In this report, we describe an auto-regulatory loop in human keratinocytes, whereby all-trans retinoic acid (retinoic acid) regulates its own biosynthesis from all-trans retinol (retinol) through regulation of retinol esterification. Retinol esterification activity was low in normal proliferating human keratinocytes, cultured in retinoid-free media. Treatment of keratinocytes with retinoic acid induced retinol esterifying activity (8-fold). Induction of retinol esterifying activity was blocked by either actinomycin D or cycloheximide. Based on substrate specificity and inhibitor sensitivity, lecithin:retinol acyltransferase (LRAT) was identified as the retinoic acid-inducible retinol esterifying enzyme. Induction of LRAT by retinoic acid reduced conversion of retinol to retinoic acid by 50%. This reduction in retinoic acid synthesis resulted from sequestration of retinol as retinyl esters, since inhibition of LRAT restored retinoic acid synthesis to control levels. In normal human skin, undifferentiated keratinocytes, in the lowest cell layer, esterified retinol 4 times greater, than differentiating keratinocytes, in upper cell layers, reflecting an induced state, under conditions of retinol sufficiency. Regulation of LRAT activity by retinoic acid provides a novel mechanism through which retinoic acid can regulate its own level by controlling availability of retinol for conversion to retinoic acid. In human skin in vivo, retinyl esters synthesized in basal keratinocytes could undergo hydrolysis during differentiation and thus serve as a source of retinol for keratinocytes in upper layers of skin.

Vitamin A, or all-trans retinol (retinol),1 is required for cellular growth and differentiation, as well as for reproduction, development, and ocular function (1). Biological activity of retinol occurs through oxidative conversion to all-trans retinoic acid (retinoic acid) (2). Retinoic acid is a ligand for nuclear retinoic acid receptors (RAR-α, -β, -γ) that bind to gene response elements and thereby regulate gene transcription (3). Most actions of retinoic acid are believed to be mediated through activation of RARs (4). Conversion of retinol to retinoic acid is a tightly controlled process (5–8). Retinoic acid synthesis in situ can be regulated directly, through regulation of oxidative enzyme activities, or by availability of substrate, dependent on relative rates of competing metabolic pathways that form 3,4-didehydroretinol, 13,14-dihydroxyretinol, and retinyl esters (9–12).

Retinyl esters are the predominant metabolites of retinol in many cells and tissues, including cultured human keratinocytes (5, 6, 13), Sertoli cells (14), intestinal Caco-2 cells (15), liver (16, 17), intestine (18), retinal pigment epithelium (19, 20), and skin (21, 22). Esterification of retinol facilitates important processes for vitamin A absorption, storage, and function. Retinyl esters function as the molecular storage form for retinol, in liver and extrahepatic tissues. Retinyl ester synthesis in retinal pigment epithelium provides energy for isomerization of retinol to 11-cis retinol for visual function (23).

Two enzyme activities catalyze retinyl ester synthesis: acyl-CoA:retinol acyltransferase (ARAT) and lecithin:retinol acyltransferase (LRAT). These two enzymes can be distinguished from each another by substrate preferences and differential sensitivity to inhibitors (16, 17, 24). ARAT utilizes acyl-CoA (25), while LRAT utilizes the acyl group at the sn-1 position of membrane phospholipids (24), as acyl donor. LRAT utilizes both free retinol and retinol bound to cellular retinol-binding protein (holoCRBP) as substrates, while ARAT catalyzes esterification of only free retinol (16, 17, 24, 26). In addition, LRAT activity is specifically inhibited by apoCRBP and by phenylmethylsulfonyl fluoride (PMSF) (16, 24, 26).

Human skin requires retinol for keratinocyte growth and differentiation (27–29). Kang et al. (22) found low levels of retinyl ester in normal human epidermis; however, following topical application of 0.4% retinol, retinyl esters were the major metabolites formed. Recently, we (5) and others (6) reported the predominant metabolites of retinol in cultures of proliferating keratinocytes were retinyl esters, and less than 1.0% of given retinol was converted to retinoic acid, regardless of the concentration of added retinol. ARAT activity has been detected in skin and cultured human keratinocytes (21); however, relatively little is known about retinyl ester formation and its regulation in human skin and in cultures of proliferating keratinocytes.

We report here that LRAT is the predominant retinol esterifying activity in human keratinocytes and that its activity is regulated by retinoic acid. Induction of LRAT activity by retinoic acid increases retinol esterification and concomitantly reduces retinol oxidation to retinoic acid. We propose that regulation of LRAT activity by retinoic acid provides a mechanism through which retinoic acid can regulate its own level by controlling availability of retinol for conversion to retinoic acid in basal keratinocytes.
EXPERIMENTAL PROCEDURES

Materials—Radioisotopes were purchased from DuPont NEN. Purified rat recombinant apoCRBP was kindly provided by Dr. William S. Blaner, Columbia University, New York. CD367 was kindly provided by Dr. Graham Shroot, Centre International De Recherches, Sophia-Antipolis, Valbonne, France. Mouse collagen type IV was purchased from Collaborative Biomedical Products. HPLC-grade organic solvents were purchased from Burdick and Jackson Laboratories, Inc. (Romulus, MI). Sepharose ODS1 high performance liquid chromatography columns were purchased from Phase Separations, Inc. (Norwalk, CT). All other chemicals used were purchased from Sigma.

Keratinocyte Cultures and Treatments—Human keratinocyte cultures were prepared from keratome biopsies of normal adult skin and maintained as described previously (5). At approximately 70% confluency, cells were placed in media supplemented with 0.1% essentially fatty acid-free bovine serum albumin and treated (under reduced light) with retinol, all-trans retinaldehyde (retinaldehyde), or retinoic acid, at concentrations ranging from 1 nm to 10 μM, or vehicle (ethanol, 0.01% butylated hydroxytoluene), for 6–96 h. Retinoid-treated keratinocyte cultures were then incubated with [3H]retinol (1 μCi/ml, 27–10 μM) for 20 min. Keratinocyte cultures were harvested for extraction and chromatography of [3H]retinol metabolites as described previously (5).

Preparation of HoloCRBP—HoloCRBP was prepared by modification of a method by Timmers et al. (30). Briefly, rat recombinant apoCRBP (31), 2–4 nmol in PBS, was incubated with 3-fold excess [3H]retinol (50–80 μCi, 6–12 nmol) overnight at 4°C. [3H]Retinol bound to CRBP was separated from unbound [3H]retinol on a Lidioph-1000 microcolumn.

Preparation of Microsomes from Human Skin and Cultured Human Keratinocytes—Human skin keratome biopsies, treated for 48 h in vivo prior to biopsy with either 0.1% retinoic acid, 0.4% retinol, or vehicle (95% ethanol:propylene glycol:butylated hydroxytoluene, 70:30:0.5), were obtained and stored as described previously (22). Microsomes were prepared in 0.2M potassium phosphate buffer, pH 7.4, with 1 mM dithiothreitol (DTT) using published methods (32). Microsomal pellets were resuspended in 0.2M phosphate buffer, pH 7.4, with 1 mM DTT, at a final protein concentration of 100 μg/30–60 μl. Procedures involving human subjects received prior approval by the University of Michigan Institutional Review Board, and all subjects provided written informed consent.

Keratinocytes, treated with retinoids or vehicle, were harvested as described above and homogenized in 0.2M potassium phosphate buffer, pH 7.4, with 1 mM DTT. Homogenates were centrifuged at 800 × g for 15 min at 4°C, and resulting supernatants were centrifuged at 100,000 × g for 1 h. Pellets were resuspended in 0.2M potassium phosphate buffer, pH 7.4, with 1 mM DTT, at a final protein concentration of 100 μg/20–80 μl. Protein concentrations were determined by the Bradford method (33).

In Vitro Determination of LRAT, ARAT, and Retinol Dehydrogenase Activities—ARAT and LRAT activities in microsomes from skin or cultured keratinocytes were determined by published methods (24, 25). Microsomal retinol dehydrogenase activity was determined in 0.2M potassium phosphate, pH 7.4, with addition of NADP⁺ (2 mM) as cofactor. Reactions were started by addition of [3H]retinol in ethanol with 0.01% butylated hydroxytoluene (0.5 μCi, 125 nm-5 μM) or [3H]HoloCRBP (0.3 μCi, 125 nm-4 μM). All enzyme assays were performed under reduced light.

Extraction of Retinoids—[3H]Retinol metabolites were extracted from in vitro assay reaction mixtures in 2 volumes of hexane. Retinoid acid synthesized from retinol, in cultured human keratinocytes, was extracted with solid phase adsorbent aminopropyl columns as described previously (5). Extracts were prepared for and analyzed by either thin layer chromatography or reverse-phase HPLC as described previously (5).

Separation of Basal Keratinocytes from Suprabasal Keratinocytes—Type IV collagen-coated plates were prepared by modification of a method of Jonnes and Watt (34). Briefly, plastic Petri dishes were incubated with 1× at 37°C with mouse monoclonal collagen in 0.05N HCl (75 μg/ml, 1.5 ml/60-mm dish), followed by incubation with 0.5% heat-denatured bovine serum albumin. Epidermal cell suspensions were prepared from human skin biopsies by trypsin digestion, as described previously (35).

Epidermal cells were seeded (1,000,000 cells/60-mm dish) onto type IV collagen-coated dishes and incubated for 1 h at 37°C. Media containing nonadherent cells, were removed, and adherent cells were rinsed twice in PBS to remove additional nonadherent cells. Both adherent cells and nonadherent cells were incubated with [3H]retinol for 30 min. Adherent cells were harvested by scraping in PBS, and nonadherent cells were pelleted by centrifugation at 1000 rpm and rinsed twice in PBS. To verify that adherent and nonadherent cells represented basal keratinocytes and suprabasal keratinocytes, respectively, both populations were analyzed by flow cytometry. Expression of β1 integrin, keratin 1, and keratin 10 was determined as described previously (36).

RESULTS AND DISCUSSION

Regulation of Retinyl Ester Formation by Retinoids in Cultured Human Keratinocytes—We previously reported that retinyl esters are quantitatively the major metabolites of retinol in human keratinocytes, cultured in retinoid-free media (5). To extend these results, we investigated the regulation and properties of retinol esterification in cultured keratinocytes and human skin. We first examined whether treatment with retinoids altered retinyl esterifying activity in cultured keratinocytes. Treatment of cultured human keratinocytes with 100 nM retinoic acid for 48 h resulted in increased esterification of exogenous retinol, compared to vehicle-treated keratinocyte cultures (Fig. 1). This increased synthesis of retinyl esters in retinoid-treated keratinocytes was observed over a wide range of substrates concentrations (27 nm–10 μM).

We next examined relative potencies of retinol, retinaldehyde, and retinoic acid, for induction of retinol esterifying activity (Fig. 2). All three retinoids induced esterification activity. Retinoic acid was most potent. Retinol and retinaldehyde were similar in potency. The lower potencies of retinol and retinaldehyde, compared to retinoic acid, are consistent with our previous results demonstrating that conversion of retinol and retinaldehyde to retinoic acid is a prerequisite for their biological activity (5).

Relative Contribution of LRAT and ARAT Activities to Retinyl Ester Formation in Cultured Keratinocytes—Microsomes isolated from cultured keratinocytes treated with retinoic acid also displayed increased retinol esterifying activity, compared with microsomes from vehicle-treated cells. Treatment of keratinocytes with 100 nM retinoic acid for 48 h resulted in an approximately 8-fold increase in specific activity of microsomal retinol esterification activity, using either holoCRBP or free retinol as substrate (Fig. 3). Addition of 60 μM exogenous palmitoyl-CoA, a substrate for ARAT, did not significantly increase specific activity of retinyl ester formation in either retinoid- or vehicle-treated keratinocytes, using either ho-
fresh media and incubated for 20 min with all-indicated. After retinoid treatment, keratinocyte cultures were given retinoic acid (trans), all-hatched bars keratinocytes were treated for 48 h with either all-trans retinoic acid induction of retinyl ester formation in cultured human keratinocytes. Cultures of human keratinocytes were treated for 48 h with either all-trans retinol (wide-hatched bars), all-trans retinaldehyde (narrow-hatched bars), all-trans retinoin acid (filled bars), or ethanol vehicle, at the concentrations indicated. After retinoid treatment, keratinocyte cultures were given fresh media and incubated for 20 min with all-trans [3H]retinol (4 μCi, 10 μM). Cells were harvested, and [3H]retinol metabolites were identified as described under “Experimental Procedures.” Results are fold increase in retinyl ester formation in retinoid-treated keratinocyte cultures over vehicle-treated keratinocyte cultures. Data are means ± S.E., n = 3–4. RE, retinyl esters.

Fig. 2. Dose dependences of all-trans retinol, all-trans retinaldehyde, and all-trans retinoic acid induction of retinyl ester formation in cultured human keratinocytes. Cultures of human keratinocytes were treated with 100 nM all-trans retinol (wide-hatched bars), all-trans retinaldehyde (narrow-hatched bars), all-trans retinoin acid (filled bars), or ethanol vehicle, at the concentrations indicated. After retinoid treatment, keratinocyte cultures were given fresh media and incubated for 20 min with all-trans [3H]retinol (4 μCi, 10 μM). Cells were harvested, and [3H]retinol metabolites were identified as described under “Experimental Procedures.” Results are fold increase in retinyl ester formation in retinoid-treated keratinocyte cultures over vehicle-treated keratinocyte cultures. Data are means ± S.E., n = 3–4. RE, retinyl esters.

Fig. 3. All-trans retinoic acid induces microsomal retinyl ester formation in human keratinocytes. Cultures of proliferating human keratinocytes were treated with 100 nM all-trans retinoic acid (hatched bars) or ethanol vehicle (filled bars) for 48 h. Microsomes were isolated and retinol esterifying activity was determined as described under “Experimental Procedures.” Reactions were initiated with either 1 μM [3H]holoCRBP or all-trans [3H]retinol (with 0.1% bovine serum albumin) with or without 60 μM palmitoyl-CoA. [3H]Retinol metabolites were identified as described under “Experimental Procedures.” Results are expressed as specific activity of retinyl ester formation (pmol/min/mg of protein). Data are means ± S.E., n = 3. RE, retinyl esters; ROL, retinol.

Increasing concentrations of apoCRBP caused a dose-dependent inhibition of retinyl ester formation in microsomes from vehicle-treated and retinoic acid-treated keratinocytes (Fig. 5A). In addition, the serine protease inhibitor PMSF, which selectively inhibits LRAT activity, reduced microsomal retinol esterifying activity by 80% in both microsomes from vehicle or retinoic acid-treated keratinocytes, using either free retinol or holoCRBP as substrate (Fig. 5B).

The above data demonstrate that retinyl esterifying activity in cultured keratinocytes utilizes holoCRBP as a substrate, not stimulated by acyl-CoA, uses fatty acid substrates with chemical compositions similar to membrane phospholipids, and is inhibited by apoCRBP and PMSF. These data demonstrate that in cultured human keratinocytes, LRAT is the predominant retinol esterifying activity, and that its activity is regulated by retinoids.

In the above experiments, keratinocytes were cultured and expanded through passage, prior to use, in retinoid-free media. Under these retinoid-deficient conditions, LRAT activity was low and, upon addition of retinoids, was induced. Thus, LRAT activity was responsive to cellular retinoid nutritional status. LRAT activity in rat liver and developing chick intestine has also been shown to be regulated by retinoid nutritional status (38, 39). In rats, retinoid deficiency reduced hepatic LRAT activity, which was restored by feeding retinoic acid (40). Similarly, Wang et al. (8) reported regulation of retinol esterification by retinoic acid in ferret liver.

Fig. 4. HPLC analysis of retinyl ester formation by microsomes from all-trans retinoic acid- and vehicle-treated keratinocytes. Cultures of proliferating human keratinocytes were treated for 48 h with either vehicle (upper panel) or 100 nM all-trans retinoic acid (lower panel). Microsomes were isolated and retinol esterifying activity was determined as described under “Experimental Procedures,” with [3H]holoCRBP (0.3 μCi, 1 μM) as a substrate. [3H]Retinol metabolites were identified based on co-elution with known retinol and retinyl ester standards. 13cROL, 13-cis retinol; tROL, all-trans retinol.
were maximal at 48 h (data not shown). Retinoic acid treatment was observed 6 h after retinoic acid treatment and Earliest detectable increases in specific activity of retinyl ester induction of LRAT activity in cultured human keratinocytes. Activity—

We next determined the time course for retinoic acid activity in microsomes isolated from cultured keratinocytes. human keratinocytes treated with 100 nM all-trans retinol esterifying activity was determined in microsomes from cultured keratinocytes for 48 h as described under “Experimental Procedures.” The molar ratio of apoCRBP to holoCRBP was increased as indicated. Results are expressed as percent of control activity without addition of apoCRBP. B, microsomal retinol esterifying activity was determined following a 10-min preincubation with PMSF (5 mM) or vehicle (dimethyl sulfoxide), preceding addition of holoCRBP (0.3 μCi, 125 nm) (filled bars) or all-trans [3H]retinol (0.5 μCi, 125 nm) (hatched bars) for an additional 10 min. Results are expressed as percent of control activity without PMSF. Data are means ± S.E., n = 3. RE, retinyl esters; RA, all-trans retinoid acid.

Mechanisms of Retinoic Acid-mediated Induction of LRAT Activity—We next determined the time course for retinoic acid induction of LRAT activity in cultured human keratinocytes. Earliest detectable increases in specific activity of retinyl ester formation were observed 6 h after retinoic acid treatment and were maximal at 48 h (data not shown). Retinoic acid treatment increased apparent V_max of esterification approximately 4-fold (Fig. 6). The apparent K_m values for holoCRBP (1.7 μM) or for free retinol (4.0 μM) were not affected by retinoic acid treatment (Fig. 6). The observed apparent K_m values for both holoCRBP and free retinol are similar to those previously reported for LRAT activity in rat liver (16, 24, 41).

Treatment of keratinocytes with CD367 (100 nM, 48 h), a synthetic activator of nuclear retinoic acid receptors (42), increased LRAT activity to a similar extent as retinoic acid (data not shown). In addition, actinomycin D and cycloheximide, which inhibit gene transcription and new protein synthesis, respectively, effectively blocked retinoic acid induction of retinyl esterification activity (Fig. 7). Taken together, the above data are consistent with retinoid induction of LRAT activity occurring as a result of increased LRAT protein synthesis, which may be mediated by RAR-dependent gene transcription.

Retinoic Acid Induction of LRAT Activity Reduces Synthesis of Retinaldehyde and Retinoic Acid from Retinid—Retinoic acid induction of LRAT activity may result in reduced availability of retinol for in situ synthesis of retinoic acid. To examine this, cultures of proliferating keratinocytes were treated for 48 h with either vehicle or CD367, to induce retinol esterification, followed by incubation with 1 μM retinol for 4 h. CD367-treated keratinocytes synthesized 2-fold less retinoic acid (66 ± 11 ng), compared with vehicle-treated cultures (111 ± 11 ng) (Fig. 8A). In addition, oxidation of retinol to retinaldehyde, the rate-limiting step in retinoic acid synthesis, by microsomes from keratinocytes treated with retinoic acid, was one-half that of microsomes from vehicle-treated cells (Fig. 8B). Of note, in microsomes from retinoic acid-treated cells, addition of PMSF to inhibit LRAT activity restored retinaldehyde synthesis to levels similar to those found in microsomes from vehicle-

![Graph](Image)

**Fig. 5.** LRAT-specific inhibitors reduce retinol esterification activity in microsomes isolated from cultured keratinocytes. A, retinol esterifying activity was determined in microsomes from cultured human keratinocytes treated with 100 nm all-trans retinoid acid (open circles) or ethanol vehicle (open diamonds), for 48 h as described under “Experimental Procedures.” The molar ratio of apoCRBP to holoCRBP was increased as indicated. Results are expressed as percent of control activity without addition of apoCRBP. B, microscopic retinol esterifying activity was determined following a 10-min preincubation with PMSF (5 mM) or vehicle (dimethyl sulfoxide), preceding addition of holoCRBP (0.3 μCi, 125 nm) (filled bars) or all-trans [3H]retinol (0.5 μCi, 125 nm) (hatched bars) for an additional 10 min. Results are expressed as percent of control activity without PMSF. Data are means ± S.E., n = 3. RE, retinyl esters; RA, all-trans retinoid acid.

**Fig. 6.** All-trans retinoic acid treatment of cultured human keratinocytes increases apparent V_max, but not apparent K_m of retinyl ester formation. Graph, Lineweaver-Burk graph of retinol dependence of microsomal retinol esterification. Cultures of human keratinocytes were treated with 100 nm all-trans retinoic acid for 24 h (open triangles) or 48 h (open diamonds) or ethanol vehicle (open circles) for 48 h. Table, apparent K_m values and V_max values of retinyl esterification with holoCRBP (0.3 μCi, 125 nm) as substrate, at the times indicated. Microsomes were isolated and retinyl ester formation determined as described under “Experimental Procedures.” Data are representative of four experiments. RE, retinyl esters; ROL, all-trans retinol; RA, all-trans retinoic acid.

![Graph](Image)

**Fig. 7.** Actinomycin D and cycloheximide block all-trans retinoic acid induction of microsomal retinyl ester formation in cultured keratinocytes. Cultures of proliferating human keratinocytes were treated with actinomycin D (1 μg/ml) or cycloheximide (5 μg/ml) or vehicle (dimethyl sulfoxide) for 1 h followed by 100 nm all-trans retinoic acid (hatched bars) or vehicle (ethanol) (open bars) for an additional 14 h. Microsomes were isolated and retinyl ester formation was determined with [3H]holoCRBP (0.3 μCi, 1 μM) as substrate, as described under “Experimental Procedures.” Results are expressed as fold induction of retinyl ester formation over vehicle. Data are means ± S.E., n = 2. RE, retinyl esters; ACT D, actinomycin D; CYCLO, cycloheximide.
Auto-regulation of Retinoic Acid Synthesis through Retinol Esterification

Retinoid induction of retinyl ester formation in human keratinocytes reduces oxidation of all-trans retinol to all-trans retinaldehyde and all-trans retinoic acid. A, cultures of human keratinocytes were treated with either the synthetic retinoid 100 nM CD367 or vehicle (ethanol) for 48 h. Keratinocyte cultures were given fresh media and incubated with 1 μM all-trans retinol for 4 h. Cells were harvested and all-trans retinoic acid was quantified as described under "Experimental Procedures." Data are means ± S.E., n = 3. B, cultures of proliferating human keratinocytes were treated with either 100 nM all-trans retinol (filled bars) or vehicle (ethanol) (open bars) for 48 h. Microsomes were isolated and assayed for retinyl dehydrogenase activity (left panel) and retinol esterification activity (right panel) in the absence or presence of PMSF, as described under "Experimental Procedures." Data are means ± S.E., n = 5. RA, all-trans retinoidic acid; RAL, all-trans retinaldehyde; RE, retinyl esters.

Retinoidic acid formed from retinol activates RAR-dependent gene transcription in human keratinocytes, and the magnitude of this gene activation is proportional to the amount of retinoidic acid synthesized, which in turn is proportional to the concentration of exogenous retinol (5). Based on these data, a 50% reduction in retinoidic acid synthesis would be expected to effect a concomitant reduction in RAR-dependent gene transcription, and therefore might be of physiological significance.

Randolph and Simon (7) reported that increased retinyl ester formation, following addition of free fatty acids to the media, decreased synthesis of retinoidic acid in human keratinocytes. Evidence indicating that holoCRBP is a substrate for enzymes that oxidize retinol to retinoidic acid, as well as LRAT, provides additional support for coordinate regulation of retinol esterification and retinoidic acid synthesis (26, 43).

Retinyl Ester Formation in Human Skin—Having characterized the properties and regulation of retinol esterification in cultured human keratinocytes, we investigated retinol esterification in human skin in vivo. For these experiments, we utilized human skin from healthy adult donors that was either untreated or treated topically, in vivo, with retinoidic acid, or its vehicle. Human skin has been reported to contain ARAT activity (21) and low levels of retinyl esters, which are increased following topical treatment with retinoidic acid (22). We found that microsomes prepared from human skin esterified both free retinol and holoCRBP at approximately one-tenth the rate of cultured keratinocytes (compare Figs. 3 and 9). Free retinol was esterified 2-fold more effectively than holoCRBP by human skin microsomes. Addition of palmitoyl-CoA stimulated retinyl ester formation 2.5-fold (Fig. 9). These data indicate ARAT activity is proportionally greater in human skin than in cultured human keratinocytes (Fig. 3). Unlike cultured keratinocytes, retinol esterifying activity in human skin was not increased following treatment with retinoidic acid (Fig. 9).

These differences between cultured keratinocytes and skin (which is composed of 95% keratinocytes) might reflect differential LRAT expression as a function of keratinocyte differen-
ester stores could provide a source of retinol for synthesis of retinoic acid, showed no increase in retinol esterifying activity, compared to basal keratinocytes isolated from vehicle-treated skin (Fig. 10, inset). On a per cell basis, adherent basal keratinocytes esterified retinol at a 4-fold greater rate than nonadherent suprabasal keratinocytes (Fig. 10, main graph). Retinyl ester formation by adherent cells, which were trypsinized and assayed in suspension, was similar to that of nontrypsinized cells, indicating that adherence, per se, does not alter retinol esterifying activity (data not shown). These data suggest that low esterifying activity observed in skin, compared to cultured keratinocytes, is due in part to dilution of the more active, but smaller population of basal keratinocytes, with the less active, but larger population of differentiating keratinocytes.

We next investigated whether retinol esterifying activity in basal keratinocytes in human skin is induced by retinoic acid. Unlike cultured keratinocytes, basal keratinocytes isolated from human skin treated in vivo for 48 h with 0.1% retinoic acid, showed no increase in retinol esterifying activity, compared to basal keratinocytes isolated from vehicle-treated skin (data not shown). This lack of induction likely indicates that retinoid levels in normal human skin, in contrast to keratinocyte cultures, are adequate to maintain fully induced retinol esterifying activity in basal keratinocytes. In fact, free retinol levels in skin (approximately 1 μM) are sufficient to maximally induce LRAT activity in cultured keratinocytes. Presumably, if skin were rendered retinol-deficient, like keratinocyte cultures, then retinol esterifying activity in basal keratinocytes would be reduced and therefore inducible by retinoids.

In the above studies, esterification of retinol in basal and suprabasal keratinocytes was measured in intact cells. The small number of adherent basal keratinocytes isolated from skin biopsies was insufficient to prepare microsomes; therefore, relative LRAT and ARAT activities could not be determined directly. Basal keratinocytes, isolated by type IV collagen adherence, are the progenitors of keratinocytes propagated in culture, and, therefore, it is very likely that they express predominantly LRAT activity like their progeny.

The data presented in these studies identify LRAT as the predominant retinol esterifying activity in cultured human keratinocytes. Treatment of keratinocyte cultures with retinoic acid significantly induces LRAT activity. In addition, retinoic acid induction of LRAT activity leads to a concomitant reduction in retinol oxidation, which is restored with inhibition of LRAT activity. These observations suggest retinoic acid regulates its own synthesis from retinol in situ, by controlling relative availability of retinol as a substrate for oxidative enzymes, through sequestration by LRAT.

Retinol esterification in cultured keratinocytes and human skin differed in several ways. Total retinol esterification was lower, and the proportion of ARAT activity higher in human skin, compared with cultured keratinocytes. In addition, retinoic acid did not induce retinol esterifying activity in vivo, whereas it did in cultured keratinocytes. These differences may be explained in part by our finding that in human skin, retinol esterifying activity was predominantly localized to the relatively small population of basal keratinocytes. Since basal keratinocytes are phenotypically and functionally similar to cultured keratinocytes (cultured keratinocytes are derived from basal keratinocytes), it is very likely that basal keratinocytes, like cultured keratinocytes, express predominantly LRAT activity. In contrast, differentiating suprabasal keratinocytes express predominantly low level ARAT activity. We speculate that LRAT activity in basal keratinocytes is fully induced by retinol and retinoic acid taken up from the circulation (Fig. 11).

A high capacity of basal keratinocytes to esterify retinol provides a mechanism to regulate availability of retinol as a substrate for in situ synthesis of retinoic acid in lower layers of the skin. In addition, it has previously been demonstrated that differentiating keratinocytes possess a greater capacity to synthesize retinoic acid from retinol than basal keratinocytes (46). Therefore, as basal keratinocytes migrate upward, away from retinol supplied from the circulation, hydrolysis of their retinyl ester stores could provide a source of retinol for synthesis of retinoic acid during differentiation.

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Auto-regulation of Retinoic Acid Biosynthesis through Regulation of Retinol Esterification in Human Keratinocytes

Sara B. Kurlandsky, Elizabeth A. Duell, Sewon Kang, John J. Voorhees and Gary J. Fisher

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