PGE2 production was markedly increased, suggesting that KCMII was now being detected. Thus, KCMII appears to be released from keratinocytes in greater quantity (or with less degradation) than KCMII, although KCMII at the present stage of purification is the more potent bone-resorbing factor. KCMII also stimulated a significant, although small, increase in cAMP production by SaOS-2 cells, similar to the increase in cAMP in bones produced by the crude KCM. The increase in cAMP was inhibited completely by indomethacin, indicating that KCMII causes the increase in cAMP indirectly via the local production of cyclooxygenase products, presumably PGs.

Other investigators have reported that medium conditioned by human keratinocytes causes an increase in cAMP levels in the rat osteosarcoma cell line ROS17/2.8 (12). This increase in cAMP was inhibited by the PTH antagonist PTH(1-34), suggesting that PTH and PTH(1-34) the PTH-like molecule may act on the same receptor; however, antibody inhibition studies show the PTH-like protein to be distinct from native PTH (12). It is possible, but unlikely, that KCMII is responsible for this PTH-like activity, as it does cause an increase in cAMP levels in cultured osteosarcoma cells. However, KCMII-induced increases in cAMP are PG mediated while, in contrast, PTH-induced increases in cAMP are not mediated by PGs (22). If the PTH-like protein acts through the native PTH receptor, it is unlikely that the PTH-like protein-induced increase in cAMP is mediated via PG production. Therefore, at this stage of purification, it appears that KCMII is distinct from the PTH-like protein. Investigators studying tumors associated with the humoral hypercalcemia of malignancy have recently purified and sequenced a PTH-related protein (25-28). As many of these tumors appear to be keratinocytes.
ocyte-like (29), it is possible that the PTH-related peptide is also expressed in normal epidermal cells. Additional studies are needed to determine if KCM_{IL}, which is produced by normal human keratinocytes, is related to this PTH-like molecule or whether it is a separate and distinct bone resorption-stimulating factor produced by keratinocytes.

Alternatively, it has been suggested that human squamous cell carcinomas associated with humoral hypercalcemia of malignancy produce an IL-1α-like molecule that is responsible for in vitro bone resorption but does not PTH-like activity (30, 31). It is possible that KCM_{IL}, which is produced by normal human keratinocytes, is an IL-1α-like molecule. The two factors derived from normal human keratinocytes, KCM_{IL} and KCM_{II}, might be expressed to varying degrees by neoplastic keratinocytes. This could account for the findings of PTH-like and/or IL-1-like factors derived from different squamous cell carcinomas. Recently, human keratinocytes were shown to produce and release (32) transforming growth factor-α (TGFα). TGFα has been shown to cause bone resorption in neonatal mouse calvaria in organ culture by a PG-mediated mechanism (33). Therefore, a portion of the bone resorption-stimulating activity of KCM_{II} could be due to TGF release by keratinocytes.

It is now clear that keratinocytes are a very dynamic cell type that produce not only immunologically active cytokines but also a PTH-related peptide and other bone resorption-stimulating factors. These factors are of different mol wt and act by two different mechanisms to enhance bone resorption. Although the pathophysiologic role keratinocytes may play in bone metabolism and systemic calcium homeostasis is yet to be determined, the fact that keratinocytes produce and release multiple bone resorption-stimulating factors suggests that these cells may play a significant role in bone physiology.

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