

# Antimicrobial implications of vitamin D

Dima A. Youssef,<sup>1,2</sup> Christopher W.T. Miller,<sup>3</sup> Adel M. El-Abbassi,<sup>1,2</sup> Della C. Cutchins,<sup>1</sup> Coleman Cutchins,<sup>5</sup> William B. Grant<sup>4</sup> and Alan N. Peiris<sup>1,2,\*</sup>

<sup>1</sup>Mountain Home VAMC Medicine Service; Mountain Home; and <sup>2</sup>Department of Internal Medicine; James H. Quillen College of Medicine; East Tennessee State University; Johnson, TN USA; <sup>3</sup>Department of Psychiatry; University of Maryland; Baltimore; <sup>4</sup>Sunlight, Nutrition and Health Research Center (SUNARC); San Francisco, CA USA; <sup>5</sup>Candidate for PharmD 2011; East Tennessee State University; College of Pharmacy; Johnson, TN USA

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Evidence exists that vitamin D has a potential antimicrobial activity and its deficiency has deleterious effects on general well-being and longevity. Vitamin D may reduce the risk of infection through multiple mechanisms. Vitamin D boosts innate immunity by modulating production of anti-microbial peptides (AMPs) and cytokine response. Vitamin D and its analogues via these mechanisms are playing an increasing role in the management of atopic dermatitis, psoriasis, vitiligo, acne and rosacea. Vitamin D may reduce susceptibility to infection in patients with atopic dermatitis and the ability to regulate local immune and inflammatory responses offers exciting potential for understanding and treating chronic inflammatory dermatitides. Moreover, B and T cell activation as well as boosting the activity of monocytes and macrophages also contribute to a potent systemic anti-microbial effect. The direct invasion by pathogenic organisms may be minimized at sites such as the respiratory tract by enhancing clearance of invading organisms. A vitamin D replete state appears to benefit most infections, with the possible noteworthy exception of Leishmaniasis. Antibiotics remain an expensive option and misuse of these agents results in significant antibiotic resistance and contributes to escalating health care costs. Vitamin D constitutes an inexpensive prophylactic option and possibly therapeutic product either by itself or as a synergistic agent to traditional antimicrobial agents. This review outlines the specific antimicrobial properties of vitamin D in combating a wide range of organisms. We discuss the possible mechanisms by which vitamin D may have a therapeutic role in managing a variety of infections.

## Introduction

Vitamin D deficiency is associated with several adverse health outcomes.<sup>1</sup> A plethora of health benefits, including a boost in longevity with vitamin D replacement, is evident.<sup>2</sup> Vitamin D has an emerging role in regulating inflammation and chemokine production<sup>3</sup> as well as an important role in immunomodulation.<sup>4</sup> These anti-inflammatory and anti-infective roles of Vitamin D are becoming increasingly important in a variety of skin diseases. A modulating effect of Vitamin D on the skin immune system is

apparent from the neonatal period, with the altered regulatory T cell profile persisting to adulthood.<sup>5</sup> Vitamin D and its analogues may have effects on, melanocytes, sebocytes and keratinocytes and offer an exciting option for treating many chronic inflammatory dermatitides.<sup>6</sup> Biggs et al.<sup>7</sup> demonstrated that vitamin D dependent induction of interleukin-10 by mast cells could contribute to the mast cell's ability to suppress cutaneous inflammation. In addition, vitamin D by modulating T cell profiles and production of antimicrobial peptides in the skin can potentially improve cutaneous inflammatory and vascular response as well as reduce secondary skin infection.<sup>8</sup>

Vitamin D may also improve survival in acute illness by boosting innate immunity.<sup>9</sup> Vitamin D appears to have systemic antimicrobial effects<sup>10</sup> that may be crucial in a variety of both acute and chronic illness. The current use of antimicrobials in the United States costs billions of dollars. Moreover, the overuse of antibiotics persists despite many efforts to address this problem and contributes to resistant organisms such as methicillin resistant staphylococcus aureus.<sup>11</sup> Growing expenditures on prescription drugs represent a major challenge to many health care systems. Vitamin D use could potentially reduce inappropriate antibiotic prescription and boost therapeutic response when combined with appropriate antibiotic use. We review the possible mechanisms by which Vitamin D may modulate the antimicrobial response.

## Vitamin D as an Immunomodulatory and Antimicrobial Agent

Vitamin (1,25-D<sub>3</sub>) acts as an immune system modulator.<sup>12</sup> Nearly all cells display a specific vitamin D receptor (VDR), including B and T lymphocytes (both resting and activated), monocytes and dendritic cells.<sup>13</sup> Vitamin D exerts its immunomodulatory activity on both mononuclear and polynuclear cell lines through its effects on the VDR.<sup>14</sup> Vitamin D tends to favor a mononuclear phenotype, increasing VDR expression on monocytes and macrophages.<sup>13,15</sup> Circulating vitamin D levels have a direct influence on macrophages, increase their "oxidative burst" potential (maturation and production of cytokines, acid phosphatase and hydrogen peroxide),<sup>16</sup> and prevent excessive expression of inflammatory cytokines. Vitamin D also facilitates neutrophil motility and phagocytic function.<sup>17</sup>

Vitamin D may improve outcomes by reducing both local and systemic inflammatory responses as a result of modulating

\*Correspondence to: Alan N. Peiris; Email: Peiris@va.gov  
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cytokine responses and reducing Toll-like receptor (TLR) activation.<sup>18</sup> Studies in mice suggest that blocking TLR9 may have use in treating human sepsis.<sup>19</sup> Vitamin D also modulates the immune system by direct effects on T-cell activation and on the phenotype and function of antigen-presenting cells, especially dendritic cells.<sup>20</sup> Vitamin 1,25-D<sub>3</sub> inhibits proliferation of T helper 1 (Th1) cells [consequently impairing production of IL-2, tumor necrosis factor  $\alpha$  and interferon (IFN)], as well as T helper 17 (Th17) cells, skewing cytokine production toward a T helper 2 (Th2) phenotype.<sup>21</sup>

A major component of the antimicrobial action of Vitamin D is through the production of peptides which have antimicrobial as well as anti-endotoxin activity. Vitamin D stimulates the expression of potent antimicrobial peptides, such as cathelicidin and  $\beta$  defensin 2,<sup>22</sup> which exist in neutrophils, monocytes, natural killer (NK) cells and epithelial cells lining the respiratory tract.<sup>23</sup> Macrophages, lymphocytes and monocytes have VDRs that, with 25(OH)D stimulation, increase the expression of these antimicrobial peptides.<sup>24,25</sup> Jeng et al.<sup>18</sup> noted a positive relationship between vitamin D levels and cathelicidin levels in acutely ill patients. Grant proposed this relationship on the basis of a synthesis of the epidemiological studies of septicemia in the United States.<sup>26</sup>

Cathelicidin is effective against gram-positive and gram-negative bacteria, fungi and mycobacteria at a variety of pathogen entry sites, including the skin and the mucosal linings of the respiratory and gastrointestinal systems.<sup>22</sup> Patients with 25(OH)D levels less than 20 ng/mL may be unable to fully express cathelicidin,<sup>18</sup> which could be associated with increased susceptibility to nosocomial infections such as pneumonia, sepsis and central line infections.<sup>21</sup>

Another antimicrobial peptide, human beta-defensin-2 (HBD)-2, may have special utility in multidrug resistant microbes from *in vitro* studies,<sup>27</sup> although its overall role is less clearly defined. In lesions observed in psoriasis and atopic dermatitis, vitamin D promoted increased cathelicidin<sup>28</sup> but decreased beta-defensin-2 production.<sup>29</sup> This enhanced antimicrobial peptide production may improve skin lesions in psoriasis and atopic dermatitis.<sup>29</sup> The ability of vitamin D analogs to alter antimicrobial peptide expression in psoriatic lesions extends our understanding of dermatologic therapies.<sup>30</sup>

Human beta-defensin-3 (HBD)-3 is an antimicrobial peptide that exhibits a broad spectrum antimicrobial activity against gram-positive/negative bacteria and fungi.<sup>31</sup> It is possible that (HBD)-3 maybe more relevant than (HBD)-2 as shown in the severity of staphylococcal aureus skin infections.<sup>32</sup> Augmentation of (HBD)-3 production in keratinocytes by antifungal agents such as Itraconazole may potentiate cutaneous defense against infection.<sup>33</sup> A deficiency of antimicrobial peptide production may contribute to the susceptibility to staphylococcus aureus skin infections in patients with atopic dermatitis.<sup>8</sup> Moreover, abnormal processing of cathelicidin peptides may contribute to cutaneous inflammation and vascular response seen in Rosacea.<sup>34</sup>

In addition to antimicrobial and immune benefits, antimicrobial peptides may contribute to host defense through wound repair<sup>35</sup> and clearance of bacteria at various barrier

sites.<sup>36</sup> Vitamin D deficiency may also predispose patients to hypocalcemia, which impairs normal lymphocyte and neutrophil function.<sup>37</sup>

### Antimicrobial Efficacy Against Different Infective Agents

**Viruses.** Acute lower respiratory infections are one of the commonest reasons for hospital emergency department presentations, hospitalization and intensive care unit admissions among children.<sup>38</sup> Evidence exists that vitamin D may have a protective role in influenza<sup>16,39</sup> and other viral diseases, such as the risk of developing acquired immunodeficiency syndrome (AIDS) in human immunodeficiency virus (HIV),<sup>40</sup> hepatitis,<sup>41</sup> Avian flu,<sup>42</sup> and other viral infections.

Studies dating back to the 1940s have associated a diet poor in vitamin D with susceptibility to experimental influenza viruses in mice.<sup>43</sup> Influenza epidemics in North America and Europe generally reach peaks during December through March, the months during which ultraviolet-B (UVB) radiation exposure and serum levels of 25(OH)D are lowest in the population.<sup>44</sup> Epidemics of influenza peak in the month after the winter solstice<sup>45</sup> and have greater clinical severity with less sunlight.<sup>16</sup> Low vitamin D levels may reduce AMP synthesis, which then is less likely to impede the influenza virus.<sup>16</sup> A randomized controlled trial involving Japanese schoolchildren found a relative risk of influenza of 0.36 in those taking 1,200 IU/day compared with those taking 200 IU/day.<sup>39</sup> This result was found to be related to influenza type A, with no effect for type B.

Sabetta et al. demonstrated that maintenance of a vitamin D serum concentration of 38 ng/mL or higher should significantly reduce the incidence of acute viral respiratory tract infections, including influenza, at least during the fall and winter in temperate zones.<sup>46</sup> Similarly, variations in vitamin D production might explain the seasonality of childhood respiratory infections in Hawaii.<sup>47</sup> During the 1918–1919 influenza pandemic, in the United States, an inverse correlation emerged between the case fatality rates and the mean vitamin D status of the population which was represented by solar UVB doses.<sup>48</sup>

In Indian children younger than 5 years, subclinical vitamin D deficiency was a significant risk factor for severe acute lower respiratory tract infections.<sup>49</sup> Corroborative evidence has emerged in a study in which 94 children received vitamin D supplementation and were noted to have a lower incidence of respiratory tract infections from autumn through spring of the following year.<sup>50</sup> The serum vitamin D<sub>3</sub>-binding protein (Gc), which B-cell membranes constitutively express in association with membrane immunoglobulin, could be involved in cell activation.<sup>51</sup> It is the precursor for the principal macrophage-activating factor (MAF) and is reduced in all patients infected with the influenza virus. Sera from these patients contain  $\alpha$ -N-acetylgalactosaminidase (Nagalase) that deglycosylates Gc protein, preventing it from converting to MAF, possibly contributing to immunosuppression.<sup>52</sup>

After the outbreaks of H1N1 influenza in 2009, Edlich et al.<sup>53</sup> strongly recommended that all health care workers and patients be tested and treated for vitamin D deficiency to prevent exacerbation

of respiratory infections. Vitamin D also reduces the production of proinflammatory cytokines, which may reduce the risk of cytokine storm in H1N1 infection.<sup>48</sup>

Other respiratory viruses such as respiratory syncytial virus (RSV) and parainfluenza 1 and 2 viruses present with a similar seasonal pattern,<sup>54</sup> although the incidence of RSV seems to relate more to humidity and temperature than to UV radiation exposure.<sup>55</sup> Vitamin D appears to decrease the inflammatory response to RSV infections in airway epithelium without jeopardizing viral clearance.<sup>56</sup>

These findings suggest that the immunomodulatory properties of vitamin D influence acute lower respiratory tract disease severity<sup>38</sup> and may thus protect against asthma. A recent randomized controlled trial involving Japanese students found a reduced incidence of asthma in those taking vitamin D.<sup>39</sup> Moreover, in children with asthma, decreased vitamin D status is associated with increased use of corticosteroids.<sup>57</sup> Viral infections also constitute a major cause of recurrent otitis media. In a group of 16 young children undergoing tympanostomy tubes, suboptimal 25(OH)D levels were noted: 50% were deficient and another 31% were in the insufficient range.<sup>58</sup>

Individuals with 25(OH)D levels less than 10 ng/mL had 55% higher probability of a recent upper respiratory infection than individuals with levels greater than 30 ng/mL.<sup>23</sup> In elderly patients, this concern is of greater magnitude; levels will need to be sufficient to compensate for an age-dependent decrease in immune function. Consuming 800 IU of oral vitamin D<sub>3</sub> daily was insufficient for prevention of infection in the RECORD trial.<sup>59</sup>

One cannot extrapolate conclusions from studies with the influenza virus to other respiratory viruses. For example, AMPs do not inactivate rhinoviruses because these viruses lack a lipoprotein envelope, which appears to be prerequisite for the antimicrobial activity of most AMPs.<sup>60</sup> Epstein-Barr virus transforms B lymphocytes into immortalized lymphoblasts. A VDR protein was found to be a binding partner of Epstein-Barr virus nuclear antigen 3 (EBNA-3), a member of the EBNA-3-protein family that can regulate transcription of cellular and viral genes. This latter protein blocks the activation of VDR-dependent genes and protects lymphoblast cell lines against vitamin D<sub>3</sub>-induced growth arrest and apoptosis.<sup>61</sup>

Despite a complex relationship that remains to be fully elucidated, vitamin D appears to contribute in defense against HIV. The role is based on both direct action (by promoting release of antiviral elements such as  $\beta$ -defensin 2 and cathelicidin),<sup>62</sup> and indirect effects through VDR actions. Polymorphisms in the structure of the VDR gene directly affect host susceptibility to HIV infection, CD4 counts, immunological hyperactivity and rapidity of disease progression to full-blown AIDS.<sup>40,63</sup> The G-A-T-G-L haplotype of VDR (which confers a diminished efficacy of the vitamin D pathway) protects against transmission of HIV type 1 (possibly secondary to diminished antiviral chemokine release and an unbalanced Th2 response),<sup>40</sup> whereas in AIDS patients, the VDR BsmI BB and FokI heterozygosities are associated with a more accelerated CD4 drop and faster progression to AIDS.<sup>64,65</sup>

However, under certain circumstances, the vitamin D pathway may be implicated in promoting disease activity. Nevado et al. used U937 cells as a model for mononuclear cells (a target for HIV) and demonstrated that VDR and its ligand 1,25-D<sub>3</sub> have a role in transactivation of the long terminal repeat sequence of HIV type 1, which is a crucial element in viral replication.<sup>66</sup> The exact nature of this influence has yet to be fully elucidated. HIV entry into monocytes depends on chemokine receptor CCR5,<sup>67</sup> and vitamin D has a dual role in potentially blocking this step in the infectious pathway. Promoting a predominantly Th2 response will induce release of cytokines (such as IL-13) known to downregulate CCR5 expression.<sup>68</sup> Vitamin D also regulates production of RANTES,<sup>3</sup> a natural ligand of CCR5 whose binding helps block viral entry.

Highly active antiretroviral therapy may be linked to significant changes in vitamin D status. Median 25(OH)D levels were significantly lower in white nonnucleoside reverse transcriptase inhibitor-treated patients than in white patients treated with protease inhibitors.<sup>63</sup> Moreover, nonnucleoside reverse transcriptase inhibitor and protease inhibitor treatment puts patients at risk of elevated parathyroid hormone levels,<sup>63</sup> as shown with tenofovir-linked hyperparathyroidism, which is closely linked to vitamin D deficiency.<sup>69</sup> However, vitamin D status did not affect CD4 cell recovery after initiation of highly active antiretroviral therapy.<sup>63</sup>

Host defense against human T-lymphotropic virus type 1 (HTLV-1) hinges on an effective Th1 response. Vitamin D seems to limit the magnitude of Th1 activation, providing a more effectively orchestrated response in a similar manner to tuberculosis (as discussed later). In patients infected with HTLV-1, a greater risk of developing tropical spastic paraparesis was associated with the ApaI polymorphism of VDR (although clinical course was unaltered).<sup>70</sup>

The potential benefit of a vitamin D-replete state against hepatitis is an interesting recent development.<sup>41</sup> Chronicity of hepatitis B infection is also influenced by mutations in the VDR gene, with polymorphisms being associated with higher viral load, disease progression and severity.<sup>71</sup> Of note, the *t* allele (resulting from a dimorphism at position 352) is associated with enhanced Th1 cellular immunity and promotes more efficient clearance of several viral infections, including hepatitis B and dengue virus.<sup>72,73</sup> A potential benefit of vitamin D on the hepatitis C virus (HCV) is emerging; however, the data are preliminary. One study in patients with HCV demonstrated that vitamin D<sub>2</sub> (but not D<sub>3</sub>) inhibits viral RNA replication, supposedly by inducing oxidative stress in a manner similar to the action of cyclosporine.<sup>74</sup> Genotype 1 chronic HCV patients have low 25(OH)D serum levels, thus placing them at risk of severe fibrosis and low sustained viral response to IFN.<sup>75</sup> Another study also reproduced these findings,<sup>76</sup> where vitamin D supplementation improved the probability of achieving a sustained virological response after antiviral treatment with IFN $\alpha$  and ribavirin. Further, vitamin D-binding protein was among the three prominent candidate biomarkers of liver fibrosis, where vitamin D-binding protein levels were higher in the normal liver/mild fibrosis stage and lower in the advanced stage. Thus, vitamin D-binding protein level is potentially a way to predict the stage of liver fibrosis without biopsy.<sup>77</sup> Vitamin D is linked not only to liver fibrosis but also

to liver cirrhosis. A significant correlation exists between VDR genetic polymorphisms and the occurrence of hepatocellular carcinoma in patients with liver cirrhosis; this association is even more prominent in alcoholic patients.<sup>78</sup>

Deficient production of CCR5 has been linked to an increased susceptibility to HCV infection,<sup>79</sup> supporting a potential deleterious role for vitamin D deficiency by favoring host infection through the aforementioned Th2 influence on CCR5.

**Bacteria.** Recent discoveries have revealed the importance of the vitamin D-dependent generation of antimicrobial peptides in human host defense against *Mycobacterium tuberculosis*.<sup>80</sup> Low vitamin D levels were associated with a fivefold-increased risk for progression to tuberculosis.<sup>81</sup> The influence of vitamin D on tuberculosis can be traced back to the predrug era, when exposure to sunlight was considered an important adjuvant treatment modality. Yesudian et al. hypothesized that high-risk populations latently infected with the organism would develop full-fledged disease upon migrating from an area with high sun exposure to areas with diminished sunlight.<sup>82</sup> Since the early 19<sup>th</sup> century, both environmental (i.e., sunlight) and dietary sources (cod liver oil) of vitamin D have been identified as treatments for tuberculosis.<sup>10</sup>

More than one study has associated polymorphisms in VDR with an increased susceptibility to tuberculosis,<sup>83,84</sup> and vitamin D deficiency has been linked to a more severe form of the disease.<sup>85</sup> The lower serum levels observed in African Americans may account for deficient production of cathelicidin and a greater susceptibility to infection.<sup>86</sup> Cathelicidin is required for the 1,25-D<sub>3</sub>-triggered antimicrobial activity against intracellular *M. tuberculosis*.<sup>86</sup> *M. tuberculosis* activates TLR2/1, enhancing 1,25-D<sub>3</sub> production and VDR expression, with subsequent release of cathelicidin by monocytes.<sup>87</sup>

Vitamin D limits mycobacterial growth within macrophages and monocytes and, until recently, the precise mechanism governing this activity was subject to debate.<sup>88,89</sup> Murine models suggest that 1,25(OH)<sub>2</sub>D-mediated induction of nitric oxide release by macrophage-like cell lines is a fundamental element in host defense, but these findings may not apply to human subjects.<sup>90</sup> Vitamin 1,25(OH)<sub>2</sub>D may play an important role in limiting the pathological process in tuberculosis by downregulating the levels of matrix metalloproteinases (MMPs) and upregulating the levels of tissue inhibitor of MMP-1.<sup>91</sup>

A variety of other mechanisms have also been postulated. Autophagy also plays a crucial role in antimycobacterial resistance<sup>92</sup> and contributes to immune surveillance of intracellular pathogens and vaccine efficacy. Vitamin D<sub>3</sub> contributes to host immune responses against *M. tuberculosis* through cathelicidin.<sup>93</sup> Yuk et al.<sup>94</sup> have outlined how vitamin D induces autophagy and mediates co-localization of *M. tuberculosis* and AMPs within an autophagolysosome, leading to killing of the bacterium. Similarly, TLR2/1 activation leads to vitamin D<sub>3</sub>-dependent antimycobacterial activities. TLR2/1/CD14 stimulation by mycobacterial lipoprotein LpqH can activate antibacterial autophagy through activating VDR signaling and inducing cathelicidin.<sup>93</sup>

Analogously to HTLV-1, 1,25-D<sub>3</sub> induces important cytokine downregulation activity and actually blocks mononuclear

cell production of IFN $\gamma$  and IL-12,<sup>95</sup> modulating the immune response against *M. tuberculosis*, possibly reducing the risk of cytokine storm.

The effects of vitamin D on other mycobacterial diseases, such as leprosy, are also being studied. The presence of 1- $\alpha$ -hydroxylase in foamy macrophages (characteristic of granulomatous disease) may result in greater circulating levels of 1,25-D<sub>3</sub> and subsequent hypercalcemia. Whether these metabolic changes alter disease course is unknown. More concrete evidence has emerged linking VDR polymorphisms with host susceptibility. The *t* allele (already highlighted as a marker of prominent Th1 immunity) has been linked with greater incidence of tuberculoid leprosy.<sup>96</sup> In a highly endemic region of Brazil, the combination of the *tt* genotype and a negative Mitsuda reaction was associated with an incidence of leprosy 13 times higher than that in control subjects with a positive Mitsuda test.<sup>97</sup>

In vitro studies proved vitamin D<sub>3</sub> has inhibitory activity on strains of *Staphylococcus aureus*, *Streptococcus pyogenes*, *Klebsiella pneumoniae*, *Escherichia coli* (*E. coli*) and other bacteria. In the presence of 50,000–90,000 IU/mL of vitamin D<sub>3</sub>, the organisms were killed or demonstrated marked growth inhibition.<sup>98</sup> Gram-positive bacteria, invasive pneumococcal disease, meningococcal disease and group A streptococcal disease are more common when vitamin D levels are low, raising the possibility that pharmacological doses of vitamin D could be an effective adjuvant therapy.<sup>99</sup>

Excess wintertime mortality related to pneumococcal pneumonia has been noted for more than a century. The major predictor of invasive pneumococcal disease in Philadelphia was extended periods of low UV radiation, which may explain the observed wintertime seasonality. The virulence of the pathogen and the efficacy of host immune function boosted by 1,25-D<sub>3</sub> may determine the outcomes of invasive disease.<sup>100</sup> A double-blind individually randomized placebo-controlled trial involving young children in an inner-city hospital in Kabul showed that the risk of a repeat episode of pneumonia within 90 days of supplementation of oral 100,000 IU of vitamin D<sub>3</sub> was lower in the intervention than in the placebo group.<sup>101</sup>

Bahr et al. examined group A streptococcal infections and found a striking association between vitamin D-binding protein (Gc2) and rheumatic fever in a homogeneous Arab population.<sup>51</sup> Vitamin D plays an important role in mediating immune function of the skin through several pathways, including the enhanced release of AMPs.<sup>102</sup> Vitamin D deficiency is associated with an increased risk of methicillin-resistant *Staphylococcus aureus* nasal carriage.<sup>103</sup> Similar findings suggest that VDR polymorphisms may be associated with nasal carriage of *S. aureus* in individuals with type 1 diabetes mellitus.<sup>104</sup> However, others found no association between VDR gene variation and *S. aureus* nasal carriage.<sup>105</sup>

Moreover, vitamin D deficiency has been linked to adverse and more costly outcomes in veterans with *Clostridium difficile* and methicillin-sensitive *S. aureus* infections.<sup>106</sup> Animal studies also confirm a potential beneficial role for vitamin D treatment. In the turkey osteomyelitis complex, vitamin D treatment

resulted in significantly lower isolation of bacteria from tissues and reduced mortality.<sup>107</sup>

The *Ti* genotype of VDR is associated with periodontal disease and may mediate this through its effects on the gram-positive bacteria that constitute the oral flora.<sup>108</sup> Both dental caries and periodontal disease are caused largely by *Streptococcus mutans*.<sup>109</sup> Although eating refined carbohydrates and poor dental hygiene contribute to dental caries, vitamin D can reduce the risk of caries by inducing production of cathelicidin and defensins, which have antibacterial properties.<sup>10</sup> A cross-sectional study from the Third National Health and Nutrition Examination Survey (NHANES III) in the United States found a significant inverse correlation between periodontal disease (PD) and serum 25(OH)D concentrations.<sup>110</sup>

A study of British Bangladeshi adults, free of known diabetes or major illness, showed that a year of modest vitamin D supplementation decreased serum circulating MMP-9 levels by 69%.<sup>111</sup> Two small randomized controlled trials of vitamin D and calcium supplementation and risk of PD revealed a higher rate of PD in the nontaker groups.<sup>112,113</sup> Thus, vitamin D evidently reduces the risk of PD since it largely satisfied the criteria for causality in a biological system, as outlined by Grant and Boucher.<sup>114</sup>

Vitamin D-induced downregulation of the cytokine response may not always be associated with optimal host defense. The Th1 cytokine profile is vital for clearance of certain organisms and ancillary immune activity, and a limiting effect on this cytokine profile may result in reduced chances for overcoming infections. A study on cultured macrophages treated with 1,25-D<sub>3</sub> demonstrated deficient activity against *Listeria* spp.<sup>115</sup> This effect may be due to inhibition of IFN $\gamma$  activation of macrophages, which would preclude reactive oxygen formation and thus effective bactericidal activity.<sup>115</sup> However, Bruce et al.<sup>116</sup> indicated that clearance of *Listeria monocytogenes* was delayed in the absence of the VDR.

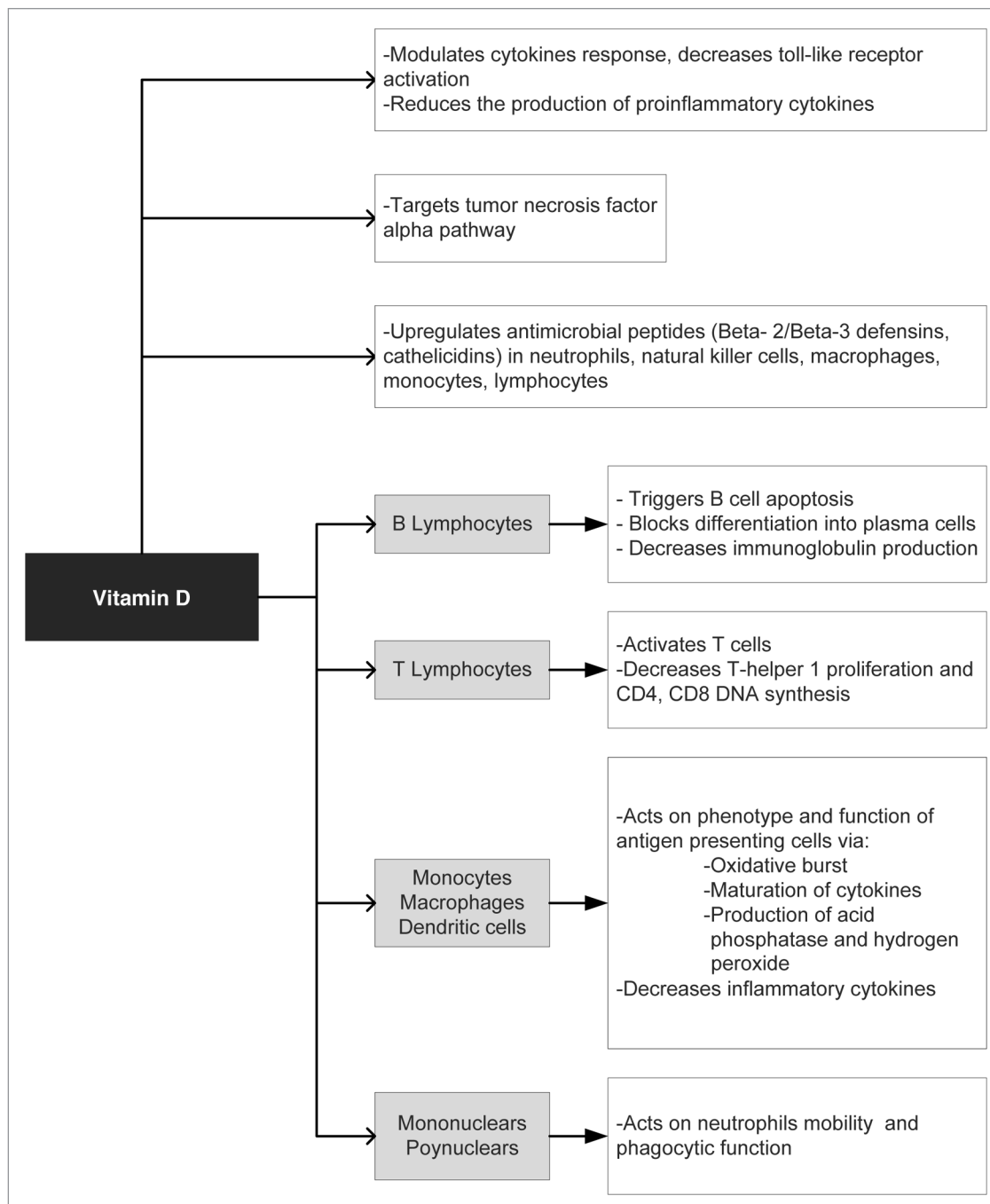
Many examples document the benefits of having an optimal vitamin D level in gram-negative bacteria. Treatment with 25(OH)D protected 3A cells of the placenta against cell death after infection with *E. coli*.<sup>117</sup> Through enhancing AMP expression, vitamin D may be a part of the defense against infections such as shigellosis, which reduce the endogenous expression of AMPs, resulting in serious infections.<sup>118</sup> Vitamin D deficiency predisposes mice to colitis by means of dysregulated colonic antimicrobial activity and impaired homeostasis of enteric bacteria.<sup>119</sup> In the same context, VDR activation reduces bacterial-induced intestinal NF $\kappa$ B activation and attenuates response to infection.<sup>120</sup> Therefore, it is an important contributor to intestinal homeostasis and host protection from bacterial invasion and infection.<sup>120</sup> Treatment with oral active vitamin D may be associated with a lower risk of peritonitis in peritoneal dialysis patients.<sup>121</sup>

Despite a decrease in mortality in the last decade, sepsis remains the tenth-leading cause of death in western countries<sup>122</sup> and one of the commonest causes of death in intensive care units. Mortality in adult intensive care units may be partially linked to severe systemic inflammatory responses and sepsis.<sup>18</sup> Vitamin D status may determine AMP levels in patients with sepsis in

the intensive care unit.<sup>18</sup> The epidemiology of septicemia in the United States and the variations of solar UVB, as well as the effects of vitamin D, support the hypothesis that both play important roles in reducing the risk of septicemia.<sup>26</sup> The risk of diseases comorbid with septicemia are generally inversely correlated with serum 25(OH)D levels.<sup>26</sup> Grant also demonstrated that vitamin D supplementation of mother and infant can reduce the risk of sepsis in infants<sup>123</sup> and neonates.<sup>124</sup> Activation of TLR4, the receptor for gram-negative bacteria's outer membrane lipopolysaccharide or endotoxin, may play a potential role in determining outcomes. Vitamin D, through its modulation, may have a role as adjunctive therapy in severe sepsis and septic shock.<sup>122</sup> In veterans admitted to the intensive care unit, higher mortality and a longer stay were significantly linked to lower vitamin D status.<sup>125</sup> A total of 17% of the intensive care patients in one study had undetectable levels of vitamin D,<sup>126</sup> which may predispose to hypocalcemia. Zaloga et al.<sup>127</sup> found that 20% of critically ill patients with bacterial sepsis had hypocalcemia and that their mortality rate was significantly higher (50%) than that of normocalcemic patients with sepsis (29%).

**Protozoa.** *Leishmania major* is an obligatory intracellular organism residing within macrophages. Effective clearance will depend on appropriate macrophage activation (which occurs through IFN $\gamma$  release by Th1 and NK cells) and production of nitric oxide.<sup>128</sup> The presence of 1,25-D<sub>3</sub> disrupts this pathway, as IFN $\gamma$  secretion is blocked, impairing macrophage activation.<sup>129</sup> Similarly, host defense against *Toxoplasma gondii* (*T. gondii*) relies on a Th1 cytokine profile response dominated by IFN $\gamma$  and IL-2 production.<sup>130</sup> The potential downregulation of this response by vitamin D can prove problematic in toxoplasmosis. Treatment with vitamin D dose-dependently inhibits both in vivo and in vitro growth of *T. gondii*, possibly by limiting tachyzoite proliferation within the parasitophorous vacuole because of activity at a cellular level.<sup>131</sup> However, no difference occurred in the total number of infected cells regardless of the presence of vitamin D, perhaps refuting a role in cell invasion. Interestingly, vitamin D may be linked to increased mortality in mice infected with toxoplasmosis, presumably because of its downregulation of the Th1 cytokine response.<sup>132</sup> This finding suggests a delicate balance in overall immune response against this organism, and further studies would need to be done to establish the role of vitamin D among other variables in determining outcomes.

Two other parasitic infections reportedly benefit from adequate circulating vitamin D levels. A murine model demonstrated that intraperitoneal injection with vitamin D conferred protection against inoculation with trypomastigotes of *Trypanosoma cruzi*, with histopathology revealing diminished tissue inflammation and parasitism.<sup>133</sup> The intraerythrocytic forms of *Plasmodium falciparum* (*P. falciparum*) produce triacylglycerols through enhanced expression of diacylglycerol acyltransferase, an essential step in sustaining their proliferation within erythrocytes.<sup>134</sup> Biosynthetic activity is particularly enhanced at the trophozoite and schizont stages, which are precisely the two forms at which higher serum levels of 25-D<sub>3</sub> and 1,25-D<sub>3</sub> have been demonstrated to hinder growth of *P. falciparum*.<sup>135</sup> It has been proposed that the activity of vitamin D resides in altering phospholipid



**Figure 1.** Potential antimicrobial mechanisms of vitamin D.

metabolism and precluding this crucial step. It is, however, not an easy hypothesis to sustain, given that erythrocytes have not been shown to have receptors for 25-D<sub>3</sub><sup>135</sup> therefore, a direct cellular effect is highly unlikely.

**Fungi.** The role of vitamin D in combating fungal infections dates back to a case series published in 1954, in which three patients with severe refractory chromoblastomycosis showed marked improvement in their skin lesions after receiving repeated treatments with 600,000 IU of calciferol.<sup>136</sup> Despite these early promising results—plus the ability of vitamin D to increase

circulating NK cells, which may contribute to host defense against fungal organisms<sup>37</sup>—subsequent findings confirming similar results have been scarce. Thus, more studies are needed to support vitamin D as an adjuvant treatment modality for fungal infection.

For *Candida albicans*, patients with end-organ resistance to 1,25-D<sub>3</sub> all had relative decrease in neutrophil fungicidal activity.<sup>137</sup> In animal studies, Cantorna et al.<sup>138</sup> demonstrated that 1,25-D<sub>3</sub> may reduce the prevalence of opportunistic infections such as candidiasis.

Murray et al. described a patient with disseminated histoplasmosis and adrenal insufficiency treated with vitamin D and calcium supplementations.<sup>139</sup> The patient was hypercalcemic (due to the granulomatous nature of the disease), and vitamin D supplements exacerbated this condition. Despite aggressive management, the patient later died. In light of this, avoiding vitamin D supplementation in invasive granulomatous fungal infections would be prudent, since it may induce or aggravate hypercalcemia and hypercalcemia may lead to impaired monocyte and neutrophil activity.<sup>140</sup>

**Parasites.** A remarkable lack of data exists on the effect of vitamin D in parasitic infections. Chowdhury et al. showed that media enriched with vitamin D<sub>3</sub> can exert a toxic effect on *Hymenolepis microstoma*, leading to degenerative changes in the worm.<sup>141</sup> Similarly, a prior study reported that a diet lacking in vitamins A, D<sub>3</sub> and E would predispose to an increase in the size of *Hymenolepis diminuta* due to host intestinal paralysis and worm migration to a position more favorable for growth.<sup>142</sup>

An 11-month trial in Kenyan children living in an endemic area for helminth infections yields some useful data. Subjects receiving supplements (consisting of 15 micronutrients, including the equivalent of 200 IU daily of vitamin D<sub>3</sub>) showed a decrease of borderline significance in the rate of reinfection with *Schistosoma mansoni* but no benefit with regards to rates of infection with *Ascaris lumbricoides*, *Trichuris trichiura* or hookworms.<sup>143</sup> The relatively low dose of vitamin D<sub>3</sub> supplementation given hinders drawing definitive conclusions.

## Conclusions

Abundant evidence is surfacing with regard to the role of vitamin D in modulating the immune and antimicrobial response. **Figure 1** summarizes the potential antimicrobial mechanisms of vitamin D. Vitamin D also has diverse and potent local and systemic activities such as enhanced production of AMPs, which generally appears to favor the host in curtailing infection. Leishmaniasis may be the possible exception to this beneficence in that vitamin D may actually favor the invading organism.

Much of the data supporting vitamin D has come from laboratory or epidemiologic settings. We urgently need clinical trials to confirm the efficacy of vitamin D as a potent antimicrobial and immunomodulating agent. Recent work suggest

that in addition to low vitamin D levels, excessive 25(OH)D levels may also increase the risk of tuberculosis.<sup>144</sup> A recently published randomized controlled trial demonstrated that the administration of four doses of 2.5 mg vitamin D(3) increased serum 25-hydroxyvitamin D concentrations in patients receiving intensive-phase treatment for pulmonary tuberculosis. Vitamin D significantly hastened sputum culture conversion in participants with the tt genotype of the TaqI vitamin D receptor polymorphism.<sup>145</sup>

Considering the plethora of general benefits that an adequate vitamin D state confers, as well as potential antimicrobial benefits, checking vitamin D status and maintaining adequate 25(OH)D levels seems prudent. It appears likely that VDR polymorphisms determine tissue responses to vitamin D.<sup>146</sup> Some individuals may need much larger replacement doses than others, emphasizing the need to customize vitamin D replacement through monitoring. While optimal Vitamin D levels are not clearly defined, there is evidence that maintaining a vitamin D level 38 ng/ml or higher should significantly reduce the incidence of upper respiratory infection.<sup>46</sup> In addition, Grant has proposed a significant reduction in mortality with a vitamin D level of 45 ng/ml.<sup>147</sup> As such we believe it is reasonable to aim for 25(OH)D levels of about 40–45 ng/ml in patients.<sup>46</sup>

Vitamin D is emerging as an important and cost-effective option in the therapeutic armamentarium in reducing many infections either as a sole agent or as an adjunct to current antimicrobial agents.

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