RESEARCH ARTICLE

The effect of 1,25-dihydroxyvitamin D_3 on TSLP, IL-33 and IL-25 expression in respiratory epithelium

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ABSTRACT. ***Background:*** **Airway epithelium is an active and important component of the immunological response in the pathophysiology of obstructive lung diseases. Recent studies suggest an important role for vitamin D_3 in asthma severity and treatment response.** ***Objective:*** **Our study evaluated the influence of an active form of vitamin D_3 on the expression of selected mediators of allergic inflammation in the respiratory epithelium.** ***Material and Methods:*** **Primary nasal and bronchial epithelial cells were exposed to$1,25D_3$ for 1 hour and were then stimulated or not with IL-4, TNF-α, LPS, and poly I:C. After 24 hours TSLP, IL-33, and IL-25 protein levels were measured in culture supernatants using ELISA and mRNA levels in cells by real time PCR.** ***Results:*** **$1,25D_3$ increased TSLP concentration in unstimulated nasal epithelial cells, but did not influence IL-33 and IL-25 expression. In IL-4-stimulated epithelial cell cultures $1,25D_3$ mostly inhibited TSLP and IL-33 expression. In LPS-treated cultures $1,25D_3$ decreased IL-33 expression. Simultaneously $1,25D_3$ augmented IL-25 production in the same model of stimulation.** ***Conclusion:*** **Our study revealed the dual nature of vitamin D_3 manifested in both pro- and anti-inflammatory properties observed in airway epithelial cells.**

Keywords: vitamin D_3, TSLP, IL-33, IL-25, airway epithelium

Airway epithelium is not only a passive anatomical structure it is also an immunologically active interface between inhaled air and the respiratory system. The role of respiratory epithelium in host defense involves the secretion of various antimicrobial peptides, reactive oxygen species (ROS), and cytokines, which are directly harmful to pathogens or initiate a specific immune response [1].

Airway epithelium also plays an important role in allergic airway diseases [2]. Interaction of the airway epithelial cells with various environmental stimuli may result in production and secretion of cytokines that are important drivers of Th2 type of immunological response. These include thymic stromal lymphopoietin (TSLP), interleukin 33 (IL-33), and interleukin 25 (IL-25). These cytokines activate dendritic cells, induce and upregulate the expression of other Th2-related cytokines and chemokines: they enhance eosinophil survival, and induce basophil and eosinophil degranulation [3]. The role of TSLP, IL-33, and IL-25 in the allergic response involves creation of a positive feedback loop between airway epithelium and inflammatory cells infiltrating the airways.

It has been shown that bronchial epithelial cells from asthmatics constitutively express more TSLP, IL-33 and IL-25 than those from healthy subjects [4-6]. Bronchial epithelium from asthma patients is more reactive and produces higher amounts of TSLP after cDNA stimulation (a surrogate for viral infection) than those from healthy controls [7]. The level of TSLP in asthmatic airways correlates with disease severity [8]. Serum IL-33 was proposed as a marker of asthma severity. Its level correlates with basal membrane thickness in asthma [9]. Increased expression of IL-33 and its receptor ST2 was observed in nasal epithelium from patients with allergic rhinitis [10]. It has also been demonstrated that allergic provocation induced IL-25 expression in asthmatic bronchial mucosa [10]. Both IL-33 and IL-25 are important mediators of rhinovirus-induced exacerbations of asthma [11, 12]. The potential therapeutic value of anti-TSLP/IL-33/IL-25 treatment is under investigation. Treatment of asthma patients with anti-TSLP antibody reduced allergen-induced bronchoconstriction and airway inflammation before and after allergen challenge [13]. It was also shown that in a mouse model, vaccination against IL-33 inhibits airway hyperresponsiveness, airway inflammation, and production of various inflammatory cytokines [14].

The secretion of Th2 cytokines by airway epithelial cells is controlled by different factors. The active form of vitamin D_3-$1,25(OH)_2D_3$ ($1,25D_3$) is a selective regulator of the immune system: data concerning this issue are complex, and often appear conflicting. It was shown that vitamin D_3 is able to influence the Th2 type of response by stimulation of IL-5, IL-4, IL-13 expression and CD4+ lymphocyte differentiation [15]. Recent findings indicating the protective role of vitamin D_3 in asthma and allergic diseases showed

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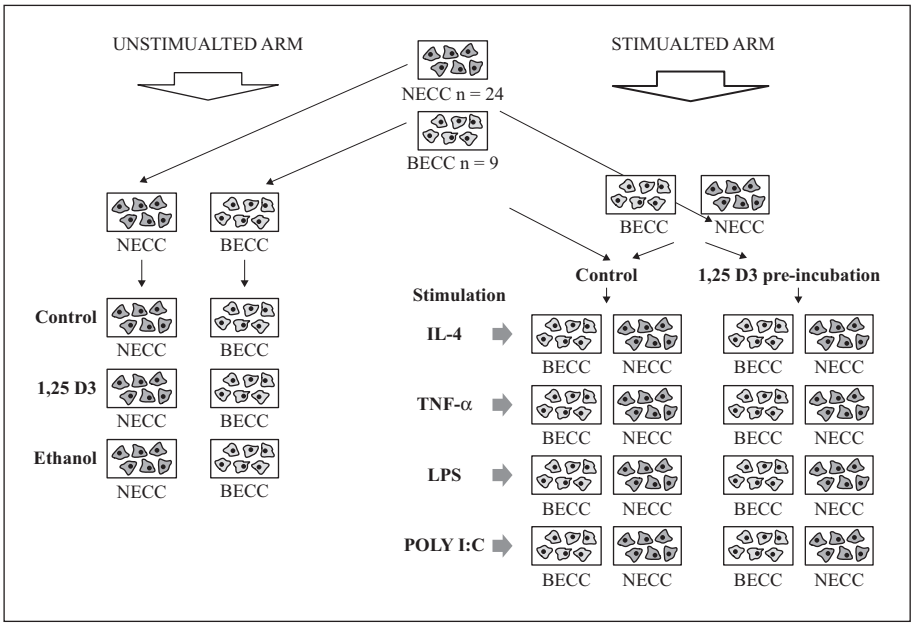

Figure 1

Study design. The unstimulated arm of the study included unstimulated nasal and bronchial epithelial cells, and cultures treated with 1,25 D_3 or ethanol only. The stimulated arm of the experiments contained nasal and bronchial epithelial cells stimulated with IL-4, TNF-α, LPS or poly I:C, with or without preincubation with 1,25 D_3.

that higher vitamin D_3 blood concentrations are associated with improved lung function, decreased airway hyperresponsiveness and increased glucocorticoid response [16].

The results of a few studies that evaluated the effect of vitamin D_3 on airway epithelium in asthma suggest its important role in airway remodeling. Vitamin D_3 induced CXCL8 and CXCL10 expression and affected epithelial cell growth and differentiation. These led to significant changes in cell morphology, including thickening of the cell layers [17]. The study by Fischer and Agrawal demonstrated that calcitriol (the active form of vitamin D_3) regulates TGF-β1-mediated epithelial-mesenchymal transition in bronchial epithelial cells [18].

Based on the above data, it may be hypothesized that vitamin D_3 plays a role in the production and secretion of TSLP, IL-33 and IL-25 by the airway epithelium. As we were not able to find data clarifying this issue, we undertook a study on the effect of 1,25D_3 on the expression of TSLP, IL-33 and IL-25 in the airway epithelium. The study was designed to evaluate the impact of vitamin D_3 on both baseline TSLP, IL-33 and IL-25 expression as well as the expression of these cytokines after the exposure of airway epithelial cells to different stimuli.

MATERIALS AND METHODS

Study design

This experimental study was performed on human nasal and bronchial epithelial cell cultures. Unstimulated and stimulated production of TSLP, IL-33 and IL-25 in epithelial cell cultures was assessed as specific mRNA expression as well as protein concentration in culture supernatants. Unstimulated TSLP, IL-33 and IL-25 production was assessed in experimental nasal and bronchial cell cultures (preincubated with 1,25D_3) and in cultures without any stimulation and in cultures preincubated with ethanol, which was used as a dissolvent for 1,25D_3. Four different stimuli of TSLP, IL-33 and IL-25 were used in the study. These included: IL-4, tumor necrosis factor α (TNF-α), lipopolysaccharide (LPS), and polyinosinic:polycytidylic acid (poly I:C). Stimulated TSLP, IL-33 and IL-25 production was assessed in experimental nasal and bronchial cell cultures (preincubated with 1,25D_3) after 24 hours of exposure to a respective compound and in control cultures, which were not preincubated with 1,25D_3, but were exposed to each of the respective stimuli (IL-4, TNF-α, LPS and poly I:C). The general study design is presented in *figure 1*.

Nasal and bronchial epithelium cultures

Nasal epithelial cell cultures used in this study included: 1) commercially available nasal epithelial cell cultures and 2) primary cultures of nasal epithelial cells obtained in our institution. The experiments with bronchial epithelium were carried out using a commercially available cell culture. The commercial primary cultures were obtained from healthy, adult, non-atopic, non-smoking, Caucasian volunteers; human nasal epithelial cells (PromoCell, Germany; C-12620; donor: female, aged 30 years) and human bronchial epithelial cells (PromoCell; C-12640; donor: male, aged 51 years). All epithelial cells were cultured in airway epithelial cell basal medium (PromoCell; C-21260) supplemented with airway epithelial cell growth medium kit (PromoCell; C-21160).

The nasal epithelial cells isolated and cultured in our department were obtained by nasal brushing (Cytobrush Plus GT, CooperSurgical, Germany) of five healthy (two men, three women) non-smoking, non-atopic volunteers with normal lung function; age 25-44 years. The study was approved by the Ethical Committee of the Medical University of Warsaw and written informed consent was obtained from all patients enrolled.

Epithelial cells were detached from the brush by gentle agitation. Cell suspensions were centrifuged (300 g, 10 min, room temperature). The cell pellets were

Table 1
Primer sequences.

	Forward primer	Reverse primer	Product size
18s rRNA	GGATGAGGTGGAACGTGTGAT	AGGTCTTCACGGAGCTTGTTG	148
IL-25	TGGTCCCTTTTTGGGAAACC	TGTGCAGAAGTGCAGGCTTT	153
IL-33	GCCTAGATGAGACACCGAATTAACA	CCAGGGTCAGAAGGGATGGT	85
TSLP	TCTTGAATTCCCGCTGCAA	CCACTGGTGTTTATAGGGTTCTGA	78

resuspended in a final total volume of 5 ml of airway epithelial growth medium (PromoCell, Germany) containing antibiotics, and seeded into sterile plastic T25 bottles (Nunc, USA). Cells were incubated in a plastic dish for two hours at 37°C. The undetached cells were removed and the cell medium changed. The adhered cells were labeled as 1st passage.

All epithelial cells used in this study were cultured with 100 U/mL of penicillin and 100 μg/mL streptomycin (PAA, Austria) at 37°C in 5% CO_2. At the time of the experimental phase, all cultured cells obtained in our institution were in their 2nd passage, and commercially available epithelial cells in their 3rd passage.

Cell stimulation

After reaching 80% confluence, cells were stimulated with IL-4 (100 ng/mL; R&D Systems, USA), TNF-α (50 ng/mL; R&D Systems), LPS from *Klebsiella pneumonia* (10 μg/mL; Sigma-Aldrich, Germany) or poly I:C (10 μg/mL , Sigma-Aldrich, Germany) for 24 h, with or without one hour of pre-incubation with 100 nM solution of 1,25D_3(Sigma-Aldrich, Germany) dissolved in 96% ethanol. The cytokines (IL-4 and TNF-α) were dissolved in phosphate-buffered saline containing 0.1% bovine serum albumin, LPS in culture medium, and poly I:C in water. Two and three independent experiments were carried out for every nasal and bronchial culture, respectively. Every experiment was performed in triplicate in the basal medium with antibiotics, without a supplement.

Protein concentration measurements

The measurements of TSLP, IL-33 and IL-25 concentrations in culture supernatants were performed with ELISA kits (R&D Systems, USA for TSLP and IL-33; EIAab, China for IL-25), according to the manufacturer's instructions. The mean minimal detectable dose of the tests was: 3.46 pg/mL, 0.519 pg/mL, and >10 pg/mL for TSLP, IL-33 and IL-25 respectively.

RNA isolation and cDNA synthesis

Total RNA was isolated from cells using Trizol (Invitrogen, USA). The purity and concentration of the isolated RNA was measured in a DU650 spectrophotometer (Beckman, USA) using the 260/280 nm absorbance ratio. One microgram of RNA was added for reverse transcription using a RevertAid Premium First Strand cDNA Synthesis Kit (Fermentas, Thermo Fisher Scientific, USA).

Real-time quantitative PCR

A real-time quantitative PCR evaluation was performed with an ABI-Prism 7500 Sequence Detector System (Applied Biosystems, USA). For real-time PCR, 0.8μL of cDNA was amplified in 16 μL PCR, containing a Power SYBR Green PCR mastermix (Applied Biosystems, USA) with 150 nM of specific primers. Sequences of applied primers are shown in *table 1*. The PCR protocol consisted of one cycle at 95°C for 10 min, followed by 40 cycles at 95°C for 15 s, and 60°C for 1 min. Each sample was measured in duplicate. 18s rRNA was applied for each sample as an internal control in order to normalize gene expression levels. The results were expressed as relative quantification units (-fold change).

Relative quantification values were calculated by the $2^{-\Delta\Delta CT}$ method. The cycle threshold (CT) for the target amplicon and the CT for endogenous control (18s rRNA) were determined for each sample. Differences were calculated between these two CTs (for the target amplicon and for the internal control) and called ΔCT, in order to account for the difference in the amount of total nucleic acid added to each reaction. The values of ΔCT for cDNA from the sputum cells of healthy patients (calibrator) were subtracted from the ΔCT of each sample and termed ΔΔ CT. The target normalized to endogenous control ratio, relative to the calibrator, was then calculated by the formula $2^{-\Delta\Delta CT}$. The calculation was made, the CT for the cDNA from stimulated and/or treated with 1,25D_3 cells was calibrated with the average CT of control (unstimulated cells).

Statistical analysis

The results are presented as median and interquartile range (IQR). Statistical analysis was performed using Statistica 12.0 software (StatSoft Inc., Tulsa, USA). Differences between continuous variables were tested using the nonparametric Mann-Whitney U test. Differences were considered statistically significant at $p<0.05$.

RESULTS

Effect of1,25(OH)2D_3 on TSLP, IL-33, and IL-25 mRNA and protein levels in nasal and bronchial epithelial cell cultures without stimulation

Bronchial epithelial cells produced significantly higher amounts of TSLP (39.8 pg/mL, IQR 26.4-56.4 pg/mL) than nasal epithelial cells (8.9 pg/mL, IQR 3.6-14.1 pg/mL) ($p = 0.003$). No other significant differences in protein concentrations were observed between unstimulated nasal and bronchial epithelial cells.

1,25D_3 increased TSLP protein concentration in nasal cell cultures compared to unstimulated controls (8.9 pg/mL IQR 3.6-14.1 pg/mL) *versus* (14.9 pg/mL IQR 11.6-21.5 pg/mL) (*figure 2*). Ethanol (the dissolvent for 1,25D_3) did not influence any of cytokine expression investigated.

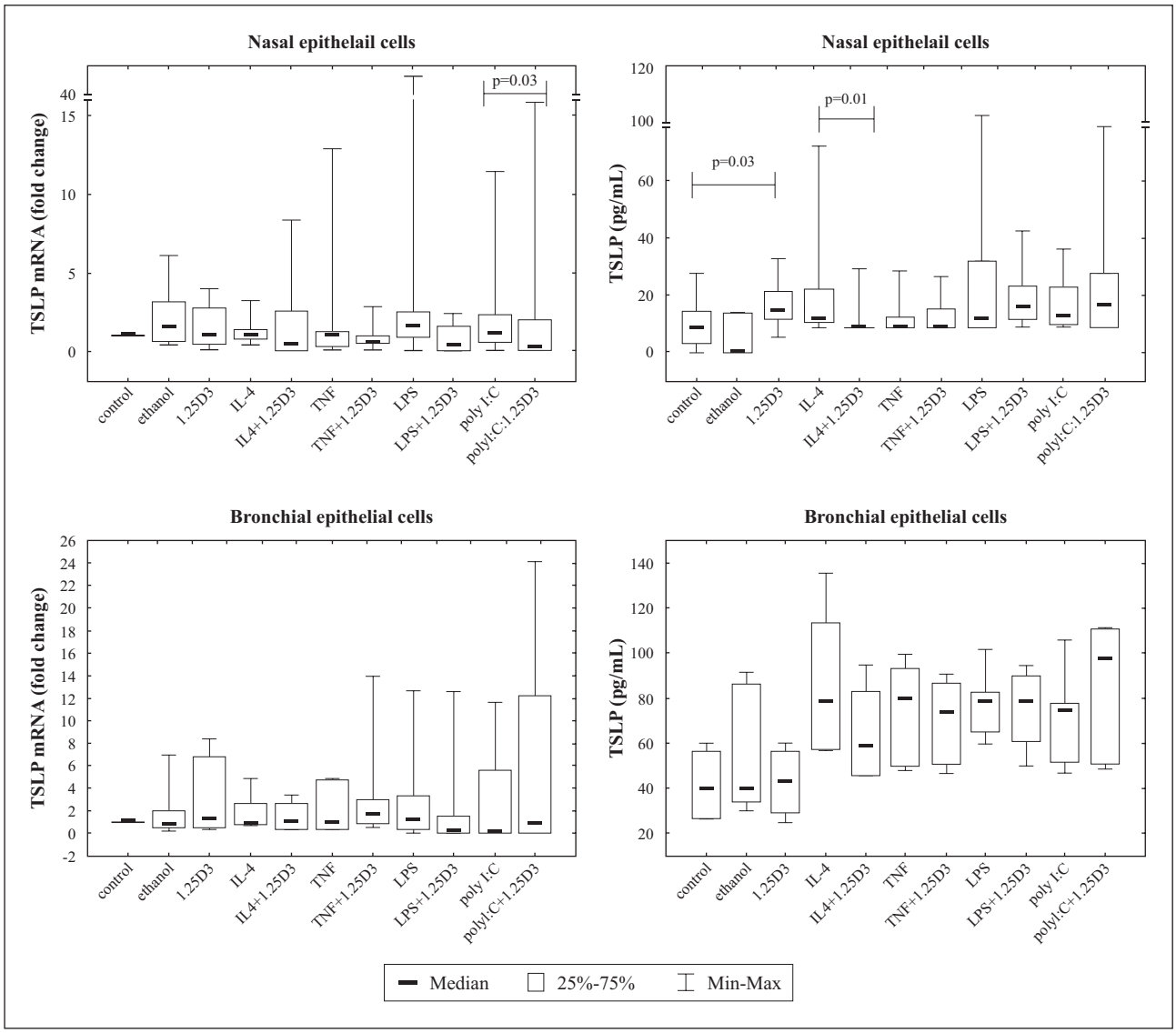

Figure 2

The effect of $1,25D_3$ on TSLP mRNA (-fold change) and protein (pg/mL) concentration in nasal and bronchial epithelial cells. Two independent experiments for six nasal epithelial cell cultures, each in duplicate (n = 24), and three independent experiments for one bronchial epithelial cell culture, each in triplicate (n = 9) were carried out.

Effect of 1,25(OH)2D$_3$ on TSLP, IL-33, and IL-25 mRNA and protein levels in nasal epithelial cell cultures after IL-4, TNF-α, LPS and poly I:C stimulation

The effects of $1,25D_3$ onTSLP, IL-33 and IL-25 mRNA expression and protein concentrations in nasal and bronchial epithelial cell cultures after IL-4, TNF-α, LPS and poly I:C stimulation are presented in *figures 2-4*. $1,25D_3$ significantly decreased the TSLP protein concentration in nasal cell cultures stimulated with IL-4 (11.8 pg/mL (IQR 10.6-22.3 pg/mL) *versus* 8.9 pg/mL (IQR 8.9-9 pg/mL) for IL-4 and IL-4+$1.25D_3$ nasal epithelial cells, respectively) (*figure 2*). The inhibition of TSLP expression after poly I:C stimulation as observed from mRNA levels in nasal cells (1.1 fold change (IQR 0.6-2.34 fold change) *versus* 0.24 (IQR 0.006-1.9 fold change) for nasal epithelium stimulated with poly I:C and pol I:C+$1.25D_3$, respectively) was not associated with a decline in TSLP protein concentration (*figure 2*). $1,25D_3$ decreased the level of IL-33 protein after IL-4 stimulation (0.89 pg/mL (IQR 0.31-1.26 pg/mL) *versus* 0 pg/mL (IQR 0-0.67 pg/mL) for IL-4 and IL-4+$1.25D_3$ nasal epithelium, respectively) (*figure 3*). A significant decrease in IL-25 mRNA levels after LPS stimulation (2.5 fold change (IQR 0.86-5.41 fold change) *versus* 0.46 fold change (IQR 0.006-2.41 fold change)) (*figure 4*) was noticed.

The effect of 1,25(OH)2D$_3$ on TSLP, IL-33, and IL-25 mRNA and protein levels in bronchial epithelial cell cultures after IL-4, TNF-α, LPS and poly I:C stimulation

The reduction in IL-33 mRNA expression after LPS (1 fold change (IQR 0.35-2.48 fold change) *versus* 0.27 fold change (IQR 0.09-1.53 fold change) for LPS and LPS+$1.25D_3$, respectively) stimulation was not associated with significant changes in IL-33 concentrations (*figure 3*). $1,25D_3$ increased IL-25 protein concentrations in LPS-treated cultures (7 pg/mL (IQR 5.9-7.3 pg/mL) *versus* 22.4 pg/mL (IQR (20.4-41.3 pg/mL) (*figure 4*).

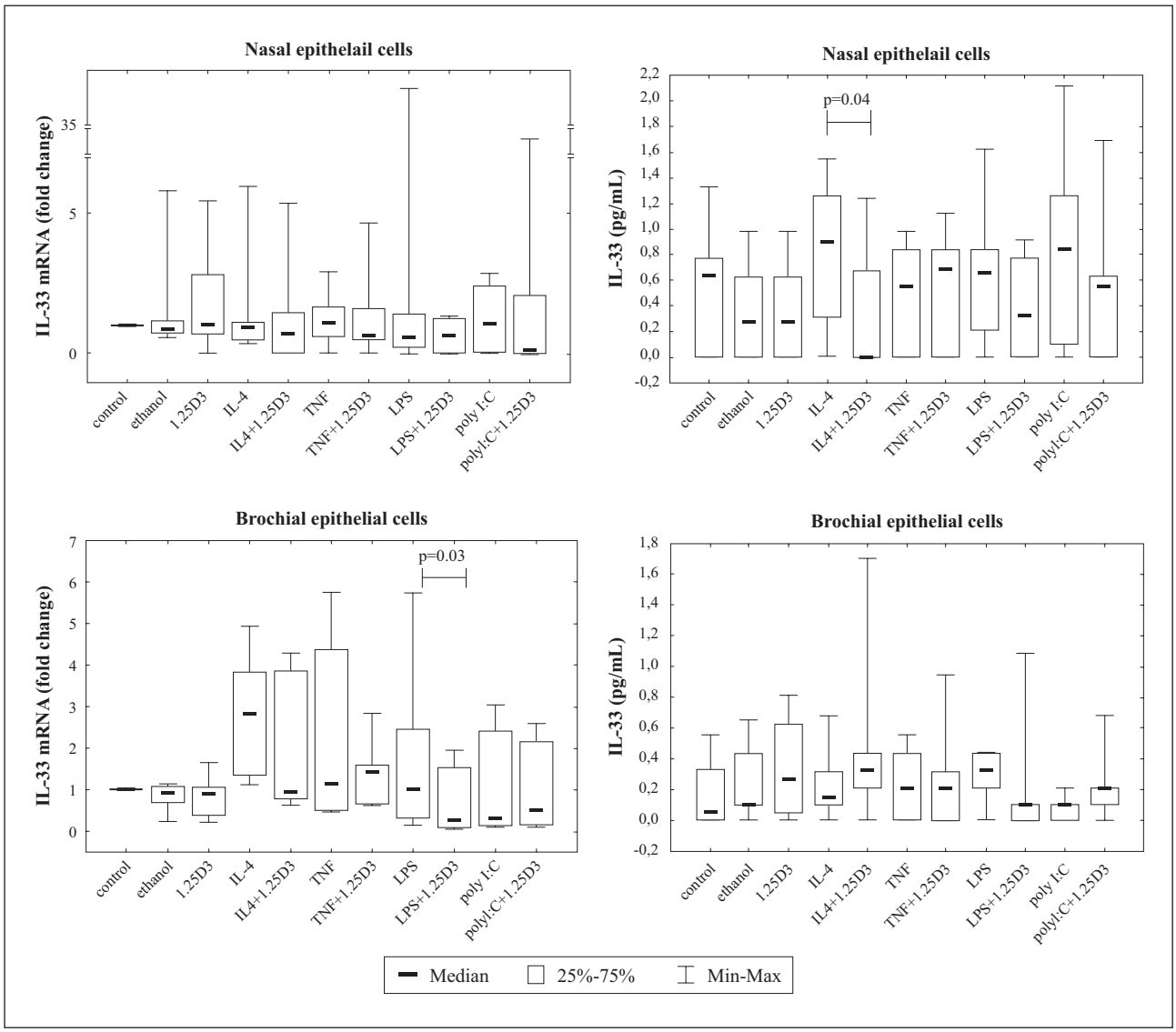

Figure 3

The effect of 1,25D_3 on IL-33 mRNA (-fold change) and protein (pg/mL) concentration in nasal and bronchial epithelial cells. Two independent experiments for six nasal epithelial cell cultures, each in duplicate (n = 24), and three independent experiments for one bronchial epithelial cell culture, each in triplicate (n = 9) were carried out.

DISCUSSION

Our study shows that 1,25D_3 regulated the expression of TSLP, IL-33 and IL-25 in airway epithelial cultures differently, depending on the experimental model. Apart from the increase of TSLP concentration in unstimulated nasal epithelial cell cultures, 1,25D_3 did not significantly alter the mRNA expression or concentrations of cytokines evaluated in either nasal or bronchial epithelial cell cultures. In IL-4-stimulated epithelial cell cultures, 1,25D_3 inhibited TSLP and IL-33 expression. In LPS-treated bronchial epithelium 1,25D_3 decreased IL-33 expression and augmented IL-25 protein production. The impact of 1,25D_3 on TSLP, IL-33 and IL-25 production in TNF-α-stimulated epithelial airway cell cultures was significantly less pronounced than in IL-4 LPS- and poly I:C-stimulated cultures. To our knowledge, this is the first "*in vitro*" study that has evaluated the effect of 1,25D_3 on IL-33 and IL-25 expression in both unstimulated and stimulated cultures of airway epithelial cells. Thus, we believe that the results of our study may add new data to the current knowledge on the relationship between vitaminD_3 exposure and pro-allergic activity of the airway epithelial cells.

In the context of the relationship between asthma and vitamin D_3, a puzzling paradox can be seen in the literature. This paradox lies in the facts that epidemiological studies have revealed an association between vitamin D_3 insufficiency and the risk of more severe asthma [19], and that vitamin D_3 supplementation in asthmatics is recommended by many clinicians, whereas some *in vitro* studies have reported that vitamin D_3 can directly promote Th2 cytokine secretion [16]. Also in our study, the upregulation of TSLP protein production in unstimulated nasal epithelial cells and the increase in IL-25 expression suggest pro-inflammatory properties of vitamin D_3. TSLP plays a role in the development (through direct stimulation of Th2 cytokines) and augmentation (through dendritic cell recruitment and activation) of the Th2 response in the allergic type of inflammation [20]. The ability of 1,25D_3 to increase TSLP expression in unstimulated epithelial cells was also reported in some other studies [21, 22].

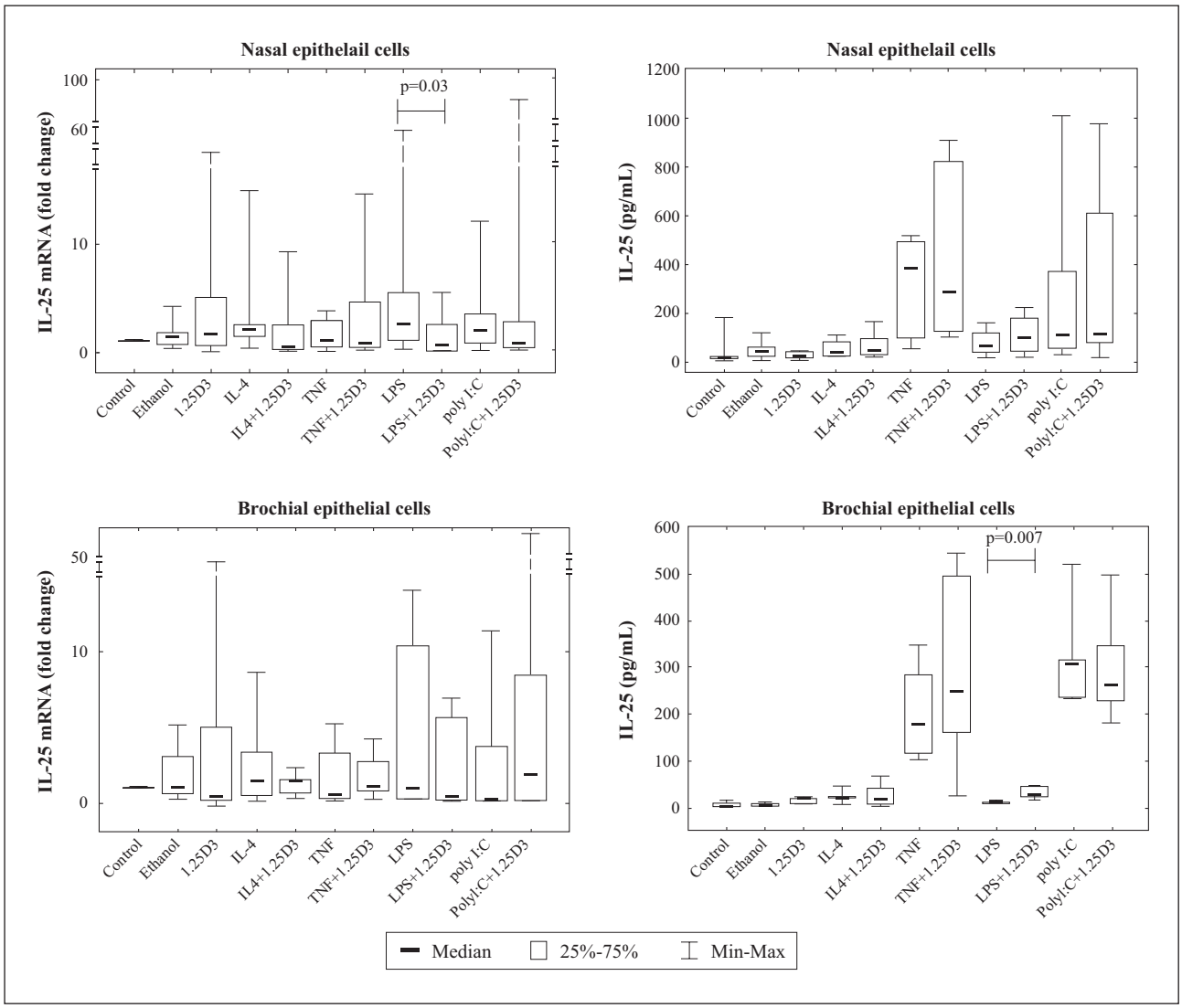

Figure 4

The effect of $1,25D_3$ on IL-25 mRNA (-fold change) and protein (pg/mL) concentration in nasal and bronchial epithelial cells. Two independent experiments for six nasal epithelial cell cultures, each in duplicate (n = 24), and three independent experiments for one bronchial epithelial cell culture, each in triplicate (n = 9) were carried out.

The differences between the effects of vitamin D_3 may be associated with its dose or concentration. Our experiments were performed with 100 nM $1,25D_3$ solution known to be the optimal physiological concentration. The commonly used *in vivo* concentrations of $1,25D_3$that promote optimal $1,25D_3$-dependent effects (the equivalent of optimal $25D_3$ concentration in blood) range from 10 to 100 nM [23].

The role of the moderate activation of TSLP by vitamin D3 found in our study (1.4-fold increase) may be equivocal. It might suggest pro-inflammatory properties of vitamin D_3. However, it might also be speculated that the slight increase in the TSLP concentration is required for the proper maintenance of the immunological balance in the respiratory tract.

On the other hand, our study revealed some anti-inflammatory actions of vitamin D_3 that included significant inhibition of TSLP and IL-33 expression in IL-4-stimulated cultures. These results are in accordance with other data demonstrating the beneficial effect of vitamin D_3 in the treatment of Th2 inflammatory response-related diseases [24]. TSLP, IL-33 and IL-25 are required for the activation of Type 2 innate lymphoid cells (ILC2s), which are reported to play an important role in bronchial inflammation as these cells are major producers of IL-13 and IL-5 during the Th2 type of immunological response [25-27]. From the three cytokines evaluated, IL-33 seems to be the most effective activator of ILC2s in the lungs. Barlow *et al.* showed that IL-33 is more potent than IL-25 in provoking IL-13 expression by ILC2s [28]. IL-33 enhanced IL-13 and IL-5 production from ILC2s [29]. Mature forms of IL-33 (cleaved by mast cells) induced massive expansion of ILC2s and eosinophils in lungs [30], which consequently exacerbated airway inflammation, and contributed to fibrosis and mucous metaplasia. In the study by Pfeffer *et al.* vitamin D_3 increased the synthesis of an inhibitor of IL-33 (a soluble decoy receptor (sST2)) in human nasal and bronchial epithelial cells, which in turn inhibited the actions of IL-33 [31]. Our results and those cited above suggest the potential benefit from vitamin D stimulation in the limitation of asthmatic mucosal inflammation.

It should also be noted that $1,25D_3$ inhibited IL-33 expression in IL-4- and LPS-stimulated airway epithelial cells, which may suggest a protective role of vitamin D_3 in asthma exacerbations during bacterial infections.

We have shown that $1,25D_3$ has the opposite, stimulating effect on IL-25 expression. Although TSLP, IL-33,

and IL-25 are usually classified together as one functionally similar group of cytokines, there are some differences between the biology and function of these proteins [3]. For example, IL-33 is stored in the nucleus, whereas IL-25 is stored and released from the extranuclear cellular compartments [32, 33].The lack of unequivocal results of other studies allows us to speculate that $1,25D_3$ is a multi-functional vitamin, influencing pro- and anti-inflammatory pathways. It cannot be excluded that upregulation of IL-25 production may be a dominant and overriding effect of vitamin D_3 compared to downregulation of TSLP and IL-33 expression, which is what seems to be demonstrated in *in vivo* studies. To date, numerous trials have evaluated the effect of vitamin D_3 supplementation on asthma symptoms and the course of the disease. However, the results are ambiguous. The results of the majority of recent studies do not support the strategy of therapeutic supplementation of vitamin D_3 in patients with symptomatic asthma because vitamin D_3 supplementation does not decrease disease exacerbation, nor improve lung function or asthma symptoms [34-36].

Our results revealed the different effects of $1,25D_3$ on nasal and bronchial epithelium, especially at the submucosal level. The epithelium showed some morphological differences at various levels of the respiratory tract (pseudofiltrated columnar cells in the nasal region; ciliated, undifferentiated columnar, secretory and basal cells in the large airways of the lower respiratory tract). We have previously shown that normal human nasal and bronchial epithelial cells are similar, but not identical in their immunological reactivity [37]. According to Comer *et al*., nasal epithelial cells from COPD patients cannot substitute for *in vitro* bronchial epithelial cells in airway inflammation studies [38]. Pringe *et al.* noticed that although nasal epithelial cells are easy to obtain, (surrogates for bronchial epithelium in children), they revealed a few differences as regards the release of some mediators, and are not identical [39]. The data concerning the effect of vitamin D_3 on epithelium-derived cytokines in other types of epithelium, such as epidermal keratinocytes in humans, are limited. No induction of TSLP expression was observed in human keratinocytes, after stimulation with calcipotriol (synthetic derivative of calcitriol), in contrast to mouse biopsies of skin [40]. Topical application of $1,25D_3$ induces TSLP expression in epidermal keratinocytes in a murine model of atopic dermatitis [41]. It was shown that intact murine epidermis is a better model for the evaluation of the role of vitamin D_3 than cultured keratinocytes [42]: we suggest that experiments using respiratory tract biopsies for serious investigation of the effects of vitamin D_3 are needed.

Some inconsistencies between the results of mRNA expression and corresponding protein concentrations have been found in our study. These inconsistencies may be explained by several different mechanisms. First, due to various post-transcriptional processes occurring within the cell, not all mRNAs are translated into proteins. Second, mRNA and proteins may differ substantially in their half-lives. Thus, the level of mRNA measured after stimulation may not necessarily reflect the protein concentration in the cell, because the process of mRNA degradation is highly dynamic. As a consequence, mRNA may already have been degraded when the protein is still present in the cell. Third, it cannot be excluded that the changes in mRNA expression were too weak to be detected at the protein level. It should be emphasized, that the lack of correlation between mRNA and protein levels has been reported in numerous other studies [43-45]. In the study by Gry *et al.* only one third of expressed mRNA (with the highest expression) correlated with the respective protein level [46].

We are aware of several limitations of our study. First, the experiments were performed with only one, physiological concentration of $1,25D_3$. Second, airway epithelial cells from healthy subjects were used in the study. It is possible that epithelial cells from patients with allergic rhinitis and/or asthma would have responded differently to $1,25D_3$ exposure. We therefore believe that research on airway epithelial cells from allergic patients is warranted and it may provide interesting new data. Third, only one bronchial epithelial cell line was evaluated, while there were six different primary nasal epithelial cell lines. However, in one earlier study we showed that nasal epithelial cells may be a good surrogate for bronchial epithelial cells and respond similarly [37]. Our results revealed different effects of $1,25D_3$ in these two types of epithelium; however, we believe that due to the limited number of bronchial epithelial cell lines used in this study, we are unable to draw such a conclusion.

CONCLUSIONS

Our study showed a multifarious effect of $1,25D_3$ on TSLP, IL-33 and IL-25 expression in respiratory epithelial cells. $1,25D_3$ increased the TSLP concentration in unstimulated nasal epithelial cells, but did not influence IL-33 and IL-25 expression. $1,25D_3$ mostly decreased TSLP and IL-33 production, especially in IL-4-stimulated cells. Simultaneously, $1,25\text{-}D_3$ augmented IL-25 production in the same model of stimulation. Our study revealed the dual nature of vitamin D_3, manifesting as both pro- and anti- inflammatory actions observed *in vitro* in airway epithelial cells. Further studies are needed for the evaluation of this two-way action of vitamin D_3.

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