Exp Dermatol 1996: 5: 218–226
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Experimental Dermatology
ISSN 0906-6705

Differential regulation of IL-1 and IL-1 receptor antagonist in HaCaT keratinocytes by tumor necrosis factor-α and transforming growth factor-β1

Kang K, Hammerberg C, Cooper KD. Differential regulation of IL-1 and IL-1 receptor antagonist in HaCaT keratinocytes by tumor necrosis factor-α and transforming growth factor-β1.
Exp Dermatol 1996: 5: 218–226. © Munksgaard, 1996

Abstract: Cytokines such as TNFα and TGFβ1 have potent effects on keratinocyte differentiation and have been implicated in cutaneous injury, immunologic reactions, and wound healing. To determine whether such conditions might alter the balance of epidermal keratinocyte IL-1 and the IL-1 receptor antagonist (IL-1ra), TNFα and TGFβ1 were added to HaCaT cells, a human adult keratinocyte cell line. mRNA levels of IL-1α, IL-1β, and IL-1Ra were detected by polymerase chain reaction (PCR) on reverse transcribed RNA extracts, followed by Southern blot of the PCR products, ^{35}S-labeled probe hybridization, and quantification against standard curves. TNFα (100 ng/ml) at the 3-h time point significantly induced increases in mRNA expression of Il-1α (9.2±2.9 fold increase) and IL-1β (2.5±0.7 fold increase) (n=7) which were concordant with increases in IL-1α protein (7.1±1.3 fold increase) and Il-β protein (4.4±1.0 fold increase) measured by ELISA 24 h after stimulation. By contrast, icIL-1Ra mRNA and protein levels were not affected by TNFα. TGFβ1 induced a mild increase in IL-1α mRNA (3.8±1.8 fold) and protein (3.5±1.2 fold). TGFβ1 did not affect IL-1β mRNA levels but caused variable increases in IL-1β protein levels. TGFβ1 did not alter icIL-1Ra mRNA or protein levels. Inhibition of RNA synthesis with actinomycin D demonstrated that the rate of degradation of IL-1β mRNA was reduced by treatment with TNFα. This stabilization of IL-1β mRNA was specific, because TGFβ1 did not stabilize IL-1β mRNA, and TGFβ1 and TNFα did not increase the stability of Il-1α mRNA. icIL-1Ra mRNA was fairly stable over a 20 hour period and its slow degradation was not affected by treatment with either TNFα or TGFβ1, indicating a higher steady state stability of icIL-1ra mRNA relative to IL-1 mRNA's. Given the high rate of degradation of IL-1α and IL-1β mRNA, levels of these mRNAs may rapidly decrease while the icIL-1ra mRNA levels remain constant, thus allowing for rapid dampening of IL-1 activity soon after the stimuli provoking an inflammatory or reparative response have abated. In conclusion, TNFα and TGFβ1, cytokines with potent effects on inflammation and differentiation, both induce keratinocyte IL-1α mRNA and protein levels, but differentially regulate IL-1β mRNA. They both exert little effect on IL-1 Ra levels, which were constitutively highly stable. Such differential regulation provides mechanisms for separately controlling the relative activity of these cytokines under normal and disordered conditions.

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Key words: Keratinocytes – TGFβ1 – TNFα – IL-1-IL-1 receptor antagonist

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Accepted for publication 11 December 1995

Abbreviations: cDNA: Complementary deoxyribonucleic acid, ELAM-1: Endothelial cell-leukocyte adhesion molecule-1, ELISA: Enzyme-linked immunosorbent assay, ICAM-1: Intercellular adhesion molecule-1, IL-1: Interleukin-1, ic IL-1ra: Intracellular IL-1 receptor antagonist, RT-PCR: Reverse transcription-polymerase chain reaction, TGF: Transforming growth factor, TNF: Tumor necrosis factor, UV: Ultraviolet, VCAM: Vascular cell adhesion molecule.

IL-1α and IL-1β have diverse functional activities that modulate immunologic, inflammatory and reparative responses and have been demonstrated to be constitutively produced by human keratinocytes (1-6).In addition, an interleukin-1 receptor antagonist (IL-1ra) which inhibits IL-1α and IL-1β biological activity, has been demonstrated to be produced by keratinocytes (7). However, as opposed to the secretory form produced by monocytes (8), human keratinocytes, both in vitro (7,9) and in vivo (10), produce a novel mRNA splice variant in which the leader sequence for secretion is replaced, such that the molecule is exclusively intracellular (icIL-1ra) (7). It has been demonstrated that IL-1α and IL-1β are differentially expressed in normal and psoriatic skin, with IL-1α decreased and IL-1β elevated in psoriatic compared to normal skin (5). Recent in vivo results showing a dramatic increase in the ratio of icIL-1ra to IL-1α in involved psoriatic skin compared with normal skin (10) suggested that IL-1α and icIL-1ra also may be discordantly regulated in keratinocytes. IL-1α, IL-1β and IL-1ra production is regulated by a select set of cytokines (11,12), including TNFα and $TGF\beta_1$, which possess potent and, in some circumstances, opposing effects on inflammation, proliferation and differentiation. TNFα, like IL-1, is a pro-inflammatory cytokine (13) that plays a major role in contact hypersensitivity reactions (14) and UV injury (15). The primary cell source for TNFα in the dermis is mast cells (16) although stromal dendrocytes (17) and basal keratinocytes (14) may also produce TNFα. TNFα induces increased production of both IL-1α and IL-1β in various cell types (11) including keratinocytes (12,18,19).

$TGF\beta_1$ has potent immunosuppresive, antiproliferative, and differentiating effects (20,21) and it also plays a critical role in wound healing (22,23). It is normally present in an inactive precursor state in suprabasal keratinocytes (24). The active form of $TGF\beta_1$ has been detected suprabasally in psoriatic lesions (24) and in epidermis undergoing a wound healing response. Its absence in knockout mice results in multifocal inflammatory disease and epidermal keratinocyte hyperproliferation (25). Like TNF. $TGF\beta_1$ also has been implicated, at least in peripheral blood mononuclear cells, in the regulation of IL-1β (26) as well as secretory IL-1ra (27).

This study addresses the question of whether the dysregulation of IL-1α, IL-1β and icIL-1ra, such as that observed in the hyperproliferative and inflammatory milieu of psoriatic skin, may be due to differences in the regulation of IL-1α, IL-1β and icIL-1ra production by IL-1-modulating cytokines. Because TNFα and TGFβ have distinct, and sometimes opposing, immunosuppressive, antiproliferative and differentiating effects on a number of cell types, we postulated that they may differentially regulate the balance of IL-1 and its receptor antagonist in keratinocytes. Because the spontaneously immortalized human keratinocyte cell line, HaCaT, is nontumorigenic and retains full epidermal cell differentiation capacity when transplanted onto nude mice, it is considered to be an in vitro cell line which models well human keratinocyte regulation (28). Our results indicate that TNFα and TGFβ1 indeed exert differential regulatory effects on IL-1α/IL-1β/icIL-1ra protein and mRNA regulation, which in turn results in increased IL-1α/IL-1β levels relative to icIL-1ra.

Material and methods

Cell culture

HaCaT cells, an immortalized and not transformed adult human keratinocyte cell line, were a gift from Dr. Fusenig (German Cancer Research Center, Heidelberg, Germany) (28). Dulbecco's Modified Eagle's Medium (DMEM), high glucose, (Irvine Scientific, Santa Ana, CA) containing 1% penicillin and stretomycin, 5mM L-glutamine and 10% heat-inactivated fetal bovine serum (FBS) was used for cell cultivation. HaCaT cells were obtained from confluent cultured cells by washing with Hanks balanced salt solution (HBSS, calcium and magnesium-free) and trypsinization at 37°C for 10 min. Cells were then adjusted in DMEM plus 10% FBS to 5×10^5/ml. A 2-ml cell suspension was distributed into 60×15mm tissue culture dishes (Falcon 3002, Becton Dickinson, NJ). After 24 h incubation, the HaCaT cells were in a subconfluent state, and fresh DMEM plus 10% FBS with TNFα (100 ng/ml) (R&D Systems, Inc., Minneapolis, MN) or TGFβ1 (1 ng/ml) (R&D) was added for further incubation. Actinomycin D (10 μg/ml) (Sigma, St Louis, MD) was added to the cultures 3 h post-stimulation with the above cytokines for determination of their mRNA stability.

RNA extraction

At the end of the incubation period, the medium was completely aspirated and RNAzol was added to the each dish with repeated pipetting as described in the manufacturer's instructions (TEL-TEST Inc., Friendswood, TX).

Reverse-transcription polymerase chain reaction (RT-PCR) amplification

RT-PCR was performed as previously described (29). The primers were chosen according to published cDNA sequences (7, 30) (Table 1), and synthesized by Biomedical Research Core Facilities,

Table 1. Cytokine primers and cDNA fragment sizes

Cytokine	Primer purpose	cDNA size(bp)	Location (5'-3')		
IL-1	PCR amplification	421	*U	+ 89–	+114
			*D	+485–	+509
	internal probe	390	U	+120–	+140
			D	+485–	+509
IL-1ß	PCR amplification	411	U	+145–	+165
			D	+532–	+555
	internal probe	391	U	+165–	+189
			D	+532–	+555
icIL-1	PCR amplification	511	U	+ 50–	+ 70
			D	+534–	+560
	internal probe	470	U	+ 91–	+114
			D	+534–	+560
sIL-1	PCR amplification and probe	492	U	+ 24–	+ 45
			D	+489–	+515
ß-actin	PCR amplification	539	U	+103–	+122
			D	+619–	+641
	internal probe	447	U	+195–	+215
			D	+619–	+641

*U:upstream
*D:downstream

University of Michigan, Ann Arbor, MI). 25 cycles were carried out in a Coy Tempcycler (Coy Laboratory Products Inc., Ann Arbor, MI) with denaturing at 94°C for 1 min, annealing at 55°C for 1 min and extension at 72°C for 2 min. β-actin was used as a control gene (31).

For quantification of cDNA (29), a standard curve of the appropriate cDNA for each mRNA was performed simultaneously. The PCR-derived cDNA product was prepared by isolating the specific cDNA band from an agarose gel, using a QIAEX agarose gel extraction kit (QIAGEN Inc., Chastsworth, CA) and were identified as the appropriate PCR-cDNA product for the particular cytokine by restriction enzyme analysis.

Southern blot hybridization

Following electrophoresis, a Posiblot Pressure Blotter (Stratagene, La Jolla, CA) was used to transfer DNA from the gel to a nylon membrane (Hybond-N, Amersham Co., Arlington Height, IL) as described in the manufacturer's instructions. After UV-crosslinking for 5 min, the non-specific DNA binding sites on the nylon membrane were blocked by incubating the nylon membrane in prehybridization buffer (50% formamide, 5× SSC, 10× Denharts, 0.1% SDS with 50 g/ml salmon sperm DNA) at 42°C for 2 h. ^{35}S-dATP radiolabeled dsDNA probes were generated by PCR using 5' primers internal to the 5' primer that was used to detect the presence of cDNA in the experimental samples (Table I). These probes were labeled with ^{35}S-dATP by random-primed labeling utilizing the DNA polymerase I Klenow fragment (Prime-a-Gene, Promega, WI). The ^{35}S-ATP-DNA probe was added (5×10^5 cpm/ml) to the nylon membrane and incubated overnight. At the end of incubation, the membrane was washed twice with 0.1× SSC containing 0.1% SDS (20 min at 65°C), then dried at room temperature.

Phosphorimager analysis of PCR cDNA products (29)

Quantification of the hybridization bands was performed with a Phosphorimager (Molecular Dynamics, Sunnyvale, CA), which determined the amount of β-emission activity in each band. The emission activity in each band was converted to concentration of cDNA PCR product by linear regression of the radioactivity of the cDNA standard curve against the cDNA concentration used as the cDNA template in the standard curve PCR reaction. Experiments were used only if the correlation coefficient was greater than 0.95.

Quantification of cytoplasmic protein by ELISA

After a 24-h incubation with TNFα (100 ng/ml) or TGFβ1 (1 ng/ml), a cell suspension was prepared by trypsinization. HaCaT cells were then counted and centrifuged, 1% Triton X-100 buffer (10mM Tris-HCl, pH 7.4, 2mM $MgCl_2$, 150mM NaCl, 2mM PMSF, 1% Triton ×100) was added to the pellet at 100 µl per 5×10^5 cells, then incubated at 4°C for 30 min. Cells were then microcentrifuged for 15 min and the supernatant collected and stored at -70°C until assayed. The amount of IL-1α, IL-1β or IL-1ra in each sample was determined by ELISA (Quantikine Kit, R&D Systems). Results are expressed as pg/10^6 HaCaT cells.

Results

HaCaT cells constitutively produce icIL-1ra and not sIL-1ra

An understanding of the effect of various cytokines upon overall HaCaT IL-1 activity requires a determination of which IL-1ra variant, sIL-1ra or icIL-1ra, is expressed by HaCaT keratinocytes. For detection of IL-1/IL-1ra mRNA by RT-PCR and Southern blot hybridization, it was found that RT of 200 ng of total cellular RNA with 25 cycles of PCR amplification was optimal. However, primers specific for sIL-1ra mRNA were unable to detect the presence of sIL-1ra mRNA from HaCaT keratinocytes. Based upon the limits of the standard curve of sIL-1ra cDNA (Fig. 1), the concentration of sIL-1ra in these keratinocytes would have to be less than

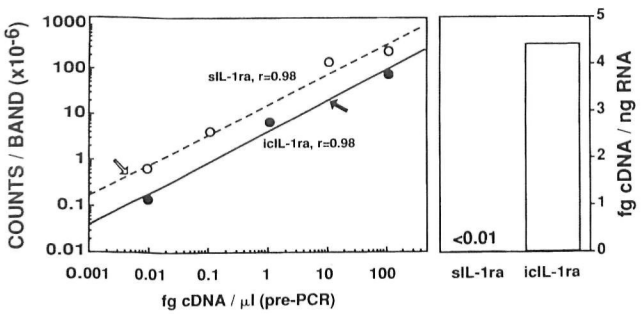

Fig. 1. Semiquantitative cytokine determination of sIL-1ra and icIL-1ra mRNA by PCR. Total RNA was extracted with RNAzol from HaCaT keratinocytes, 200 ng was reverse transcribed to cDNA, and a standard curve of corresponding cDNA (sIL-1Ra = open circles) (icIL-1Ra = closed circles) with a series of 10-100 fold dilutions (112 fg/µl→ 0.01 fg/µl) was simultaneously amplified by PCR for 25 cycles. ^{35}S labeled internal probes were hybridized to Southern blotted PCR products, and quantified by phosphorimager. sIL-1ra was less than 0.004 fg cDNA/ng RNA, (left panel, open arrow) and icIL-1ra was 4.4 fg cDNA/ng RNA (left panel, closed arrow and right panel, right bar).

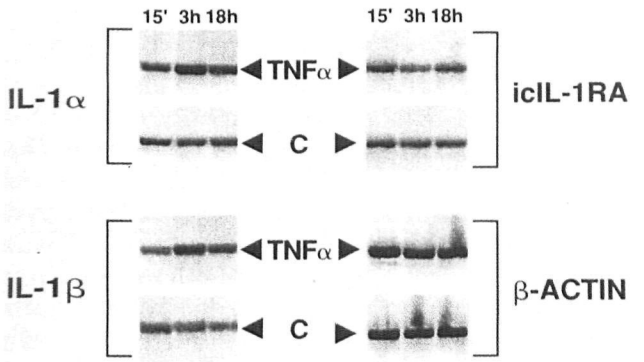

Fig. 2. TNFα induces IL-1α and IL-1β mRNA but not icIL1ra. Total RNA was harvested from HaCaT keratinocytes at 15 min, 3 hours and 18 h following TNFα (100 ng/ml) stimulation or from control cells (C) that did not receive TNFα. The RNA was then reverse transcribed to cDNA and PCR-amplified using primers for IL-1α, IL-1β, icIL-1ra and β-actin. Southern blot hybridization with 35S probes was quantified by Phosphoimager.

0.004 fg cDNA/ng RNA. By contrast, RT cDNA of icIL-1ra was usually greater than 0.4 fg/ng RNA (Fig.1). In addition, neither TNFα nor TGFβ1 induced expression of sIL-1ra (data not shown).

TNFα induces IL-1α and β, but not icIL-1ra mRNA expression and protein production

In preliminary experiments, TNFα at a concentration of 100 ng/ml was found to be the optimal dose for induction of both IL-1α and IL-1β protein (data not shown) and thus used to study the effect of TNFα upon IL-1α, IL-1β and icIL-1ra mRNA induction. A time course study (Fig. 2) showed that IL-1α mRNA levels were markedly increased by TNFα with a peak response occurring at 3 h relative to control HaCaT keratinocytes cultured in parallel (Fig. 2, upper left panel). Thus, after quantification of counts of $S^{35}\beta$-emission per band and comparison to the IL-1α cDNA standard curve, HaCaT keratinocytes stimulated for 3 h with TNFα contained 1.19 fg IL-1α cDNA/ng total RNA, as compared to control HaCaT keratinocytes at this time point which exhibit only 0.05 fg/ng RNA. Induction of IL-1β mRNA levels by TNFα also peaked at 3 h (Fig. 2, lower left panel). Thus, 3 h after TNFα, there were 54 fg IL-1β/ng RNA versus 22 fg/ng RNA in the control culture. By contrast, TNFα-stimulated HaCaT keratinocyte icIL-1ra mRNA levels did not demonstrate an increase in band intensity (3.3 fg/ng RNA at 3 h) relative to control cells (6.5 fg/ng RNA) even at the peak response time (3 hours) for IL-1α and β mRNA increases (Fig 2, upper right panel). A similar lack of changes in actin mRNA levels at all time points was observed (Fig. 2, lower right panel).

In order to make grouped data comparisons using replicate experiments, band radioactivity was quan-

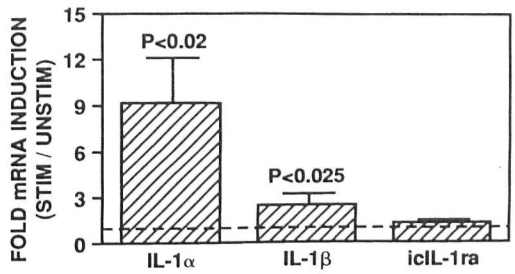

Fig. 3. TNFα induced increases in IL-1α and IL-1β, but not icIL-1Ra, mRNA levels at the 3-h time point. Messenger RNA levels determined by semi-quantitative PCR are expressed as the mean±SEM of 7 separate experiments. Statistically significant differences of IL-1α ($p<0.02$) IL-1β ($p<0.025$) and are relative to icIL-1ra.

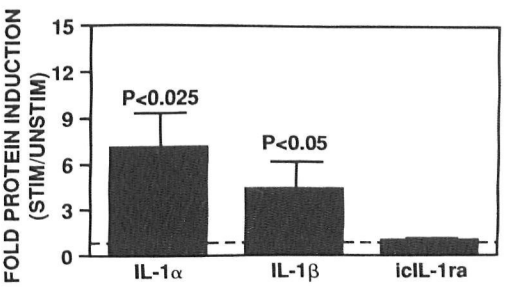

Fig. 4. TNFα induced increases in IL-1α,IL-1β protein levels at the 24-h time point. Protein levels determined by ELISA are expressed as the mean±SEM of 3 experiments. Statistically significant differences of IL-1α ($p<0.025$) and IL-1β ($p<0.05$) are relative to icIL-1ra.

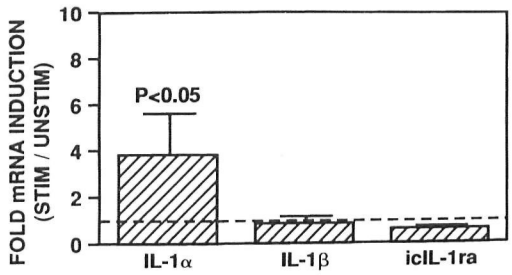

Fig. 5. TGFβ1 induced increases in IL-1α mRNA levels at the 3-h time point but not IL-1β and icIL-1ra mRNA levels. mRNA levels determined by semiquantitative PCR are expressed as the mean±SEM of 6 (IL-1α) and 8 (IL-1β and icIL-1ra) experiments. Statistically significant differences of IL-1α is relative to icIL-1ra mRNA levels.

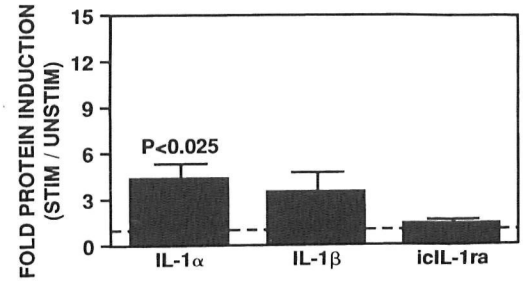

Fig. 6. TGFβ1 induced increases in IL-1α protein levels at the 24-h time point, but not icIL-1ra protein levels and variably induced IL-1β protein levels. Protein levels determined by ELISA are expressing as mean±SEM of 8 (IL-1α, IL-1β) or 5 (icIL-1ra) experiments. Statistically significant differences of IL-1α ($p<0.025$) but not IL-1β are relative to icIL-1ra.

tified by phosphorimager analysis and expressed as the fold increase in mRNA levels of TNFα-stimulated HaCaT keratinocytes over unstimulated cells cultured in parallel for the same time period (Fig. 3). Thus, TNFα induced a mean 9.2+2.9 fold increase in IL-1α mRNA at the 3-h time point (n=7 experiments). IL-1β mRNA was also induced in each of the 7 experiments, but to a lesser degree (2.5+0.7 fold increase at 3 h). However, icIL-1ra did not exhibit even a modest increase in any of the experiments. The increases in IL-1α and IL-1β mRNA were statistically significant relative to icIL-1ra

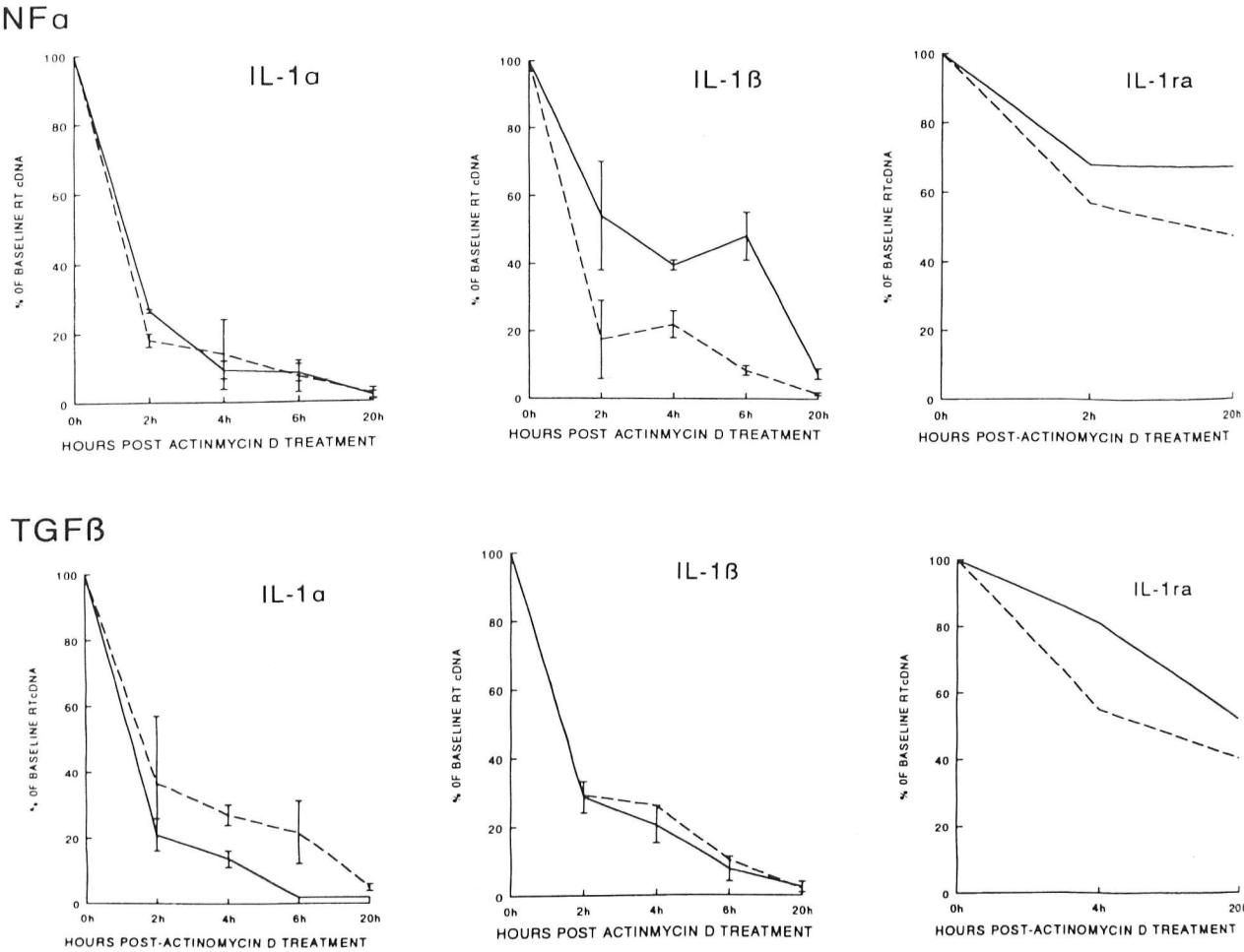

Fig. 7. Degradation rate of IL-1α, IL-1β, and IL-1ra mRNA post TNFα (100 ng/ml) and TGF1 (1 ng/ml) stimulation (3 h) followed by actinomycin D treatment (10 μg/ml). Dash line - control; solid line - after TNFα or TGFβ1 stimulation.

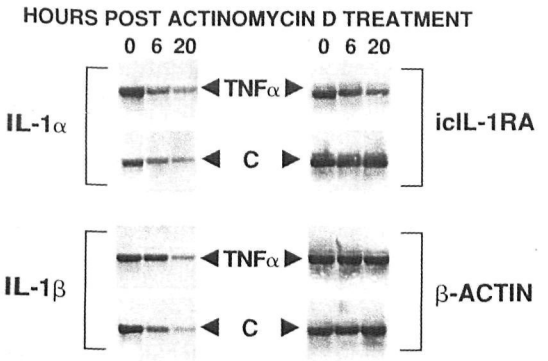

Fig. 8. A representative experiment showing actinomycin D-treated HaCaT keratinocyte mRNA degradation. 3 h after TNFα stimulation, cells were treated with actinomycin D, then harvested at 0, 6 and 20 h post-actinomycin D treatment. mRNA levels were determined by semi-quantitative PCR. TNFα did not increase the stability of IL-1α mRNA. IL-1β mRNA levels showed only 60% reduction in TNFα treated cells at 6 h relative to 90% reduction in control cells. icIL-1ra and β-actin mRNA levels exhibited slow degradation rates that were basically unaffected by TNFα.

($p<0.02$ and $p<0.025$, respectively) (Fig. 3) and relative to actin (not shown).

Quantification of cytoplasmic IL-1α, IL-1β, and icIL-1ra protein extracted from HaCaT cells stimulated for 24 hours with TNFα showed that protein levels reflected fairly closely the mRNA levels (Fig. 4). ELISA values for unstimulated HaCaT keratinocyte IL-1α (105±49 pg/ml) rose to 613±199 pg/ml after TNFα stimulation. Similarly, IL-1β protein rose from 124±55 to 415±91 pg/ml, whereas icIL-1ra protein levels were at 19931+1168 pg/ml (unstimulated) and 22365±1030 pg/ml (stimulated) (n=3). Converted to fold increase, IL-1α, IL-1β and icIL-1ra protein levels increased 7.1±1.3, 4.4±1.0, and 1.1±0.03-fold respectively (Fig. 4). Both IL-1α and IL-1β protein inductions were significantly increased over that of icIL-1ra ($p<0.025$ and $p<0.05$ respectively) (Fig. 4).

TGFβ1 induces IL-1α, but not IL-1β or icIL-1ra mRNA

Based upon a dose response study of TGFβ1 stimulation of HaCaT keratinocyte IL-1α and IL-1β protein levels (data not shown), an optimal stimulating dose of 1ng/ml TGFβ1 was selected. A time course study showed that TGFβ1 induced increases in IL-1α mRNA levels at 3 h (3.8±1.8 fold increase, n=6), at 6 h (3.4±1.7 fold increase, n=4) and at 24 h (2.8±0.7 fold increases, n=4). However, TGFβ1 did not affect IL-1β mRNA levels at any of these time points: at 3 h the fold increase was 0.9±0.3, (n=8), at 6 h it was 0.7±0.2, (n=4), and at 24 h it was 1.6±0.5, (n=5). TGFβ1 also did not affect the expression of icIL-1ra mRNA, with fold increases of 0.6±0.1 (n=8), 1.1±0.4 (n=4), and 0.8±0.3 (n=4) at 3 h, 6 hours, and 24 h, respectively. The increase in IL-1α mRNA levels, but not IL-1β, relative to icIL-1ra mRNA levels at 3 h was statistically significant ($p<0.05$) (Fig. 5).

TGFβ1 induces not only IL-1α protein, but also IL-1β protein, despite a lack of induction of IL-1β mRNA

The TGFβ1-stimulated increase of IL-1α mRNA levels was paralleled by a statistically significant increase in IL-1α cytoplasmic protein levels in HaCaT keratinocytes 24 h after stimulation with TGFβ1. Interestingly, IL-1α cytoplasmic protein levels also increased, albeit somewhat variably, resulting in an overall 3.5±1.2 fold increase. Concordant with the lack of TGFβ1 induction of icIL-1Ra mRNA, icIL-1ra protein demonstrated only a 1.4±0.2 fold increase (Fig. 6).

Differential stability of steady-state and regulated levels of IL-1α, IL-1β, and icIL-1ra mRNA

Actinomycin D, a specific inhibitor of DNA-dependent RNA synthesis, was used to determine whether TNFα or TGFβ1 induced changes in the stability of IL-1α, IL-1β and icIL-1ra mRNA. Actinomycin D was added to HaCaT keratinocytes after exposure for 3 h to either TNFα or TGFβ1. Cytokine mRNA levels were then measured at various time intervals thereafter to determine the mRNA rate of degradation. HaCaT IL-1α mRNA was extremely unstable, with a 70% reduction in IL-1α mRNA levels in untreated HaCaT keratinocytes occurring by 2 h post-actinmycin D treatment (Fig. 7). Exposure of HaCaT keratinocytes to TNFα (Fig. 7, 8) or TGFβ1 (Fig. 7) did not increase the stability of IL-1α mRNA. IL-1β mRNA was also highly unstable, with a 70-80% reduction in IL-1β mRNA levels in control HaCaT keratinocytes occurring as soon as 2h after actinomycin D treatment (Fig. 7). However, the rate of degradation of IL-1β mRNA was retarded by treatment with TNFα (Fig. 7) but not TGFβ1 (Fig. 7). Thus, treatment with TNFα resulted in a slower rate of degradation of IL-1β mRNA with only a 30% reduction at 2 h (relative to 80% reduction in unstimulated cells) and a 60% reduction at 6 h (relative to 90% reduction in unstimulated cells) post actinomycin D exposure, respectively (Fig. 7, 8).

Interestingly, HaCaT keratinocyte icIL-1ra mRNA was more stable than either IL-1α or IL-1β mRNA, with less than a 40% reduction in icIL-1ra

occurring with the first 4 h post actinomycin D exposure and less than 50% degradation 20 h post-actinomycin D addition. Neither TNFα (Fig. 7) nor TGFβ1 (Fig. 7) significantly affected the rate of degradation of icIL-1ra mRNA.

Discussion

Our experiments indicate that the profile of IL-1 family mRNA and protein production of HaCaT keratinocytes is consistent with that observed in normal human and murine keratinocytes, with the dominant IL-1/IL-1ra species being IL-1α and icIL-1ra (3, 5, 7, 9, 10, 18, 32). The constitutive levels of these cytokines in HaCaT keratinocytes are very similar to constitutive levels in normal human keratinocytes(10), thus, it is feasible to use HaCaT keratinocytes as an in vitro model for these cytokines. These data further indicate that, whereas IL-1α and IL-1β are both responsive to important regulatory cytokines of the skin such as TNFα and TGFβ1, icIL-1ra is remarkably resistant to cytokine stimulation. The stability of icIL-1ra in keratinocytes was further emphasized by its resistance to degradation under both unstimulated and stimulated conditions. High mRNA abundance and stability would suggest that maintenance of a stable level of icIL-1ra is important to the homeostatic function of the cell.

TNFα-induced increases in IL-1β mRNA or protein levels were less prominent than the IL-1α increases. Because IL-1α is biologically active whereas IL-1β is not in cultured keratinocytes (3) as well as in in vivo human epidermis (5), the net result of TNFα and TGFβ1 stimulation would be expected to be an increase in bioactive IL-1 material.

The effect, if any, of TGFβ1 upon IL-1β expression was subtle. IL-1β protein levels were increased, but inconsistently, whereas IL-1β mRNA levels at 3 h were unaffected by TGFβ1 treatment. The dissociation between IL-1β mRNA expression at the 3-h time point and inconsistent IL-1β protein increases stimulated by TGFβ1 could possibly be attributed to a more delayed effect of TGFβ1 upon IL-1β mRNA levels (1.6±0.5 fold increase at 24 h versus 0.9±0.3 at 3 h) and/or an effect on the efficiency and stability of protein production at the translational and post-translational level (33).

The present observation on the degradation rate of IL-1α mRNA showed that IL-1α mRNA normally was unstable (70% decay by 2 h) and IL-1α stability was unaffected by treatment with either TNFα or TGFβ1. IL-1β mRNA was also unstable in unstimulated cells and the degradation rate was unaffected by TGF. However, less decay of IL-1β mRNA occurred after exposure to TNFα, suggesting that the IL-1β mRNA increase, but not the IL-1α mRNA increase, was due, at least in part, to TNFα-mediated stabilization of IL-1β transcripts. These results imply differential sites of regulation of keratinocyte IL-1α and IL-1β mRNA expression by TNFα.

Unlike both IL-1α and IL-1β mRNA, the icIL-1ra mRNA was relatively stable with a $t_{1/2}$ turnover rate longer than 20 h. The differences in the rate of degradation between IL-1α/IL-1β mRNA and icIL-1ra mRNA can be attributable to the IL-1α/IL-1β having an AUUUA sequence within the 3' noncoding region, which is a common nucleotide sequence in the 3'-untranslated region of mRNAs which encode proteins related to the inflammatory response (34, 35). In contrast, although the 3'-untranslated region of the icIL-1ra mRNA is A-U rich, this region lacks the AUUUA motif. This latter motif is the recognition signal for an mRNA processing pathway and specially targets the mRNA for degradation (36).

Although transcription of the icIL-1ra gene is not an early (primary) response (2-24 h) gene for TNFα and TGFβ1 (Fig. 2), icIL-1ra mRNA levels may modulate in response to stimulation by these cytokines, but at a later time point, suggesting secondary effects. A delayed response could account for the slight increase in IL-1ra protein levels observed in HaCaT keratinocytes 48 h post TGFβ1 treatment (37) and in the induction in icIL-1ra mRNA after TNFα stimulation of cultured keratinocytes at 48 h (12). However, even at this later time point, cultured keratinocytes in the latter study retained a differential response of IL-1α (3.3 fold increase) relative to IL-1ra (1.8 fold increase) protein levels (12).

The large quantities of icIL-1ra protein relative to a small amount of IL-1α protein in normal human keratinocytes have physiological and pathophysiological significances. In vivo, in normal skin the ratio of icIL-1ra/IL-1α was 123(10)and a similar ratio of 190 was observed in unstimulated HaCaT keratinocytes. After TNFα stimulation the icIL-1ra/IL-1α ratio dropped to 36.5 due solely to the increase in IL-1α production. Changes in icIL-1ra/IL-1α ratio in vivo in psoriatic lesions were also due primarily to changes in IL-1α and not icIL-1ra levels (10).The present study reveals that the relatively small amount of IL-1α may be of significance since recent studies have established that IL-1ra must be present in 10-100 times excess over IL-1 in order to block cell stimulation by IL-1. Such a large excess of IL-1ra over IL-1 is required because the target cells possess 200-5000 or more IL-1 receptors per cell, and cells can exhibit a full biological response when only 1-2% of these receptors are occupied by IL-1(9). Large quantities of stable IL-1ra are thus necessary if IL-1ra is to role in the buffering and dampening of the overall effect of IL-1, once an inflammatory process is initiated.

The differential effects of TNFα and TGFβ$_1$ upon IL-1α, IL-1β and icIL-1ra mRNA and protein levels demonstrate that the members of the IL-1 family in keratinocytes can be discordantly regulated, as occurs in psoriasis (10). The anti-proliferative effect of TNFα and TGFβ$_1$ psoriatic keratinocytes has been suggested for the treatment of psoriasis (38-40). In accordance with this line of thought, IL-1α is decreased in psoriatic epidermis (5) and the serum level of IL-1α correlated negatively with clinical disease severity (41). Thus, we speculate that induction of IL-1α (i.e., through TNFα or TGFβ1) may normalize the aberrant IL-1/IL-1ra relationship in psoriasis, and provide clinical benefit. UVB induces IL-1α in keratinocytes, and improves psoriasis, consistent with such a concept.

In conclusion, our data document a mechanism for regulating the effects of the pleiotropic cytokine, IL-1, upon production by keratinocytes in the skin. icIL-1ra mRNA and protein levels are not immediately affected by TNFα or TGFβ1, and the high stability of icIL-1ra mRNA may serve as an effective mechanism for buffering IL-1α effects as IL-1 levels rise and fall in response to external stimuli such as TNFα and TGF. Such a mechanism is likely important to minimize and localize the toxic effects of IL-1.

Acknowledgements

Supported by the Medical Research Service, Department of Veterans Affairs, and NIH RO1 AR 41642-01A1 and NIH RO1 AR 41707-01-GMA1. We are grateful to Patricia Hopkins for her expertise in preparing the manuscript, and to Lawrence S. Chan, M.D., Northwestern University for his technical input.

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