Retinoic Acid Receptor Gene Expression in Human Skin

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Human skin exhibits a characteristic, pleiotropic response to topical retinoic acid. In attempting to understand this response at the molecular level, we have used fast protein liquid chromatography (FPLC) and RNA blot hybridization to characterize the expression of the nuclear retinoic acid receptor (RAR) alpha, beta, and gamma genes in adult human epidermis. Size exclusion FPLC of 0.6 M NaCl nuclear extracts prepared from keratome biopsies revealed two peaks of specific [3H] retinoic acid (RA) binding at M, 45 and 18 kDa, in agreement with the expected sizes of RAR and cellular RA binding protein. Blot hybridization analysis of total RNA extracted from keratome biopsies revealed that RAR-gamma was the predominant RAR species expressed in human epidermis, as RAR-alpha transcripts were detectable only at low levels and RAR-beta transcripts were undetectable. RAR transcripts were not induced by topical treatment with 0.1% RA cream under occlusion for 4 h or 4 d. Moreover, there was no significant difference in RAR-gamma transcript levels in normal and psoriatic epidermis. RAR-gamma transcripts were constitutively expressed not only in cultured human keratinocytes, but also in human dermal and lung fibroblasts. RAR-beta was induced by RA in dermal fibroblasts, but not in keratinocytes. RA induced IL-1beta transcripts in keratinocytes rapidly (2 to 4 h) and at low concentrations (3 x 10^-10 M), consistent with activation of the IL-1beta gene via RAR. These results demonstrate constitutive expression of RAR-gamma in human epidermis, and suggest that RAR-gamma is a molecular target of RA action in adult human skin. J Invest Dermatol 96:425–433, 1991

Retinoic acid (RA) induces a characteristic set of biochemical and histologic alterations when applied to human or murine skin. In the rhino mouse, which displays an altered pattern of follicular keratinization, topical RA markedly reduces the size of keratinizing follicles, the epidermal filaggrin content, and the wrinkled appearance of the skin [1]. Comedonal acne in man is also characterized by altered follicular keratinization [2], which is reversed by topical RA [3,4]. Topical RA also reduces the appearance of fine wrinkling in photoaged human skin [5,6], coincident with increased epidermal thickness and mitotic counts, compaction of the stratum corneum, increased alcin blue-positive epidermal mucin, and increased numbers of dermal anchoring fibrils [5,7]. Very similar effects are seen in UV-irradiated mouse skin [8,9]. However, the cellular and molecular mechanism(s) by which topical RA treatment affects these cutaneous phenotypes are not well understood.

At the molecular level, the discovery of a family of nuclear retinoic acid receptors (RAR) belonging to the steroid receptor superfamily has provided a possible explanation for the pleiotropic effects of retinoids on the epidermis. [10–14]. These receptors modulate gene transcription by ligand-dependent binding to response elements in the S' regulatory regions of ligand-responsive genes [15]. Thus, a 46-base pair retinoid response element (RRE) has been described in the S' flanking region of the laminin B1 gene [16], which undergoes RA-dependent transcriptional activation in P9 embryonal carcinoma cells [17]. This element functions in HeLa as well as F9 cells [16], indicating that RA can affect transcription directly in epithelial cells. RA also directly stimulates transcription of the tissue transglutaminase gene in macrophages [18].

Retinoic acid has pleiotropic effects on gene expression when applied topically to mouse skin or when used to treat skin-derived keratinocytes or fibroblasts in vitro [19–25]. However, at this time, no genes have been described that are directly activated by RA in normal human keratinocytes or fibroblasts at concentrations consistent with the known affinities of RAR-alpha, -beta, and -gamma for ligand [10–14]. RA demonstrates several biologic effects that are difficult to ascribe to direct gene activation (see [26] for review). In this regard, it has been suggested that the high doses of retinoic acid required to reduce fibroblast collagen synthesis [24,25] could reflect inhibition of ascorbate-stimulated lipid peroxidation rather than binding to nuclear receptors [27]. Therefore, it is important to determine whether the biologic effects of RA on the skin are primarily mediated by RAR, as is the granulocytic differentiation of HL-60 myeloid leukemia cells [28].

As an initial step toward determining the role(s) of RAR in mediating the cutaneous effects of RA, we have determined whether intact human epidermis expresses RAR at the protein level, and have characterized the expression of the three known RAR genes in

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Abbreviations:
CRABP: cellular retinoic acid-binding protein
di-CR: dioctanoyl glycerol
EGF: epidermal growth factor
FPLC: fast protein liquid chromatography
IL-1: interleukin 1
IL-8: interleukin 8
kDa: kilodalton
KGM: keratinocyte growth medium
RA: retinoic acid
RAR: nuclear retinoic acid receptor
RAR-alpha, -beta, -gamma: retinoic acid-receptor-alpha, beta, gamma
RRE: retinoid response element
TGF-alpha: transforming growth factor-alpha
TGF-beta: transforming growth factor-beta
TPA: 12-O-tetradecanoyl phorbol 13-acetate
Table I. Retinoic Acid Binding Activities in Extracts from Human Epidermis and HL-60 cells

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Total (cpm ± SD)</th>
<th>Non-Specific* (cpm ± SD)</th>
<th>Specific (cpm ± SD)</th>
<th>Specific Binding/10^6 cells</th>
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</thead>
<tbody>
<tr>
<td>HL-60 (cell extract)</td>
<td>6465 ± 218</td>
<td>4451 ± 76</td>
<td>2014 ± 218</td>
<td>883</td>
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<tr>
<td>Epidermis (nuclear extract)</td>
<td>6933 ± 703</td>
<td>2316 ± 313</td>
<td>4617 ± 703</td>
<td>1465</td>
</tr>
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</table>

* Extracts were incubated overnight at 4 °C in the presence of 3 nM [3H]retinoic acid and bound ligand was separated from free ligand by filtration as described in Materials and Methods. Assays of HL-60 cells and epidermis contained 58 and 13 μg protein, respectively. Results are means of triplicate determinations from three experiments.

**Table 1.** Retinoic Acid Binding Activities in Extracts from Human Epidermis and HL-60 cells

...normal, psoriatic, and retinoid-treated human epidermis, and in keratinocyte and fibroblasts cultured under various conditions. Our results demonstrate cutaneous expression of RAR protein, indicate that the RAR-γ gene is the most likely source of that protein in human epidermis, cultured keratinocytes and dermal fibroblasts, and identify a keratinocyte gene response consistent with mediation of RA action by RAR.

**MATERIALS AND METHODS**

**Skin Biopsies** After obtaining informed consent, keratome biopsies consisting primarily of epidermis were obtained from the buttocks region of normal volunteers or psoriatic patients as previously described [29]. For studies involving in vivo retinoid acid treatment, 0.1% Retin-A cream (Ortho Pharmaceutical Corp. Raritan, NJ, USA) was applied once to buttocks skin and maintained under occlusion with plastic wrap for 4 h or 4 d prior to biopsy. An adjacent site was identically treated with Retin-A vehicle (Ortho Pharmaceutical Corp, Raritan, NJ, USA). Biopsies were immediately frozen in liquid nitrogen after removal and stored at −70 °C until use.

**Cell Culture** Primary cultures of normal human keratinocytes were prepared as described [30], except that keratome biopsies, rather than punch biopsies, served as the starting material. Subcultures were maintained in a commercially available modification of MCDB153 medium (KGM) optimized for high density keratinocyte growth (Clonetics, San Diego, CA, USA), and used in the second to fourth passage. Keratinocyte cultures were actively growing and subconfluent (50–80% confluent) when treated. Human dermal fibroblast cultures were prepared from papillary dermis, middermis, or reticular dermis obtained from punch biopsies as described [31], propagated in modified McCoy's 5A medium (Sigma Chemical Company, St. Louis, MO, USA) containing 10% fetal calf serum and antibiotics, and used in the third through tenth passages. Human lung fibroblasts were obtained from the American Type Culture Collection and grown in the same medium. Fibroblasts were treated at confluence, two days after the last change of medium. Cells were treated with all-trans RA (Ortho Pharmaceuticals), triacetin, and sodium selenite (Sigma), 12-O-tetradecanoyl phorbol 13-acetate (TPA, Sigma), 1,2-diacylglycerol (diC8, Avanti Polar Lipids), recombinant human IL-1α (Dainippon), transforming growth factor-α (Collaborative Research) or transforming growth factor-β (TGF-β, from bovine bone, provided by L. Ellingsworth, Collagen Corp) for the times and at the doses indicated in the text. Retinoic acid solutions were prepared in dimethylsulphoxide (DMSO), stored under liquid nitrogen, and handled under subdued light.

**Preparation of Nuclear Extracts** For molecular weight determination of RA-binding proteins, fresh keratome biopsies (200–500 mg wet weight) were homogenized by 15 strokes of a loose-fitting Dounce homogenizer in 2 ml of buffer A (15 mM KCl, 3.75 mM NaCl, 0.0375 mM spermine, 0.125 mM spermidine, 0.5 mM EDTA, 3.75 mM Tris–HCl, pH 7.4) containing 0.1% (w/v) digitonin (Sigma) at 4 °C as described [32]. The homogenate was centrifuged at 1000 × g for 5 min and homogenization was repeated once. The resulting nucleic-enriched pellet was resuspended in 1 ml buffer A and NaCl was added to a final concentration of 0.6 M. The sample was then sonicated (3 × 10 sec) on ice and centrifuged at 15,000 × g for 5 min. The supernatant was used for subsequent analysis of RA binding. For comparison of RA binding in epidermal and HL-60 cells, nuclear extracts were prepared from an epidermal cell suspension prepared by trypsinization of fresh keratome biopsies in 0.25% trypsin, 0.1% ethylenediaminetetraacetic acid for 30 min at 37 °C, while total cellular extracts were prepared from HL-60 cells as described [33]. For normalization of RA binding activity to cell number, cells were counted in a hemacytometer prior to preparation of extracts.

**RA Binding Assay** Binding of [3H]RA [11,12-3H] all-trans RA, 50.2 Ci/mmol, New England Nuclear) to nuclear extracts from human epidermis and HL-60 cells was measured by a modification of a previously described filtration method [34,35]. For comparison of RA binding activities in epidermal and HL-60 cells, RAR-containing extracts (10 to 20 μg protein) were incubated overnight at 4 °C with 3 nM [3H]RA. Bound and free ligands were separated by passage through mixed cellulose filters (HAWP, Millipore). Filters were washed 3 times with 1 ml phosphate-buffered saline, followed by 1 ml 25% ethanol, then counted by liquid scintillation spectrometry. Size fraction of epidermal RA binding proteins was performed on a GF 250 column (9.4 × 250 mm, DuPont Pharmaceuticals, Wilmington, DE, USA) connected to an FPLC system (Pharmacia Inc, Piscataway, NJ, USA). Epidermal nuclear extracts (50 to 60 μg protein) were incubated with 100 nM [3H]RA over...
night at 4°C in the presence or absence of 10 μM unlabeled RA, in a total volume of 200 μl. An aliquot (100 μl) was applied to the column and elution was performed with 0.3M KH₂PO₄ (adjusted to pH 7.8 with KOH) at a flow rate of 0.5 ml/min. Fractions (0.35 ml) were collected and analyzed by scintillation counting as previously described [34,35].

To compare the elution positions of [³H]RA binding proteins from human epidermis with that of RA, in vitro-translated, [³⁵S]methionine labeled human RAR-γ (provided by Dr. Anders Åström, University of Michigan, Ann Arbor, MI) was chromatographed under conditions identical to those described above for human epidermal extracts. Fractions were collected and analyzed for radioactivity by scintillation spectroscopy.

RNA and DNA Isolation and Blot Hybridization RNA was extracted from frozen keratome biopsies and cell cultures by guanidinium isothiocyanate lysis and ultracentrifugation exactly as previously described [30,36]. In some experiments, RNA was extracted from cell cultures using RNAzol (Biotex) as directed by the manufacturer. Polyadenylated RNA was prepared by oligo-dT chromatography [37]. Quantitation of specific RNA transcripts was by densitometry of RNA blot autoradiograms after hybridization to various [³²P]-labeled probes as described [36]. Genomic DNA was prepared from various human cells and tissues by proteinase K digestion and phenol-chloroform extraction as described [38] and digested with various restriction endonucleases as recommended by the supplier (Bethesda Research Laboratories). DNA blots were prepared by alkaline capillary transfer from 1% agarose gels to derivatized nylon membranes (Zeta-Probe, Bio-Rad) as described by the manufacturer and hybridized in parallel with the RNA blots.

Plasmids and Hybridization Probes Plasmid DNA was prepared by alkaline lysis followed by polyethylene glycol precipitation [39]. Hybridization probes (specific activity 1–4 × 10⁶ cpm/μg DNA) were prepared by random priming [36] of insert fragments prepared from the plasmids pA3 (IL-1α) [40] and pΔ (IL-1β) [41], containing human interleukin 1 (IL1-α) and β sequences, respectively; psp65C17N3 [42], containing human transforming growth factor-α (TGF-α) sequences; pMDNCF [43], containing human IL-8 sequences; pB15 [44], containing rat cyclophilin sequences, and pNL1-6XB [45], containing lipocortin II sequences. Selection of insert fragments from the plasmids p63 [10], pRAR-β-ER-CAS [12], and phRARγ 0 [13], containing human RAR-α, β, and γ sequences, respectively, is described below.

Data Analysis As appropriate, densitometric data were compared by paired or unpaired Student t tests using a two-tailed hypothesis.

RESULTS

RA Binding Proteins To identify RA binding proteins in human epidermis, nuclei were enriched by low speed centrifugation of keratome homogenates, extracted with 0.6 M KCl, incubated with [³H]RA and subjected to fast protein liquid chromatography (FPLC) size exclusion chromatography as described in Materials and Methods. The results are shown in Fig 1. Peaks of radioactivity were found at eluent volumes consistent with RA binding to proteins of M,45 kDa and 18 kDa, as determined by comparison to the elution volumes of known protein standards. A third peak eluted at an M, of

Table II. Effects of RA Treatment on RAR-γ and Cyclophilin mRNA Levels in Human Epidermis In Vivo

<table>
<thead>
<tr>
<th>Transcript</th>
<th>n</th>
<th>Minimum</th>
<th>Maximum</th>
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<td>RAR-γ</td>
<td>14</td>
<td>0.146</td>
<td>2.13</td>
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* Integrated autoradiographic intensity of RA-treated skin divided by the integrated autoradiographic intensity of vehicle-treated skin.

† p = 0.046 by paired, two-tailed t test, RAR-γ versus cyclophilin.

Figure 3. RAR expression in adult human skin. A, autoradiographs of RNA blots (40 μg total RNA per lane) extracted from keratome biopsies (paired biopsies from two individuals are shown in each panel). Left, RAR-α probe, 3-d exposure; open arrows, mobilities of the 3.8- and 2.8-kb RAR-α transcripts. Center, RAR-β probe, 3-d exposure. Right panel, same blot shown in the center panel was rehybridized against the RAR-γ probe. A 20-h exposure is shown. Solid arrow, mobility of the 3.3-kb RAR-γ transcript. Approximate mobilities of 18S and 28S ribosomal RNA are shown to the left. a, autoradiographs of genomic DNA blots (10 μg per lane). DNA was digested with Bam H1 (lane 1), Eco RI (lane 2), Hind III (lane 3), and Pst I (lane 4). Approximate mobilities of molecular weight markers (in kb) are shown to the left. Left, RAR-α probe, 1-d exposure; center panel, RAR-β probe, 3-d exposure; right panel, RAR-γ probe, 20-h exposure. C, autoradiographs of 40 μg total RNA (left 4 lanes) or 5 μg polyadenylated RNA (right 4 lanes) extracted from keratome biopsies hybridized against RAR-β probe (left, exposure time 3 d) or RAR-γ probe (right, exposure time 36 h). Mobility of the 3.3-kb RAR-γ transcript is shown by the solid arrow.

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less than 10 kDa. The elution positions of the 45- and 18-kDa peaks in human epidermis are essentially identical to those previously demonstrated for murine and human RAR [32–35] and cellular retinoic acid-binding protein (CRABP) [46], respectively. These two peaks of [3H] RA binding were reduced by at least 95% by a 100-times molar excess of unlabeled RA, whereas the less than 10-kDa peak was reduced to a lesser extent (approximately 75%). The presence of a peak similar in size to CRABP in the epidermal extracts probably reflects cytosolic contamination of the nuclear preparations. The identity of the less than 10 kDa peak is not certain, but probably represents free retinoic acid or retinoic acid complexed to other small molecules.

To determine whether the 45 kDa [3H] RA binding peak observed in human epidermis could be RAR, [35S] methionine-labeled RAR-γ was obtained by in vivo translation of RAR-γ mRNA transcribed from the plasmid phRAR-γ-0. Analysis of the in vivo translation reaction by SDS polyacrylamide gel electrophoresis and autoradiography indicated that the major radioactive materials were a protein of approximately 50 kDa and free methionine (data not shown). Chromatography of the in vivo-translation product, under conditions identical to those employed for nuclear epidermal extracts, yielded three peaks of radioactivity: a small peak (2000 cpm), eluting with an apparent molecular weight of 65 kDa, and two large peaks (35000 cpm each) eluting with apparent molecular weights of 45 kDa and less than 10 kDa, respectively (data not shown). The two later peaks most likely are RAR-γ (45 kDa) and free [35S] methionine (less than 10 kDa). The identity of the small 65 kDa peak observed in the in vitro translated material is unknown. Thus, in vitro-translated human RAR-γ co-chromatographs with the 45 kDa [3H] RA binding peak observed in human epidermis.

The specific RA binding activity of epidermal keratocyte extracts was compared to that of HL-60 myeloid leukemic cells, which are known to express RAR proteins but lack CRABP [33]. As shown in Table I, specific RA binding activity of epidermal keratinocyte nuclear extracts was 66% higher than that of total cellular extracts prepared from HL-60 cells, as measured on a per cell basis. Because, as discussed above, epidermal extracts appear to contain CRABP, the amount of RAR in epidermal cells and HL-60 cells is likely to be similar.

### Hybridization Probes
To determine which of the three known human RAR genes might be expressed to yield the 45 kDa peak of [3H] RA binding activity shown in Figure 1, we have prepared cDNA probes from recombinant plasmids containing hRAR-α, -β, and γ containing inserts. To minimize the possibility of cross-hybridization between these related sequences, restriction fragments mapping in the least-conserved 3' regions of the RAR-α and RAR-γ cDNA plasmids were selected for use as probes (Fig 2). Thus, region F of the RAR (Fig 2) displays less than 20% amino acid homology between hRAR-α, -β, and γ genes [13]. Because RAR-β sequences proved to be very difficult to detect in human epidermis and cultured keratinocytes (see below), a longer, 790 bp Xhol-BamHI fragment of the plasmid pRAR-β-ER-CAS [12], containing all of the more highly conserved E region in addition to the entire F region, was used as a probe in an attempt to increase detection sensitivity.
Figure 5. Expression of RAR-γ in cultured adult keratinocytes. A, RAR-α, RAR-β, and RAR-γ are not induced by RA in cultured keratinocytes. The same RNA blot (20 μg total keratinocyte RNA) was repeatedly hybridized to the probes indicated on the right. Expected positions of RAR-α and RAR-β transcripts are indicated by open arrows; RAR-γ, closed arrow. All probes yielded bands of comparable intensity when hybridized against genomic Southern blots (data not shown). B, induction of keratinocyte cytokine and growth factor transcripts by RA. Only the relevant bands of multiple rehybridizations of the same RNA blot (20 μg total RNA) are shown. Probes used are identified to the right. RNA was harvested 24 (lanes a, e, g) or 48 h (lanes b, d, f) after treatment with the concentrations of retinoic acid shown at the bottom of the figure. RA (1000 × in DMSO) was added during a normal refeeding of the cells. C, induction of IL-1β by low concentrations of RA. RNA blots (20 μg total RNA) from cultured KC treated for 4 h with DMSO vehicle (a), or RA at 3 × 10^{-10} M (b), 3 × 10^{-9} M (c), 3 × 10^{-8} M (d), 3 × 10^{-7} M, or 3 × 10^{-6} M (f). Probes are indicated to the right, and mobilities of 18S and 28S ribosomal RNA to the left. –HCZ indicates that the cells were grown in KGM lacking hydrocortisone for 1 week prior to treatment, +HCZ indicates growth in complete KGM.
keratinocytes, and were not induced by various concentrations of RA at 4 or 48 h. Figure 5A, B demonstrates that addition of RA under various conditions resulted in less than 1.5 fold changes in RAR-γ mRNA levels. Figure 5B shows that after 24 or 48 h of RA treatment, other keratinocyte transcripts, including transforming growth factor-α (TGF-α), interleukin-1β (IL-1β), and IL-8, were increased more than threefold over untreated controls. Of the transcripts analyzed, IL-1β was distinctive in that it was consistently induced by low concentrations of RA (as low as $3 \times 10^{-10}$ M) within 4 h (Fig 5C). Clear induction of IL-1β transcripts was seen at 2 h but not at 30 min after RA treatment (data not shown).

Keratinocyte RAR-γ transcript levels were also not detectably increased by treatment with the protein kinase C (PKC) acti-
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vators di-C8 and TPA or purified human recombinant IL-1α under conditions [48,49] in which IL-8 transcripts were markedly induced (Fig 6). TGF-α (20 ng/ml, 4 or 24 h), transforming growth factor-β (TGF-β) (1 to 10 ng/ml, 3 to 48 h), triamcinolone acetonide (10 to 10-4 M, 4 or 24 h), and triiodothyronine (3 x 10-8 M) also did not increase keratinocyte RAR-γ transcripts (data not shown).

We also analyzed the expression of RAR-α, -β, and -γ transcripts in secondary cultures of human dermal fibroblasts (Fig 7). As found for human epidermis and cultured keratinocytes, fibroblast RAR-β transcripts were easily detectable and not induced by the addition of RA (Fig 7A). RAR-α and -β transcripts were not detectable under basal conditions in fibroblasts. In contrast to cultured keratinocytes, however, RAR-β transcripts were induced by RA in fibroblasts after 24 to 48 h but not at 4 h (Fig 7A). In the experiment shown, RAR-α transcripts were transiently induced at 4 h in response to fresh medium alone, and this response was not potentiated by RA (Fig 7A). However, this response was observed in only one of five experiments. On an RNA basis, RAR-γ transcripts were found in similar amounts of keratinocytes, in fibroblasts derived from reticular, mid, and papillary dermises, and in three strains of human lung fibroblasts (Fig 7B).

DISCUSSION

The discovery of nuclear retinoic acid receptors possessing ligand-dependent transcriptional enhancer activity [10-12] provided an important new insight into the understanding of the molecular basis of retinoid action. It was paradoxical then, that at the time of their discovery, the RAR-α and RAR-β genes were found to be expressed only at fairly low levels in adult human skin [13], a highly retinoid-responsive tissue [2-7]. This paradox was apparently resolved with the description of the RAR-γ gene, which is well-conserved and expressed at high levels (as determined by steady-state transcript levels) in adult human [13] and murine [14] skin.

We have confirmed these results at the mRNA level (Fig 2), and extended them to demonstrate the presence of one or more 45-kDa specific RA binding protein(s) in normal human epidermis (Fig 1). Because the RAR-α and -β transcripts are only minimally expressed in these skin samples (Fig 3 and 4), it is likely, if not proved directly, that the 45-kDa RA binding activity represents the RAR-γ gene product. Our in vitro results (Fig 5 and 7) indicate that RAR-γ is also the predominant RAR species expressed in cultured keratinocytes and in fibroblasts derived from reticular and papillary dermises. It is interesting that the RAR-α gene appears to be expressed at higher levels in fetal than in adult skin in vivo [13,47] and at detectable levels in neonatal human keratinocytes in vitro [47]. Differential expression of RAR-α in fetal versus adult skin could reflect developmental regulation of retinoic acid-responsive genes.

We have begun to examine the regulation of RAR gene expression in adult human skin and in cultured keratinocytes. No autoinduction of any of the RAR genes was observed after 4 h or 4 d of topical RA treatment. In fact, a statistically significant decrease in RAR-γ transcripts was observed after 4 d of treatment (p = 0.046, Table I), probably due to rather marked decreases in selected individuals (Fig 4A). Similarly, in cultured adult keratinocytes, RAR-γ gene expression was not markedly increased between 4 and 48 h of RA treatment, whereas transcripts for TGF-α, IL-1β, and IL-8 were clearly induced (Fig 5). IL-1β mRNA was consistently induced at short times (2 to 4 h) and at low RA concentrations (3 x 10^{-10} M). This dose response corresponds well with the affinity of various RAR for RA [10-14], strongly suggesting that RA exerts at least some of its action on keratinocytes via RAR.

Expression of RAR-γ in human dermal fibroblasts (Fig 7) suggests that the known responses of dermal cells to topically applied retinoic acid [8,9] could reflect the direct action of RA on RAR in those cells rather than secondary responses to RA treatment of keratinocytes. Here we show that RAR-β is induced by RA in human dermal fibroblasts, albeit with delayed kinetics (24 to 48 h). Induction of RAR-β by RA has been reported in hepatoma [50] and teratocarcinoma [51] cell lines, and a retinoid response element (RRE) has been described in the 5' flanking region of the RAR-β gene [52]. The regulation of RAR-β gene expression clearly depends on the cell type analyzed because RA failed to induce RAR-β transcripts in keratinocytes (Fig 5A and data not shown) and the induction of RAR-β in hepatocytes and teratocarcinoma cells occurs much more rapidly than in fibroblasts [50,51]. Consistent with the report of RAR-γ expression in mouse lung [14], we find that RAR-γ is also the principal RAR transcript expressed in human lung fibroblasts (Fig 7B). Moreover, RAR-γ expression was comparable in fibroblasts derived from papillary, mid, and reticular dermises, suggesting that RAR expression is not an important determinant of the different proliferative capacities of fibroblasts derived from these sources [31].

We found little evidence for regulation of RAR-γ mRNA levels in response to activation of various signal transduction pathways. The PKC activators di-C8 and TPA, and the cytokine hrIL-β had no effect on RAR-γ transcript levels, whereas IL-8 mRNA was strongly induced by these treatments as previously described [48,49]. TGF-β, which is strongly synergistic with RA in the inhibition of murine keratinocyte proliferation [53], also did not influence keratinocyte expression of RAR-γ (data not shown). Therefore, the synergistic interaction of TGF-β and RA [53] appears not to be due to induction of RAR gene expression. Similarly, treatment with epidermal growth factor (EGF) or TGF-α under conditions that markedly stimulate keratinocyte growth and gene expression had no effect on RAR-γ transcript levels (data not shown). Triamcinolone acetonide and triiodothyronine, which bind to nuclear receptors related to RAR [15], also had no effect on RAR transcript levels, and the omission of hydrocortisone from KGM did not influence RAR expression in response to other stimuli (Fig 5C). TGF-α, IL-1β, and IL-8 transcripts were induced in response to RA treatment of cultured keratinocytes; of these, the induction of TGF-α and IL-8 required exposure to high doses of RA for prolonged periods. The induction of IL-1β mRNA by low concentrations of RA after short times is evidence that at least some of these inductions are the direct result of RAR-RRE interactions.

These studies have demonstrated a 45-kDa specific RA binding activity, consistent with RAR protein, in human epidermis. RNA blot hybridization experiments identify RAR-γ as the likely source of this 45-kDa nuclear RA binding activity in human epidermis, keratinocytes, and dermal fibroblasts. As mRNA levels do not necessarily correlate with protein abundance, proof of this point will require the development of high affinity, isoform-specific antibodies and further biochemical characterization of the 45-kDa peak. From the available in vivo and in vitro data, the RAR-γ gene appears to be constitutively expressed, and its transcript is relatively abundant in human epidermis. When considered in the context of its selective expression in the skin [13,14], the expression of RAR-γ in two cutaneous cell types—keratinocytes and fibroblasts—suggests that RA action via RAR-γ could be an important mediator of dermal-epidermal interactions. Taken together, these results strongly suggest that RAR-γ is a major mediator of retinoid action in human skin. The direct implication of RAR-γ as the molecular mediator of cutaneous retinoid action remains a challenge for future research.

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