THE PENNSYLVANIA STATE UNIVERSITY
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DEPARTMENTS OF Biochemistry and Molecular Biology and Veterinary and Biomedical Sciences

THE EFFECT OF VITAMIN D DEFICIENCY ON VDR AND ZO-1 EXPRESSION IN THE COLON DURING A CITROBACTER RODENTIUM INFECTION OF WILD TYPE AND CY27B1KO MICE

KELLY MCGILL
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Reviewed and approved* by the following:

Margherita Cantorna
Distinguished Professor of Molecular Immunology
Thesis Supervisor

Sarah Ades
Associate Professor of Biochemistry and Molecular Biology
Honors Adviser

Robert Van Saun
Professor of Veterinary Science
Honors Advisor
* Signatures are on file in the Schreyer Honors College.

ABSTRACT

Though traditionally and historically associated with bone metabolism and calcium absorption, recent developments in the field of immunology have demonstrated an important role for vitamin D with regard to immune function. The Cantorna laboratory has studied the interactions of vitamin D with the immune system in face of an immune challenge. They used a bacterial enteric infection and mice that were incapable of responding to vitamin D as an indirect way of assessing the potential role of vitamin D with regard to an immune challenge. The experiment determined that the vitamin D receptor knock out (VDR KO) mice had decreased *Citrobacter rodentium* fecal loads and a quicker recovery compared to wild-type (WT) mice. The VDR KO mice also had a stronger immune response; they had more interleukin-22 (IL-22)-producing innate lymphoid cells (ILCs) and increased expression of antibacterial peptides compared to the WT mice. The inability of the mice to respond to vitamin D without the vitamin D receptor (VDR) suggested that the down regulation of vitamin D may benefit the animal by enabling a stronger response to the immune challenge. Contradictorily, a different study examined vitamin D deficiency's influence on infection-induced changes in intestinal epithelial barrier by challenging WT mice that were ether vitamin D sufficient or vitamin D-deficient with an enteric infection. The researchers determined that the vitamin D deficient mice infected with *C. rodentium* had increased epithelial barrier dysfunction. They concluded that vitamin D deficiency increased susceptibility by allowing for greater intestinal injuries. The aim of the experiment was to determine if the VDR and ZO-1 were down regulated in the colon tissue during an enteric infection in order to better understand how the mice responded to the immune challenge. The expected outcome was that the VDR and ZO-1 expression in the colon would be decreased compared to baseline levels in order to allow a stronger immune response in the face

of an enteric infection. *C. rodentium* was used to infect three different mice groups in the experiment: vitamin D sufficient wild type C75BL/6; vitamin D deficient wild type C57BL/6 mice; and vitamin D deficient cytochrome 27B1 (CYPKO) mice. The distal colon tissue was harvested at the peak day of infection (day 14) and carried out RNA extraction using TRIzol RNA protocol, a reverse transcription reaction, and quantitative PCR to measure the VDR RNA and ZO-1 RNA levels. The three groups were compared with a two-way ANOVA statistical analysis with a $p<0.05$ as significant. The VDR expression at peak infection or day 14 was down regulated significantly compared to baseline day 0 expression levels in all three groups with a p value <0.001. There was no statistical difference ($P>0.05$) in down regulation between the vitamin D deficient or vitamin D sufficient mice or between the WT or cyp27B1 KO mice. In addition the VDR colonic expression, the normalized ZO-1 colonic expression level changed significantly between the WT D- and CYPKO D- mice on day 14 with a p-value <0.05. The vitamin D status of the mouse did not have statistical significance between day 0 and day 14. The type of mouse, WT or CYPKO, had a p-value less than 0.05 when the day 0 and day 14 normalized ZO-1 colonic expression. This down regulation may have occurred in order to promote the production of antimicrobial peptides or a stronger Th1 cell response. A kinetic study of this down regulation will take place to determine if it occurs earlier on in the infection, gradually throughout or closer to peak day of infection.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

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everyone who inspired me to continue learning, brainstorming, and imagining a world where I could make a difference through hard work, dedication, and intellectual discoveries.

Chapter 1

Background Information

Discovery of Vitamin D

The Boston University biochemist and science fiction author Isaac Asimov once said "The most exciting phrase to hear in science, the one that heralds new discoveries is not 'Eureka' but 'That's funny…" Indeed, his profound statement illustrates the discovery process of vitamin D. Vitamin D was discovered in a similar process to other vitamins with a deficiency induced disease fixed by dietary intervention. Besides its discovery, the metabolism of vitamin D precursors into the active hormone form of vitamin D creates a unique structure, different from the other steroid hormones and vitamins. This rare structure gives vitamin D a powerful structure-function relationship that enables vitamin D to perform its significant physiological actions on the immune system.

The medical and scientific community discovered vitamin D due to a deficiency-induced disease: rickets[1]. From the 1600's to the 1800's, rickets plagued many communities as the Industrial Revolution rapidly grew and spread across the world[1]. Rickets involves the softening of bones, physical deformities, bowed legs, muscle spasms, and seizures[1]. Due to the success of curing scurvy with vitamin C rich foods, scientists began the search for foods that could cure or alleviate rickets[1]. They found that cod liver oil, a rich source of vitamin D, cured and prevented rickets[1]. Dr. Scheutte became one of the first doctors to prescribe cod liver oil as a cure for rickets in 1824[2]. In 1922 in Vienna, Henrietta Chick fed children a diet of either whole fat milk

or cod liver oil in an attempt to prevent the development of rickets and successfully gathered support that dietary components of certain foods could stop the development of rickets[2]. Moreover, in 1922, Elmer McCollum and his research team named the fat soluble factor found in cod liver oil vitamin D because it followed on the heels of the discovery of vitamin B and C[2]. Furthermore, they differentiated vitamin D from fat soluble vitamin A, heat inactivation eliminated vitamin A activity but not vitamin D, the heat treatments still effectively treated rickets[2]. Like other vitamins at that time, scientists became aware of vitamin D and its importance to human health through a diet and disease relationship.

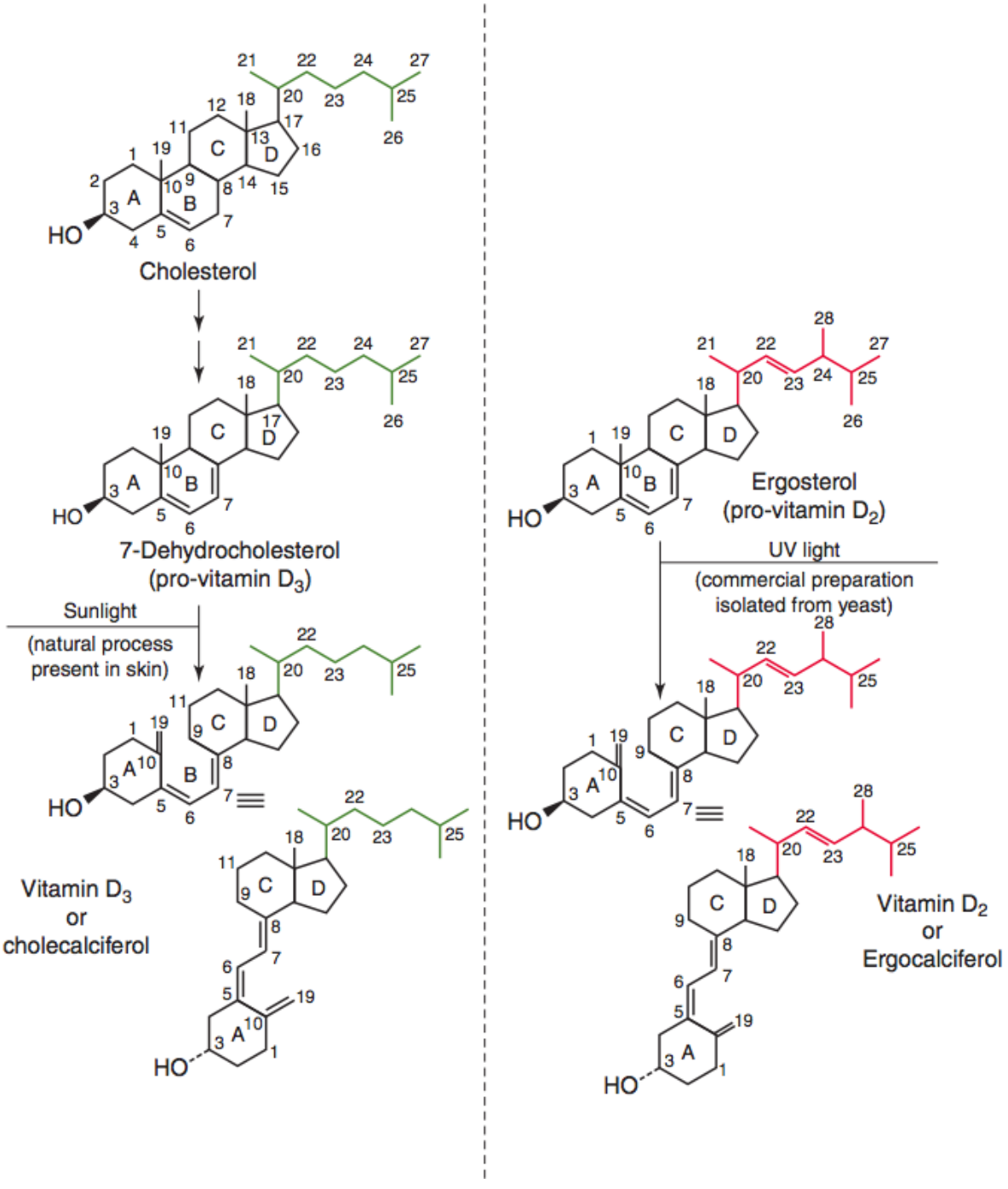
Vitamin D's Structure

Besides carrying out research on the dietary aspects of rickets, scientists also observed the environmental influence of sunlight with its ability to prevent and cure rickets[1]. Henrietta Chick discovered that sunlight could alleviate rickets in children[2]. In the 1920's, scientists showed that irradiation of vegetables was required to prevent rickets whereas milk and cod liver oil did not require irradiation[1], [2]. Another scientist named A.F. Hess identified the difference in the vegetable fats that could cure rickets compared to those that could not cure rickets after irradiation, as sitosterol[2]. Hess then proposed that sunlight could activate cholesterol in skin (7-dehydrocholesterol to distinguish it from other types of cholesterol in the skin) to prevent and cure rickets[2]. The essential fact that both sunlight and food could prevent and cure rickets sets vitamin D apart as a unique kind of vitamin.

To gather more data and support for his proposal about the pre-existing form of vitamin D in animal products and human skin, Hess asked Adolf Windaus, a well-known German

chemist, who researched steroids, to help unravel the structure of vitamin D formed from UV radiation of cholesterol[2]. Physical purification methods like recrystallization were ineffective and the researchers used chemical purification procedures to isolate the cholesterol from 7-dehydrocholesterol[2]. Otto Rosenheim and Thomas A. Webster analyzed the three typical components of steroid structures, which include a hydroxyl group, at least one carbon-carbon double bond, and four reduced rings but found that these structures did not distinguish the vitamin D precursor from pure cholesterol (Figure 1)[3]. Additionally, the vitamin D precursor molecule had three peaks of maximum absorbance at 269, 280, and 293 nanometers suggesting the presence of multiple double bonds as opposed to cholesterol's single double bond (Figure 1)[4]. They then realized that pure cholesterol did not form the precursor to vitamin D[2.] Furthermore, Rosenheim and Webster also demonstrated that UV radiation could not activate open ring systems to become antirachitic (curing rickets) even if they possessed three double bonds[4]. After analysis of 30 different cholesterol structures, the research group of Hess, Windaus, Webster, and Rosenheim determined that ergosterol formed a precursor to vitamin D in plants (Figure 1)[2]. In 1933, they renamed the purified compound califerol or vitamin D_2[2]. It took another six years for Windaus and his research team to discover the animal or human precursor, 7-dehydrocholesterol that they renamed vitamin D_3 or cholecaliferol (Figure 1)[2]. The animal or human precursor form differed from ergosterol in that 7-dehydrocholesterol has two double bonds rather than three double bonds (Figure 1)[5]. Over a period of about twenty years, scientists determined the structures of both the dietary or plant form and animal or physiological forms of vitamin D precursors.

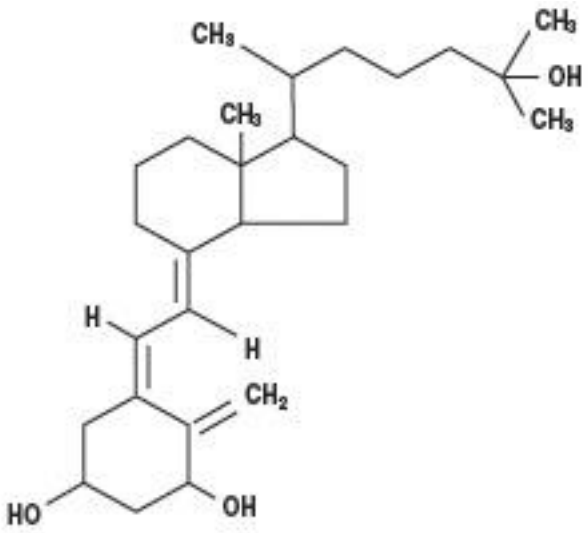
Figure 1 The Different Structures of Vitamin D and its Precursors[5]



Of equal if not greater importance than the structure determination, the structure-function relationship of vitamin D differentiates vitamin D from other steroid hormones[6]. Compared to steroid hormones like cortisol and aldosterone, the active vitamin D has a secosteroid structure

since one of the four rings has a broken carbon-carbon single bond or in other words, an open ring (Figure 2)[7]. The open ring resulted in a more flexible structure compared to other steroid hormones[7]. The A-ring in vitamin D adopts the α conformation for the hydroxyl group on the first carbon while the seco-B-ring prefers a trans arrangement of its double bonds that overall contributes to less steric stress while lengthening the molecule (Figure 2)[6]. These preferences that create the remarkable structure of vitamin D and bear responsibility for vitamin D's different behavior and functions compared to other steroid hormones and vitamins.

Figure 2 1,25-dihydroxyvtitamin D_3 Structure[8]



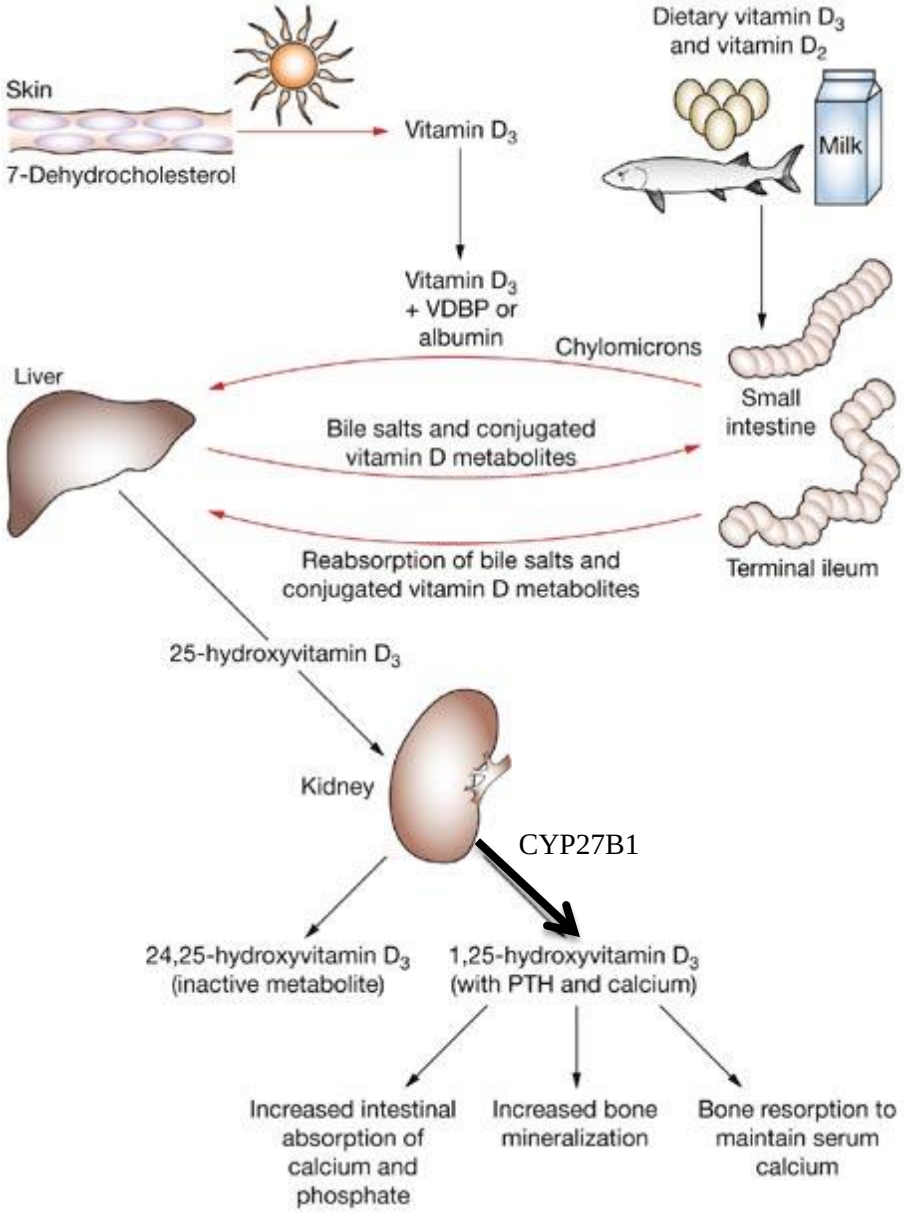
Vitamin D Metabolism and Regulation

Vitamin D Metabolism

The structure function relationship is a circular one where function determines structure based on what needs to be carried out while structure also influences function with how easily certain tasks are performed. The formation of vitamin D structure that begets the key structure-

function relationship develops from the metabolism of vitamin D precursors: vitamin D_2 and vitamin D_3. The small intestine absorbs each of the precursor forms[9]. Both vitamin D_2 and vitamin D_3 bind the vitamin D binding protein (DBP) to travel by the bloodstream to the liver[9]. Once in the liver, the enzyme cytochrome P450 vitamin D 25 hydroxylase adds a hydroxyl group at the C25 position to form 25-hydroxyvitamin D[9]. This form circulates through the blood with the aid of DBP to the kidney or other tissues[9]. The cytochrome P450 monooxygenase 25(OH)D 1α hydroxylase otherwise known as CYP27B1 converts 25-hydroxyvitamin D to $1,25(OH)_2D$, the biologically active form[9]. The flexible structure of vitamin D also sets it apart from the steroid hormones because it has both a nuclear genomic receptor to allow transcription control and a membrane bound receptor for non-genomic responses[10]. Thus, vitamin D can influence transcription of certain molecules and also signal molecules. The metabolism of vitamin D precursors generates the remarkable structure of the hormone form of vitamin D to engineer the crucial structure-function relationship that permits the physiological actions of vitamin D.

Figure 3 Vitamin D Metabolism Pathway[11]



Vitamin D Regulation

Due to its importance in the metabolism of vitamin D, the human body tightly regulates the renal CY27B1 enzyme[5]. The product, $1,25(OH)_2D$, serves as a homotropic allosteric inhibitor of the enyzme[5]. A high concentration of the active form of vitamin D inhibits

production of the enzyme while a low concentration increases the kidney's production of the CYP27B1 enzyme[5]. Additionally, calcium and phosphate operate as heterotropic allosteric inhibitors of renal CYP27B1[9]. When their concentrations drop, the CYP27B1 enzyme experiences enhanced activity[9]. The lowered concentration of calcium increases the parathyroid hormone (PTH) that in turn increases the transcription of CYP27B1[9]. The tight regulation of the key step in activating vitamin D enables control over the calcium level to prevent both hypocalcaemia and hypercalcaemia[9].

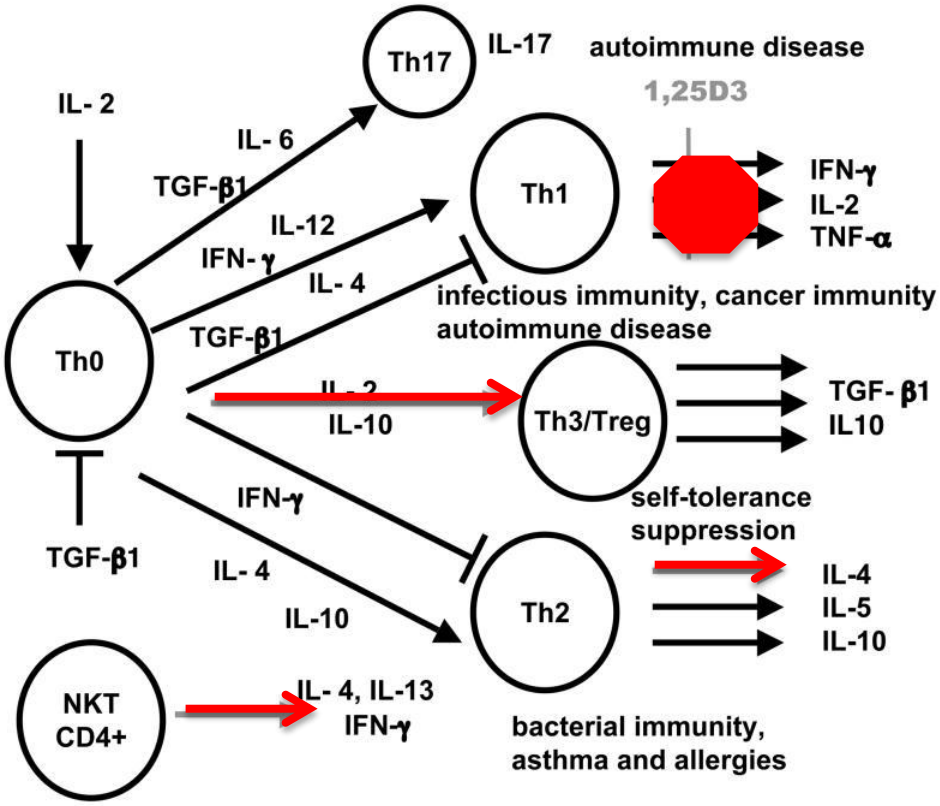
Vitamin D and Autoimmunity

Of further interest, $1,25(OH)_2D$ influences the development of autoimmune disorders[12]. Studies have demonstrated that low vitamin D status can increase risk of developing T cells (T_{h1}) mediated autoimmune disorders such as inflammatory bowel disease, type I diabetes, asthma, and multiple sclerosis[12]. On the other hand, research studies have also exhibited that the biologically activated form of vitamin D ($1,25(OH)_2D_3$ or hormone form of vitamin D) can prevent the development of these autoimmune disorders[12]. Autoimmune disorders result when the body's T cells lose control and start attacking the body's own cells and produce excessive inflammation[12]. The overproduction and over activation of T cells was associated with low vitamin D status[12]. The VDR allows 1,25-dihydroxyvitamin D to regulate the immune system cells like macrophages, dendritic cells, and regulatory T cells[12]. Vitamin D increases the production of macrophages while inhibiting the differentiation of T helper cells[12]. These actions reduce the inflammatory cytokine production of TNF-α and IL-12 that characterizes autoimmune disorders[12]. Additionally, $1,25(OH)_2D$ prevents the maturation of dendritic cells in order to limit

their ability to prime T_{h1} cells[12]. More importantly, the biologically active hormone form of vitamin D induces the production of regulatory T cells by up-regulating the Fox3 gene in T cells[12]. Regulatory T cells help to prevent the development of autoimmunity by inducing apoptosis in self reactive T cells as well as limiting the number of active effector T cells present in the body[12]. $1,25(OH)_2D$ influences the development of autoimmune disorders through its interactions with macrophages, dendritic cells, T cells[12]. In sum, $1,25(OH)_2D_3$ interacts with the immune system cells to suppress the development of autoimmune disorders by inhibiting effector T cells and increasing regulatory T cells[12].

Figure 4 Vitamin D's Effect on T Cell Development[12]

1,25D3 (actions show in red) is shown inhibiting the development of Th1 cells and promoting the development of Th3/Treg and Th2 cells to aid with tolerance and prevent autoimmune disease. The cytokines such as IL-2 are also shown in the T cell development pathway.



According to the National Institute of Health, around 23.5 million Americans suffer from autoimmune disorders and doctors make many new diagnoses every day[13]. Thus, research that explores the molecular aspects of autoimmune disease holds a relevant, central role for the future of the biomedical and biochemical field. Inflammatory bowel diseases (IBD), the umbrella term for a group of autoimmune diseases, causes over 700,000 doctor's visits and 100,000 hospitalizations[14]. The estimated health care expenses involved with the treatments of autoimmune disease for Americans costs over 100 billion dollars, which about doubles cancer treatment costs[13]. Autoimmune diseases cause enough bodily harm to merit a spot on the list of the top ten causes of death in girls and middle-aged women[14]. Around 34,000 people died from

IBD in 2010 where as only 29,500 people died from IBD in 1990[15]. Scientists must continue to perform more innovative research on IBD since current treatments have not lowered the mortality rate. Furthermore, other countries have surpassed the U.S in terms of research performed on autoimmune disease[16]. Many factors such as genetics, gut flora antigens, and environmental triggers can impact the development and remission of IBD[13]. The goal of this set of experiments is to contribute to better therapies and treatments for IBD by exploring the different environmental influences that can influence the development and severity of IBD.

VDR Expression Studies

The Cantorna laboratory has shown that vitamin D_3 plays in a pivotal role in the outcome of experimental IBD models by suppressing IBD development[17]. Specifically, $1,25(OH)_2D_3$ interacts with the adaptive immune system to regulate the T cell response inhibiting the overactive T helper one (Th_1) response and stimulating the development of regulatory T cells (T_{reg})[12]. Beyond vitamin D_3's interactions with the immune system, other studies have determined that vitamin D_3 influences gut epithelial barrier integrity[18]. Juan Kong and other researchers performed a study that compared the intestinal barrier between wild type mice and mice with their vitamin D receptor (VDR) knocked out (KO) in all the body cells[18]. They found that the VDR KO mice developed more severe colitis and suffered a greater loss of transepithelial electric resistance (TER)[18]. TER provides a way to measure the integrity of the epithelial barrier, the greater the resistance, the more intact the epithelial barrier[18]. Furthermore, their study also revealed a loss of tight junction proteins and thus disrupted tight junctions in gut epithelium of the VDR KO mice but not in the wild type mice[18].

An additional study published in the Journal of Clinical Investigation explored the mechanism of vitamin D_3's interactions with the gut epithelial barrier integrity in greater detail[19]. The researchers found that the expression of the VDR in human patients with either ulcerative colitis or Crohn's disease was lower than the normal expression levels[19]. They used transgenic mice that over-expressed the human form of the vitamin D receptor hVDR in the gut epithelial cells in their experiments[19]. The hVDR over-expression preserved epithelial integrity and protected mice from developing chemically induced colitis[19]. The researchers also determined that the transgenic hVDR mice produced lower levels of inflammatory cytokines and maintained expression of tight junction proteins[19]. Their group generated VDR KO mice with hVDR induced expression specifically in the gut epithelial cells and found that these mice had stronger resistance to developing colitis in the experimental colitis models than wild type mice[19]. The researchers determined that vitamin D_3 inhibited the signal molecule p53 upregulator modulator of apoptosis (PUMA) by blocking the nuclear gene transcription factor NF-κB which serves as an activator of PUMA[19]. 1, 25$(OH)_2D_3$ blocked the binding site of NF-κB and prevented TNF-α induced transcription of PUMA[19]. In other words, the active form of vitamin D_3 inhibited the apoptosis of intestinal epithelial cells and preserved the membrane integrity. Thus, this research group determined that over-expression of the human VDR in mice served a protective function by decreasing the apoptosis of intestinal epithelial cells during two different models of IBD[19].

Citrobacter rodentium Studies

Citrobacter rodentium is an enteric pathogen[20]. It starts by colonizing the cecum and then proceeds to the distal colon by day 2 or 3[21]. The infection model produces inflammation and a mucosal T_{h1} and T_{h17} mediated response[20].

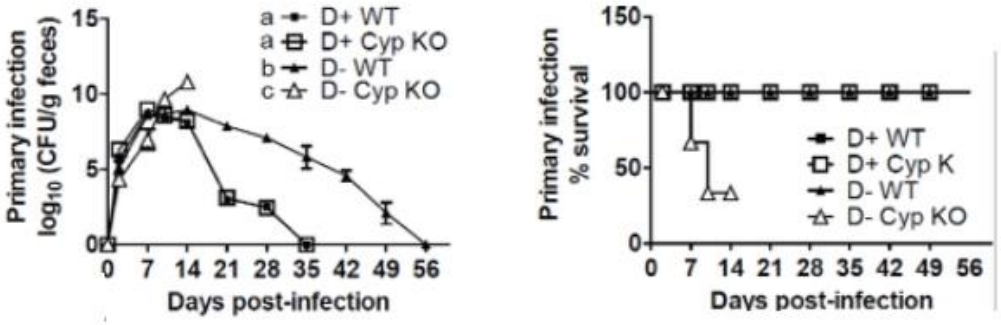
A study conducted by the Cantorna laboratory studied the effects of a *Citrobacter rodentium* infection on VDR KO mice[22]. They determined that the VDR KO mice had fewer *C. rodentium* in the feces than wild-type (WT) mice coupled with a faster clearance rate[22]. Additionally, the research group found that the VDR KO mice had more interleukin-22 (IL-22)-producing innate lymphoid cells (ILCs) and more antibacterial peptides compared to the WT mice due to the deficiency of VDR in the VDR KO[22]. The VDR expression regulates ILC frequencies, IL-22, dysbiosis, all of which influence susceptibility to *C. rodentium* infection[22]. Thus, VDR expression may play a role the severity of a *C. rodentium* infection.

The effect of vitamin D deficiency on infection-induced changes in intestinal epithelial barrier function was determined in a *C. rodentium* infection in mice[23]. Four groups of mice were studied: wild type C57BL/6 mice that were vitamin D sufficient and wild type C57BL/6 mice that were vitamin D-deficient, CYPKO vitamin D deficient mice and CYPKO vitamin D deficient mice[23]. They determined that the vitamin D deficient mice infected with *C. rodentium* had increased colonic hyperplasia and epithelial barrier dysfunction ($P < .0001$ and $P < .05$, respectively). The vitamin D sufficient mice, WT and CYKPKO, cleared the infection faster than the vitamin D deficient mice (Figure 5). They were free of bacteria colony forming units (CFU) in their feces by day 35 while the WT D- mice weren't free of CFU until day 56 (Figure 5). The CYPKO mice died or had to be euthanized before the experiment was over due to the severity of

the infection (Figure 5). Thus, they concluded that D- WT mice were more susceptible than D+ WT or D+ CYPKO to C. rodentium infection, and that D- CYPKO were the most susceptible[23].

Figure 5[22] Vitamin D deficient mice are more susceptible to a *Citrobacter rodentium* infection. CYPKO vitamin D deficient mice are more susceptible than WT deficient mice. There are no noticeable differences vitamin D sufficient mice between WT and CYPKO mice.

Unpublished data from Jing Chen and Yang Ding



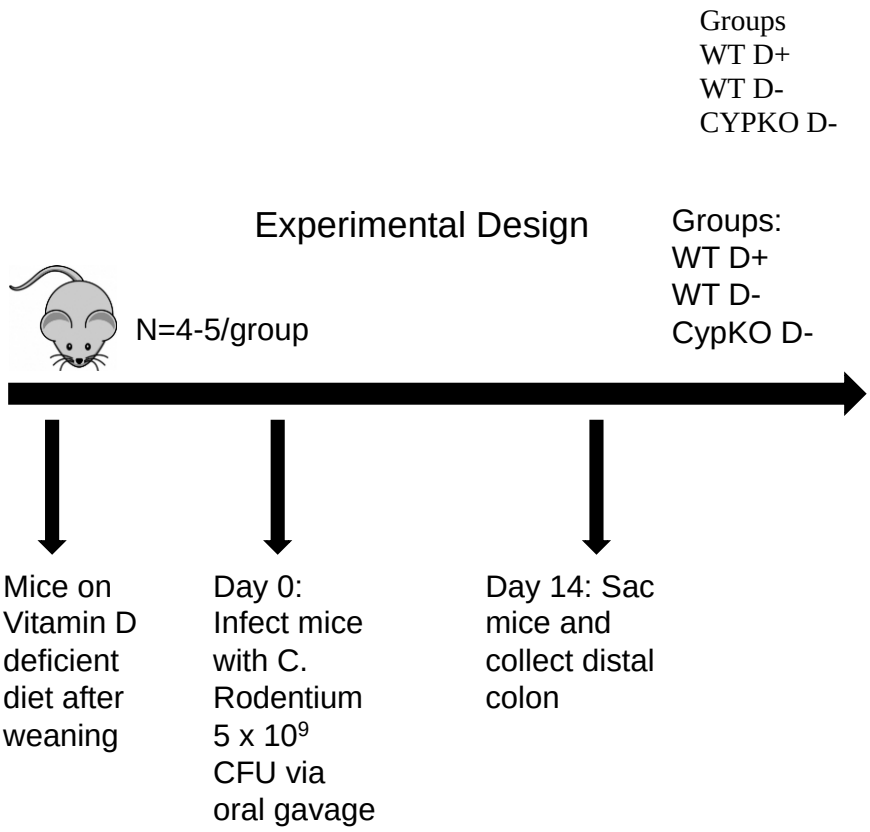
Chapter 2

Experiment Rationale and Methods

Rationale

Three different groups of mice were used: vitamin D sufficient WT C75BL/6; mild vitamin D deficient WT C57BL/6 mice; and extreme vitamin D deficient CYPKO mice. The CYPKO mice are unable to produce $1,25(OH)_2D_3$ and thus display a more severe vitamin D deficiency compared to WT deficient mice. Based on the results in Figure 5 that show there were no differences in susceptibility between WT D+ and CYPKO D+ mice, we used three groups of WT D+, WT D- and CYPKO D-. Like in Figure 5, *C. rodentium* infection was carried out by Yang-Ding Lin (Cantorna lab) on the three groups of mice. Uninfected (day 0) and peak infection (day 14) the mice were euthanized. The distal colon was harvested and RNA extraction using TRIzol RNA protocol was done followed by reverse transcription and quantitative PCR to measure the VDR RNA and ZO-1 RNA levels. The three values from the 3 groups and 2 time points were compared by two-way ANOVA with a $p<0.05$ as significant. The house keeping gene used was hypoxanthine-guanine phosphoribosyltransferase (HPRT). Values from the uninfected chow fed colons served as the control and were set to 1.

Figure 6 Experimental Design Outline. The groups of mice included WT vitamin D sufficient and vitamin D deficient WT mice and CYPKO vitamin D deficient mice.



Methods

Mice

The Cyp271 knockout (CYPKO) breeders were a gift from Dr. Hector DeLuca (University of Wisconsin). The vitamin D receptor knock out (VDRKO) breeders were purchased from Jackson Laboratories (Bar Harbor ME). C57BL/6 mice were produced and housed at the Pennsylvania State University, University Park. The CYP or VDR breeders were used to generate KO mice from the same breeders for all experiments. The Office of Research

Protection, Institutional Animal Care and Use Committee at the Pennsylvania State University approved all experimental procedures.

Vitamin D Deficiency[24]

For vitamin D deficiency experiments, VDRKO mice were fed TD.04179, a calcium rescue diet (Harlan Teklad) and CKYPKO mice were fed chow diet from birth before mating with mice of the same background. The offspring from these mice were fed using the vitamin D-deficient diet throughout the experiment after weaning. The mice were made vitamin D deficient in order to increase the VDR prior to infection to make any changes easier to see. To confirm the vitamin D deficient status, Vitamin D analysis serum was performed using both a $25(OH)D_3$ ELISA following the manufacturer's instructions.

C. rodentium infection[22]. The *C. rodentium* strain ICC169 was a gift of Gad Frankel (London School of Medicine and Dentistry, London, UK). *C. rodentium* was cultured in Luria-Bertani (LB) broth containing 20 μg ml−1 nalidixic acid (EMD Chemicals, Gibbstown, NJ). Mice were infected by oral gavage with 200 μl of C. rodentium suspension that contained 5×10^9 colony-forming units. Mice were housed one per cage to prevent mouse-to-mouse transmission of *C. rodentium*. The *C. rodentium* numbers in the feces were determined by plating on LB agar plates with 50 μg ml−1 nalidixic acid.

RNA Extraction.

A portion of the distal colon (0.5-1.0 cm) tissue and kidney were harvested from the mice. The TRIzol RNA protocol by Ambion Life Technologies (Grand Island, NY) was

followed to extract and purify the RNA. The colon RNA was re-suspended in 50 microliters of RNase free water. The concentration was determined by spectroscopy on NanoDrop8.1.

cDNA synthesis by Reverse Transcription and quantitative PCR[22]: Complementary DNA was synthesized using the TaqMan reverse transcription reagents kit (Applied Biosystems, Carlsbad, CA) with the oligo(dT) primer. One-fifth volume of the reverse transcription reaction cDNA mixture was used in qPCR reaction with SYBR green mix (Bio-Rad) by MyiQ Single-Color Real-Time PCR machine (Bio-Rad). Expression levels of vitamin D receptor (VDR) and tight junction protein ZO-1 in the colon were normalized by hypoxanthine-guanine phosphoribosyltransferase (HPRT) The sequences used are as follows: VDR forward primer CCCCTTCAATGGAGATTGC; VDR reverse primer CTGCACCTCCTCATCTGTGA; ZO- forward primer CCACCTCTGTCCAGCTCTTC; ZO-1 reverse primer CACCGGAGTGATGGTTTTCT; HPRT forward primer CAGACTGAAGAGCTATTGTAATG; and HPRT reverse primer CCAGTGTCAATTATATCTTCCAC. Threshold values from the standard curve were obtained using PE Biosystem software, and RNA was quantified. The specificity of the PCR was confirmed by melting-curve analysis with Bio-rad program.

Statistics.

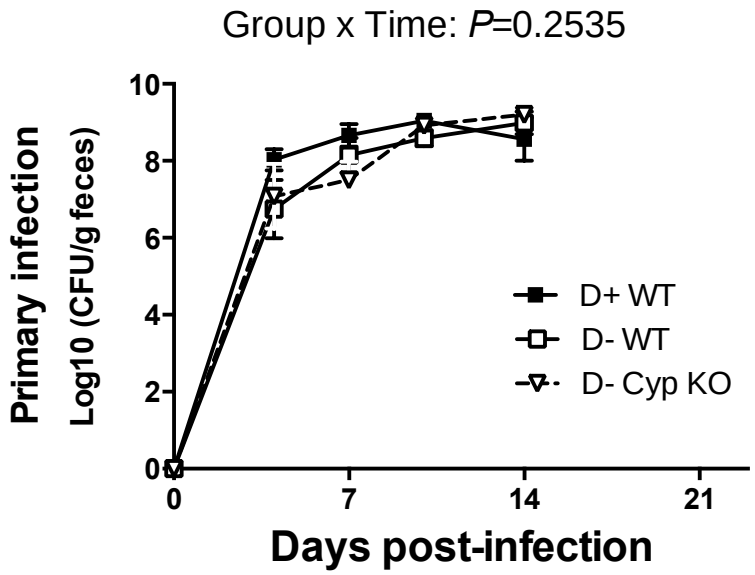
The data are presented as the means within the standard error of the mean (SEM). Two groups were compared together with a two-way ANOVA determine statistical significance by the GraphPad Prism software (version 6.01) and a p value < 0.05 of significance.

Chapter 3

Results

The course of the *C. rodentium* infection was monitored by the bacterial colony forming units (CFU) in the feces (Figure 7).

Figure 7: Fecal Shedding Curve During *C. rodentium* Infection



Data from collaboration with Yang-Ding (unpublished results)

There were no differences in the *C. rodentium* shedding kinetics between the D+ WT, D- WT and D- Cyp KO mice (Fig. 7). This result is consistent with the early shedding kinetics observed previously (Fig. 5).

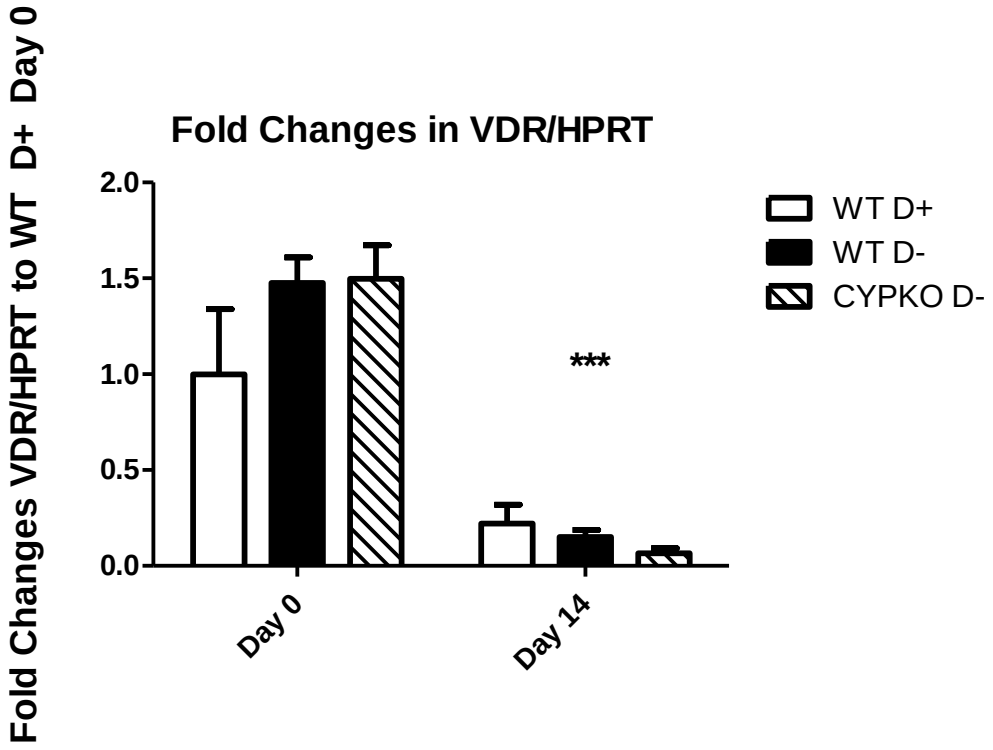
The VDR is highly expressed in the kidney and so to establish the protocol, kidney samples were used to test the extraction, isolation, and qPCR methods (Appendix 1). The same procedures were repeated for the colon and kidney samples from the infected mice on day 14. Day 14 was used because it is the peak day of the infection for *C. rodentium*[25]. The VDR and

ZO-1 expression were normalized to the HPRT expression to account for the differences in the cellular expression levels and the amount of RNA extracted from the samples. The normalization calculation involved dividing the VDR or ZO-1 expression levels by the HPRT level for each sample. The HPRT colonic expression levels ranged from $4.08x10^{-7}$ to $9.65x10^{-7}$. This tight range showed that consistency in the mRNA extraction and PCR values. Colon expression of the VDR ranged from $3.67x10^{-11}$ to $1.24x10^{-6}$ which indicated a change in expression level during the course of the infection (Appendix 2). The normalized VDR colonic expression levels ranged from $6.71x10^{-5}$ to $1.94x10^{0}$ further supported that the change in expression level (Appendix 2). The ZO-1 levels ranged from $1.47x10^{-7}$ to $6.83x10^{-6}$(Appendix 2). The normalized ZO-1 expression levels ranged from 5.90×10^{-1} to $9.66x10^{0}$(Appendix 2).

Statistical analysis was carried out on the normalized VDR colonic expression levels to determine if a trend existed and if so, based on what variable (Figure 8). The two-way ANOVA statistical test for variance was used to determine if vitamin D deficiency influenced the VDR expression change by comparing the WT D+ and WT D- groups. The vitamin D deficient diet did not have a statistically significant effect at day 0 or day 14 (p value >0.05). Along those same lines, the type of mouse, WT or CYPKO did not have a statistically significant effect at day 0 or day 14 (p value >0.05). However, the two-way ANOVA analysis revealed that the uniform decrease from day 0 to day 14 in all three groups was very significant (p value <0.001). In sum, there were no statistically significant differences between any of the groups based on the variables of vitamin D status or mouse type (Figure 8). The only variable that appreciably influenced the VDR expression in the colons was the time of the infection, day 0 or day 14 (Figure 8). All three groups of mice had a significant decrease in the VDR expression in the colon at day 14 (Figure 8).

Figure 8 Colonic Expression of VDR normalized to HPRT RNA in vitamin D deficient CYPKO and WT mice and vitamin D sufficient WT mice.

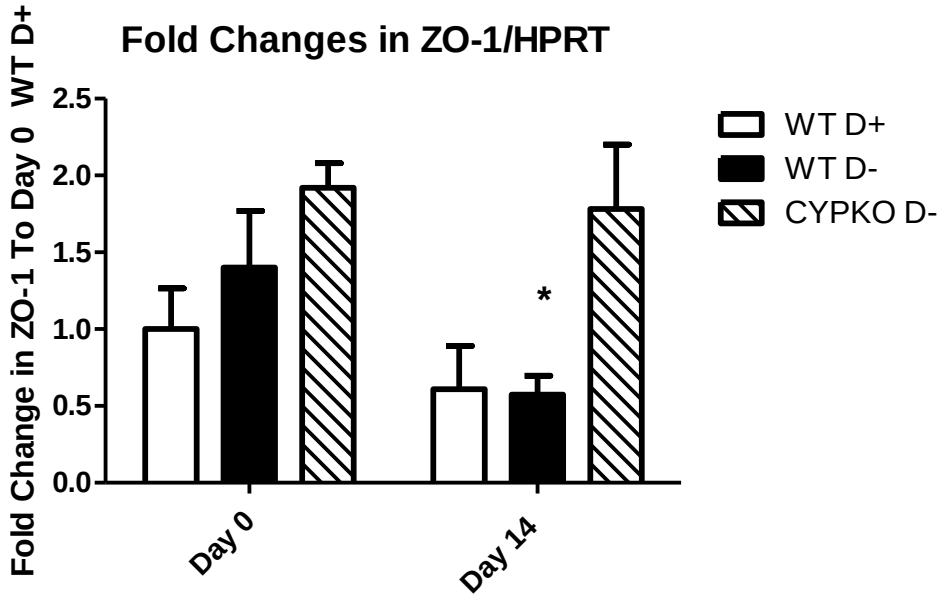
The data was plotted as the average mean value of the fold changes for the normalized VDR to HPRT ratio compared to the average normalized VDR to HPRT ratio for the day 0 WT D+ mice. Two-way ANOVA revealed no significant differences between Day 0 and Day 14 VDR expression for WT D+ v. WT D-or CYPKO D- v. WT D- There was a significant difference with p<0.001 for day 0 and day 14 time point.



Besides analyzing the VDR colonic expression changes over the course of the infection, the ZO-1 colonic expression levels were also analyzed. The vitamin D status, deficient or sufficient, did not change the ZO-1 expression significantly at day 0 or day 14 (p value <0.05; Figure 9). The time of infection (day 0 or day 14) also did not change the ZO-1 expression for any of the three groups (p value >0.05). However, the ZO-1 expression was significantly different between the WT D- and CYPKO D- mice on day 14 (p value <0.05; Figure 9).

Figure 9 Colonic Expression of ZO-1 normalized to HPRT RNA in vitamin D deficient CYPKO and WT mice and vitamin D sufficient WT mice.

The data was plotted as the average mean value of the fold changes in normalized ZO-1 expression to the WT D+ day 0 value. Two-way ANOVA revealed no significant differences between Day 0 and Day 14 ZO-1 expression for WT D+ v. WT D-. A Two-way ANOVA did show a significant difference between CYPKO D- v. WT D- with a p value of 0.01. * indicates p<0.05



Chapter 4

Discussion

The WT D+, WT D- and CYPKO D- had comparable concentrations of *C. rodentium* in their feces indicating a successful infection and that there were not differences in the *C. rodentium* in the D+ and D- mice. Thus, the decrease in the VDR levels on day 14 can be attributed to an effect of the *C. rodentium* infection on VDR expression in the colon. There were no significant changes in VDR expression as a function of diet (D- versus D+) or genotype (D- WT versus D- CYPKO) (Figure 8). The absence of an effect of the diet on VDR expression contrasts with what has been reported in the literature for the kidney expression of the VDR[26]. $1,25(OH)_2D_3$ was shown to increase renal VDR expression 5 fold in the presence of calcium[26]. The renal VDR mRNA increased 10 fold in the presence of $1,25(OH)_2D_3$ and calcium[26]. However, the expression of duodenal VDR mRNA has not been shown to be affected by $1,25(OH)_2D_3$[26]. The active form of vitamin D regulates VDR expression in the kidney[26]. In contrast, the VDR expression in the colon may not be regulated by dietary vitamin D.

The decreased expression of the VDR that occurs following infection is in agreement with the effect of inflammation and colitis on VDR expression in the colon[27]. IBD patients and colitis associated colon cancer patients have lower VDR protein levels[27]. Vitamin D deficiency has also been associated with acute respiratory infection (ARI)[28]. In an experiment involving a similar infection (chlamydia) that involves both bacterial colonization and inflammation, pre-treatment with $1,25(OH)_2D_3$ inhibited the replication of the bacteria[29]. Supplementation in IBD patients and children at risk for ARI improved the symptoms and decreased the chances of

development respectively[28]. However, lowered VDR expression in patients with acute infections or inflammatory diseases weakens their ability to respond to the vitamin D. Levels above the dietary intake recommendations may be needed to account for the lower levels of VDR. A better approach may be to supplement patients with some of vitamin D's products that carry out the anti-bacterial effects or anti-inflammatory effects such as antimicrobial peptides or leukocyte elastase inhibitor LEI which defends the host by inhibiting neutrophil proteases[27, 29].

In a different aspect besides the adaptive immune response and cytokine levels of the infection, the permeability of the gut barrier is also vital to fighting off the infection. *C. rodentium* has the ability to regulate the epithelial barrier integrity, inflammation levels and healing ability of the gut[21]. During the course of the infection, bacteria attach to the epithelial cell surface[30]. The infection is also accompanied by transmissive crypt hyperplastic mucosa following this attachment[21]. The IgG antibody mediates the clearance of the *C. rodentium* after passing into the gut lumen[31]. Normally, the IgG cannot pass through the intact gut barrier; however, during the infection, the gut barrier can become leaky which results in increased permability[31].

The effect of vitamin D deficiency on infection-induced changes in intestinal epithelial barrier function using a *C. rodentium* infection in mice has been analyzed in a different experiment[23]. The researchers determined that the vitamin D deficient mice infected with *C. rodentium* had significant changes in the colon epithelial tissue including increased colonic hyperplasia and barrier dysfunction. They concluded that vitamin D deficiency increased susceptibility by enabling greater intestinal injuries in a *C. rodentium* infection[23]. The researchers determined that vitamin D_3 inhibited the signal molecule PUMA by blocking the transcription factor NF-κB[19]. Thus, $1,25(OH)_2D_3$ inhibited the apoptosis of intestinal epithelial cells and

preserved the membrane integrity to protect the colon from injury. However, inflammation during the *C. rodentium* infection decreases the expression of VDR[23]. The gut would no longer be able to respond to vitamin D. Thus, supplementation with vitamin D would not help the host avoid the loss of gut barrier integrity. Decreasing the inflammation that accompanies the infection and the immune response could potentially restore or at least increase the VDR expression.

Another study involving vitamin D deficiency and tight junction proteins like ZO-1 in the gut revealed that $1,25(OH)_2D_3$ increased tight protein junction expression[18]. Thus, the decrease in ZO-1 expression of WT D- mice from day 0 to day 14 could be a side effect of the vitamin D deficiency or the decrease in the VDR expression. A different study looked specifically at *C. rodentium*'s effect during the peak of the infection and found that the bacteria, not the inflammation disturbed localization of tight junction protein[32]. The inflammation in the gut persisted after the bacteria were removed from the gut but the tight junction proteins were unchanged indicating a functional gut barrier despite the inflammation[32]. However, this study does not help to explain why the CYPKO D- mice exhibited no significant change in their ZO-1 expression between day 0 and day 14 of the *C. rodentium* infection since the bacteria colonized both the WT and the CYPKO mice. It could be that a different tight junction protein such as claudin is affected due to the different phenotype of the mice. Or, the absence of $1,25(OH)_2D_3$ induced expression of tight junction proteins in the gut barrier caused the tight junction proteins to respond differently to immune challenges. It is also possible that decrease in the VDR expression in the colon renders the effects of $1,25(OH)_2D_3$ negligible during the infection.

In terms of future experiments, a kinetic study of the VDR and tight junction protein expression would be interesting. By tracking the kinetics of the changes in VDR expression in

the colon, the factors influencing the expression may become clearer. If the VDR expression decreases early in the course of infection around day 3 or 4, the *C. rodentium* bacteria colonization may activate decrease in the VDR. Or, innate immune system may initiate the decrease or some combination of the two. If the VDR expression decreased later on during the course of the infection, it could be due to the adaptive immune response. If the decrease in VDR expression occurs gradually throughout the infection, then the *C. rodentium* may play the dominant role. In terms of the tight junction protein, it would also be interesting to see if the tight junction expression was restored with the clearance of the infection or with restoration of vitamin D activity.

In addition to tracking the VDR expression change, it would also be interesting to look in other tissues like the small intestine to see if the VDR or tight junction protein expression changes there as well. If a similar decrease in the VDR expression occurred in the small intestine, then the integrity of the gut barrier may be a dominant factor in determining the VDR expression. It would also be important to look at the protein levels in addition to the mRNA levels of both the VDR and tight junction protein expression. Though mRNA is usually a good representation of expression, it is not the same as looking at the actual protein levels. Along those same lines, the experiment should be repeated with more mice in order to better see the differences between the three groups.

Appendix 1

Kidney qPCR VDR Data

Table 1 qPCR VDR and HPRT Expression in Kidney

Sample	HPRT	VDR	VDR/HPRT
WT kidney	2.68E-07	1.13E-06	4.22E+00
	5.11E-08	7.57E-07	1.48E+01
	1.02E-07	2.57E-07	2.52E+00
	2.45E-07	1.95E-09	7.96E-03
	3.82E-07	1.14E-06	2.98E+00
	1.14E-07	2.71E-09	2.38E-02
	5.25E-07	4.76E-09	9.07E-03
	2.22E-07	1.12E-09	5.05E-03
	3.58E-07	1.21E-06	3.38E+00
VDRKO kidney	1.98E-11	0.00E+00	0.00E+00
MQ water	0.00E+00	0.00E+00	0.00E+00
WT kidney	1.92E-07	5.37E-08	2.80E-01

The table shows the renal expression of HPRT and VDR of control WT mice and VDRKO mouse. The positive control was the last WT kidney listed in the table from unpublished data by Yang Ding. The negative control for VDR was a kidney from a VDRKO mouse. The negative control for the cDNA synthesis and aPCR amplification was the MQ water.

Appendix 2

VDR and ZO-1 qPCR Data

Table 2 qPCR VDR and HPRT Expression in Colon Tissue

	Sample	HPRT	VDR	Normalized VDR	Fold Change from WT D+ Day 0 Average
Day 0 WT D+	1	7.52E-07	6.29E-07	8.36E-01	7.78E-01
	2	6.39E-07	1.24E-06	1.94E+00	1.80E+00
	3	6.29E-07	8.23E-07	1.31E+00	1.22E+00
	4	5.93E-07	1.28E-07	2.16E-01	2.01E-01
Day 0 WT D-	5	6.26E-07	1.07E-06	1.71E+00	1.59E+00
	6	4.57E-07	8.30E-07	1.82E+00	1.69E+00
	7	6.12E-07	1.10E-06	1.80E+00	1.67E+00
	8	6.26E-07	6.53E-07	1.04E+00	9.70E-01
	9	7.38E-07	1.16E-06	1.57E+00	1.46E+00
Day 0 CYPKO D-	10	4.08E-07	8.99E-07	2.20E+00	2.05E+00
	11	6.34E-07	8.83E-07	1.39E+00	1.30E+00
	12	6.84E-07	9.51E-07	1.39E+00	1.29E+00
	13	5.54E-07	1.04E-06	1.88E+00	1.75E+00
	14	6.73E-07	7.93E-07	1.18E+00	1.10E+00
Day 14 WT D+	15	7.13E-07	3.93E-07	5.51E-01	5.13E-01
	16	8.86E-07	9.14E-08	1.03E-01	9.59E-02
	17	8.58E-07	9.54E-08	1.11E-01	1.03E-01
	18	8.60E-07	1.57E-07	1.83E-01	1.70E-01
Day 14 WT D-	19	6.32E-07	1.81E-07	2.86E-01	2.66E-01
	20	9.65E-07	8.16E-08	8.46E-02	7.86E-02
	21	6.74E-07	1.00E-07	1.48E-01	1.38E-01
	22	7.46E-07	6.14E-08	8.23E-02	7.65E-02
	23	6.68E-07	1.42E-07	2.13E-01	1.98E-01
Day 14 CYPKO D-	24	6.25E-07	9.09E-08	1.45E-01	1.35E-01
	25	7.66E-07	8.65E-08	1.13E-01	1.05E-01
	26	6.99E-07	6.47E-08	9.26E-02	8.61E-02
	27	5.47E-07	3.67E-11	6.71E-05	6.24E-05
	28	8.64E-07	0.00E+00	0.00E+00	0.00E+00
WT kidney	29	2.49E-07	3.22E-08	1.29E-01	1.20E-01
VDRKO kidney	30	2.09E-07	0.00E+00	0.00E+00	0.00E+00
WT kidney	Positive		1.01E-07		
MQ water	Negative		0.00E+00		

The table shows the colonic expression of HPRT and VDR of day 0 control mice and day 14 infected mice. The vitamin D sufficient wild type mice are samples 1-4 for the day 0 mice and samples 15-18 for the day 14 infected mice. The vitamin D deficient wild type mice are samples 5-9 for the day 0 control mice and samples 19-23 for the day 14 infected mice. The CYPKO vitamin D deficient mice are samples 10-14 for the day 0 mice and samples 24-28 for the day 14 infected mice. Sample 29 was a WT kidney positive control for VDR expression whereas sample 30 was a VDR KO kidney negative control for VDR expression. The positive control for the qPCR run was a WT kidney and the negative control was MQ water.

Table 3 qPCR ZO-1 and HPRT Expression in Colon Tissue

	Sample	HPRT	ZO-1	Normalized ZO-1	Fold Changes from WT D+ Day 0 Average
Day 0 WT D+	1	7.52E-07	1.32E-06	1.76E+00	5.34E-01
	2	6.39E-07	3.05E-06	4.77E+00	1.45E+00
	3	6.29E-07	Outside limit of detection	N/A	2.69E-06
	4	5.93E-07	1.98E-06	3.34E+00	1.02E+00
Day 0 WT D-	5	6.26E-07	2.49E-06	3.98E+00	1.21E+00
	6	4.57E-07	1.98E-06	4.33E+00	1.32E+00
	7	6.12E-07	1.96E-06	3.20E+00	9.74E-01
	8	6.26E-07	1.43E-06	2.28E+00	6.95E-01
	9	7.38E-07	6.83E-06	9.25E+00	2.81E+00
Day 0 CYPKO D-	10	4.08E-07	2.52E-06	6.18E+00	1.88E+00
	11	6.34E-07	3.39E-06	5.35E+00	1.63E+00
	12	6.84E-07	5.02E-06	7.34E+00	2.23E+00
	13	5.54E-07	2.77E-06	5.00E+00	1.52E+00
	14	6.73E-07	5.18E-06	7.70E+00	2.34E+00
Day 14 WT D+	15	7.13E-07	1.25E-06	1.75E+00	5.33E-01
	16	8.86E-07	4.86E-07	5.49E-01	1.67E-01
	17	8.58E-07	2.75E-06	3.21E+00	9.74E-01
	18	8.60E-07	3.20E-06	3.72E+00	1.13E+00
Day 14 WT D-	19	6.32E-07	1.02E-06	1.61E+00	4.91E-01
	20	9.65E-07	6.75E-07	6.99E-01	2.13E-01
	21	6.74E-07	1.49E-06	2.21E+00	6.72E-01
	22	7.46E-07	1.29E-06	1.73E+00	5.26E-01

	23	6.68E-07	2.13E-06	3.19E+00	9.69E-01
Day 14 CYPKO D-	24	6.25E-07	2.64E-06	4.22E+00	1.28E+00
	25	7.66E-07	2.18E-06	2.85E+00	8.65E-01
	26	6.99E-07	2.79E-06	3.99E+00	1.21E+00
	27	5.47E-07	4.65E-06	8.51E+00	2.59E+00
	28	8.64E-07	8.44E-06	9.77E+00	2.97E+00
WT kidney	29	2.49E-07	1.47E-07	5.90E-01	1.79E-01
VDRKO kidney	30	2.09E-07	1.64E-07	7.85E-01	2.39E-01
WT kidney	positive		4.87E-07		
MQ water	negative		0.00E+00		

The table shows the colonic expression of HPRT and ZO-1 of day 0 control mice and day 14 infected mice. The WT D+ mice are samples 1-4 for the day 0 mice and samples 15-18 for the day 14 infected mice. The WT D- are samples 5-9 for the day 0 control mice and samples 19-23 for the day 14 infected mice. The CYPKO D- mice are samples 10-14 for the day 0 mice and samples 24-28 for the day 14 infected mice. Sample 29 was a WT kidney positive control for VDR expression whereas sample 30 was a VDR KO kidney. The positive control for the qPCR run was a WT kidney and the negative control was MQ water.

BIBLIOGRAPHY

[1](2002). A dose of vitamin D history. *Nature Structural Biology*. 9:77.

[2]Wolf, George. (2004) The Discovery of Vitamin D: The Contribution of Adolf Windaus. *Journal of Nutrition*. 134 (6):1299-1302.

[3]Rosenheim, Otto, and Webster, Thomas Arthur. (1928) The parent substance of vitamin D. *Journal of Biochemistry*. 22 (3):762-766.

[4]Rajakumar, Kumaravel, Greenspan, Susan L., and Holick, Micheal F. (2007). SOLAR Ultraviolet Radiation and Vitamin D. 97 (10):1748-1754.

[5]Vitamin D. http://vitamind.ucr.edu/about/ (Accessed 15 November 2013).

[6]Yamamoto, Keiko, Masuno, Hiroyuki, and Yamada, Sachiko. (2000). Three dimensional modeling of and ligand binding to vitamin D receptor ligand binding domain. *Proceeding of National Academy of Science*. 97 (4):1467-1472.

[7]Norman, Anthony W. (2008). From vitamin D to hormone D: fundamentals of the vitamin D endocrine system essential for good health. *American Journal of Clinical Nutrition*. 88:491S-499S.

[8]Kabi, Francis. (2012). "Calcitriol." MedLibrary.org. http://medlibrary.org/lib/rx/meds/calcitriol-12/ (Accessed 1 March 2015).

[9]Christakos Sylvia, Ajibade, Christakos, Dare V., and Mady, Leila J. (2010). Vitamin D: metabolism. *Endocrinology and metabolism clinics of North America*. 39 (2):243-253.

[10]Falkenstein, Elisabeth, Tillmann, Hanns-Christain, Christ, Michael, Feuring, Martin, and Wehlgin, Martin. (2000). Multiple Actions of Steroid Hormones-A Focus on Rapid, Nongenomic Effects. *Pharmacological Review*. 52 (4):513-556.

[11]Bronwyn A Crawford, Eternity D Labio, Simone I Strasser and Geoffrey W McCaughan. (2006). "Vitamin D replacement for cirrhosis-related bone disease." *Nature Clinical Practice Gastroenterology & Hepatology*. 3:689-699.

[12]Cantorna, Magherita T, Yu, Sanhong, and Bruce, Danny. (2008). The paradoxical effects of vitamin D on type 1 mediated immunity. *Molecular Aspects of Medicine*. 29 (6):369–375.

[13]American Autoimmune Related Diseases Association. http://www.aarda.org/autoimmune_statistics.php. (Accessed 25 July 2013).

[14]Center for Disease Control and Prevention. http://www.cdc.gov/ibd/. (Accessed 24 July 2013).

[15]Lozano, R. (2012). Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet*. 380 (9859):2095–128.

[16]"What causes Inflammatory Bowel Diseases (IBD)?" *Division of Gastroenterology and Hepatology UNC Multidisciplinary Center for IBD Research and Treatment*. UNC School of Medicine, n.d. Web. 10 July 2013.

[17]Froicu, Monica and Cantorna, Margherita. (2007). Vitamin D and the vitamin D receptor are critical for control of the innate immune response to colonic injury. *BMC Immunology* 8:5.

[18]Kong, Juan, Zhang, Zhongyi, Musch, Mark W., Ning, Gang, Sun, Jun, Hart, John, Bissonnette, Marc, Li, and Ya Chan. (2007). Novel Role of the vitamin D receptor in maintaining the integrity of the intestinal mucosal barrier. *American Journal of Physiology- Gastrointestinal Liver Physiology*. 294 (1):G208-16.

[19]Liu Weincheng, Chen, Yunzi, Golan, Maya Ahanroni, Annunziata, Maria L., Du, Jie, Dougherty Urszula, Kong, Juan, Musch, Mark, Huang, Yong, Pekow, Joel, Zheng, Changquing, Bissonette, Marc, Hanauer, Stephen B, and Li, Yan Chun. (2013). Intestinal epithelial vitamin D receptor signaling inhibits experimental colitis. *Journal of Clinical Investigation.* 123 (9):3983-96.

[20]Raczynski Arkadiusz R., Muthupalani Sureshkumar, Schlieper Katherine, Fox James G, Tannenbaum Steven R, Schauer David B. (2012). Enteric infection with Citrobacter rodentium induces coagulative liver necrosis and hepatic inflammation prior to peak infection and colonic disease. *PLoS One*. 7 (3):e33099.

[21]Koroleva, Ekaterina P., Halperin, Sydney, O. Gubernatorova, Ekaterina, Spencer, Cody M., and Tumanov, Alexei V. (2015). Chapter 8 *Citrobacter rodentium*-induced colitis: A robust model to study mucosal immune responses in the gut. *Journal of Immunological Methods*. doi:10.1016/j.jim.2015.02.003

[22]Chen, Jing, Waddell, Amanda, Lin, Yang Ding, and Cantorna, Margherita T. (2014). Dysbiosis caused by vitamin D receptor deficiency confers colonization resistance to Citrobacter rodentium through modulation of innate lymphoid cells. *Mucosal Immunology*. doi:10.1038/mi.2014.94

[23]Assa, Amit, Vong, Linda, Pinnell, Lee J., Avitzur, Naama, Johnson-Henry, Kathene C., and Sherman, Philip M. (2014). "Vitamin D deficiency promotes epithelial barrier dysfunction and intestinal inflammation." *Journal of Infectious Diseases*. 210 (8):1296-1305.

[24]Whitcomb, J. P., DeAgostino, M., Ballentine, M., Fu, J., Tenniswood, M., Welsh, J., … McDowell, M. A. (2012). "The Role of Vitamin D and Vitamin D Receptor in Immunity to Leishmania major Infection." *Journal of Parasitology Research*. 134645:1-10.

[25] Collins, James W., Keeney, Kristie M., Crepin, Valerie F., Rathinam, Vijay A. K.. Fitzgerald, Katherine A., Finlay, B. Brett, Frankel, Gad. (2014). Citrobacter rodentium: infection, inflammation and the microbiota. *Nature Reviews Microbiology*. 12 (9):612-623.

[26]Healy, Kevin D., Frahm, Marc A., and De Luca, Hector. (2005). 1,25-Dihydroxyvitamin D3 up-regulates the renal vitamin D receptor through indirect gene activation and receptor stabilization. *Archives of Biochemistry and Biophysics*. 433 (2):466-473.

[27]Sun, Jun. (2010). Vitamin D and muscoal immune function. *Current Opinion in Gastroenterology*. 26 (6):591-595.

[28]Yin K, Agrawal Devendra K. (2014). Vitamin D and inflammatory diseases. *Journal of Inflammation Research*. 7:69-87.

[29]He, Q., Ananaba, Godwin. A., Patrickson, John., Pitts, Sidney., Yi, Yeming., Yan, Fengxia, Eko, Francis O., Lyn, Deborah, Black, Carolyn M., Igietseme, Joseph U., and Thierry-Palmer, Myrtle. (2013). Chlamydial infection in vitamin D receptor knockout mice is more intense and prolonged than in wild-type mice. *The Journal of Steroid Biochemistry and Molecular Biology* 135:7–14.

[30]Luperchio Steven A. and Schauer David B. (2001). Molecular pathogenesis of Citrobacter rodentium and transmissible murine colonic hyperplasia. *Microbes and Infection*. 3:333–340.

[31]MacDonald, Mundy, R., Dougan, T. T., Frankel G., and Wiles, S. (2005), Citrobacter rodentium of mice and man. *Cellular Microbiology*. 7:1697–1706.

[32]Guttman Julian A, Samji Fereshte N, Li Yuling, Vogl A. Wayne, Finlay B. Brett. (2006). Evidence that Tight Junctions Are Disrupted Due to Intimate Bacterial Contact and Not Inflammation during Attaching and Effacing Pathogen Infection In Vivo. *Infection and Immunity*. 74 (11):6075-6084.

ACADEMIC **VITA**

Kelly McGill, BS

478 East Calder Way Apt 406 State College, PA 16801;

Phone: 724-992-2943 (cell) Email: kellymcgill53@gmail.com

Education

Bachelor of Sciences, Biochemistry and Molecular Biology/Nutrition Minor

The Pennsylvania State University: University Park, University Park, PA

08/2011 – projected 05/2015

Research

Summer Intern, Department of Public Health Sciences

Penn State Hershey College of Medicine, Hershey, Pennsylvania

PI: Dr. Robin Taylor Wilson; Mentor: Dr. Eric Battaglioli 05/2014 – 07/2014: 40 hours/week

- Participated in journal club and attended research presentations and discussions
- Attended professional development seminars
- Designed a primer and performed anisotropy reactions to analyze protein and DNA interactions
- Cultured and passaged MCF-7 and Hek293 cells lines
- Carried out transfection reactions and luciferase assays in cell lines
- Presented research results with a talk and poster at Symposium

Undergraduate Researcher, Department of Veterinary and Biomedical Sciences,

The Pennsylvania State University, University Park, Pennsylvania

PI: Dr. Margherita Cantorna 01/2013 – present: 20 hours/week

- Attended laboratory meetings and research symposiums
- Ran PCR, performed gel assay, prepared diet, and assisted with basic laboratory maintenance
- Learned and practiced mouse biomethodology and stool collection
- Performed the ELISA technique for cytokines for cell cultures and stimulations
- Practiced intracellular staining and column purification of splenocytes and serum
- Isolated, purified, and ran cell samples through Flow Cytometer and analyzed the results
- Performed Genotyping and learned the Cre-Lox breeding technique

- Designed, carried out, and presented independent research projects
- Established model and protocol for chronic DSS induced colitis in mice

Laboratory Volunteer, Medical Laboratory

Grove City Medical Center, Grove City, Pennsylvania

Supervisor: Billie Shepard, MS MT 05/2012 – 08/2012: 15 hours/week

- Assisted laboratory technicians
- Recorded patient information and observed pathologist
- Independently helped to prepare specimens for analysis

Leadership Experience

Teacher's Assistant, Department of Biochemistry and Molecular Biology

The Pennsylvania State University, University Park, Pennsylvania, Principles of Immunology

Instructor: Dr. Surojit Sarkar 08/2014 – 12/2014: 8 hours/week

- Provided homework help
- Created exam study guides and chapter outlines
- Organized and oversaw study sessions
- Led exam reviews
- Graded homework and honor papers

Teacher's Assistant, Department of Chemistry

The Pennsylvania State University, University Park, Pennsylvania, Organic Chemistry

Instructor: Dr. Steven Weinreb 08/2013 – 12/2013: 4 hours/week

- Organized and oversaw study sessions
- Led exam reviews

Vice President, Nittany Chemical Society

The Pennsylvania State University, University Park, Pennsylvania 08/2013-05/2014

- Assisted president, secretary, and treasurer
- Filled in for president during meetings
- Lead club meetings and organized Halloween Demonstration Show and Lab Tour
- Recruited new members and helped organize Faculty-student lunches

Shift-leader, Dairy Queen Orange Julius

Grove City Premium Outlets Grove City, Pennsylvania 04/2009-present: 40 hours/week (breaks)

- Delegated tasks and efficiently ran the store while manager was off-duty
- Handled the cash register and maintained a clean store at all times
- Trained new employees and earned a perfect company inspection multiple occasions

Technical Skills

- Excel - proficient (4+ years)
- Microsoft Office suite (Word, PowerPoint) – expert (4+ years)
- Data Prism- satisfactory (2+ years)

Professional Skills

- Determination and ability to complete difficult tasks successfully and creatively
- Self-motivated
- Team player
- Emotionally intelligent
- Leadership skills necessary for group cohesiveness
- Ability to manage multi-disciplinary projects and time wisely
- Effective communication and didactic skills

Relevant Coursework

- Honors Biochemistry
- Honors Immunology
- Molecular Biology of the Gene and Laboratory component
- Laboratory in Proteins, Nucleic Acids, and Molecular Cloning
- Laboratory in Protein Purification Techniques
- Nutrition Metabolism and Assessment
- Nutrition Diet and Disease
- Physical Chemistry
- Honors Organic Chemistry and Laboratory component
- Honors Inorganic Chemistry and Laboratory component
- Microbiology and Laboratory component
- Honors Molecular and Cell Biology