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The Effects of Vitamin D on Allergen-Induced Expression of Interleukin-13 and Interleukin-17 in Cord Blood $CD4^+$T Cells

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ABSTRACT

Cytokine production in response to allergens may influence the development of atopy-predisposing immune responses, initializing the early programming of allergy and asthma. Vitamin D intake may be protective due to its immunoregulatory properties, that may contribute to influence the expression of the atopic phenotype initiated in early life. The objective of our study was to investigate the effects of 1,25-$(OH)_2D_3$ on allergen-stimulated expression of asthma related cytokines in cord blood T cells.

Cord blood samples were collected from the umblilical vein of 24 term deliveries during labor, $CD4^+$T cells derived from cord blood mononuclear cells (CBMCs), were cultured for 72 hours with ovalbumin (OVA), β-lactoglobulin (β-LG), respectively, in presence or absence of 1,25-$(OH)_2D_3$ to detect the levels of interleukin-13 (IL-13) and interleukin-17 (IL-17) in culture supernatants and the mRNA expressions in $CD4^+$T cells.

After allergens stimulation, $CD4^+$T cells showed an increase of IL-13 and IL-17 production, while cultured in the presence of 1,25-$(OH)_2D_3$ displayed a statistically significant down-regulation of allergen-induced expression of IL-13 and IL-17 in $CD4^+$T cells.

These results indicated that allergens may induce changes in $CD4^+$T cell function to increase inflammatory cytokine production. 1,25-$(OH)_2D_3$ modulated the capacity of $CD4^+$T cells in response to allergens, which might be protective for allergy.

Keywords: 1,25-$(OH)_2D_3$; Allergens; CD4-Positive T-Lymphocytes; Interleukin-13; Interleukin-17

INTRODUCTION

The ability of cord blood mononuclear cells (CBMCs) to proliferate and produce cytokines in response to a variety of environmental allergens has been suggested by several studies.[1-5]

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Moreover, the magnitude of these responses has been linked with risk of subsequent expression of allergy. $CD4^+$T cells, upon activation and expansion, develop into different T helper cell subsets with different cytokine profiles and distinct effector functions. Interleukine (IL)-13 is a T helper (Th)2-type cytokine that possesses IL-4-like activity, inhibitting the production of proinflammatory cytokines derived from monocytes and inducing B-cell proliferation and differentiation (including IgE production)

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independently.[6] Therefore IL-13 production by the cord blood $CD4^+$T cells was investigated to develop understanding of the Th1/Th2 cytokine balance in early life. The third subset of IL-17-producing effector Th cells, called Th17 cells, has been discovered and characterized. IL-17 now referred to as IL-17A, has been shown to induce neutrophil induction and maturation,[7] possibly as a central player in noneosinophilic inflammation, in some of extent IL-17 could be a therapeutic target.

In the last years, it has been recognized that in addition to its classical function in calcium and phosphorus metabolism, Vitamin D (VitD) modulates a variety of processes and regulatory systems including immunity and inflammation.[8] Hereby, we have investigated the effects of 1,25-$(OH)_2D_3$ during early life upon asthma related cytokine production, using the cord blood CD4+T cell model in vitro induced by ovalbumin (OVA) and β-lactoglobulin(β-LG).

PATIENTS AND METHODS

Subjects

Cord blood samples were collected from the umbilical vein of 24 term deliveries during labor from November 2011 to June 2012, in the Shanghai First People's Hospital, and divided into two groups: OVA group (n=12) and β-LG group (n=12). CBMCs were prepared using density gradient centrifugation (Lymphoprep, Cedarlane, Canada) and washed in culture medium.

Isolation, Culture and Stimulation of $CD4^+$T Cells

For the isolation of $CD4^+$T cells, magnetic cell separation technology (MACS) was used according to the manufacturer's instructions (Miltenyi Biotec., Germany). $CD4^+$T cells were cultured in 96-well plates (2×10^5/200µl/well), with RPMI 1640 supplemented with 10% Charcoal Stripped FCS, 100U/ml penicillin, 100µg/ml streptomycin. Cells were stimulated with optimal stimulatory concentrations of OVA (100µg/ml,Sigma), β-LG (100µg/ml,Sigma), or with addition of 1,25-$(0H)_2D_3$ (10^{-8}M,Sigma) based on above allergens respectively, for 72 hours at 37 C° in a humidified atmosphere with 5% CO_2. Unstimulated negative blank cultures were also set up.

ELISA for IL-13 and IL-17

Cytokines were quantified in the cell-culture supernatant via ELISA after 72 hours of culture. The amounts of IL-13 and IL-17 were measured using IL-13 or IL-17A detection kits from R&D Systems according to the manufacturer's instructions.

Gene Expression Analysis

$CD4^+$T cells were harvested from culture and total RNA was extracted using TRIzoL Reagent (Invitrogen,USA). The cDNA was reverse-transcribed applying "PrimeScript RT Master Mix Perfect Real Time" (Takara, Japan) according to the manufacturer's instructions. We performed real-time PCR to quantify IL-13 and IL-17A mRNA expressions in a continuous Fluorescence Detector (BIO-RAD, USA) with a SYBR premix ex taq (Takara, Dalian, China). The amplification reaction volume was 20µl, which comprised 10µl SYBR Premix Ex Taq, 4µl primes, 2µl cDNA and 4µl H_2O. The PCR conditions included denaturation at 95˚C for 30s, followed by 40 cycles consisting of 95˚C for 5s, 60˚C for 30s. All experiments were run with the same thermal-cycling parameters. cDNA samples were normalized relative to the expression of housekeeping gene, β-actin.

Primers and probes were designed with Sangon Biotech (Shanghai). The primers and probes used to quantitate genes were as follows:
IL-13, atcctctcctgttggcactg (forward),
Ctggttctgggtgatgttgac (reverse);
IL-17A, tgtcactgctactgctgctgag (forward),
ccttttgggattggtattggta (reverse);
β-actin, agcgagcatcccccaaagtt (forward),
gggcacgaaggctcatcatt (reverse).

Statistical Analysis

Data were analyzed using SPSS 13.0 version. The results were presented as mean±standard deviation (SD). Homogeneity of variance in each group was tested first. If the data showed homogeneity, analysis was performed using one way analysis of variance (ANOVA) followed by Student Newman Keuls (SNK) test. When heteroscedasticity was present, we did Kruskal Wallis rank test. *P*-value of less than 0.05 was considered statistically significant for all analyses.

RESULTS

Allergens Induced IL-13 and IL-17 Production in Cord Blood $CD4^+$T Cells

Significant amounts of IL-13 and IL-17 secretion

were observed after allergen (OVA/β-LG) stimualtion, the levels of IL-13 and IL-17 in culture supernatant and the mRNA expressions in the cord blood $CD4^+$T cells increased with statistically significant differences, when compared with blank group (Fig1,2).

Effect of 1,25-$(0H)_2D_3$ on the Cytokine-Producing by Allergen-Stimulated Cord Blood $CD4^+$T Cells

Compared with OVA/β-lactoglobulin-stimulated cord blood CD4+T cells (Fig.2) decreased significantly group, the levels of IL-13 and IL-17 in culture supernatant (Fig.1) and the mRNA expressions in the while cultured in presence of 1,25-(OH)2D3, but sill higher than blank group. In CD4+T cells, 1,25-(OH)2D3 generated a statistically significant downregulation of OVA/β-lactoglobulin-induced production of IL-13 and IL-17.

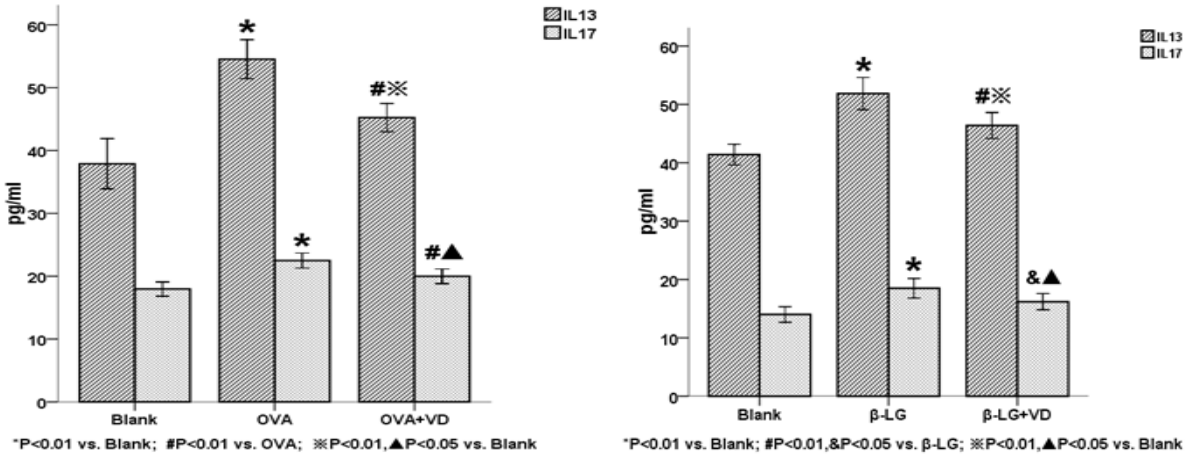

Figure 1. Cell culture supernatant levels of IL-13 and IL-17 in OVA/β-LG group

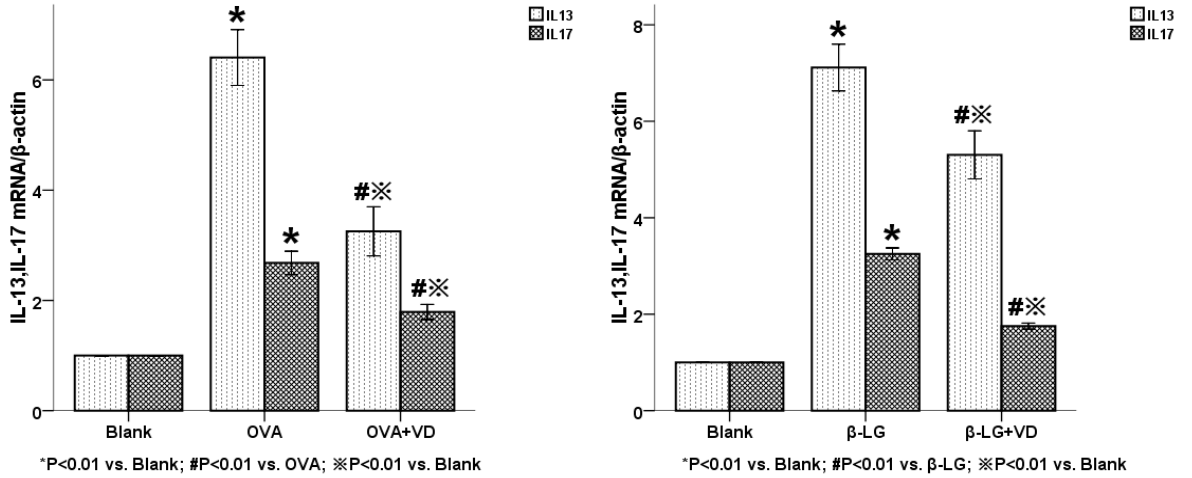

Figure 2. The mRNA expressions of IL-13 and IL-17 in OVA/β-LG group

OVA: ovalbumin; β-LG: β-lactoglobulin; VD:1,25-$(OH)_2D_3$

DISCUSSION

T helper cells can generate an antigen specific, T cell response to an allergen, which plays a key role in the earliest stages of the establishment of allergic sensitization. Kopp *et al.*[9] observed the influence of the maternal Th2-associated cytokine pattern on the naive fetal T cell phenotype, and data suggested that maternal sensitization to allergens was associated with the elevated production of the Th2 cytokine IL-13 in the CBMC. Bianca *et al.*[10] detected increased IL-17 secretion after allergen stimulation, which highly correlated with IL-13 secretion, Our findings showed that the production of Th2-type cytokine IL-13 and Th17-type cytokine IL-17 from cord blood $CD4^+$T cells could be up-regulated when exposed to allergens, so it can be predicted that antigenic stimulation could promote Th0 to Th2 and Th17 polarization in early stage of cell differentiation. Thus, we presume that elevated Th2 cell and Th17 cell response induced by allergens in early life could be associated with the occurrence of asthma. However, the allergen specific immune response in cord blood is poorly understood and specificity of allergen proliferation regarding the detection of clinically relevant effector T cells is still questioned. There is increasing evidence to suggest that allergens can indeed traverse the human placenta,[11,12] so this investigation has generated much interest in the possibility that priming of the fetal immune system to allergen occurs before birth, and has focused attention on the impact that this might have on the development of allergic disease.

In recent decades, the prevalence of asthma and allergy has increased in many industrialized countries. A number of hypotheses have been proposed to explain this trend, including a decline in exposure to infection and microbial constituents and an increase in exposure to environmental pollutants. More recently, immunological and epidemiological studies have suggested a role for vitamin D in the development of asthma and allergy. However, recent studies have reported conflicting data on association between maternal intake of vitamin D during pregnancy and asthma.[13-16] To some extent, our data showed that advisable level of vitamin D to be associated with decreased markers of allergy and asthma. In our study, we observed that, after exposure of umbilical cord blood $CD4^+$T cells to allergens, the level of IL-13 was increased significantly, and addition of 1,25-$(0H)_2D_3$ effectively ameliorated allergen-induced IL-13 expression in $CD4^+$T cells. This is consistent with previous studies, which have also demonstrated effects of 1,25-$(0H)_2D_3$-induced downregulation of Th2 cytokine expression and inhibition of eosinophilic inflammation.[17,18] Moreover, we also investigated that 1,25-$(0H)_2D_3$ could also suppress allergen-induced production of IL-17, which suggested that 1,25-$(0H)_2D_3$ as a kind of immunomodulator, decreased neutrophilic inflammation and might have auxiliary therapeutic effects in the treatment of asthma, especially in steroid-resistant asthma.

Our study had several limitations. We did not have data on maternal intake of vitamin D nor did we have data on maternal plasma $25(OH)_2D_3$ concentration during pregnancy, both of which would have helped to give a more complete estimate of prenatal vitamin D status. We also did not have data on other dietary factors, which could be correlated with vitamin D and potentially could have confounded the relationships examined if such micronutrients were also related to immune function at birth. Finally, our sample size was rather small.

In summary, our results showed that 1,25-$(OH)_2D_3$ inhibited expression of IL-13 and IL-17 in cord blood $CD4^+$T cells after exposure to allergens , that may be one of the underlying mechanisms of early intervention in asthma management. We provided evidence that, for the current used vitamin D, an improved characterization of immunoregulatory function in early life is necessary and feasible. However, further studies are required to clarify the regulatory pathways and mechanisms.

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