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A LITERATURE REVIEW ON THE ROLE OF VITAMIN D IN HOST RESISTANCE TO INFECTION AND MEMORY

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ABSTRACT

Vitamin D is an essential nutrient to the body and its role as a major player in calcium homeostasis has been well established. Interestingly, vitamin D has been receiving increased attention in the immunology field. 1,25D3 has been shown to ameliorate symptoms of autoimmune disorders like inflammatory bowel disease (IBD), multiple sclerosis (MS), and rheumatoid arthritis (RA), through immunosuppression of auto reactive cells of the immune system. How this immunomodulatory capability of vitamin D could influence the outcome of an immune response in the event of an infection forms the crux of this review. A better response to an infection increases the host resistance to that infection. A robust response could be looked upon as the one that not only helps in effective and timely clearance of that infection, but also leaves a good memory repertoire in the event of a secondary infection. The immunomodulatory function of this vitamin can be addressed in two phases: Its effect on innate immunity and its effects on the adaptive immunity. The review attempts to look at studies that are targeted at the different branches of the immune system and how they could influence the behavior of these cells before, after or during the course of an infection.

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Chapter 1

Introduction

Vitamin D_3 also known as cholecalciferol , whose structure is closely related to steroids, features under a separate category called secosteroids, that slightly differ from the molecular structure of conventional steroid hormones[1] . Vitamin D is hydroxylated in the liver and the kidney to finally yield the active form of vitamin D, 1, 25$(OH)_2D_3$ (1, 25 D3), that executes its functions. Vitamin D_3 signaling proceeds through the Vitamin D receptor (VDR) that heterodimerizes with the intracellular receptor, retinoid X receptor (RXR). Together, the receptor complex binds through DNA binding elements[1], to vitamin D response elements (VDRE) thereby influencing gene expression. Apart from its widespread effect on calcium homeostasis in the body, it is increasingly being recognized as a potential immunomodulator in both autoimmune disease and infection. Indeed, vitamin D levels have been correlated with susceptibility or resistance to infection. Low vitamin D levels have been implicated in susceptibility to infections of the human immunodeficiency virus(HIV)[2], *Mycobacterium tuberculosis*[3], and viral infections of the respiratory tract[4].The active form of vitamin D has been reported as an anti-mycobacterial agent[5]. The protective role of vitamin D in *Mycobacterium tuberculosis*infection was addressed by Liu et al, 2006 and they reported that individuals with low levels of vitamin D[6] are more susceptible to *Mycobacterium tuberculosis*infections, thereby increasing the incidence of tuberculosis. Vitamin D has also been shown to activate facets of the innate immune response, like production of antimicrobial peptides in response to an infection. Moreover, recent findings have also proposed a role of vitamin D in modulation of adaptive immune responses, related to B cells and T cells. This review focuses on evidence for the evidence for the action of this hormone 1, 25$(OH)_2D_3$ on the branches of the immune system - innate and adaptive. In response to an infection, the

response is initiated by the innate immune system, which subsequently activates the adaptive immune system. The review also discusses data on Vitamin D and signals that are likely to play a role in memory cell generation.

Chapter2

Innate immune system and vitamin D

The innate immune system is the first facet of the immune response that gets activated in response to an infection and forms one of the main mediators of developing host resistance to infection. It contains a repertoire of effector cells that are not specific to any one pathogen, but rather broadly recognize general pathogen associated patterns (PAMPs, pathogen associated molecular patterns) that are similar between various pathogens through receptors known as pattern recognition receptors (PRRs). Toll-like receptors (TLRs) are one of the well-known PRRs. The main components of the innate immune system are the phagocytic cells (neutrophils, monocytes, macrophages). In addition to the phagocytic cells, there are also cells that belong to the innate immune system but originate from lymphoid lineages. The cells that originate from the lymphoid lineage but perform innate functions are the natural killer cells, γδ T cells and natural killer T cells which recognize viral antigens in cells. The innate immune system is also capable of recognizing unconventional antigens like phosphate antigens, expressed by pathogens throughnatural killer cells, γδ T cells and natural killer T cells. The innate immune system is concerned with recruiting various effector cells to the site of infection as defined by a specific order. The first cells (neutrophils) secrete important molecular cues or cytokines and recruit other cells. Neutrophils and macrophages are crucial in anti-pathogenic activity, phagocytose antigen and activate killing mechanisms within them.The other cells of the innate immune system are important in secreting cytokines. And lastly, the innate immune system also serves to trigger the adaptive facet of the immune system. It serves to “present” antigen through professional antigen presenting cells (APCs) to activate antigen specific B cells and T cells through MHC molecules. The following sections focus on the function of each cell type and discuss evidence for the immunomodulatory function for vitamin D on these cell types. This could help in the understanding of the role of vitamin D in the outcome of an infection.

Vitamin D and pathogen recognition

Pathogen recognition through PRRs is the most crucial initial step in initiating any kind of immune response. PRRs mediate the recognition of pathogens through various subtypes of receptors, the ones mostly in discussion are TLRs, expressed by many cell types, including DCs and macrophages. 1,25D3 has been traditionally shown to downregulate TLR expression in 1, 25 D3 treated monocytes[25], which could be detrimental in pathogen recognition. On the contrary, there is evidence that 1, 25 D3 is likely to not downregulate TLR expression or signaling, wherein pulsation of DCs with TLR ligand and 1, 25 D3 was not shown to decrease DC function[5774]. Moreover, there is evidence that supports a likely role for vitamin D in facilitating pathogen recognition though CD14, a comolecule mediating detection (along with the associated TLR) of LPS[8].An inhibition of CD14 has been implicated in the development of Crohn's disease[7]. Incidentally, a study published in 2007, shows that 1, 25 D3 drives the expression of CD14[8]. More recently, 1, 25 D3 has also been shown to increase the expression of another kind of PRR, NOD2[9]. NOD2 is an intracellular PRR, and recognizes bacterial peptidoglycans, leading to proinflammatory responses through NfKB mediated signaling[10]. Though there is evidence for TLR downregulation, there is increasing evidence that 1, 25 D3 is likely not to antagonize PRR associated signaling. Interesting evidence for the role of 1, 25 D3 in inducing other PRRs like NOD2 seems worthy of investigation.

Vitamin D and neutrophils

Neutrophils are the first phagocytic cells to reach the site of infection. These recognize and respond to various chemokines and other inflammatory mediators (secreted by tissue resident DCs and macrophages) and reach the site of infection post activation to exert phagocytic activity. Neutrophils are known to express TLRs on their surface and get activated. Different TLR ligands elicit different activation mechanisms in neutrophils[11]. Upon TLR-mediated signaling neutrophils get activated to exhibit increased phagocytic properties and carry out various effector functions such as releasing antimicrobial peptides, and generation of ROS upon phagocytosis, secrete various chemokine and cytokines, all facilitating pathogen killing[1213]. Takahashi K et alshow evidence for VDR upregulation in sorted and purified human neutrophils in the same levels as 1,25D3 treated monocytes, thereby suggesting a likely responsiveness of the neutrophil to 1,25D3[14]. Moreover, there was curtailed expression of IL-1β by neutrophils that were treated with 1,25D3[14]. Neutrophils are one of the pillars of the inflammatory response and IL -1β is important in activating lymphocytes and recruiting other cells of the immune system[15]. VDRE elements have been shown to exist in neutrophils, in the promoter of anti-microbial peptide genes[16]. Hence, it is likely that expression of antimicrobial peptides in neutrophils proceeds in the same way as macrophages and in macrophages 1,25D3 and VDR signaling is crucial in the expression of antimicrobial peptide expression[16].

Macrophages, vitamin D

Macrophages are important phagocytic cells of the immune system, and serve to internalize the pathogen, through cell surface receptors like TLRs and CD14. Under normal conditions, these circulate as monocytes and upon phagocytosis following pathogen challenge, monocytes get activated and form mature macrophages. The principal function of macrophages is to kill internalized pathogen through reactive oxygen species and other antimicrobial molecules like nitric oxide (NO) that serve to kill the pathogen. Nitric oxide production is catalyzed by the enzyme, iNOS (nitric oxide synthase). Moreover, macrophages also serve as effector cells of the innate and adaptive immune system, where they are activated by antigen-antibody complexes in a process called opsonization, which increases phagocytic activity manifold. Macrophages are instrumental in secreting proinflammatory cytokines like IL-12 and IFNɣ and various other cytokines that aid in activating other cells of the immune system[17]. Macrophages are known to produce antimicrobial peptides in response to infections, like cathelicidin and defensin[18]. Macrophages also serve in adaptive immune responses by processing and presenting antigens to T helper cells. It is worthy to examine the effect of vitamin D in the activation of macrophages, as this activation is one of the steps that aid in orchestrating pathogen killing and other immune responses. 1,25D3 has been shown to suppress production of iNOS in vivo in animal models for allergy[1920] and also in macrophage cell lines upon LPS challenge[21]. 1,25D3 is suggested to mediate differentiation of pluripotent hematopoietic stem cells into monocytic characteristics[2223]. CD34+ bone marrow hematopoietic cells have been shown to upregulate monocyte markers like CD11b and CD14 *in vitro*, with physiological concentrations of1,25D3 thereby suggesting that 1, 25D3 induces monocyte differentiation [23]. 1,25D3 has been proposed in promoting macrophage differentiation from leukemia cells[24]. 1,25D3 treated mouse leukemia cells exhibited macrophage characteristics[24]. It is likely that appropriate vitamin D levels are involved in maintaining healthy monocyte numbers in the body. However, more recent studies have suggested an

immunosuppressive role of vitamin D on macrophages. 1,25D3 has been reported to cause a donwnregulation of TLR in response to an infection in macrophages that were isolated from healthy human donors and cultured *in vitro*[25]. 1,25D3 treated macrophages, on stimulation with LPS or lipoteichoic acid, showed marked downregulation of TLRs coupled with impaired localization of Nf-κB in the nucleus[25]. Nf-κB is crucial as a transcription factor and is involved in inducing the expression of various proinflammatory cytokines like TNF alpha and other effectors that mediate the innate immune response[26]. Jan Ehrchen *et al* investigate the effect of ablation of the vitamin D receptor (VDR) in macrophages, in a *Leishmania major* infection model[27].*Leishmania major*-infected macrophages that were treated with 1,25D3 were shown to have a reduced capacity to clear off the infection[27]. Interestingly, studies on the associated signaling pathways seem to suggest that inflammatory pathways and VDR signaling pathways seem to converge to induce defensin expression in response to infection[28]. There is evidence that expression of the antimicrobial peptide, defensin, requires the combined action of the IL-1β and the VDR signaling pathways. The VDR signaling pathways and Nf-κB localization seem to act in conjunction, Nf-κB localization being equally indispensable in induction of defensin expression[28]. Indeed, the defensin gene consists of both Nf-κB and VDR response elements[28]. Moreover, since Nf KB is predominantly involved in inducing inflammatory responses, the data suggests that VDR signaling does not necessarily antagonize the inflammatory pathway through IL-1β and the subsequent Nf-κB localization to the nucleus, in the context of defensin expression. Previous results have indicated a possible blockade of Nf-κB signaling through 1,25D3 in other cell types[293031]. On one hand, there is evidence that 1, 25 D3 is immunosuppressive in monocytes thus controlling inflammatory responses elicited by monocytes[3225]. On the other hand, VDR signaling and inflammatory Nf-κB signaling do not seem to necessarily antagonize each otherin monocytes[33].These seemingly contrasting observations indicate that there could be more intricacies involved between 1,25D3 and VDR signaling. A recent study by Edfeldt *et al*focuses

on the effect of vitamin D and the 1alpha hydroxylase in infection through production of antimicrobial peptides like cathelicidin and defensin[34]. The studies suggest that predominantly inflammatory cytokines like IFN γ increase 1,25D3 levels through upregulating CYP27B1 expression in monocytes[34]. On the contrary, enhanced production of IL-4 seemed to induce the expression of CYP24A1, the catabolic gene that encodes the protein to clear off the active form of vitamin D[34]. The observation that Th1 and Th2 cytokines regulate 1,25D3 levels inversely suggests a feedback regulatory loop between cytokines and vitamin D.

Studies on macrophages have been done mostly *in vitro*, more *in vivo* information is necessary to elucidate the physiological role of vitamin D in the event of an infection, since an *in vivo* model has a lot of temporal and spatial variations in vitamin D availability and its signaling, which could serve as a better simulation to predict the exact role of vitamin D. In conclusion, it could be said that the seemingly contrasting observations of 1,25D3 in macrophages can be attributed to undiscovered intricacies involved in the regulation of the VDR signaling pathway.

Vitamin D and the autophagy pathway in macrophages: Autophagy is a mechanism through which damaged intracellular components are scavenged through autophagy-specific compartments or vesicles known as autophagosome in macrophages.Gutierrez *et al*reported autophagy has a mechanism that assisted in reducing mycobacterial loads, by assisting fusion of microbe-containing phagosomes with autophagosomes[35]. It would be incomplete to conclude the discussion on macrophages without discussing autophagy, a host defense mechanism that has been shown to be crucial in tackling bacterial invasions like *Mycobacterium tuberculosis*[36].Given the fact that autophagic mechanisms are only recently being focused upon, the role of vitamin D in autophagy induction-related molecular mechanisms, has garnered much interest. Edfeldt *et al* studied autophagy formation in relation to vitamin D following LPS challenge[34].Monocytes treated with 1, 25 D3 showed were significantly exhibited more number of autophagosomes, implying more autophagy[34]. Recently, there have been some studies that suggest a likely

divergence in autophagy mechanisms between primates (human) and non-primates (prime). There is no VDRE element in the promoter of CAMP in non –primates like murine models[37]. Thus, this suggests a difference inautophagy induction mechanisms between murine models and human models of mycobacterial infection, given that autophagy has been shown to be regulated by the vitamin D cathelicidin pathway in humans[38]. On the other hand, antimicrobial activity in the event of a *Mycobacterium tuberculosis* infection in a murine model requires activation pathway preceded by NO and its related intermediates[3940], which interestingly is suppressed by vitamin D as discussed earlier. In HIV1 infected human macrophages, 1,25 D3 induced autophagy[41]. Autophagy has been shown to inhibit HIV1 replication[41]. As a side note, HIV infection is curious in the sense that its viral packaging mechanism cross intersects with the autophagy induction mechanism[4243]. More recently, Dong-Min Shin *et al* investigate the mechanism of CYP27B1(Gene that codes for 1α hydroxylase to synthesize 1,25D3) expression in the context of autophagy induction and TLR signaling[4445]. CYP27B1 expression inhibition reduces autophagosome formation, increasing evidence for a substantial role for 1,25D3 in inducing autophagy following pathogen recognition[44]. Though there seems to be a possibility of various mechanisms in the induction of autophagy, the data nevertheless suggests a protective role for vitamin D in infections that require more intervention of innate immune mechanism like autophagy that respond to an infection.

Dendritic cells, vitamin D

Dendritic cells are a heterogeneous population that plays crucial roles in initiating immune responses to adaptive and immune responses. Upon pathogen invasion, DCs recognize them through pathogen recognition receptors (PRRs) and start secreting chemokines that recruit other innate immune cells like neutrophils, NK cells[46], thus aiding in containing the spread of infection. Upon this initial secretion, they attain a mature phenotype wherein they downregulate PRRs and exhibit upregulation of MHC and various costimulatory molecules that serve to activate B cells and T cells.Various kinds of dendritic cells have been isolated and studied, and these have been classified on the basis of the markers they express. An extensive review of the classification of dendritic cells has been done by Sao et al[47]. Broadly these can be classified as myeloid DCs and plasmacytoid DCs based on their myeloid and lymphoid lineages, respectively[47]. DCs express the VDR and also have been shown to express CYP27B1[48]. There is considerable evidence that vitamin D is immunosuppressive in DCs and has an inhibitory effect on DC maturation and promotes a tolerogenic phenotype[49]. 1,25D3 mediated DC functional inhibition has been shown to alleviate symptoms of autoimmune diseases like multiple sclerosis (MS) and inflammatory bowel disease (IBD)[5051], though a lot remains to be done on its role as a potential host defense agent[52].A universally immunosuppressive action of vitamin D on DC function, while beneficial for autoimmune diseases, could turn detrimental in the event of an infection. Lyakh *et al*have investigated the role of vitamin D in suppressing the maturation of human monocytes into CD83+ DCs[53].Monocytes upon pathogen challenge get activated and are known to mature into macrophages or dendritic cells depending upon the molecular cues they respond to. Monocytes cultured with 1,25D3 in the presence of LPS downregulated markers for maturity in DCs like CD83+ and costimulatory molecules CD80 and CD86, CD40, which could be detrimental during an infection[53]. Costimulatory molecules are important in triggering the adaptive arm of the immune system, failure of which could result in a weak immune response, and poorer host

resistance to infection. Moreover, secretion of inflammatory cytokines like IL-12 is shown to be reduced in the presence of 1,25D3 treatment and upon stimulation with LPS[53]. IL-12 is important in recruiting Th1 responses[54] and also mediated NK cell killing[55], which are both crucial in innate and adaptive immune responses to viral infections, like HIV[56]. A reduced secretion of IL-12 could mean crippled trigger of lymphocyte responses, in the presence of 1,25D3. More recently, there is evidence that vitamin D3 function might differ depending upon its temporal presence in the event of an infection[57]. There is evidence that the immunomodulatory activity of this 1, 25 D3 differs between its *pre*-antigen challenge (with LPS) and its *post*-antigen challenge (with LPS)[57]. Treatment of bone marrow dendritic cells with 1, 25 D3 *prior* to LPS challenge, crippled the expression of various pro inflammatory genes like IL-1β, IL-6, and also resulted in reduced expression of iNOS levels (nitrogen-oxide synthase, catalyzes NO production) which are important in recruiting other cells of the immune system during the infection[57]. This in vitro data seems to suggest that prior treatment with 1, 25 D3 is likely to "desensitize" DCs to produce curtailed functions, whereas treatment with 1, 25D3 *post* antigen challenge does not seem to have an effect on the expression of IL-1β, IL-6 and iNOS, suggesting that treatment with 1, 25 D3 might not have a suppressive effect after antigen –mediated signaling has commenced[57]. Thus, DC response in vivo upon differential stimulation with antigen or 1,25D3 is a question worthy to investigate.

1,25D3 has been shown to induce chemokine expression (CCL18) in dendritic cell in response to various antigen challenges like LPS, influenza virus, also jointly inhibiting the maturation process of dendritic cells[58]. CCL18 is a chemokine that is shown to attract naïve T lymphocytes[59]. Interaction of naïve T lymphocytes with an immature DC is shown to confer a regulatory phenotype on the naïve T lymphocytes[60]. Increased T regulatory cell activity curtails the extent of an immune response. This suggests that 1,25D3 treatment of DCs could mediate reduction of the immune response at various levels. It is worthy to mention vitamin D mediated

DC modulation is likely to depend on the specific subset of DC involved. 1,25 D3 has been shown to mediate tolerogenicity in myeloid dendritic cells and is likely to not have an effect on plasmacytoid DCs[61]. In a study that looks at 1, 25 D3 treatment of myeloid and plasmacytoid DCs, chemokines that have been known to activate effector T cells (CCL17) was downregulated due to 1, 25 D3 treatment of myeloid DCs, not plasmacytoid DCs[61]. Downregulation of IFN gamma and other costimulatory molecules was observed in myeloid but not plasmacytoid DCs[61]. However, some evidence does suggest that P-DCs are likely to be intrinsically tolerogenic in allergy models as well as infection[6263]. There is again evidence Plasmacytoid DCs are also crucial in eliciting immune functionthrough antigen presentation[64]. It is intriguing that 1, 25 D3 has a different or no effect on DC subpopulations, but it is difficult to predict that if such a differential behavior would be beneficial during an infection.

Current data suggests that 1, 25 D3 does have an inhibitory effect on DC maturation. Indeed, VDR deficient mice have been shown to have higher numbers of mature DCs[65]. Immature DCs have been shown to have enhanced chemokine secreting ability that results in the recruitment of other phagocytic effector cells of the innate immune system, like neutrophils and macrophages[46]. This could be helpful in certain scenarios because some of the pathogens necessitate the intervention of the innate immune system like mycobacterial infections[66]. It could be said that 1,25D3 is likely to have an effect on the innate part of the immune response orchestrated by DCs, but is likely to curtail the full spectrum on the ensuing responses.

Vitamin D and Natural killer cells

Natural killer cells have been implicated in being cytotoxic to tumor cells and virus infected cells. These do not express the conventional antigen recognition receptors expressed by T cells and thus are different from NKT cells and conventional T cells[67]. Balogh*et al*show that 1, 25 D3 mediated NK –cell cytotoxicity through protein kinase C, a signaling intermediate which was shown to be crucial in the exocytotic process on the NK cell granules[68]. This suggests that vitamin D might have an active role in NK cell activity, thereby contributing hose defense mechanisms mediate through NK cells. Though, there has not been much follow up to this observation, more studies on this light could have implications on the levels of vitamin D in viral infections where low levels could compromise NK- cell mediated cytotoxicity.

Vitamin D and γδ T cells

As reviewed by Bonneville et al[69], cells that are part of the lymphoid lineage but still exert their innate immune effector functions, namely, γδ T cells and iNKT cells play indispensable roles in innate immune mechanisms. They home to different tissues through specific homing receptors and serve as surveillance cells, also recognizing pathogen through PRRs[69]. They exert cytotoxic responses against virus-infected cells, and have also been known to produce defensins against microbial infections .γδ T cells bifurcate early in the T cell developmental pathway and develop independently of the conventional αβ T cells[69]. To this date, the functional aspect of these cells, in the context of antigen recognition has a lot to be ascertained. Both vitamin D and γδ T cellshave been implicated in the autoimmune disorders like MS[70]. Chen *et al*investigate the role of vitamin D in γδ T cell function[71]. Cultured γδ T cells that were induced with appropriate phosphate ligands upregulated their VDR expression and had an active VDR signaling[71]. Moreover, this 1,25D3 signaling led to suppression of IFN γ production[71]. This is in the same line as the immunosuppressive action of 1,25D3 in other cell types. This suggests that 1,25D3 could be

responsible for regulating the response of ɣδ T cells in response to phosphate antigens, which are expressed by pathogens.

Vitamin D and iNKT

iNKTcells are a unique set of lymphocytes that constitute the innate immune genre , and bridge the adaptive and innate immune responses. Deficiencies in iNKT cells and other related NKT cell subsets have been implicated in susceptibility to infections like lyme disease and *Mycobacterium tuberculosis*[72]. A study by Cantorna *et al*shows that vitamin D is important in the development of iNKT cells, in vivo[73]. VDR KO mice showed fewer less responsive iNKT cells[73]. This suggests that inadequate vitamin D levels compromise on optimal functions of iNKT cells during an infection.

Chapter 3

Adaptive immune responses

A classical adaptive immune response consists of antigen uptake and processing and the subsequent presentation to antigen specific CD4 or CD8 T cells of either the Th1 or the Th2 lineages through MHC class I or MHC class II molecules respectively. Antigen specific (CD4 and CD8) T cells that have been formed in the thymus through random T cell receptor gene rearrangements find their cognate APCs and activate a signal through their TCRs and co receptor molecules and get "primed" to proliferate in response to an antigen. CD4 T cells of the Th1 lineages specifically stimulate CD8 T cells that recognize MHC class I plus antigenic peptide from intracellular antigens. This forms the basis of cell-mediated immunity. B cells on the other hand, mediate humoral immunity or antibody mediated humoral immunity. B cells have cell surface immunoglobulin receptors that recognize antigens as specific conformational epitopes on pathogens, internalize them and present them to the CD4 Th2 cell specific for the *same antigenic peptide* being presented. This facilitates priming of the B cell and proliferates and increases its affinity towards the antigen through somatic hyper mutation and affinity maturation. A typical immune response , cell-mediated or humoral, consists of a peak where the number of immune cells reaches the maximum and then goes through a waning phase, where effectors (about 95%) die, leaving behind a long lived memory repertoire. This memory cell repertoire is capable of swift action, upon rechallenge with the same pathogen, and usually is able to contain the pathogen before the appearance of adverse disease symptoms, forming the basis of vaccine design. Memory cell generation forms the crux of vaccine design. There have been studies that implicate vitamin D signaling in the efficacy of vaccine[74], wherein 1,25D3 and antigen-pulsed activated DCs showed better CD4+ T cell proliferation. Moreover, VDR polymorphisms or single nucleotide variations in individuals have been associated with variations in adaptive cytokine responses to viral vaccines, which suggests that variations in VDR signaling could cause

individual increase or decrease in immune responses[75] depending upon the polymorphism and more importantly, memory. In vivo experiments in cattle suggest a role for vitamin D in secondary infection with antigen, that is, memory responses[76], since cattle deficient for vitamin D elicited crippled recall responses upon antigen rechallenge . This part of the article focuses on vitamin D and its possible direct/indirect roles in shaping the adaptive primary immune responses and also shaping the memory cell repertoire.

Vitamin D and antigen presentation

As mentioned earlier, the foundation of a good adaptive immune response lies in antigen presentation. DCs have been shown to modify their migratory pathways in the presence of 1,25D3[77]. This diversification of migration in response to vitamin D3 has been suggested to induce a more robust induction of adaptive immune responses because of the antigen specific B cell and T cells in lymphoid organs[77]. Cultured DCs have been shown to exhibit reduced costimulatory molecule expression like CD80, CD86, and reduced expression of MHC molecules when treated with 1,25D3 and LPS[78]. 1,25D3 and LPS treated DCs also exhibited less T cell proliferation compared to non 1, 25 D3 treated DCs when challenged with tetanus toxoid[78] . 1,25D3 also caused less chemotactic ability for DCs against chemokines, as was shown through a chemotaxis assay that is well described[78]. Additionally, the addition of the 1α-hydroxylase antagonist ketoconazole reduced the synthesis of 1, 25 D3 and rescued the expression of the costimulatory molecules[79]. This suggests that 1,25D3 suppresses antigen presentation.

It is also worthy to discuss calreticulin as it is known to be crucial in antigen presentation[80] , especially in the transport and presentation of MHC class I associated peptides to elicit peptide specific CD8 T cell responses. Efficient antigen presentation and less antigen duration have all been shown to play a crucial role in the generation of good memory responses[81]. Indeed, Sarkar *et al*[81], have shown that the generation of antigen specific memory CD8 T cells improved when their duration of encounter with the antigen was reduced. Fu H et al have shown that calreticulin reduces the peptide concentration required for MHC class I associated peptide presentation, an important consideration in the field of CD8 T cell memory[80]. Interestingly, it has been proposed that calreticulin actually antagonized Vitamin D signaling[82]. VDR and other nuclear receptors contain a consensus domain which is required for binding to DNA[82]. Calreticulin has been shown to bind to this consensus domain thereby inhibiting the action of

vitamin D[82]. This finding could have good research potential in elucidating the role of vitamin D in antigen specific immune responses and the ensuing recall responses, if memory B or T cell generation is to be studied.

It is now well established that professional APCS express the VDR and are also capable of synthesizing their own active 1, 25D3 post TLR ligand induced activation. *In vitro* data discussed in this section suggest that 1,25D3 is likely to curtail antigen presentation function, with some light on the mechanism that involved calreticulin. On the contrary, *in vivo* experiments on BALB/c mice that were immunized with both TLR ligand(OVA peptide) and 1,25D3 showed increased proliferation of CD4+ T cells as was seen with CFSE experiments that involved the transfer of CFSE-labeled CD4+ T cells followed by immunization of antigen + 1,25D3[74] . CD4+ T cell activity requires antigen presentation by professional APCs since it is necessary that the response remains antigen –specific, one of the mechanisms that keeps the immune system leashed. Thus, it could be said that though 1,25D3 seems to curb the antigen presentation as is evinced by in vitro data, however, *in vivo* data suggest otherwise.

Vitamin D and T cell priming/proliferation

Post antigen presentation, antigen specific T cells transit from their naïve antigen inexperience state to antigen primed state upon TCR stimulation. Antigen primed T cells are more proliferative, and upregulate their cytokine secreting abilities[83]. Various signaling molecules are operative to transit the naïve T cell into a primed T cell. TCR signaling studies have received a lot of attention and there is considerable evidence for the existence of two pathways that operate alternatively following TCR trigger[848586].A study by von Essen MR et al, 2010 investigates a role of the VDR in the activation of T cells[87]. A crucial mediator in this transition seems to be PLCɣ1, a signaling moleculenot expressed in naïve cells[87]. Previous studies have identified an induction role of 1,25D3 in activation of PLCɣ1[88] and also the presence of a VDR response element in the promoter of PLCɣ1[89]. The use of VDR antagonists and inhibitors of 1α-hydroxylase resulted in impaired proliferation of primed T cells, thereby suggesting a functional role of 1,25 D3 and the subsequent VDR signaling in T cell activation, a crucial step in an immune response to infection[87]. On the other hand, the 1,25 D3 treatment on DCs that have been pulsed with viral peptides or LPS has been shown to cause a decrease in antigen specific T cell proliferation[90]. 1,25D3 treatment has also been shown to curtail T cell proliferation in mixed lymphocyte reaction which serves to measure the alloreactive potential of T cells[91]. Moreover, 1, 25 D3 has been specifically shown to inhibit antigen specific CD4+ T cell proliferation that aided to alleviate symptoms of multiple sclerosis[92]. Thus, it could be said that the role of 1, 25 D3 in T cell proliferation to be contradictory. Given that T cells express the VDR[50], the role of 1, 25 D3 is a puzzle that needs to be solved completely.

The "memory" stage

After the peak of the immune response, there is a typical waning period, where about 95% of the effector cells die, leaving about 5% of long lived memory cells.How memory cells develop and acquire long lived characteristics is a topic of avid research. Many factors including antigen duration[81], cytokines like IL 2[93] and IL -12 and proinflammatory/anti-inflammatory responses have been postulated to play a decisive role in the fate of expanding CD8 T cells in the context of whether they will form short lived effector cells (SLECs) or Memory progenitors (MPs) .Two models of memory cell differentiation have been proposed and these have been extensively reviewed by Kalia et al[94]. In any case, a considerable amount of molecular factors have been shown to play a role in memory B /T cell differentiation. There are few scattered studies that look at the effect of vitamin D on these factors though there have been none to study the direct immunomodulatory effect of vitamin D on memory CD8 T cell differentiation. Unfortunately, there is no substantial evidence that look for the factors that look at the effect of vitamin D on B cell specific memory factors. Hence, the following sections attempt to address CD8 T cell memory.

Vitamin D and IL 2: IL 2 has an effect of memory cell differentiation[93]. For instance, cells that are low for their expression of the IL-2 receptor have been shown to be long lived and exhibit higher chances of developing into a memory cell[95]. IL-2 signaling in T cells has been shown to be inhibited in stimulated T cells and also in various T cell lines by 1, 25 D3[9697]. The gene for IL- 2 has been shown to contain a VDRE(Vitamin D Responses Element) [97],thereby suggesting a link for the transcriptional regulation of IL-2 by 1,25 D3. Moreover, IL-2 mRNA levels have been shown to go up in T reg cells (CD4+CD25+), when these were treated with topical 1,25 D3[98]. It is likely that 1, 25 D3 could stimulate production of IL-2 in T reg cells. It is nevertheless intriguing that vitamin D3 while shown to repress Il-2 signaling in T cell has been shown to induce the production of IL-2 in T reg cells. Whether this decreased IL-2 signaling, or

in other words, cells that have been made less responsive to IL-2 by vitamin D3 results in a significantly higher memory repertoire remains to be seen.

CTLA 4, vitamin D : Cytotoxic T lymphocyte antigen 4(CTLA-4) is an important inhibitory signaling molecule that has been originally shown to keep a check on autoimmunity[99]. Mice deficient for CTLA4 develop an autoimmune phenotype99. It has also been shown to curtail T cell proliferation and suppress the production of proinflammatory cytokines like IL-6, by signaling through Dendritic cells (DCs) during an infection model using the lymphocytic choriomeningitis virus (LCMV)[100]. The role of CTLA 4 in memory cells has also been investigated. In agreement with previous data, increased expression of CTLA4 on T regs dampens the primary immune response, but increases the memory cell repertoire, in an in vivo ovalbumin immunization model[101]. Additionally, it also has been shown that CTLA 4 signaling induces the production of Indoleamine 2,3-dioxygenase (IDO) that catabolizes tryptophan , resulting in T-cell cytotoxicity, thereby curbing its proliferation[101]. Vitamin D has been suggested to operate in conjunction with IL-2 to induce a regulatory phenotype in CD4+ T cells that serve to dampen the primary immune response, through increased expression of CTLA 4[102]. This suggests a role of vitamin D in programming the memory cell repertoire, also since there is evidence that early and timely curbing of the primary response results in an efficient memory repertoire[81].

Chapter 4

How does this all of this reflect in vivo?

A majority of the studies that have been discussed earlier are in vitro. However, in vitro and in vivo environment vary considerably. Many factors such as serum vitamin D levels, regulation of the vitamin D binding protein, which is responsible for transporting vitamin D to target tissues are factors that increase the complexity of vitamin D functions that have not been explored in vitro. Indeed, vitamin D binding protein and other trafficking mechanisms of vitamin D have been implicated in infections[103]. There have been relatively few in vivo studies that have looked at vitamin D and host response to infection.. For example, a *Listeriamonocytogenes* model of infection between WT and VDR KO mice showed that there is no appreciable difference in the clearance of infection between the two groups[104].*Leishmania major* infection studies onVDR KO mice showed that clearance was improved compared to WT[27]. 1, 25 D3 treated mice that had no change in susceptibility to Candida albicans and herpes simplex virus-1 infections which seems contrary to many of the in vitro data discussed above[105]. More studies thus need to be done in vivo to exactly simulate the multifactorial environment that exists in the body and regulates VDR signaling.

Chapter 5

Conclusion

The review discussed the immunomodulatory role of vitamin D3 in a new light: infection. At this juncture, it can be said that vitamin D and its signaling pathways play immunomodulatory roles in both innate and adaptive immune response. At first glance, vitamin D3 runs into the risk of being deemed immunosuppressive. There is considerable evidence to support the previous statement. but, taking more recent evidence into account, it can be said that previously unknown intricacies are coming into light. Vitamin D deficiencies interestingly denote a state of poor health, with increased susceptibility to diseases, both infection mediated and autoimmune. The innate immune system is the frontline defense mechanism that comes into effect during an infection. vitamin D is instrumental in inducing autophagy and antimicrobial peptides. Interestingly, 1, 25 D3 is shown in many instances to inhibit DC maturation. But, immature DCs have been known to facilitate innate immune responses, like recruiting other effector cells of the innate immune system. Macrophages and vitamin D seem to have a very complex relationship. Though vitamin D3 is thought to inhibit functional responsiveness of macrophages, with impaired NfKB localization, some studies that VDR and NfKB work in conjunction to induce defensins. It is likely that 1, 25 D3 plays a positive role in inducing non specific innate immune response and its regulation.

On the other hand, it is likely that the inhibitory nature of 1, 25 D3 could cause attenuated adaptive immune responses, given that 1, 25 D3 has been shown to promote regulatory T cell differentiation. Moreover, there is contradictory data on the role of 1, 25 D3 in T cell priming. However, recent data suggests that 1, 25 D3 enhanced CD4+ T cell responses in vivo when DCs were pulsed with VD3 and antigen. Efficacy of vaccine lies in good memory cell generation. No study till date has looked at the direct effect of vitamin D on the mechanisms that generate memory. Vitamin D does seem to inhibit IL-2 and increase CTLA4, both known to increase memory CD8 T cell generation. Timely attenuation of an immune responses following a robust

start that include proper inflammation is thought to favor memory cell generation. Through , the few papers discussed , it is likely that vitamin D could have a positive effect on memory response, but its role in eliciting in a primary response is currently unclear mainly because of the contradictions in T cell priming. Ultimately, one can conclude that 1,25 D3 is a hormone that is involved in mediating innate immune response and its regulation, keeps autoimmunity under check , and could have a potential role in adaptive immunity which needs more elucidation.

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