EFFECTS OF IN VITRO 1,25 DIHYDROXYVITAMIN D ON MATURATION OF DENDRITIC CELLS IN GRAVES’ DISEASE PATIENTS

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ABSTRACT

Objective: The autoimmune reaction in Graves’ disease (GD) is induced by self-antigen, which is presented by dendritic cells (DCs). DCs in GD have more active immune responses than those in healthy subjects. The ability of DC as antigen-presenting cell is determined by its maturity level. In GD, vitamin D level is inversely proportional to antibody titer and proportionally associated with remission status. Studies on healthy subjects and autoimmune patients (systemic lupus erythematosus (SLE), multiple sclerosis (MS), and Crohn’s disease) have demonstrated immunoregulatory effects of vitamin D, mainly through inhibition of DC maturation, which may decrease the DC’s immunogenic profile. This study aims to identify the effect of 1,25-D3 in vitro on DC maturation in patients with GD.

Methods: This is an experimental study, which was conducted in 12 GD patients with thyrotoxicosis. Monocyte-derived DC of GD patients was cultured, with or without 1,25-D3 in vitro at monocytic phase. The DC maturation was then stimulated by lipopolysaccharide (LPS) and evaluated based on the expression of DC markers (human leukocyte antigen-D-related [HLA-DR], CD80, CD40, CD83, CD14, and CD206) and the ratio of cytokine interleukin-12 (IL-12)/IL-10 levels in the supernatants.

Results: Following the LPS stimulation, DC with 1,25-D3 showed lower expressions of HLA-DR, CD80, CD40, and CD83, and higher expressions of CD14 and CD206 compared to DC without 1,25-D3. DC with 1,25-D3 had lower ratio of IL-12/IL-10 levels than those without 1,25-D3.

Conclusion: In vitro 1,25-D3 supplementation inhibits DC maturation in patients with GD.

Keywords: Vitamin D, Graves’ disease, Dendritic cells.
1,25-D3 on the expression of DC markers in GD

The flow cytometry analysis was performed using FACSCalibur (BD Bioscience Pharmingen). The culture plate was then incubated at 37°C with CO₂ for 5 days.

Although the expression of HLA-DR in the cultures with 1,25-D3 decreased on the 5th day, the expression of HLA-DR in the cultures with 1,25-D3 was lower than those cultures without 1,25-D3 (p<0.01). The expression of CD206 in the cultures with 1,25-D3 was lower than those cultures without 1,25-D3 (p<0.01; p=0.016), while the expression of CD206 in the cultures with 1,25-D3 was significantly lower than those cultures without 1,25-D3 (p=0.016; Fig. 1). On the 7th day, the expression of IL-12/IL-10 in the cultures with 1,25-D3 was significantly lower than those cultures without 1,25-D3 (p<0.01; p=0.016; Fig. 2).

DISCUSSION

As a natural immunomodulator, vitamin D increases innate immune response and regulates excessive adaptive immune response such as found in autoimmune disease [17]. Dcs are immune cells that initiate and maintain the autoimmune response [5]. One of the important effects of vitamin D in autoimmune is inhibition of DC maturation. The present study provides additional information regarding the immunoregulator effect of vitamin D, particularly on inhibition of DC maturation in GD patients. This is the first study reporting the effect of in vitro vitamin D on inhibition of DC maturation in GD.

Effects of 1,25-D3 on the expression of DC markers in GD patients

This study shows that in vitro 1,25-D3 supplementation inhibits DC maturation in MDDC cultures of GD patients, which is characterized by lower expression of major histocompatibility complex class II and IL-10.
the expressions of CD80, CD40, CD83, and CD206 showed no difference (p>0.05).

HLA-DR molecule is very important since polymorphism or genetic mutation of HLA-DR has a role in the pathogenesis of some autoimmune diseases including GD [18,19]. The complex HLA-DR gene is the major genetic factor in AITD in addition to the gene of Treg cells (CTLA4) and specific genes of thyroid [19]. It explains why the expression of HLA-DR in MDDC culture has been affected since the iDC phase by in vitro 1,25-D3 supplementation, while the expression of CD80, CD40, CD83, and CD206 was not affected. The role of CD14 in GD still cannot be explained as clearly as the role of HLA-DR. The change of CD14 expression is earlier and probably due to the essential role of the molecule in the GD progression. It needs further studies considering that there is still no study reporting the correlation between the genetic defect of CD14 molecule and GD.

Other findings in our study are the change of DC molecules expression on the 5th day. At iDC phase, the expressions of HLA-DR and CD14 have already changed but other markers have not. In vitro 1,25-D3 in monocytic phase reduced the expression of HLA-DR (p<0.01) and increased the expression of CD14 (p<0.01) of iDC on the 5th day, while the expressions of CD80, CD40, CD83, and CD206 showed no difference (p>0.05).

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Effects of in vitro 1,25-D3 on the ratio of cytokines IL-12/IL-10 levels in MDDC cultures of GD patients

The balance between cytokines IL-12 and IL-10 is required to maintain tolerance [20]. This study used IL-12/IL-10 ratio to provide a better description of both cytokines interaction [20]. It shows that in vitro vitamin D supplementation in MDDC cultures of GD patients decreases the ratio of IL-12/IL-10 cytokines. This is the first study which demonstrates the effect of in vitro 1,25-D3 supplementation on IL-12/IL-10 ratio in patients with autoimmune disease. Lower IL-12/IL-10 ratio suggests lower DC immunogenicity.

If each cytokine is evaluated separately in this study, in vitro 1,25-D3 supplementation decreases IL-12 cytokine level significantly, while IL-10 cytokine level is relatively maintained. It indicates that in vitro 1,25-D3 supplementation can suppress IL-12 production and maintain the role of IL-10 on immune response initiated by DC. Low inflammatory cytokines level will reduce T-cells activation leading to prevention of persistent immune response in autoimmune cases [5,20].

The effect of in vitro vitamin D supplementation on IL-12 or IL-10 cytokines has been previously reported for SLE, MS, and Crohn's disease. Studies on Crohn's disease did not demonstrate the effect of in vitro vitamin D supplementation on the decrease of IL-12 level because they used less sensitive ELISA kit. Studies on SLE and MS only evaluated either IL-12 or IL-10, therefore, may not provide balanced description of inflammatory and anti-inflammatory cytokines produced by DC [12,13].

CONCLUSION

In vitro 1,25-D3 supplementation inhibits DC maturation in patients with GD.

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