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Interleukin-13 stimulates interleukin-6 production by human keratinocytes

Similarity with interleukin-4

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Abstract

Interleukin-13 (IL-13) is a recently described human lymphokine which is produced by activated T-cells. Its effect on the production of IL-6 by normal keratinocytes and keratinocyte cell lines of human origin was studied and compared to that of IL-4. IL-13, similarly to IL-4, stimulated IL-6 expression by these cells in a dose- and time-dependent manner. Contamination with endotoxin was excluded by the use of polymyxin B and heat-inactivated cytokines. Further, we showed that IL-13, like IL-4, not only stimulated IL-6 production but also was able to induce overexpression of this cytokine in response to an inflammatory signal such as lipopolysaccharide (LPS). In a previous study, we demonstrated that IL-13, by inhibiting IL-6 and other cytokines produced by monocytes, exhibited an 'anti-inflammatory profile' comparable to that displayed by IL-4. In contrast, we show here that IL-13, by stimulating IL-6 production by keratinocytes, may favour the installation of an inflammatory process at a local level and, here again, it acted like IL-4. Therefore, according to the type of target cell, these two 'TH2 type' cytokines induce similar opposing effects on IL-6 production and are likely to be important cytokines in the regulation of inflammation at both systemic and local levels.

Key words: Interleukin-4; Interleukin-6; Interleukin-13; Keratinocyte; Inflammation

1. Introduction

Epidermal keratinocytes which represent a major constituent of the skin, produce various cytokines such as transforming growth factor α, tumor necrosis factor α, interferon γ, granulocyte-macrophage colony-stimulating factor, interleukin-1 (IL-1), IL-6 and IL-8 [1]. Of these, IL-6 is interesting in that it is a multifunctional cytokine involved in immune and inflammatory reactions, which stimulates proliferation of both epidermal cells and lymphocytes [2–4]. These properties make it a possible key component of the interaction between the epidermis and the immune system in inflammatory and autoimmune skin diseases such as psoriasis or scleroderma.

In these diseases, T-cell infiltrates are frequently observed and thought to play an important role in sustaining a chronic inflammatory process. IL-4, a product of T-helper cells, has been shown to upregulate IL-6 production in endothelial cells [5], in skin fibroblasts [6] and in keratinocytes [7], whereas it downregulates IL-6, IL-1 and TNFα expression in human monocytes [8,9]. IL-13 is a novel T-helper cell lymphokine we have recently described [10]. It has been shown that IL-13, like IL-4, inhibits IL-6 and other inflammatory cytokines produced by human monocytes [10] as well as pyrogen-induced procoagulant activity in endothelial cells and monocytes [11].

In the present work, we sought to determine whether IL-13 behaves like IL-4 in displaying a totally opposing effect, with respect to IL-6 secretion, when the target cells are of nonhematopoietic origin such as keratinocytes. We show that, similarly to IL-4, exposure to IL-13 stimulates keratinocytes to produce IL-6. This is the first report describing the ability of IL-13 to stimulate human keratinocytes. It therefore adds a new target cell for this novel pleiotropic cytokine. It also suggests that IL-13 plays with IL-4 a critical role in activating epidermal cells during inflammation and certain autoimmune diseases.

2. Materials and methods

2.1. Cells

Keratinocyte cell lines SVK14 (established by J. Taylor-Papadimitriou et al. [12]) and A431 (ATCC CRL 1555, [13]) were maintained in DMEM with 10% fetal calf serum (FCS). Normal human keratinocytes (NHK) were prepared from the skin of human female breast sections by Biopredic (Rennes, France) and grown in the same medium supplemented with 10% FCS, 10 ng/ml epidermal growth factor and 50 μg/ml bovine pituitary gland extract. The medium was changed every other-day. For IL-6 induction, cells were plated in triplicate at a density of 5×10^5 cells/ml in the presence of the stimuli. Supernatants were har-

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vested at the indicated times, centrifuged at 400 × g and stored at −20°C until determination of cytokine content.

2.2. Reagents and cytokines

LPS (*E. coli* 055:B5) and polymyxin B were purchased from Sigma Chemicals (Saint-Quentin Fallavier, France). Human recombinant IL-4 (ARM 18005) was obtained from Amersham (Les Ulis, France) and human recombinant IL-13 was cloned, produced and purified as described [10].

2.3. Measurement of cytokines

IL-6, IL-1α, IL-8 and TNFα were quantified using ELISA kits from Amersham (Biotrak RPN 2145, 2140, 2147, and 2148, respectively) according to the specifications of the manufacturer. The lower limit of detection was 3 pg/ml. The levels of biologically active IL-6 in culture supernatants were also measured using the B9 bioassay [14] as described [10]; values were determined by comparison with an IL-6 standard.

2.4. Northern blot analysis for measurement of IL-6 mRNA

SVK14 keratinocytes were cultured 4 h in culture flasks in the presence or absence of 10 μg/ml LPS with fresh medium supplemented with 10 ng/ml IL-4 or IL-13. As control, parallel flasks were similarly treated without the addition of cytokines. Total cellular RNA was extracted using guanidium isothiocyanate and purified by CsCl gradient ultracentrifugation [15]. Fifteen μg RNA aliquots were electrophoresed in 1% agarose gel using denaturating conditions and blotted onto nylon membranes. Hybridysation was carried out at 37°C in hybridisation buffer (Quick Hyb Stratagene) for 5 h with ^{32}P-radiolabeled human IL-6 cDNA probe. Final washes were at 43°C in 2 × SSC (1 × SSC = 0.3 M NaCl, 0.05 M NaCitrate, pH 7.0) and membranes were exposed for 24 h to Kodak X-Omat film with an intensifier screen at −70°C.

2.5. Statistical analysis

Data were analysed by Student's *t*-test. A cut-off value of $P \leq 0.05$ was used to indicate statistical significance.

3. Results

3.1. Effect of IL-13 on IL-6 production by keratinocytes

The production of IL-6 by the SVK14 keratinocyte cell line was first evaluated using ELISA after a 24 h exposure with the indicated concentrations of IL-13 (Fig. 1). A comparison with IL-4 was done over an identical range of concentrations within the same experiment. The results showed that both cytokines dose-dependently increased the production of IL-6 by keratinocytes. An exposure to 10 to 30 ng/ml led to a 3-fold stimulation of IL-6 secretion ($P \leq 0.05$). IL-4 was slightly more active displaying an EC_{50} of 0.8 ng/ml versus 3 ng/ml for IL-13.

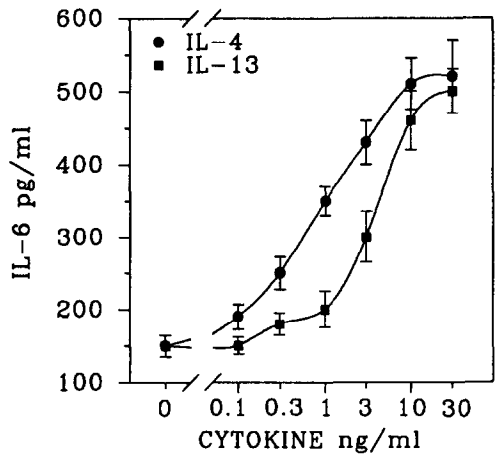

Fig. 1. Effects of IL-13 and IL-4 on IL-6 production by SVK14 keratinocytes. Cells were incubated with the indicated concentrations of IL-4 or IL-13 for 24 h at 37°C. IL-6 levels were measured by ELISA. Values represent the mean ± S.E.M. of triplicate cultures. The figure is representative of four separate experiments.

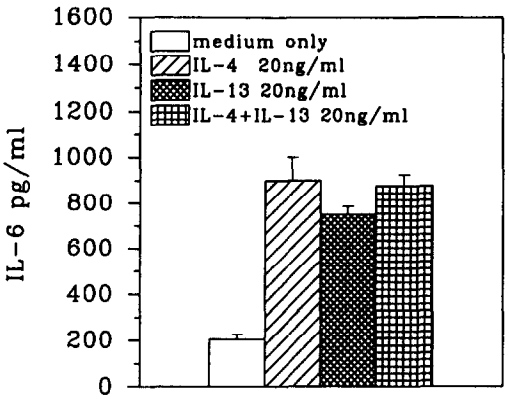

Fig. 2. Effect of the associaton of IL-4 and IL-13 on IL-6 production by SVK14 keratinocytes. Cells were treated 24 h with the indicated concentrations of IL-4 or IL-13 or both. IL-6 was evaluated by ELISA. Values represent the mean ± S.E.M. of triplicate cultures. The figure is representative of three separate experiments.

As shown in Fig. 2, when IL-4 and IL-13 were used in combination at the optimal concentration of 20 ng/ml for 24 h, neither synergistic nor additive effects were found on the stimulation of IL-6. The association of IL-4 and IL-13 led to an increase of IL-6 production similar to what was observed with each cytokine taken alone (800–900 pg/ml in all cases in treated cells versus 200 pg/ml in control cells).

The kinetics of IL-13- and IL-4-induced IL-6 production were then compared using the B9 proliferation assay (Fig. 3). IL-4 and IL-13 at 10 ng/ml similarly induced a significant increase of IL-6 secretion ($P \leq 0.05$) as soon as 6 h after exposure which continued until 24 h, at which time the stimulated SVK14 cells were producing approximately 3-fold more IL-6 than were the unstimulated cells.

The property of IL-13 to stimulate IL-6 production by keratinocytes is a general phenomenon observed on another cell line (A431) and on normal human keratinocytes (NHK) as well. Table 1 shows that both IL-13 and IL-4 after a 24 h treatment at 10 ng/ml induced a significant 2.5- to 3-fold increase in IL-6 production whatever the type of keratinocytes.

Considering the complex network of cytokines involved in inflammation, we sought to determine whether IL-13 was also able to stimulate keratinocytes to produce other inflammatory cytokines such as IL-8, IL-1α and TNFα. In all cases, using immunoassays, the values obtained after exposure to either IL-13 or IL-4 (24 h at 10 ng/ml) were below detection limit and we failed, under our conditions, to find any stimulating effect of IL-13 or

IL-4 whereas control cells treated with 10 μg/ml of LPS produced significant amounts of these proinflammatory cytokines, well above detection limit of the assay (data not shown).

3.2. Absence of endotoxin contamination

Endotoxin is known to be a potent inducer of IL-6 secretion by keratinocytes [16,17]. Therefore, it was important to know whether the phenomenon observed could be due to a contamination of the recombinant proteins with endotoxin. Pre-incubating IL-13 and IL-4 with polymyxin B which binds LPS, did not affect their stimulating effect whereas, in the same conditions, the level of IL-6 produced by LPS-stimulated cells was brought down to basal level (Fig. 4). Heating IL-13 and IL-4 to 70°C for 1 h before use completely abrogated their bioactivity while the stimulating effect of LPS remained unchanged (Fig. 4).

3.3. Amplification by IL-13 and IL-4 of IL-6 mRNA and protein induced by LPS

As shown in Fig. 5, using LPS (10μg/ml) as an inflammatory stimulus, the level of IL-6 raised from 516 pg/ml in cells treated with LPS alone to 1290 and 1030 pg/ml in cells treated with a simultaneous combination of LPS + IL-4 or LPS + IL-13, respectively ($P \leq 0.05$). A Northern blot analysis (Fig. 6) showed that similar to IL-4, the ability of IL-13 to induce an overexpression of IL-6 in LPS stimulated human keratinocytes was related to a higher accumulation of IL-6 transcripts. In both cases, a densitometric quantitation by an image analyser indicated an almost 2-fold increase of IL-6 mRNA compared to LPS control cells. In the absence of LPS, the stimulating effect of IL-13 and IL-4, demonstrated at the level of IL-6 protein, was not detectable at the level of mRNA probably because of the low sensitivity of the Northern blot analysis.

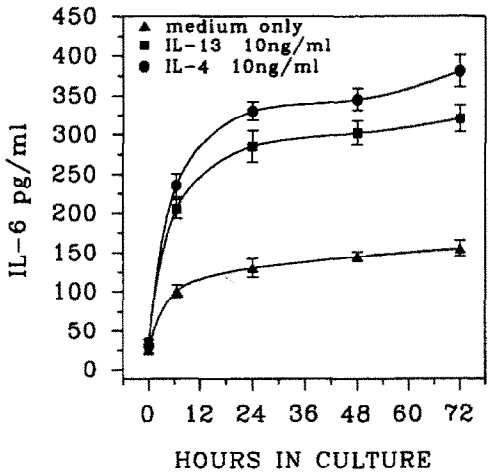

Fig. 3. Kinetics of IL-13- and IL-4-induced IL-6 production. SVK14 keratinocytes were cultured with medium only or medium supplemented with 10ng/ml IL-4 or IL-13. At the times indicated, the levels of biologically functional IL-6 in culture supernatants were measured using the B9 hybridoma proliferation assay. Values represent the mean ± S.E.M. of triplicate cultures. The figure is representative of three separate experiments.

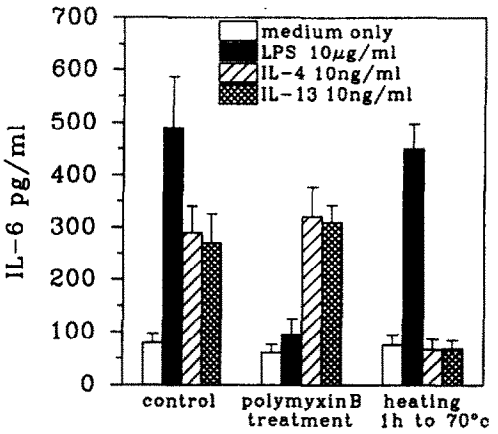

Fig. 4. Stimulation of IL-6 production is not due to endotoxin contamination. SVK14 keratinocytes were incubated 24 h with medium only or medium supplemented with one of these agents: LPS , IL-4 , IL-13. The absence of endotoxin contamination was evaluated by pre-incubating these agents with polymyxin B (100 μg/ml) for 1 h or by heating them to 70°C for 1 h before addition. IL-6 production was measured as indicated in Fig. 1. The figure i representative of two separate experiments.

In the experiments of Figs. 5 and 6 we clearly showed that IL-4 and IL-13 were also able to act in concert with an already potent inducer leading to an overexpression of IL-6 at both transcriptional and translational levels.

4. Discussion

The results presented here show a great similarity between IL-13 and IL-4 in stimulating IL-6 production by human keratinocytes although IL-4 seemed to be slightly more potent. IL-4 and IL-13 not only stimulated IL-6 production per se but also amplified IL-6 expression induced by a primary inflammatory signal such as LPS. This was observed at the level of RNA and protein. This is the first report of such a biological effect of IL-13, a recently described lymphokine [10], on this type of cells.

Table 1
Effects of IL-4 and IL-13 on the production of IL-6 by normal keratinocytes and keratinocyte cell lines. Normal human keratinocytes (NHK) or keratinocyte cell lines (A431 and SVK14) were cultured with IL-4 or IL-13 for 24 h at 37°C. IL-6 content in cell-free supernatants was measured by ELISA. Values represent the mean ± S.E.M. of triplicate cultures.

	IL-6 (pg/ml)		
Treatment	NHK	A431	SVK14
Control	65 ± 5	16 ± 2	98 ± 10
IL-4 10 ng/ml	180 ± 15	52 ± 7	325 ± 22
IL-13 10 ng/ml	156 ± 12	45 ± 5	276 ± 18

This study further reinforces the homology found between the biological activities of IL-4 and IL-13. We previously demonstrated that IL-4 and IL-13 displayed comparable abilities to inhibit inflammatory cytokine synthesis in LPS stimulated monocytes [10]. More recently, it was shown that both IL-4 and IL-13 inhibit the procoagulant activity of monocytes and endothelial cells exposed to inflammatory mediators [11]. This similarity of action could be explained, at least in part, by the fact that these two lymphokines share a common receptor component that is important for signal transduction [18]. In line with this finding, when IL-4 and IL-13 were used in combination on keratinocytes , neither synergistic nor additive effects were found on the upregulation of IL-6 production, suggesting a common transduction pathway in this model . Indeed, cross-competition for receptor binding is seen between IL-4 and IL-13 on A431 keratinocytes (N. Vita, personnal communication).

IL-6 which is an important mediator in normal and pathological immune reactions [19–21], like other cytokines, does not function in isolation. We therefore sought to evaluate whether IL-4 and IL-13 were able to stimulate keratinocytes to produce other proinflammatory cytokines such as TNFα, IL-1, and IL-8. Under our conditions, we failed to find any stimulating effect of IL-4 and IL-13 on these cytokines. A more sensitive approach such as the polymerase chain reaction is needed to totally rule out any influence of IL-4 and IL-13 on the expression of these mediators by human keratinocytes.

IL-6 expression can be downregulated or upregulated by various cytokines in a cell type and tissue specific manner. For exemple TNFα [22] and IL-4 [6] stimulate IL-6 gene expression in fibroblasts. IL-4 downregulates IL-6 production by human monocytes 9 but upregulates the same cytokine in B lymphocytes [23], endothelial cells [5] and keratinocytes [7]. IL-13 shows the same profile of action than IL-4: downregulation of IL-6 in monocytes [10], upregulation on nonhematopoietically-derived cells of the skin such as keratinocytes (this study).

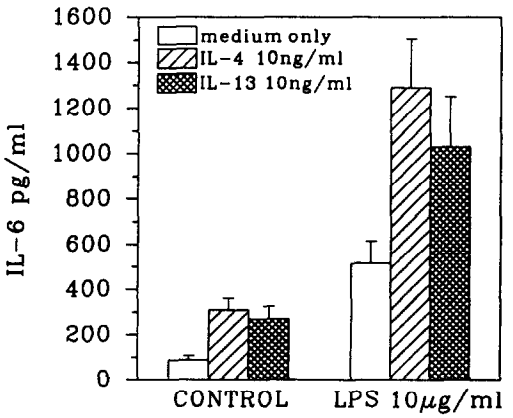

Fig. 5. Amplification of LPS-induced IL-6 production by IL-4 or IL-13. SVK14 keratinocytes were incubated for 24 h with IL-4 or IL-13 in the presence or absence of LPS . IL-6 levels were measured as described in Fig. 1. The figure is representative of three separate experiments.

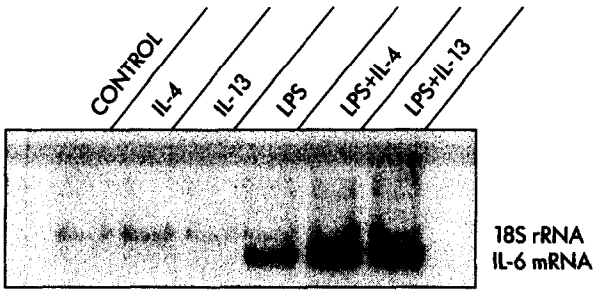

Fig. 6. Effects of IL-4 and IL-13 on IL-6 mRNA levels. SVK14 keratinocytes were incubated in the presence or absence of 10 μg/ml LPS with medium only or medium supplemented with 10 ng/ml IL-4 or IL-13. Total RNA was prepared and used in Northern blot analysis as described in section 2. The joint bands above IL-6 mRNA corresponded to cross hybridization of IL-6 probe with 18S rRNA which confirmed equal amounts of RNA in each lane. The figure is representative of two separate experiments.

The role of IL-4 and IL-13 in stimulating IL-6 in epidermal cells may be directly relevant in vivo in autoimmune skin diseases such as scleroderma [24] and psoriasis [25] where overproduction of IL-6 has been implicated and activated T-cell infiltrates with a helper-inducer phenotype have been observed [1,26,27]. Thus IL- 4 and IL-13 which both are products of helper T-cells, may be directly involved in the pathogenesis of these diseases by locally increasing the IL-6 production in affected sites. More generally, IL-4 and IL-13 are pleiotropic cytokines which seem to play a crucial role in the regulation of inflammatory processes at a local and systemic level. The biological significance of the apparent redundancy displayed by these two regulators remains to be elucidated.

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