Original Article

Chemokines Induced in Human Respiratory Epithelial Cells by IL-1 Family of Cytokines

(epithelial cells / A549 / chemokines / IL-1β / IL-33 / IL-18)

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Abstract. IL-1-related cytokines share similarities in their receptor distribution and signalling pathways; however, overlapping actions of these cytokines have not been clearly demonstrated. The aim of our study was to compare the capacity of different IL-1-related cytokines to stimulate production and release of multiple CC and CXC chemokines by epithelial cells. The chemokine gene expression was studied using a cDNA array system in human alveolar type-II like cells A549 stimulated by IL-1β, IL-18, and IL-33. The chemokine levels in culture supernatants were measured using multiplex immunoluminometric assay or by ELISA. In repetitive experiments, in response to IL-1β epithelial cells expressed mRNA for CCL2, CCL5, CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL8, and CXCL11. In contrast, induction of epithelial cells by IL-33 and IL-18 resulted only in moderate up-regulation of a few CC or CXC chemokines compared to the potent effect of IL-1β stimulation. We conclude from our data that individual members of the IL-1 family, although related in molecular structure and signalling pathways, widely differ in their capacity to stimulate epithelial production of both CXC and CC chemokines.

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Abbreviations: ANOVA – analysis of variance, ATCC – American Tissue Culture Collection, BAL – bronchoalveolar lavage, EBS – Earle's Balanced Salt Solution, FCS – foetal calf serum, I/R – ischaemia/reperfusion, IFN-γ – interferon γ, IMDM – Iscove's modified Dulbecco's medium, IP-10 – interferon-inducible protein 10, LPS – lipopolysaccharide, MIG – monokine induced by interferon γ, NK – natural killer, PE – phycoerythrin, PGD – primary graft dysfunction, Th – T-helper, TNF – tumour necrosis factor, x-MAP – x-multianalyte profiling technology.

Introduction

Respiratory epithelial cells play an important role in defence mechanisms of the lung by releasing chemokines responsible for recruitment of immune cells. Their receptor structures are able to recognize different signals from the environment and in the case of injury respond immediately by up-regulation of pro-inflammatory genes. A large scale of chemokines may be released from lung epithelial cells in response to bacterial motifs (Gudmundsson and Hunninghake, 1999; Koyama et al., 1999b; Graham and Paton, 2006; Mendez-Samperio et al., 2006; Sachse et al., 2006; Kohwiwattanagun et al., 2007), viral infections (Carpenter et al., 2002; Jang et al., 2006; Kanzawa et al., 2006), fungi (Zhang et al., 2005), but also to non-infectious injuries such as cyclic stretch (Li et al., 2003; dos Santos et al., 2004), oxygen tension (Wendel et al., 2008) or inhaled stimuli from the environment like allergens (Asokananthan et al., 2002; Adam et al., 2006; Kauffman et al., 2006), exhaust particles (Lee et al., 2005), or mineral particles (Ovrevik et al., 2006). In addition to these immediate responses to external stimuli, respiratory epithelial cells actively communicate with immune cells in the frame of the cytokine network. Among the pro-inflammatory cytokines, tumour necrosis factor (TNF) α (Krunkosky et al., 2005) and IL-1β (Koyama et al., 1999a; O'Gorman et al., 2005; Thomas et al., 2007) are the most potent inducers of chemokines in the respiratory epithelium. These cytokines are released within minutes after reperfusion following organ transplantation, and thus play an important role in driving the immune response (Walsh et al., 2004).

The role of other members of the IL-1 family of cytokines such as IL-18 and IL-33 in the activation of epithelial cells has been much less characterized. Although they share structural similarities and several biological properties overlap for these cytokines (Barksby et al., 2007), differences do exist in signalling pathways (Lee et al., 2004; Schmitz et al., 2005). In an animal model, instillation of IL-18 was associated with a significant increase in peribronchial eosinophil accumulation together with a significant increase in eotaxin, but not other eosinophil chemotactic factors (Campbell et al., 2000). Furthermore, IL-18 with IL-2 promotes Th2 cytokine

production from T cells and natural killer (NK) cells and IL-18 along with IL-3 also stimulates basophils and mast cells towards Th2 cytokine responses in a mouse model of bronchial asthma (Ishikawa et al., 2006). On the other hand, a recent study demonstrated that the combination of IL-2 and IL-18 might prevent airway hyperreactivity and airway inflammation, likely through IL-12-mediated induction of interferon γ (IFN-γ) production in NK cells (Matsubara et al., 2007). IL-18 may also be involved in enhancing the lipopolysaccharide (LPS)-induced neutrophilic inflammation of the lung but does not affect the resolution of inflammation (Harris et al., 2007). Similarly to IL-18, IL-33 promotes production of Th2-associated cytokines (Ho et al., 2007; Iikura et al., 2007) and seems to also be a selective Th2 chemoattractant (Komai-Koma et al., 2007). The role of IL-33 in allergic inflammation is supported by an experimental study showing inhibition of Th2 cytokine production by soluble IL-33 receptor ST2 (Hayakawa et al., 2007). It has become quite clear that epithelial surfaces play an active role in immune and immunopathological reactions in the lung (Schleimer et al., 2007); however, the mechanisms by which respiratory epithelial cells regulate the influx of immune cells in response to the IL-1 family of cytokines has not yet been fully elucidated. The aim of our study was to compare IL-1β, IL-18, and IL-33 with respect to their capacity to induce multiple chemokines in the epithelial tissue culture model.

The importance of studying chemokines is obvious from the number of clinical studies. Hoffman et al. (2009) have demonstrated elevated levels of MIG (CXCL-9) and interferon-inducible protein 10 (IP-10) (CXCL-10) in plasma samples of patients with primary graft dysfunction (PGD) after lung transplantation. Cumulative elevation of monokine induced by interferon-γ (MIG) (CXCL-9) and IP-10 (CXCL-10) measured in bronchoalveolar lavage (BAL) has been associated with a significant risk of graft failure after transplant (Neujahr et al., 2012). Furthermore, IP-10 plays a key role in early injury after cardiac and kidney transplantation (Hoffman et al., 2009). Although the most potent inducer in chemokine expression is ischaemia/reperfusion (I/R) injury, chemokines also play an important role in autoimmune diseases such as psoriasis vulgaris (Ekman et al., 2013) or systemic lupus erythematosus (Hrycek et al., 2012).

Material and Methods

Cell culture technique

The A549 alveolar type II-like cell line was purchased from American Tissue Culture Collection (ATCC, Manassas, VA). Cells were cultured in Iscove's modified Dulbecco's medium (IMDM) (Sigma-Aldrich, St. Louis, MO) containing 5% foetal calf serum (FCS) and L-glutamine, penicillin, streptomycin (Sigma-Aldrich) until confluence in 6-well tissue culture plates (Costar, Corning, NY). The medium was removed and the cells were washed twice with Earle's Balanced Salt Solution (EBS) (Sigma-Aldrich) to exclude the effect of different serum factors and then stimulated in serum-free IMDM medium with IL-1β (1–100 ng/ml), IL-18 (1–100 ng/ml) purchased from R&D Systems (Minneapolis, MN) and IL-33 (1–100 ng/ml) obtained from Alexis Biochemicals (San Diego, CA). After 24 h the supernatants were collected and tested for concentrations of selected chemokines by Luminex technology (R&D Systems) and the cells for isolation of total RNA were harvested by trypsinization with Trypsin-EDTA Solution for Endothelial Cell Cultures (Sigma-Aldrich). After cell detachment, the effect of trypsin was inhibited by FCS. Cells were centrifuged and the cell pellet was resuspended in the RNA stabilization reagent RNAprotect Cell Reagent (Qiagen, Hilden, Germany). Before RNA isolation cells were centrifuged at 5,000 *g* for 5 min to remove the stabilization reagent. RNA was isolated using a column-based commercial kit RNeasy Plus Mini Kit (Qiagen) according to manufacturer's instructions. Spectrophotometric measurement was performed for checking the quality of nucleic acid isolation.

Gene expression of chemokines and chemokine receptors

The expression of genes that encode 26 different chemokines and 16 chemokine receptors as well as 44 other related genes was analysed using RT2 PCR Profiler kit – Human Chemokines & Receptors PCR Array (SABiosciences, Frederick, MD, Table 1) according to manufacturer's instructions. Twelve primer pairs for quality control of reverse transcription and PCR synthesis efficacy and contamination with genomic DNA are included in the gene array.

The starting amount of RNA for cDNA synthesis was 0.5 μg in each sample. Measurement of each sample was repeated three times. Evaluation of relative gene expression was performed with web-based software PCR array analysis (http://www.sabiosciences.com/pcrarraydataanalysis.php). The algorithm is based on relative quantification of a certain gene in the sample compared to the same gene in the control sample using the $2_T^{-\Delta\Delta C}$ equation. *t*-Test statistics was used for assessing the differences in gene expression. Genes with at least 4-fold change with P value less than 0.05 were considered statistically significant.

Determination of selected chemokines by a multiplex immunoluminometric assay

The A549 cells were stimulated with different concentrations of pro-inflammatory cytokines IL-1β, IL-18, and IL-33. After 24 h the supernatants were simultaneously tested for the presence of different CC (CCL2, CCL3, CCL4, CCL5) and CXC (CXCL5, CXCL8) chemokines using the x-multianalyte profiling technology (x-MAP) in a Luminex machine. The device is based on laser analysis of analyte-specific antibody-

Table 1. List of analysed genes

Abbreviation	Name
APLNR	Apelin receptor
BDNF	Brain-derived neurotrophic factor
CXCR5	Chemokine (C-X-C motif) receptor 5
C5	Complement component 5
C5AR1	Complement component 5a receptor 1
CCBP2	Chemokine binding protein 2
CCL1	Chemokine (C-C motif) ligand 1
CCL11	Chemokine (C-C motif) ligand 11
CCL13	Chemokine (C-C motif) ligand 13
CCL15	Chemokine (C-C motif) ligand 15
CCL16	Chemokine (C-C motif) ligand 16
CCL17	Chemokine (C-C motif) ligand 17
CCL18	Chemokine (C-C motif) ligand 18 (pulmonary and activation-regulated)
CCL19	Chemokine (C-C motif) ligand 19
CCL2	Chemokine (C-C motif) ligand 2
CCL3	Chemokine (C-C motif) ligand 3
CCL4	Chemokine (C-C motif) ligand 4
CCL5	Chemokine (C-C motif) ligand 5
CCL7	Chemokine (C-C motif) ligand 7
CCL8	Chemokine (C-C motif) ligand 8
CCR1	Chemokine (C-C motif) receptor 1
CCR10	Chemokine (C-C motif) receptor 10
CCR2	Chemokine (C-C motif) receptor 2
CCR3	Chemokine (C-C motif) receptor 3
CCR4	Chemokine (C-C motif) receptor 4
CCR5	Chemokine (C-C motif) receptor 5
CCR6	Chemokine (C-C motif) receptor 6
CCR7	Chemokine (C-C motif) receptor 7
CCR8	Chemokine (C-C motif) receptor 8
CCRL1	Chemokine (C-C motif) receptor-like 1
CCRL2	Chemokine (C-C motif) receptor-like 2
CKLF	Chemokine-like factor
CMTM1	CKLF-like MARVEL transmembrane domain containing 1
CMTM2	CKLF-like MARVEL transmembrane domain containing 2
CMTM3	CKLF-like MARVEL transmembrane domain containing 3
CMTM4	CKLF-like MARVEL transmembrane domain containing 4
CMKLR1	CHEMOKINE-LIKE RECEPTOR 1
CSF3	Colony-stimulating factor 3 (granulocyte)
CX3CL1	Chemokine (C-X3-C motif) ligand 1
CX3CR1	Chemokine (C-X3-C motif) receptor 1
CXCL1	Chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, α)
CXCL10	Chemokine (C-X-C motif) ligand 10
CXCL11	Chemokine (C-X-C motif) ligand 11
CXCL12	Chemokine (C-X-C motif) ligand 12
CXCL13	Chemokine (C-X-C motif) ligand 13
CXCL2	Chemokine (C-X-C motif) ligand 2
CXCL3	Chemokine (C-X-C motif) ligand 3
CXCL5	Chemokine (C-X-C motif) ligand 5
CXCL6	Chemokine (C-X-C motif) ligand 6 (granulocyte chemotactic protein 2)
CXCL9	Chemokine (C-X-C motif) ligand 9
CXCR3	Chemokine (C-X-C motif) receptor 3
CXCR4	Chemokine (C-X-C motif) receptor 4
CXCR6	Chemokine (C-X-C motif) receptor 6
CYFIP2	Cytoplasmic FMR1 interacting protein 2
TYMP	Thymidine phosphorylase
GDF5	Growth differentiation factor 5
GPR31	G protein-coupled receptor 31
HCAR1	Hydroxycarboxylic acid receptor 1
HIF1A	Hypoxia inducible factor 1, α subunit (basic helix-loop-helix transcription factor)
IL13	Interleukin 13
IL16	Interleukin 16
IL18	Interleukin 18 (interferon γ-inducing factor)
IL1A	Interleukin 1, α
IL4	Interleukin 4
IL8	Interleukin 8
CXCR1	Chemokine (C-X-C motif) receptor 1
LTB4R	Leukotriene B4 receptor
MMP2	Matrix metallopeptidase 2 (gelatinase A, 72 kDa gelatinase, 72 kDa type IV collagenase)
MMP7	Matrix metallopeptidase 7 (matrilysin, uterine)
MYD88	Myeloid differentiation primary response gene (88)
NFKB1	Nuclear factor of κ light polypeptide gene enhancer in B-cells 1
AIMP1	Aminoacyl tRNA synthetase complex-interacting multifunctional protein 1
SDF2	Stromal cell-derived factor 2
SLIT2	Slit homologue 2 (Drosophila)
TCP10	T-complex 10 homologue (mouse)
TLR2	Toll-like receptor 2
TLR4	Toll-like receptor 4
TNF	Tumour necrosis factor
TNFRSF1A	Tumour necrosis factor receptor superfamily, member 1A
TNFSF14	Tumour necrosis factor (ligand) superfamily, member 14
TREM1	Triggering receptor expressed on myeloid cells 1
VHL	Von Hippel-Lindau tumour suppressor

coated microparticles (beads) that differ in fluorescent intensity. For our measurement we used the Luminex^{100} System (Luminex B.V., Oosterhout, The Netherlands) supplied with two lasers, a 635 nm diode laser exciting the red and infrared fluorochromes associated with microparticles and the second laser, 523 nm neodymium/yttrium-aluminium-garnet laser exciting fluorochrome phycoerythrin (PE) that is conjugated with streptavidin.

Briefly, during the first 3 h, 50 µl of supernatants were incubated at room temperature with gentle agitation on a horizontal orbital shaker together with chemokine-specific antibody-coated microparticles, and after washing off the unbound substances the secondary antibodies conjugated with biotin were added and samples were then incubated for another 1 h. After incubation and washing off the unbound secondary antibodies, streptavidin-PE was added and bound to biotin on a secondary biotinylated antibody, and after 30 min incubation and washing the microparticles were finally resuspended in 100 µl of wash buffer and then evaluated by the Luminex analyser. Standards and samples were incubated in a special filter-bottomed 96-well plate from which the fluid was drained out using a microplate vacuum manifold. The concentrations of individual chemokines were calculated by interpolation from particular standard curves.

Statistics

Paired-sample *t*-test, one-way analysis of variance (ANOVA), Kruskal-Wallis test, and Spearman rank correlations were used for the statistical analysis of differences in the chemokine levels in supernatants of stimulated cells; P values below 0.05 were considered statistically significant.

Results

The effect of IL-1β, IL-18, and IL-33 on chemokine gene induction in A549 cells

Stimulation with 50 ng/ml of IL-1β for 24 h resulted in up-regulation of nine chemokine genes with the highest increase in mRNA expression found for *CXCL1* (GROα), *CCL5* (RANTES), *CXCL5* (ENA-78), *CXCL8* (IL-8), *CXCL6* (GCP-2), *CXCL11* (I-TAC), *CXCL2* (GROβ), *CXCL3* (GROγ) and *CCL2* (MCP-1) (Fig. 1A). Stimulation of A549 cells with 50 ng/ml of IL-18 slightly up-regulated expression of only two chemokine genes, *CXCL5* (ENA-78) and *CXCL6* (GCP-2). IL-33 was a more potent stimulator as its stimulation resulted in a moderate increase of five chemokine mRNAs, *CXCL1* (GROα), *CCL5* (RANTES), *CXCL5* (ENA-78), *CXCL6* (GCP-2) and *CXCL2* (GROβ) as compared to unstimulated cells. Neither IL-18 nor IL-33 was able to induce *CXCL8*, *CXCL11*, *CXCL3* and *CCL2*.

All three cytokines down-regulated expression of *CCL17* (TARC), *CCR7*, *CCRL1*, *CCRL2*, *CX3CL1* (Fractalcine), *CXCL12* (SDF), *CXCR5* and *CXCR6* (Fig. 1B).

Chemokine concentrations in culture supernatants of stimulated epithelial cells

In order to confirm mRNA data obtained from qRT-PCR, a panel of selected CC and CXC chemokines was evaluated by using the multiplex luminometric system Luminex. In agreement with mRNA data, stimulation of A549 cells with IL-1β (50 ng/ml) for 24 h induced high levels of CCL2 (MCP-1) (Fig. 2A) and CCL5 (RANTES) (Fig. 2D). Despite a high concentration of CCL3 (MIP-1α) (Fig. 2B) and CCL4 (MIP-1β) (Fig. 2C) proteins in the supernatant, mRNA data did not show any significant up-regulation of the corresponding genes. This might be due to the fact that mRNA expression was assessed at a certain time point, while measurement of protein concentration came from a cumulative effect of the chemokine in the supernatant during the incubation, and thus mRNA expression did not necessarily correspond with the protein concentration. In the case of CCL5, borderline up-regulation was also found in cells stimulated with 50 ng/ml of either IL-18 or IL-33. Similarly to CC chemokines, in CXC chemokines IL-1β was also found to be the most potent stimulus inducing

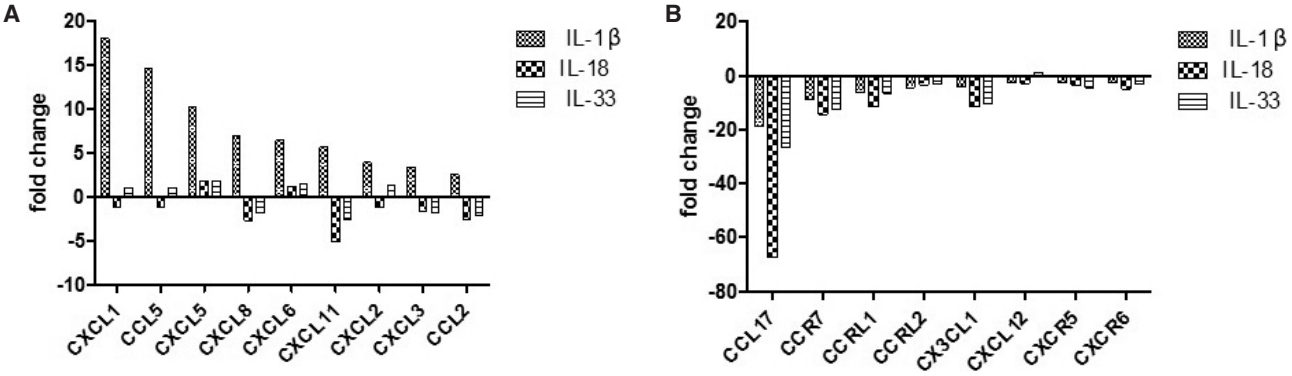

Fig. 1. The cells were cultured until confluence and then stimulated with cytokines at 50 ng/ml concentrations. The expression of genes encoding 26 different chemokines and 16 chemokine receptors as well as the 44 other related genes were analysed using the RT2 PCR Profiler kit. Stimulation of A549 cells with IL-1β resulted in increased (1A) or decreased (1B) expression of mRNA in some chemokine genes. Both IL-18 and IL-33 showed only a limited effect on chemokine gene expression.

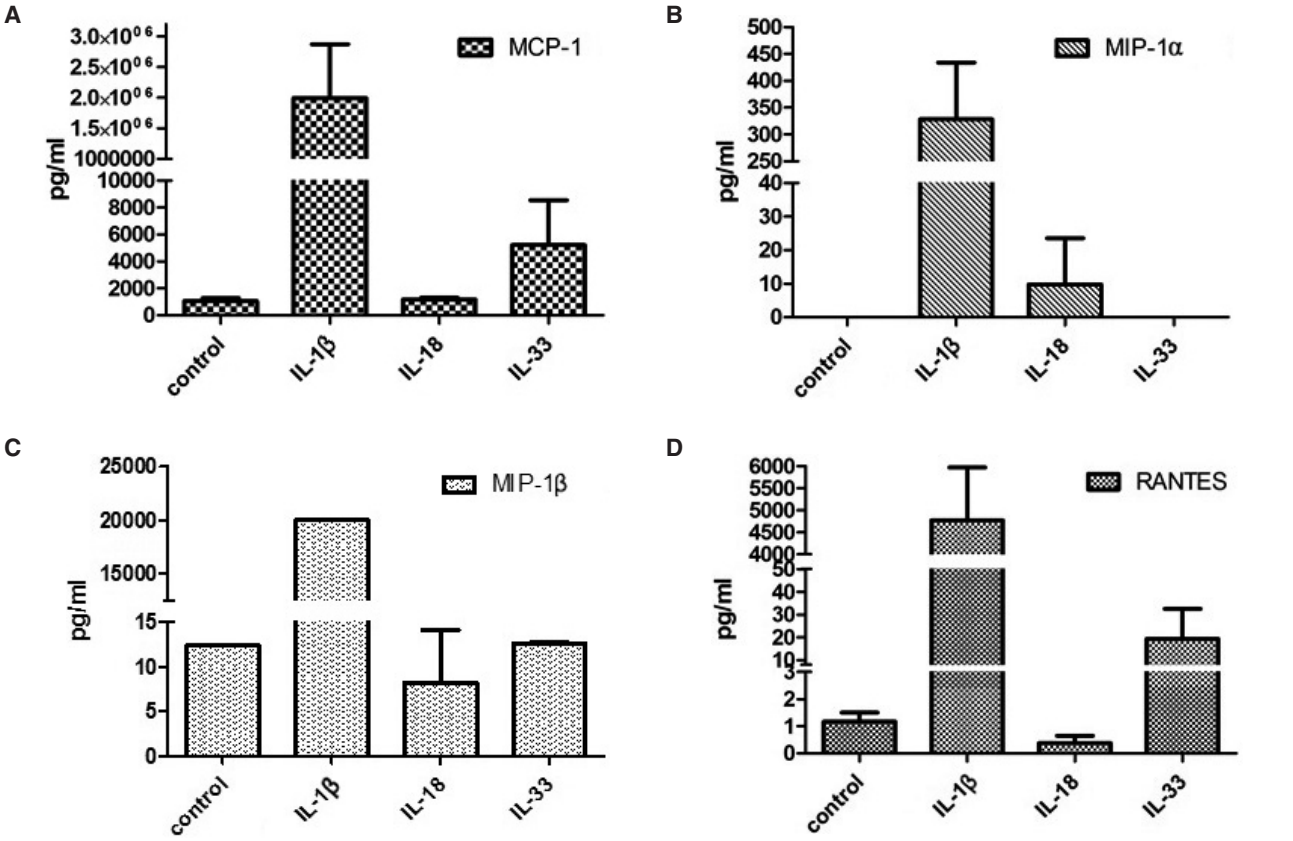

Fig. 2. The cells were cultured until confluence and then stimulated with cytokines IL-1β, IL-18, and IL-33 at 50 ng/ml concentrations. The supernatants were collected after 24 h and the levels of A) CCL2 (MCP1), B) CCL3 (MIP-1α), C) CCL4 (MIP-1β) and D) CCL5 (RANTES) were measured by Luminex. Only IL-1β but not IL-18 or IL-33 induced significant release of the chemokine.

both CXCL5 (ENA-78) and CXCL8 (IL-8) (Fig. 3). Neither IL-18 nor IL-33 showed significant induction of chemokine release to the culture media of A549 cells. In order to exclude the possibility that IL-18 and IL-33 require higher concentrations than IL-1β to be able to stimulate the cells, the dose dependency of CXCL8 release was tested for all three stimuli. The CXCL8 release from A549 cells reached the plateau at 10 ng of IL-1β/ml, and neither IL-18 nor IL-33 showed an adequate effect up to 100 ng/ml concentrations (Fig. 4).

Discussion

Our data demonstrated respiratory epithelial cells as an important source of both CC and CXC chemokines and support their role as key elements in regulating immune and inflammatory reactions in the lung (Schleimer et al., 2007). The differences in the capacity of individual members of the IL-1 family of cytokines to induce chemokines might be partially explained by the receptor expression or binding properties. In this respect, our

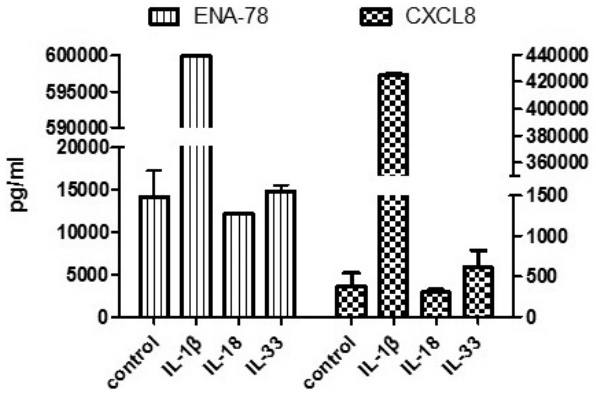

Fig. 3. Similarly to CC chemokines, also in CXC chemokines IL-1β was found to be the most potent stimulus inducing both CXCL5 (ENA-78) and CXCL8 (IL-8).

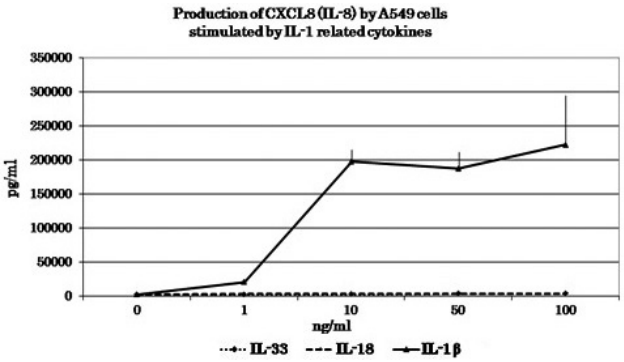

Fig. 4. In order to exclude the possibility that IL-18 and IL-33 require higher concentrations than IL-1β to be able to stimulate the cells, the dose dependency of CXCL8 release was tested in all three stimuli.

previous study showed detectable basal expression of IL-18α chain receptors in A549 cells and a moderate response to recombinant IL-18 (Krasna et al., 2005). Also, the expression of the IL-33 receptor gene has been demonstrated in A549 cells (Tajima et al., 2007). Signalling differences are another possibility for the fact that IL-1β is a much more potent inducer of chemokine genes than other members of the IL-1 family (Lee et al., 2004). The cell-specific effects should be taken into account as well. The role of IL-18 or IL-33 in inflammatory responses has not yet been completely elucidated and their main effect might rather be relatively specific targeting of T lymphocytes (Ishikawa et al., 2006; Komai-Koma et al., 2007) than induction of local inflammation with tissue damage, like in the case of TNF-α or IL-1β. Our data are in agreement with a recent study showing that IL-1β, but not IL-18, induces transcription-dependent lower airway epithelial cell-specific CCL5 secretion (Thomas et al., 2007) and extend these observations to multiple chemokines. With respect to T-lymphocyte chemoattraction by factors derived from respiratory cells, the previous study documented a key role of IFN-γ in the induction of CXCL9 (MIG), CXCL10 (IP-10), and CXCL11 (I-TAC), where IL-1β was involved only as a co-stimulatory factor (Sauty et al., 1999). In our experiments, IL-1β itself was shown to be a potent inducer of the *CXCL11* gene and did not require pre-stimulation with IFN-γ. The induction of *CXCL9* and *CXCL10* genes was not clearly demonstrated in our study, possibly due to a different time point needed for analysis of mRNA expression.

Chemokines released from respiratory epithelial cells in response to pro-inflammatory cytokines are undoubtedly the key regulators regulating the influx of either neutrophils, monocytes, NK cells, dendritic cells, mast cells, or T lymphocytes to the site of mucosal injury. Recent pharmacological strategies in progress involving chemokine antagonists should also be tested by using inhalatory routes to interrupt epithelial communications with other immune cells.

References

Adam, E., Hansen, K. K., Astudillo Fernandez, O., Coulon, L., Bex, F., Duhant, X., Jaumotte, E., Hollenberg, M. D., Jacquet, A. (2006) The house dust mite allergen Der p 1, unlike Der p 3, stimulates the expression of interleukin-8 in human airway epithelial cells via a proteinase-activated receptor-2-independent mechanism. *J. Biol. Chem.* **281**, 6910-6923.

Asokananthan, N., Graham, P. T., Stewart, D. J., Bakker, A. J., Eidne, K. A., Thompson, P. J., Stewart, G. A. (2002) House dust mite allergens induce proinflammatory cytokines from respiratory epithelial cells: the cysteine protease allergen, Der p 1, activates protease-activated receptor (PAR)-2 and inactivates PAR-1. *J. Immunol.* **169**, 4572-4578.

Barksby, H. E., Lea, S. R., Preshaw, P. M., Taylor, J. J. (2007) The expanding family of interleukin-1 cytokines and their role in destructive inflammatory disorders. *Clin. Exp. Immunol.* **149**, 217-225.

Campbell, E., Kunkel, S. L., Strieter, R. M., Lukacs, N. W. (2000) Differential roles of IL-18 in allergic airway disease: induction of eotaxin by resident cell populations exacerbates eosinophil accumulation. *J. Immunol.* **164**, 1096-1102.

Carpenter, L. R., Moy, J. N., Roebuck, K. A. (2002) Respiratory syncytial virus and TNF α induction of chemokine gene expression involves differential activation of Rel A and NF-κB1. *BMC Infect. Dis.* **2**, 5.

dos Santos, C. C., Han, B., Andrade, C. F., Bai, X., Uhlig, S., Hubmayr, R., Tsang, M., Lodyga, M., Keshavjee, S., Slutsky, A. S., Liu, M. (2004) DNA microarray analysis of gene expression in alveolar epithelial cells in response to TNFα, LPS, and cyclic stretch. *Physiol. Genomics* **19**, 331-342.

Ekman, A. K., Sigurdardottir, G., Carlstrom, M., Kartul, N., Jenmalm, M. C., Enerback, C. (2013) Systemically elevated Th1-, Th2- and Th17-associated chemokines in psoriasis vulgaris before and after ultraviolet B treatment. *Acta Derm. Venereol*. **93**, 527-531.

Graham, R. M., Paton, J. C. (2006) Differential role of CbpA and PspA in modulation of in vitro CXC chemokine responses of respiratory epithelial cells to infection with Streptococcus pneumoniae. *Infect. Immun.* **74**, 6739-6749.

Gudmundsson, G., Hunninghake, G. W. (1999) Respiratory epithelial cells release interleukin-8 in response to a thermophilic bacteria that causes hypersensitivity pneumonitis. *Exp. Lung. Res.* **25**, 217-228.

Harris, J. F., Aden, J., Lyons, C. R., Tesfaigzi, Y. (2007) Resolution of LPS-induced airway inflammation and goblet cell hyperplasia is independent of IL-18. *Respir. Res.* **8**, 24.

Hayakawa, H., Hayakawa, M., Kume, A., Tominaga, S. (2007) Soluble ST2 blocks interleukin-33 signaling in allergic airway inflammation. *J. Biol. Chem.* **282**, 26369-26380.

Ho, L. H., Ohno, T., Oboki, K., Kajiwara, N., Suto, H., Iikura, M., Okayama, Y., Akira, S., Saito, H., Galli, S. J., Nakae, S. (2007) IL-33 induces IL-13 production by mouse mast cells independently of IgE-FεRI signals. *J. Leukoc. Biol.* **82**, 1481-1490.

Hoffman, S. A., Wang, L., Shah, C. V., Ahya, V. N., Pochettino, A., Olthoff, K., Shaked, A., Wille, K., Lama, V. N., Milstone, A., Ware, L. B., Orens, J., Weinacker, A., Demissie, E., Bellamy, S., Kawut, S. M., Hancock, W. W., Christie, J. D. (2009) Plasma cytokines and chemokines in primary graft dysfunction post-lung transplantation. *Am. J. Transplant.* **9**, 389-396.

Hrycek, E., Franek, A., Blaszczak, E., Dworak, J., Hrycek, A. (2012) Serum levels of selected chemokines in systemic lupus erythematosus patients. *Rheumatol. Int*. **33**, 2423-2424.

Iikura, M., Suto, H., Kajiwara, N., Oboki, K., Ohno, T., Okayama, Y., Saito, H., Galli, S. J., Nakae, S. (2007) IL-33 can promote survival, adhesion and cytokine production in human mast cells. *Lab. Invest.* **87**, 971-978.

Ishikawa, Y., Yoshimoto, T., Nakanishi, K. (2006) Contribution of IL-18-induced innate T cell activation to airway inflammation with mucus hypersecretion and airway hyperresponsiveness. *Int. Immunol.* **18**, 847-855.

Jang, Y. J., Kwon, H. J., Lee, B. J. (2006) Effect of clarithromycin on rhinovirus-16 infection in A549 cells. *Eur. Respir. J.* **27**, 12-19.

Kanzawa, N., Nishigaki, K., Hayashi, T., Ishii, Y., Furukawa, S., Niiro, A., Yasui, F., Kohara, M., Morita, K., Matsushima, K., Le, M. Q., Masuda, T., Kannagi, M. (2006) Augmentation of chemokine production by severe acute respiratory syndrome coronavirus 3a/X1 and 7a/X4 proteins through NF-κB activation. *FEBS Lett.* **580**, 6807-6812.

Kauffman, H. F., Tamm, M., Timmerman, J. A., Borger, P. (2006) House dust mite major allergens Der p 1 and Der p 5 activate human airway-derived epithelial cells by protease-dependent and protease-independent mechanisms. *Clin. Mol. Allergy* **4**, 5.

Kohwiwattanagun, J., Kawamura, I., Fujimura, T., Mitsuyama, M. (2007) Mycobacterial mammalian cell entry protein 1A (Mce1A)-mediated adherence enhances the chemokine production by A549 alveolar epithelial cells. *Microbiol. Immunol.* **51**, 253-261.

Komai-Koma, M., Xu, D., Li, Y., McKenzie, A. N., McInnes, I. B., Liew, F. Y. (2007) IL-33 is a chemoattractant for human Th2 cells. *Eur. J. Immunol.* **37**, 2779-2786.

Koyama, S., Sato, E., Nomura, H., Kubo, K., Miura, M., Yamashita, T., Nagai, S., Izumi, T. (1999a) Monocyte chemotactic factors released from type II pneumocyte-like cells in response to TNF-α and IL-1α. *Eur. Respir. J.* **13**, 820-828.

Koyama, S., Sato, E., Nomura, H., Kubo, K., Miura, M., Yamashita, T., Nagai, S., Izumi, T. (1999b) The potential of various lipopolysaccharides to release monocyte chemotactic activity from lung epithelial cells and fibroblasts. *Eur. Respir. J.* **14**, 545-552.

Krasna, E., Kolesar, L., Slavcev, A., Valhova, S., Kronosova, B., Jaresova, M., Striz, I. (2005) IL-18 receptor expression on epithelial cells is upregulated by TNF α. *Inflammation* **29**, 33-37.

Krunkosky, T. M., Maruo, K., Potempa, J., Jarrett, C. L., Travis, J. (2005) Inhibition of tumor necrosis factor-α-induced RANTES secretion by alkaline protease in A549 cells. *Am. J. Respir. Cell Mol. Biol.* **33**, 483-489.

Lee, C. C., Cheng, Y. W., Kang, J. J. (2005) Motorcycle exhaust particles induce IL-8 production through NF-κB activation in human airway epithelial cells. *J. Toxicol. Environ. Health A* **68**, 1537-1555.

Lee, J. K., Kim, S. H., Lewis, E. C., Azam, T., Reznikov, L. L., Dinarello, C. A. (2004) Differences in signaling pathways by IL-1β and IL-18. *Proc. Natl. Acad. Sci. USA* **101**, 8815-8820.

Li, L. F., Ouyang, B., Choukroun, G., Matyal, R., Mascarenhas, M., Jafari, B., Bonventre, J. V., Force, T., Quinn, D. A. (2003) Stretch-induced IL-8 depends on c-Jun NH2-terminal and nuclear factor-κB-inducing kinases. *Am. J. Physiol. Lung Cell Mol. Physiol.* **285**, L464-475.

Matsubara, S., Takeda, K., Kodama, T., Joetham, A., Miyahara, N., Koya, T., Swasey, C. H., Okamoto, M., Dakhama, A., Gelfand, E. W. (2007) IL-2 and IL-18 attenuation of airway hyperresponsiveness requires STAT4, IFN-γ, and natural killer cells. *Am. J. Respir. Cell Mol. Biol.* **36**, 324-332.

Mendez-Samperio, P., Miranda, E., Vazquez, A. (2006) Expression and secretion of CXCL-8 and CXCL-10 from mycobacterium bovis BCG-infected human epithelial cells: role of IL-4. *Mediators Inflamm.* **2006**, 67451.

Neujahr, D. C., Perez, S. D., Mohammed, A., Ulukpo, O., Lawrence, E. C., Fernandez, F., Pickens, A., Force, S. D., Song, M., Larsen, C. P., Kirk, A. D. (2012) Cumulative exposure to γ interferon-dependent chemokines CXCL9 and CXCL10 correlates with worse outcome after lung transplant. *Am. J. Transplant.* **12**, 438-446.

O'Gorman, M. T., Jatoi, N. A., Lane, S. J., Mahon, B. P. (2005) IL-1β and TNF-α induce increased expression of CCL28 by airway epithelial cells via an NFκB-dependent pathway. *Cell. Immunol.* **238**, 87-96.

Ovrevik, J., Refsnes, M., Namork, E., Becher, R., Sandnes, D., Schwarze, P. E., Lag, M. (2006) Mechanisms of silica-induced IL-8 release from A549 cells: initial kinase-activation does not require EGFR activation or particle uptake. *Toxicology* **227**, 105-116.

Sachse, F., von Eiff, C., Stoll, W., Becker, K., Rudack, C. (2006) Induction of CXC chemokines in A549 airway epithelial cells by trypsin and staphylococcal proteases – a possible route for neutrophilic inflammation in chronic rhinosinusitis. *Clin. Exp. Immunol.* **144**, 534-542.

Sauty, A., Dziejman, M., Taha, R. A., Iarossi, A. S., Neote, K., Garcia-Zepeda, E. A., Hamid, Q., Luster, A. D. (1999) The T cell-specific CXC chemokines IP-10, Mig, and I-TAC are expressed by activated human bronchial epithelial cells. *J. Immunol.* **162**, 3549-3558.

Schleimer, R. P., Kato, A., Kern, R., Kuperman, D., Avila, P. C. (2007) Epithelium: at the interface of innate and adaptive immune responses. *J. Allergy Clin. Immunol.* **120**, 1279-1284.

Schmitz, J., Owyang, A., Oldham, E., Song, Y., Murphy, E., McClanahan, T. K., Zurawski, G., Moshrefi, M., Qin, J., Li, X., Gorman, D. M., Bazan, J. F., Kastelein, R. A. (2005) IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. *Immunity* **23**, 479-490.

Tajima, S., Bando, M., Ohno, S., Sugiyama, Y., Oshikawa, K., Tominaga, S., Itoh, K., Takada, T., Suzuki, E., Gejyo, F. (2007) ST2 gene induced by type 2 helper T cell (Th2) and proinflammatory cytokine stimuli may modulate lung injury and fibrosis. *Exp. Lung Res.* **33**, 81-97.

Thomas, L. H., Wickremasinghe, M. I., Friedland, J. S. (2007) IL-1 β stimulates divergent upper and lower airway epithelial cell CCL5 secretion. *Clin. Immunol.* **122**, 229-238.

Walsh, P. T., Strom, T. B., Turka, L. A. (2004) Routes to transplant tolerance versus rejection; the role of cytokines. *Immunity* **20**, 121-131.

Wendel, M., Giessmann, U., Behrend, P., Augstein, A., Koslowski, R., Haufe, D., Kasper, M., Koch, T. (2008) Inflammatory-activated microvascular endothelial cells regulate interleukin-8 and monocyte chemoattractant protein-1 expression of A549 cells in a paracrine fashion. *Exp. Lung Res.* **34**, 85-100.

Zhang, Z., Liu, R., Noordhoek, J. A., Kauffman, H. F. (2005) Interaction of airway epithelial cells (A549) with spores and mycelium of Aspergillus fumigatus. *J. Infect.* **51**, 375-382.