Effects of vitamin D on expression of Toll-like receptors of monocytes from patients with Behçet’s disease

J. E. Do¹, S. Y. Kwon¹, S. Park² and E.-S. Lee¹

Objectives. Recent studies have shown the immunomodulatory effect of vitamin D₃ through down-regulation of Toll-like receptor (TLR) expression in human monocytes. To understand the implication of innate immunity with the role of vitamin D affecting TLR expression in Behçet’s disease (BD), we focused on the association between the TLR expression and the serum vitamin D concentration in BD.

Methods. The expression of TLR2, TLR4 and CD16 on monocytes was detected by flow cytometric analysis and RT-PCR. Serum 25-hydroxyvitamin D [25(OH)D] levels were measured in the patients with BD, psoriasis and healthy controls, and then the expression of TLRs was correlated with the value of serum 25(OH)D levels. To assess the influence of vitamin D₃ on expression and function of TLRs in vitro, human monocytes were treated with increasing concentrations of 1,25(OH)₂D₃.

Results. We found that the monocytes of active BD patients showed higher expressions of TLR2 and TLR4 than those of controls, and serum 25(OH)D levels tended to be lower in active BD. Furthermore, 25(OH)D levels were inversely correlated with the expressions of TLR2, TLR4 and clinical indicators. In vitro analysis showed that vitamin D₃ was found to dose-dependently suppress the protein and mRNA expressions of TLR2 and TLR4. TNF-α synthesis was also decreased upon TLR ligand stimulation in vitamin D₃-treated monocytes.

Conclusion. These results suggest that the inflammation triggered through TLR2 and TLR4 is important in the pathogenesis of BD. And it seems possible that vitamin D may be used as a therapeutic option by modulating TLR2 and TLR4 expression of monocytes in BD.

KEY WORDS: Behçet’s disease, Monocyte, Toll-like receptors, Vitamin D.

Introduction

Toll-like receptors (TLRs) are crucial players in the innate immune response to microbial invaders, enabling vertebrates to detect the pathogen-associated molecular patterns (PAMPs) early and subsequently activating the adaptive immune response [1]. Among 11 members of the TLR family, TLR2 and TLR4 have been identified as signalling receptors activated by bacterial wall components, such as lipopolysaccharide (LPS) from Gram-negative bacteria and lipoteichoic acid (LTA) from Gram-positive bacteria. It has been shown that endogenous molecules such as HSP60 and fragmentation products of fibronectin can also trigger an inflammatory response via TLR2 and TLR4 [2].

TLR plays a major role in the initiation of protective immune responses. However, the extensive release of TLR-triggered pro-inflammatory mediators may harm the host as in cases of sepsis or chronic inflammatory disease [3]. Recent studies demonstrate higher constitutive expression of TLR2 and TLR4 in blood monocytes derived from chronic inflammatory disease patients (RA, inflammatory bowel disease) than that from healthy controls [4, 5]. As TLRs in monocytes are instrumental in both launching innate immune responses and influencing adaptive immunity, the regulation of TLR expression in chronic inflammatory diseases could be an important therapeutic target for the disease activity.

Behçet’s disease (BD) is a chronic, multi-systemic, inflammatory disease characterized by recurrent attack of oral aphthae, genital ulcers, uveitis and erythema nodosum. Although of unknown aetiology, both genetic and environment factors are considered to determine the inflammatory background [6]. Furthermore, it has become clear that the susceptible infection of Streptococci and Mycoplasma, and immunological cross-reaction with HSP60 act as a ‘Danger signal’ in the initiation and progression of BD [7]. Since HSP60 and infectious agents act via innate immune system of TLRs, the expression status of TLR2 and TLR4 on monocytes may reflect the intrinsic activation potential that is responsible for initiating or perpetuating inflammation [2]. Recent studies have disclosed the involvement of excessive Th1 cell functions in the pathogenesis of BD [8, 9]. However, much remains unanswered about the role of TLRs that connects the recognition of PAMP and intracellular signal transduction for T-cell activation in BD.

A growing body of evidence supports the hypothesis that vitamin D is an environmental factor important in the aetiology of T-cell-mediated autoimmune diseases. The net effect of 1,25(OH)₂D₃ treatment in vivo is a reduction in the autoimmune Th1 response targeting DCs, CD4+ T cells and regulatory T cells, and an amelioration of symptoms of inflammatory bowel disease, RA, insulin-dependent diabetes mellitus and multiple sclerosis [10–13]. Recently, the immunomodulatory effect of 1,25(OH)₂D₃ through down-regulation of the expressions of TLR2 and TLR4 was demonstrated in human monocytes in vitro model [14]. Based on these evidences, we tried in the present study to determine TLR expressions of monocytes and serum 25-hydroxyvitamin D [25(OH)D] levels in BD patients, and explored possible association between those variables. In vitro study was performed to confirm the effect of 1,25(OH)₂D₃ on the expression and function of TLR2 and TLR4 from BD monocytes, healthy controls and human monocyte cell line. It would be of great importance to clarify the roles of TLRs and vitamin D in the pathogenesis and to determine therapeutic targets in BD.

Patients and methods

Patients and samples

The patient population consisted of 41 patients with BD (25 women and 16 men; mean ± s.d. age, 39.07 ± 9.15 yrs), who presented for the first time or were monitored at the Department of Dermatology, Ajou University Hospital. According to the International Study Group for BD criteria [15], the presence of any two of the following symptoms in addition to recurrent oral ulceration is diagnostic: recurrent genital ulceration, uveitis,
large-vessel vasculitis, cutaneous erythema nodosum and a positive pathergy test. Active group patients had at least one of the BD symptoms despite treatment and inactive group patients were in well-controlled states by taking anti-inflammatory medication. The control groups consisted of 19 newly diagnosed psoriasis patients without any other evident disease and 15 healthy volunteers as disease and healthy control groups, respectively. Informed consent was obtained from patients prior to enrolling them into the study. This study was approved by the Institutional Review Board (IRB no.: AJIRB-GN3-06-206).

Flow cytometry

Samples were collected during winter to early spring, and the daily sun exposure time was estimated to be <1 h for all subjects. Peripheral blood mononuclear cells (PBMCs) were prepared from heparinized blood samples by centrifugation over Ficoll Hypaque density gradients (Ficoll paque™ plus, StemCell Technologies, Vancouver, BC, Canada). CD14+ monocytes were separated by means of magnetic cell sorting method (EasySep™, StemCell Technologies, Vancouver, BC, Canada), and the purity of positively selected monocytes was usually >95%, as determined by flow cytometry. CD14+ monocytes (1 x 10^6) were incubated for 30 min with 20 μl of phycoerythrin-labelled anti-human TLR4 (HTA125, mouse IgG2a, eBioscience, San Diego, CA, USA), TLR2 (TL2.1, mouse IgG2a, eBioscience, San Diego, CA, USA) or FITC-labelled anti-CD16 antibody (BD Biosciences Pharmingen, Heidelberg, Germany). The labelled mononuclear cells were analysed by Vantage flow cytometer (FACS, Becton Dickinson Immunocytometry Systems, Mountain View, CA, USA), and results were expressed as mean fluorescence intensity (MFI).

Semiquantitative RT-PCR

Total RNA was extracted from 1 x 10^6 CD14+ monocytes using the RNeasy Mini kit (Qiagen, Valencia, CA, USA) according to the manufacturer’s instructions. A total of 1 μg of RNA in a 13 μl volume was transformed to cDNA by using dNTP and OligodT primer (Invitrogen, Carlsbad, CA, USA). The samples were incubated for 5 min at 65°C. The cDNA was then amplified in a 20 μl final volume using Superscript™ III (Invitrogen, Carlsbad, CA, USA) following the recommendations of the manufacturer. RT-PCR analysis was carried out in a reaction mixture (Bioneer, Daejeon, Korea) containing 1U Taq polymerase, 250 μM dNTP, 10 mM Tris–HCl (PH 9.0), 40 mM KCl, 1.5 mM MgCl2, 200 μM of each primer for TLR2 or TLR4. For the co-amplification primer, glyceraldehyde-3-phosphate (GAPDH) was used. The reaction was carried out in a DNA Thermal Cycler (RTC-200, MJ Research, MA, USA). The sequences of the sense and anti-sense primers used for amplification were as follows: TLR2, 5'-GGGCGAGCAATTCATTACGGTGTG-3' and 5'-AGGCC GGACATCCTGAACCT-3'; TLR4, 5'-GGCATATCGAGGCTT AAGCCA-3' and 5'-AAAGGCTCAGGAGTTTACCT-3'; GAPDH, 5'-AGTCAACCCAGATGGTGGTGA-3' and 5'-GGAAA CATGAAACCATGTGA-3'. To quantify the transcripts, the intensities of the PCR bands were measured by densitometry using Image-Pro Plus Version 4.5 (Media Cybernetics Co, MD, USA) and were expressed as intensities relative to GAPDH.

Measurement of serum 25(OH)D

Serum concentrations of 25(OH)D were assayed with a radioimmunoassay kit (Dia-Sorin, Stillwater, MN, USA). Following extraction of 25(OH)D using donkey anti-goat precipitating complex, the treated sample was assayed according to the equilibrium radioimmunoassay procedure. The reference range for the assay was 9.0–37.6 ng/ml.

Cell culture and measurement of TNF-α in supernatants

Functional assessments were performed on the cultured monocytes from active stage of BD patients, healthy controls and THP-1 cell line (TLR-expressing human monocytic leukaemia cell line). CD14+ monocytes (5 x 10^5 cells) were resuspended in culture medium (RPMI 1640 medium supplemented with 2 mM l-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin) with 10% FBS for 2 h. Then, adherent monocytes at a density of 5 x 10^5 cells were incubated in 10% FBS/culture medium in the presence or absence of indicated concentrations (10^-7 M, 10^-8 M and 10^-11 M) of 1,25(OH)2D3 (Sigma Chemical Co., St Louis, MO, USA) for 48 h. Subsequently, the protein and mRNA expression levels of TLR2 and TLR4 were measured as described earlier.

For cytokine analysis, pre-treated monocytes were incubated with either 10 ng of LPS or 10 μg of LTA for 4 h, and the cell culture supernatants were collected. TLR blocking experiments were performed using 20 μg/ml anti-TLR2 (clone TL2.1) and 20 μg/ml anti-TLR4 (clone HTA125) antibodies (functional grade, endotoxin-tested, eBioscience, San Diego, CA, USA) for 1 h prior to the addition of LPS or LTA. TNF-α concentrations were measured using the sandwich ELISA method with Ab set (R&D Systems Inc., Minneapolis, MN, USA). The rates of LPS/LTA-stimulated TNF-α production were compared between normal and BD monocytes, and their values were also monitored in THP-1 monocyte cell line.

To evaluate the effect of vitamin D on TLR2 and TLR4 in the HSP60-induced TNF-α response, pre-treated monocytes were stimulated with 1 μg/ml HSP60 (StressGen, Victoria, British Columbia, Canada) and 10 μg/ml of Polymyxin B (Sigma Chemical Co., St Louis, MO, USA) by the same ways.

Statistical analysis

Data were expressed as mean± standard deviation (s.d.). To compare variables among the different groups, we used one-way analysis of variance and the post hoc Tukey test. Pearson’s correlation analysis was performed to determine the correlation between the TLRs and serum 25(OH)D levels. Differences in the rates of low serum 25(OH)D levels between active and inactive BD groups were analysed using chi-square test, and they were presented with estimated odds ratio (OR; 95% CIs). Clinical values were analysed using multiple regression analysis to identify independent predictors of serum 25(OH)D levels. Additionally, to determine the effect of systemic corticosteroid treatment on serum 25(OH)D in BD patients, independent t-test was used between the steroid and non-steroid group. A two-sided P-value <0.05 were considered statistically significant.

Results

Characteristics of Participants

Baseline demographics and clinical characteristics of BD patients (n = 41, active 23, inactive 18) are summarized in Table 1. Of the 41 BD patients, 23 patients (14 females, 9 males) were clinically active and 18 patients (11 females, 7 males) were inactive. Mean disease duration was 10.58 ± 6.84 yrs, and 37% of the patients (n = 15, active 12, inactive 3) had been taking systemic corticosteroids for at least 6 months. Evaluation of the patients in the active BD group (n = 23) showed that six patients had more than three major symptoms, three patients had two major and one minor symptoms, six patients had two major symptoms and eight patients had only oral aphthae. All patients (100%) in the active BD group had oral aphthae, 10 (43%) had genital ulcer, 11 (48%) had erythema nodosum-like lesion and 4 (17%) had arthralgia.
**Increased expression of TLR2 and TLR4 on monocytes from patients with active BD**

The cell surface expressions of TLR2, TLR4 and CD16 (FcγRIIIA) on CD14+ monocytes from BD patients and controls were determined by flow cytometric analysis. As shown in Fig. 1A, the MFI of TLR2 (mean ± s.d., 61.48 ± 22.92) was significantly increased in active BD patients compared with inactive BD patients and control groups (P < 0.05), and the intensity of TLR2 expression on BD monocytes was found to be correlated with the clinical disease activity (ESR level) of active and inactive BD patients (Fig. 1C, r = 0.245, P < 0.05). The intensity of TLR4 expression was also increased on monocytes from active BD patients (mean ± s.d., 21.04 ± 6.91) compared with healthy volunteers; however, the difference was not statistically significant (Fig. 1B).

Because CD16+ CD14+ monocytes have been known as the pro-inflammatory phenotype, the mean frequency of CD16+ cells in all CD14-expressing blood monocytes was measured. The CD16+ monocyte frequency was found to be significantly increased (P < 0.05) in patients with active BD (mean ± s.d., 26.39 ± 21.19), compared with healthy controls (mean ± s.d., 13.16 ± 9.49). However, the intensity of TLR2 and TLR4 expression on CD16+ and CD16− monocyte subsets from both BD and control groups was similar.

**Increased expression of TLR2 and TLR4 mRNA in active BD patients**

To quantify the expression of TLR2 and TLR4 transcripts among those groups, we performed semiquantitative RT-PCR. As TLR2 and TLR4 are constitutively expressed on monocytes, we could detect TLR2 and TLR4 expressions in all the samples. Figure 2 shows that the levels of TLR2 and TLR4 mRNA were significantly increased in monocytes of active BD group compared with those of inactive BD, psoriasis controls and healthy controls.

**Vitamin D level**

We found a trend towards lower serum 25(OH)D levels in BD patients than in psoriasis and healthy controls, after controlling for age, gender and season. However, the mean differences of serum 25(OH) levels were not significantly different (P = 0.07) between the groups (Table 2). Using a cut-point of 10 μg/ml, critically low vitamin D levels (<10 μg/ml) were found in 11 cases...
(47.8%) in the BD-active group, while 2 cases (13.3%) were found in the healthy controls \((P < 0.05; \text{OR} \ 7.09; 95\% \ CI \ 1.30, 38.77)\). None of the persons who had critically low vitamin D levels showed abnormal values in serum calcium and inorganic phosphate levels. Our effort to find other variables in multiple regression model failed to observe any significant association between serum 25(OH)D concentration and age, gender, disease duration or previous medication. Furthermore, the separate BD group analysis to determine the effect of systemic corticosteroid medicine did not reveal any significant difference in the mean serum 25(OH)D concentration between the two subgroups \((\text{mean} \pm \text{s.d. in steroid group, 11.97} \pm \text{4.91; in non-steroid group, 11.78} \pm \text{3.51, } P = 0.88)\).

**Correlation between TLR expression and serum vitamin D levels**

Interestingly, a significant inverse correlation between 25(OH)D and TLR2 and TLR4 expressions was found in BD patients (Fig. 3; vitamin D to TLR2, \(r = -0.37, P < 0.05\) and vitamin D to TLR4, \(r = -0.43, P < 0.05\)). The serum vitamin D levels were also found to be inversely correlated with the serum CRP level (CRP; \(r = -0.41, P < 0.05\), however, not with age, gender, disease duration, previous medication and frequency of CD16+ cells.

**In vitro effect of vitamin D on TLR2 and TLR4 expressions**

To assess the effect of 1,25(OH)\(_2\)D\(_3\) on TLR2 and TLR4 expressions, human monocytes were exposed to increasing concentrations of 1,25(OH)\(_2\)D\(_3\), ranging from 10\(^{-11}\) to 10\(^{-7}\) M, for 48 h. As seen in Fig. 4, the results showed that 1,25(OH)\(_2\)D\(_3\) significantly down-regulated TLR2 and TLR4 expressions in concentration-dependent manner, demonstrated by flow cytomteric analysis. These findings were also consistently observed in human monocytes from healthy volunteers, active BD patients and TLR-expressing human monocyctic leukaemia cell line (THP-1).

To assess TLR2 and TLR4 gene expression profiles in response to 1,25(OH)\(_2\)D\(_3\), we performed semiquantitative RT-PCR (Fig. 4C). The expressions of TLR2 and TLR4 mRNA were

![Graph showing the correlation between TLR expression and serum vitamin D levels](image)

![Graph showing in vitro effect of vitamin D on TLR2 and TLR4 expressions](image)

**Table 2. Vitamin D levels of the individual groups**

<table>
<thead>
<tr>
<th></th>
<th>BD-active (n=23)</th>
<th>BD-inactive (n=18)</th>
<th>Psoriasis control (n=19)</th>
<th>Normal control (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean yrs ± s.d.)</td>
<td>39.77 ± 10.56</td>
<td>38.30 ± 7.51</td>
<td>34.06 ± 12.11</td>
<td>27.07 ± 10.19</td>
</tr>
<tr>
<td>Male: Female</td>
<td>9:14</td>
<td>8:10</td>
<td>11:7</td>
<td>12:3</td>
</tr>
<tr>
<td>Vitamin D (ng/ml)</td>
<td>10.88 ± 4.32</td>
<td>12.45 ± 3.65</td>
<td>14.92 ± 6.09</td>
<td>14.01 ± 3.57</td>
</tr>
<tr>
<td>P-value</td>
<td>0.78</td>
<td>0.05</td>
<td>0.21</td>
<td>0.21</td>
</tr>
<tr>
<td>Vitamin D &lt;10 ng/ml (percentage of cases)</td>
<td>47.8</td>
<td>27.8</td>
<td>15.8</td>
<td>13.3</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>7.09 (1.30, 38.77)</td>
</tr>
</tbody>
</table>
significantly down-regulated in monocytes, depending on the concentration of vitamin D used.

In vitro cytokine analysis to confirm the effect of vitamin D on the function of TLR2 and TLR4

LPS/LTA. Monocytes were pre-incubated with increasing concentrations of 1,25(OH)2D3 (10^{-11} to 10^{-7} M) for 48 h and were then treated with either 10 ng/ml of LPS or 10 µl/ml of LTA for 4 h. Subsequently, TNF-α released in the supernatants was determined using ELISA method. In human monocytes from healthy volunteers, TNF-α synthesis was decreased in a dose-dependent manner upon LPS and LTA stimulation, with the lowest TNF-α level at the highest 1,25(OH)2D3 concentration, which was in accordance with down-regulation of TLR2 and TLR4. In order to examine the TLR specificity of LPS and LTA, we pre-incubated monocytes with either anti-TLR2 or anti-TLR4 antibodies. As shown in Fig. 5A, both clones were found to have TNF-α expression inhibited by 70.8% with anti-HTA125 (TLR4), and by 65.3% with anti-TL2.1 (TLR2), respectively. To define the responsive pattern of TNF-α production in active BD compared with healthy volunteer, we challenged the BD monocytes with LPS and LTA. Unstimulated cells produced hardly detectable levels of TNF-α, similar to normal monocytes, and

![Figure 4. In vitro effect of vitamin D on TLR2 and TLR4 expression. Flow cytometric analysis shows that 1,25(OH)2D3 dose-dependently down-regulated (A) TLR2 and (B) TLR4 expression in monocytes. (C) Semiquantitative RT-PCR for human TLR2 and TLR4 was conducted, and the densities of PCR bands are expressed as intensities relative to GAPDH gene expression. Down-regulation of TLR mRNA was observed after incubation with 1,25(OH)2D3 (asterisk represents P < 0.05).](image-url)
FIG. 5. *In vitro* cytokine analysis confirming the effect of vitamin D on functional consequences of TLR2 and TLR4. (A) TNF-α synthesis in normal human monocytes was dose-dependently decreased upon LPS or LTA stimulation with the lowest TNF-α level at the highest 1,25(OH)2D3 concentration. Diminished TNF-α production was observed in monocytes that were pre-incubated with either anti-TLR2 (by 70.8%) or anti-TLR4 (by 65.3%) antibodies. TNF-α production in active BD monocytes was significantly increased to higher levels following 4 h of stimulation with (B) LPS or (C) LTA compared with normal monocytes (*P < 0.05*). In contrast to the results of mature monocytes, 1,25(OH)2D3 dose-dependently increased TNF-α production in (D) LPS, or (E) LTA-stimulated THP-1 cell line (a human myelomonocytic cell line).
TNF-α synthesis in BD monocytes was also dose-dependently decreased upon LPS or LTA stimulation. On the other hand, TNF-α production in active BD monocytes showed significantly higher levels following 4 h of stimulation with LPS or LTA than normal monocytes (Fig. 5B and C). In contrast to the results of peripheral blood monocytes of healthy volunteer and BD patients, THP-1 cell line showed an opposite pattern of TNF-α production (Fig. 5D and E).

**HSP60.** Local over-expression of HSP60 in inflamed, necrotic skin lesions is considered to have pathogenic significance in BD [16]. In this study, therefore, we determined the immunomodulatory effects of 1,25(OH)2D3 on HSP60-derived TNF-α production in normal and BD monocytes. Consistent with the previous finding, TNF-α production induced by HSP60 in normal and BD monocytes was inhibited by 75.8 and 86.3% with anti-TLR2 antibody, and by 69.8 and 81.9% with anti-TLR4 antibody, respectively. TNF-α production in active BD monocytes was higher in normal controls after stimulation with HSP60. Furthermore, the HSP60-induced response was also dose-dependently decreased by vitamin D (Fig. 6).

**Discussion**

The alteration of innate immune systems could critically be involved in the pathogenesis of BD [7]. Imamura et al. [17] found that the expression of HSP60 was enhanced in necrotic mucocutaneous lesions of BD and specific epitopes of human HSP60-stimulated PBMCs of BD patients, resulting in excess production of Th1 cytokine. HSP60 can induce a potent inflammatory response in the innate immune system via activation of TLR2 and TLR4 [2]; however, it is not clear what kinds of stimuli and mechanisms are responsible for the up-regulation of TLRs in vivo. The ability of certain individuals to respond properly to TLR ligands may be impared by nucleotide polymorphisms within the TLR genes, resulting in an altered susceptibility to infectious or inflammatory diseases [18]. Bacani et al. [19] could not detect any significant difference in the TLR2 Arg753Gln polymorphism between BD and normal volunteers; nevertheless, the possibility of TLR involvement in the aetio-pathogenesis of BD could not be eliminated. In the present study, we explored the possibility that alteration of TLR expression could be involved in abnormal activation of innate immune-mediated inflammation in BD, and the results showed that monocytes from BD patients had significantly increased TLR2 expression compared with that of controls. Although TLR4 intensities in flow cytometric analysis did not show significant difference in BD monocytes compared with controls, TLR4 mRNA levels in BD monocytes were found to be significantly increased. It is uncertain whether this discrepancy is due to relatively lower fluorescence of anti-TLR4 antibody compared with anti-TLR2 or CD16 antibody on monocyte surface, or anti-CD14-antibody attached to magnetic particles, which are used in selection process of CD14+ monocytes, partially interfering TLR4–CD14 interaction. If not, as Skinner et al. [20] suggested, TLR4 may be down-regulated along with CD14, while monocytes mature and acquire CD16+ pro-inflammatory phenotype, even though the mRNA transcript is up-regulated.

Iwahashi et al. [5] demonstrated that CD16+ monocytes express TLR2 more strongly than CD16− monocytes in RA patients as well as healthy controls. We found that CD16+ monocytes subset was increased in BD monocytes compared with normal monocytes; nevertheless, the MFI of TLRs in CD16+ monocytes was similar in both monocyte subsets from BD patients. Because TLR induction is associated with maturation of CD16+ macrophage-lineage cells, the expression of TLRs could be enhanced on CD16+ macrophages at the site of inflammation.

Several studies indicate possible physiological immune function of vitamin D, suggesting that vitamin D deficiency could lead to immune malfunctioning [10–13]. Although the exact mechanisms of lower vitamin D levels in chronic inflammatory states are not yet elucidated, the prevalence of autoimmune diseases has been correlated with vitamin D, and inverse correlation between the supplements of vitamin D and risk of chronic inflammatory disease has also been reported [11, 12]. Until lately, the lack of sun exposure was widely accepted as the primary cause of epidemic low vitamin D status [21–23]. However, recent promising evidence shows variable responsiveness among individuals to UV radiation, causing some to have low vitamin D status despite abundant sun exposure [24, 25]. These findings suggest that genetically predisposed individuals, who either do not maintain adequate vitamin D levels or perhaps have polymorphisms in genes important for vitamin D metabolism, catabolism or function, have an increased likelihood of developing vitamin D deficiency, rather than environmental limitations such as inadequate sun exposure. Kandi et al. [26] showed that there was no significant difference between BD patients and healthy controls in terms of serum vitamin D levels, although the mean value in BD patients was lower than that in controls. In the present study, we also could not establish a significant difference in serum 25(OH)D levels. This might be due to a relatively mild organ involvement in small number of BD population, some of which received immunomodulatory medication. Also, the fact that this study was conducted in wintertime, when there is the least amount of sun exposure, might be responsible for low 25(OH)D status amongst the populations without significant differences. Nevertheless, the present study showed a trend towards lower serum 25(OH)D levels in active BD patients compared with controls; a higher prevalence of critically low 25(OH)D levels among active BD patients, suggesting vitamin D deficiency as a possible risk factor for disease activation and dysregulated inflammatory status. To elucidate the immunomodulatory effects of vitamin D on antigen-presenting cells in vivo, we determined TLR expressions and parameters of systemic inflammation such as ESR and CRP in active and inactive BD monocytes, and found that the serum 25(OH)D level was negatively correlated with monocyte TLR expressions and disease activity markers.

As seen in in vitro analysis, BD monocytes produced TNF-α about 2-fold higher than normal monocytes, and this was in line with the study by Mege et al. [27] who demonstrated that LPS strongly induced TNF-α, IL-6 and IL-8 production in monocytes from both active and inactive BD compared with healthy volunteers. How much the different cell subpopulations contribute to the marked increase of TNF-α production in active BD patients remains unknown; however, minor population of CD4+CD16+...
monocyes, whose TLR2 and TLR4 were up-regulated, could be candidates for major TNF-α producers in human blood.

The present study on the modulation of TNF-α by 1,25(OH)2D3 revealed opposing effects between peripheral monocyes and monocyte cell line: TNF-α production was inhibited by 1,25(OH)2D3 in mature peripheral blood monocyes, whereas they increased TNF-α production in LPS/LTA-stimulated THP-1 cell line (a human myelomonocytic cell line). As Hakim and Bar-Shavit [28] noticed, the differentiation/maturatation status of the cells seems to play a role in determining their response to 1,25(OH)2D3. The hormone increases TNF-α production in immature cells (cell lines and bone marrow cells), whereas it decreases the cytokine production in more mature cells (peripheral blood monocyes and peritoneal macrophages).

Psoriasis is another T-cell mediated chronic inflammatory skin disease involving both innate and adaptive immunity [29–31]. Considering the possible role of TLRs in psoriasis pathogenesis [32] and a recent evidence to implicate vitamin D and vitamin D receptor (VDR) in psoriatic patients [33–35], it is worth to investigate the influence of vitamin D on TLR expression in psoriasis. It is of interest to note that our present study showed that the expression of both TLRs in psoriasis controls was lower than that of normal monocyes. Since psoriasis is an inflammatory disease that is mainly confined to skin, peripherally shifted mononuclear cells could exhibit higher expression of TLRs, if it was observed in lesional skin. Together with our present data, immunohistopathological correlation from the psoriatic tissue specimen could provide more detailed proof about the role of TLRs in chronic inflammation of psoriasis.

This is a cross-sectional study. Therefore, a causal effect of 25(OH)D3 concentrations could not be established on the basis of these data. Intervention studies are needed to establish whether increased intake of vitamin D can reduce TLR levels, biologic clinical markers and inflammatory symptoms. Since BD is a chronic inflammatory multisystemic disorder, we measured TLR2 and TLR4 in peripheral blood monocytes and synovial tissue macrophages: involvement of vitamin D response element. J Cell Biochem 2006;98:292–33.

In this study, we showed higher expressions of TLR2 and TLR4 with a trend towards lower 25(OH)D3 levels in active BD patients. The facts that these variables were inversely correlated and that vitamin D dose-dependently regulated TLRs in vitro could strengthen the notion about immunomodulatory effects of vitamin D on innate immunity-mediated inflammation in BD. However, to consider the clinical use of vitamin D as a biomarker of disease activity or therapeutic application in BD, more studies are needed to clarify the following: mechanisms by which vitamin D regulates TLR2 and TLR4; the optimal amount of vitamin D necessary for immune-regulation; and whether there are any genetic reasons of why either production of 1,25(OH)2D3 from vitamin D or signalling through the VDR is altered in certain BD patients.

Rheumatology key messages
- The higher expression of monocyte TLRs in active BD showed inverse correlation with serum 25(OH)D3 levels.
- Vitamin D may have an immunomodulatory effect on innate immunity-mediated inflammation in BD.

Role of vitamin D in affecting TLR expression in BD

Acknowledgements

Funding: This work was supported by a grant No. R01-2004-000-10731-0 from the Basic Research Program of the Korea Science & Engineering Foundation.

Disclosure statement: The authors have declared no conflicts of interest.

References


