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Title: 25-hydroxide-vitamin D induces TSLP expression in human bronchial epithelial cells via the VDUP1 pathway

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Body: Background: Airway epithelial cells (AEC) express 1α -hydroxylase and are able to convert 25-hydroxide-vitamin D (25[OH]D) to an active form that plays a role in mucosal immunity. Thymic stromal lymphopoietin (TSLP), a cytokine mainly produced by AECs during allergic asthma reactions, plays a critical role in the activation of Th2 inflammatory responses. Therefore, we hypothesized that 25(OH)D would enhance the expression of TSLP in airway epithelia (16HBE cell line) and that vitamin D3 upregulated protein 1 (VDUP1) could be involved in this process. Methods: 16HBE cells were cultured with 25(OH)D, and TSLP and VDUP1 mRNA and protein expression were then determined by means of quantitative PCR, ELISA, and Western blot analysis, as appropriate. The role of VDUP1 on TSLP expression was assessed in untreated and 25(OH)D-treated 16HBE cells where VDUP1 levels were manipulated via overexpression or siRNA-mediated silencing. The effect of 10^{-6} M itraconazole, a chemical inhibitor of 1α -hydroxylase, on the expression of TSLP was also determined. Results: 25(OH)D significantly induced TSLP and VDUP1 mRNA expression in 16HBE cells. Silencing of VDUP1 dramatically inhibited 25(OH)D-mediated induction of TSLP, and overexpression of VDUP1 upregulated baseline TSLP expression and 25(OH)D-induced TSLP expression in 16HBE cells. Inhibition of 1α -hydroxylase reduced 25(OH)D-induced TSLP expression in 16HBE cells. Conclusion: These data demonstrated that vitamin D increased TSLP expression in 16HBE cells via upregulation of VDUP1.