Response of lung epithelial cells to inflammatory stimuli following exposure to the active form of vitamin D

R. Norton\textsuperscript{1}, D. W. Sexton\textsuperscript{2}, I. M. Clark\textsuperscript{2}, A. M. Wilson\textsuperscript{2}, D. A. Hughes\textsuperscript{3} and M. A. O’Connell\textsuperscript{1}

\textsuperscript{1}School of Pharmacy, University of East Anglia, Norwich NR4 7TJ, UK, \textsuperscript{2}Biomedical Research Centre, University of East Anglia, Norwich NR4 7TJ, UK and \textsuperscript{3}Research Consultant, Wymondham, Norfolk NR18 0SJ, UK

The active form of vitamin D (1,25(OH)\textsubscript{2}D\textsubscript{3}) is now of huge interest to the scientific community with numerous beneficial effects beyond Ca homeostasis and rickets. Inflammation plays a central role in the pathogenesis of numerous pulmonary diseases and recent evidence suggests vitamin D exerts immunomodulatory effects in the lung. Serum concentrations of inactive 25(OH)D\textsubscript{3} have been shown to directly correlate with FEV\textsubscript{1}\textsuperscript{(1)} and inversely with upper respiratory tract infection\textsuperscript{(2)}. More recent studies have shown potential beneficial effects of high dose vitamin D improving treatment for some tuberculosis patients\textsuperscript{(3)}. It is clear from this evidence that vitamin D may play an important role in the lung, but due to the low levels of vitamin D available from the diet, it is unlikely that levels of vitamin D required to be of benefit are achievable through diet alone. However, on a cellular level, respiratory epithelial cells have been shown to be capable of activating vitamin D\textsuperscript{(4)} but as yet it is unknown what concentrations of active vitamin D can be achieved locally given sufficient serum concentrations of 25(OH)D\textsubscript{3}. Vitamin D inadequacy is a global problem, especially among elderly patients\textsuperscript{(5)} and with an ever increasing ageing population and lifestyles that reduce exposure to sunlight, it is crucial to identify mechanisms by which vitamin D improves function and to identify optimal levels. In this study, the effects of 1,25(OH)\textsubscript{2}D\textsubscript{3} on pro-inflammatory mediator production were investigated in primary human small airway epithelial cells (SAEC). Cells were incubated with 100 nM 1,25(OH)\textsubscript{2}D\textsubscript{3} for up to 48 h and the effects on basal mRNA expression of cytokines (IL-1\textbeta, IL-6, IL-8, TNF\alpha, IL-10 and IL-12p70) were investigated using qRT-PCR. 1,25(OH)\textsubscript{2}D\textsubscript{3} treatment significantly reduced the expression of IL-6 mRNA from just 1 h of treatment (results at least in triplicate, one way ANOVA, $P<0.001$), but had no effect on basal mRNA of the other cytokines. IL-10 and IL-12p70 mRNA and protein were below the limit of detection. Both 10 ng/ml TNF\alpha and 10 \mu g/ml lipopolysaccharide (LPS)-induced expression of IL-1\textbeta, IL-6, IL-8 and TNF\alpha mRNA. Pre-treatment of SAEC with 1, 10 or 100 nM 1,25(OH)\textsubscript{2}D\textsubscript{3} for 1 h before stimulation with TNF\alpha or LPS led to a dose-dependent reduction of IL-6 mRNA induction that was significant at 100 nM with TNF\alpha treatment (results at least in triplicate, $t$ test, $P<0.05$). Induction of IL-6 protein expression by TNF\alpha was significantly reduced by 1 h pre-incubation with 100 nM 1,25(OH)\textsubscript{2}D\textsubscript{3} as assessed by flow cytometry (results in triplicate, one way ANOVA, $P<0.01$).

IL-6 is a key cytokine involved in the initiation and extension of the inflammatory response both locally and systemically in lung diseases. This data suggests that vitamin D may be of benefit in reducing IL-6 production by primary human small airway epithelial cells. More research is required into further effects and the mechanism of action of vitamin D on lung epithelium, to determine potential benefits for preventing or treating lung diseases and to define optimum levels.

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