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Interleukin-13 acts as an apoptotic effector on lung epithelial cells and induces pro-fibrotic gene expression in lung fibroblasts

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Clinical and Experimental Allergy

Summary

Background IL-13 promotes acute allergic asthma and is discussed to play a role in late asthmatic features such as fibrotic processes and airway remodelling. The contributions of IL-13-mediated mechanisms to subepithelial events related to fibrosis are not yet settled.

Objective We investigated the impact of IL-13 on lung epithelial cells as apoptotic effector and on lung fibroblasts as inducer of pro-fibrotic gene expression.

Methods Using the two lung epithelial cell lines A549 and BEAS-2B as well as primary lung epithelial cells, we investigated the capability of IL-13 to induce apoptosis by both flow-cytometry and ELISA. The ability of IL-13 to increase the expression of pro-fibrotic genes and to exert influence on the expression of its own receptor was investigated by real-time quantitative PCR measurement of mRNAs encoding collagen I, collagen III, basic fibroblast growth factor (bFGF), α -smooth muscle actin (α -SMA) and the IL-13 receptor α 1 (IL-13R α 1) chain in human primary lung fibroblasts. The specificity of IL-13-mediated cellular responses was confirmed by means of an inhibitory monoclonal antibody directed to the IL-13 receptor. **Results** IL-13 induces apoptosis in lung epithelial cell lines as well as in primary lung epithelial cells. Furthermore, IL-13 increases the expression of mRNA for α -SMA and collagen III, but not for bFGF in human primary lung fibroblasts. The susceptibility of lung fibroblasts to IL-13-induced up-regulation of pro-fibrotic genes is associated with the regulation of IL-13 receptor expression. IL-13-dependent fibrosis-associated effects could be inhibited by antibody-mediated blockade of the IL-13R α 1 subunit.

Conclusion Our findings indicate a function of IL-13 as a mediator in fibrotic processes leading to loss of functional airway tissue in asthma. They also highlight the therapeutic potential of specifically targeting the interaction between IL-13 and its receptor.

Keywords apoptosis, asthma, fibrosis, IL-13 receptor, IL-13

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Introduction

Allergic asthma is an inflammatory disorder of the airways, which is, in its early stage, characterized by the accumulation of T helper type 2 (Th2) cells, macrophages and eosinophils in the bronchial submucosa [1].

Airway remodelling is a hallmark of chronic asthma and of major importance for the worsening progression of the disease [2]. Various fibrotic processes lead to a loss of structure, elasticity and function of the lung and enhance airway hyperresponsiveness as well as the development of fixed airflow obstruction [3]. A key feature of the syn-

drome is the thickening of airway walls caused by the deposition of extracellular matrix proteins such as collagens, fibronectin and laminin [4–6].

Throughout the airway remodelling, bronchial epithelium in asthma shows a characteristic loss of columnar epithelial cells, increased apoptosis and impaired repair capacity [4, 7]. Damaged epithelium is able to release fibroproliferative and fibrogenic growth factors such as basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF) and TGF- β , which in turn stimulate the differentiation of submucosal fibroblasts to myofibroblasts and smooth muscle cells and induce increased

production of extracellular matrix proteins in the lung [8–11].

The Th2-like cytokines IL-13 and IL-4 are critically involved in the pathogenesis of allergic asthma [1]. IL-13 and IL-4 share a common receptor subunit and have overlapping functions, but, interestingly, in mouse models IL-13 has been shown to elicit most of the symptoms of asthma independently of IL-4 [12, 13]. While the importance of IL-13 for symptoms of acute allergic asthma is well appreciated, its contribution to the progression into chronic persistent asthma with its airway fibrosis and airway remodelling has not yet been precisely defined.

In transgenic mice, increased expression of IL-13 leads to submucosal remodelling and enhances collagen deposition, mucus cell metaplasia and emphysema [14, 15]. In addition, it also enhances fibrosis indirectly by inducing TGF- β in macrophages [16]. In line with these findings, the absence of IL-13 prevents asthmatic thickening of subepithelial basal lamina in the murine model [17].

Studies on the role of IL-13 in chronic asthma, lung remodelling and fibrosis in the human system are scarce and results as well as their interpretation are in part controversial. Batra *et al.* [18] reported that IL-4, but not IL-13, stimulation leads to increased protein levels of both collagen III and α -smooth muscle actin (α -SMA) in human lung fibroblasts, whereas collagen I production was not substantially enhanced by neither cytokine. In contrast, work by Saito *et al.* [19] showed both IL-13 and IL-4 as efficient inducers of α -SMA protein.

Apoptosis of epithelial cells is a further potential mechanism contributing to chronic tissue damage in asthma. Although it has been shown that IL-4 can promote apoptosis of airway epithelial cell lines via induction of 15-lipoxygenase [20], the role of IL-13 in this process has not been addressed. An indication for apoptosis-promoting effects of IL-13 on epithelia in general comes from results obtained with gut-derived epithelial cells [21, 22].

In this report, we analyse systematically in model cell lines as well as in primary cells from surgical airway specimens the effects of IL-13 on lung epithelial apoptosis and on the expression of pro-fibrotic genes in lung fibroblasts. Our results strongly support the notion that IL-13 is not only of primary relevance for acute asthma, but might also be an important player in the chronic phase of the disease and, thus, an attractive target for pharmaceutical intervention in acute as well as chronic asthma.

Materials and methods

Cell lines

A549 (DSMZ, Braunschweig, Germany) cells were established from a human alveolar epithelial carcinoma, and

have characteristics of well-differentiated alveolar epithelial cells type II (AEC-II). BEAS-2B (ECACC, Salisbury, UK) cells are adenovirus-transformed cells, which had been established from normal human bronchial epithelium. Both cell lines were cultured in 75 cm² tissue culture flasks in Dulbecco's modified Eagle's medium (DMEM; Biochrom AG, Berlin, Germany) supplemented with 10% fetal calf serum (FCS) and 50 μ g/mL Gentamycin (both from PAA, Cölbe, Germany) at 37 °C in a humidified atmosphere with 5% CO₂. Growing cells were passaged when >80% confluent by treatment with trypsin/ethylenediaminetetraacetic acid (EDTA) (0.05%/0.02%; Biochrom AG). For experiments, passage numbers 15–25 were used.

Human primary alveolar epithelial cells type II

Samples from macroscopically tumour-free lung tissue were cut from the surgical specimens and used for the cell isolation procedure as described previously [23]. In brief, the lung tissue was sliced, slices were washed three times at 4 °C in phosphate-buffered saline (PBS) and then incubated in sterile dispase solution at 37 °C for 45 min. After dispase digestion, slices were cut into small, pipettable pieces, and thoroughly pipetted for several minutes. Crude tissue and cell suspensions were filtered through nylon gauze with meshes of 100, 50 and 20 μ m. The resulting single cell suspension was placed on Ficoll separating solution and centrifuged at 800 $\times$ g for 20 min. AEC-II-enriched cells from the interphase were incubated in 100 mm plastic dishes at 37 °C in humidified air containing 5% CO₂ for 15, 20 and 30 min with seeding of non-adherent cells on fresh dishes for each time interval to remove adherent cells (alveolar macrophages, monocytes, fibroblasts and endothelial cells). To remove remaining monocytes/macrophages and lymphocytes, cells were incubated with CD45-coated magnetic beads (Miltenyi Biotec, Bergisch Gladbach, Germany) and separated using magnetic activated cell sorting system as suggested by the supplier (Miltenyi Biotec). AEC-II were collected and cultured in RPMI1640 (Cell Concepts, Umkirch, Germany) containing 10% FCS and 1% of streptomycin and penicillin. Identity and purity of isolated AEC-II were confirmed by a modified Papanicolaou staining and alkaline phosphatase activity directly after isolation (purity >92% in both tests; data not shown). Viability of the AEC-II after isolation was >97% as determined by trypan blue exclusion 24 h after stimulation. CD3 and CD14 mRNA were detected in four of 12 samples by RT-PCR (data not shown). In order to avoid false positive results from contaminated cells, these four AEC-II preparations were excluded in further experiments. Informed consents were obtained from all subjects. Cells were cultured directly after isolation for 2–3 days in six-well plates with a density of 10⁶ cells/mL before they

were seeded in 96-well plates for stimulation with cytokines. Alternatively, cells were frozen after isolation and after thawing cultured in RPMI 1640 containing 10% FCS and 1% of streptomycin and penicillin for 2–3 days in a six-well plate. After positive test of viability, cells were stimulated with cytokines in 96-well plates. The study was approved by the ethics committees of the involved institutions.

Human primary lung fibroblasts

Primary lung fibroblasts were obtained from surgical specimens of seven bronchial carcinoma patients. Macroscopically tumour-free tissue was sliced and washed three times in PBS at 4 °C. The washed slices were placed into six-well plates containing Quantum medium (PAA) supplemented with 1% penicillin/streptomycin. The slices were cultured at 37 °C in a humidified atmosphere with 5% CO₂ for 2–3 weeks. Media was replaced every 3 days. Cells grown from these tissue slices were serially passaged several times in 150 cm² cell culture flasks to yield pure populations of lung fibroblasts. The primary fibroblasts were assessed by cellular morphology and immunolocalization of vimentin detected by flow-cytometry and immunofluorescence microscopy. Purity was >99%. For experiments, 2 × 10⁵ cells per well were cultured in six-well plates at 37 °C and 5% CO₂ in Quantum medium. Twenty-four hours before stimulation media were replaced by DMEM supplemented with 1% FCS and 50 µg/mL Gentamycin. Primary fibroblasts were used at passages 2–7 in the experiments described below.

Apoptosis assays

Apoptosis rates were measured by ELISA detection of active caspase-3/7 (Tebu-Bio, Offenbach, Germany) and by flow-cytometric determination of cleaved poly(adenosine diphosphate-ribose) polymerase (PARP).

For ELISA, 10⁴ cells/well (A549 and BEAS-2B) or 4 × 10⁴ cells/well (primary AEC-II cells) were incubated with varied concentrations of IL-4 or IL-13 for 72 h in 96-well plates. Specific inhibitors were added optionally 1 h before cytokine stimulation. Fluorescence was measured at 442 nm. For flow-cytometric analysis, a fluorescein isothiocyanate-conjugated antibody to cleaved PARP (Cell Signaling Technology, Denver, CO, USA) was used. Samples of 5 × 10⁵ cells were incubated in the presence or absence of IL-4 and IL-13 (1 nM) in six-well plates for 72 h. Subsequently, cells were detached with trypsin/EDTA, washed twice and fixed in 2% formaldehyde for 15 min, permeabilized by 0.1% Saponin (Roth GmbH, Karlsruhe, Germany) in PBS for 1 h and incubated with anti-cleaved-PARP antibody according to the manufacturer's protocol for 30 min. For control, cells were cultured in the

presence of 80 µM apoptosis inducer tetrandrine (Sigma Aldrich, Steinheim, Germany) for 24 h [24]. Cytometric measurements were performed using a FACScan (Becton Dickinson, Heidelberg, Germany) and CELL Quest Pro software.

Inhibitors of interleukin-13 and interleukin-4 signalling

Generation of the antagonistic antibody GM1E7 to the IL-13 receptor α1 (IL-13Rα1) chain [25] and the antagonistic IL-4 mutant R121D/Y124D/S125D [26] have been described previously. Both proteins were employed in purified form.

Real-time quantitative polymerase chain reaction

Total RNA was extracted from samples of 10⁶ fibroblasts using Trizol reagent (Invitrogen, Karlsruhe, Germany) according to the manufacturer's protocol. Equal RNA amounts were incubated with oligo-dT primers and M-MLV reverse transcriptase (Stratagene, La Jolla, CA, USA) for 1 h at 37 °C according to the supplier's protocol to generate cDNA. PCR amplification for detecting fibrosis-related gene products was carried out using the following specific primer pairs. bFGF: 5'-AGAAAACCCGAGCGAGTAGG-3' and 5'-GGTTCACGGATGGGTGTCT-3'; collagen I: 5'-CCCTGTCTGCTTCTGTAACT-3' and 5'-CATGTTCGGTTGGTCAAAGATA-3'; collagen III: 5'-CTCCCCTTTGCACAAAGCTCA-3' and 5'-AGGGCAGGGAACAACCTGTG-3'; α-SMA: 5'-CATCATGCGTCTGGATCTGG-3' and 5'-GGACAATCTCACGCTCAGCA-3'; glyceraldehyde 3-phosphate dehydrogenase (GAPDH) control: 5'-CACCA GGGCTGCTTTAACT-3' and 5'-GATCTCGCTCTGGAA GATG-3'. For PCR amplification of IL-13Rα1 mRNA in correlation with β-actin, the following specific primer pairs were used: IL-13Rα1: 5'-CAAGAAATGAGTATAGGTAAGA-3' and 5'-TAAAGCAGGAGTACTATGAT-3'; β-actin: 5'-GGACTTCGAGCAAGAGATGG-3' and 5'-GCAGTGATCTCCTTCTGCATC-3'.

Primers were designed using Primer3 software (Whitehead Institute for Biomedical Research, Cambridge, MA, USA). All primers were intron-spanning and obtained from MWG-Biotech (Ebersberg, Germany). Real-time quantitative PCR was performed using QPCR SYBR Green Mix (Abgene, Hamburg, Germany), using an iCycler and iQ 3.0 software (Bio-Rad Laboratories GmbH, München, Germany). To verify specificity of the amplification products, a melting curve analysis was performed. No amplification of non-specific products was observed in any of the reactions. Results of real-time measurements were normalized to 'house keeping' GAPDH message. Results are expressed as fold induction of stimulated in relation to unstimulated samples.

Statistical analysis

Statistical analysis was performed using SPSS software version 11.0 (SPSS, Chicago, IL, USA). Non-parametric tests were used if asymmetric distribution of data was suggested by the Shapiro–Wilks test. Data are expressed as boxplots including median, quartiles ($Q_{0.25}/Q_{0.75}$) and range. Outliers (all cases more distant than 1.5 interquartile ranges from the upper or lower quartile) were omitted in the graphs. To calculate significance levels between samples, data were compared using the Wilcoxon test for paired samples and Mann–Whitney test for unpaired samples, respectively. Probability values of $P < 0.05$ were used as the generally accepted level of statistical significance.

Results

Interleukin-13 induces apoptosis in human airway epithelial cell lines as well as in human primary alveolar epithelial cells

The potential of IL-13 to induce apoptosis in two human epithelial cell lines, A549 and BEAS-2B, was tested by ELISA. We revealed a dose-dependent induction of apoptosis by IL-13 in BEAS-2B cells. The half maximal effect was achieved at concentrations around 0.5 nM; a plateau of maximal apoptosis induction (factor about 2.5) was reached at concentrations above 1 nM (Fig. 1a). The specificity of pro-apoptotic signal release by the IL-13 receptor was confirmed by blockade of IL-13R α 1 with GM1E7, an inhibitory antibody directed to this subunit [25]. IL-4, whose pro-apoptotic activity was assessed in a parallel experiment, showed comparable kinetics of apoptosis induction. The effect of IL-4 could be specifically inhibited by inclusion of 250 nM of the antagonistic IL-4 mutant RYS [26]. Similar results were obtained using A549 cells as responder cells (Fig. 1b). In addition, BEAS-2B cells were assayed for IL-13- and IL-4-dependent apoptosis by flow-cytometry using cleaved PARP as marker for apoptosis (Fig. 1c). Although IL-13 was not as efficient in inducing apoptosis as tetrandrine, which is a very potent pro-apoptotic agent in lung epithelial cells [27], IL-13 evoked a robust degree of apoptosis in BEAS-2B, which was at least comparable to that of IL-4.

The cell line-derived results were furthermore proven in *ex vivo* experiments using primary AEC-II isolated from areas of macroscopically tumour-free lung tissue of surgical specimens taken by lobectomy or pneumectomy from seven different lung tumour patients. Primary epithelial cells were incubated with concentrations of IL-13 between 0.05 and 1 nM, and apoptosis was quantified by ELISA for cleaved caspase 3/7 (Fig. 2a). To confirm specificity, IL-13 was antagonized by the inhibitory antibody GM1E7. For comparison, a similar experiment with

IL-4 was performed, here using IL-4 RYS as a specific inhibitor (Fig. 2b).

Interleukin-13 induces expression of pro-fibrotic genes in human primary lung fibroblasts

In order to test the potential of IL-13 to increase the expression of pro-fibrotic genes in human primary lung fibroblasts we stimulated them for 24 h with IL-13 or, for comparison, with IL-4 and assessed the resulting changes in the mRNA levels of collagen I (Fig. 3a), collagen III (Fig. 3b), α -SMA (Fig. 3c) and bFGF (Fig. 3d) by real-time quantitative PCR. To confirm specificity of the stimulation, we used GM1E7 or IL-4 RYS as specific inhibitors.

Expressions of all genes except for bFGF were up-regulated by both IL-13 and IL-4. Levels of relative gene activation, however, varied over a large range within the collection of cell samples. Specific mRNA encoding for both collagen III and α -SMA showed a significant IL-13-dependent increase, the IL-13-induced elevation of collagen I mRNA failed to reach statistical significance ($P = 0.054$). Levels of α -SMA mRNA responded to IL-13 to the highest extent with an induction index of >100 for 1 nM IL-13 compared with unstimulated cells (Fig. 3).

Interleukin-13-driven transcriptional regulation of pro-fibrotic genes in lung fibroblasts is linked to the expression level of the interleukin-13 receptor α 1 chain

The pronounced diversity in our collection of fibroblast samples regarding the degree of IL-13-dependent gene regulation prompted us to address possible mechanisms underlying this finding. In a previous study, we observed that stimulation of eosinophils with IL-13 can influence the expression of the IL-13 receptor [28]. To test if a similar phenomenon is also operative in fibroblasts, we correlated basal and IL-13-dependent expression levels of fibrosis-related genes with that of the IL-13R α 1 chain.

Five primary lung fibroblast samples were incubated with 1 nM IL-13 and stimulation indices for collagen I (Fig. 4a), collagen III (Fig. 4b) and α -SMA (Fig. 4c) mRNA were individually determined by real-time PCR. Interestingly, the transcriptional responses of all three pro-fibrotic genes to IL-13 showed comparable patterns in the individual cell samples. Whereas sample 297KA responded to IL-13 by a drastical gene induction, 300SB, 298SE and 306DJ showed a moderate induction. Fibroblast sample BonGio responded only marginally by a minor induction of collagen I mRNA.

We then determined the relative levels of IL-13R α 1 mRNA in quiescent and IL-13-treated fibroblast samples. Surprisingly, the pattern of IL-13R α 1 mRNA abundance in non-stimulated samples was roughly inverse to that observed for the inducibility of pro-fibrotic genes (Fig. 4d). However, upon IL-13 stimulation, the IL-13 receptor

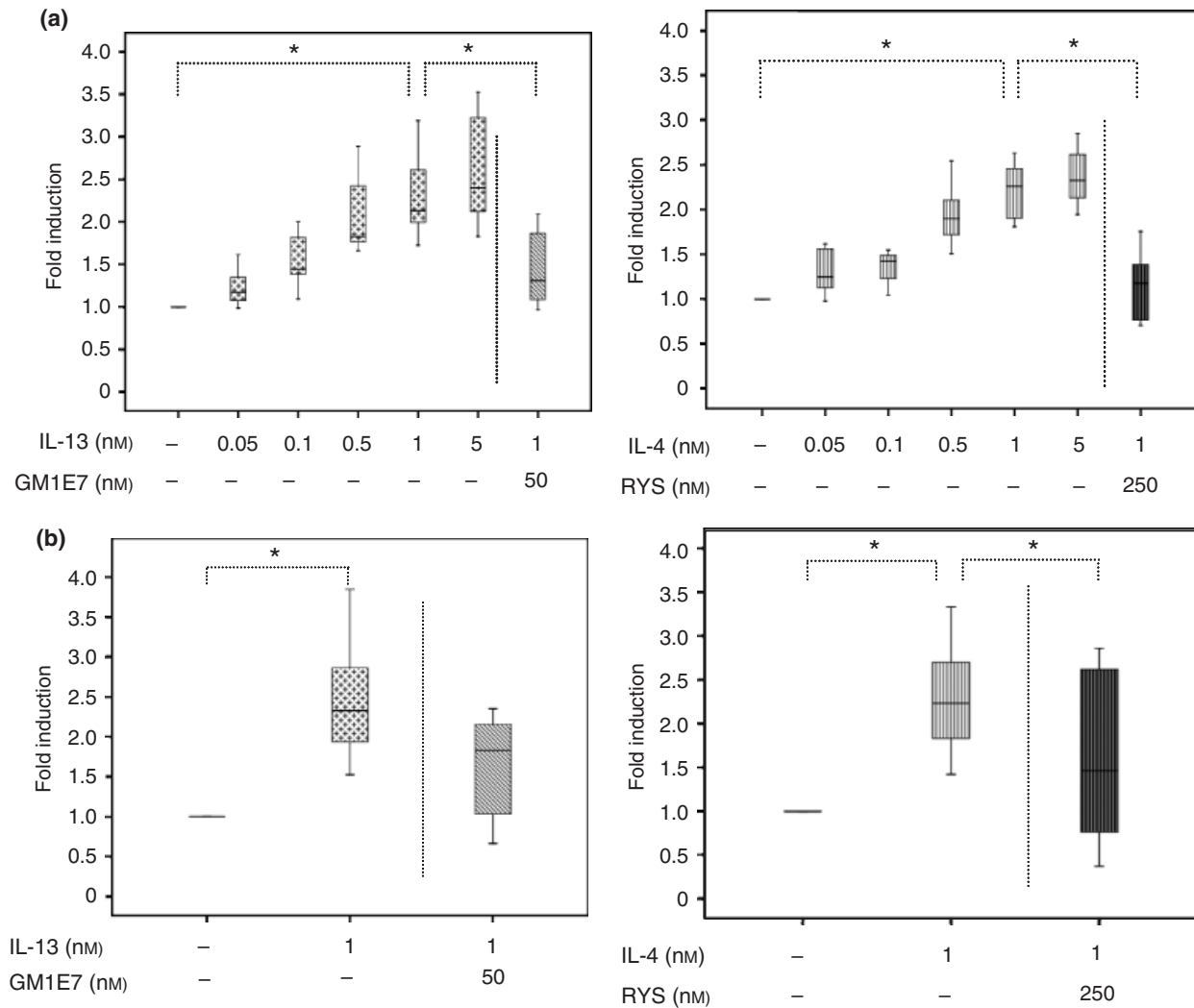


Fig. 1. Analysis of apoptosis rates in human lung epithelial cell lines treated with IL-13 and IL-4. Apoptotic effect of IL-13 and IL-4 in BEAS-2B (a) and A549 (b) cells determined by ELISA for caspase 3/7 activity. 10^4 cells were treated with IL-13 or IL-4 at the indicated concentrations for 72 h. For specificity controls, inhibitors (50 nM antagonistic antibody GM1E7 to the IL-13 receptor $\alpha 1$ chain; 250 nM antagonistic IL-4 mutant R121Y124S125D) were added 1 h before cytokine stimulation. Apoptosis rates were analysed by an ELISA assay detecting the concentration of proteolytically cleaved Ac-DEVD-AMC fluorescence dye generated by active caspase 3/7. For each cell line, results of five independent experiments are shown as box-plots and represent median, interquartile range and range. All experiments were performed in triplicate. Differences with P -values < 0.05 were considered statistically significant and are marked with an asterisk. Flow-cytometric analysis of apoptosis in BEAS-2B cells induced by IL-13 and IL-4 (c). 4×10^5 cells were cultured in the presence or absence of IL-13 or IL-4 for 72 h. In both experiments, cells treated with $80 \mu\text{M}$ tetrandrine for 24 h were employed as positive control for induced apoptosis. Apoptotic cells were detected by flow-cytometry upon staining of permeabilized cells with a fluorescein isothiocyanate-conjugated antibody to cleaved poly(adenosine diphosphate-ribose) polymerase. In both cases, one representative of three independent experiments is shown. Results of untreated, cytokine-treated and tetrandrine-treated cells were overlaid.

mRNA increased by two orders of magnitude in fibroblast sample 297KA, which had been characterized as particularly susceptible to IL-13-dependent up-regulation of pro-fibrotic genes. Samples 300SB and 298SE, on the other hand, responded to IL-13 by a considerable down-regulation of IL-13R $\alpha 1$ mRNA by factors of more than 10. These results suggest that the inducibility of pro-fibrotic genes in lung fibroblast is, at least in a relevant percentage of individuals, functionally connected to IL-13-mediated control of IL-13 receptor expression.

Discussion

It has been demonstrated that IL-4 can favour apoptotic processes in epithelial cell lines derived from human airways [20]. Considering the essential role of IL-13 in asthma pathogenesis and the fact that IL-13 shares one receptor subunit with IL-4, we addressed the potency of IL-13 to induce apoptosis in two human cell lines. We revealed in this study a massive apoptotic potential of IL-13 indicated by our findings that this Th2-type

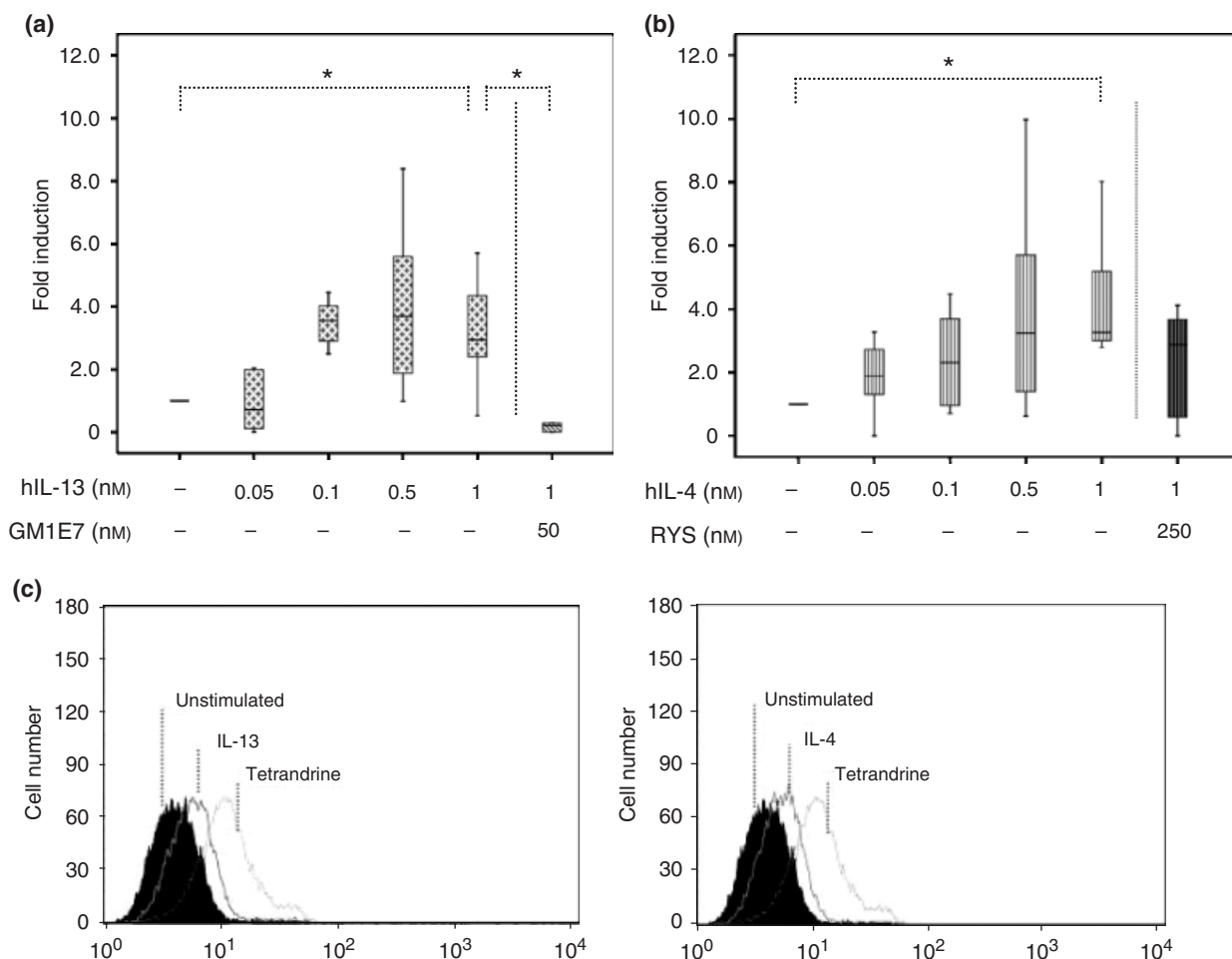


Fig. 2. Effects of IL-13 and IL-4 on apoptosis in primary alveolar epithelial cells type II. Epithelial cells were obtained from areas of macroscopically tumour-free lung tissue of surgical specimens taken by lobectomy or pneumectomy from seven different lung tumour patients. 4×10^5 cells were incubated with IL-13 (a) and IL-4 (b) at the indicated concentrations for 72 h at 37 °C and assayed for apoptosis by ELISA for caspase 3/7 activity as described in Fig. 1. For specificity controls, inhibitors (50 nM antagonistic antibody GM1E7 to the IL-13 receptor $\alpha 1$ chain; 250 nM antagonistic IL-4 mutant RYS) were added 1 h before cytokine stimulation. Results represent the means from independent assays with cell populations from seven individual subjects. Data were reported as described in Fig. 1. * Differences with P -values < 0.05 were considered statistically significant.

cytokine specifically induces in a dose-dependent manner apoptosis in BEAS-2B and A549 cells, representing two different types of epithelial cells with relevance for lung fibrosis. While the effects on apoptosis of BEAS-2B cells are rather unequivocal, our findings in A549 cells are more heterogeneous, possibly reflecting differences in the cell origin.

To assure the accuracy of our test system, we used IL-4 as previously described apoptotic effector for comparison [20] and achieved equivalent results, indicating that AEC-II respond by apoptosis to both IL-13 and IL-4. In order to assure the general significance of our findings and to extrapolate our results derived from studies with cell lines we confirmed the results by performing *ex vivo* experiments using primary AEC-II from the human lung. These results suggest that IL-13 released in the course of allergic asthma might exert a pro-apoptotic influence on cells of

the airway epithelium *in vivo*. Interestingly, in primary AEC-II, both IL-13 and IL-4 evoked even stronger apoptotic responses than in epithelial cell lines. We attribute this observation to the fact that cell lines derived from cancer cells or viral transfected cells frequently show increased growth rates and a decreased propensity for apoptosis. In our experiments, human lung epithelial cells responded to IL-13 and IL-4 with similar changes in apoptotic behaviour. The unique role of IL-13 in asthma pathogenesis may, however, still be reflected also in epithelial cells by other aspects of distinguishable responses to the two cytokines. It has, for instance, recently been shown that up-regulation of mucin MUC4 in the respiratory epithelium upon IL-4 stimulation requires activation of JAK3, a kinase that is not activated through the IL-13 receptor [29]. Global expression analysis of lung tissue performed to address possible reasons for the dominant

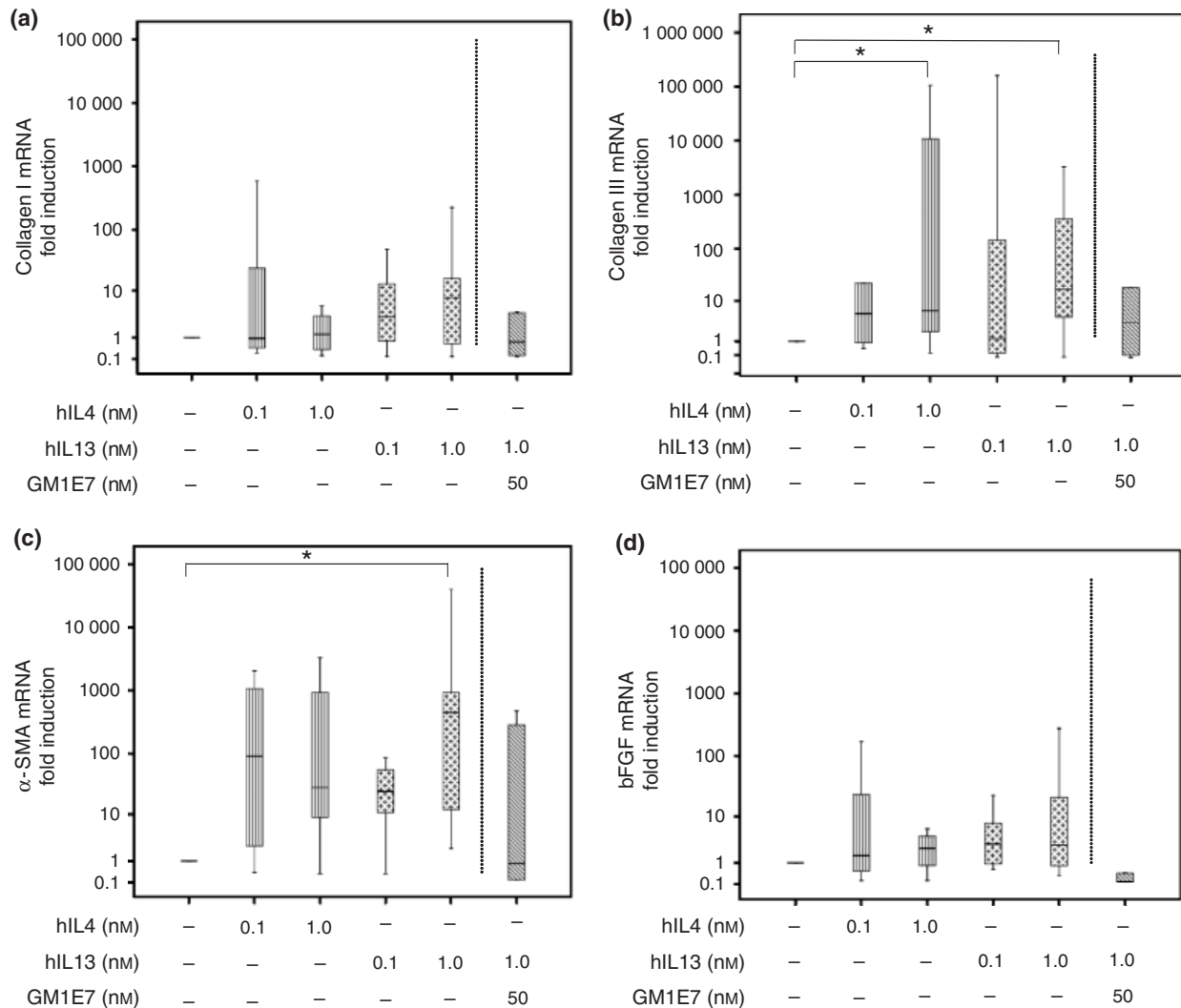


Fig. 3. Effect of IL-13 on the expression of pro-fibrotic genes in primary human lung fibroblasts. Quantitative analysis of specific mRNAs encoding for collagen I (a), collagen III (b), α -smooth muscle actin (α -SMA) (c) and basic fibroblast growth factor (bFGF) (d) in human primary lung fibroblasts in response to stimulation with IL-13 and IL-4. Samples of 5×10^5 cells obtained from lung biopsies were treated with IL-13 and IL-4 for 24 h at 37 °C, optionally in the presence of specific inhibitors (50 nM antagonistic antibody GM1E7 to the IL-13 receptor α 1 chain; 250 nM antagonistic IL-4 mutant RYS) as indicated were added 1 h before cytokine stimulation IL-13 and IL-4. After isolation of total RNA, cDNA was synthesized and real-time PCR assays were performed for the four specific mRNAs as described detailed in 'Materials and Methods'. Results are expressed as relative increase of mRNA levels upon cytokine stimulation normalized relation to glyceraldehyde 3-phosphate dehydrogenase. Experiments were performed three times for cell preparations from seven (α -SMA and bFGF) or 10 (collagen I and III) donors in total. Data were reported as described in Fig. 1. * Differences with *P*-values < 0.05 were considered statistically significant.

pro-asthmatic effects of IL-13 compared with IL-4 in mice revealed further differences: Three small proline-rich protein genes were induced strikingly more by IL-13 than by IL-4. Moreover, IL-4, but not IL-13, induces IL-12 and IFN- γ , both of which in turn suppress many effects of IL-4 [30].

Loss of airway epithelium has been listed as a characteristic feature of bronchial asthma. Recently, mechanisms associated with this loss of airway epithelium and the subsequent attempts of repair have been associated with a propagation of subepithelial airway fibrosis. To address a potential contribution of IL-13 to this process, we studied

its influence on the expression of pro-fibrotic genes in human lung fibroblasts. As the closest possible approximation to the *in vivo* situation, we employed primary fibroblasts isolated from macroscopically tumour-free tissue of bronchial biopsies of seven carcinoma patients. Upon IL-13 treatment, we observed a significantly increased expression of the genes encoding α -SMA and collagen III, and a trend towards a significant increase in mRNA level for collagen I, but no change in mRNA level for bFGF. It is interesting to note that IL-13 was more potent than IL-4 in the induction of α -SMA-specific mRNA in primary human lung fibroblasts. These findings

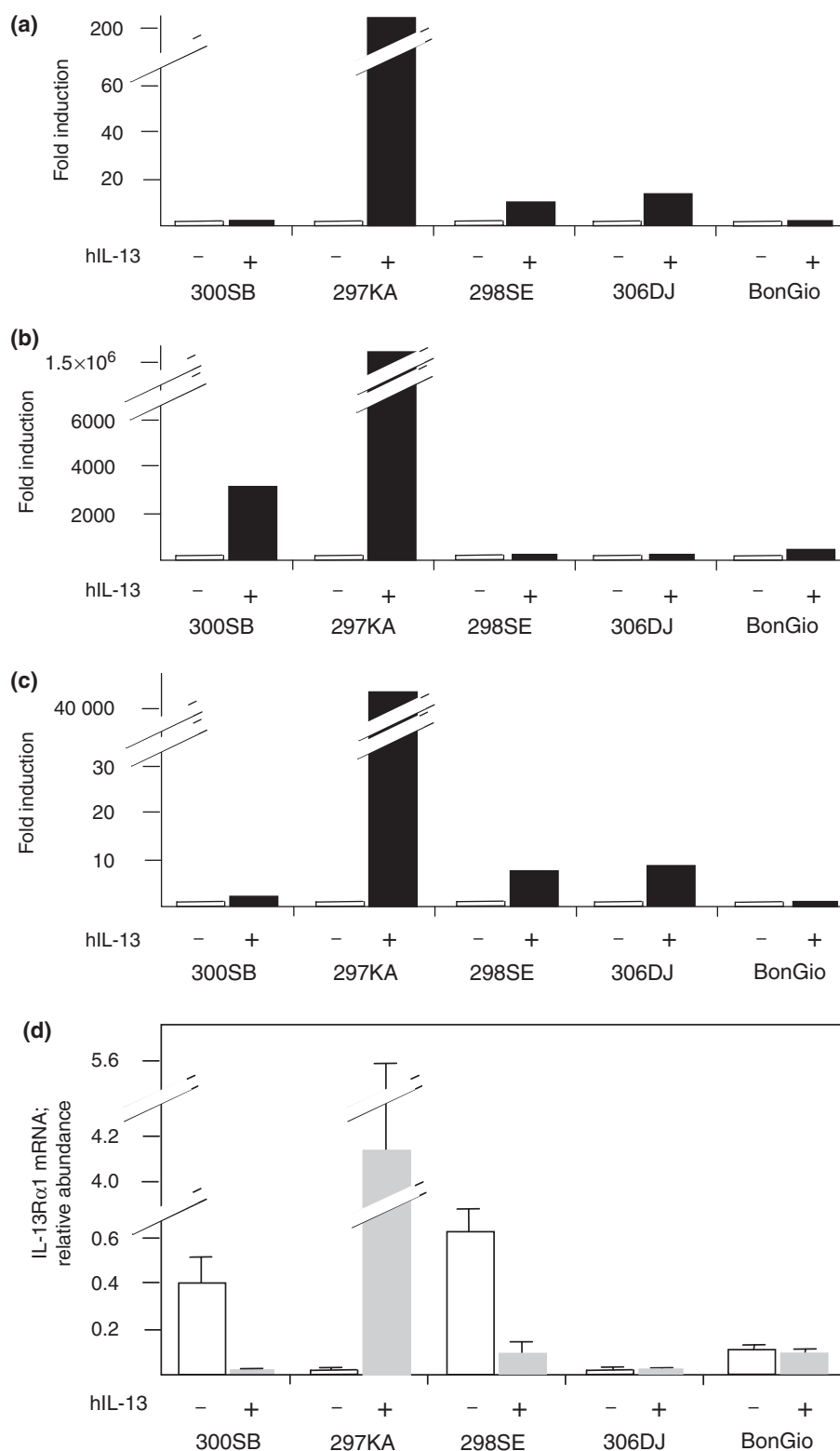


Fig. 4. Effect of IL-13 treatment on the expression of pro-fibrotic genes and the IL-13 receptor $\alpha 1$ chain in individual samples of primary lung fibroblasts. IL-13-dependent changes in the mRNA levels for collagen I (a), collagen III (b) and α -smooth muscle actin (α -SMA) (c). Samples of 5×10^5 cells obtained from individual lung biopsies were treated with IL-13 (1 nM) for 24 h at 37 °C. After mRNA isolation and cDNA synthesis real-time PCR assays were performed as described in 'Materials and methods'. mRNA levels determined in non-treated cells (white bars) were set as 1, black bars show relative changes upon IL-13 stimulation. Results for IL-13 receptor $\alpha 1$ (IL-13R $\alpha 1$) expression (d) are expressed as relative abundance of mRNA levels related to the respective levels of β -actin message.

are in line with the notion of IL-13 as a trigger for the formation of a specific 'non-haematopoietic' functional receptor in fibroblast consisting of the IL-4R α chain and both the IL-13R α 1 and γ c chain [31]. Alternatively, distinct signal transduction qualities mediated by the respective receptors for IL-13 and IL-4 may result in distinct patterns of transcription regulation, ultimately explaining the unique role of IL-13 in asthma pathogenesis. For instance, the IL-13 receptor complex elicits a different pattern of JAK/STAT activation than the IL-4 receptor complex in Ba/F3 cells [32]. In addition to its direct impact on the fibrosis-associated behaviour of lung fibroblasts, IL-13 apparently also mediates fibrosis indirectly, for instance via TGF- β released by macrophages in response to stimulation by IL-13 [33] or through PDGF-AA produced by IL-13-stimulated lung fibroblasts [34].

Our results are not consistent with those by Batra et al. [18], who reported that IL-13 does not induce collagen III and α -SMA in human lung fibroblasts, whereas IL-4 does. These authors employed a different approach and studied protein levels, whereas we analysed expression on the mRNA level. Although there is no published hint to such a phenomenon, it cannot be excluded that IL-4 and IL-13 have distinct, indirect effects on the translation of specific mRNAs and/or the stability of the respective gene products/proteins.

A particularly interesting result in this study was that the response of primary lung fibroblasts to IL-13 is probably functionally linked to IL-13-dependent regulation of receptor expression. These findings are reminiscent of previous results by our group, which showed that in eosinophils, another cell type critically involved in asthma pathogenesis, IL-13 down-regulates its own receptor [28]. The mechanisms underlying this effect are presently obscure. There are, however, other examples for the regulation of cytokine receptor expression by their own ligand on the level of transcription. The abundance of the IL-2 receptor on T cells, for instance, increases in response to IL-2 upon T cell activation [35].

Although the role of the IL-13 receptor in the development of asthma symptoms is apparently complex, both its up- and down-regulation may be involved in pathogenic events, rendering it an interesting target for pharmaceutical intervention. Consistent with the multifaceted function of IL-13 in fibrotic progression of chronic asthma, its specific blockade is an interesting option. Notably, an inhibitory antibody to murine IL-13 proved capable of reducing not only acute asthma symptoms, but also airway remodelling in a mouse asthma model [36].

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