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Vitamin D modulates the allergic phenotype of dendritic cells in children with atopic dermatitis

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Author contribution statement:

FC, GPM, CVK, SSV, CI, RHB, CND, LC, AB performed the research

FC, GPM, CVK, SSV, CI, LC, CAC, AMK, and AB designed the research study

FC, GPM, CAC, AB analysed the data

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Abstract

Vitamin D (VD) deficiency has been associated with increased incidence and severity of atopic dermatitis (AD), but the mechanisms through which VD may ameliorate AD are unclear. We compared the phenotypic characteristics of circulating myeloid and plasmacytoid dendritic cells (mDCs and pDCs, respectively) of children with AD vs. healthy controls (HC), and evaluated if VD can modulate the allergic phenotype of circulating DCs in AD patients. Although there was no difference in frequency of circulating DCs between groups, among children with AD there was an inverse correlation between SCORAD and circulating total DCs and mDCs. In AD, serum IgE concentration correlated with FcεRI and surface-bound IgE expression on mDCs and pDCs; pDCs expressing FcεRI and IgE were significantly

increased compared to HC. *Ex vivo*, 1,25(OH)₂D₃ significantly decreased FcεRI expression on mDCs and surface-bound IgE on mDCs and pDCs. Oral VD supplementation reduced expression of surface-bound IgE on pDCs in children with AD. In summary, VD decreases the allergic phenotype of circulating DCs in children with AD, a potential mechanism for how VD supplementation may improve AD severity. Future studies are needed to further assess the role of VD supplementation as an immunomodulatory therapy for AD.

Keywords:

vitamin D; atopic dermatitis; dendritic cells; IgE; immune tolerance

Background

Atopic dermatitis (AD) is a common inflammatory skin disease with a complex pathogenesis that combines genetic and environmental factors.^[1,2] In recent years, vitamin D (VD) deficiency has been associated with increased incidence and severity of AD.^[3,4] In addition, VD supplementation was shown to decrease AD severity in several studies,^[5-8] a result that was confirmed by a recent systematic review.^[9] However, the mechanisms through which VD may ameliorate AD remain unclear. VD has widespread effects on immune cells, including monocytes, lymphocytes and dendritic cells (DCs), all of which express the VD receptor (VDR).^[10] Of these, DCs are professional antigen-presenting cells (APCs) that are key in initiating and orchestrating immune responses in AD. Skin-resident DCs, such as Langerhans cells and dermal DCs, facilitate the recognition and capture of allergens and foreign antigens, and activate innate and adaptive immunity eventually leading to IgE-mediated allergy. Abnormalities in skin DCs from AD patients may contribute to AD

pathogenesis, allergen hyper-responsiveness, and increased susceptibility to infections.^[11,12]

Alterations in circulating DCs have also been shown in AD. The main subtypes of circulating DCs are myeloid DCs (mDCs) and plasmacytoid DCs (pDCs). While mDCs are most efficient as APCs capable of priming naïve T cells, pDCs specialize in antiviral immune responses. Circulating DCs in AD patients express IgE and its high-affinity receptor FcεRIα, show compartmental imbalances, and have aberrant immune function.^[13,14] Furthermore, circulating pDCs expressing FcεRIα have lower levels of skin-homing receptors, and impaired secretion of IFN-α and IFN-β upon CPG stimulation.^[15] These data suggest that DCs are a potential target for AD treatment.

DC modulation to induce immunological tolerance to antigens and decrease immune activation is an attractive therapeutic approach that is being studied in multiple immune-mediated diseases.^[16] The active form of VD, 1,25(OH)₂D₃, inhibits DC differentiation, maturation and activation, promotes their apoptosis, and modulates T cell phenotype and function.^[17] However, the effect of VD on DCs of AD patients is unknown.

Questions addressed

The present study compared the phenotypic characteristics of circulating mDCs and pDCs of children with AD vs. healthy controls (HC), and investigated if VD can modulate the allergic phenotype of circulating DCs in AD patients.

Experimental design

We first performed a cross-sectional study, in which we evaluated peripheral blood mDCs and pDCs by flow cytometry in 16 children with AD diagnosed by Hanifin and Rajka criteria^[18] (age 7 ± 5 years [range=3-16]; 50% male; SCORAD 41 ± 15) and 9 non-atopic healthy controls (age 11 ± 3 years [range=6-17]; 70% male) from Santiago, Chile, evaluated during summer and fall seasons. Given the findings of this cross-sectional study, we performed two interventional studies on the effect of VD on DC phenotype in AD subjects: a) *ex vivo* culture with $1,25(\text{OH})_2\text{D}_3$, the active form of VD or vehicle (ethanol), and b) *in vivo* study of 8 children with AD who were supplemented weekly cholecalciferol (VD3) for 6 weeks, with an age-adjusted dosing protocol (8000 IU/week for 2-5.9 years; 12000 IU/week for 6-11.9 years; 16000 IU/week for 12-18 years). Inclusion criteria were pediatric age and active AD (i.e. SCORAD ≥ 10). Patients receiving topical corticosteroids or calcineurin inhibitors 7 days prior to inclusion, systemic corticosteroids, antibiotics, immunosuppressors, VD or fish oil supplements, or phototherapy one month prior to inclusion were excluded from the study. The Scientific Ethics Committee at the Pontificia Universidad Católica de Chile approved the study.

The frequency and phenotype of mDCs and pDCs were assessed in whole blood by multicolor staining flow cytometry; mDCs were Lineage⁻HLA-DR⁺CD11c⁺ and pDCs were Lineage⁻HLA-DR⁺CD123⁺. Gating strategy is shown in Figure s1. Surface expression of FcεRI, IgE, CD86, and the cutaneous lymphocyte antigen (CLA) were assessed on DCs. Serum 25-hydroxyvitamin D (25(OH)D) was evaluated by liquid chromatography tandem mass spectrometry (LC-MS/MS). Normal range of 25(OH)D was considered at ≥ 30 ng/ml.

All values are expressed as mean $\pm$ standard deviation unless otherwise stated. A two-sided $p < 0.05$ was considered statistically significant. Student's T test or Mann-Whitney U test were used to analyze differences between groups. Wilcoxon signed-rank test was used to assess differences in DC phenotypic markers after *ex vivo* treatment, and before and after *in vivo* VD3 supplementation. The association between continuous variables was evaluated by Spearman's rank correlation coefficient. Data were analyzed with SPSS 23.0 (IBM Corp, Armonk, NY).

Results

In the cross-sectional evaluation of AD vs. HC, serum IgE (normal range < 100 IU/L) was significantly higher in AD vs. HC (513 ± 379 vs. 44 ± 53 IU/L, $p < 0.001$), but serum 25(OH)D did not differ between groups (30 ± 7 vs. 28 ± 6 ng/ml, $p = 0.56$). There was no significant difference in frequency of circulating DCs, mDCs and pDCs between AD and HC (Fig.1a). However, among AD patients, we observed an inverse correlation between SCORAD and circulating total DCs ($r = -0.75$, $p = 0.001$) and mDCs ($r = -0.52$, $p = 0.04$) (Fig.1b).

We then evaluated the allergic and activation phenotype of mDCs and pDCs by analyzing expression of surface markers Fc ϵ RI, IgE, CD86 and CLA. In children with AD, serum IgE concentration positively correlated with Fc ϵ RI mean fluorescence intensity (MFI) on mDCs ($r = 0.82$, $p < 0.001$) and pDCs ($r = 0.78$, $p < 0.001$) and IgE MFI on mDCs ($r = 0.85$, $p < 0.001$) and pDCs ($r = 0.45$, $p = 0.02$) (Fig.s2). In children with AD, mDCs expressing CLA and pDCs expressing Fc ϵ RI and IgE were significantly increased compared to HC (Fig.1c). In turn, Fc ϵ RI and IgE MFI on mDCs, but not pDCs, were significantly increased in AD vs. HC (Fig.1d).

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To assess the relation between VD status and DCs, we analyzed if serum 25(OH)D levels correlated with DC number and phenotype. AD children with suboptimal VD status (25(OH)D <30 ng/ml) had significantly increased FcεRI+ mDCs vs. VD-sufficient children (79±95 vs. 69±14, p=0.03) (Fig.s3). However, the FcεRI MFI in mDCs did not vary by 25(OH)D concentration. VD status was not associated with AD severity assessed by SCORAD, IgE level, frequency or phenotypic markers of DCs.

To determine the effect of VD on DCs from children with AD, we first treated whole blood from 8 AD patients with 10 nM 1,25(OH)₂D₃ or vehicle (ethanol) for 24 hours, after which DC phenotype was assessed. *Ex vivo*, 1,25(OH)₂D₃ significantly decreased FcεRI expression on mDCs and surface-bound IgE on mDCs and pDCs (Fig.2a). We then evaluated *in vivo* whether oral VD₃ supplementation induced changes in DC phenotype in 8 children with AD. After supplementation with VD₃, serum 25OHD significantly increased (17±5 to 37±8 ng/ml, p<0.0001), and expression of surface-bound IgE on pDCs significantly decreased (p=0.01) (Fig.2b). Additionally, we observed a non-significant decrease in FcεRI expression and surface-bound IgE on mDCs, similar to the *ex vivo* culture findings. Although this study was not powered to detect significant clinical improvement, median SCORAD decreased non-significantly after VD supplementation (36.2 [IQR 26.9-42.5] to 31.1 [IQR 25.9-46.2], P=0.78).

Conclusions

Our data show phenotypic abnormalities in circulating DCs from children with AD, with differential expression of allergic and activation markers involved in AD pathogenesis. The results confirm that circulating DCs from children with AD express FcεRI and have high levels of surface-bound IgE, as shown previously in adults with AD.^[13,15] We also show that circulating mDCs inversely correlate with SCORAD, confirming previous studies and suggesting that circulating mDCs may be a biomarker of AD severity.^[14,19,20]

Targeting DC allergic phenotype is an appealing strategy against AD and other allergic diseases. Our study showed that low VD in children with AD might be associated with increased mDC allergic activation. Moreover, *ex vivo* VD treatment as well as *in vivo* oral VD supplementation reduced the allergic phenotype in DCs from children with AD, suggesting VD may produce tolerogenic effects on DCs in this population. The effect of VD on DCs has been previously studied, although in other settings. Penna et al. showed that 1,25(OH)₂D₃ preferentially modulates tolerogenic properties in mDCs rather than pDCs.^[21] However, pDCs also express VDR and VD modulates pDC function and decreases their capacity to activate T cells.^[22] Stimulation of FcεRI in pDCs reduces their capacity to produce type I interferons and enhances Th2 immune responses.^[15] Our data suggest that VD may decrease the allergic phenotype of both DC types, but particularly reduce surface-bound IgE on pDCs both *ex vivo* and *in vivo*. Thus, it is possible that VD modulation of pDC phenotype may contribute to boosting innate immunity and ameliorating Th2 responses, providing an additional mechanism of how VD supplementation may improve AD severity.

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A limitation of this study is that whole blood was used for DC treatment instead of purified DCs; due to the patients' young age, large blood draws for DC purification were not feasible. Another limitation is that due to recruitment seasons (summer/fall) most patients were not VD-deficient, so evaluating the effect of VD deficiency on DC number and phenotype was not possible. Lastly, this study was underpowered to evaluate the effect of VD on AD severity or other clinical outcomes.

In conclusion, our study shows that VD decreases the allergic phenotype of circulating DCs in children with AD, constituting a potential mechanism of how VD may decrease disease severity. Future studies are needed to evaluate whether VD may be used for induction of DC tolerance against allergens in AD, and further assess the role of VD supplementation as an immunomodulatory therapy for decreasing AD severity.

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Figure Legends

Figure 1. Frequency and phenotype of circulating dendritic cells (DCs), myeloid dendritic cells (mDCs) and plasmacytoid dendritic cells (pDCs) in children with atopic dermatitis (AD) and healthy controls.

a) Frequency of circulating DCs, mDCs, and pDCs in children with AD and healthy controls. Symbols represent DC percent of control (■) or AD (▲) subjects.

b) Correlation of circulating DCs and mDCs and SCORAD in children with AD. Black circles represent AD subjects. Black line shows linear trend. $p < 0.05$ was considered significant.

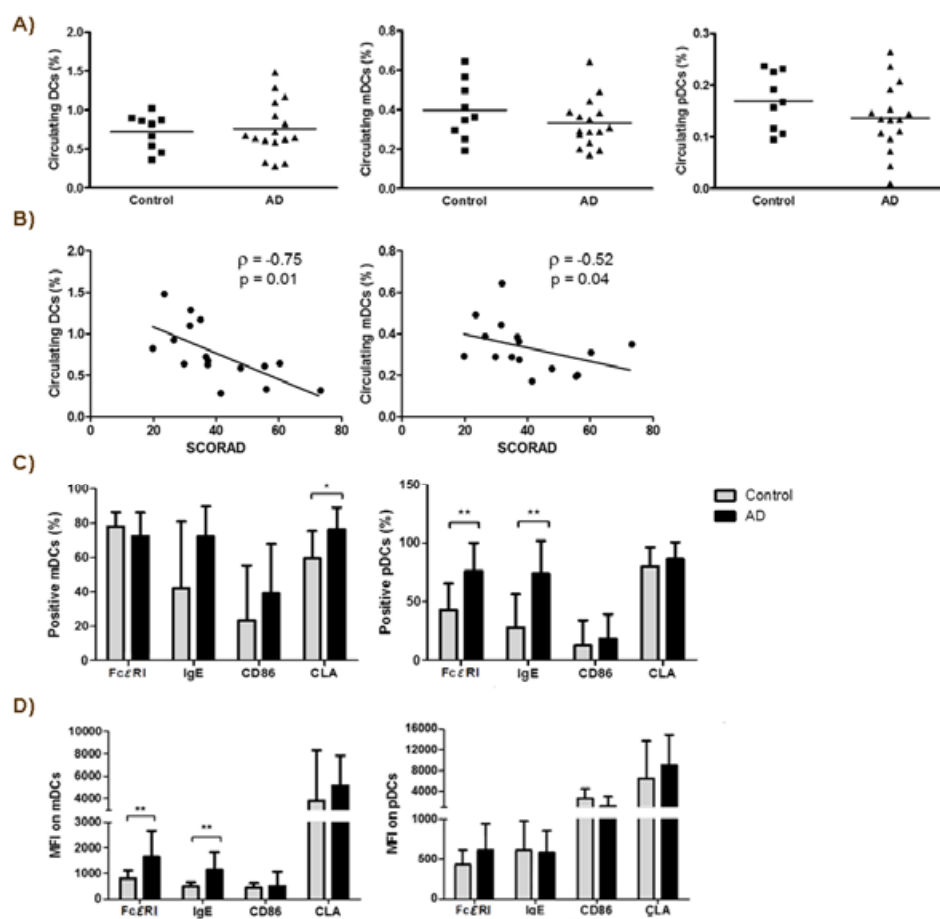
c) Phenotypic characterization of mDCs and pDCs in children with AD compared with healthy controls, analyzed for surface marker positivity. Columns represent mean DC percent. Error bars represent 2 standard deviations. * $p < 0.05$, ** $p < 0.01$.

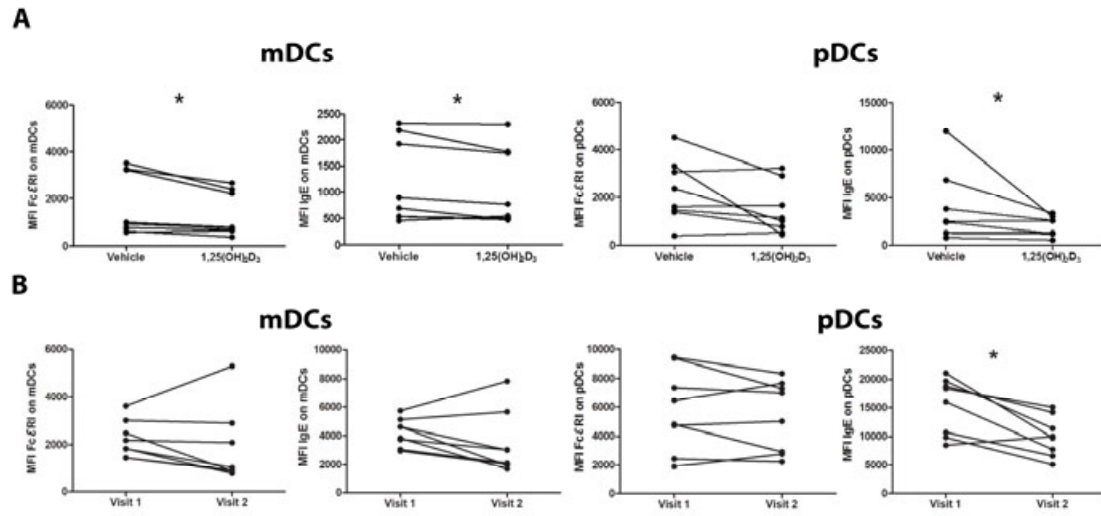
d) Phenotypic characterization of mDCs and pDCs in children with AD compared with healthy controls, analyzed by mean fluorescence intensity. Columns represent average DC mean fluorescence intensity. Error bars represent 2 standard deviations. ** $p < 0.01$.

Figure 2. Expression of allergic surface-markers of FcεRI and surface-bound IgE on circulating myeloid and plasmacytoid dendritic cells in response to vitamin D in 8 children with atopic dermatitis. Black circles represent mean fluorescence intensity (MFI) of each patient's cells. Lines represent change in MFI. * $p < 0.05$.

a) After 24-hour culture *ex vivo* of whole blood with $1,25(\text{OH})_2\text{D}_3$ or vehicle (ethanol).

b) Before and after a 6-week course of oral VD3 supplementation.





Supplementary material

Vitamin D modulates the allergic phenotype of dendritic cells in children with atopic dermatitis

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Figure S1. Gating strategy for multicolor flow cytometry analysis of myeloid dendritic cells (mDCs) and plasmacytoid dendritic cells (pDCs). Fluorescence-minus-one controls were used to determine gating boundaries. **A.** In whole blood, live cells were selected. **B.** Live cells were further gated for Lineage (Lin) and HLA-DR staining, taking the Lin⁻ HLA-DR⁺ population as DCs. **C.** DCs were gated for expression of CD11c to identify mDCs and CD123 to identify pDCs. To identify dendritic cells we used a 4-color dendritic kit (#340565, BDBiosciences), which includes Lineage cocktail 1 (LIN1) FITC, HLA-DR PerCP, CD123 PE and CD11c APC. To analyze DC phenotype, we used the following antibodies: FcεR1α PE-Cy7 (#334620, Biolegend), IgE PE-Cy7 (#325510, Biolegend), CD86 PE-Cy7 (#305422, Biolegend) and CLA FITC (#321306, Biolegend). Data were analyzed using FlowJo software version 7.6.1 (Tree Star, Ashland, OR, USA).

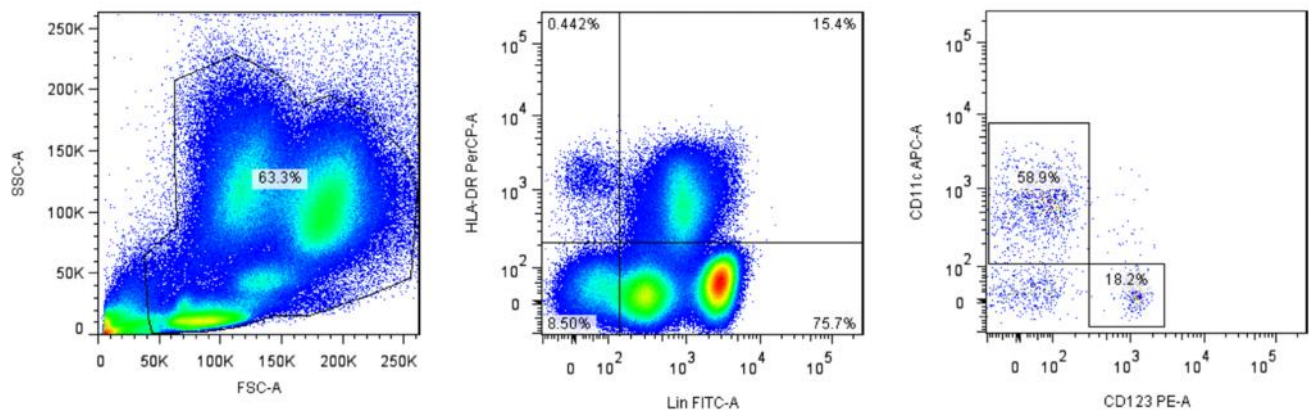


Figure S2. Correlation of serum IgE with MFI of FcεRI and surface-bound IgE in myeloid and plasmacytoid dendritic cells from children with AD. Black circles represent AD subjects. Black line shows linear trend. $p < 0.05$ was considered significant.

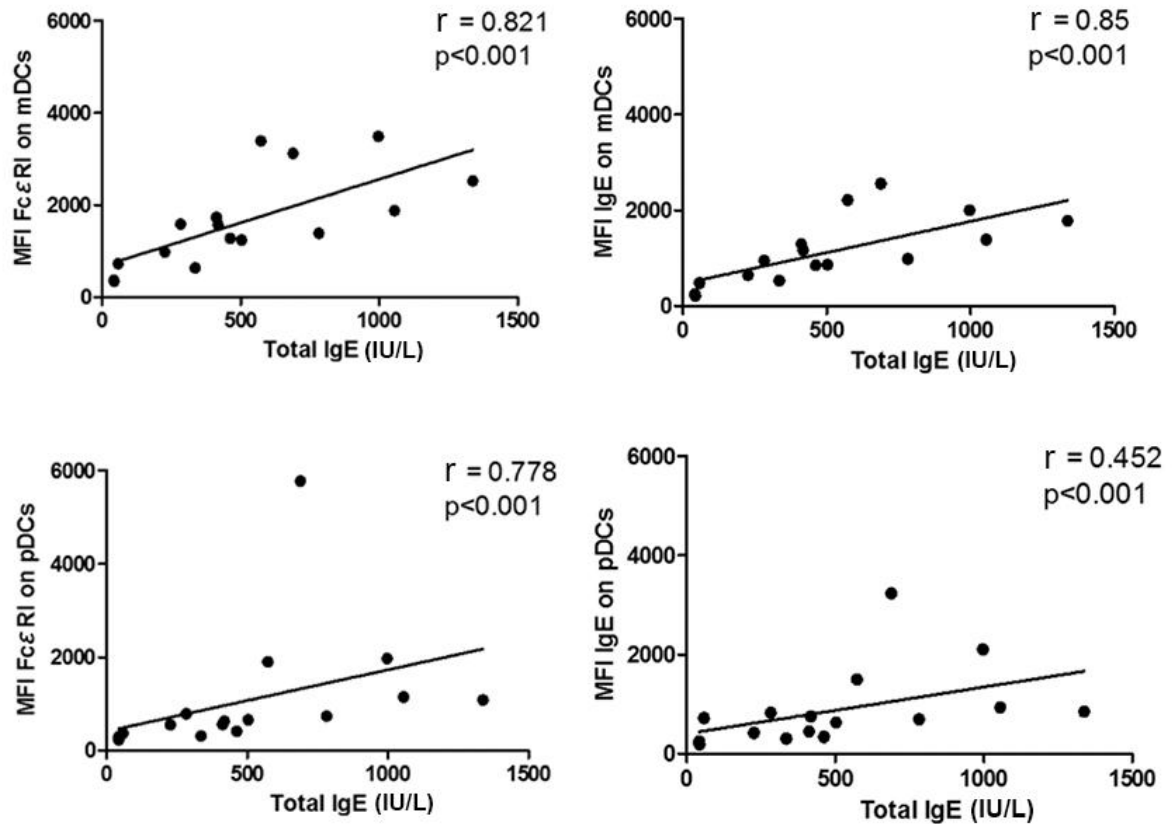


Figure S3. Low serum 25(OH)D is associated with higher circulating FcεRI+ myeloid dendritic cells in children with atopic dermatitis. * p<0.05.

