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

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The active form of vitamin D (1,25(OH)₂D₃) 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)D₃ have been shown to directly correlate with FEV1⁽¹⁾ and inversely with upper respiratory tract infection⁽²⁾. More recent studies have shown potential beneficial effects of high dose vitamin D improving treatment for some tuberculosis patients⁽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⁽⁴⁾ but as yet it is unknown what concentrations of active vitamin D can be achieved locally given sufficient serum concentrations of 25(OH)D₃. Vitamin D inadequacy is a global problem, especially among elderly patients⁽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)₂D₃ on pro-inflammatory mediator production were investigated in primary human small airway epithelial cells (SAEC). Cells were incubated with 100 nM 1,25(OH)₂D₃ for up to 48 h and the effects on basal mRNA expression of cytokines (IL-1β, IL-6, IL-8, TNFα, IL-10 and IL-12p70) were investigated using qRT-PCR. 1,25(OH)₂D₃ 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 TNFα and 10 μg/ml lipopolysaccharide (LPS)-induced expression of IL-1β, IL-6, IL-8 and TNFα mRNA. Pre-treatment of SAEC with 1, 10 or 100 nM 1,25(OH)₂D₃ for 1 h before stimulation with TNFα or LPS led to a dose-dependent reduction of IL-6 mRNA induction that was significant at 100 nM with TNFα treatment (results at least in triplicate, *t* test, *P*<0.05). Induction of IL-6 protein expression by TNFα was significantly reduced by 1 h pre-incubation with 100 nM 1,25(OH)₂D₃ 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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