

Tumour necrosis factor- α induced CD70 and interleukin-7R mRNA expression in BEAS-2B cells

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Tumour necrosis factor- α induced CD70 and interleukin-7R mRNA expression in BEAS-2B cells. K. Wolf, C. Schulz, G.A.J. Riegger, M. Pfeifer. ©ERS Journals Ltd 2002.

ABSTRACT: Over the past few years, evidence has emerged for the potential role of the human bronchial epithelial cell in the initiation and progress of inflammation of the airway.

Thus, the aim of this study was to investigate the expression pattern of cytokines and immunomodulatory factors in the human bronchial epithelial cell. To elucidate this highly complex expression and regulation pattern, the simian virus-40 transformed human bronchial-epithelial cell line BEAS-2B was stimulated with human recombinant tumour necrosis factor (hrTNF)- α ($10 \text{ ng} \cdot \text{mL}^{-1}$ (specific activity, $2.86 \times 10^7 \text{ U} \cdot \text{mg}^{-1}$)) and messenger ribonucleic acid (mRNA) expression pattern was analysed by complementary deoxyribonucleic acid (cDNA) array analysis.

Among 375 arrayed cDNA clones, 173 (46%) were detected in BEAS-2B cells. The levels of expression of 17 genes, including those of monocyte chemoattractant protein (MCP)-1, intercellular adhesion molecule (ICAM)-1, growth-related oncogene (GRO) α , β , γ , interleukin (IL)-7 receptor, CD70, IL-6, IL-8, granulocyte-macrophage colony-stimulating factor (GM-CSF) and regulated in activation, normal T-cell expressed and secreted (RANTES) were elevated after TNF- α stimulation. The differential character of 12 clones was further characterised and verified by real time polymerase chain reaction (PCR) analysis of total ribonucleic acid (RNA) isolated from BEAS-2B cells after 4 or 16 h incubation with increasing TNF- α concentrations (1 pg – $10 \text{ ng} \cdot \text{mL}^{-1}$). The authors semiquantified concentration-dependent mRNA upregulation of cytokines and immunology factors identified in the array and could determine threshold values of mRNA increases at $10 \text{ pg} \cdot \text{mL}^{-1}$ – $1 \text{ ng} \cdot \text{mL}^{-1}$ TNF- α by real-time PCR.

For CD70 (CD27 ligand) and interleukin-7 receptor, which to the best of the author's knowledge have not yet been described in the human bronchial epithelial cell, a rapid and continuous messenger ribonucleic acid increase induced by $100 \text{ pg} \cdot \text{mL}^{-1}$ tumour necrosis factor- α after only 60–90 min was shown. A potential role for these genes in the inflammatory process in the human bronchial epithelial cell is proposed.

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Airway inflammation is characterised by accumulation of activated leucocytes. The bronchial epithelial cell may be involved in this process by secreting cytokines or expressing surface molecules that trigger adhesion and/or activation of recruited leucocytes [1–3]. Until a few years ago airway epithelial cells were considered to be a physical barrier between the external and internal environment. Today it is known that epithelial cells are specific targets of exogenous or endogenous stimuli. The investigation of specific environmental effects on the epithelial cell and the response of the epithelial cell to different environmental agents is still in its infancy. Bronchial epithelial cells have been reported to express major histocompatibility complex (MHC) class II molecules and to be able to induce T-cell proliferation [4]. As with other epithelial cells, the BEAS-2B cell is able to internalise immunoglobulin (Ig)-G immune complexes [5]. In this context the expression of specific cell-surface molecules involved in antigen presenting was investigated.

Expression of costimulatory molecules CD80 and CD86 was determined by flow cytometry [5]. Thus, there is increasing evidence that airway epithelial cells are not a simple barrier but may function as mediators of a "crosstalk" between the environment and the organism. The recent detection of quite new sequences in these cells, by a subtraction library based strategy, demonstrates that this field is still open for investigation [6].

The simian virus-40 transfected BEAS-2B cell line (ATCC No. CRL-9609) is a widely accepted model for investigation of the human bronchial epithelial cell. To obtain a general idea of the cytokine expression in this type of cell a human cytokine expression array (R&D Systems, Wiesbaden, Germany) containing a collection of 375 cytokines, chemokines, immunomodulatory factors and their receptors was used. To determine possible candidate genes involved in the initiation or processing of airway inflammation, BEAS-2B cells were stimulated with tumour necrosis

factor (TNF)- α and the expression pattern of the arrayed genes analysed.

Based on reports that describe the epithelial cell as a source and target of cytokines and cytokine-related genes this comprehensive approach of cytokine-expression analysis was favoured. Although some previous studies investigating the BEAS-2B cell line after TNF- α stimulation exist [7–9] this array-based strategy led to the detection of regulated CD70 and interleukin (IL)-7 receptor(R) transcripts. To the best of the present author's knowledge, neither have been described previously in the human bronchial epithelial cell and this confirms the suitability of the experimental procedure in this study.

Methods

Cell culture

A human bronchial epithelial cell line BEAS-2B was plated onto collagen-coated flat-bottom tissue-culture plates (6-well). Roswell Park Memorial Institute (RPMI) 1640 (GIBCO BRL) culture medium containing 10% foetal calf serum was used. For stimulation 1×10^6 cells \cdot well $^{-1}$ were washed in serum-free medium and incubated for 4 or 16 h with human recombinant TNF- α (Sigma, Deisenhofen, Germany) in increasing concentrations from 1 pg \cdot mL $^{-1}$ –10 ng \cdot mL $^{-1}$. In the time-course experiment 1×10^6 cells \cdot well $^{-1}$ were stimulated with 1 ng \cdot mL $^{-1}$ TNF- α for 5, 15, 30, 1, 1.5, 2 and 3 h.

cDNA array analysis

After stimulation (10 ng \cdot mL $^{-1}$ TNF- α) cells were collected and total ribonucleic acid (RNA) was isolated using a commercially available total RNA extraction kit (Roche Diagnostics, Mannheim, Germany).

A human cytokine complementary deoxyribonucleic acid (cDNA) array was used (R&D Systems). Five micrograms of total RNA were deoxyribonuclease (DNase) digested according to the manufacturers instructions. Two micrograms of DNase digested total RNA of TNF- α stimulated and control cells were reverse transcribed into α - 33 P dCTP labelled cDNA. Arrays were hybridised in parallel and exposed overnight on Kodak BioMax LE intensifying screens (Eastman Kodak Company, Rochester, NY, USA) at -80°C. A second exposure lasted 2 weeks. Between the two autoradiographs the arrays were exposed on Storage Phosphor screens for analysis in the Cyclone Phosphorimager (Canberra-Packard, Dreieich, Germany). Arrays were normalised against the nine housekeeping genes presented on the array (fig. 1).

Real-time PCR with light-cycler system

Real time polymerase chain reaction (PCR) was performed with Light Cycler System (Roche). All PCR experiments were performed using the Light

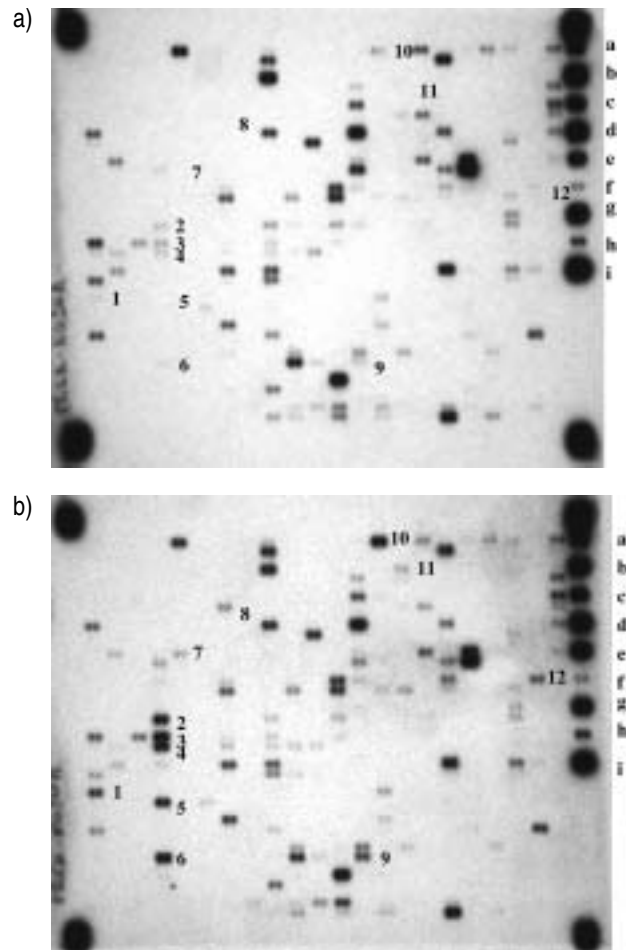


Fig. 1. – Autoradiograph of cytokine array analysis of a) control BEAS-2B and b) tumour necrosis factor (TNF)- α stimulated BEAS-2B cells. Each double spot represents a gene product. Gene products tested in real-time PCR were marked with numbers. The numbered spots represent signals for: intercellular adhesion molecule-1 (1), growth-related oncogene (GRO)- α (2), - β (3), - γ (4), interleukin (IL)-8 (5), monocyte chemoattractant protein-1 (6), regulated on activation, normal T-cell expressed and secreted (RANTES) (7), granulocyte-macrophage colony-stimulating factor (8), IL-1 β (9), IL-6 (10), IL-7R (11) and CD70 (12). Normalisation was performed using a set of nine high abundant house-keeping genes: β_2 microglobulin (A), β -actin (B), Cyclophilin A (C), glyceraldehydes-3-phosphate dehydrogenase (GAPDH) (D), histocompatibility leucocyte antigen-A heavy chain (E), phosphoribosyltransferase (F), ribosomal protein L19 (G), transferrin receptor (H) and α -tubulin (I).

Cycler-FastStart DNA Master SYBR Green I kit (Roche). For verification of the correct length of amplification product, PCR reactions were analysed on an ethidium bromide stained 2% agarose gel. Conditions for real-time PCR were as follows: 15 s melting at 95°C, 10 s annealing at 57–60°C, 15 s amplification at 72°C. Forty-five cycles were performed followed by melting-curve analysis to verify the correctness of the amplicon. In initial experiments amplification efficiency was determined for all primer pairs. Efficiency of the PCR was by determined analysing a dilution series of control cDNA. Using the analysis mode of Light Cycler software package, the slope of log concentrations of the control cDNA

dilutions was calculated. Efficiency was calculated as follows:

$$\text{Eff} = 10^{-1/\text{slope}} \quad (1)$$

Only primer pairs that showed an amplification efficiency between 1.94–2.03 were used for quantification. The measured efficiencies were used for calculation of the single mRNA values. Cytoplasmic β -actin, a stable housekeeping gene in this cell system and under TNF- α incubation, was analysed in parallel in every PCR. The resulting actin values were used as standard for presentation of the mRNA data of the different transcripts. Analysis of data was performed according to the manufacturers instructions using Light Cycler software version 3.5.3. Oligonucleotide primers were designed according to the published sequences (table 1).

Statistics

An unpaired t-test and Bonferroni adjustment was used for comparisons between different concentrations or time points. A $p < 0.05$ was considered significant.

Results

Cytokine cDNA array

Human epithelial BEAS-2B cells were stimulated with TNF- α for 16 h, total RNA was isolated and analysed with a human cytokine array to obtain information about expression and possible regulation of cytokines or cytokine-related genes in the human bronchial epithelial cell. Figure 1 shows an autoradiograph of the parallel analysis of control and TNF- α stimulated BEAS-2B cells after overnight exposure. Signals of 12 genes, which were further characterised by real-time PCR, are numbered on the autoradiographs of the array. The two charged nylon membranes were analysed by phosphoimaging and relative changes were determined using OptiQuantTM software

Table 2. – Transcriptionally regulated genes in tumour necrosis factor (TNF)- α stimulated BEAS-2B cells revealed by cDNA array analysis after normalisation of the two arrays against housekeeping genes represented on the array membrane

TNF- α upregulation	x-fold
IL-6	9.8 [#]
IL-8	16.5 [#]
MCP-1	18.0 [#]
GRO- α	10.1 [#]
GRO- β	9.2 [#]
GRO- γ	6.1 [#]
RANTES	10.9 [#]
ICAM-1	11.1 [#]
GM-CSF	6.1 [#]
IL-7R	4.2 [#]
IL-1 β	5.4 [#]
CD70	5.7 [#]
Integrin 5 α	3.9
M-CSF	2.3
HCC1	2.3
IP10	2.8
Eotaxin	2.5

Values represent x-fold regulation *versus* control as measured by OptiQuantTM phosphoimaging software by Packard Instruments Company. IL: interleukin; MCP: monocyte chemoattractant protein; GRO: growth-related oncogene; ICAM: intercellular adhesion molecule; GM-CSF: granulocyte-monocyte colony-stimulating factor; M-CSF: monocyte colony-stimulating factor; HCC: human CC cytokine; IP: interferon-gamma inducible protein; RANTES: regulated on activation, normal T-cell expressed and secreted. [#]: regulation was further characterised and verified by real-time polymerase chain reaction.

(table 2). From the 375 genes present on the array membrane, a positive expression for 173 genes (46% of the displayed genes on the array) could be detected. Seventeen of them were upregulated >three-fold after TNF- α incubation with 10 ng·mL⁻¹ TNF- α (table 2). Upregulation was found for IL-8, IL-6, monocyte chemoattractant protein (MCP)-1, CD70, regulated on activation, normal T-cell expressed and

Table 1. – Accession numbers of the investigated genes and oligonucleotide sequences of primers used for real time polymerase chain reaction based quantification of messenger ribonucleic acid

Gene	Accession no.	Fwd primer	Rev primer
β -actin	X00351	cgccccaggcaccagggc	gctggggtgttgaaggt
IL-8	Y00787	tgccaaggagtgtctaaag	ctccacaacctctgcac
IL-6	M14584	tctccacaagcgcttcg	ctcagggtgagatgccg
GRO- α	J03561	gccagtgtctgcagacc	gatgtcacaacacattag
GRO- β	M36820	tgccagtgtctgcagac	tcttaacctggggcgatgc
GRO- γ	M36821	tggtcactgaactgcgt	atgcgggggttgagacaag
MCP-1	S69738	atcaatgccccagtcacc	agtcttcggagtgtggg
RANTES	M21121	taccatgaaggtctccgc	gacaaagacgactgctgg
GM-CSF	M10663	acctgagtagagacactg	tcaaagggtgatagtctgg
IL-1 β	M15330	aagctgaggaagatgctg	atctacactctccagctg
IL-7R	M29696	tgacctgagtgtcatcta	catctccctgagctatt
CD70	L08096	tacgtatccatcgtgatg	gttggtgcagagtgtgtc
ICAM-1	J03132	ctgcagacagtaccatc	gtccagtttcccgacaa

IL: interleukin; GRO: growth-related oncogene; MCP: monocyte chemoattractant protein; GM-CSF: granulocyte-macrophage colony-stimulating factor; ICAM: intercellular adhesion molecule; RANTES: regulated on activation, normal T-cell expressed and secreted.

secreted (RANTES), granulocyte-macrophage colony-stimulating factor (GM-CSF), intercellular adhesion molecule (ICAM)-1, growth-related oncogene (GRO)- α , - β , - γ , IL-7 receptor, IL-1 β , macrophage colony-stimulating factor (M-CSF), integrin 5 α , human CC chemokine (HCC) 1, interferon- γ inducible protein (IP) 10 and eotaxin. Further analysis was carried out with genes that showed a >three-fold upregulation after normalisation against nine housekeeping genes and background subtraction of the arrays carried out with the OptiQuantTM acquisition and analysis software.

Real-time PCR analysis of IL-8, IL-6, MCP-1, GM-CSF, RANTES, IL-1 β , ICAM-1, GRO- α , - β , and - γ mRNAs after 4 and 16 h TNF- α incubation

To verify the induction on the transcriptional level seen in the cDNA array, real-time PCR experiments were performed with total RNA isolated from cells that were stimulated with increasing concentrations of TNF- α for 4 and 16 h. In the array analysis only two conditions (control *versus* 10 ng·mL⁻¹ TNF- α) were compared and exact quantification of transcripts according to the signals obtained by phosphoimaging is not reliable. Real-time PCR allows for mRNA measurements in total RNA isolated from cells kept under increasing concentrations of TNF- α . Values were expressed relative to those of β -actin determined in the same sample in parallel. Cytoplasmic β -actin mRNA, which is a stable housekeeping gene in this system, was measured. Measured mRNA values for β -actin did not vary significantly (not shown). Table 3 presents the mRNA levels of different cytokines as determined by real-time PCR. In general, strength of signals on the array reflects the mRNA abundance as determined by real-time PCR. Comparing control and maximum TNF- α values, 21-fold (4 h) and 83-fold (16 h) induction was measured for IL-8, eight-fold (4 h) and 72-fold (16 h) for RANTES, 45-fold (4 h) and 66-fold (16 h) for MCP-1, 20-fold (4 h) and 41-fold (16 h) for GM-CSF, 18-fold (4 h) and 36-fold (16 h) for IL-6, 19-fold (4 h) and 13-fold (16 h) for ICAM-1, 17-fold (4 h) and seven-fold (16 h) for GRO- α , three-fold (4 h) and seven-fold (16 h) for GRO- β and four-fold (4 h) and eight-fold for GRO- γ (table 3). As expected the induction of the mRNAs investigated was dependent on TNF- α concentrations. Significant changes *versus* control for IL-8, IL-6, GRO- α , - β , and - γ at very low TNF- α concentrations (10 pg·mL⁻¹) were detected. Significant changes of mRNA levels for RANTES were detected after incubation with 100 pg TNF- α . mRNA values for ICAM-1, GM-CSF, IL-1 β , IL-7 receptor and MCP-1 did not alter at concentrations <1 ng·mL⁻¹ TNF- α (table 3). Upregulation for integrin 5 α and HCC-1 (both positive in the array) could not be verified by reverse transcriptase (RT)-PCR (not shown).

Time and concentration dependent induction of CD70 and IL-7R mRNAs

Significant changes in mRNA levels for CD70 and RANTES were detected after incubation with 100 pg

Table 3. – mRNA values of tumour necrosis factor (TNF)- α inducible genes after 4 h and 16 h under increasing TNF- α concentrations

	Control			1 pg·mL ⁻¹			10 pg·mL ⁻¹			100 pg·mL ⁻¹			1 ng·mL ⁻¹			10 ng·mL ⁻¹		
	4 h	16 h		4 h	16 h		4 h	16 h		4 h	16 h		4 h	16 h		4 h	16 h	
IL-8	4.4±0.7	2.7±0.4		3.0±0.4	3.5±0.3		3.3±1.5	16.4±6.5*		30.1±7.8*	13.8±4.1*		93±15.2*	41.0±7.0*		94.5±22.4*	249±45*	
IL-6	2.3±0.5	1.3±0.3		1.8±0.4	2.4±0.6		3.6±0.9*	4.1±1.1*		42±10.2*	4.7±1.4*		63±12.5*	13.3±3.9*		40.4±10*	47.0±9.0*	
RANTES	0.21±0.8	0.1±0.04		0.21±0.02	0.1±0.01		0.3±0.08	0.25±0.07		1.3±0.2*	0.68±0.1*		2.3±1.2*	1.8±1.0*		1.6±0.3*	8.1±3.1*	
GM-CSF	0.11±0.06	0.17±0.11		0.09±0.03	0.13±0.08		0.18±0.7	0.08±0.04		2.9±1.0*	0.21±0.7*		4.5±1.2*	0.9±0.22*		2.2±1.1*	7.0±2.7*	
MCP-1	16.3±2.4	11.3±1.5		13.1±4.2	8.7±2.3		19.2±3.5	11.3±4.1		275±45*	17.0±5.0		654±78*	172±64*		632±140*	722±186*	
GRO- α	3.4±0.5	3.0±0.1		2.5±0.8	4.3±1.5		2.3±0.4	6.9±1.2*		8.5±2.9*	7.7±2.5*		25.9±6.4*	16.7±0.7*		61.6±12.0*	23.2±4.8*	
GRO- β	4.6±1.2	3.7±1.1		5.2±0.3	3.4±0.1		4.1±1.8	7.1±2.9*		20.1±6.4*	6.3±2.3*		13.3±2.9*	10.2±0.8*		11.8±1.9*	20.0±2.0*	
GRO- γ	1.3±0.5	0.7±0.3		1.5±0.4	1.6±0.9		1.4±0.6	0.9±0.5		1.9±0.3	1.1±0.1		4.7±2.2*	2.1±1.0*		5.9±1.2*	5.5±2.1*	
ICAM-1	5.4±0.9	5.2±0.9		4.4±1.4	16.6±11.7		6.9±2.3	16.3±10.3*		105±32*	14.0±3.2*		85±2.1*	32.3±10.1*		104±22*	152±52*	
IL-1 β	ND	0.8±0.2		ND	1.7±0.6		ND	1.9±0.6		ND	1.9±1.0		ND	2.8±0.2*		ND	5.1±0.4*	

Data are presented as % of cytoplasmic β -actin expression measured in the same sample in parallel, mean±SEM of three individual Light Cycler experiments. IL: interleukin; GM-CSF: granulocyte-macrophage colony-stimulating factor; MCP: monocyte chemoattractant protein; GRO: growth-related oncogene; ICAM: intercellular adhesion molecule; RANTES: regulated on activation, normal T-cell expressed and secreted. *: $p < 0.05$ *versus* control.

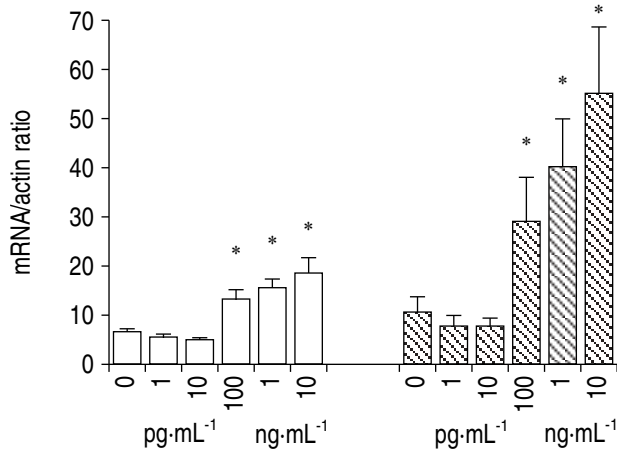


Fig. 2. –Real-time polymerase chain reaction (PCR) analysis of CD70 messenger ribonucleic acid (mRNA) upregulation. BEAS-2B cells were treated with tumour necrosis factor (TNF)- α for 4 h (□) and 16 h (▨). Data are presented as mean \pm SEM of three individual experiments. *: $p < 0.05$ versus control.

TNF- α . Comparing control and maximum TNF- α values (10 ng·mL⁻¹), three-fold (4 h) and eight-fold (16 h) induction of mRNA levels were measured for CD70 (fig. 2). For IL-7 receptor a seven-fold (4 h) and an eight-fold (16 h) mRNA increase was found (fig. 3). mRNA values for IL-7 receptor in the 16 h experiments did not alter at concentrations < 1 ng·mL⁻¹ TNF- α .

Time-course experiments were performed with IL-8 as a positive control of TNF- α action and CD70 and IL-7R. 1×10^6 cells were stimulated with 1 ng·mL⁻¹ TNF- α for 5 min up to 12 h. Again β -actin was used as a reference gene that did not change after different time points. Values measured for β -actin in the real-time PCR experiments were consistent in all total RNA samples isolated from cell samples of BEAS-2B cells with different TNF- α concentrations and after different time periods. IL-8 first showed a significant

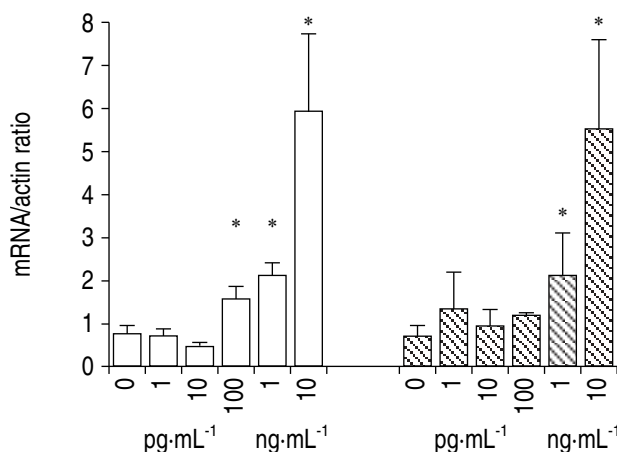


Fig. 3. –Real-time polymerase chain reaction analysis of interleukin-7 receptor messenger ribonucleic acid (mRNA) upregulation. BEAS-2B cells were treated with tumour necrosis factor (TNF)- α for 4 h (□) and 16 h (▨). Data are presented as mean \pm SEM of three individual experiments. *: $p < 0.05$ versus control.

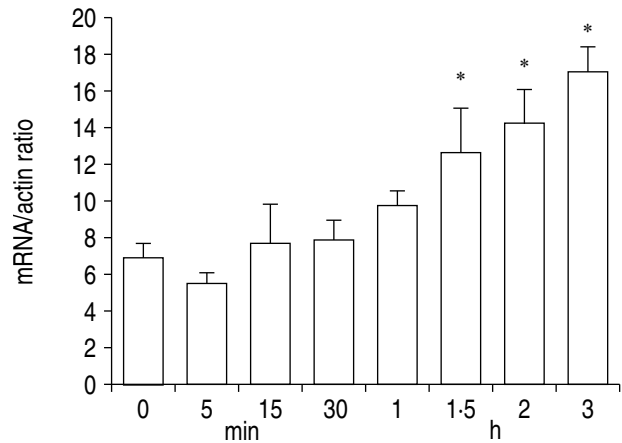


Fig. 4. –Real-time polymerase chain reaction (PCR) analysis of CD70 messenger ribonucleic acid (mRNA) upregulation with 1 ng·mL⁻¹ tumour necrosis factor (TNF)- α at different time points in BEAS-2B cells. Data are presented as mean \pm SEM of four experiments. Time course of TNF- α stimulation was repeated once and each ribonucleic acid sample of both time courses was analysed by two real-time PCR experiments. *: $p < 0.05$ versus control.

increase (four-fold *versus* control) after 60 min. Increases in mRNA levels after 60 min for IL-7 receptor and for CD70 were found, reaching the level of significance after 90 min for both gene products (figs 4 and 5).

Discussion

Using cDNA array analysis followed by real-time PCR based mRNA quantification several cytokines and other immunomodulatory factors that were upregulated after TNF- α stimulation in the human bronchial epithelial cell line BEAS-2B were found. Approximately 46% of the genes displayed on the array were detected in the author's experimental

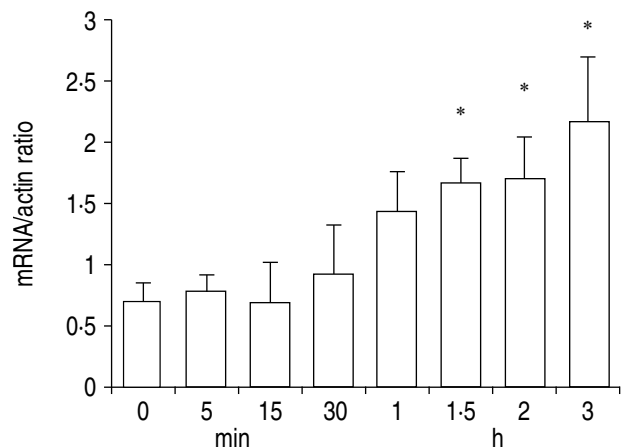


Fig. 5. –Real-time polymerase chain reaction (PCR) analysis of interleukin-7 receptor messenger ribonucleic acid (mRNA) upregulation with 1 ng·mL⁻¹ tumour necrosis factor (TNF)- α at different time points in BEAS-2B cells. Values are presented as mean \pm SEM of four experiments. Time course of TNF- α stimulation was repeated once and each ribonucleic acid sample of both time courses was analysed by two real-time PCR experiments. *: $p < 0.05$ versus control.

approach. As only a single cell type was investigated, this appears to be a realistic number. The results of several studies which have shown expression and mRNA upregulation of MCP-1, GRO- α , - β , - γ , IL-6, IL-8, RANTES and GM-CSF after stimulation in BEAS-2B cells can be confirmed. TNF- α mediated induction in BEAS-2B cells has been demonstrated for IL-6 [8–11], IL-8 [8–10, 12], GM-CSF [12–15], RANTES [9, 16] and ICAM-1 [17, 18]. MCP-1 was found to be upregulated by BECKER *et al.* [7]. The GRO- α , - β , - γ levels were determined by VAN DER VELDEN [19]. Transcriptional activation of these genes is, at least in part, mediated by transcription factor nuclear factor- κ B (NF- κ B). The NF- κ B pathway has been proposed to be the first critical step in the inflammatory cascade followed by exposure to antigenic challenges [10]. The intracellular signalling pathway for ICAM-1 in BEAS-2B cells seems to be protein kinase C and NF- κ B mediated [17]. A common inflammatory pathway seems conceivable since other stimuli such as diesel exhaust particles or soot cause release of IL-8 and IL-6 [8]. Constitutive expression of mRNAs for GRO- α , GRO- β , GRO- γ and MCP-1 belonging to the family of 8–20 kDa cytokines with chemotactic properties for granulocytes and monocytes has been demonstrated by BECKER *et al.* [7] in bronchial epithelium.

The question arises whether the array results presented here are due to direct TNF- α action or are a secondary phenomenon initiated by TNF- α but occurring late in the 16 h incubation phase. For that reason 4 h TNF- α incubation experiments were performed which, for the most part, paralleled the results of the 16 h experiments. For the genes investigated, an increase in the mRNA levels after 4 h could also be shown. Interestingly, a 4 h TNF- α incubation at a concentration of 100 pg·mL⁻¹ was necessary to induce significant mRNA changes for most of the investigated genes. On the contrary IL-6, IL-8, GRO- α , GRO- β and ICAM-1 were found to be upregulated with 10 pg·mL⁻¹ TNF- α in the 16 h experiment. These results suggest that the degree of the observed gene induction is a consequence of a combination of TNF- α concentration and the time of treatment.

The current authors were also interested in the time-course for CD70 and IL-7R induction. The results of mRNA increases after 60–90 min for CD70 and IL-7R provide evidence that these two gene products may be directly involved in the inflammatory response of the BEAS-2B cells. To the best of the authors knowledge there is no data on expression or regulation of these genes products in this human bronchial epithelial cell line. CD70 is a TNF-related transmembrane protein induced upon activation on T- and B-cells [20]. CD27/CD70 interaction plays a key role in T-dependent B-cell responses and is responsible for plasma-cell differentiation. CD70 expression has been ascribed to cells of the lymphoid lineage. Its function can be summarised as a costimulatory molecule for a regulatory cascade initiating differentiation of B-cells to antibody producing plasma cells.

As for CD70 a potential role for IL-7R in the bronchial epithelial cell needs to be clarified. In the last few years a major role for IL-7 receptor-dependent

signalling in T-cell development has been established by knockout studies [21]. IL-7 receptor is comprised of two chains and after ligand binding and receptor crosslinking rapid activation of several classes of kinases occurs [22]. The observations in this study suggest that airway epithelial cells are strongly involved in regulation of the inflammatory response. This has been confirmed by a large comprehensive flow-cytometry analysis demonstrating constitutive expression of a large number of surface molecules including CD23, CD33, CD40, CD48 and CD50 by ATSUTA *et al.* [18]. SALIK *et al.* [5] propose a role in local antigen presentation for the bronchial epithelial cell and describe airway epithelial cells being capable of supporting antigen-specific T-cell proliferation. SALIK *et al.* [5] found expression of the costimulatory molecules CD80/CD86 on BEAS-2B cells. CD40 and interferon- γ dependent T-cell activation has been recently shown by TANAKA *et al.* [23].

It is important to mention that apart from the array technique, new techniques exist which allow the identification of new sequences. Therefore, COOPER *et al.* [6] used the subtraction hybridisation technique to investigate TNF- α stimulated primary cells. COOPER *et al.* [6] showed upregulation of some genes described here but also another distinct set of genes. This may reflect either the sensitivity of the techniques used, the time point studied, or, perhaps most probably, differences between the BEAS-2B cell line and primary cells.

Taken together, the human bronchial epithelial cell might play an important role in the pathogenesis of airway disorders and may become a target for therapeutic interventions. The results of this investigation focus interest on new screening techniques such as, for example, nylon-based arrays or glass chips with even more genes displayed. Nevertheless the ribonucleic acid present may not always be translated into protein and also other regulatory mechanism beyond transcription might be involved in the biological response to tumour necrosis factor- α . Taking this into consideration, the role of CD70 and interleukin-7R should not, at present, be speculated upon. Thus, expression and regulation of CD70 and interleukin-7R messenger ribonucleic acid and/or protein in the human BEAS-2B cell and their potential involvement in the inflammatory process should be investigated further.

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