The immunoregulatory effects of vitamin D3 on Mycobacterium infection

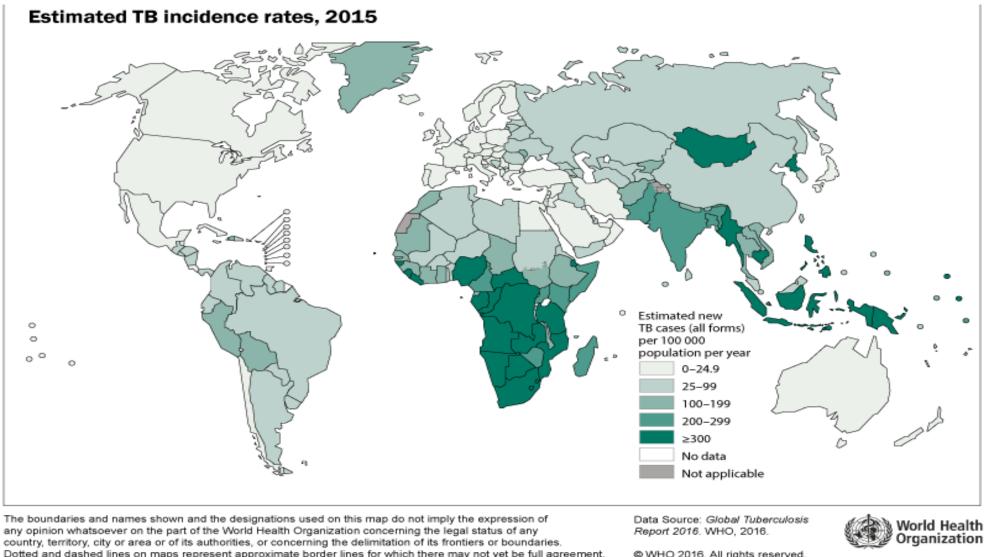Maya E. Gough[1], Edward A. Graviss[2], & Elebeoba E. May[1]*

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Objective: Examine the effects of vitamin D3 on the production of immune related cytokines, chemokines, and effector molecules at varying severities of *Mycobacterium* infection.
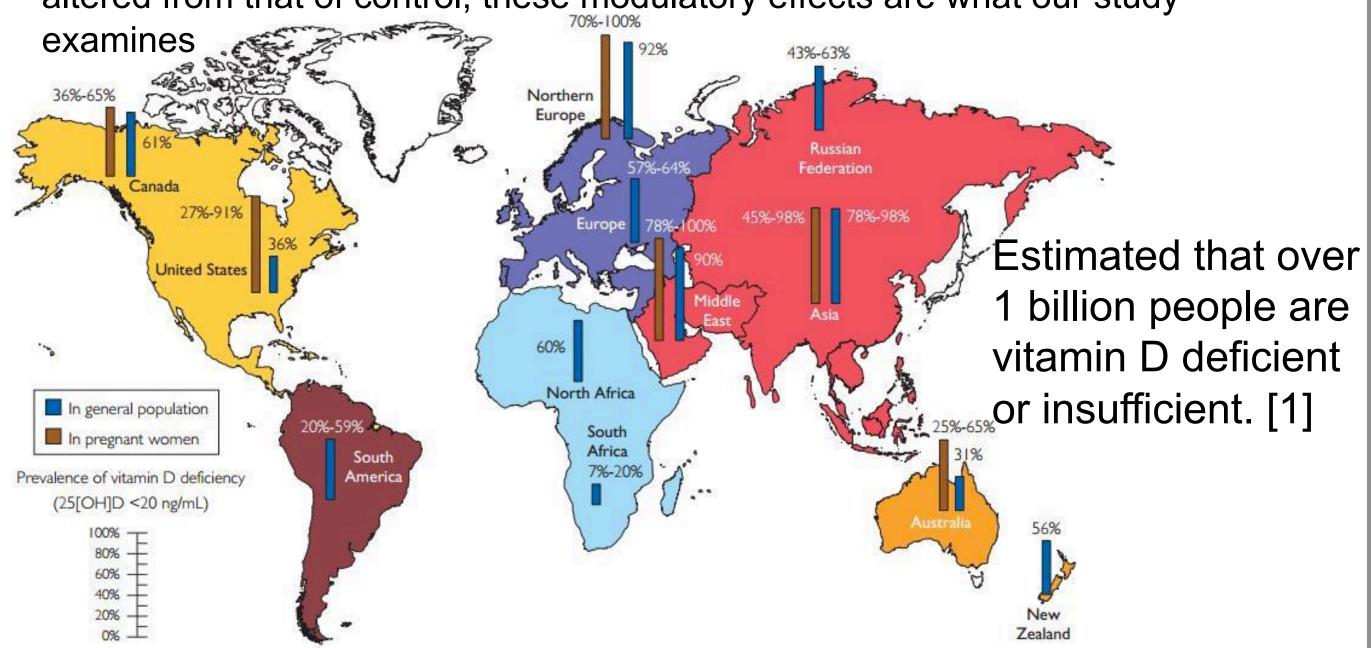
Introduction & Background

- *Mycobacterium tuberculosis* is a global health concern, infecting 10.4 million people and causing 1.8 million deaths a year.
- Infection primarily takes place in the lungs but can spread throughout the body to other organs, such as spleen, liver.
- In a healthy person TB can present with no symptoms and it is estmated that 1/3 people have latent TB. [4]
- Vitamin D_3 deficiency has been closely tied to incidence of TB and outcome of TB infection.

In 2015:
10.4 million TB cases   1.8 million deaths due to TB [1]
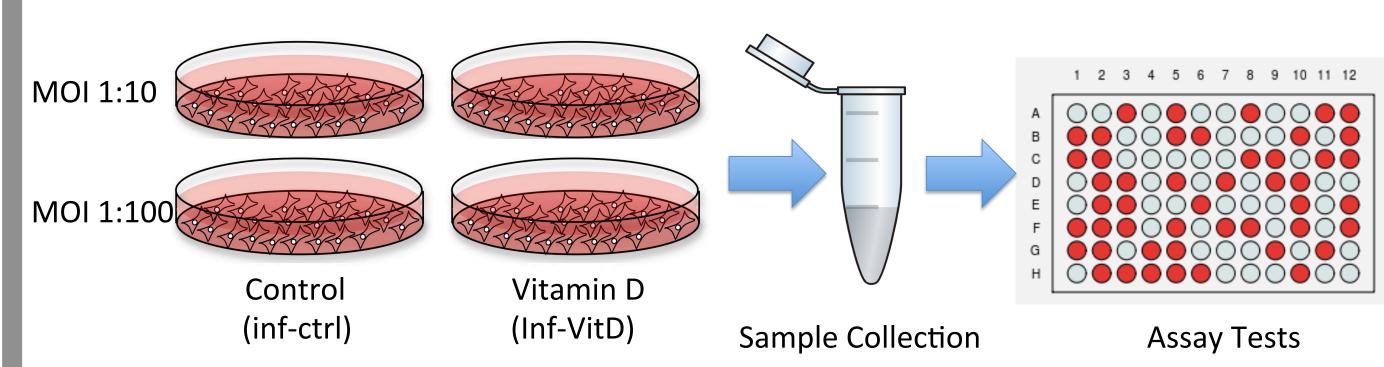
Estimated TB incidence rates, 2015

- Vitamin D3 is produced by the human body through exposure to UV light or obtained through the consumption of a limited number of plants, fungi, fish, and manufactured oral supplements.
- Classical Functions – Aid in the absorption of calcium and phosphorous.
- Non-Classical Functions – Modulate innate and adaptive immune response.[3]
- Through the vitamin D response element (VDRE), vitamin D_3 when bound to its receptor, is thought to act as a transcription factor and modulate production of certain cytokines and effector molecules, including IL-10, IL-12,TNF-a, NO, H2O2, and many more.
- In the presence of vitamin D3 the phenotypic response of macrophages is altered from that of control; these modulatory effects are what our study examines
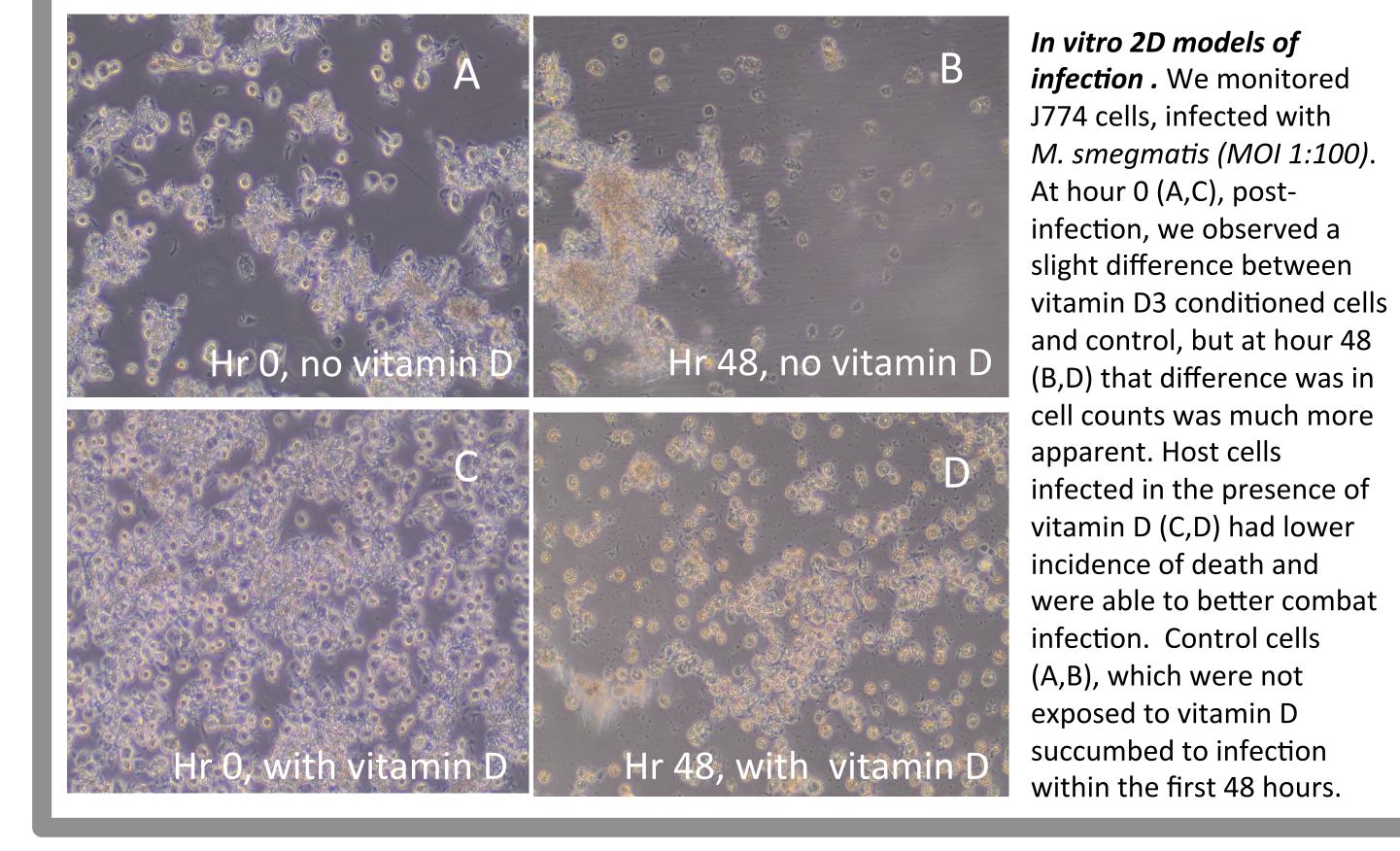
Estimated that over 1 billion people are vitamin D deficient or insufficient. [1]

Prevalence of vitamin D deficiency (25[OH]D <20 ng/mL)

References: 1. World Health Organization. **2.**Center for Disease Control. **3.** Holick MF. Vitamin D Deficiency. N Engl J Med 2006; 357:266-281. **4.** Todar K; Todar's online textbook of bacteriology. 2008. **5.** http://bigtomato.org/endo/bcp/vitamind.php. **6.** Flynn JL, Chan L. Immune evasion by Mycobacterium tuberculosis: living with the enemy. Current Opinion in Immunology 2003;15:450-455. **7.** Waters WR, Palmer MV, Nonnecke BJ, et. al. Mycobacterium bovis infection of vitamin D-deficient NOS2-/- mice. Microbial Pathogenesis 2004; 36:11-17. **8.**Chun, R. F., Liu, P. T., Modlin, R. L., Adams, J. S. & Hewison, M. Impact of vitamin D on immune function: lessons learned from genome-wide analysis. *Frontiers in Physiology* **5,** (2014).
Acknowledgements: Research supported in part by University of Houston/Houston Methodist Research Institute Graduate Fellowship in Clinical Translational Research and DARPA, Cirilo Lab at Texas A&M, Carson Lab Sandia National Labs.

Methods

- *M.smegmatis*, an analog for *M.tuberculosis*, was grown to late growth stage and used to infect the J774 mouse cell line.
- The infection was administered at a ratio of host to bacterial cell, 1:10 and 1:100.
- We monitored cells over three days, allowing for the investigation of short and long-term effects of vitamin D_3 on infection outcome.
- Cells were conditioned with vitamin D3 from time of infection onward.
- Uninfected cells of all conditions were maintained to ascertain overall health of cells, as well as infected unconditioned cells, and infected vitamin D_3 treated.
- Samples were taken for analysis over 72 hours.
- LDH, H2O2, Greiss Reagent, and ELISA assays were utilized to analyze samples.
- 7H11 agar plates were inoculated with supernatant and cell lysate to ascertain colony forming units per milliliter.

MOI 1:10   MOI 1:100

Control (inf-ctrl)   Vitamin D (Inf-VitD)   Sample Collection   Assay Tests

A   B   C   D
Hr 0, no vitamin D   Hr 48, no vitamin D
Hr 0, with vitamin D   Hr 48, with vitamin D

In vitro 2D models of infection . We monitored J774 cells, infected with *M. smegmatis* (MOI 1:100). At hour 0 (A,C), post-infection, we observed a slight difference between vitamin D3 conditioned cells and control, but at hour 48 (B,D) that difference was in cell counts was much more apparent. Host cells infected in the presence of vitamin D (C,D) had lower incidence of death and were able to better combat infection. Control cells (A,B), which were not exposed to vitamin D succumbed to infection within the first 48 hours.

Conclusion

Results suggest that the efficacy of immune response is significantly enhanced in the presence of 1,25-dihydroxyvitamin D_3. Vitamin D_3 supplementation was directly correlated to a significant increase in production of antibacterial product NO and a slight increase in H2O2. The modulation of these immune effector molecules, as well as the pro- and anti-inflammatory cytokines IL-12 and IL-10, respectively, corresponded to increased clearance of extracellular and intracellular bacterial load, while minimizing host cell cytotoxicity (LDH). The effect of vitamin D_3 during the infection is unique when compared to control cells but also differs between severity of infection, the response of vitamin D3 conditioned cells during MOI 1:10 infection is vastly different from that of MOI 1:100. This allows us to conclude that severity of infection plays an important roll in the modulatory prowess of vitamin D3.

Results

Vitamin D3 decreases extracellular and intracellular bacterial load and host cell cytotoxicity (death), it also allows for a more gradual production/increase of IL-10; in the presence of vitamin D3 IL-12 and NO production is decreased at low level of infection (MOI 1:10) and increased at high level of infection (MOI 1:100) possibly to decrease damage to host cell caused by vigorous inflammatory response.

To enable comparison of the immune response across experimental conditions (unconditioned control, vitamin D3 conditioned, and ethanol conditioned) the grand median was determined for each assay across all experimental conditions, and used as the common normalization factor (50). Grand median normalized data was used to perform Welch's t test for overlapping time intervals of 0-16, 8-24, 16-34, 24-44, 34-54, 44-64, 54-74 hours to identify variations in immune response between experimental conditions. These sliding intervals allow us to elucidate differences that otherwise may be minimized due to grouping.

Overlapping Time Interval t-Test		Hours						
Low MOI 1:10		0-16	08-024	16-34	24-44	34-54	44-64	54-74
il10_ic.l	il10_id.l		3.05	2.98 **	2.54 ***	1.98 **		
il12_ic.l	il12_id.l		1.45	1.45 **	1.34	1.35		
ldh_ic.l	ldh_id.l	4.43 ***	3.82 **		2.46	1.97 *	1.89 *	
no_ic.l	no_id.l				1.34	1.32		
High MOI 1:100		0-16	08-024	16-34	24-44	34-54	44-64	54-74
extra_c.h	extra_d.h			1.63 *			5.87 *	
il10_ic.h	il10_id.h		1.57	1.49				
il12_ic.h	il12_id.h	0.54 **		0.88	0.90 **		0.89 **	
no_ic.h	no_id.h	0.88 **	0.87 **	0.88	0.90 **	0.89	0.90 **	

Statistically significantly different   (p≤0.05*; p≤0.01**; p≤0.001***)