Vitamin D Nuclear Receptor and Periodontal Disease: A Review

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Abstract
The immune system has acquired an intricate mechanism for recognizing and eliminating pathogens. Situated in the nucleus of numerous cells, Vitamin D nuclear receptor represents an important component of this mechanism. Correspondingly pathogens have also acquired complex mechanisms of evading and manipulating the host immune response to their benefit, thereby increasing their ability to cause disease. One such pathogen is Porphyromonas gingivalis which is able to exploit several components of the innate and adaptive immune system and persist in macrophages. This manuscript reviews current concepts regarding immune responses to periodontal pathogens and provides a link between Vitamin D nuclear receptor and P. gingivalis. The authors offer a rational for further investigations of Vitamin D nuclear receptor agonists as potential therapeutic agents for resolution of inflammation in periodontal disease coupled with enhanced intracellular clearance of bacteria.

Keywords: Vitamin D nuclear receptor; Periodontal inflammation; VDR agonist; Periodontopathogens; P. gingivalis

Introduction
Vitamin D has received increasing attention in recent years beyond its customary role in bone metabolism. There has been an explosion of scientific evidence which suggests the profound role that vitamin D plays in immune homeostasis. Vitamin D nuclear Receptor (VDR) has been identified as a ligand-dependent transcription factor which, in concert with its endogenous ligand, 1,25 hydroxivitamin D (1,25(OH)2D3), is involved in the expression of countless genes [1]. In view of recent research which revealed that most immune cells express the Vitamin D nuclear receptor (VDR) [2-5], the authors reviewed the current knowledge regarding the immunomodulatory effects of vitamin D through its VDR on periodontal disease.

This manuscript provides a viable link between immune responses to periodontal pathogens and VDR and proposes a possible immunomodulatory treatment strategy for fine-tuning the host’s immune response against periodontopathogens that would diminish the destructive aspects of the response, while boosting the defensive responses to periodontal disease.

Materials and Methods
Data collection for this review manuscript was web-based. Literature review was conducted systematically using search engines such as PubMed, J-STOR, EBSCO, Elsevier, Medline Science Direct and other search engines.

Vitamin D nuclear receptor

Brief history of Vitamin D and Vitamin D synthesis: Numerous scientists played a role in the lengthy discovery of Vitamin D from 1919 to 1945 [6]. Amongst these, Windaus in collaboration with Hess, Rosenheim and Webster played a crucial role in determining the chemical properties of ergosterol-D3 [6] After being awarded the Nobel Prize for chemistry in 1928 "for his studies on the constitution of the sterols and their connections with vitamins" [7], Windhaus and Bock went on to isolate a compound 7-dehydrocholesterol in 1935 and deciphered the photochemical reaction of cholecalciferol-D3 [8].

Vitamin D3 is synthesized from 7-dehydrocholesterol present in the skin, which is hydroxylated to 25-hydroxycholecalciferol (25(OH)D3) in the liver by mitochondrial cytochrome P450 enzyme also known as (CYP450) encoded by CYP27A1 gene. This is a sunlight dependent process, by ultraviolet light B (UVB) at 270-300 nm [9]. The other source of vitamin D is obtained from food or dietary supplements in the form of vitamin D2 ergocalciferol. Both forms of vitamin D are hydroxylated in the liver [9]. The next process takes place in the proximal tubules of the kidney, where 1-α-hydroxylase, encoded by CYP27B1 gene, transform 25(OH)D3, into the hormonal active form 1,25-dihydroxycholecalciferol (1,25(OH)2D3) [10] Resident immune cells and cells of the gastrointestinal tract, amongst others, express the gene CYP27B1 [11].

The VDR gene is positioned on human chromosome 12q and consists of a promotor, a regulatory region and eight axons responsible for encoding 427 amino acids that compose the VDR protein [10,12]. VDR consists of several domains which facilitate translocation to the nucleus [13]. Following passive diffusion across the cell membrane, 1,25(OH)2D3 exerts immunoregulatory and anti-inflammatory properties by binding to vitamin D response elements (VDRE) in vitamin D responsive genes, also known as DNA binding domains (DBD) which recognize a particular DNA sequence present in regulatory regions of primary 1,25 (OH)2D3 responsive genes which belong to the nuclear receptor superfamily [10].

VDR is thus a ligand-dependent secosteroid transcription factor that functions as a heterodimer with retinoid X receptor (RXR) for which it has a very high affinity, even though it is able to dimerize with other nuclear receptors [10,14]. It has been established that VDR-RXR heterodimers have the capacity to either induce or repress gene transcription [15].

VDR expression in innate and adaptive immunity: As more evidence emerges, it is becoming clear that VDR is involved in...
synchronizing interaction between the innate and adaptive immune systems by tightly regulating their activities. The innate immune system is regarded as the first line of defense against bacterial pathogens, including periodontopathogens [16]. VDR inhibits the differentiation, maturation and immune stimulating ability of dendritic cells by down-regulating the expression of Major Histocompatibility Complex class II molecules [17]. This inhibition maintains dendritic cells in an immature state that present phenotypes that promote T-cell tolerance [17]. At the same time, VDR suppresses interleukin (IL)-12 production and enhances IL-10 production in dendritic cells [17]. VDR regulates the production of T-cell helper 1 and 2 cytokines and IL-17, by which it influences adaptive immunity and inflammation [18]. Furthermore specific T-cell cytokines are able to influence the TLR-induced VDR-dependent antimicrobial pathway in human monocytes. The TH1 cytokine interferon γ enhances the TLR2/1 induction of CYP27B1, cathelicidin [18]. Cathelicidins have significant broad antimicrobial activity against both gram-positive and gram-negative bacteria [17].

Th1/Th2/Th17 paradigm: For decades the Th1/Th2 paradigm was utilized to explain chronic inflammation associated with periodontal disease [19]. Comprehensive descriptions regarding the role of Th1 and Th2 cytokines in periodontal inflammation are available elsewhere and will thus not be reviewed in this manuscript [20,21]. More recently, another subset of the Th cells, identified as Th17 has been added to this paradigm and has helped in elucidating some of the weaknesses in the original Th1/Th2 theory [19,22]. Th17 cells have been detected in periodontal epithelia and these cells secrete IL-17 which, in turn, induces the production of IL-6, IL-8 and prostaglandin, as well as the activation of nuclear factor kappa B ligand (RANKL) in osteoblasts, thereby inducing osteoclastic bone resorption [19,23]. Following binding to its receptor, RANK, RANKL steers the differentiation to a mature osteoclast that displays resorptive capacity in bone [19]. To counteract this, Osteoprotegerin (OPG) receptor acts as a “decoy receptor” that is able to directly bind to RANKL, thereby inhibiting osteoclast maturation through inhibition of RANKL/RANK complex [19]. As a result, OPG acts as shielding cytokine that prevents bone resorption [19]. Worthy of note is the finding that IL-17 favours bone loss through modulation of RANKL/OPG ratio in favour of RANKL expression in osteoblasts [24]. To sum up, Th17 in unison with Th1 cells participate in destruction of tissue and bone in periodontal disease.

VDR thus plays a crucial immunomodulatory role by inhibiting antigen induced T-cell proliferation such as Th1 and Th17 as well as cytokine production [10].

Periodontal disease and immune subversion strategies of periodontopathogens: Periodontal disease is a chronic inflammatory disease that causes tissue damage and steadily leads to alveolar bone loss and ultimately, tooth loss [25]. This gradual destruction of bone is owed to the periodontopathogens that produce an immune response [26]. Traditional management of chronic periodontal disease was directed towards the mechanical removal of the bacterial agents; however this approach did not provide considerable clinical improvements [26]. More recently, management of chronic periodontal disease has been focused on augmented vitamin D supplementation owing to the fact that 1,25(OH)2D3 has been recognized as an essential immunomodulatory agent [17]. Clinical observations that 25(OH)D levels were reduced in periodontal disease and the assumption that low levels of 25(OH)D must be the cause of periodontal disease prompted its clinical administration [17,27]. In fact, the general administration of 25(OH)D in infectious disease may disturb the immune response by hindering the functions of vitamin D nuclear receptor (VDR) [28,29]. Elevated 1,25(OH)2D3 have also been shown to bind to pregnane X nuclear receptor thereby impeding the capacity of CYP24A1 to control the levels of 1,25(OH)2D [28,29]. Consequently conversion of 25(OH)D in the liver is inhibited which leads to the clinical expression of low levels of 25(OH)D encountered in inflammatory diseases [28,29].

In order to achieve the desired long-term therapeutic effects, development of immunomodulatory treatment strategies are required. Periodontal epithelium provides the primary barrier to infection and has an active role in the innate host defences against periodontopathogens [30]. The "red-complex" group of periodontal pathogens exhibits the strongest correlation to periodontitis [31]. This group comprises of Porphyromonas gingivalis, Tannerella forsythia and Treponema denticola. The front-runner of this group is considered to be Porphyromonas gingivalis that is assumed to be a “keystone species” as it “serves an essential function for the entire community, similar to a differentiated cell serving a function for an entire tissue” [32]. P. gingivalis is an obligate anaerobe, biofilm producing bacterium that presents several virulence factors, amongst others, atypical lipopolysaccharides (LPS), fimbria and cysine proteins also known as gingipains [33]. P. gingivalis is typically known for its ability to form a subgingival bacterial biofilm within the periodontal pocket [34]. P. gingivalis is able to persist within macrophages by evolved strategies that subvert the host’s immunity and secures a niche by manipulating key features of innate immunity, particularly Toll-like receptors and the complement system which have direct roles in adaptive immunity [34-36]. It has been demonstrated that P. gingivalis has the capacity to cross epithelial barriers and has been detected in remote sites in the human body [37]. It is thus strongly implicated in distant systemic inflammatory responses in several chronic diseases, such as atherosclerosis [38]. P. gingivalis express unfamiliar LPS molecules that can directly antagonize TLR4 [39]. This in turn impedes the expression of antimicrobial peptides such as β-defensins in the host’s epithelial cells [39]. P. gingivalis also has the capacity to hinder TLR4-mediated immune response against other similar bacteria thereby facilitating a niche for other periodontopathogens [39].

P. gingivalis is recognized by the innate immune system mainly through TLR2, but P. gingivalis is unable to antagonize TLR2 directly [32]. Interestingly, P. gingivalis has developed undermining interactions between TLR2 and other receptors of the innate immune system which allows it to indirectly diminish the TLR2 antimicrobial response [32]. Through complex inside-out signalling of TLR2, P. gingivalis binds to transactivated CR3 of the compliment system through its fimbria, thereby stimulating phagocytosis by macrophages [40]. CR3 mediated phagocytosis is not associated with a robust antimicrobial mechanism and thus does not stimulate lysing of P.gingivalis once inside the macrophage and, as a result it is able to chronically persist within these immune cells [41].

P. gingivalis and Th1/Th2 response: CD4+ T-cells mediate immunity against pathogens by modulating the Th1 or Th2 type response [19]. P. gingivalis appears to primarily induce a Th-1 cell type response [14]. In addition to the Th-1 cell type response, it has been shown that the protein located on the membrane of P. gingivalis also has the ability to significantly increase the secretion of IL-17, which represents the principal cytokine secreted by TH17 cells within the periodontium [19,33]. P. gingivalis is able to induce Th-17 differentiation by various mechanisms [33]. One such mechanism is the expression a specific virulence factors which, through TLR2 ligands signalling, activate NFKB-dependent proinflammatory pathways in monocytes and dendritic cells [33]. P. gingivalis also influences the release of Th17 cytokines, namely IL-1β, IL-6 and IL-23 [33]. Th-17 development is driven predominantly by monocytes and dendritic cells [33].

Thus insufficient clearance of intracellular bacteria coupled with a deregulated release of pro-inflammatory cytokines in considered as a trigger of periodontal disease [42].

VDR and \textit{P. gingivalis}

It has been demonstrated that VDR plays an important role in both the innate and the adaptive immune response to \textit{P. gingivalis} [14]. Of significance is the fact that VDR deficient mice are increasingly more susceptible to infection with \textit{P. gingivalis} and are unable to mediate intracellular lysis of bacteria and hence show an increased bacterial burden [14]. Both VDR gene polymorphism and Vitamin D resistant rickets has been linked to a greater susceptibility to aggressive periodontitis and VDR gene risk marker is being used as a vulnerability test to periodontal disease [14]. VDR has also been shown to inhibit IL-8 expression induced by \textit{P. gingivalis} in periodontal ligament cells and thus has anti-inflammatory effects in periodontal disease [43].

VDR agonists

It is currently recognized that VDR has a modulatory effect on the immune response against \textit{P. gingivalis} through its anti-inflammatory role and the fact that it aids in intracellular clearance of the bacteria. The biological activity of Vitamin D is thus dependent both on the adequate production and distribution of 1, 25 (OH)\_2 D\_3 as well as adequate expression of VDR [1]. In individuals with functioning VDR, 1,25(OH)\_2 D\_3 has been considered a therapeutic agent for the treatment of periodontal inflammation[43]. But the high levels of 1,25(OH)\_2 D\_3 that are needed in order to bring about an adequate immunomodulatory response, result in unwanted hypercalcemia due to up-regulation of calcium ion channel transient receptor potential vanilloid type 5 and 6 (TRPV 5-6) through VDRE [9,43]. Duodenal TRPV6 has been identified as an important mediator of 1,25(OH)\_2 D\_3, leading to calcium absorption from the intestine [9]. Investigations carried out on inflammatory and autoimmune diseases have elucidated a VDR agonist, elocalcitol, as a potent VDR agonist and potential therapy that is able to ameliorate abnormal inflammation without calcium increasing capacity [9,44]. Elocalcitol exerts an anti-inflammatory as well as a regulatory effect on both the innate immune response and the adaptive immune response [9]. Elocalcitol interferes with the differentiation of dendritic cells, thereby preventing their maturation and modulates Th17 cells by inhibiting the production of IL-17 [9]. Furthermore, elocalcitol down-regulates pro-inflammatory Th\_1 cells and has the potential to augment the development of Th2 cells thereby moderating tissue damage in chronic inflammation [9]. Such or similar agonists may be used in ameliorating the inflammatory response in periodontitis due to the fact that VDR has been directly implicated in immune response to \textit{P. gingivalis} [14]. Moreover, inflammatory cytokines, amongst others, IL-17 provide a viable link between periodontal disease and systemic disease [19]. Periodontal disease is also considered a risk factor for an assortment of systemic diseases ranging from exacerbated cardiovascular disease, to rheumatoid arthritis [19,45]. Understanding the degree to which VDR agonists are capable of regulating inflammatory cytokines involved in both oral and systemic diseases whilst enhancing the intracellular lysing capacity of cells in the immune system would thus be of great therapeutic significance in periodontal disease as well as a wide-range of systemic disease.

Summary

Regulation of VDR is a shared defence mechanism against periodontopathogen \textit{P. gingivalis} of both the innate and adaptive immune system. It regulates the functions of dendritic cells and stimulates the secretion of antimicrobial peptides at the site of infection and appears to largely inhibit pro-inflammatory pathogenic T- cells subsets and thus mediate inflammation and tissue damage.

Chronic inflammation of the periodontal tissues has been demonstrated to have significant negative effect on the overall health of individuals suffering from the disease. It is thus apparent that adjusting the activity of VDR clinically may play an important role in shaping the immunomodulatory effects of vitamin D as well as on the overall health of patients by decreasing the activity of cytokines such as IL-17 which are important links between oral and systemic disease.

Periodontitis is a chronic disease confounded by several factors, including environmental and genetic factors that play a part in disease pathogenesis. The current understanding of the link between VDR and \textit{P. gingivalis} highlighted in this article could nevertheless facilitate the development of effective designs for augmenting VDR expression and activity through potent VDR agonists, fine-tuned for minimizing periodontal associated inflammation.

These observations provide a new research arena for VDR agonists with potent anti-inflammatory properties without adverse systemic effects to be used in the anti-inflammatory treatment of periodontal disease.

References
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