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IL-1 and IL-1 receptor antagonist regulation during keratinocyte cell cycle and differentiation in normal and psoriatic epidermis

Abstract Changes in the levels of IL-1 (IL-1α, IL-1β, and its receptor antagonist, IL-1RA) occur upon keratinocyte differentiation in vitro and are associated in vivo with abnormal differentiated and hyperproliferative states of psoriatic keratinocytes. A flow cytometric procedure, capable of detecting changes in the intracellular levels of IL-1, was used to determine whether intracellular IL-1/IL-1RA levels in psoriatic and normal keratinocytes alter during in vivo differentiation and the cell cycle. Increases in the IL-1RA levels and IL-1α levels were observed as both normal and psoriatic keratinocytes differentiated from basal stem cells (β_1 integrin$^+$, small size) into transient amplifying cells (TAC; β_1 integrin$^+$, large size). Upon further differentiation (β_1 integrin$^-$, large size) both IL-1RA and IL-1α levels dropped. However, while psoriatic IL-1β levels increased as cells differentiated into TACs, little change occurred in the IL-1β levels of normal keratinocytes during differentiation. Changes in IL-1/IL-1RA levels were also detected as keratinocytes progressed through the cell cycle. Within the basal stem cell population of both normal and psoriatic keratinocytes, the IL-1α and IL-1RA levels increased between G0/G1 and S but not between S and G2/M. However, psoriatic basal keratinocyte IL-1β levels differed from those of normal keratinocytes by showing no increase between S and G2/M. The IL-1/IL-1RA levels of normal TAC increased throughout the cell cycle. However, in psoriatic TAC, a slight decrease in IL-1α and IL-1RA levels was observed between G0/G1 and S followed by a delayed increase between S and G2/M. IL-1β levels in psoriatic TAC varied little throughout the cell cycle. Thus, we were able to detect precisely the regulation of IL-1/IL-1RA intracellular levels during the keratinocyte cell cycle and differentiation, showing notably decreased IL-1β upregulation in psoriatic keratinocytes progressing through the cell cycle.

Key words TGFβ · Psoriasis · Cell cycle · Differentiation · IL-1

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Introduction

Localization and quantitation of the expression of the IL-1 family of proteins within the epidermis has been the subject of considerable interest. IL-1 released by basal epidermal keratinocytes may influence papillary dermal inflammatory processes, such as endothelial adhesion molecule expression (Lawley and Kubota 1991; Pober et al. 1986), whereas IL-1 released by upper epidermal keratinocytes may have effects primarily on other keratinocytes, such as IL-8 induction or prostaglandin production (Mielke et al. 1990; Kristensen et al. 1991; Pentland and Mahoney 1988). Furthermore, the balance of IL-1 family molecules appears to be affected by epidermal differentiation and growth. Exposure of cultured keratinocytes to such modifiers of differentiation as retinoic acid or phorbol 12-myristate 13-acetate increases the levels of IL-1 biology activity (Blanton et al. 1989). In addition, induction of differentiation in human cultured keratinocytes by increasing the calcium concentration of the medium results in an increase in the ratio of intracellular IL-1 receptor antagonist (icIL-1RA) to IL-1 (Bigler et al. 1991).

However, numerous studies using immunohistochemistry to localize IL-1α and IL-1β within specific epidermal cell compartments have not been able to establish a clear consensus of opinion on the differential epidermal cell compartment expression, and quantitation is limited by the subjective nature of the technique (Anttila et al. 1990;

Debets et al. 1997; Didierjean et al. 1989; Oxholm et al. 1988; Tron et al. 1988). Quantitation by ELISA of IL-1 levels in whole epidermal extracts has been more successful, and there is general agreement that IL-1α levels are higher than IL-1β in normal epidermis (Cooper et al. 1990a; Gruaz et al. 1990). This approach has also revealed that IL-1α levels are decreased and IL-1β levels elevated in psoriatic epidermis (Cooper et al. 1990a; Cooper et al. 1990b; Gruaz et al. 1989). Recenty IL-1β levels have been examined in freshly isolated whole epidermal cell suspensions and have also been found to be elevated in psoriatic epidermal cells (Debets et al. 1995). Extraction of total epidermal tissue may permit quantitation over the whole epidermis, but does not account for preferential localization of IL-1 expression to specific regions of the epidermis, or even whether the changes in IL-1 levels are due to changes in the proportion of intercellular and intracellular IL-1. The ability to quantitate differential IL-1 expression within the various epidermal compartments is especially important given the detection of icIL-1RA in in vitro cultured keratinocytes (Bigler et al. 1992; Gruaz-Chatellard et al. 1991; Haskill et al. 1991) and in vivo in epidermal extracts (Hammerberg et al. 1992). Our finding of dramatic changes in the IL-1RA to IL-1α ratios between normal and psoriatic epidermis (Hammerberg et al. 1992) has established the importance of developing methodology to assess whether distinct epidermal cell compartments account for the aberrant balance of IL-1RA and functional IL-1 in human disease states.

In this study we first demonstrated the feasibility of using flow cytometry and specific antibodies to determine, on an individual cell basis, quantitative changes in in vivo keratinocyte cytoplasmic IL-1α, IL-1β and IL-1RA protein levels. Flow-based values of individual HaCaT cells were compared with those of parallel cultures of HaCaT cells in which IL-1/IL-1RA protein levels were determined by Triton X-100 extraction and IL-1/IL-1RA ELISA. A strong correlation was observed between the IL-1/IL-1RA values obtained by ELISA and those by flow cytometric analysis. Using the established flow cytometric analysis, we examined the IL-1/IL-1RA levels in keratinocyte subpopulations freshly obtained from normal and psoriatic skin. It was found that the transient amplifying cells (TAC) of both psoriatic and normal keratinocyte populations contained the highest amounts of IL-1RA and IL-1α. IL-1β levels of normal keratinocytes did not vary as much upon differentiation but were elevated in psoriatic TAC keratinocytes. Greater changes in IL-1/IL-1RA levels between normal and psoriatic keratinocytes were observed as the cells progressed through the cell cycle.

Materials and methods

Human subjects

Keratome biopsies were taken from the buttock area of normal volunteers and psoriasis vulgaris patients. Oral medication was not taken by either group during the month preceding the keratome biopsy. In addition, external treatment of the psoriatic lesion was not allowed during the 2 weeks before the procedure. All lesional tissue consisted of inflamed but relatively stable psoriatic plaques. The keratome procedure was approved by the Institutional Review Board of the University of Michigan and informed consent was obtained from each patient.

Materials

The HaCaT cell line was generously supplied by Dr. N. E. Fusenig (German Cancer Research Center, Heidelberg, Germany). Hank's balanced salt solution (phenol red-free, HBSS), L-glutamine and penicillin-streptomycin were obtained from Irvine Scientific (Santa Anna, Calif.). Dulbecco's modified Eagle's medium (high glucose, DMEM) was obtained from Gibco/BRL (Grand Island N.Y.). Fetal bovine serum (FBS) was purchased from HyClone (Logan, Utah). Trypsin, EDTA, Tris-HCl, Triton X-100, bovine serum albumin (BSA), propidium iodide (PI), RnaseA, fluorescein isothiocyanate (FITC) and the IgG1 isotype control immunoglobulin were acquired from Sigma Co. (St. Louis, Mo.). 7-Amino-actinomycin D (7AAD) was obtained from Calbiochem Novabiochem Corporation (San Diego, Calif.). The IgG1 monoclonal antibodies against IL-1α (clone A8) and IL-1RA (clone I4), which demonstrated no cross-reactivity to other IL-1 molecules, were provided by Dr. Ann Berger (UpJohn Co., Kalamazoo, Mich.). The anti-IL-1RA antibody detected both secretory IL-1RA and icIL-1RA (Firestein et al. 1992; Krzesicki et al. 1993). The IgG1 monoclonal antibody against IL-1β (clone 8516.3) was obtained from R&D Systems, Minneapolis, Minn.). The anti-β1 integrin (clone 4B4, mouse IgG1) directly conjugated with phycoerythrin (PE) was obained from Coulter Immunology (Hialeah, Fl.). The FITC-conjugated goat antimouse IgG1 was obtained from Boehringer Mannheim (Indianapolis, Ind.). The ELISA kits detecting IL-1α, IL-1β and IL-1RA were purchased from R&D Systems (Minneapolis, Minn.).

Cytoplasmic IL-1α/β and IL-1RA staining and flow cytometric analysis of HaCaT cells

HaCaT cells were grown in DMEM supplemented with 10% FBS, L-glutamine and penicillin-streptomycin. HaCaT cells for IL-1/IL-1RA analysis were taken from either confluent cultures or cultures stimulated with TGFβ1 (1 ng/ml). HaCaT cells were fixed and permeabilized with cold 70% ethanol. After storage at least overnight in 70% ethanol at –20°C HaCaT cells were resuspended in HBSS, centrifuged at 3000 rpm for 10 min, then transferred through a 30-G needle to small Eppendorf tubes to obtain approximately 5×10^5 cells in each tube, and the tubes centrifuged (3000 rpm, 5 min). Monoclonal antibody (100 µl) against IL-1α, IL-1β or IL-1RA or an IgG1 isotype control diluted in phosphate-buffered saline (PBS) plus 1% BSA and 0.01% sodium azide to a final concentration of 33 µg/ml was added and cells incubated for 45 min at 4°C. After three washes in HBSS, FITC-conjugated goat antimouse IgG1 was added and cells incubated for 30 min at 4°C. Cells were washed three times and kept in the dark at 4°C for less than 24 h before flow cytometric analysis.

Flow cytometry was performed using an Epics Elite Flow Cytometer (Coulter Cytometry, Hialeah, Fl.). Data were collected on a logarithmic scale for FITC and stored in Listmode. Fluorescence data were analyzed using Coulter Elite software. Data were initially recorded as mean channel fluorescence (MCF) intensity of the population. Specific staining with the anticytokine monoclonal antibodies was quantified as the difference between the MCF intensity of the cells stained with the monoclonal antibody and the MCF intensity of the cells stained with the isotype control (ΔMCF).

ELISA determination of cytoplasmic HaCaT IL-1/IL-1RA levels

Commercial ELISA determination kits (R&D Systems) for IL-1α, IL-1β, and IL-1RA were used to measure the IL-1/IL-1RA levels

in HaCaT cell lysates. The IL-1α and the IL-1β ELISAs were sensitive to 0.3 pg/ml and the IL-1RA ELISA sensitive to 6.5 pg/ml. The assays were performed according to the kit instructions except that we included 0.5% Triton X-100 in both standards and samples. The results are expressed as picograms cytokine/per 10^6 cells.

Flow cytometric determination of IL-1/IL-1RA levels in freshly isolated normal and psoriatic keratinocytes

Epidermal cell suspensions were prepared as described previously (Bata-Csorgo et al. 1993; Bata-Csorgo et al. 1995). Briefly, after treatment of the skin with dispase to obtain an epidermal sheet, epidermal cell suspensions were obtained by trypsin digestion. Epidermal cells were filtered through a 112–μm nylon mesh and washed. Cells were then fixed and permeabilized in 70% cold ethanol and kept at –20° C in ethanol at least overnight. Epidermal cells were stained with anti-β₁ integrin directly conjugated with PE (clone 4B4, mouse IgG1) in combination with unconjugated anti-IL-1α (mouse IgG1), IL-1β (mouse IgG1) or IL-1RA (mouse IgG1). The latter three monoclonal antibodies were visualized using goat antimouse IgG1-FITC. Protein was stained by reacting separate aliquots of cells with FITC (1.0 μg/ml) for 30 min at room temperature in the dark (Crissman and Steinkamp 1973). DNA content was determined by staining the cells either with 50 μg/ml PI plus 100 U/ml Rnase A or with 25 μg/ml 7AAD. Flow cytometry was performed as described above using an Epics Elite Flow Cytometer.

Results

Changes in HaCaT cytoplasmic IL-1α/IL-1β protein levels were detected equivalently by both ELISA and flow cytometric analysis

In order to compare flow cytometric analysis and ELISA-based detection systems, it was necessary to generate a range of cytoplasmic HaCaT IL-1α or IL-1β protein levels. This was accomplished because we found that cytoplasmic IL-1α and IL-1β protein levels drop in confluent and postconfluent cultured HaCaT cells (Fig. 1), and that IL-1α and IL-1β protein levels increase in 24-h TGFβ₁-stimulated HaCaT cells (Kang et al. 1993). We then correlated the changes in IL-1α and IL1-β cytoplasmic protein levels obtained by flow cytometric analysis of anti-IL-1α- and IL-1β-stained HaCaT cells with the results obtained by ELISA on cell extracts.

HaCaT cultures were initiated at subconfluency, allowed to grow to confluence (48 h of culture) and cultured for another 48 h in the confluent state. Cells to be used for antibody staining and flow cytometric analysis were fixed and permeabilized in 70% ethanol prior to staining. Prefixation in 0.5% paraformaldehyde or the use of saponin as a cell permeant did not increase MCF intensity or improve the correlations between the flow analysis results and the ELISA results (data not shown). At least six different combinations of fixation procedures involving paraformaldehyde, ethanol or saponin, were used, with the ethanol fixation repeated twice to ensure reproducibility.

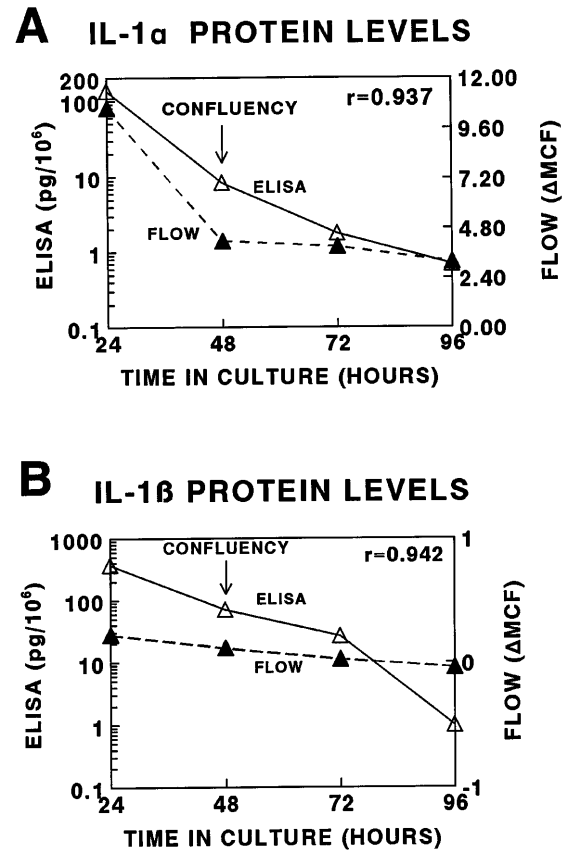IL-1α and IL-1β soluble cytoplasmic protein levels both dropped abruptly as the HaCaT cells entered confluence (Fig. 1, 48 h) and continued to drop as the cells were cultured in the confluent state (Fig. 1, 72 h and 96 h). The results from both the flow cytometric analysis (expressed as ΔMCF) and the ELISA (expressed as picograms per 10^6 cells) also showed a parallel drop in expression of cytoplasmic IL-1α and IL-1β. The correlation coefficient resulting from linear regression of the values obtained by ELISA and flow cytometric analysis of monoclonal antibody-stained cells were 0.937 for IL-1α (Fig. 1 A) and 0.942 for IL-1β (Fig. 1 B), indicating that flow cytometric analysis was capable of detecting changes in intracellular IL-1α in the range 100 pg/10^6 cells down to 1 pg/10^6 cells and in IL-1β in the range 300 pg/10^6 cells down to 30 pg/10^6 cells.

We have previously found that TGFβ₁ stimulates an increase in IL-1α mRNA and protein expression and IL-1β protein levels by HaCaT cells (Kang et al. 1993). The TGFβ₁-stimulated increase was used to compare flow cytometric analysis with ELISA for their ability to detect cytokine-stimulated increases in cytoplasmic IL-1α and IL-1β protein levels (Fig. 2). The IL-1α and IL-1β cytoplasmic levels obtained simultaneously by flow cytometric
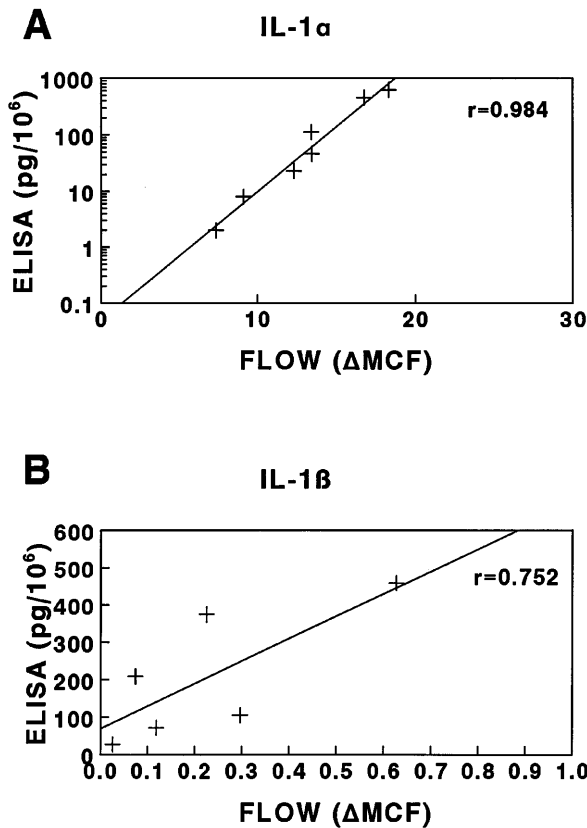

A IL-1α PROTEIN LEVELS

B IL-1β PROTEIN LEVELS

Fig. 1 A, B Levels of intracellular IL-1α (**A**) and IL-1β (**B**) protein in HaCaT cells determined either by ELISA *(open triangles)* or IL-1 staining and flow cytometric analysis *(closed triangles)* in cells cultured to confluence. ELISA-determined IL-1 levels are expressed as picograms per 10^6 HaCaT cells and are shown on the left y-axis. IL-1 levels determined by IL-1 staining and flow cytometric analysis are expressed as ΔMCF and are shown on the right y-axis. The respective IL-1 values were then plotted against time in culture as linear data and linear regression analysis performed

A

IL-1α

B

IL-1β

Fig. 2A, B Correlation between $TGF\beta_1$-induced changes in IL-1α and IL-1β protein levels as detected by flow cytometric analysis and ELISA. Intracellular IL-1α (**A**) and IL-1β (**B**) protein levels were determined simultaneously by ELISA and flow cytometry in HaCaT cells grown with or without $TGF\beta_1$ for 96 h. The data were subjected to linear regression analysis

Fig. 3 Increased IL-1RA expression in HaCaT cells cultured for 96 h with $TGF\beta_1$ (1 ng/ml). IL-1RA cytoplasmic levels determined by ELISA *(open triangles)* are expressed as picograms per 10^6 HaCaT cells and are shown on the left y-axis. IL-1RA levels determined by flow cytometric analysis *(closed triangles)* are expressed as ΔMCA and are shown on the right y-axis. The data were subjected to linear regression analysis

analysis and ELISA in parallel cultures of HaCaT cells grown with or without $TGF\beta_1$ over a period of 96 h were compared by linear regression. A strong correlation was found for both IL-1α (Fig. 2A, $r = 0.984$) and IL-1β (Fig. 2B, $r = 0.752$).

Changes in cytoplasmic IL-1RA levels were detected by both ELISA and flow cytometric analysis

HaCaT expression of IL-1RA, unlike that of IL-1, did not decrease as the cells were grown to confluence and maintained in a confluent state (data not shown). In order to obtain alterations in IL-1RA cytoplasmic levels which could be used to compare the flow cytometric analysis with ELISA-based analysis, it was necessary to stimulate HaCaT cells with $TGF\beta_1$ over a period of 96 h (Fig. 3). Incubation of the HaCaT cells in the presence of $TGF\beta_1$ over a period of 96 h resulted in a 2.5-fold increase in ELISA-detectable cytoplasmic IL-1RA, which was paralleled by a 1.6-fold increase in IL-1RA as determined by staining cytoplasmic IL-1RA with a monoclonal antibody to IL-1RA and flow cytometric analysis. The correlation coefficient resulting from linear regression of the ΔMCF
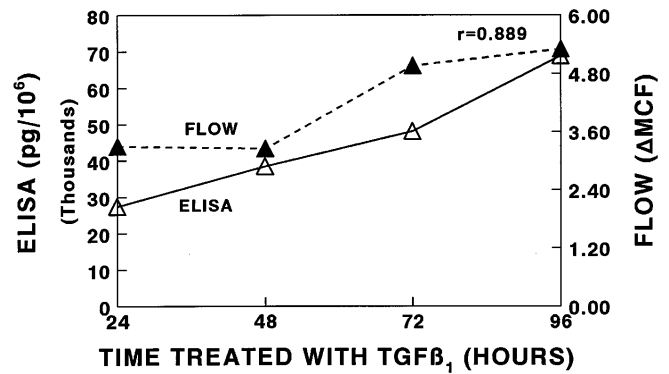
values and ELISA data over this time period was 0.889, indicating good agreement between the IL-1RA detected by flow cytometric analysis and soluble IL-1RA measured by ELISA.

Flow cytometric analysis of IL-1/IL-1RA protein levels in keratinocyte subpopulations from freshly isolated normal or psoriatic involved epidermal cells

Epidermal cell suspensions were prepared from keratome biopsies taken from either normal or psoriatic involved skin. After fixation in 70% ethanol, epidermal cells were stained with anti-IL-1α, IL-1β, or IL-1RA antibodies in combination with anti-β1 integrin antibody directly conjugated with PE. Reactivity of the antibodies to IL-1/IL-RA was visualized using goat antimouse IgG1-FITC. DNA content and thus cell cycle position were determined by staining with PI or 7AAD. Cells were analyzed by flow cytometry and the basal cells were electronically isolated by their expression of the β_1 integrin and their small size (low-angle 90° light scattering) (Bata-Csorgo et al. 1993). The second proliferative population in the epidermis, which, despite its very active proliferative status, has begun terminal differentiation (TAC population), was identified by retention of expression of β_1 integrin but a larger size than basal stem cells (Bata-Csorgo et al. 1993). The ΔMCF for each anti-IL-1/IL-1RA antibody was determined and then related to the ΔMCF of the basal cell population to obtain a fold increase in IL-1/IL-1RA expression (Fig. 4).

The expression of both IL-1RA and IL-1α increased three- to fourfold as the basal cell population differentiated into TAC in both normal and psoriatic keratinocytes. This would represent a larger increase in IL-1α relative to IL-1RA based upon our HaCaT comparison studies. Then, as the larger keratinocytes further differentiated as represented by the loss of the β1 integrin, both IL-1α and IL-1RA showed a drop in expression. Although IL-1β in
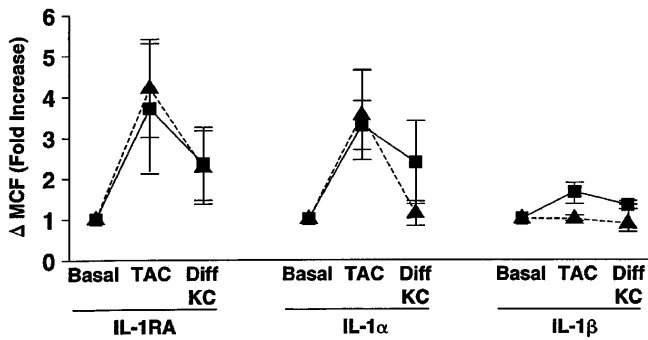
Fig. 4 In both psoriatic and normal in vivo keratinocytes maximum IL-1/IL-1RA levels are observed in the TAC subpopulation. Freshly isolated ethanol-fixed epidermal cells from normal and psoriatic skin stained for IL-1RA, IL-1α and IL-1β and for β_1 integrins were analyzed by flow cytometry and the results expressed as ΔMCF. Basal keratinocytes (β_1 integrin⁺, small size) ΔMCF served as the basal level for comparison of the ΔMCF of the TAC and differentiated keratinocyte *(Diff KC)* populations (*solid squares* normal keratinocytes, *solid triangles* psoriatic keratinocytes) Values are means ± SEM; $n = 3$ for normal IL-1α and IL-1RA, $n = 4$ for psoriatic IL-1α, IL-1RA and normal IL-1β, and $n = 5$ for psoriatic IL-1β

Fig. 5 A–D Differential expression of IL-1α/IL-1RA and IL-1β in basal keratinocytes (β_1 integrin⁺, small size) basal keratinocytes progress from S to G2/M. Ethanol-fixed freshly isolated epidermal cells from normal or psoriatic epidermis were stained for IL-1RA (**A**), IL-1α (**B**) and IL-1β (**C**), or total protein (**D**), as well as β_1 integrin, and DNA content (*solid squares* normal keratinocytes, *solid triangles* psoriatic keratinocytes). The ΔMCF in the G0/G1 population represents the basal level to which the ΔMCF in the S and G2/M stages are compared. Values are means ± SEM; $n = 3$ for normal IL-1RA and IL-1α, $n = 4$ for psoriatic IL-1RA, IL-1α, and IL-1 β, and $n = 5$ for psoriatic IL-1β

psoriatic keratinocytes showed changes similar to those observed for IL-1α and IL-1RA during differentiation, normal keratinocytes showed little change between basal stem cells and TAC (Fig. 4). However, the differences in the changes in IL-1β levels observed between normal and psoriatic keratinocytes were not statistically significant.

IL-1/IL-1RA levels changed as keratinocytes progressed through the cell cycle

Because it has been suggested that intracellular IL-1 may be involved in regulation of cellular proliferation (Maier et al. 1990) we examined the levels of IL-1/IL-1RA at different stages of the cell cycle of the keratinocyte subpopulations. The basal cell population in both normal and psoriatic keratinocytes showed an increase in IL-1RA (Fig. 5 A) and IL-1α levels (Fig. 5 B) between G0/G1 and S with no further increase occurring between S and G2/M. The lack of further increase in IL-1α and IL-1RA between S and G2/M is significant, because normally total protein increases with further progression through the cell cycle (Fig. 5 D). As observed in other cell types (Crissman and Steinkamp 1973; Crissman et al. 1985), as basal keratinocytes go from G0/G1 to S to G2/M, the overall protein content, as revealed by FITC staining, steadily increases. Thus, there is a downregulation of IL-1α and IL-1RA, relative to total protein, at the S to G2/M transition point in keratinocytes. Interestingly, this downregulation may be specific to IL-1α and IL-1RA, because IL-1β levels (Fig. 5 C), like the general protein levels, in normal keratinocytes increased at each stage of the cell cycle. As with differentiation, IL-1β levels exhibited differences from the changes occurring in normal basal keratinocytes during the cell cycle but the changes were similar to those occur-
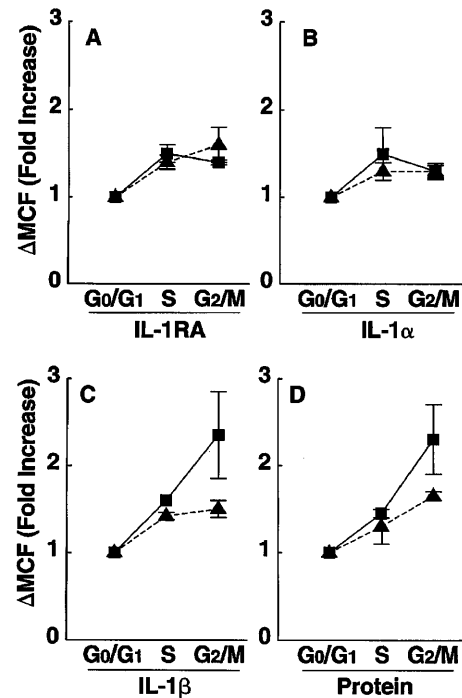
ring in psoriatic IL-1α/IL-1RA levels; IL-1β levels in psoriatic basal stem keratinocytes also increased between S and G2/M equivalent to the increase shown by IL-1α/IL-1RA, but not between S and G2/M (Fig. 5 C).

The suprabasal TAC population showed a different pattern of IL-1/IL-1RA from the basal cell population (Fig. 6). Normal TAC IL-1/I-1RA levels increased at all stages of the cell cycle, similar to the increases observed in the overall protein level (Fig. 6 D). However, the psoriatic TAC showed a radically different pattern during the cell cycle. Both IL-1α (Fig. 6 B) and IL-1RA (Fig. 6 A) decreased slightly between G0/G1 and S before undergoing a delayed increase between S and G2/M. This difference at S between normal and psoriatic IL-1RA levels was statistically significant. Psoriatic TAC IL-1β levels did not decrease between G0/G1 and S and failed to show a significant increase between S and G2/M (Fig. 6 C). Normal TAC IL-1β levels showed a progressive increase through the cell cycle and were significantly different from psoriatic TAC IL-1β levels at the S phase.
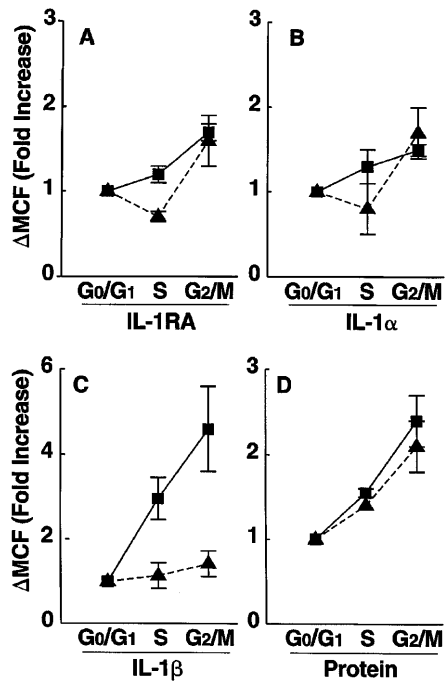
Fig. 6 A–D Psoriatic IL-1/IL-1RA levels differ from normal values in S phase of the cell cycle of TAC keratinocytes (β_1 integrin+, large size). Ethanol-fixed freshly isolated epidermal cells from normal or psoriatic epidermis were stained for IL-1RA (**A**), IL-1α (**B**), IL-1β (**C**) or total protein (**D**), as well as β_1 integrin and DNA content (*solid squares* normal keratinocytes, *solid triangles* psoriatic keratinocytes). The ΔMCF in the G0/G1 population represents the basal level to which the ΔMCF in the S and G2/M stages are compared. Values are means ± SEM; $n = 3$ for normal IL-1RA and IL-1α, $n = 4$ for psoriatic IL-1RA, IL-1α, and normal IL-1β, and $n = 5$ for psoriatic IL-1β

Discussion

Increased IL-1β levels characteristic of psoriatic lesions have been demonstrated by numerous groups (Cooper et al. 1990a; Debets et al. 1995; Gruaz et al. 1989). In fact, decreased IL-1β levels early after treatment initiation may be predictive of whether a treatment will result in improvement of psoriatic lesions (Elder et al. 1993). In addition decreased expression of IL-1α has been reported in whole psoriatic lesions (Cooper et al. 1990a; Gruaz et al. 1989), a situation that results in an increased ratio of IL-1RA to IL-1α, and a net inhibition of IL-1 bioactivity intracellularly (Hammerberg et al. 1992). With the technical ability now to determine IL-1/IL-1RA family protein levels on a single-cell basis by flow cytometry, intracellular IL-1 regulation in the different anatomic keratinocyte compartments of the epidermis can be determined as well as the cell cycle status within these compartments. The enabling technology was the development of an antibody staining method for intracellular IL-1/IL-RA that was verified with ELISA-detectable cytoplasmic IL-1/IL-1RA protein levels.

The immortalized keratinocyte cell line HaCaT (Boukamp et al. 1988) was used to establish a method, utiliz-

ing flow cytometry, by which changes in IL-1α and IL-1β can be related to changes in their antagonist, IL-1RA, in individual cells. Under normal culture conditions, as HaCaT cells approach confluence and are maintained at confluence, which induces them to differentiate (Ryle et al. 1989), both IL-1α and IL-1β, but not IL-1RA, soluble cytoplasmic protein levels drop. A comparison of changes in IL-1α and IL-1β protein levels detected by ELISA in cell extracts with levels detected by quantitation of fluorescence intensity by flow cytometry demonstrated a highly significant correlation (IL-1α $r = 0.937$ and IL-1β $r = 0.942$) between the two methods. Similarly, a comparison of changes in IL-1RA levels in HaCaT cells stimulated with TGFβ_1, as determined by ELISA with monoclonal antibody also showed a highly significant correlation ($r = 0.889$). These results demonstrate that flow cytometric analysis is capable of detecting changes in IL-1α, IL-1β and IL-1RA levels in keratinocytes at relevant concentration ranges that correspond to the ELISA-determined cytoplasmic values.

Using the verified flow cytometric method for measuring cellular IL-1/IL-1RA levels, the changes in IL-1/IL-1RA protein levels within specific subpopulations of keratinocytes were examined. Normal and psoriatic keratinocytes were divided into different populations based upon their expression of β_1 integrin and their size and cytoplasmic complexity as determined by the light scattering properties of the cells. We have previously demonstrated that basal (stem) keratinocytes are smaller and less complex than suprabasal cells (TAC), which are distinctly larger and exhibit a more complex cytoplasm, and both express β_1 integrins. Terminally differentiated keratinocytes lack β_1 integrins and are large and complex (Bata-Csorgo et al. 1993). The ΔMCF of the basal keratinocyte population was used as the basal IL-1/IL-1RA level for comparative purposes. In psoriasis, IL-1RA, IL-1α, and IL-1β all showed dramatic increases as the keratinocytes differentiated to the TAC stage, and then decreased with the onset of the terminally differentiating stage. These changes were also observed for IL-1RA and IL-1α as normal keratinocytes differentiated. By contrast, the IL-1β levels did not increase in normal TACs relative to basal (stem) cells. However, the results of prior studies showing elevated IL-1β levels in psoriatic epidermis (Cooper et al. 1990a; Cooper et al. 1990b; Debets et al. 1995; Gruaz et al. 1989) would suggest that IL-1β is particularly elevated in psoriatic TAC subpopulations compared with normal TAC.

Comparison of these results with in vitro results provides information on what stages of differentiation the in vitro system really models. An increase in IL-1RA was observed upon induction of differentiation of cultured keratinocytes by increasing the calcium concentration. Under these conditions IL-1α is unchanged and IL-1β is decreased (Bigler et al. 1992). We have previously observed that most cultured keratinocytes a week after plating express K1/K10 (Bata-Csorgo et al. 1995) so the initial stage of passaged keratinocytes in cultures would be the TAC population; increasing calcium would result in

the development of a terminally differentiated population. Based upon our present in vivo observations a drop in IL-1α, IL-1β, and IL-1RA protein levels would be expected to occur if culture differentiation models in vivo differentiation. Thus, the increase in IL-1RA protein levels observed in normal cultured keratinocytes (Bigler et al. 1992) is not expected based on the drop we observed in vivo as keratinocytes terminally differentiated. This suggests that cultured keratinocytes are not necessarily a good model for changes in IL-1/IL-1RA levels in the different in vivo keratinocyte subpopulations.

The development of intracellular IL-1/IL-1RA analysis by flow cytometric methods allows changes in IL-1/IL-1RA levels as keratinocytes progress through the cell cycle to be examined; such an analysis has not previously been possible and has not been reported. We examined the two proliferating keratinocyte subpopulations basal, β_1 integrin$^+$ small (stem), keratinocytes and β_1 integrin$^+$ large, (transiently amplifying) keratinocytes. Overall protein changes as determined by FITC binding (Crissman and Steinkamp 1973; Crissman et al. 1985) increased as basal keratinocytes went from G0/G1 to S and the G2/M. Both IL-1α and IL-1RA levels increased between G0/G1 and S but not between S and G2/M, suggesting that either IL-α and IL-1RA are not needed for the S to G2/M transition or they may serve as a negative regulator and must be downregulated before cells can progress to the G2/M stage. Interestingly, IL-1β differed from IL-1α and IL-1RA in that it did increase between S and G2/M in normal basal keratinocytes. However, in psoriatic basal keratinocytes this increase in IL-1β levels did not occur between S and G2/M. Since these cells are in a hyperproliferative state (Bata-Csorgo et al. 1993), the decreased expression of IL-1β in the S to G2/M transition point may reflect the removal of a molecule involved in downregulation of basal keratinocyte proliferation.

Unlike the basal keratinocyte subpopulation of both normal and psoriatic keratinocytes, the TAC subpopulation of normal keratinocytes showed an increase in their IL-1α, IL-1β and IL-1RA levels through the cell cycle with IL-1β levels showing the greatest increase, similar to that of the overall protein levels. By contrast, in the psoriatic TAC population the greatest difference from the normal was observed. First, no increase was observed between G0/G1 and S in the IL-1α, IL-1β and IL-1RA levels. However, this was followed by an S to G2/M increase that brought the IL-1α and IL-1RA levels up to the normal keratinocyte values. Again, IL-1β differed from the other two IL-1 family molecules in that in psoriatic TAC IL-1β levels did not increase between S and G2/M. These changes may be secondary effects resulting from the hyperproliferative state of their progenitor basal stem cells. In addition they could be a result of or contributing to the abnormal differentiated state of psoriatic keratinocytes (Bernard et al. 1985; Bernard et al. 1986; Bernard et al. 1988; Bernerd et al. 1992).

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