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Role of fibroblasts in the regulation of proinflammatory interleukin IL-1, IL-6 and IL-8 levels induced by keratinocyte-derived IL-1

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Abstract In the epidermis, the keratinocytes are the first cells to be encountered by external stimuli and they are able to promote the inflammatory response by increased production and release of various cytokines. In their turn, these cytokines may directly affect the production of proinflammatory cytokines in human dermal fibroblasts. In addition, in both epithelial and mesenchymal cells cytokine production may be modulated by their mutual interaction, and thereby regulate the inflammatory response. The present study aimed to examine the role of fibroblasts in the regulation of proinflammatory IL-1, IL-6 and IL-8 levels induced by keratinocyte-derived IL-1. The data show that in fibroblasts exposed to conditioned media derived from cultures of normal human keratinocytes or squamous carcinoma cells (SCC-4), both the IL-8 and IL-6 mRNA expression as well as protein production were elevated. In addition, it was shown that these effects were induced by IL-1α. The IL-1α-induced increase in IL-8 and IL-6 production, both on the protein level as well as on the mRNA level, were concentration dependent and occurred almost simultaneously. While the induction of IL-6 and IL-8 occurred simultaneously, the IL-6 mRNA remained elevated for longer. In contrast to increased IL-6 and IL-8 production the IL-1α levels markedly decreased upon culturing of fibroblasts in keratinocyte-derived conditioned medium. From internalization experiments it could be concluded that binding of IL-1 to IL-1 receptors, and its subsequent internalization and intracellular degradation is the most likely mechanism involved in the reduction of IL-1 levels by fibroblasts. Comparing the rate of IL-1 reduction in the presence of various cell types indicated that the rate of IL-1 reduction is directly related to the number of IL-1 receptors found on these cell types. In conclusion, these results indicate that the release of IL-1α by activated keratinocytes may act as an inducer of IL-8 and IL-6 production in neighbouring fibroblasts. This may be an important pathway for the amplification of the inflammatory response. The amounts of both cytokines produced by fibroblasts were at least two to three orders of magnitude higher than those produced by keratinocytes, suggesting an important role of fibroblasts in the general inflammatory response. Furthermore, fibroblasts might be involved in turning off this inflammatory response by reducing IL-1 levels, most likely via IL-1 receptor-mediated uptake.

Key words Interleukin-8 · Interleukin-1 · IL-1 receptor · Keratinocyte – Fibroblast interaction

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Introduction

There is increasing evidence that the release and production of cytokines, either preformed or newly synthesized, by keratinocytes and fibroblasts is altered after certain events, such as skin injury [1]. The keratinocytes become 'activated' and release, amongst other things, the proinflammatory cytokine interleukin-1 (IL-1) [2]. It has been shown that exposure of skin cells to IL-1 leads to an increased production of IL-6 and IL-8 [2–7]. Proinflammatory cytokines, such as IL-8 and IL-6 are pleiotropic cytokines and act on multiple cell types. In addition, both IL-8 and IL-6 are thought to play an important role in both the cellular and the humoral immune responses via different pathways [8]. At low concentrations IL-8 possesses neutrophil, lymphocyte and basophil chemotactic properties, while at higher concentrations IL-8 can activate neu-

trophils and T cells [9]. The major effects of IL-6 with respect to immune response are the stimulation of the final differentiation of B cells into antibody-producing plasma cells and the induction of acute phase proteins in hepatocytes [10]. Since the humoral and cellular pathways are complementary, the presence of both IL-6 and IL-8 will potentiate the immune response.

We have recently shown that in keratinocyte–fibroblast cocultures, IL-1α derived from epidermal cells increases IL-6 production in fibroblasts [11]. It has been shown that the transcription factors NF-IL-6 and NF-kB are needed for IL-1-induced IL-6 and IL-8 gene expression [12]. The present study aimed to examine the kinetics of induction of IL-6 and IL-8 production in human dermal fibroblasts by conditioned medium derived from keratinocyte cultures and by IL-1α. We have previously reported that IL-1 activity is strongly reduced in cocultures of keratinocytes and 3T3 fibroblasts [11]. Since this might represent a negative feedback mechanism we further investigated the processes involved in the reduction of IL-1 levels by fibroblasts.

Materials and methods

Cell culture

Human fibroblasts (HFF), derived from surgical specimens of female breast skin, and 3T3 cells were cultured in Dulbecco's Modification of Eagle's Medium (DMEM) supplemented with 5% (v/v) fetal calf serum (FCS), penicillin (100 U/ml) and streptomycin (100 μg/ml). Normal human keratinocytes (NHK) derived from juvenile foreskin and squamous carcinoma cells (SCC-4) [13] were cultured using the Rheinwald-Green feeder technique [14], as described previously [15].

Preparation of conditioned medium

HFF (1.25×10^4/cm^2) and SCC-4 were seeded as monocultures in DMEM/F12 (3:1) supplemented with 5% (v/v) FCS, and the medium was refreshed after 3 days. The supernatants (conditioned medium) of the 3–6-day culture period were collected and kept at –20°C until use. To prepare conditioned medium of 3T3-free NHK monocultures, 1.25×10^4/cm^2 NHK were seeded together with 1.8×10^4/cm^2 lethally irradiated 3T3 cells and grown to subconfluence in medium as described previously [15]. Then 3T3 cells were removed by 0.02% (w/v) ethylenediaminetetraacetic acid treatment [16] and NHK were then grown to confluence in DMEM/F12 (3:1) supplemented with 5% (v/v) FCS. NHK cultures were then refed with fresh medium and reincubated for 3 days. This supernatant was collected and kept at –20°C until use. For experiments, a mixture (1:1) of fresh and conditioned medium derived from HFF, SCC-4 and NHK was used. These media were termed CM-HFF, CM-SCC-4 and CM-NHK, respectively.

Stimulation of fibroblast cultures with either IL-1α or with conditioned medium in the presence or absence of antibodies against IL-1

Fibroblasts, HFF or 3T3 cells were grown to confluence in DMEM/F12 supplemented with 5% (v/v) FCS. When HFF were exposed to conditioned medium the cells were incubated for 4, 8 and 24 h in CM-HFF, CM-SCC-4, CM-NHK or in control medium. For IL-1 stimulation, the HFF were refed with fresh medium. After 24 h 0, 10 or 100 pg/ml IL-1α (Hoffman la Roche, Basel, Suisse) was added and the cells were reincubated for another 4, 8, 24 or 48 h. At various time points supernatants were harvested and stored at –20°C and the cells were lysed for RNA isolation. When antibodies against IL-1 were included in the experiment, HFF were incubated for 20 h in control medium or in a mixture of control medium and CM-SCC-4 or CM-NHK (4:1 v/v), the latter two media were preincubated for 1 h at 37°C either in the absence or the presence of anti-IL-1α and/or IL-1β (kindly provided by S. Poole, National Institute for Biological Standards and Control, Potters Bar, UK). Supernatants were harvested and stored at –20°C until use. Two independent experiments were performed for all conditions described above.

Determination of IL-6 and IL-1 bioactivity

IL-6 and IL-1 levels were determined using the B9 hybridoma growth assay [17] and D10 growth assay [18], respectively, using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) for the determination of cell numbers [19]. The levels of IL-6 and IL-1 are expressed as picograms per millilitre by reference to standard curves obtained with recombinant human IL-6 or recombinant human IL-1α, respectively. All assays were performed in biological duplicate (two culture dishes per experimental condition) ($n = 2$) and the cytokine levels in the supernatants were assayed in duplicate using five serial dilutions. Because the experiments were performed in duplicate no standard deviation is given. The internal variation of the mean values of the cytokine levels in the two independent cultures did not exceed 13%.

Determination of IL-1 antigen levels

IL-1α was measured using a radioimmunoassay (RIA) in which rabbit polyclonal antibodies to human recombinant IL-1α were used. The detection limit was 5 pg IL-1α/ml. This antibody did not crossreact with human IL-1β, IL-2, TNF, α-IFN, γ-IFN, GM-CSF or C5a. The RIA was carried out as described by Lonnemann et al. [20] with minor modifications.

Determination of IL-8 levels

IL-8 was measured using an enzyme-linked immunosorbent assay (ELISA) in which monoclonal antibodies (MAbs) and polyclonal antibodies to human recombinant IL-8 were used, as described by Hack et al. [21]. The detection limit was 4 pg IL-8/ml. Standards and antibodies were a gift from Dr. L. A. Aarden (Red Cross Central Laboratory for Blood Transfusion, Amsterdam, The Netherlands).

Analysis of mRNA

RNA was isolated according to the method of Chomczynski and Saechi [22] and samples were analysed using the Northern blotting technique [23]. cDNA probes specific for human IL-1β (443 bp), IL-6 (819 bp) and IL-8 (585 bp) were prepared by reverse-transcriptase polymerase chain reaction [24] (kindly provided by Dr. R. A. de Paus, Department of Haematology, Leiden, the Netherlands). Hybridization was performed as described by Quax et al. [25]. The intensity of the bands of the autoradiograms was quantified photodensitometrically (Desaga CD60) and normalized for total mRNA using 28S rRNA as a control (kindly provided by Dr. C. Backendorf, Leiden, The Netherlands).

IL-1 receptor binding assay

Triplicate confluent cultures of 3T3 cells and fibroblasts were washed twice with cold (0°C) 'assay medium' (DMEM containing 0.1% bovine serum albumin, buffered with 25 m*M* *N*-2-hydroxyethylpiperazine-*N*^{1}-ethane sulphonic acid (HEPES), pH 7.4). The cells were then incubated in assay medium containing increasing amounts of ^{125}I-IL-1α (0–4 ng/ml, specific activity 80 μCi/μg) in the presence or absence of a 500-fold excess of nonlabelled IL-1α. After a 5-h incubation at 4°C, the cells were washed twice with cold phosphate-buffered saline supplemented with 0.5% bovine

serum albumin. The cells were then scraped and collected using a cotton-wool stick and total cell-associated radioactivity was determined. The number of molecules specifically bound to the cell surface was determined after subtraction of ^{125}I-IL-1α bound in the presence of an excess of nonlabelled IL-1α. Scatchard analysis was performed in two independent experiments to determine the maximum number of binding sites and the Kd values.

Internalization and degradation of IL-1α was studied as described by Van Oosterhout et al. [26]. Briefly, cells were incubated for various times at 37° C in the presence of ^{125}I-IL-1α. The cells were then treated or not treated with ice-cold isotonic glycine buffer (pH 2) for 10 min to discriminate between intracellular activity and the total amount of associated radioactivity, respectively. Furthermore, 75 µl of culture supernatant was treated with 1 ml 25% trichloroacetic acid (TCA) and 75 µl 1% bovine serum albumin at 4° C for 30 min, centrifuged at 18000 *g* for 15 min and the radioactivity in the supernatant and pellet determined. All experiments (n = 2) were performed in triplicate and each sample analysed in duplicate.

Results

Modulation of IL-8, IL-6 and IL-1 levels and mRNA expression in HFF

Effects of conditioned media derived from epidermal cell cultures

The effects of conditioned media derived from epidermal cell cultures on the IL-8, IL-6 and IL-1 protein levels and mRNA expression in HFF cultures were examined. Confluent HFF cultures were incubated for various times in control medium or in the conditioned media CM-HFF, CM-NHK or CM-SCC-4, and the supernatants were collected and RNA was isolated.

When HFF were grown in CM-NHK or CM-SCC-4, levels of both IL-8 and IL-6 markedly increased with time (Fig. 1 a). The increase was more pronounced in HFF cultures grown in CM-SCC-4 than in CM-NHK. Exposure of HFF to CM-HFF did not affect either IL-6 or IL-8 production (data not shown). In contrast to increased IL-8 and IL-6 levels, IL-1 activity decreased within 24 h from 0.12 to 0.03 ng/ml in HFF grown in CM-NHK and from 0.4 to 0.3 ng/ml in HFF grown in CM-SCC-4. In HFF cultures grown for 24 h in control medium or in CM-HFF no IL-1 activity was detected.

Northern blot analysis indicated that exposure of HFF to CM-SCC-4 and CM-NHK also resulted in an increase in IL-8 and IL-6 mRNA levels (Fig. 1 b). This increase persisted for longer in cells grown in CM-SCC-4 than in CM-NHK. Transiently elevated levels of IL-8 and IL-6 mRNA were also observed in HFF grown in control medium, probably resulting from medium renewal at the beginning of the experiment. In addition, an increased expression of IL-1β mRNA was seen upon exposure of HFF to CM-SCC-4 and CM-NHK. No IL-1α mRNA could be

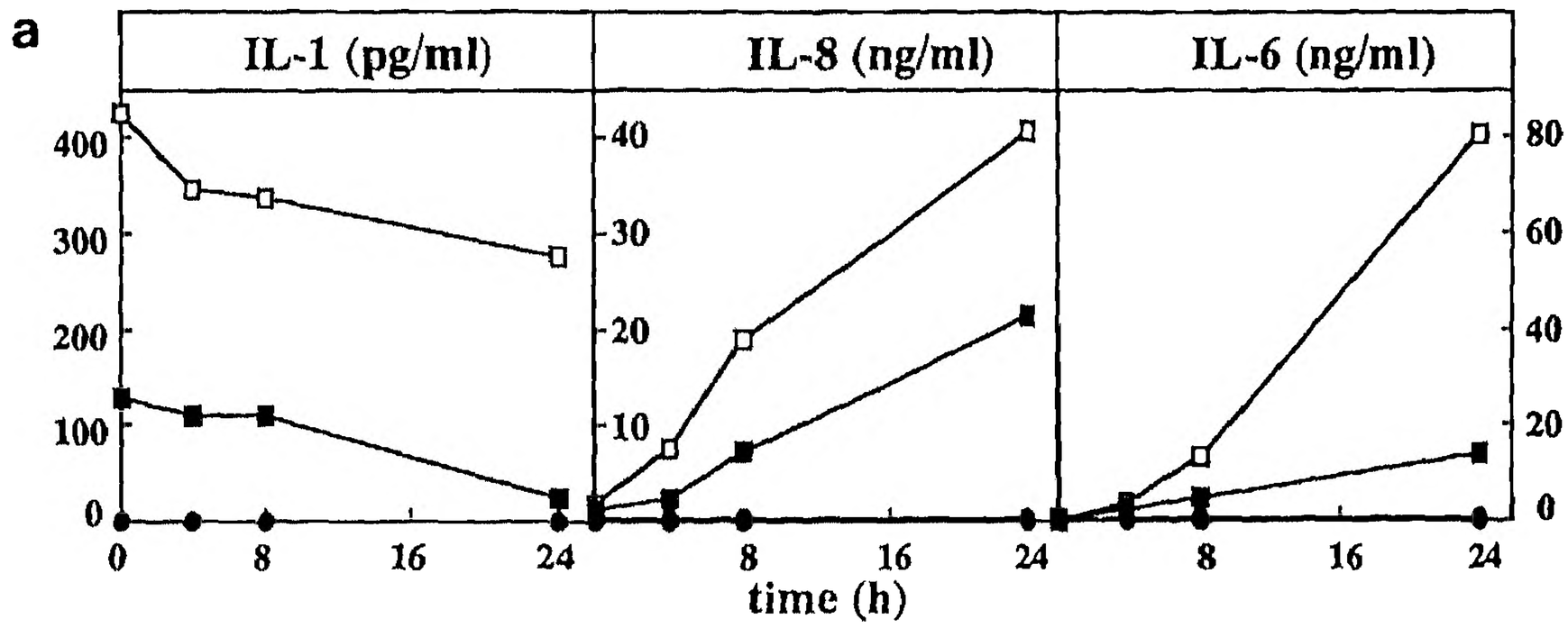

Fig. 1 a, b Modulation of IL-8, IL-6 and IL-1 levels and mRNA expression in HFF cultures by conditioned medium derived from epithelial cultures. Confluent HFF cultures were grown in control medium (○), CM-HFF (●), CM-SCC-4 (□) or CM-NHK (■) for 4, 8 or 24 h. **a** At the indicated time points supernatants were collected and analyzed for levels of IL-8, IL-6 and IL-1 as described Materials and methods. **b** At the indicated time points RNA was isolated and analysed using ^{32}P-labelled cDNA probes for human IL-8, IL-6, IL-1β and 28S rRNA

detected either in stimulated or in nonstimulated cells (data not shown).

IL-1α present in supernatants derived from SCC-4 or NHK was found to be responsible for the observed increase in IL-8 expression and production in HFF, since after preincubation of CM-NHK and CM-SCC-4 with antibodies directed against IL-1α the IL-8 production in fibroblasts was markedly decreased (Table 1). The IL-8 production was not affected by the neutralizing IL-1β antibody, indicating that there was hardly any IL-1β activity in CM-NHK or CM-SCC-4.

Table 1 Effect of IL-1α and IL-1β antibodies on IL-8 production in fibroblasts induced by conditioned media derived from SCC-4 and NHK cultures. HFF were cultured in medium supplemented with 5% FCS to confluence. The cells were then incubated for 20 h in control medium or in a mixture of control medium and CM-SCC-4 or CM-NHK (4:1 v/v), the latter two media were preincubated for 1 h at 37° C either in the absence or in the presence of anti-IL-1α and/or anti-IL-1β. The supernatants were collected and IL-8 assayed using the IL-8 ELISA. Values are means ± SD

Additives	IL-8 levels (ng/ml)		
	Control medium	CM-SCC-4	CM-NHK
None	0.08	5.04 ± 0.6	4.40 ± 1.1
Anti-IL-1α	0.14	0.97 ± 0.3	0.86 ± 0.1
Anti-IL-1β	0.14	5.42 ± 0.9	3.17 ± 0.6
Anti-IL-1α/β	0.12	1.08 ± 0.2	0.72 ± 0.4

Effects of IL-1α

We investigated whether the induction of the IL-8 and IL-6 production in HFF by supernatants derived from epidermal cultures was comparable to the effects induced by purified IL-1α. HFF were exposed for various times to increasing concentrations of IL-1α, and the IL-8 and IL-6 production was monitored. As shown in Fig. 2a, an increase in IL-8 and IL-6 levels was observed in culture supernatants derived from HFF exposed to IL-1α. Concomitantly with the increase in IL-8 and IL-6 levels, a reduction in IL-1 activity was observed in supernatants of HFF cultures exposed to IL-1α. IL-1 activity decreased within 48 h from the initial value of 100 pg/ml to 14 pg/ml, whereas at an initial concentration of 10 pg/ml, no IL-1 activity could be detected after 24 h.

Changes in IL-8 and IL-6 mRNA expression in HFF exposed to IL-1α were also time- and concentration-dependent. Detectable levels of IL-8 and IL-6 mRNA were measured as early as 4 h after stimulation of HFF with 10 pg/ml IL-1α (Fig. 2b). By prolongation of the incubation time to 24 h, the IL-8 mRNA expression decreased to un-

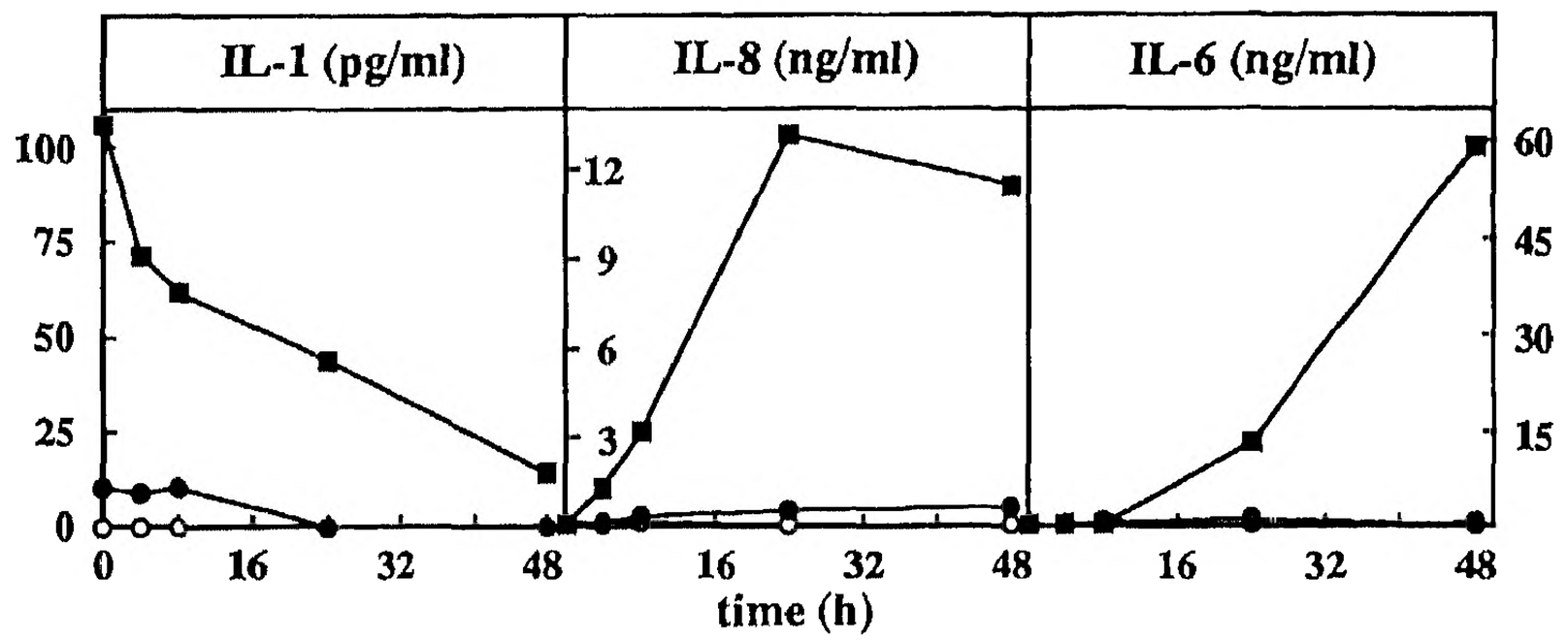

Fig. 2a b Effect of IL-1α on the IL-8, IL-6 and IL-1 levels and mRNA expression in HFF cultures. Confluent HFF cultures were refed with 20 ml fresh medium and after 24 h 20 μl IL-1α was added to reach a final concentration of 0, 10 or 100 pg/ml (○, ● and ■, respectively). **a** Supernatants were collected after 4, 8, 24 and 48 h after stimulation and analysed for levels of IL-8, IL-6 and IL-1 as described in Materials and methods. **b** RNA was isolated at the indicated time points and analysed using ^{32}P-labelled cDNA probes for human IL-8, IL-6, IL-1β and 28S rRNA

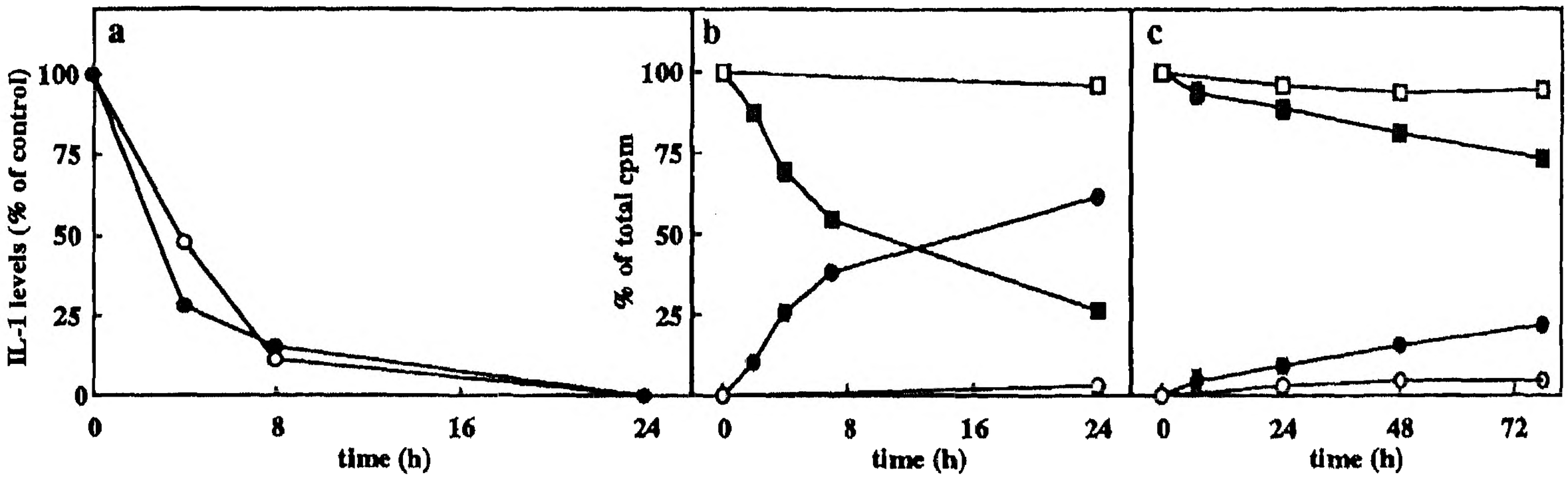

Fig. 3 a–c Time course of the reduction in IL-1 protein. **a** Confluent 3T3 cultures were grown in control medium or CM-SCC-4 for 4, 8 or 24 h. At the indicated time points supernatants were collected and the IL-1 levels determined by bioassay (○) and by radioimmunoassay (●). **b, c** Confluent 3T3 (**b**) and HFF (**c**) cultures were incubated with ^{125}I-IL-1α (150 pg/ml) for the indicated times and in the supernatants collected. These supernatants were then treated with TCA, as described in Materials and methods. In the absence of fibroblasts (control) hardly any spontaneous degradation of ^{125}I-IL-1α at 37° C was found (□ intact molecule, TCA insoluble; ○ degradation product, TCA soluble). In the presence of fibroblasts degradation of ^{125}I-IL-1α was observed (■ intact molecule, TCA insoluble; ● degradation product, TCA soluble). The standard deviation is given in **c**, but was usually smaller than the plot symbol

detectable levels, indicating that transcription of the IL-8 gene was temporarily enhanced. In contrast, IL-6 mRNA expression remained elevated during the 24 h period. After stimulation with 100 pg/ml IL-1α, enhanced IL-8 mRNA levels were detected for a longer period. Elevation in the IL-1β mRNA levels was observed only after stimulation of HFF with 100 pg/ml IL-1α for 8 and 24 h. No IL-1α mRNA could be detected either in stimulated or in nonstimulated cells (data not shown).

Fibroblast-induced decrease in IL-1 protein levels

As described above, exposure of confluent cultures of human dermal fibroblasts to CM-SCC-4 (Fig. 1 a) or IL-1α (Fig. 2 a) resulted in a decrease in IL-1 bioactivity. To examine whether this decrease was caused by the loss of IL-1α protein, a RIA specific for human IL-1α was used. Since the decrease in IL-1α levels in the presence of 3T3 cells is more rapid than with HFF [11], 3T3 cells were used in these experiments. When 3T3 cells were exposed to CM-SCC-4, the time course of the reduction in IL-1 immunoreactivity closely paralleled the reduction in bioactivity (Fig. 3 a). Apparently the observed decrease in IL-1 bioactivity was not caused by mere transformation of IL-1 to its inactive form, but by a decrease in IL-1α protein levels. In addition, we found that in SCC-4/3T3 cocultures the extracellular levels of immunoreactive and bioactive IL-1 remained low, while in SCC-4 monocultures both IL-1 immunoreactivity and bioactivity increased with time (data not shown).

To examine the reduction in IL-1 protein in the presence of fibroblasts in more detail, confluent HFF or 3T3 cultures were incubated with ^{125}I-IL-1α (150 pg/ml) for various times at 37° C and the radioactivity in the supernatants present in TCA-soluble (degraded IL-1) and TCA-insoluble (intact IL-1) fractions were determined. In the presence of HFF the amount of degraded IL-1 increased within 78 h to 22.3 ± 1.5% of total added IL-1. The rate of IL-1 degradation was much higher in 3T3 cultures than in HFF (61.8 ± 0.7% versus 9.2 ± 2.3% after 24 h). Hardly any spontaneous degradation of ^{125}I-IL-1α at 37° C was found in the absence of fibroblasts (4.6 ± 0.5% after 78 h). These findings were confirmed by running these supernatants on an 18% SDS-PAGE gel followed by autoradiography (data not shown).

It has been demonstrated that IL-1 can enhance the production and secretion of proteolytic enzymes by fibroblasts [27]. Therefore, we further investigated whether degradation of IL-1 was due to enhanced proteolytic activity. To this end ^{125}I-IL-1α (600 pg/ml) was added to supernatants derived from 3T3 cell and HFF cultures stimulated or not with 1 ng/ml IL-1 for 20 h and incubated for 20 h at 37° C. After TCA precipitation the distribution of the radioactivity over the two fractions was not significantly different from the control (unconditioned medium). This indicates that there is no evidence for enhanced extracellular degradation. However, when 3T3 cells were first incubated with ^{125}I-IL-1α (5ng/ml) at 4° C, washed and subsequently incubated at 37° C, it could be shown that cell surface-bound IL-1α was rapidly internalized, degraded and excreted. As early as 1 h after the start of the experiment, detectable amounts of radioactivity were present in the supernatants in the TCA-soluble fraction; this amount increased to 85.7 ± 3.3% within 20 h (data not shown).

IL-1 receptor binding

Since the reduction in extracellular IL-1 levels occurs only in the presence of fibroblasts, presumably via IL-1 receptor-mediated uptake [28, 29], the number of IL-1 binding sites and Kd values were determined by Scatchard analysis. The results of these experiments revealed that the number of IL-1 binding sites on 3T3 cells

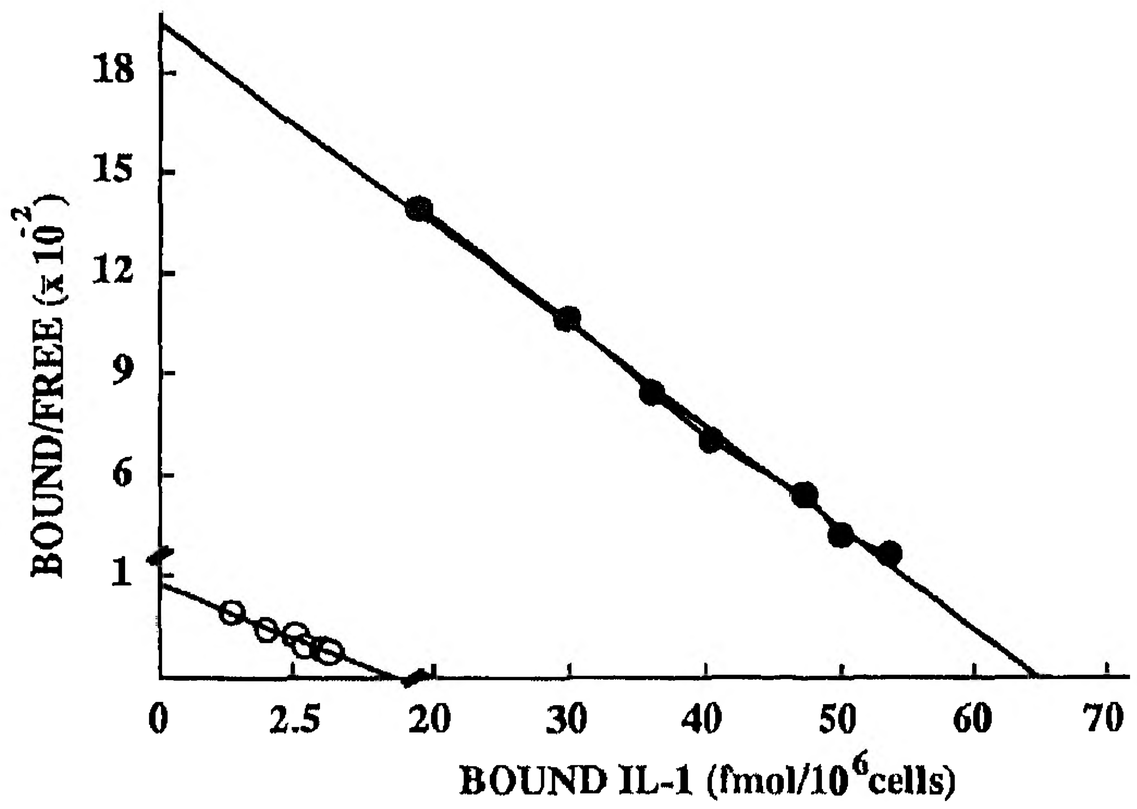

Fig. 4 Scatchard analysis of IL-1 binding to 3T3 cells and HFF. Increasing concentrations of ^{125}I-IL-1α were incubated with cultures of 3T3 cells (●) and HFF (○) in the presence or absence of a 500-fold excess of unlabelled IL-1α as described in Materials and methods. The data are plotted in the Scatchard format. The Kd obtained in the experiment was 60 p*M* for both 3T3 cells and HFF, whereas the numbers of binding sites were approximately 38×10^3 and 2.5×10^3, respectively

was more than 15 times higher than on HFF, approximately 38×10^3 and 2.5×10^3, respectively. This is in line with the observation that IL-1 degradation by 3T3 cells was more rapid than by HFF. The affinity of the receptors on both cell types for IL-1α was similar (approximately 60 p*M*; Fig. 4).

Discussion

There is increasing evidence for the existence of a complex network of interacting cytokines mediating immune and inflammatory reactions in the skin [30, 31]. Fibroblasts of the mesenchymal compartment and keratinocytes of the epithelial compartment participate actively and mutually in this network [32, 33]. IL-1 released from keratinocytes seems to play an important role in the initiation of a cytokine cascade, because IL-1α derived from the keratinocytes is responsible for markedly enhanced IL-6 [11] and IL-8 (this study) production in fibroblasts. The present study further elucidates the mechanism of IL-1, IL-6 and IL-8 modulation. It was shown that the protein and mRNA levels of both IL-6 and IL-8 increase almost simultaneously in a dose-dependent manner. The finding that both cytokines were induced simultaneously is in line with the results of a recent study by Matsusaka et al. [12] showing that the IL-1-induced gene transcription of both IL-6 and IL-8 is synergistically activated by similar transcription factors, namely NF-IL-6 and NF-kB.

As was observed for IL-6 and IL-8 mRNA, IL-1β mRNA expression was also inducible in HFF by IL-1α in a dose-dependent fashion. The findings that total (added and secreted) bioactive IL-1 was reduced over time and that the IL-1-induced stimulation of IL-8 or IL-6 production in HFF was not affected by the presence of a neutralizing antibody against IL-1β, strongly suggest that the amount of IL-1β produced by fibroblasts was too low to exert an additional effect to exogenously administered IL-1α.

In contrast to increased levels of IL-6 and IL-8 measured during the exposure of HFF to IL-1α-containing media (conditioned media or IL-1α-supplemented medium), IL-1α bioactivity and immunoreactivity markedly decreased. The reduction in IL-1α levels can be fully ascribed to the action of fibroblasts, since in the absence of fibroblasts no spontaneous decrease in IL-1 protein was observed. Since this might represent a negative feedback mechanism we further investigated the processes involved in the reduction of IL-1 levels by fibroblasts. Various mechanisms may be responsible for the observed fibroblast-induced decrease in IL-1. The results of the present study show that it is unlikely that the decrease in IL-1 levels is due to binding of IL-1 to an IL-1 inhibitor [34], because the IL-1-inhibitor complex should have been detected in the RIA by the polyclonal antibody, which recognizes different epitopes on IL-1α [20] and such a complex should be TCA-precipitable. It is also unlikely that the decrease in IL-1 activity is caused by an increase in IL-1 receptor antagonist [35–37], since the reduction in the IL-1 bioactivity paralleled a decrease in IL-1 immunoreactivity. Furthermore, we can exclude the involvement of extracellular degradation of IL-1 by proteolytic enzymes, since ^{125}I-IL-1 was not degraded when incubated in the presence of supernatants derived from IL-1-stimulated fibroblast cultures. We present evidence that ^{125}I-IL-1 was only degraded in the presence of fibroblasts by receptor-mediated uptake. A higher rate of reduction in IL-1 was observed in 3T3 cells as compared to HFF. This observation is in line with the 15-fold higher number of IL-1 receptors on 3T3 cells than on HFF. The affinity of the IL-1-receptor for IL-1 was similar in both cell types. The finding that the number of IL-1 binding sites on 3T3 cells was higher than on HFF is in agreement with the results of Mizel et al. [28] and Akahoshi et al. [29]. In line with the hypothesis that the fibroblast-regulated decrease in IL-1 levels may function as a negative feedback mechanism, is the observation that the disappearance of extracellular IL-1 is paralleled by a decrease in IL-6 and IL-8 mRNA expression in fibroblasts.

It is generally accepted that in the epidermis the keratinocytes are the first cells to be encountered by external stimuli, initiating the immune response by increased production of various cytokines. However, in comparison with the amounts of IL-1 produced and secreted by keratinocytes, the production of IL-6 and IL-8 in these cells is very low [5, 11]. In contrast, fibroblasts produce 100 to 1000 times more IL-6 and IL-8 than keratinocytes. Therefore, the release of IL-1α by activated keratinocytes may act as an inducer of IL-8 and IL-6 production in neighbouring fibroblasts, which may be a potent pathway for the amplification of the signal and initiation of a more general inflammatory response. On the other hand, fibroblasts may also be involved in the overall regulation of

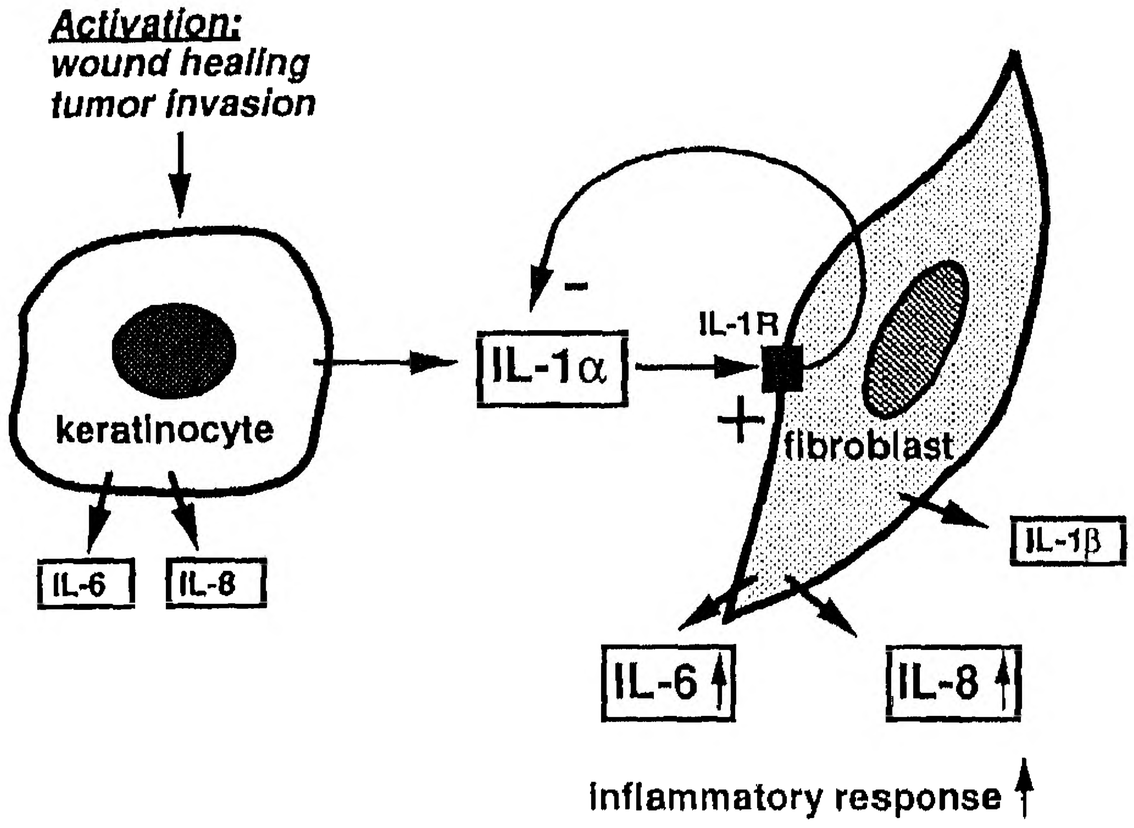

Fig. 5 Role of fibroblasts in the regulation of proinflammatory IL-1, IL-6 and IL-8 levels induced by keratinocyte-derived IL-1: a working hypothesis. The activated keratinocyte releases high amounts of IL-1α and low amounts of IL-6 and IL-8. The IL-1α released markedly enhances the IL-8 and IL-6 production in neighbouring fibroblasts. This may be a potent pathway for the amplification of the signal and initiation of a more general inflammatory response. Fibroblasts may turn off this pathway by reducing the IL-1 activity via receptor-mediated uptake and degradation

cytokine production by turning off this pathway by reducing the IL-1 activity via a receptor-mediated uptake and degradation (schematically presented in Fig. 5). It remains to be established whether or not interactive circuits in skin tissue involving signalling by diffusible cytokines such as IL-1, IL-6 and IL-8 also occur in vivo.

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