The Insulin-like Growth Factor I Receptor Is Overexpressed in Psoriatic Epidermis, but Is Differentially Regulated from the Epidermal Growth Factor Receptor

By Jeffrey F. Krane, Alice B. Gottlieb, D. Martin Carter, and James G. Krueger

From the Laboratory for Investigative Dermatology, The Rockefeller University, New York, New York 10021

Summary

Insulin-like growth factor I (IGF-I)/somatomedin C is an important mediator of keratinocyte growth in vitro, and the expression of IGF-I receptors in the basal layer of normal epidermis suggests that this growth pathway may function in the regulation of keratinocyte growth in vivo as well. The pattern of IGF-I receptor expression in normal skin is distinct from that of the epidermal growth factor (EGF) receptor, suggesting that these receptors might be differentially regulated. The purpose of this study was to obtain a better understanding of IGF-I receptor function in the skin by examining IGF-I receptor expression in psoriatic epidermis and in cultured human keratinocytes. Our findings indicate that IGF-I receptor expression is increased in psoriasis as measured by protein tyrosine kinase assays of biopsy extracts and by immunohistochemical staining with an IGF-I receptor–specific monoclonal antibody. Unlike EGF receptor expression, which is also increased in psoriatic epidermis, the pattern of IGF-I receptor expression corresponds closely with the increased size of the keratinocyte proliferative compartment in psoriasis. Biochemical agents that diminish EGF receptor ligand binding (phorbol ester or calcium ionophore treatment) produce opposite effects on the IGF-I receptor. These results suggest that cellular expression and differential regulation of both growth factor receptor systems may control critical aspects of epidermal proliferation or function.

Insulin-like growth factor I (IGF-I),[1] previously termed somatomedin C, is a 7.5-kD polypeptide that circulates in plasma in high concentrations and is detectable in most tissues (1). IGF-I, insulin, and IGF-II comprise a family of structurally related hormones that, nonetheless, produce distinct metabolic effects through interactions with unique cell surface receptors (2, 3). IGF-I functions predominantly as a mitogenic factor for a variety of cell and tissue types, unlike insulin and IGF-II, which serve predominantly as anabolic hormones, regulating glucose and mannose-6-phosphate intracellular transport, respectively (3). The importance of IGF-I to growth of postembryonic tissues is suggested by increasing plasma concentrations throughout adolescence, reaching a plateau in adults, and in the requirement of most mammalian cell types for IGF-I for sustained proliferation (1).

The IGF-I receptor is composed of two subunits: α, a 125-kD protein that is entirely extracellular and functions in ligand binding, and β, a 95-kD transmembrane protein, with extracellular and cytoplasmic domains. The IGF-I receptor is synthesized as a single chain propeptide that undergoes glycosylation, proteolytic cleavage, and assembly into a 350-kD heterodimer of both subunits ($\alpha_2\beta_2$) (4). The IGF-I receptor exhibits high affinity for binding of IGF-I, low affinity for binding of insulin, and intermediate affinity for binding of IGF-II (2). Thus, depending on ligand concentration, each of these factors has the potential to activate the IGF-I receptor after binding. Mitogenic signaling after ligand binding is produced by activation of tyrosine kinase enzymatic activity associated with the cytoplasmic portion of the IGF-I receptor β subunit. Although the insulin receptor possesses a structurally similar tyrosine kinase domain, the mitogenic properties of the IGF-I receptor are specific to unique sequences in its tyrosine kinase region (5). Like expression of the IGF-I ligand, tissue expression of the IGF-I receptor is also developmentally regulated (6).

The roles of IGF-I and its receptor in regulation of skin structure and function are only partly understood. Epidermal

[1] *Abbreviations used in this paper:* BB, binding buffer; EGF, epidermal growth factor; FGF, fibroblast growth factor; IGF-I, insulin-like growth factor I; KBM, keratinocyte basal medium; KGF, keratinocyte basal medium.

 J. Exp. Med. © The Rockefeller University Press • 0022-1007/92/04/1081/10 $2.00
Volume 175 April 1992 1081–1090

keratinocytes (7–9) and a variety of dermal cell types, such as fibroblasts and vascular elements, express receptors for IGF-I (10, 11). In connective tissue cells, such as fibroblasts, IGF-I regulates cell proliferation in conjunction with platelet-derived growth factor or other mitogens (12). In keratinocytes, IGF-I regulates proliferation via synergistic interactions with epidermal growth factor (EGF) or fibroblast growth factor (FGF)-like hormones (9, 13). The IGF-I receptor is localized in the basal epidermal layer of normal human skin, where it could serve to regulate keratinocyte proliferation in conjunction with EGF or other mitogens (9). However, the expression of the IGF-I receptor in human epidermis is distinct from that of the EGF receptor, which is located in both spinous and basal epidermal compartments (9, 14, 15). To better understand the regulation and function of the IGF-I receptor in human skin, we have studied IGF-I receptor expression in psoriatic tissue and in cultured keratinocytes. We report that IGF-I receptor expression is increased in psoriatic epidermis, paralleling the increased size of the epidermal proliferative compartment, and that IGF-I receptor expression is differentially regulated in human keratinocytes compared with expression of the EGF receptor.

Materials and Methods

Reagents. Reagents were obtained from Sigma Chemical Co. (St. Louis, MO), except where noted.

Receptor Binding Studies. Receptor binding assays were done as previously described (9). Briefly, cultured human neonatal foreskin keratinocytes were grown in keratinocyte growth medium (KGM; Clonetics Corp., San Diego, CA) to ~75% confluence in 24-well tissue culture plates. For experiments performed in KGM, compounds were added in KGM for the indicated times and the plates were then washed twice for 90 min at 37°C with keratinocyte basal medium (KBM; Clonetics Corp.) before the assay. For analysis of receptor binding in KBM, plates were transferred to KBM for 24 h before the addition of compounds in KBM. Added compounds were 10 ng/ml PMA (LC Services Corp.; Woburn, MA), 10 ng/ml 4α-PMA (LC Services Corp.), 1.15 mM $CaCl_2$, and the Ca^{2+} ionophores ionomycin (1 μM) and A23187 (0.1 μM). Plates were placed on ice and washed once with cold binding buffer (BB: KBM, 5 mg/ml BSA, 20 mM Hepes, pH 7.3) followed by the addition of 0.25 ml/well of ^{125}I-EGF (Amersham Corp., Arlington Heights, IL; sp act, 100 μCi/μg) or ^{125}I-IGF-I (Amersham Corp.; sp act, 2,000 Ci/mmol) in BB to each well. Plates were transferred to a rocking incubator at 4°C for 6 h and then washed four times with BB. Cells were lysed in 0.1 M NaOH/0.1% Triton X-100, and counts were read in a gamma counter. Nonspecific binding was measured with a 5,000-fold excess of unlabeled EGF or IGF-I (PeproTech Corp., Rocky Hill, NJ) and did not exceed 20% of total binding. Receptor binding inhibition experiments were performed as described above with 1 ng/ml ^{125}I-IGF-I mixed with unlabeled IGF-I, insulin (Clonetics Corp.), or mAb α-IR-3. mAb α-IR-3 was a generous gift of Dr. Steven Jacobs (Wellcome Research Laboratories, Research Triangle Park, NC). Each value represents mean specific binding of triplicate measurements.

Immunohistochemistry. Immunoperoxidase staining of cryostat sections of skin from 18 psoriatic patients was performed with mouse mAb α-IR-3, as described previously (9), using 3-amino-9-ethylcarbazole as the developing reagent.

Tyrosine Kinase Assays. Split-thickness skin biopsies from three psoriatic patients were homogenized in 0.5 ml of lysis buffer (LB, 20 mM Tris, pH 8.0, containing 150 mM NaCl, 10% glycerol, 1% Triton X-100, 1 mM PMSF, 0.15 U/ml aprotinin, and 1 mM sodium orthovanadate) using a motor-driven Teflon pestle. After homogenization, insoluble material was removed by centrifugation in a microfuge for 15 min at 4°C. Protein concentration was determined using the protein assay kit according to the manufacturer's instructions (Bio-Rad Laboratories, Richmond, CA), and 60 μg of each protein sample was immunoprecipitated with 1 μl α-IR-3 or 1 μl rabbit anti-EGF receptor antibody (a generous gift of Dr. Stuart Decker, The Rockefeller University) at 4°C overnight. Subconfluent cultured neonatal keratinocytes grown in KGM were treated for 24 h with KBM before lysis. 30 min before lysis, the medium was changed to KBM alone or containing 100 ng/ml IGF-I and reincubated at 37°C. Cultured keratinocytes were then lysed in 1 ml LB/plate, and ~300 μg protein was immunoprecipitated with 2 μl α-IR-3 overnight. For EGF receptor immunoprecipitation from cultured keratinocytes, cells grown in KGM were lysed as described above and immunoprecipitated with 1 μl rabbit anti-EGF receptor at 4°C overnight. Immunoprecipitates were then bound for 4 h to protein A–Sepharose beads alone (for EGF receptor immunoprecipitations) or that had been preincubated with rabbit anti–mouse IgG (Organon Teknika-Cappel; Malvern, PA). Immunoprecipitates were washed once with cold PBS, twice with 0.5 M LiCl in 20 mM Tris, pH 8.0, and once with kinase buffer (50 mM Tris, pH 7.4, 10 mM $MnCl_2$). The beads were resuspended in 40 μl kinase buffer and the kinase reaction was allowed to proceed for 15 min at room temperature after the addition of 1 μl γ-[^{32}P]ATP (Amersham Corp., sp act, 6,000 Ci/mmol). The reaction mixtures were washed twice with 1 ml kinase buffer and then boiled 5 min in 50 μl 2× SDS sample buffer. Proteins were subjected to electrophoresis in a 7.5% SDS-PAGE gel, and gels were fixed, dried, and exposed at −70°C using XAR-5 film (Eastman Kodak Co., Rochester, NY) and Cronex intensifying screens (DuPont Co., Wilmington, DE). Gels of psoriatic skin sample kinase reactions after immunoprecipitation with EGF receptor and IGF-I receptor antibodies were both exposed for 2 d.

Results

mAb α-IR-3 Recognizes the IGF-I Receptor in Human Keratinocytes. The mAb α-IR-3 was raised to IGF-I receptors purified from human placental membranes (16). α-IR-3 reacts with an antigenic site on the extracellular α subunit (16), and its specificity has been demonstrated in a variety of cell types (16–19). The reactivity of α-IR-3 with basal keratinocytes in normal human epidermis has been previously reported (9). To further substantiate the ability of α-IR-3 to recognize the IGF-I receptor in human keratinocytes, radioreceptor competition and immunoprecipitation experiments were performed with cultured normal human keratinocytes. Fig. 1 displays results obtained with radioreceptor competition experiments. In these experiments, the ability of IGF-I, insulin, and α-IR-3 to inhibit binding of ^{125}I-IGF-I was measured on keratinocyte monolayers. Unlabeled IGF-I displaced 90% binding of radiolabeled IGF-I, whereas a 1,000-fold higher insulin concentration only displaced 57% of binding of IGF-I, consistent with the lower affinity of insulin for the IGF-I receptor and results obtained previously in other cell types

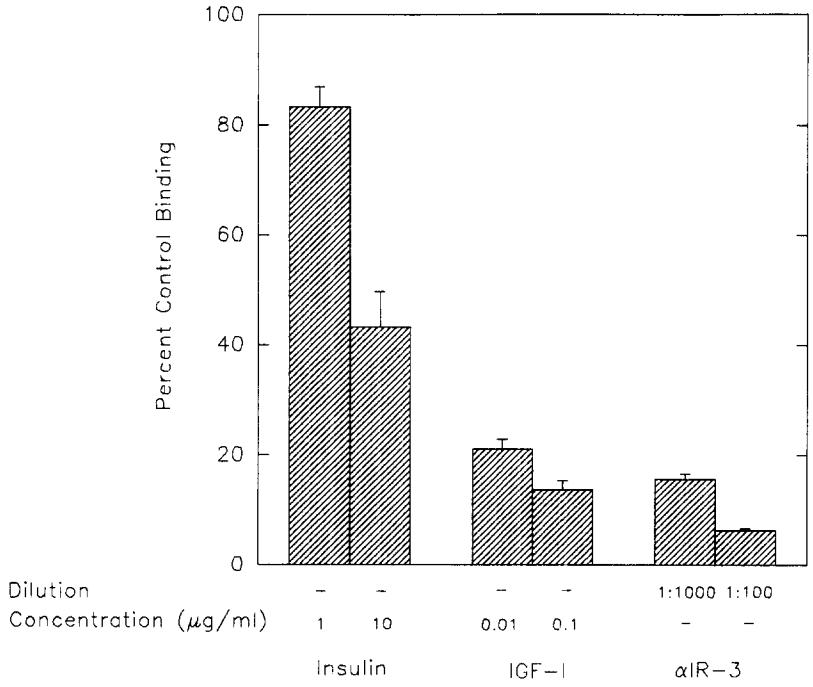

Figure 1. mAb α-IR-3 inhibits binding of ^{125}I-IGF-I to cultured human keratinocytes. ^{125}I-IGF-I (1 ng/ml) binding was measured in the presence of the indicated concentrations of insulin, IGF-I, and α-IR-3. Data are expressed as the percentage of counts bound in the condition tested/counts bound in KBM alone.

(2). α-IR-3 displayed an ability to inhibit binding of ^{125}I-IGF-I to its receptor to nearly the same extent as IGF-I (Fig. 1).

The specificity of α-IR-3 binding to the IGF-I receptor was also confirmed by immunoprecipitation of the receptor from keratinocyte lysates followed by autophosphorylation in an in vitro kinase reaction (Fig. 2). The 95-kD β subunit of the IGF-I receptor was detected only when cells were briefly exposed to IGF-I before immunoprecipitation (Fig. 2, lanes *B* and *C*).

Analysis of IGF-I Receptor Expression in Psoriatic Epidermis by Immunohistochemistry. Since activation of the IGF-I receptor by IGF-I or supraphysiological insulin concentrations is obligate for keratinocyte mitogenic activation by EGF (9, 20) or bFGF (20), the expression of the IGF-I receptor may define the potential proliferative pool of keratinocytes in epidermal tissue (9). As the proliferative keratinocyte population extends to suprabasal keratinocytes in lesional psoriatic epidermis (21), the expression of IGF-I receptors was compared in lesional and nonlesional psoriatic epidermis by immunohistochemistry with the α-IR-3 antibody. Biopsy samples of active psoriatic plaques and normal-appearing nonlesional skin from 18 patients were sectioned on a cryostat microtome and were reacted with α-IR-3 antibody. Representative results from this analysis are shown in Fig. 3. In normal human epidermis, plasma membrane staining by α-IR-3 (which presumably represents receptors capable of ligand binding or activation) is confined to basal keratinocytes (9). In contrast, acanthotic epidermis from lesional psoriatic plaques shows plasma membrane staining with α-IR-3 in both basal keratinocytes and in lower spinous keratinocytes (Fig. 3, *B–D*), corresponding to the increased proliferative keratinocyte compartment in lesional psoriatic epidermis (21). Biopsy tissue from 18 individuals with active psoriatic lesions consistently showed this pattern of IGF-I receptor staining at cell surfaces of basal and lower spinous epidermal layers. Diffuse, cytoplasmic staining was variably seen in mid and upper spinous keratinocyte layers. IGF-I receptor expression was also studied in normal appearing, nonlesional skin from 15 of these patients with active psoriatic lesions (Fig. 3 *A*). Cell surface IGF-I receptor expression in 13 of 15 tissue samples (87%)

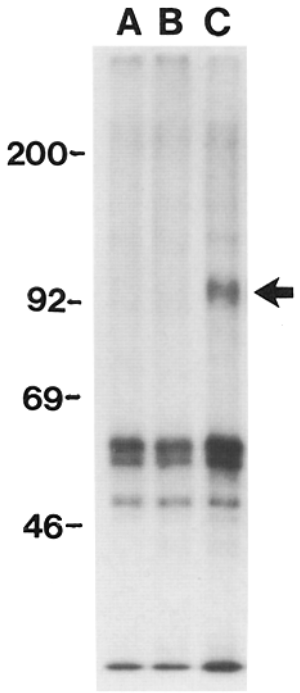

Figure 2. mAb α-IR-3 immunoprecipitates the IGF-I receptor in cultured human keratinocytes. Cells were grown in basal medium for 24 h before lysis, and the sample in lane *C* was treated with 100 ng/ml IGF-I for the last 30 min. Cell lysates were immunoprecipitated with α-IR-3 (lanes *B* and *C*) or an isotypic control antibody (lane *A*) and then autophosphorylated by an in vitro kinase assay in the presence of γ-[^{32}P]ATP. The arrow indicates the 95-kD β subunit of the IGF-I receptor.

was confined to the basal epidermal layer, as previously reported with skin from normal individuals (9). Diffuse, cytoplasmic staining of IGF-I receptors was also seen in some suprabasal keratinocytes. Two other samples of nonlesional skin from psoriatics showed focal areas of suprabasal membrane staining for the IGF-I receptor in addition to basal staining.

Analysis of IGF-I Receptor Expression in Psoriatic Epidermis by Immunoprecipitation and Kinase Assay. To confirm the apparent increase in IGF-I receptor expression in psoriatic epidermis, IGF-I receptor kinase activity was assessed in psoriatic tissue. Split-thickness biopsies consisting predominantly of epidermal tissue were obtained from lesional and nonlesional skin from three different individuals. Equal quantities of homogenized tissue protein were immunoprecipitated with α-IR-3, and receptor autophosphorylation was then initiated by the addition of γ-[^{32}P]ATP. Kinase reaction samples were separated by SDS-PAGE with the results shown in Fig. 4 *a*. In all three paired samples, IGF-I receptor kinase activity as

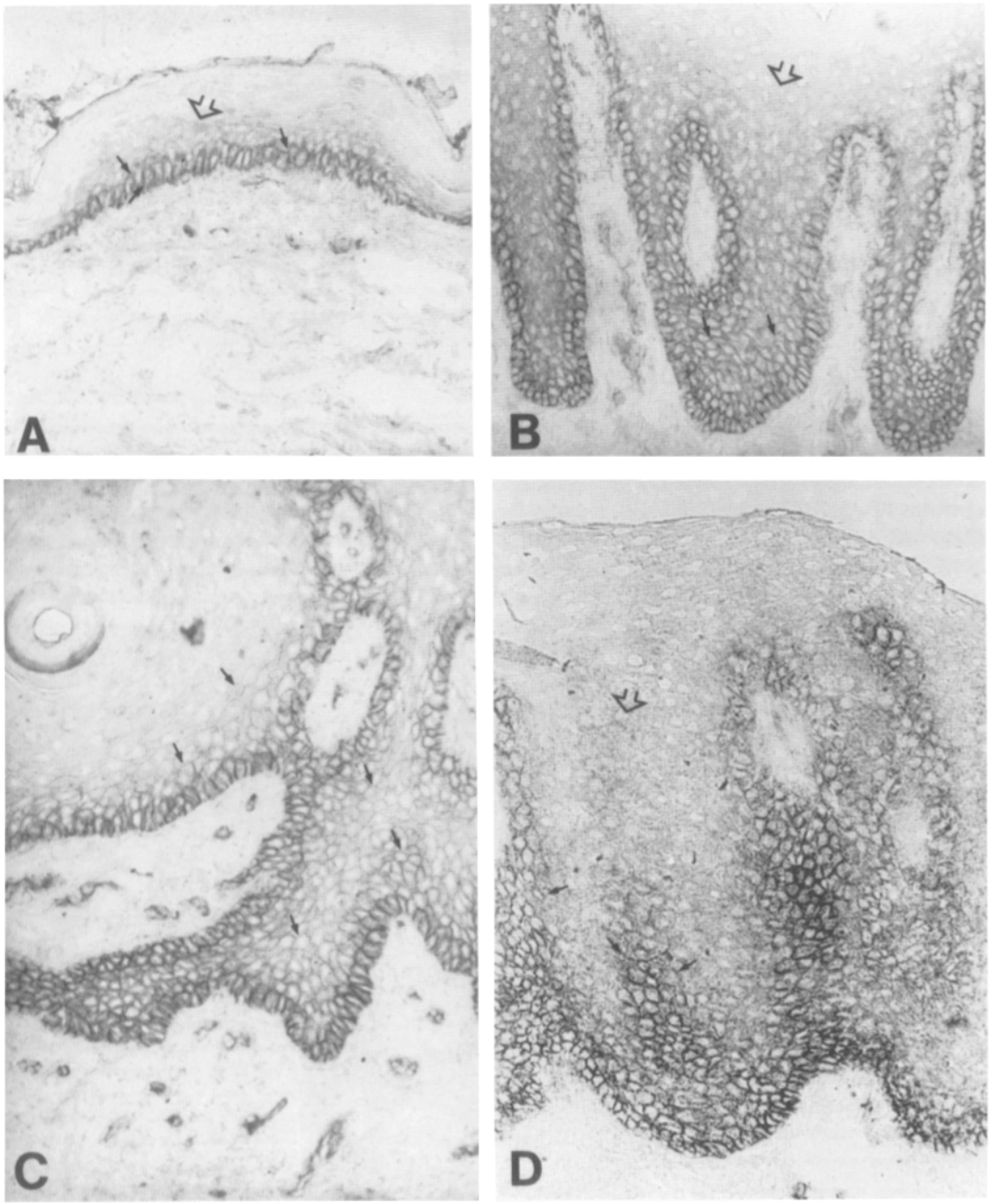

Figure 3. Immunohistochemical reactivity of psoriatic epidermis with the anti-IGF-I receptor mAb, α-IR-3. Intense membrane staining (*arrows*) of both basal and suprabasal keratinocytes is observed in psoriatic lesional epidermis (*B–D*), while plasma membrane reactivity is confined to the basal layer of keratinocytes in normal appearing, nonlesional skin (*A*). Weaker cytoplasmic staining of spinous keratinocytes with the mAb is also detected as indicated by the open arrowheads.

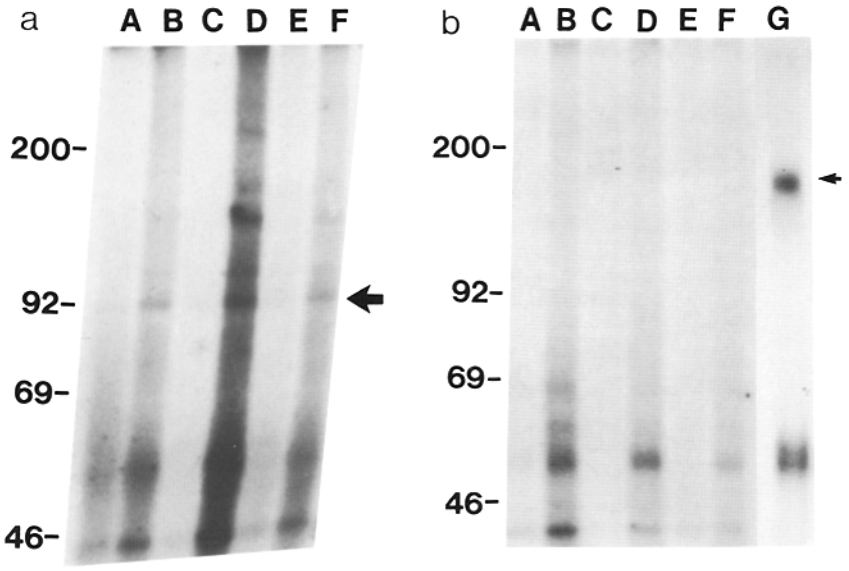

Figure 4. IGF-I receptor kinase activity is increased in psoriatic epidermis. Paired immunoprecipitation and kinase reactions on tissue extracts were performed with IGF-I receptor antibody α-IR-3 (*a*) or an EGF receptor antibody (*b*). Samples used for immunoprecipitation consisted of 60 μg of protein from nonlesional (lanes *A*, *C*, and *E*) and lesional (lanes *B*, *D*, and *F*) split-thickness biopsies of patients 1 (lanes *A* and *B*), 2 (lanes *C* and *D*), and 3 (lanes *E* and *F*). (*b*) Lane *G* is an immunoprecipitation of proteins from cultured human keratinocytes with the EGF receptor antibody as a positive control. After immunoprecipitation, samples were then autophosphorylated with $\gamma[^{32}P]$. The arrow in *a* indicates the 95-kD β subunit of the IGF-I receptor, while the arrow in *b* denotes the 170-kD EGF receptor.

measured by receptor autophosphorylation was greater in psoriatic tissue than in the paired nonlesional specimen. Densitometry measurements showed a 5–20-fold increase in kinase activity in psoriatic epidermis compared with paired nonlesional specimens. In contrast with the kinase assay on cultured keratinocytes in Fig. 2, IGF-I receptor kinase activity is detectable in psoriatic tissue without the addition of exogenous IGF-I (despite the use of approximately fivefold less total protein from the biopsy samples than with the cultured keratinocytes). A control for antibody specificity is shown in Fig. 4 *b*, where an immunoprecipitation/kinase reaction was performed in parallel with EGF receptor antibody. Autophosphorylation of the EGF receptor is readily seen in immunoprecipitated receptors from cultured human keratinocytes (lane *G*). In contrast, no phosphorylated products were seen in the size range of the EGF receptor (170 kD) or the IGF-I receptor β subunit (95 kD) in psoriatic tissue samples. The lower molecular mass phosphorylated products (69–46 kD) in Fig. 4 *a* and *b* are probably keratins and are not specifically precipitated by either antibody.

The increased kinase activity of IGF-I receptors is consistent with the immunohistochemical finding of increased expression of IGF-I receptors in psoriatic epidermis and further indicates that these receptors are both biologically functional and activated in vivo. Even with the apparent increase in IGF-I receptor expression in lesional psoriatic epidermis, it should be noted that the immunohistochemical findings indicate that there is a much more restricted expression of the IGF-I receptor in normal or psoriatic skin compared with expression of the EGF receptor, which is present throughout the stratum malpighii in psoriatic epidermis (9, 14, 15). This suggested that expression of the IGF-I receptor and the EGF receptor might be regulated by different biochemical pathways in cultured keratinocytes or in epidermal tissue.

Differential Modulation of IGF-I Receptor and EGF Receptor Expression in Human Keratinocytes. Expression of IGF-I and EGF receptors was examined in cultured human keratinocytes to better understand potential similarities or differences in regulation of these growth factor receptor systems. Fig. 5 shows binding of ^{125}I-IGF-I or ^{125}I-EGF over a range of ligand concentrations to keratinocyte monolayers maintained in serum-free MCDB 153 medium with and without added exogenous growth factors. This figure also displays a Scatchard transformation of the binding data. Keratinocytes maintained in growth factor–supplemented medium (KGM) expressed approximately twice as many EGF receptors as cells maintained for 24 h in unsupplemented basal medium (KBM). We have previously determined that IGF-I or supraphysiological concentrations of insulin (present in KGM) can transmodulate EGF receptor expression on human keratinocytes to the same degree as shown in this experiment (9). Thus, presumably the insulin component in KGM causes increased EGF receptor expression under these experimental conditions. In contrast, expression of the IGF-I receptor was not affected by growth factor supplementation of basal medium over a 24-h period (Fig. 5). These data suggest that the IGF-I receptor might be less sensitive than the EGF receptor to transmodulation by unrelated growth factors. Note that the binding curve for IGF-I can be resolved into high and low affinity receptor components. The high affinity binding certainly represents binding of IGF-I to its receptor, but it is possible that all or part of the low affinity component could be binding of ^{125}I-IGF-I to the insulin or IGF-II receptors. Based solely on the high affinity receptor component, it appears that human keratinocytes have fewer IGF-I receptors than EGF receptors under identical culture conditions. If low affinity receptor sites of IGF-I are considered, then keratinocytes grown in KGM would express approximately equal numbers of IGF-I and EGF receptors.

A number of pharmacological agents have significant effects on binding of EGF to its receptor, either through effects on cell surface receptor number or through an altered binding

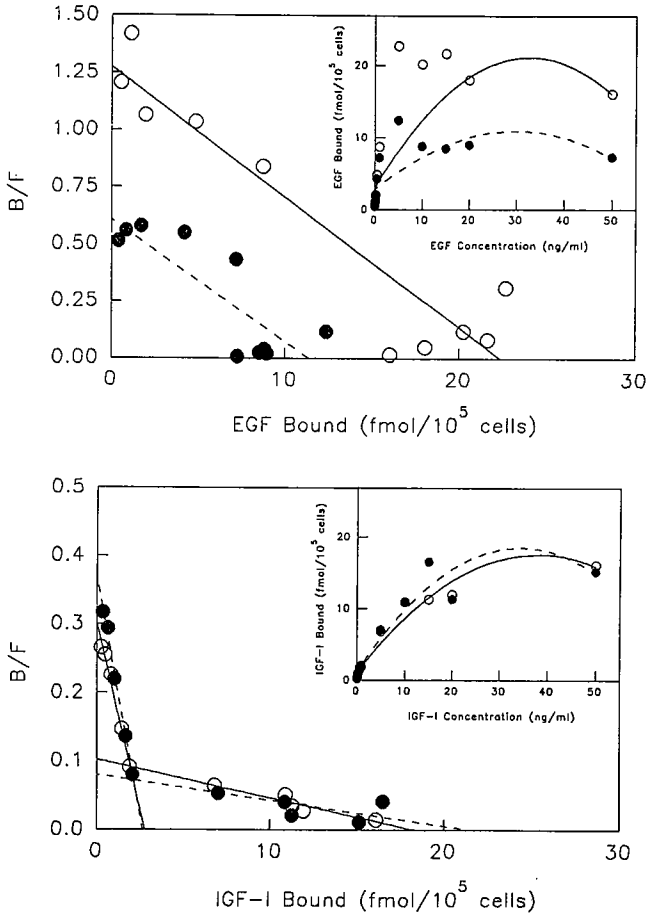

Figure 5. Growth factor supplementation increases EGF receptor binding sites on cultured keratinocytes, but does not affect IGF-I receptor binding. Scatchard analysis was performed with ^{125}I-EGF (*top*) and ^{125}I-IGF-I (*bottom*) for keratinocytes grown in KGM and then transferred to KBM (*filled circles*) or KGM (*open circles*) for 24 h before the assay. The insets in each panel are plots of the concentration of ligand added versus specific binding of the ligand.

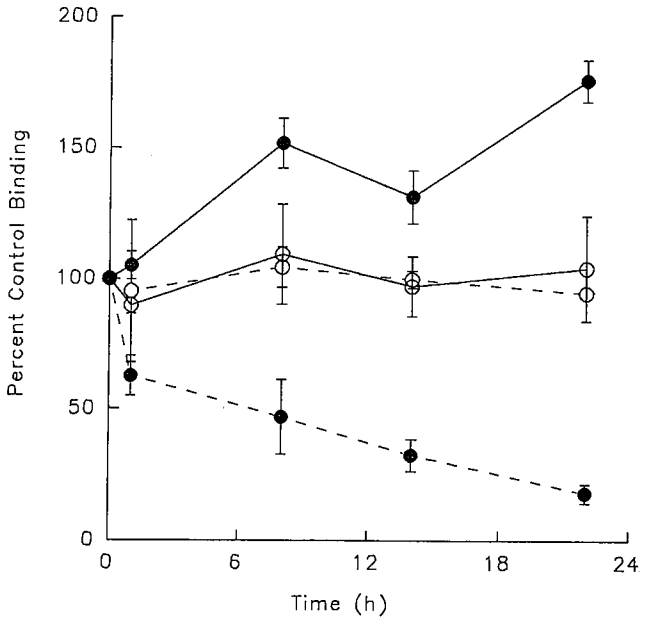

Figure 6. PMA treatment of keratinocyte monolayers has opposite effects on ^{125}I-EGF and ^{125}I-IGF-I binding. Keratinocytes in KBM were treated for 0–22 h with PMA (*filled circles*) or the inactive phorbol analogue 4α-PMA (*open circles*). Solid lines indicate ^{125}I-IGF-I binding, while the dashed lines denote ^{125}I-EGF binding. Control binding was performed on cells treated with KBM containing solvent alone.

affinity of the receptor for EGF. Given differences in the tissue distribution of IGF-I and EGF receptors in human epidermis, we sought to compare effects of potent pharmacological agents on expression of IGF-I and EGF receptors. Activation of protein kinase C (PKC) by PMA rapidly decreases EGF binding to keratinocytes by altering EGF receptor binding affinity (22). In the experiment shown in Fig. 6, binding of ^{125}I-IGF-I or ^{125}I-EGF to keratinocyte monolayers was determined after treatment with PMA for times up to 22 h. As a control for non-specific effects of phorbol compounds that are unrelated to PKC activation, binding has been compared with treatment with 4α-PMA, an inactive isomer of PMA. As expected, treatment of keratinocytes with PMA produced a rapid decrease in ^{125}I-EGF binding, which was maximally depressed at 22 h of treatment. In contrast, opposite effects on binding of ^{125}I-IGF-I were produced by PMA treatment, with in-

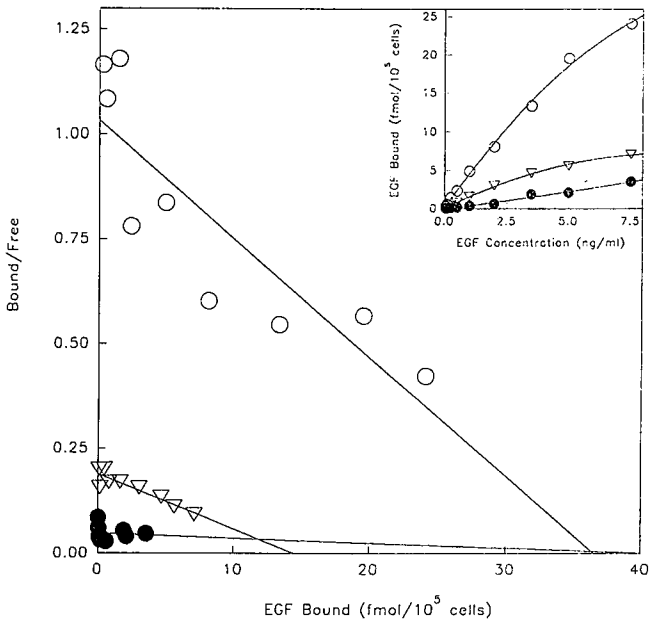

Figure 7. Scatchard analysis of the effects of PMA and Ca^{2+}/A23187 on EGF receptor binding to cultured human keratinocytes. Keratinocytes were treated for 24 h with KGM alone (*open circles*) or containing 10 ng/ml PMA (*filled circles*) or 1.15 mM $CaCl_2$/0.1 μM A23187 (*open triangles*). The inset is a plot of EGF concentration added versus specific binding of the ligand.

creased binding of ^{125}I-IGF-I evident by 8 h of treatment. Treatment of keratinocytes over this period with 4α-PMA produced no significant change in ^{125}I-EGF or ^{125}I-IGF-I binding. To determine whether these changes in ligand binding were due to alterations of receptor number or binding affinity, binding of these ligands to PMA-treated cells was examined over a range of ligand concentrations, and binding data were transformed to a Scatchard plot. PMA treatment decreased EGF receptor binding affinity from K_d ~0.4 to ~10 nM, with no apparent change in receptor number (Fig. 7). In contrast, PMA treatment increased the affinity of high affinity ^{125}I-IGF-I binding sites from K_d ~2 to ~0.6 nM (Fig. 8) with no significant alteration of low affinity ^{125}I-IGF-I binding sites (data not shown).

As PKC, as well as a number of other enzymes, can be activated by increased intracellular calcium ions, keratinocytes were treated with increased extracellular calcium (1.15 mM) or ionophore A23187 with and without increased extracellular calcium. The effect of these treatments was then measured on binding of ^{125}I-IGF-I and ^{125}I-EGF to keratinocyte monolayers (Fig. 9). Treatment of keratinocytes with increased extracellular calcium and with ionophore A23187 produced a time-dependent decrease in EGF binding, though the overall effect was not as large as that produced by PMA treatment. In contrast, a time-dependent increase in ^{125}I-IGF-I binding was produced by all treatments, though ionophore A23187 with increased extracellular calcium produced the largest increase. Scatchard analysis of the ionophore A23187 effect on EGF and IGF-I binding (Figs. 7 and 8) showed that the decreased EGF binding was largely a function of reduced receptor number, whereas the increased IGF binding was produced by an increased affinity of high affinity binding sites. Increased affinity of high affinity IGF-I receptors was also produced by treatment of keratinocyte monolayers with ionomycin in the presence of increased (1.15 mM) extracellular calcium. Ionomycin is an ionophore with higher selectivity for intracellular calcium transport than A23187 (23), suggesting that the effect of A23187 on IGF-I receptor expression is mediated through increased calcium transport and not that of another divalent cation.

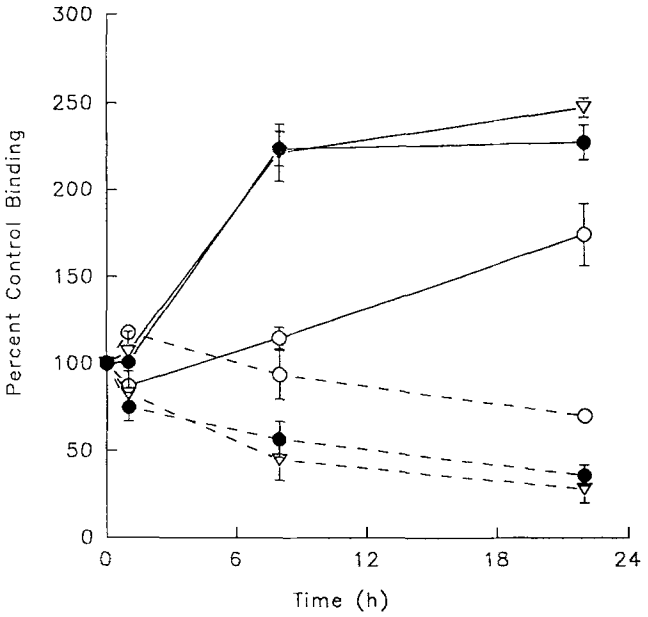

Figure 9. Calcium and/or calcium ionophore treatment of keratinocyte monolayers has opposite effects on ^{125}I-EGF and ^{125}I-IGF-I binding. Keratinocytes in KBM were treated for 0–22 h with 1.15 mM $CaCl_2$ (*filled circles*), 0.1 μM A23187 (*open circles*), or both (*open triangles*). Solid lines indicate ^{125}I-IGF-I binding while the dashed lines denote ^{125}I-EGF binding. Control binding was performed on cells treated with KBM containing solvent alone.

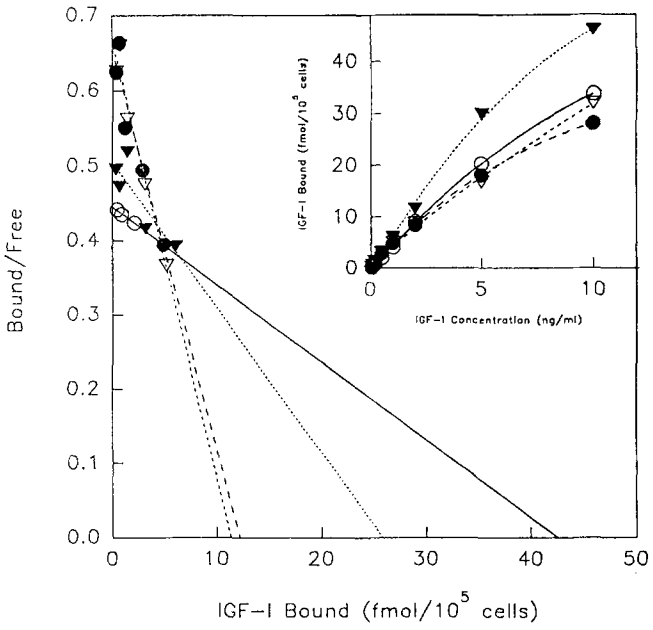

Figure 8. Scatchard analysis of the effects of PMA, Ca^{2+}/A23187, and Ca^{2+}/ionomycin on high affinity IGF-I receptor binding to cultured human keratinocytes. Keratinocytes were treated for 24 h with KBM alone (*open circles, solid line*) or containing 10 ng/ml PMA (*filled triangles, dotted line*) or 1.15 mM $CaCl_2$ with either 0.1 μM A23187 (*filled circles, long-dashed line*) or 1 μM ionomycin (*open triangles, short-dashed line*). The inset is a plot of IGF-I concentration added versus specific binding of the ligand.

Discussion

IGF-I, which mediates the growth-promoting effects of growth hormone, is itself an important hormone in determining structure and function of adult tissues (1). While the primary function of IGF-I is to regulate growth-related cellular metabolism, it could also have effects on intracellular hexose transport and anabolic metabolism through crossreaction with the insulin or IGF-II receptors. IGF-I may be an especially important hormone for development and homeostasis of adult tissues, since its plasma level increases throughout adolescence to a peak concentration of ~40 nM and levels plateau at ~20 nM in adults (1). These concentrations are far above the K_d of the IGF-I receptor for IGF-I (~1 nM),

but IGF-I access to tissues is restricted by serum binding proteins for IGF-I. Tissue responses to IGF-I in adults may also be tightly regulated by IGF-I receptor expression, which decreases in many tissues throughout development and is at low levels in many adult tissues (6). Although IGF-I could potentially bind to insulin or IGF-II receptors in adult tissues, the mitogenic regulation of cellular growth is specifically mediated by the β subunit tyrosine kinase of the IGF-I receptor and not that of the insulin receptor (5). In this context, it is interesting that little change is seen in expression of the IGF-I receptor in basal epidermal keratinocytes of neonatal vs. adult skin (9). The IGF-I receptor may thus play an important role in sustained proliferation of keratinocytes throughout life.

Keratinocytes have previously been shown to possess IGF-I receptors functionally and biochemically equivalent to those in other tissues (7, 8). IGF-I is a strong mitogen for keratinocytes in culture and its presence may be essential for in vitro keratinocyte proliferation; however, IGF-I appears to act as a mitogen primarily in synergistic combination with either EGF-related (9, 13, 20) or FGF-related (13, 20) peptides. Given the probable role of IGF-I as an epidermal mitogen, the restriction of IGF-I receptor expression to the basal epidermal compartment may largely limit proliferation to basal keratinocytes in normal skin. In this regard, IGF-I receptor localization is more specific for keratinocytes with proliferative potential than is EGF receptor distribution, since EGF receptors can be detected at cell surface membranes of all viable epidermal layers (9, 14, 15). This suggests a potentially important difference in differentiation or developmental regulation of IGF-I and EGF receptor expression in human skin.

There may also be important differences in biochemical regulation of ligand-receptor binding interactions in the EGF and IGF-I receptors. In situ binding of ^{125}I-EGF is primarily limited to basal epidermal keratinocytes in epidermis, even though suprabasal keratinocytes also express EGF receptors. This finding suggests that regulation of the binding availability of existing EGF receptors may be an important determinant of receptor-ligand interactions in vivo (15). Based on our observations of diminished EGF binding to its receptor after treatment of keratinocytes with PMA, calcium, or ionophores, it appears that EGF receptor–ligand binding can be highly modulated in a negative fashion, possibly through PKC-mediated threonine phosphorylation (22). Alternatively, increased cell surface EGF receptor number in cultured keratinocytes can be rapidly induced by an IGF-I receptor–mediated transmodulation (9). In contrast, the ability of the IGF-I receptor to bind IGF-I is not negatively affected by PMA, calcium, or ionophore treatment of keratinocytes. Thus, expression of the IGF-I receptor is regulated differently from that of the EGF receptor in cultured keratinocytes. These data, combined with details of the IGF-I receptor gene promoter sequence (24) and the pattern of IGF-I receptor expression in epidermis, suggest that IGF-I receptor–ligand interactions in tissue may be more a function of differentiation-specific control of receptor expression rather than biochemical modification of ligand binding potential. In preliminary experiments, binding of ^{125}I-IGF-I to epidermal tissue in situ closely paralleled IGF-I receptor expression defined by immunohistochemistry with α-IR-3, fulfilling one prediction of this IGF-I receptor–ligand binding scheme.

The increased expression of IGF-I receptors in suprabasal keratinocytes of lesional psoriatic skin could be important in regulating the keratinocyte hyperplasia associated with this disorder. The IGF-I receptor distribution closely parallels that of the increased keratinocyte proliferative compartment in active psoriatic epidermis (21). The potential need for IGF-I receptor activation to trigger keratinocyte proliferation might help to explain the relative confinement of keratinocyte proliferation to basal and suprabasal epidermal layers in psoriasis, despite increased expression of TGF-α (25, 26) and EGF receptors (14, 15) capable of ligand binding over the entire stratum malpighii in psoriasis. Indeed, mitogenic responsiveness to IGF-I in some epithelial cell types appears to be controlled through regulation of cell surface IGF-I receptors and not the available ligand concentration (27). Even so, free IGF-I would need to be available to activate IGF-I receptors in psoriatic skin if this receptor system is relevant to psoriatic epidermal hyperplasia. A relative abundance of IGF-I might be available to psoriatic epidermis through leaky, fenestrated capillaries in the papillary dermis in this disorder (28), or by secretion of IGF-I in a paracrine fashion from dermal fibroblasts that overexpress platelet-derived growth factor receptors in active psoriatic lesions (29). There is even the possibility that IGF-I could be supplied to the epidermis in an autocrine fashion, since IGF-I immunoreactivity can be induced in the epidermis under some conditions (30).

Our ability to detect IGF-I receptor kinase activity in psoriatic lesional keratinocytes in the absence of exogenously added IGF-I indicates that the IGF-I receptor pathway is activated in psoriatic epidermis. Our findings suggest a 5–20-fold increase in IGF-I receptor kinase activity in lesional psoriatic epidermis compared with uninvolved skin from the same individuals. A role for participation of the IGF-I receptor–ligand system in the pathogenesis of psoriasis is further suggested from clinical improvement produced by somatostatin-like drugs that diminish plasma IGF-I concentrations (31). Furthermore, activation of the increased IGF-I receptors present in lesional psoriatic skin might increase EGF receptor expression (a characteristic of psoriatic epidermis) via IGF-I-mediated transmodulation of the EGF receptor (9). We should note, however, that minimal EGF receptor kinase was detected in normal and lesional psoriatic skin samples, consistent with previous observations of EGF receptor–dependent tyrosine phosphorylation in tissue samples (32). Since ligand-receptor binding, tyrosine kinase activation, and receptor downregulation are normally linked, one explanation for the abundance of EGF receptors and TGF-α in psoriatic skin is that little in vivo ligand-receptor interaction actually occurs.

While much remains to be learned about the function and regulation of the IGF-I receptor in human skin, our results suggest that the IGF-I receptor is likely to be a key hormone receptor that influences the growth of epidermis in normal and pathological states. Since the independent mitogenic pathways stimulated by EGF and bFGF appear to converge through the IGF-I receptor system, it could be an important target for therapy of hyperproliferative epidermal diseases.

This work was supported in part by a General Clinical Research Center grant (M01-RR-00102) from the National Institutes of Health to The Rockefeller University Hospital; by a grant from the Dana Foundation; by grants from the Skin Disease Society, New York; by The Carl Herzog Foundation; by a Thomas J. Fitzpatrick Research Award from the Kao Corporation of Tokyo, Japan, to J. G. Krueger; by a 1989 grant from the Dermatology Foundation to A. B. Gottlieb; and with general support from the Pew Trusts. J. F. Krane is supported by a Medical Scientist Training Program grant to The Rockefeller University-Cornell University Medical College.

Address correspondence to J. G. Krueger, Laboratory for Investigative Dermatology, The Rockefeller University, 1230 York Avenue, New York, NY 10021.

Received for publication 27 September 1991 and in revised form 17 January 1992.

References

1. Daughaday, W.H., and P. Rotwein. 1989. Insulin-like growth factors I and II. Peptide, messenger ribonucleic acid and gene structures, serum, and tissue concentrations. *Endocr. Rev.* 10:68.
2. Massague, J., and M.P. Czech. 1982. The subunit structures of two distinct receptors for insulin-like growth factors I and II and their relationship to the insulin receptor. *J. Biol. Chem.* 257:5038.
3. Czech, M.P. 1989. Signal transmission by the insulin-like growth factors. *Cell.* 59:235.
4. Ullrich, A., A. Gray, A.W. Tam, T. Yang-Feng, M. Tsubokawa, C. Collins, W. Henzel, T. Le Bon, S. Kathuria, E. Chen, S. Jacobs, U. Francke, J. Ramachandran, and Y. Fujita-Yamaguchi. 1986. Insulin-like growth factor I receptor primary structure: comparison with insulin receptor suggests structural determinants that define functional specificity. *EMBO (Eur. Mol. Biol. Organ.) J.* 5:2503.
5. Lammers, R., A. Gray, J. Schlessinger, and A. Ullrich. 1989. Differential signalling potential of insulin- and IGF-1-receptor cytoplasmic domains. *EMBO (Eur. Mol. Biol. Organ.) J.* 8:1369.
6. Werner, H., M. Woloschak, M. Adamo, Z. Shen-Orr, C.T. Roberts, Jr., and D. LeRoith. 1989. Developmental regulation of the rat insulin-like growth factor I receptor gene. *Proc. Natl. Acad. Sci. USA.* 86:7451.
7. Misra, P., B.J. Nickoloff, V.B. Morhenn, R.L. Hintz, and R.G. Rosenfeld. 1986. Characterization of insulin-like growth factor-I/somatomedin-C receptors on human keratinocyte monolayers. *J. Invest. Dermatol.* 87:264.
8. Nickoloff, B.J., P. Misra, V.B. Morhenn, R.L. Hintz, and R.G. Rosenfeld. 1988. Further characterization of the keratinocyte somatomedin-C/insulin-like growth factor I (SM-C/IGF-1) receptor and the biological responsiveness of cultured keratinocytes to SM-C/IGF-1. *Dermatologica (Basel).* 177:265.
9. Krane, J.F., D.P. Murphy, D.M. Carter, and J.G. Krueger. 1991. Synergistic effects of epidermal growth factor (EGF) and insulin-like growth factor I/somatomedin C (IGF-I) on keratinocyte proliferation may be mediated by IGF-I transmodulation of the EGF receptor. *J. Invest. Dermatol.* 96:419.
10. Pfeifle, B., and H. Ditschuneit. 1983. Two separate receptors for insulin and insulin-like growth factors on arterial smooth muscle cells. *Exp. Clin. Endocrinol.* 81:280.
11. Rosenfeld, R.G., and L.A. Dollar. 1982. Characterization of the somatomedin-C/insulin-like growth factor I (SM-C/IGF I) receptor on cultured human fibroblast monolayers: regulation of receptor concentrations by SM-C/IGF I and insulin. *J. Clin. Endocrinol. & Metab.* 55:434.
12. Pardee, A.B. 1989. G_1 events and regulation of cell proliferation. *Science (Wash. DC).* 246:603.
13. Ristow, H.-J., and T.O. Messmer. 1988. Basic fibroblast growth factor and insulin-like growth factor I are strong mitogens for cultured mouse keratinocytes. *J. Cell. Physiol.* 137:277.
14. Nanney, L.B., C.M. Stoscheck, M. Magid, and L.E. King, Jr. 1986. Altered [^{125}I]epidermal growth factor binding and receptor distribution in psoriasis. *J. Invest. Dermatol.* 86:260.
15. Green, M.R., and J.R. Couchman. 1985. Differences in human skin between the epidermal growth factor receptor distribution detected by EGF binding and monoclonal antibody recognition. *J. Invest. Dermatol.* 85:239.
16. Kull, F.C., Jr., S. Jacobs, Y.-F. Su, M.E. Svoboda, J.J. Van Wyk, and P. Cuatrecassas. 1983. Monoclonal antibodies to receptors for insulin and somatomedin-C. *J. Biol. Chem.* 258:6561.
17. Jacobs, S., S. Cook, M.E. Svoboda, and J.J. Van Wyk. 1986. Interaction of the monoclonal antibodies α-IR-1 and α-IR-3 with insulin and somatomedin-C receptors. *Endocrinology.* 118:223.
18. Catanese, V.M., F. Grigorescu, G.L. King, and R.C. Kahn. 1986. The human erythrocyte insulin-like growth factor I receptor: characterization and demonstration of ligand-stimulated autophosphorylation. *J. Clin. Endocrinol. & Metab.* 62:692.
19. Furlanetto, R.W., J.N. DiCarlo, and C. Wisehart. 1987. The type II insulin-like growth factor receptor does not mediate deoxyribonucleic acid synthesis in human fibroblasts. *J. Clin. Endocrinol. & Metab.* 64:1142.
20. Aaronson, S.A., J.S. Rubin, P.W. Finch, J. Wong, C. Mar-

chese, J. Falco, W.G. Taylor, and M.H. Kraus. 1990. Growth factor-required pathways in epithelial cell proliferation. *Am. Rev. Respir. Dis.* 142:S7.
21. Van Scott, E.J., and T.M. Ekel. 1963. Kinetics of hyperplasia in psoriasis. *Arch. Dermatol.* 88:373.
22. Davis, R.J. 1988. Independent mechanisms account for the regulation by protein kinase C of the epidermal growth factor receptor affinity and tyrosine-protein kinase activity. *J. Biol. Chem.* 263:9462.
23. Liu, C., and T.E. Hermann. 1978. Characterization of ionomycin as a calcium ionophore. *J. Biol. Chem.* 253:5982.
24. Werner, H., B. Stannard, M.A. Bach, D. LeRoith, and C.T. Roberts. 1990. Cloning and characterization of the proximal promoter region of the rat insulin-like growth factor I (IGF-I) receptor gene. *Biochem. Biophys. Res. Commun.* 169:1021.
25. Gottlieb, A.B., C.K. Chang, D.N. Posnett, B. Fanelli, and J.P. Tam. 1988. Detection of transforming growth factor α in normal, malignant, and hyperproliferative human keratinocytes. *J. Exp. Med.* 167:670.
26. Elder, J.T., G.J. Fisher, P.B. Lindquist, G.L. Bennett, M.R. Pittelkow, R.J. Coffey, Jr., L. Ellingsworth, R. Derynck, and J.J. Voorhees. 1989. Overexpression of transforming growth factor α in psoriatic epidermis. *Science (Wash. DC).* 243:811.
27. Stewart, A.J., M.D. Johnson, F.E.B. May, and B.R. Westley. 1990. Role of insulin-like growth factors and the type I insulin-like growth factor receptor in the estrogen-stimulated proliferation of human breast cancer cells. *J. Biol. Chem.* 265:21172.
28. Braverman, I.M., and A. Yen. 1977. Ultrastructure of the human dermal microcirculation: II. The capillary loops of the dermal papillae. *J. Invest. Dermatol.* 68:44.
29. Krane, J.F., D.P. Murphy, A.B. Gottlieb, D.M. Carter, C.E. Hart, and J.G. Krueger. 1991. Increased dermal expression of platelet-derived growth factor receptors in growth-activated skin wounds and psoriasis. *J. Invest. Dermatol.* 96:983.
30. Hansson, H.-A., R. Jonsson, and K. Petruson. 1988. Transiently increased insulin-like growth factor I immunoreactivity in UVB-irradiated mouse skin. *J. Invest. Dermatol.* 91:328.
31. Camisa, C. 1989. Somatostatin and a long-acting analogue, octreotide acetate. Relevance to dermatology. *Arch. Dermatol.* 125:407.
32. Gentleman, S., T.A. Martensen, J.J. Digiovanna, and G.J. Chader. 1984. Protein tyrosine kinase and protein phosphotyrosine phosphatase in normal and psoriatic skin. *Biochim. Biophys. Acta.* 798:53.