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Expression of Human Toll-Like Receptor Genes and Vitamin D Receptor Gene Variants in Behçet's Disease

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Abstract: Behcet's Disease is a multifactorial condition, to which several genes may confer susceptibility. Several studies indicate possible physiological immune function of vitamin D, suggesting that vitamin D deficiency could lead to immune malfunctioning. The variation in the ability to synthesize vitamin D, including polymorphisms in the VDR gene, may be a contributing factor to increase BD susceptibility. Recent studies demonstrate higher constitutive expression of TLR2 and TLR4 in blood monocytes derived from chronic inflammatory disease patients that from healthy controls. In vitro analysis showed that vitamin D3 was found to dose-dependently suppress the protein and mRNA expressions of TLR2 and TLR4.
Keywords: Toll-Like Receptor, Vitamin D Receptor, Behcet Disease

1-Introduction

Behcet's Disease (BD) is a chronic inflammatory disease with exacerbations and remissions characterized by recurrent orogenital ulcerations, ocular manifestations, arthritis, and vasculitis. In addition, neurological and large vessel involvement can occur in some cases (1). The etiology and pathogenesis of BD have not been clearly defined. However, several genetic, environmental, and immunological factors have been suggested as causative factors in this disease (2). Cases of BD seem to cluster along the ancient Silk Road, which extends from eastern Asia to the Mediterranean basin. The prevalence is 80 to 370 cases per 100,000 populations in Turkey, 10/100,000 in Japan and 0.6/100,000 in Yorkshire. European cases are more often described, not exclusively in the migrant population. BD is a multifactorial condition, to which several genes may confer susceptibility. These genes have not yet been fully identified. In common with ankylosing spondylitis and psoriatic arthropathy, BD has MHC class I

associations. HLA-B51 is the most strongly associated known genetic factor to BD (3). One hypothesis is that an infection contributes to the development of BD in individuals with genetic susceptibility factors, leading to Th1 stimulation with abnormal activity of neutrophils and endothelial cells [8-28]. In patients with BD, CD4+ and CD8+ T cells produce proinflammatory cytokines such as IL2, IL6, IL8, IL12, and TNF, which are found in high concentrations in the serum. Peripheral blood Th1 counts are increased in correlation with disease activity. Th1 infiltrates are found within damaged tissues (4)

2-Vitamin D

Vitamin D has long been known to be important for bone health and turnover (5). In recent studies, vitamin D has been demonstrated to play a significant role in malignancy immune system functioning, and cardiovascular events (6-7). The pathogenesis of vitamin D deficiency is not clearly understood. Several factors, such as impaired calcium and vitamin D intake, malnutrition, aging, smoking, alcohol consumption, inadequate sunlight exposure, hypogonadism, corticosteroid therapy, and impaired physical activity, may play an important roles in the etiopathogenesis of vitamin D deficiency.Increased metabolism or impaired 25-hydroxylation inducedby medication or by disease involvement may affect vitamin Dvalues. In addition, the inhibition of 1-a-hydroxylase by TNFamay contribute to lower vitamin D levels (8) Several studies indicate possible physiological immune function of vitamin D, suggesting that vitamin D deficiency could lead to immune malfunctioning (9) 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 (10-11).

3-Toll-like receptors

Toll-like receptors (TLRs) are crucial players in the initiate immune response to microbial invaders, enabling vertebrates to detect the pathogen-associated molecular patterns (PAMPs) early and subsequently activating the adaptive immune response (12). Among 11 members of the TLR family, TLR2 and TLR4 have been identified as signaling receptors activated by bacterial wall components, such as lipoteichoic acid (LTA) from Gram-positive bacteria and lipopolysaccharide (LPS) from Gram-negative bacteria and. 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 (13) The extensive release of TLR-triggered pro-inflammatory mediators may harm the host as in cases of sepsis or chronic inflammatory disease (14) 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 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 (15,16). human TLRs are type I transmembrane proteins with an extracellular leucine-rich repeat (LRR) domain and a cytoplasmic carboxy-terminal Toll-interleukin 1 receptor (TIR) domain Based on the chromosomal localization, genomic structure and amino acid sequences, the human TLRs can be divided into five subfamilies: TLR2, TLR3, TLR4, TLR5, and

TLR9. The TLR2 subfamily consists of TLR1, TLR2, TLR6, and TLR10, the TLR9 subfamily is composed of TLR7, TLR8, and TLR9. TLR3, TLR4, and TLR5 are represented only by one family member, respectively. TLR1 and TLR6 genes are located closely to 4p14, TLR2 maps to 4q32, while TLR3 is located near TLR2, at 4q35. TLR4 resides on 9q33-35, whereas TLR5 is at 1q33.3 TLR7 and TLR8 are located as a tandem in Xp22, TLR9 maps to 3p21TLRs are classified as members of the IL-1R (IL-1 receptor) superfamily on the basis of a shared cytoplasmic region known as the TIR (Toll/IL-1R) domain. The extracellular portions of TLRs are rather diverse comprising varying numbers of leucine-rich repeats. Following encounter with a microbe, TLRs trigger a complex cascade of events that lead to the induction of a range of proinflammatory genes (17-19). There is convincing evidence that common TLR SNPs regulate cellular signaling events and cytokine production. The best evidence includes studies of TLR2 R753Q, TLR5 R392*, TLR1 I602S and TIRAP S180L. Signaling effects from SNPTLR4D299G have varied, possibly due to the use of different cell types and assays. A number of genetic association studies suggest that TLR polymorphisms may be associated with susceptibility to different diseases (20). Among TLR family members, TLR4 has been the most exhaustively investigated; it has been shown to be a principal receptor for lipopolysaccharide (LPS)-recognition. Single nucleotide polymorphisms (SNPs) in TLR4 have been reported to be associated with endotoxin hypo-responsiveness and Gram-negative infections, and they affect the risk for various inflammatory diseases, such as atherosclerosis, Crohn's disease, ulcerative colitis, prostate cancer, RA and BD (21-28). A markedly higher expression at the mRNA and protein level of TLR2, TLR3, TLR4, and TLR8 was observed in active BD patients as compared with controls. Significantly higher levels of IL-1β and IL-23 were detected in the supernatants of monocytes stimulated with LPS or PGN. A significantly higher level of IL-17 was observed in the supernatants of naïve T cells and monocytes stimulated with LPS or PGN in BD patients as compared with controls. Upon stimulation with R848 or PolyI:C, the levels of IL-17 in the supernatants of naïve T cells and monocytes and IL-23 levels in the supernatants of monocytes were not different between BD patients and controls.(29) The TLR4 gene has been well investigated in the context of RA (26,30,31), and arthritis classified as one of the minor symptoms of BD. In fact, 40% of the BD patients have the complication of arthritis, and it is the most common minor symptom (32). 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 impaired by nucleotide polymorphisms within the TLR genes, resulting in an altered susceptibility to infectious or inflammatory diseases (26) however, Boiardi et al show that the TLR4 gene polymorphisms are not associated with susceptibility to clinical expression and severity of BD in Italian patients(33).Also, there were no any association between TLR2 polymorphisms and individual's susceptibility to BD in Japanese patients, and Ben Dhifallah et al found that SNPs in the TLR2, 4 and 9 genes were not significantly associated with susceptibility to BD. Bacanli et al. (27) could not detect any significant difference in the TLR2 Arg753Gln polymorphism between BD and normal volunteers; nevertheless, the possibility of TLR involvement in the aetiopathogenesis of BD could not be eliminated (33). Do et al. 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.

4-Vitamin D receptor gene polymorphisms

The VDR belongs to the steroid and thyroid hormone receptor family of ligand-activated transcription factors. The VDR mediates the effects of 1,25-dihydroxyvitamin D (1,25(OH)2D) on gene expression (34). The gene encoding the VDR is located on chromosome 12cen-ql2 (35), contains 14 exons (36), and spans approximately 75 kilobases of genomic DNA (37). Exons IA through IF encode the 5' untranslated region, exons II and III encode the DNA-binding domain, and exons FV-IX encode the ligand-binding region. The expression of the human VDR is under complex transcriptional control by multiple tissue-specific promoters (36.38). Several common allelic variants have also been identified in the VDR gene and are the focus of the present review. The presence of a T/C transition polymorphism (ATG to ACG) at the first of two potential translation initiation sites in exon II (39) has been defined using the Fokl restriction endonuclease (40). Individuals with the C allele (designated F) initiate translation at the second ATG site and lack the three NH2~ terminal amino acids of the full-length VDR protein). In contrast, individuals with the T allele (designated f) initiate translation at the first ATG site and synthesize the full-length (427 amino acids) VDR protein (41). Bsml (42) and Apal (43) restriction site polymorphisms occur in the intron separating exons VIII and IX. A T/C nucleotide substitution (ATT to ATC) leading to a synonymous change at codon 352 (isoleucine) in exon IX has also been described (44) and is detected by the restriction enzyme Taql. Even more recent attention has focused on the possible role of these variants in the development of auto inflammatory and other disease related to immune system. The association or not of BsmI,ApaI and TaqI VDR polymorphisms withBD in several geographic areas was discussed.(45-54)BsmI,ApaI and TaqIVDR polymorphisms do not appear to change VDR gene expression or VDR function. Diseases associated with these polymorphisms are therefore most probable caused by linkage disequilibrium with other functional variation within the VDR gene or with another closely linked gene or genes (55). The FokIVDR polymorphisms was associated with susceptibility to BD in Iranian population, similar to the study of association between type 1 diabetes mellitus and the FokI VDR polymorphism in Italy (56) and with Autoimmune Addison's disease in Germany (57).In IranianAzari population, a significant relationship was observed between f allele and Behçet's disease. However, no associations were found between Multiple sclerosis and the FokIVDR polymorphism in Netherlands (58) type 1 diabetes mellitus and the FokI VDR polymorphism in Iran (59), Portugal (60) and Germany (61).

5-Conclusions

BD is a multifactorial condition, to which several genes may confer susceptibility. These genes have not yet been fully identified. BD is classified among the vasculitides .The FokI site polymorphism in exon 2 of the hVDR gene has two variants differing from each other by three amino acids: the f/ M1 variant has 427 amino acids and the F/M4 variant 424 amino acids. The shorter (F/M4) receptor seems to be originated from the divergence of hominids from apes and has been called a“neomorph”, yet it actually includes about 65 Percent of VDR gene alleles inhuman subjects. This predominance of the F/M4 allele indicates an evolutionary advantage

inhuman (62-71). Accordingly, the FokI variant remains acandidate functional polymorphism; the f allele isoform interacts with the basal transcription factor HB less efficiently than does the F allele isoform, providing a possible mechanism for the reducedtransactivation associated (connected) with this allele (72). Also, Colin et al, confirm the higher activity of the 424 aa short VDR variant, found thatphytohemoglutinin stimulated growth of peripheralblood monocytes differs by FokI polymorphism. They found that the one-half maximal concentrationfor 1,25(OH)2 vitamin D inhibition of phytohemoglutinin-stimulated growth was noticeably higher for cells containing the full-length VDR isoform (i.e., Ff and ff genotypes) than for those with the shorter isoform (FF genotype) (73). Noticing the fact that recent studies have shown the immunemodulatoryeffect of vitamin D3 through the downregulation of Toll-like receptor (TLR) expression in human monocytes. Inflammation triggered through TLR2 and TLR4 is important in the pathogenesis of BD. Nevertheless, serum 25-hydroxyvitamin D levels are decreased in patients with BD (74). Thismay be linked to vitamin D receptors functional polymorphisms. These factors together may Increase susceptibility and promote the development ofclinical manifestations of BD. Recent studies demonstrate higher constitutive expression of TLR2 and TLR4 in blood monocytes derived from chronic inflammatory disease patients than that from healthy controls (75,76). monocytes of active BD patients show 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. (77). It seems that vitamin D may be useful as a therapeutic option for inflammation triggered through TLR2 and TLR4, which are important in the pathogenesis of Behcet's Disease.

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