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COMMENTARY

Vitamin D and Lung Infection

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Available data suggest that vitamin D plays a role in controlling inflammation in the lungs. However, to date vitamin D-induced production of cathelicidin has not been shown to have an effect on the burden of either viruses or bacteria. Future work should continue to determine the effects of vitamin D-regulated mechanisms in the lung and the possible role of cathelicidin against different pulmonary pathogens *in vivo*.

In this issue of *Infection and Immunity*, Niederstrasser and colleagues report that there were no effects of vitamin D deficiency on the susceptibility of mice to pulmonary infection with *Streptococcus pneumoniae* or *Pseudomonas aeruginosa* (1). The authors suggest that, because of differences in the responses of mice and humans to vitamin D, mice might not be useful for studying the role of vitamin D in human lung infection (1). This conclusion is based on two assumptions: (i) regulation of cathelicidin is the critical factor underlying possible anti-infective properties of vitamin D, and (ii) vitamin D protects against a diverse array of pulmonary infections in humans.

VITAMIN D AND CATHELICIDIN

The antibacterial peptide cathelicidin is induced by the active form of vitamin D [1,25$(OH)_2$D] in human but not mouse cells (2). The murine equivalent of cathelicidin lacks a vitamin D response element and therefore is refractory to addition of 1,25$(OH)_2$D (2). In addition, human macrophage cells express the gene for the 1-alpha hydroxylation that produces 1,25$(OH)_2$D from its vitamin D precursor [25(OH)D] (3). *In vitro*, human macrophages can produce 1,25$(OH)_2$D, which induces cathelicidin that in turn inhibits growth of *Mycobacterium tuberculosis* (3). Human macrophages have been shown to produce 1,25$(OH)_2$D in patients with sarcoidosis (4). However, extrarenal macrophage production of 1,25$(OH)_2$D can lead to hypercalcemia, which has been observed in patients with mycobacterial diseases, including those caused by *M. tuberculosiss*, *Mycobacterium leprae*, and *Mycobacterium avium* (5). The hypercalcemia and presumed local production of 1,25$(OH)_2$D have been treated with glucocorticoids, which suppress macrophage activation (5). It may be that macrophages normally produce local 1,25$(OH)_2$D during an immune response but that hypercalcemia occurs due to extrarenal production of 1,25$(OH)_2$D in the setting of excessive immune activation and overexpression of the vitamin D 1-alpha hydroxylating enzyme.

Cathelicidin has been shown to have direct antibacterial, antifungal, antiviral, and immunoregulatory properties when added to infected cells and cultures *in vitro* (6). It kills bacteria by disrupting bacterial membranes and blocks viral entry by interacting with viral proteins, suppressing viral entry and/or replication (6). Cathelicidin can both inhibit the growth of and increase the virulence of *P. aeruginosa* via mutation, resulting in chronic infection (7). In addition, it modulates the host immune response by inducing production of chemoattractants and cytokines (6). Cathelicidin may also be associated with immune-mediated diseases like rheumatoid arthritis and psoriasis (6). Although the contribution of cathelicidin to the host response to different pathogens *in vivo* is still not well understood, it could be beneficial for both microbial clearance and immune regulation but detrimental if it induces pathogen mutation or contributes to immune system-mediated disease.

VITAMIN D AND THE IMMUNE SYSTEM

The effects of vitamin D and 1,25$(OH)_2$D on immunity include regulation of the innate and adaptive immune responses. In macrophages, 1,25$(OH)_2$D induces interleukin-10 (IL-10) production and inhibits IL-12 production (8). Dendritic cells treated with 1,25$(OH)_2$D become tolerogenic and induce fewer T cells to proliferate both *in vitro* and *in vivo* (9, 10). 1,25$(OH)_2$D inhibits gamma interferon (IFN-γ), tumor necrosis factor alpha (TNF-α), IL-2, and IL-17 production and induces regulatory T (T reg) cells that produce IL-10 (11, 12). Thus, the effects of vitamin D inhibit/suppress type 1-mediated immunity (11, 12). Type 1 immunity and IFN-γ/IL-17 are important for host defense against *M. tuberculosis* and influenza virus (11, 12), and 1,25$(OH)_2$D-induced production of T reg cells and IL-10 production are associated with poorer outcomes in human infection with *M. tuberculosis* (13). Thus, the ability of vitamin D and 1,25$(OH)_2$D to inhibit Th1/Th17 responses and induce T reg cells might result in more severe infection with *M. tuberculosis*.

Animal models of infection have generated mixed results on the role of vitamin D in host defense. Because of the inhibitory effects of 1,25$(OH)_2$D on Th1/Th17 responses, it might be predicted that pathogens which require a Th1/Th17 response for resistance would be more severe in 1,25$(OH)_2$D-treated and less severe in vitamin D-deficient or vitamin D receptor (VDR)-knockout (KO) mice. This was not the case for either *Candida albicans* or herpes simplex virus, each of which was unaffected by 1,25$(OH)_2$D treatment (14). VDR-KO mice were slower to clear *Salmonella* and *Listeria monocytogenes* than wild-type (WT) mice (15, 16). Feeding mice a vitamin D-deficient diet was associated

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with increased barrier dysfunction, dysbiosis of the microbiota, and more intestinal inflammation following *Citrobacter rodentium* infection (17), whereas administration of $1,25(OH)_2D$ decreased the Th17 response and increased *C. rodentium* shedding on day 10 postinfection (18). Dietary vitamin D treatment in mice was beneficial for resolving inflammation but did not alter their *M. tuberculosis* bacterial burdens (19). The experiments performed by Niederstrasser et al. demonstrated that vitamin D deficiency had no effect on acute infection and/or bacterial burdens 3 to 5 days postinfection (1). As such, the main effect of vitamin D in mice seems to be as an immune system regulator that may or may not affect the bacterial (viruses have not been well studied) burden *in vivo*.

VITAMIN D AND HUMAN PULMONARY INFECTION

The bulk of the evidence that vitamin D status is linked to host resistance to pulmonary infections in humans is associative and comes from observational studies (reviewed in references 20 and 21). Given that it is difficult to design effective interventions to test whether vitamin D supplementation would be beneficial in protecting humans from respiratory infections, it is not surprising that studies so far have yielded mixed effects (21). A disparate group of pathogens, including viruses and bacteria, have been studied in clinical interventions that range from acute (influenza/colds) to chronic (tuberculosis) infection (21). The mechanisms that control a virus, such as influenza virus versus an intracellular organism, like *M. tuberculosis*, or an extracellular organism, like *P. aeruginosa*, are different. In fact, the immune response is not always protective and can be detrimental. For example, during the 2009 H1N1 influenza epidemic, young individuals who mounted too strong an immune response were the sickest and the most likely to be admitted to hospital critical care units (22). In one human study, vitamin D supplementation inhibited IFN-γ and accelerated recovery in patients with *M. tuberculosis* by suppressing immune responsiveness (23). These and other data support the idea that the importance of vitamin D status in humans might be a function of its ability to dampen inflammation stemming from the host response and prevent inflammation-related injury.

Animal models have been incredibly useful for understanding the effects of vitamin D on the communication between cells *in vivo*. Mice have been good models to dissect the functioning of the immune system, and this includes our understanding of the mechanisms by which vitamin D regulates immunity. Comparative analyses of the effects of $1,25(OH)_2D$ on human and mouse cells have been performed. The inhibitory effects of $1,25(OH)_2D$ on T cell production of IL-17, IFN-γ, and IL-2 are identical in human and mouse cells (11, 12, 24, 25). The induction of $FoxP3^+$ T reg cells by $1,25(OH)_2D$ has also been demonstrated to occur in human and mouse T cells (11, 12, 24, 25). Some pathways are not identical; for example, the $1,25(OH)_2D$-induced production of cathelicidin occurs only in human but not mouse macrophages (2). Conversely, there are limitations to studying purified human cells in isolation and *in vitro* that do not apply to mouse cells. Nonetheless, overall data from animal models largely confirm the available data in humans that support a role for vitamin D and $1,25(OH)_2D$ in resolving inflammation following infection. The role of cathelicidin induction by vitamin D in bacterial/viral burdens and/or immune function *in vivo* is as yet unclear.

CONCLUSIONS

The scientific community needs to use all of the available tools to continue to determine the mechanisms by which vitamin D regulates host immune responses to pathogens and to translate the findings to improving outcomes in humans. Transgenic mice that express the human cathelicidin gene and regulatory elements (vitamin D response element) are being proposed as a way to utilize mice to demonstrate the *in vivo* role of vitamin D-induced cathelicidin following infection. At present, there is strong evidence in humans and animal models that suggests that improving vitamin D status might be beneficial for improving outcomes of lung infection with a variety of microbes. It may be that vitamin D will have different effects depending on the nature of the host response and the relevant pathogen. The study by Niederstrasser et al. demonstrates that in mice neither the innate immune response nor early clearance of either *P. aeruginosa* or *S. pneumoniae* is affected by vitamin D status (1). Future work should continue to determine the mechanisms of vitamin D action in the lung and the potential role that cathelicidin plays *in vivo* in controlling pathogen burden, host immune response, and regulation of inflammation.

REFERENCES

1. **Niederstrasser J, Herr C, Wolf L, Lehr CM, Beisswenger C, Bals R.** 2016. Vitamin D deficiency does not result in a breach of host defense in murine models of pneumonia. Infect Immun **84:**3097–3104. http://dx.doi.org/10.1128/IAI.00282-16.
2. **White JH.** 2010. Vitamin D as an inducer of cathelicidin antimicrobial peptide expression: past, present and future. J Steroid Biochem Mol Biol **121:**234–238. http://dx.doi.org/10.1016/j.jsbmb.2010.03.034.
3. **Liu PT, Stenger S, Li H, Wenzel L, Tan BH, Krutzik SR, Ochoa MT, Schauber J, Wu K, Meinken C, Kamen DL, Wagner M, Bals R, Steinmeyer A, Zugel U, Gallo RL, Eisenberg D, Hewison M, Hollis BW, Adams JS, Bloom BR, Modlin RL.** 2006. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. Science **311:**1770–1773. http://dx.doi.org/10.1126/science.1123933.
4. **Adams JS, Sharma OP, Gacad MA, Singer FR.** 1983. Metabolism of 25-hydroxyvitamin D3 by cultured pulmonary alveolar macrophages in sarcoidosis. J Clin Invest **72:**1856–1860. http://dx.doi.org/10.1172/JCI111147.
5. **Zhang JT, Chan C, Kwun SY, Benson KA.** 2012. A case of severe 1,25-dihydroxyvitamin D-mediated hypercalcemia due to a granulomatous disorder. J Clin Endocrinol Metab **97:**2579–2583. http://dx.doi.org/10.1210/jc.2012-1357.
6. **Bandurska K, Berdowska A, Barczynska-Felusiak R, Krupa P.** 2015. Unique features of human cathelicidin LL-37. Biofactors **41:**289–300. http://dx.doi.org/10.1002/biof.1225.
7. **Limoli DH, Rockel AB, Host KM, Jha A, Kopp BT, Hollis T, Wozniak DJ.** 2014. Cationic antimicrobial peptides promote microbial mutagenesis and pathoadaptation in chronic infections. PLoS Pathog **10:**e1004083. http://dx.doi.org/10.1371/journal.ppat.1004083.
8. **Verma R, Kim JY.** 2016. 1,25-Dihydroxyvitamin D3 facilitates M2 polarization and upregulates TLR10 expression on human microglial cells. Neuroimmunomodulation **23:**75–80. http://dx.doi.org/10.1159/000444300.
9. **Griffin MD, Kumar R.** 2003. Effects of 1alpha,25(OH)2D3 and its analogs on dendritic cell function. J Cell Biochem **88:**323–326. http://dx.doi.org/10.1002/jcb.10335.
10. **Griffin MD, Lutz W, Phan VA, Bachman LA, McKean DJ, Kumar R.** 2001. Dendritic cell modulation by 1alpha,25 dihydroxyvitamin D3 and its analogs: a vitamin D receptor-dependent pathway that promotes a persistent state of immaturity in vitro and in vivo. Proc Natl Acad Sci U S A **98:**6800–6805. http://dx.doi.org/10.1073/pnas.121172198.
11. **Bruce D, Ooi JH, Yu S, Cantorna MT.** 2010. Vitamin D and host resistance to infection? Putting the cart in front of the horse. Exp Biol Med **235:**921–927. http://dx.doi.org/10.1258/ebm.2010.010061.
12. **Cantorna MT, Waddell A.** 2014. The vitamin D receptor turns off chron-

ically activated T cells. Ann N Y Acad Sci **1317:**70–75. http://dx.doi.org/10.1111/nyas.12408.
13. **Parkash O, Agrawal S, Madhan Kumar M.** 2015. T regulatory cells: Achilles' heel of Mycobacterium tuberculosis infection? Immunol Res **62:**386–398. http://dx.doi.org/10.1007/s12026-015-8654-0.
14. **Cantorna MT, Hullett DA, Redaelli C, Brandt CR, Humpal-Winter J, Sollinger HW, Deluca HF.** 1998. 1,25-Dihydroxyvitamin D3 prolongs graft survival without compromising host resistance to infection or bone mineral density. Transplantation **66:**828–831. http://dx.doi.org/10.1097/00007890-199810150-00003.
15. **Wu S, Liao AP, Xia Y, Li YC, Li JD, Sartor RB, Sun J.** 2010. Vitamin D receptor negatively regulates bacterial-stimulated NF-kappaB activity in intestine. Am J Pathol **177:**686–697. http://dx.doi.org/10.2353/ajpath.2010.090998.
16. **Bruce D, Whitcomb JP, August A, McDowell MA, Cantorna MT.** 2009. Elevated non-specific immunity and normal Listeria clearance in young and old vitamin D receptor knockout mice. Int Immunol **21:**113–122. http://dx.doi.org/10.1093/intimm/dxn129.
17. **Assa A, Vong L, Pinnell LJ, Avitzur N, Johnson-Henry KC, Sherman PM.** 2014. Vitamin D deficiency promotes epithelial barrier dysfunction and intestinal inflammation. J Infect Dis **210:**1296–1305. http://dx.doi.org/10.1093/infdis/jiu235.
18. **Ryz NR, Patterson SJ, Zhang Y, Ma C, Huang T, Bhinder G, Wu X, Chan J, Glesby A, Sham HP, Dutz JP, Levings MK, Jacobson K, Vallance BA.** 2012. Active vitamin D (1,25-dihydroxyvitamin D3) increases host susceptibility to Citrobacter rodentium by suppressing mucosal Th17 responses. Am J Physiol Gastrointest Liver Physiol **303:**G1299–G1311. http://dx.doi.org/10.1152/ajpgi.00320.2012.
19. **Reeme AE, Robinson RT.** 2016. Dietary vitamin D3 suppresses pulmonary immunopathology associated with late-stage tuberculosis in C3HeB/FeJ Mice. J Immunol **196:**1293–1304. http://dx.doi.org/10.4049/jimmunol.1500931.
20. **Jolliffe DA, Griffiths CJ, Martineau AR.** 2013. Vitamin D in the prevention of acute respiratory infection: systematic review of clinical studies. J Steroid Biochem Mol Biol **136:**321–329. http://dx.doi.org/10.1016/j.jsbmb.2012.11.017.
21. **Bartley J, Garrett J, Grant CC, Camargo CA, Jr.** 2013. Could vitamin D have a potential anti-inflammatory and anti-infective role in bronchiectasis? Curr Infect Dis Rep **15:**148–157. http://dx.doi.org/10.1007/s11908-013-0321-9.
22. **Louriz M, Mahraoui C, Azzouzi A, El Fassy Fihri MT, Zeggwagh AA, Abidi K, Ferhati D, Echcherif El Kettani S, Tachinante R, Belayachi J, Zekraoui A, Sefiani Y, Charif Chefchaouni AM, Abouqal R.** 2010. Clinical features of the initial cases of 2009 pandemic influenza A (H1N1) virus infection in an university hospital of Morocco. Int Arch Med **3:**26. http://dx.doi.org/10.1186/1755-7682-3-26.
23. **Coussens AK, Wilkinson RJ, Hanifa Y, Nikolayevskyy V, Elkington PT, Islam K, Timms PM, Venton TR, Bothamley GH, Packe GE, Darmalingam M, Davidson RN, Milburn HJ, Baker LV, Barker RD, Mein CA, Bhaw-Rosun L, Nuamah R, Young DB, Drobniewski FA, Griffiths CJ, Martineau AR.** 2012. Vitamin D accelerates resolution of inflammatory responses during tuberculosis treatment. Proc Natl Acad Sci U S A **109:**15449–15454. http://dx.doi.org/10.1073/pnas.1200072109.
24. **Ooi JH, Chen J, Cantorna MT.** 2012. Vitamin D regulation of immune function in the gut: why do T cells have vitamin D receptors? Mol Aspects Med **33:**77–82. http://dx.doi.org/10.1016/j.mam.2011.10.014.
25. **Cantorna MT, Snyder L, Lin YD, Yang L.** 2015. Vitamin D and 1,25(OH)2D regulation of T cells. Nutrients **7:**3011–3021. http://dx.doi.org/10.3390/nu7043011.