

Vitamin D Receptor: An Underrated Hero

SMART Team members: Catherine Minter, Chenoah Gad, Elizabeth Merkel, Holly Van Gorden, Jenna Amro, Noah Amro, Sylvia Stuebs

Teacher: Mrs. Karen O'Donnell

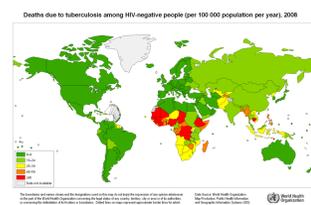
Mentor: Allison Reeme, Medical College of Wisconsin

I. Abstract

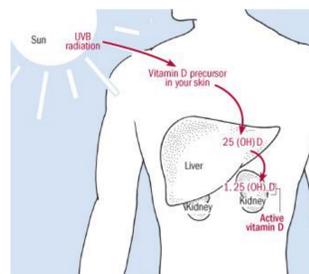
For over a century, vitamin D (vit. D) has been used as therapy for the bacteria *Mycobacterium tuberculosis* (Mtb) due to its effects on the immune system. Interest has risen for vit. D's ability to modulate immune responses by signaling through the vitamin D receptor (VDR). Vit. D is obtained through dietary sources, like seafood, or exposure to sun's UVB rays. Vit. D in its active form can passively diffuse into multiple cell types, such as lymphocytes, while the VDR, a transcription factor for vit. D regulated genes, can regulate the effects of the hormone in these cells. Vit. D has been shown to modulate the immune response during Mtb infection by controlling production of cytokines and antimicrobial peptides and its interactions with the VDR is critical for these effects. In order to recognize VDR's role during the immune response to Mtb, the Wisconsin Virtual learning SMART Team (Students Modeling A Research Topic) is using 3D printing technology to model the structure, primarily highlighting amino acids Arg274 and His305 which are required for ligand binding to the VDR. Scientists, recognizing vit. D's positive role during an immune response, will continue to investigate vit. D as a therapeutic agent to treat this significant plight.

II. The World and Vitamin D: A Global Phenomenon

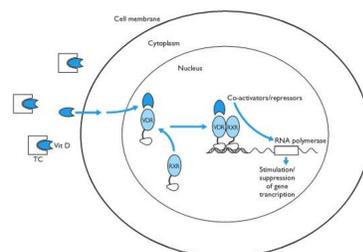
- *Mycobacterium tuberculosis* (Mtb) is a bacterial pathogenic disease
- Mtb is second deadliest disease (one third of population infected)
- Current strains of Mtb are becoming resistant to treatments
- New discoveries that UVB light exposure may alleviate symptoms of Mtb
- New treatments require continued research into Vitamin D's influence on immune responses during Mtb infection



Metabolism of Vitamin D



- Body receives Vitamin D from sources such as seafood, dairy, and light exposure
- Vitamin D Receptor (VDR) is necessary for Vitamin D metabolism
- Vitamin D undergoes hydroxylation in liver and then is converted to its active form



- Vitamin D can passively diffuse into multiple cell types
- VDR is a transcription factor for vit. D-regulated genes
- VDR regulates effects of hormones in cells

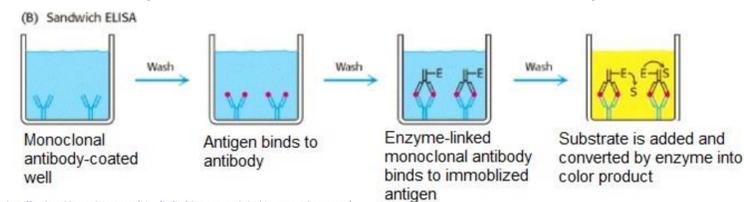
III. Hypothesis

Vitamin D supplementation during *Mycobacterium tuberculosis* infection will play a beneficial role to the host by decreasing the number of bacteria in the lungs, which is dependent upon the vitamin D receptor.

IV. Examining Vitamin D in Mtb-infected Mice

- Put mice on special diets (High vitamin D 20000 IU/No vitamin D) and aerosol infect the mice with Mtb
- At various time points after infection, asphyxiate mice with CO₂.
- Collect blood from the heart and place in a centrifuge to separate out the serum, perform an Enzyme-Linked Immunosorbent Assay (See chart below) to calculate vitamin D levels
- Collect lungs and spleen from the mice and homogenize the tissues
- Place collected tissues on agar plates and calculate the amount of Mtb colonies.

The Enzyme-linked Immunosorbent Assay (ELISA)



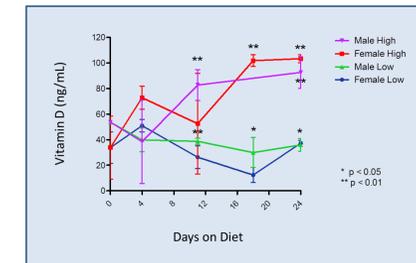
<http://exploreable.wordpress.com/2011/05/25/elisa-enzyme-linked-immunosorbent-assay/>

ELISA is the process in which scientists use to identify the presence of Vitamin D in samples taken from mice. The intensity of the color change in the final product correlates to how much Vitamin D is present within the sample.



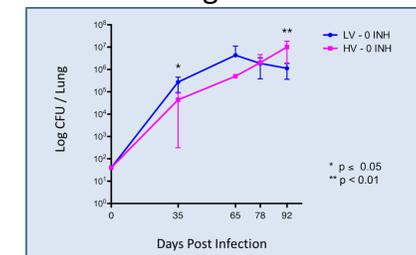
V. Results

Figure 1



Mice fed diets containing different levels of Vitamin D have varying serum Vitamin D levels after 24 days. Blood was collected once a week for 4 weeks and serum Vitamin D had been determined using ELISA.

Figure 2



The mice on both the high and low Vitamin D diets showed increased numbers of Mtb colonies in the lungs. Colonies were counted on agar plates, sometimes requiring dilutions in order to accurately count the bacteria.

VI. Conclusions and Future Direction

- The Vitamin D Receptor is important for the effects we see in the treatment of tuberculosis.
- Vitamin D levels can be manipulated in mice through diet
- High levels of Vitamin D are protective with mice infected with Mtb early in infection
- Determine if the effects of vitamin D are in hematopoietic cells (bone marrow cells) using VDR knockout mice

VII. References

1. N.Rochel, J.M. Wurtz, A. Mitschler, B. Klaholz, D. Moras, The Crystal Structure of the Nuclear Receptor for Vitamin D Bound to Its Natural Ligand, *Molecular Cell*, 2000 January; 5(1): 173-179
2. 10 Facts About Tuberculosis. *WHO | World Health Organization*. 2013 October.