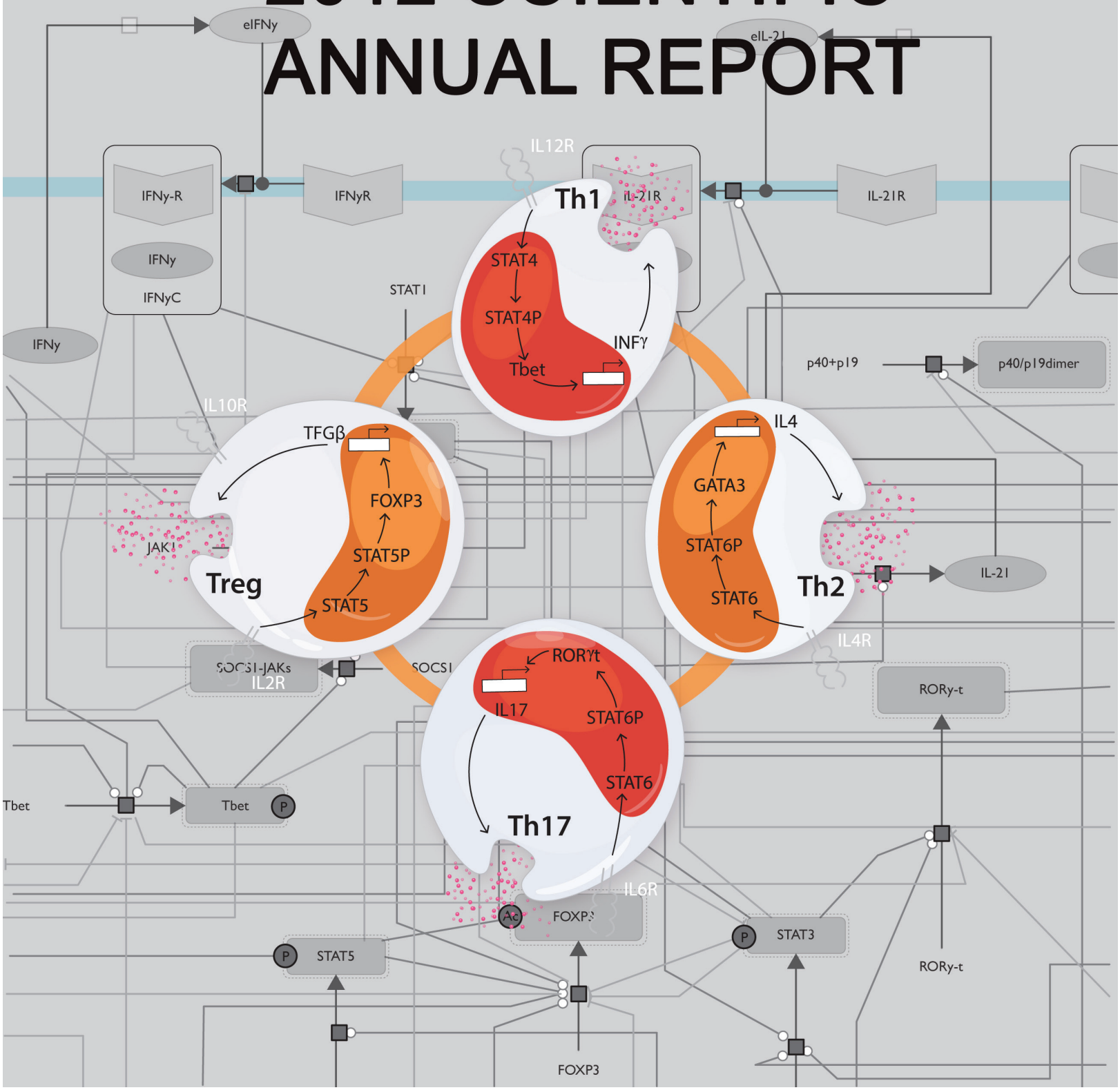
NIMML

Nutritional Immunology & Molecular Medicine Laboratory

2012 SCIENTIFIC ANNUAL REPORT



Contents

DIRECTOR'S REPORT

We appreciate your taking the time to read the Nutritional Immunology and Molecular Medicine Laboratory (NIMML) scientific annual report. This report marks the 10th anniversary of establishing the Laboratory by Dr. Hontecillas and I. The NIMML was founded in September of 2002 at the interface of nutritional and immunology research and discovery.

Over the past several years, the mission of NIMML has evolved to comprehensively characterize infectious, metabolic and immune-mediated diseases at the systems level and develop novel therapeutics. Finding cures for the most devastating human diseases necessitates a translational focus that combines expertise in nutrition, immunology, chemistry, bioinformatics, genomics, mathematics, pharmacology and toxicology into a novel transdisciplinary research thrust in Immunoinformatics and Therapeutics without any kind of disciplinary constraints or boundaries.

Our 10th year marks an important milestone at NIMML. Our faculty, students and staff have been extremely productive as shown by the portfolio of new publications, patents, computational models, modeling tools, invited presentations, funded projects and ever-increasing collaborations. The NIMML's Immunoinformatics and Therapeutics thrust is best illustrated by the $10.6 million National Institute of Allergy and Infectious Disease-funded Center for Modeling Immunity to Enteric Pathogens (MIEP). Under this program, the NIMML has developed computational and mathematical models of immune responses to pathogens. These integrated translational efforts under MIEP or the planned Center for Translational Influenza Research (CETIR) will help to accelerate the development of safer and more effective treatments for human diseases.

The NIMML has established a partnership with the Carillion Clinic, resulting in the hiring of Dr. Dario Sorrentino, a world renowned Gastroenterologist with expertise in inflammatory bowel disease. Dr. Sorrentino will lead the expansion of the clinical efforts at NIMML. Also, the NIMML faculty will be involved in the new Gastroenterology Fellowship program led by Dr. Paul Yeaton, Section Chief of Gastroenterology at Carilion. Indeed, this new partnership builds upon our established pre-clinical and clinical expertise and will help to grow the NIMML's clinical informatics capabilities.

We are proud and excited about past accomplishments and future prospects in contributing to solve important public health problems.

Josep Bassaganya-Riera, Ph.D. DVM

Director, Nutritional Immunology and Molecular Medicine Laboratory and Center for Modeling Immunity to Enteric Pathogens

10.6M

Modeling Immunity

In 2010 NIAID/NIH awarded $10.6M to Dr. Bassaganya-Riera for establishing the Center for Modeling Immunity to Enteric Pathogens (MIEP). The Center combines computational modeling and animal studies to support immunoinformatics and therapeutics research.

MIEP

MODELING IMMUNITY
TO ENTERIC PATHOGENS

Center for Modeling Immunity to Enteric Pathogens

The MIEP program is a first step in developing comprehensive and extremely detailed models, with a focused application on gut inflammation and well-integrated computational and laboratory research teams. An important goal is to allow immunologists to use these sophisticated tools to study the mechanisms of immunoregulation underlying immune responses to gut pathogens without becoming computing experts.

The NIMML team leads the NIH-funded Center for Modeling Immunity to Enteric Pathogens (www.modelingimmunity.org), a $10.6 million program Directed by Dr. Bassaganya-Riera with the goal of modeling the regulatory mechanisms underlying immune responses to gastroenteric pathogens. The MIEP engages 30 scientists and is part of a $45 million infectious disease program (Modeling Immunity for Biodefense) including four National Centers in Modeling Immunity for Biodefense (Mount Sinai/Yale, Rochester and Duke are the homes for the other three Centers).

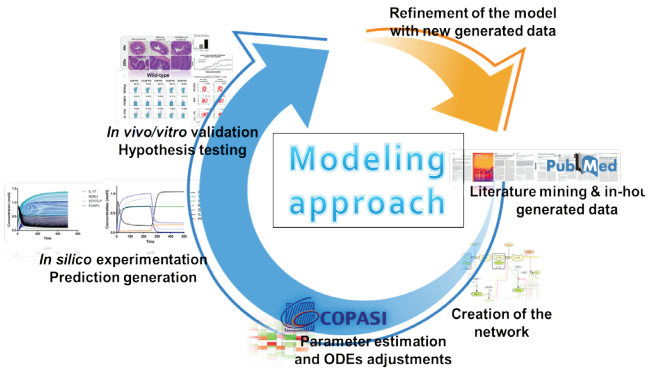


Figure 1. Model refinement and validation process

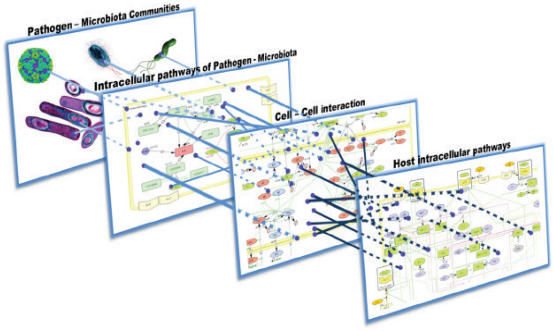


Figure 2. Multiscale modeling of immune responses representing different linked expression levels

The long-term goal of the MIEP program is to characterize the mechanisms of immunoregulation underlying mucosal immune responses to gastroenteric pathogens. More specifically, we have characterized new host pathways controlling gut inflammation during infection with *Helicobacter pylori, Enteroaggregative Escherichia coli* and *Clostridium difficile*. We integrated computational and experimental approaches to investigate the role of these gut bacteria as harmless or beneficial commensals versus pathogens. be user-friendly, predictive and systems-based.

CD4

CD4+ T cell differentiation

The NIMML team engineered a comprehensive model of the signaling pathways controlling CD4+ T cell differentiation. The new model predicted a novel role of PPARγ in controlling how gut-associated T helper cells change from pro-inflammatory to anti-inflammatory types.

“This is an example on how modeling can be predictive”

MIEP demonstrated for the first time that activation of PPARγ results in reprogramming of the CD4+ T cell molecular pathways that control the Th17 phenotype, leading to the induction of an iTreg phenotype. This phenotype switch is associated with protection from CD4+ T cell-induced colitis during adoptive transfer experiments in mice. The balance between Th17 and Treg cells helps delineate the outcome of immunological processes from effector inflammation to regulatory tolerance.

The CD4+ T cell differentiation process activates the transcriptional and secretory cellular machinery that helps orchestrate immune modulation in infectious, allergic and immune-mediated diseases. Upon antigen presentation, naïve CD4+ T cells become activated and undergo a differentiation process controlled by the cytokine milieu in the tissue environment. For instance, interleukin-6 (IL-6) in combination with transforming growing factor β (TGF-β) trigger a naive CD4+ T cell to become a T helper 17 (Th17) cell. In contrast, TGF-β alone can activate regulatory pathways leading to differentiation of naive CD4+ T cells into an induced regulatory CD4+ T cell (iTreg) phenotype, which in turn tightly dampens effector and inflammatory responses. Due to the complexity of this process, MIEP has constructed a computational and mathematical model with 60 ordinary differential equations representing 52 reactions and 93 species that makes a CD4+ T cell differentiating into either Th1, Th2, Th17 or iTreg (Fig. 3). Our model includes cytokines, nuclear receptors and transcription factors that define fate and function of CD4+ T cells. The model was calibrated with experimental data and it properly computes the four phenotypes with up- or down-regulation of cytokines and transcription factors that are a trademark of each phenotype. Therefore, the user can choose how the model can be initialized and thus, induce the system into a phenotype or another. Alternative inductions can also be tested and internal parameters and concentrations can be read.

The first set of validation studies were focused on the switch from Th17 to iTreg in differentiated wild type or PPARγ null Th17 cells upon activation of PPARγ. Our computational simulations showed that increasing concentrations of PPARγ in the Th17 cell in silico led to downregulation of RORγt and IL-17 and upregulation of FOXP3 (Fig. 4A).

In line with this result, following induction of Th17 and PPARγ activation, IL-17, STAT-3 and RORγt were dramatically downregulated, whereas FOXP3 was upregulated, thereby demonstrating a phenotypic switch from a Th17 to an iTreg phenotype (Fig. 4B).

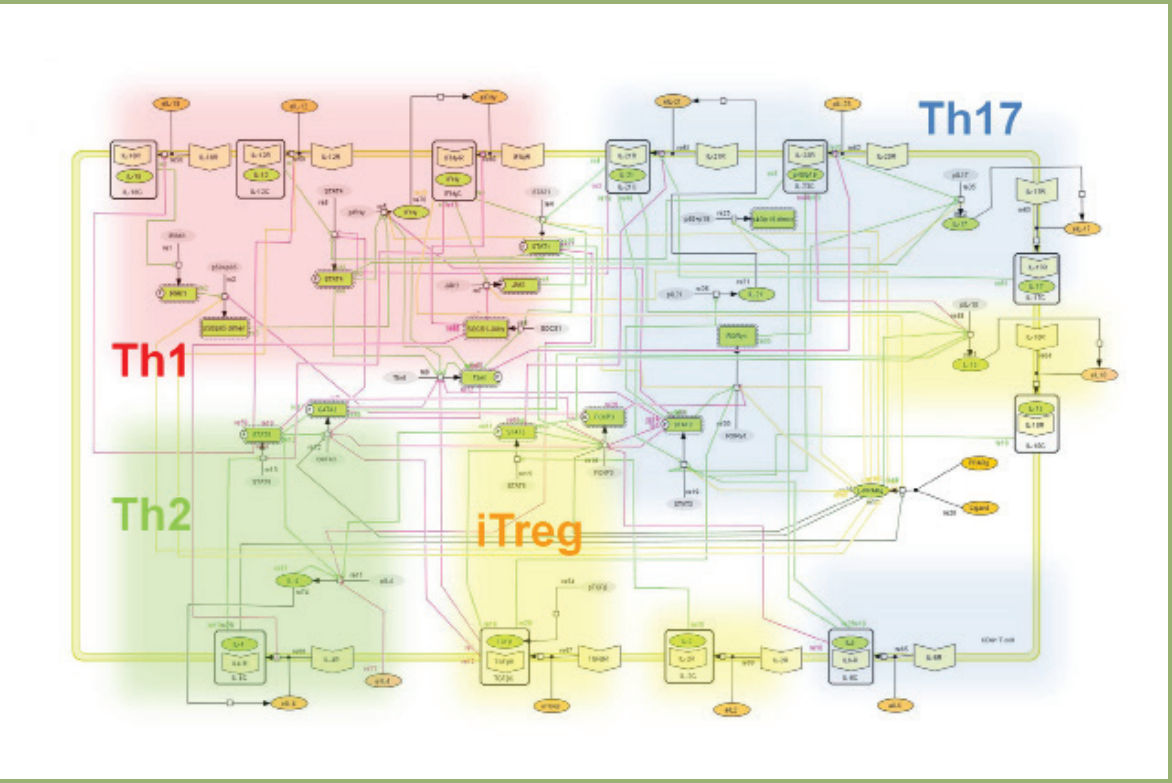


Figure 3. Signaling pathways controlling function of T helper cells

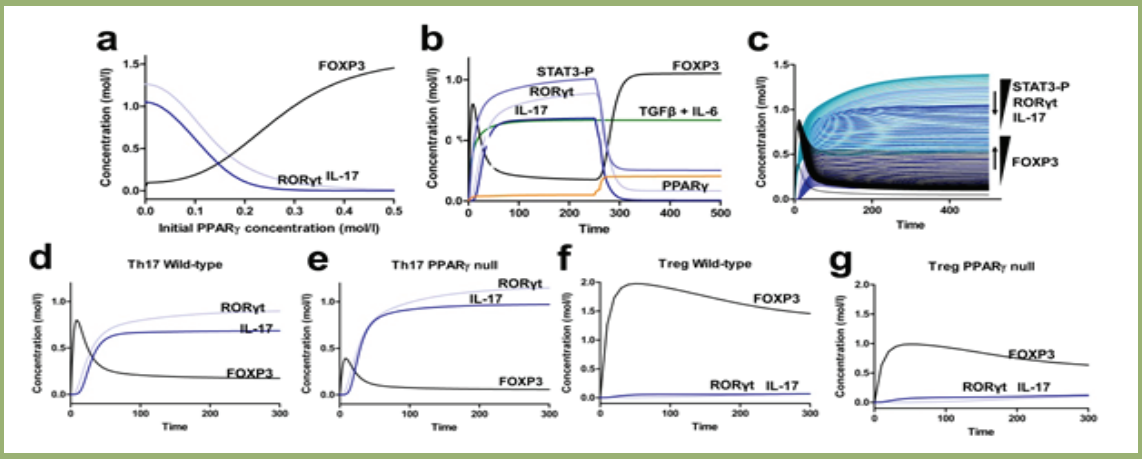


Figure 4. Computational simulations in the CD4+ T cell differentia-

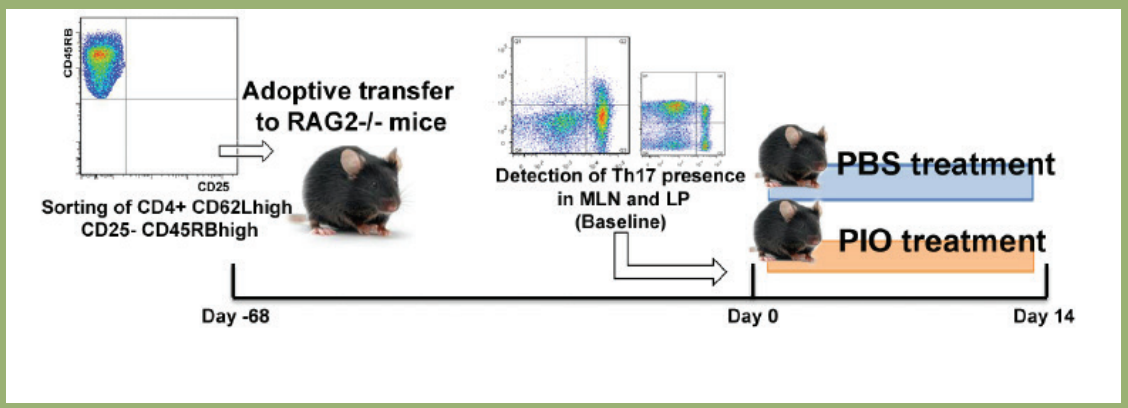


Figure 5. In vivo pharmacological validation study set up

An extra combination of both time course and scan was run, reiterating the phenotype switch with increasing concentrations of PPARγ over time (Fig. 4C).

The *in vivo* studies were focused on elucidating the effect of the loss of PPARγ in CD4+ T cells to validate our knock out *in silico* models and to validate the therapeutic role of pioglitazone on the plasticity context on adoptive transfer animal models given by the predictions *in silico*.

To determine whether the loss of T cell PPARγ favors Th17 and impairs iTreg cell differentiation we conducted computational simulations and *in vivo* studies of PPARγ deletion in T cells. Chronologically, a PPARγ-deficient naïve CD4+ T cell was created *in silico* by blocking PPARγ downstream signaling. The loss of PPARγ *in silico* caused upregulation of RORγt and IL-17 in Th17 cells (Fig. 4E) and down-regulation of FOXP3 in iTreg cells (Fig. 4G) compared to wild-type CD4+ T cells (Fig. 4E and 4F). To validate this computational prediction, we sorted naïve T cells from spleens of donor wild-type and T cell-specific PPARγ null mice and adoptively transferred them to SCID recipients. Cells from the colonic lamina propria (LP), spleen and mesenteric lymph nodes (MLN) of recipient mice were assayed for expression of FOXP3, RORγt and IL-17A by intracellular flow cytometry. The transfer of CD4+ T cells lacking PPARγ resulted in significantly greater accumulation of IL-17-producing Th17 cells and lower levels of FOXP3+ iTreg cells in spleen, MLN and colonic LP of recipient mice.

To validate the computational prediction that pharmacological activation of PPARγ leads to a phenotypic switch from Th17 to Treg (Fig. 4B), we sorted naïve T cells from wild-type donor spleens and transferred those to RAG2-/- recipients. When clinical signs of disease and colitis appeared, a subset of mice was sacrificed and spleen, MLN and colons were extracted to examine Th17 and Treg levels (baseline results). After verifying the presence of Th17 cells, half of the mice received a daily treatment of 70 mg/kg of pioglitazone (PIO) given orally to activate PPARγ (Fig. 5).

During the treatment period, mice treated with PIO recovered weight and their disease activity scores dropped significantly. Untreated mice maintained a predominant Th17 response characterized by increased levels of CD4+ T cells expressing RORγt and IL-17A. In contrast, PIO-treated mice not only recovered from colitis and its associated weight loss, but also showed a switch from a predominant Th17 into an iTreg phenotype characterized by increased expression of FOXP3 and decreased IL17-A and RORγt in CD4+ T cells of the colonic LP (not shown) and MLN (Fig. 6).This data supports the *in silico* prediction that activation of PPARγ in Th17 cells favors differentiation into iTreg cells.

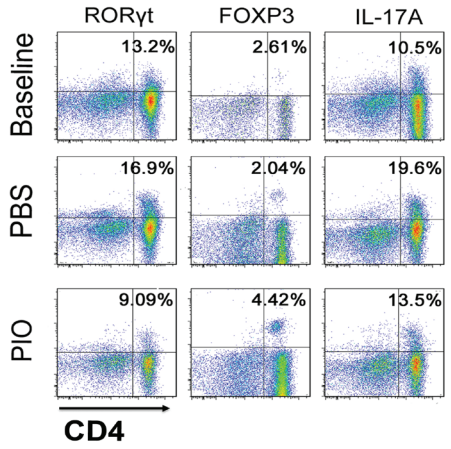


Figure 6. Flow cytometry results for model validation

Our modeling approaches allowed us to narrow the design of experiments and to better understand the molecular mechanisms of action controlling CD4+ differentiation. This new mechanistic knowledge is broadly applicable to the development of immune therapeutics for infectious, allergic and immune-mediated diseases. More specifically, we propose that PPARγ is a promising therapeutic target for chronic inflammatory and infectious diseases where Th17 cells contribute to the gut immunopathogenesis.

EAEC

Enteroaggregative

E. coli

Enteroaggregative E. coli (EAEC) causes persistent diarrhea and life threatening disease in many populations worldwide, particularly in malnourished children. New research from the NIMML provides novel insight into how EAEC interacts with its host. This new knowledge could be used by scientists to devise new therapeutic strategies against *E.coli*.

“We have developed the first host-targeted approach for treating *E. coli* infections”

In the human gut, *E. coli* are the predominant aerobic commensal inhabitants and remain involved in a key symbiotic relationship with their host beginning at infancy. Over time, some *E. coli* strains have acquired pathogenic genes enabling them to cause a broad spectrum of diseases. Under the MIEP program we study mechanisms that pathogenic *E. coli* utilize to cause infection and disease to develop safer and more efficacious therapies that target the host and not the bacterium.

EAEC is one of the most common causes of intestinal inflammation and persistent diarrhea worldwide, especially in malnourished children and immunosuppressed individuals. Malnutrition significantly dampens immune function and is, in fact, the most common cause of immunodeficiency worldwide. Infectious diseases that cause diarrhea, such as EAEC, account for more approximately 17% of deaths in children under the age of 5 years. The relationship between malnutrition and infection is a vicious cycle of altered nutrient absorption, severe growth retardation, compromised immune integrity, and chronic bacterial burden. In 2011, the largest EAEC outbreak ever recorded originated in northern Germany and received international attention when it sickened more than 3,000 people and caused over 50 deaths. Alarmingly, 90% of the victims caused by EAEC-induced illness during the 2011 outbreak were healthy adults highlighting how new EAEC strains are becoming hyper-virulent and more pathogenic. Currently, no FDA approved therapeutics exist to specifically combat EAEC infection. As an empirical effort, many medical doctors have administered antibiotics to treat EAEC-induced diarrhea. Importantly, this bacterium-targeted approach is strongly associated with enhanced release of bacterial toxins increasing disease and can pose life-threatening risks to the infected individual. An ongoing high demand exists for safe and effective therapeutics to treat EAEC infections. The NIMML team created a malnourished mouse model of EAEC infection to investigate host-bacteria interactions and areas of potential therapeutic intervention.

Consistent with reported clinical cases, malnourished mice clearly portrayed weakened immune responses accompanied by persistent infection. Since the malnourished mice were unable to mount an effective immune response, EAEC was able to colonize in the colon and cause chronic disease including growth retardation. In order to alter mucosal inflammation and immunity toward EAEC we targeted peroxisome proliferator activated receptor (PPAR)γ: a potent anti-inflammatory immune mediator. When PPARγ function was blocked in EAEC-infected mice, the animals developed stronger innate and effector immune responses and recovered significantly faster. Soon after infection (day 5 post infection), mice lacking PPARγ function had heightened levels of potent pro-inflammatory cytokines, such as IL-6, IL-1β, CXCL1,

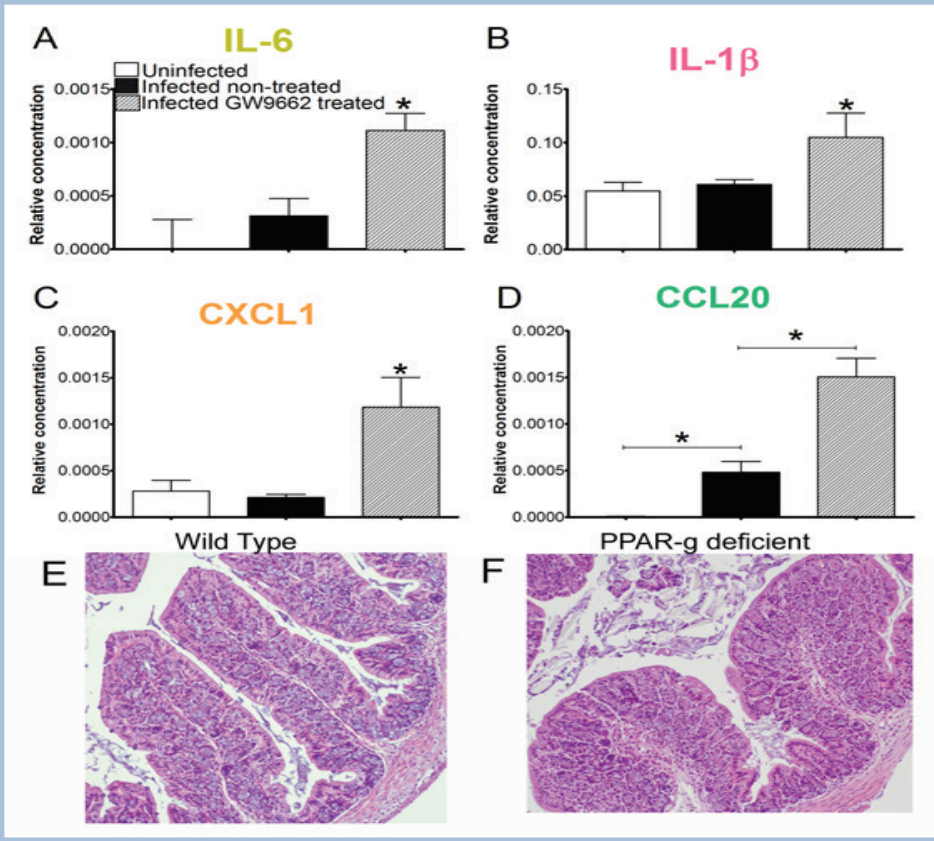


Figure 7. PPARγ blockade promotes beneficial inflammation

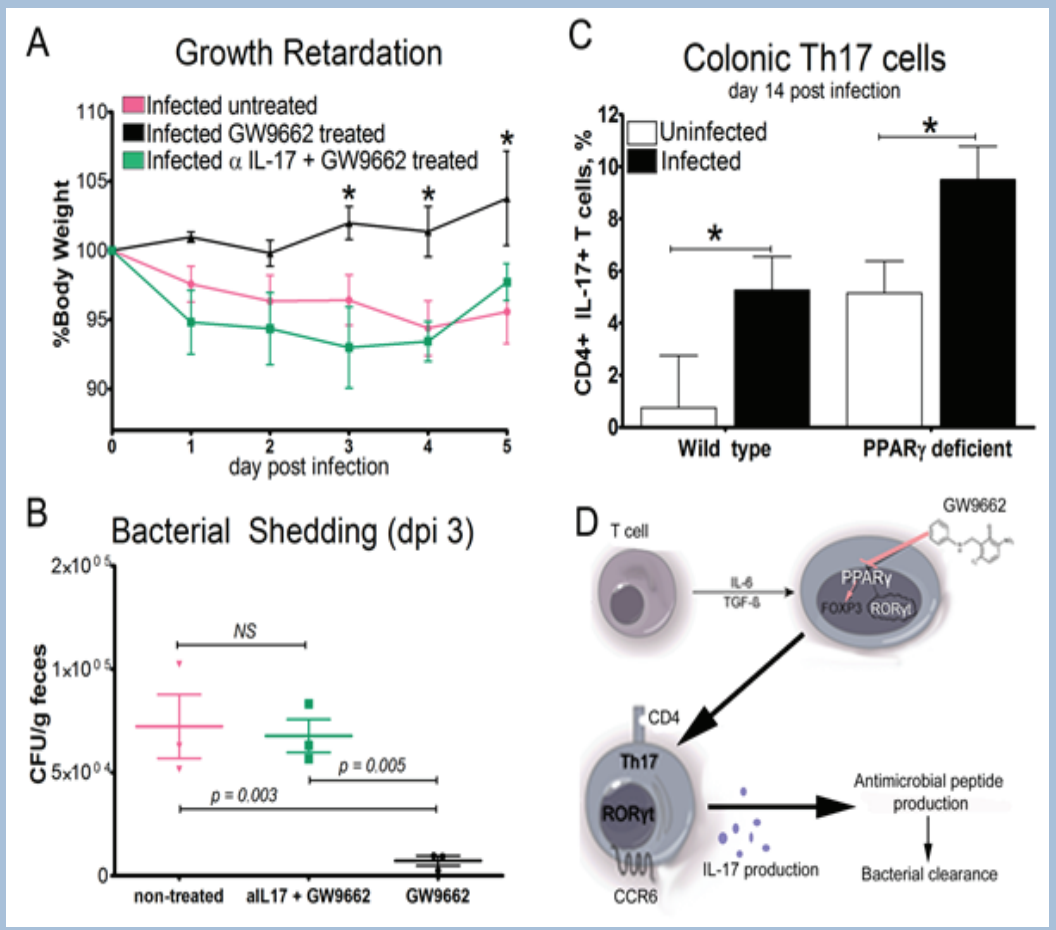


Figure 9. Th17 cells mediate immunity towards EAEC

and CCL20 and enhanced secretion of the antimicrobial peptides (Fig. 7). A computational model was developed using this data to expose key pathways involved in EAEC infection.

The protective immune responses to EAEC were further characterized as predominantly T helper 17 effector cells. Th17 cells promote antimicrobial immunity and facilitate faster bacterial clearance during infection. These findings were also associated with fewer colonic lesions and faster body weight recovery. Interleukin 17A (IL-17A) is an inflammatory mediator secreted by immune cells including CD4+ Th17 cells. IL-17A has been linked to both the pathogenesis and resolution of inflammatory, infectious, and autoimmune diseases in the gut. Cytokines IL-6, TGF-β, and IL-17 were upregulated in the colon through day 14 post infection promoting sustained Th17 responses (Fig. 8). During EAEC infection, IL-17A regulated the protective effects of PPARγ blockade. Specifically, when mice simultaneously lacked IL-17A and PPARγ function, the animals experienced significantly reduced growth rates interrelated with elevated bacterial counts that resembled the same patterns observed in untreated mice (Fig. 9).

Studying EAEC and ways in which therapeutic targets modulate the immune responses in the colonic mucosa promotes the development and discovery of new drugs to fight EAEC-induced disease. Future studies will focus on the innate sources of IL-17 early following infection and how IL-17 is mediating beneficial effects during disease. While the malnourished mouse model has provided novel insight, this animal model does not reproduce some of the key aspects of clinical EAEC manifestation observed in healthy adults. Researchers in NIMML aim to develop a robust animal model of EAEC infection that replicates clinical symptoms of disease and provides insight for treating infections in adults.

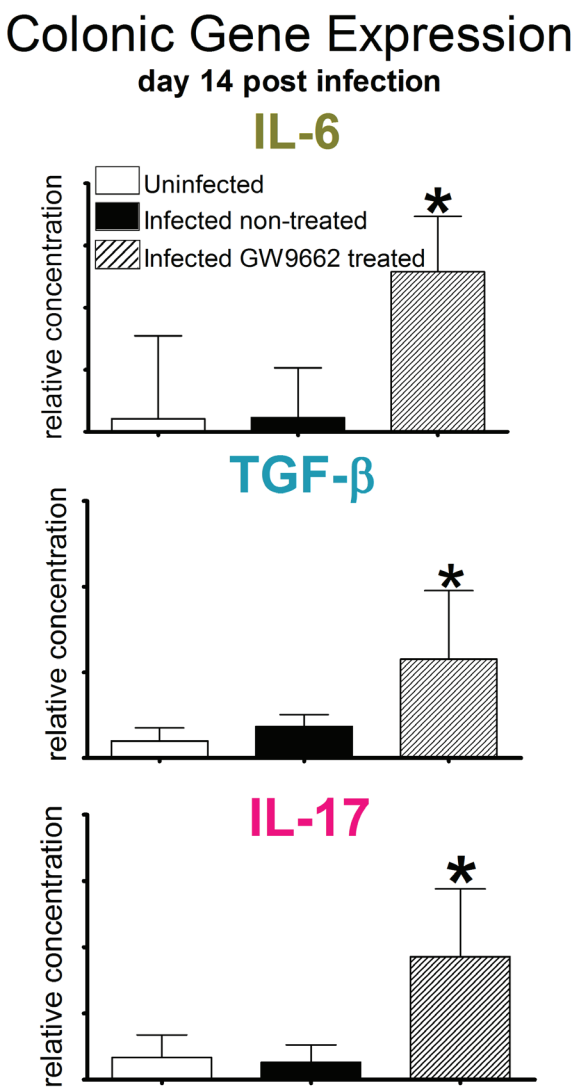


Figure 8. Cytokines promoting T helper 17 cell differentiation during EAEC infection

In conclusion, NIMML reported for the first time the importance of Th17 and IL-17A in EAEC disease amelioration. PPARγ blockade significantly enhanced beneficial proinflammatory responses, especially those related to IL-17A production. Increased IL-17A was associated with antimicrobial peptides and decreased EAEC levels in mouse feces. Thus, PPARγ antagonism represents a novel host-targeted therapeutic approach for EAEC infections.

Host Response

Host *H. pylori* interactions

We engineered novel computational models of the gastric mucosal immune system in response to *Helicobacter pylori* infection. Our results illustrate the importance of helper T (Th) cell subsets in the modulation of host responses to *H. pylori*. The models predict increased numbers of effector and regulatory Th cells in the gastric mucosa of *H. pylori*-infected mice.

“The computational model of *Helicobacter pylori* within two different modeling platforms”

Our modeling studies predicted that during the chronic phase of infection, the major contributor to gastric inflammatory lesions is not *H. pylori* itself but the mucosal immune response driven mainly by IFNγ-producing Th1 cells and inflammatory macrophages.

Given the complexity of the host-*H. pylori* interaction and to facilitate a better understanding of the gastric mucosal immune response during *H. pylori* infection, we constructed a computational and mathematical model (Fig. 10). The structural network of the model is comprised of three different compartments representing the effector sites: the gastric lumen, the epithelium and the gastric lamina propria plus a fourth inductor compartment representing the gastric lymph nodes (GLN). This network was used for equation-based and agent-based modeling efforts.

Mathematical modeling provides novel means of synthesizing cellular, molecular and tissue-level data into a common systems-level framework. Herein, we used two complementary types of modeling to study the impact of *H. pylori* infection in mucosal effector and regulatory pathways. In ODE-based modeling, the variables of the equations represent average concentrations of the various components of the mathematical model whereas ABM takes into consideration the rules and mechanisms of behavior of the individual components of the system and distribution of agents within the system. In contrast to ODE models which have fully developed and automated systems of parameter estimation, a key limitation of ABM is that sensitivity analysis and parameter estimation methods are immature. To investigate how the interplay between CD4+ T cells and other immune and epithelial cells in the gastric mucosa contributes to driving gastric pathology, we formalized a computational model of *H. pylori* infection using ODE and ABM approaches sequentially.

Our computational simulations show a distinct time-dependent behavior in Th cell phenotypes (i.e., Th1, Th17 and iTreg) represented in the model during *H. pylori* infection. Whereas Th17 is crucial at an early stage of the infection, Th1 predominates over Th17 and is key for the chronicity of the infection in the gastric LP. Together with these responses, there is a regulatory T cell upregulation peaking at day 30 and being persistent over the infection.

To determine and track the main responsible subsets triggering gastric inflammatory lesions during *H. pylori* infection, sensitivity analysis methods were applied. Results showed how at the early stage of infection, the epithelial cell damage is mainly caused by the bacterium itself (Fig. 11A).

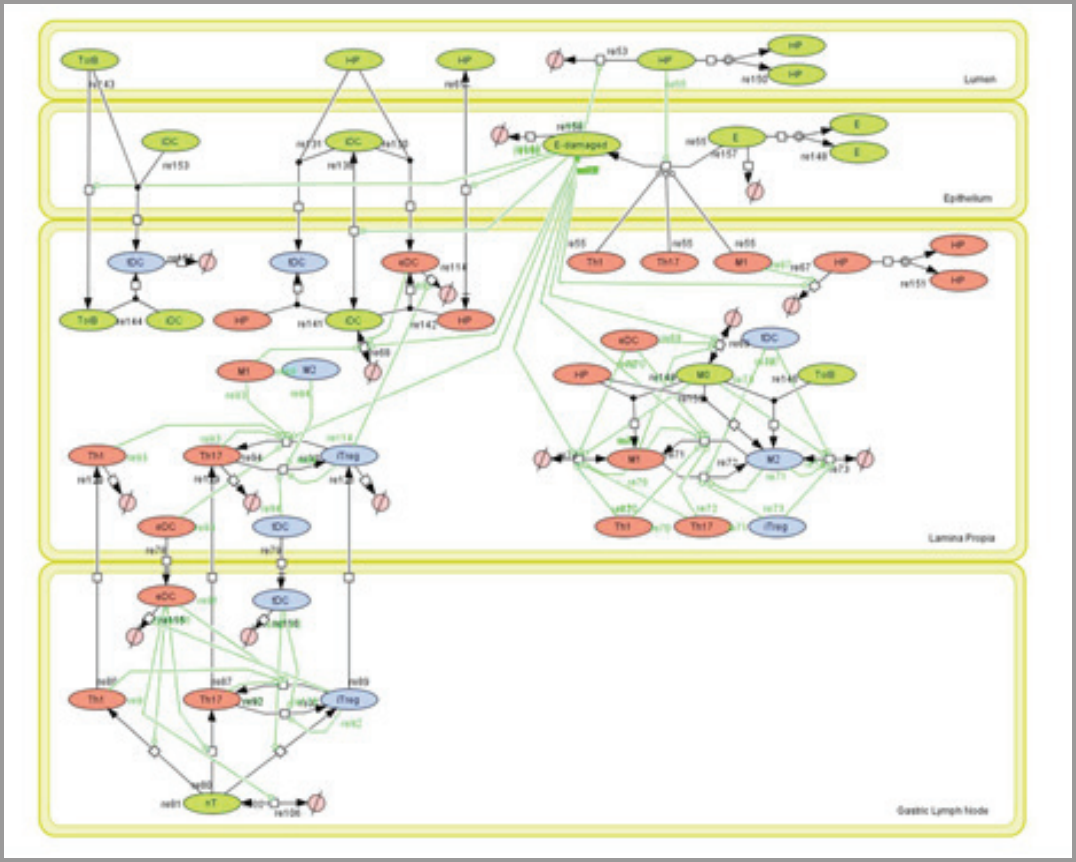


Figure 10. Computational modeling of host responses to *Helicobacter pylori*

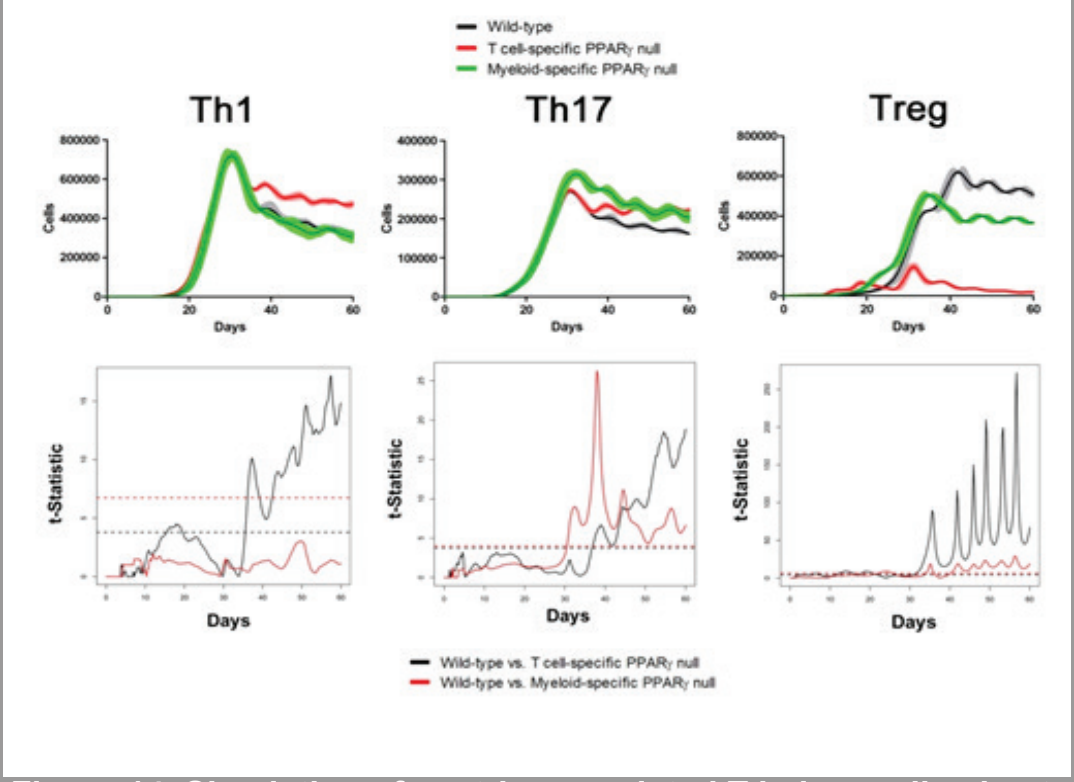


Figure 14. Simulation of gastric-associated T helper cell subsets during *Helicobacter pylori* infection

Interestingly, as the infection progresses, a trend towards Th1 cells triggering epithelial cell damage (Fig. 11B) is observed. At the chronic phase of the infection, results showed a dramatic increase of Th1- and Th17-inducing epithelial cell damage (Fig. 11C). Of note, SA performed in the deterministic model at day 60 post infection also showed how Th1 and Th17 in both LP and GLN were contributing to the formation and accumulation of damaged epithelial cells as well as M1 macrophage differentiation, whereas *H. pylori* exhibited no impact on such formation (Fig. 11D).

To study the mucosal immune responses to *H. pylori* at the systems level locally in the gastric mucosa, we used ODE and ABM sequentially. First, our deterministic ODE model shed some new light on CD4+ T cell distribution after infection as well as the role of PPARγ during infection. Secondly, the ODE model provided a set of parameter values that were after used as a starting point for our ABM modeling, where the strategies for parameter estimation are not fully developed or automated. ABM adds randomness to the biological systems, which can help to better represent complex cellular responses and to take into account the individual behaviors of cells, such as the differential in number of gastric-associated T helper cell subsets during *H. pylori* infection (Fig. 12). Furthermore, one can assesstheir role in a spatiotemporal manner. Thus, stochastic models can provide novel insights into the effect of cognate and non-cognate interactions, representing entire systems with a greater granularity and capturing cell-cell interactions. By simulating individual behaviors of agents, ABM better represents cross-linked, complex and nonlinear processes with multiple feedback loops and, provides a more comprehensive and interactive modeling of mucosal immune responses to *H. pylori*. The ability of ABM to encompass multiple scales of biological processes and incorporate spatiotemporal considerations, coupled with an intuitive modeling paradigm, underscores the added value of this modeling framework in translational systems immunology and immunoinformatics research. Our combined modeling ODE and ABM approaches provided evidence suggesting that the cause for gastric lesions during the chronic stage of the infection were effector Th1 and Th17 cell subsets as well as inflammatory macrophages. Future studies will investigate the spatiotemporal progression of lesions in relation to immune cell trafficking in the tissue space.

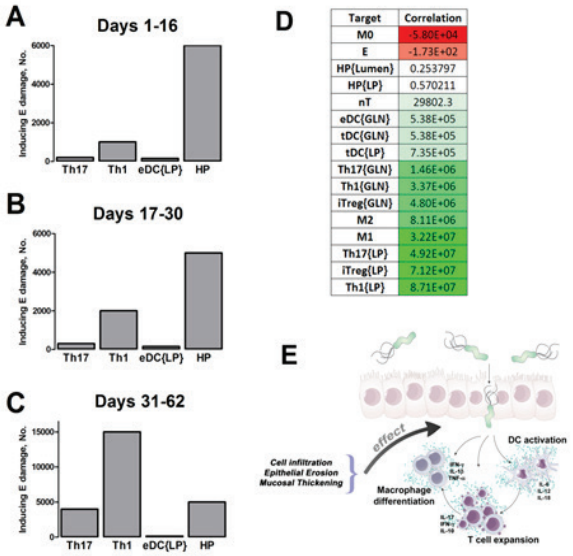


Figure 12. Tracking immune cell-mediated tissue damage in *Helicobacter pylori* infection

In summary, we combined computational modeling approaches and mouse challenge studies to investigate how CD4+ T cells and other immune cell subsets are distributed in the gut mucosa during *H. pylori* infection. Our model simulated T cell responses to *H. pylori* by using both platforms: ODE and ABM. Our modeling efforts predicted higher levels of effector responses in both the gastric mucosa and the lymph nodes when deleting PPARγ, thus highlighting the role of PPARγ activation as a potential mechanism for modulating CD4+ T cell responses during bacterial infection and positioning PPARγ as a candidate for immunotherapeutics development. Future studies will more fully realize the potential of multiscale modeling to understand mucosal immunity.

miRNA

Clostridium difficile

therapeutics

NIMML applied computational and mathematical modeling approaches in combination with RNA-sequencing and mouse challenge studies to characterize the disruption of an important regulatory pathway during *Clostridium difficile* infection.

“A novel therapeutic approach for *C. difficile* infection”

Clostridium difficile typically is a harmless anaerobic bacterium, but it has recently re-emerged as a significant enteric pathogen implicated in nosocomial diarrhea, colitis and even death, particularly after antibiotic treatment. We have found that tissue damage and disease severity in *C. difficile* infection is associated with a disruption of the peroxisome proliferator-activated receptor gamma (PPARγ) pathway, including modulation of T cell responses by microRNAs.

Clostridium difficile typically is a harmless environmental sporulated Gram-positive anaerobic bacterium, but it has recently re-emerged as a significant enteric pathogen implicated in antibiotic-associated diarrhea, colitis and even death. *C. difficile* bacteria naturally reside in the gut of 2-5% of the adult population without showing any symptoms. However, it can also grow in the intestine of individuals with altered commensal microflora due to treatment with antimicrobials, immunosuppressants, cytostatic agents or proton pump inhibitors. Non-carrier people can accidentally ingest *C. difficile* spores, especially in hospitals and other healthcare-related facilities. Once in the colon, such spores can germinate and *C. difficile* starts growing. When in small concentrations, *C. difficile* infection does not result in any significant disease. However, *C. difficile* flourishes and overruns the colon when the normal gut flora has been destroyed, usually after antibiotic treatment. *C. difficile* infection can result in a wide range of clinical symptoms including asymptomatic colonization, mild to severe diarrhea, colitis with or without the presence of pseudomembranes, colonic perforation and toxic megacolon. Some of these complications can be life-threatening and result in death in some cases. The outcome of the infection depends on the *C. difficile* strain and the production of toxins, which are mainly released by pathogenic strains and are responsible for the diarrhea and inflammation.

Several countries have reported an increase in both incidence and severity of *C. difficile*-associated disease (CDAD) over the last years, correlating with the emergence of new hypervirulent strains such as NAP1/BI/027. Previously, CDAD was a concern in older or severely ill patients, but the emergence of these new *C. difficile* strains has resulted in increased morbidity and mortality for other age groups in the developed countries. The increased virulence of *C. difficile* is attributed to greater sporulation and production of toxins or a higher resistance to antibiotics. Persistent or severe CDAD is currently being treated with discontinuation of the antibiotic therapy that led to the disease, and vancomycin therapy. Nevertheless, these therapeutic approaches do not restore the normal microflora and are not effective in eliminating the bacterium, but further prolong *C. difficile* growth and destroy other beneficial gut anaerobic bacteria. To date, it seems that targeting the bacterium and its toxins directly has not been successful in ameliorating CDAD,

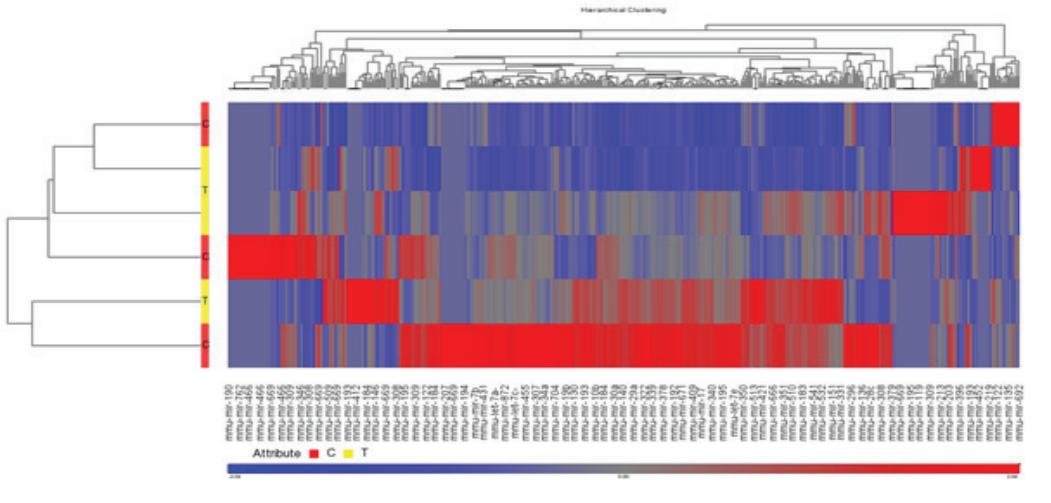


Figure 14. Modulation of miRNAs by *Clostridium difficile* infection

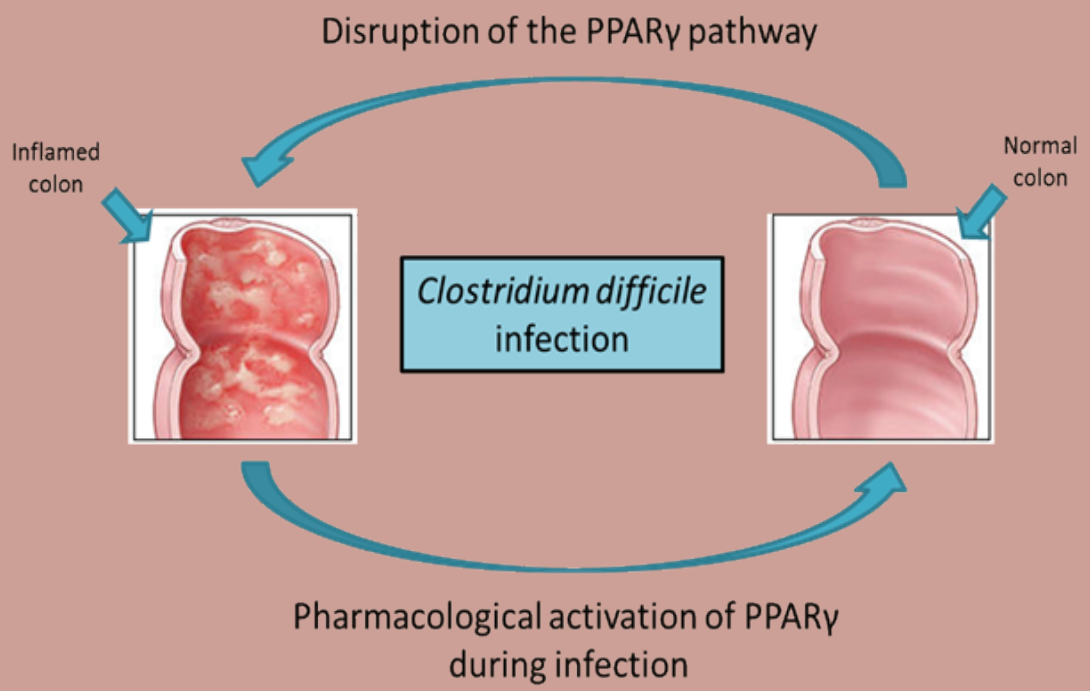


Figure 15. PPARγ regulates *Clostridium difficile* associated disease

thus emphasizing the importance of developing broad-based, host-targeted approaches to control the disease as opposed to just relying on anti-microbial therapies that target the bacterium and can stimulate the spread of resistance.

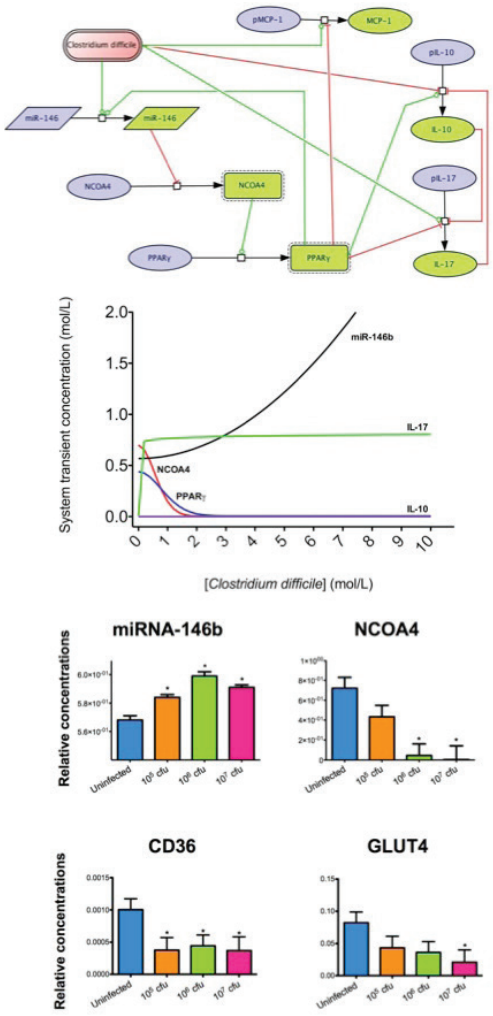


Figure 13. Computational modeling of PPARγ disruption during *Clostridium difficile* infection

The NIMML has recently reported new findings that suggest a different host-targeted approach for treating *C. difficile* infection. We applied computational and mathematical modeling approaches (Fig. 13) in combination with RNA-sequencing (Fig. 14) and mouse challenge studies to characterize the disruption of an important regulatory pathway during *C. difficile* infection. We have found that tissue damage and disease severity in *C. difficile* infection is associated with a disruption of the peroxisome proliferator-activated receptor gamma (PPARγ) pathway, including a possible relationship between this nuclear receptor and microRNAs (Fig. 14). The human intestine must peacefully coexist with trillions of beneficial bacteria while swiftly responding to pathogens such as *C. difficile*. Sometimes the immune system will go into overdrive when responding to pathogens, causing more damage in an attempt to clear the infection. Activation of PPARγ by using a diabetes drug ameliorated *C. difficile*-related gut pathology and disease in mouse challenge studies. Thus, PPARγ helps keep the immune response in check, allowing the body to heal but also allowing the immune cells that fight infection to do their work in a controlled manner (Fig. 15). When PPARγ was absent or not activated, disease was more rampant and colonic lesions from *C. difficile* were much worse.

In conclusion, we have used loss-of-function approaches in combination with pharmacological activation of PPARγ and computational modeling to investigate the critical role of PPARγ in regulating immune responses and disease severity following *C. difficile* infection. Our data suggests that overexpression of miRNA-146b in the colon might exacerbate inflammatory responses by suppressing PPARγ activity through a mechanism possibly involving suppression of NCOA4, a co-activator molecule required for activation of PPARγ. This research demonstrates that the integration of powerful computer simulations of host responses with systems immunology experimentation not only contributes to a better understanding of the immunoregulatory processes in the gut mucosa during *C. difficile* infection, but it also advances the discovery of broad-based therapeutic targets in the host for infectious diseases.

The NIMML will continue developing new drugs targeting this pathway with fewer side effects and greater efficacy than those currently on the market. Moreover, we will investigate the possible role of other nuclear receptors such as vitamin D receptor during CDAD, as well as the protective effects of the commensal microflora during *C. difficile* infection.

ENISI

Enteric Immune Simulator

ENteric Immunity SImulator (ENISI) is an agent-based model designed to simulate the cell movement and interactions in the gastroenteric mucosa. Here we present the current 1.5 version of ENISI. In ENISI each individual cell is modeled and acts independently.

"ENISI is the first agent-based modeling tool for Enteric Immunity"

ENISI (ENteric Immunity Simulator) is the first agent-based modeling tool we have been developing for enteric immunity. It has both HPC (high-performance computing) version and single-machine version. The HPC version runs with our super computer ShawdowFax and can scale up to 10^8 cells. We have also developed an online job submission system for performing in silico experiments. The single machine version has smaller scale but provides greater visualizations.

ENISI is an interaction-based model where individual cells are modeled, along with their movement though different tissues, and the probabilistic outcomes of cell-cell interaction. ENISI has the ability to simulate at least 10^7 individual cells. With ENISI, mucosal immunologists will be able to test and generate hypotheses for enteric disease pathology and propose interventions through experimental infection of an *in silico* gut. This is done by using a simple scripting language to assign parameter values that conform to one's knowledge and assumptions of the experimental scenario they wish to simulate. Simulation outcomes given different experimental conditions allow observation of in silico behaviors that are not readily seen through in vitro and in vivo techniques. This information can then be used to generate novel treatment strategies that can be tested in the laboratory. The ENISI modeling environment has already been illustrated by developing (i) a *in silico* model and dynamic simulation of *H. pylori* and (ii) a simulation of dysentery resulting from *Brachyspira hyodysenteriae* infection so as to identify aspects of the host immune pathways that lead to continued inflammation-induced tissue damage even after pathogen elimination. The ENISI modeling environment has been designed from the start for: (i) scalability; (ii) efficiency; and (iii) the use of large-scale parallel computing systems. The underlying message-passing middleware is based on the *EpiSimdemics* modeling environment. Our recent work has demonstrated scaling to between 10^7-10^8 cells using 600-800 cores of a parallel cluster. To our knowledge this is the first individual agent based model of an immune system that achieves this kind of scalability.

We have studied the performance issues of ENISI and developed several improvements at the algorithmic and programming levels. We describe the improved results in light of the previous version (v1) which was published in the IPDPS 2012 conference. Also we improved many of the C++ functions, streamlining the code to provide programmatic improvements. In the following figure we show the improvements in terms of speed-up, execution time and scaling. As the model is large enough to be run on a single cluster, we compared the speed-up with a 12-core simulation. Here we tested ENISI for 10^7 cells on up to 700 processing

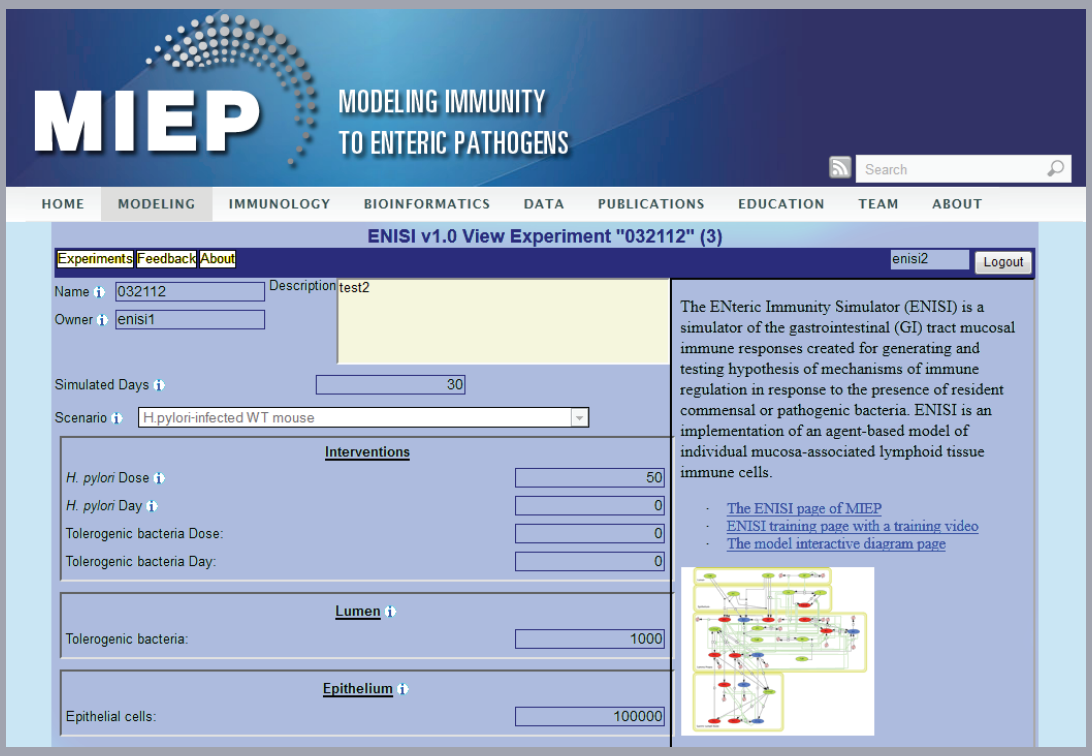


Figure 16. ENISI HPC user interface through Modeling Immunity to Enteric Pathogens website

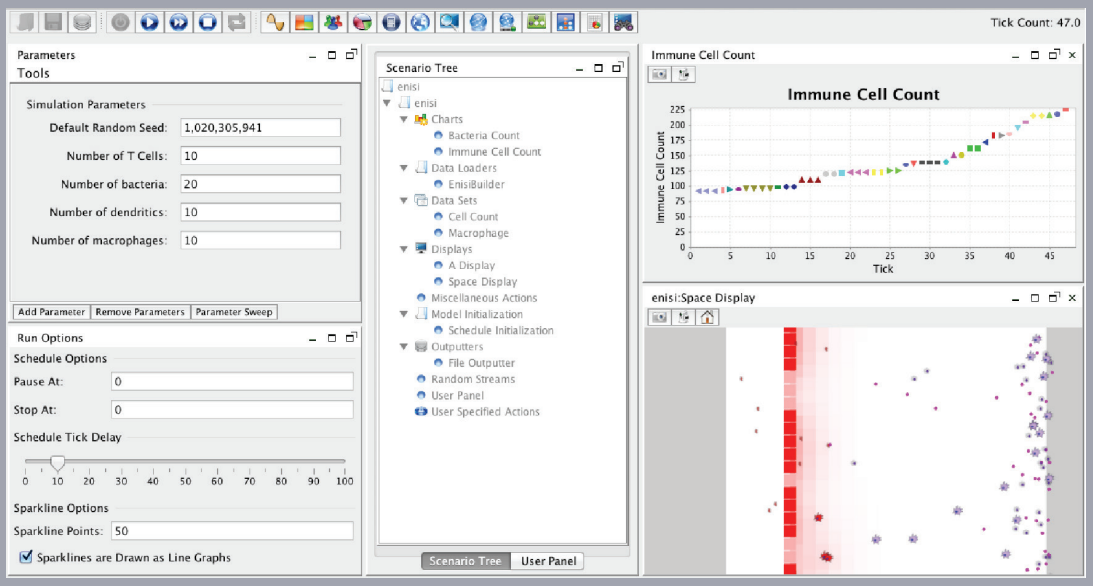


Figure 17. ENISI Visual User Interface

nodes. We have seen a remarkable increase in speed which allows us to make more runs of the simulation than the previous version. We can now do multiple runs of each simulation, which will facilitate sensitivity analysis.

We integrated the ENISI Tool into our website (www.modelingimmunity.org), which allows the scientific community to study the behavior of *H. pylori* infection in a mouse stomach (Fig. 16). The users are able to request an account and set up their own *in silico* experiments, which can be submitted to our High Performance Computing cluster. The user is informed by email of job completions and can view the results on the MIEP website. We integrated the statistical results calculated by multiple simulations of the ENISI *H. pylori* model into the MIEP Website, which are accessible via the simulation results under the ENISI tool.

ENISI Visual

There are two versions of ENISI: ENISI High Performance Computing (HPC), described above, and ENISI Visual. ENISI Visual is implemented using the REPAST platform and can be used to develop and calibrate ENISI models at a smaller scale before running them in our HPC cluster.

ENISI Visual is adapted from the HPC implementations of ENISI and emphasizes quality user interfaces and visualizations (Fig. 17). ENISI Visual allows users to specify the initial concentrations of cells, the pathogen infectious dose and simulation time. From the interface, users can control the animation speed and specify the output charts, figures, real-time animations, snapshots, and videos. ENISI Visual has integrated secretion and propagation of cytokines and chemokines, and the cell movement models. The real-time animations during *in silico* experiments allow users to quickly test hypotheses and discover novel phenomena. ENISI Visual will be enhanced to include host-pathogen-microbiota interactions. ENISI Visual provides rich graphic user interfaces. Users can control initial cell concentrations, simulation speed, data and graphic outputs. A video of ENISI Visual is available at http://www.modelingimmunity.org/modeling-tools/enisi-visual/

We plan to extend our current work on ENISI Visual in the following ways:

- To simulate more complex shapes of compartments.
- To extend the simulatrion from 2-D to 3-D
- To run parameter estimation and model calibration
- To develop mechanistic models based upon experimental data
- To integrate with ODE-based models for multi-scal modeling

ENISI Stochastic Differential Equations

Stochastic differential equations (SDE) is a modeling and simulation technology for stochastic modeling. ODE-based models are deterministic. Agent-based models are stochastic, but they require highly on computational resources such as high-performance computing clusters. SDE can simulate stochasticity of various resources with relatively low computational resources. SDE has been widely used in the economy and social studies. However, its uses in biology especially immunology are very limited. We believe this is due to lack of appropriate SDE modeling tools for biologists. There are a few existing SDE modeling tools existing in the forms of Matlab or R modules. However, they requires highly on mathematical knowledge and programming skills. We are developing a biologists-friendly SDE modeling tool, named ENISI SDE. ENISI SDE will require no mathematical knowledge or programming skills and will provide quality user interfaces so that biologists can use it with very little learning curve.

H. pylori

Obesity & Diabetes

H. pylori is the dominant member of the gastric microbiota and infects about half of the world population. While *H. pylori* infection can be associated with severe disease, it helps control chronic inflammatory, allergic, or autoimmune diseases. The NIMML demonstrated for the first time that gastric colonization with *H. pylori* exerts beneficial effects in mouse models of obesity and diabetes.

A stomach bacterium believed to cause health problems such as gastritis, ulcers, and gastric cancer may play a dual role by balancing the stomach's ecosystem and controlling body weight and glucose tolerance. Usually viewed as a pathogen in studies of gastric cancer and peptic ulcers, *Helicobacter pylori* infect about half of the world's population although most infected individuals don't get sick. The bacterium's dwindling numbers coincide with the epidemic of obesity and diabetes.

Helicobacter pylori is a Gram-negative, microaerophilic bacterium of the Epsilonproteobacteria that colonizes the stomach as a dominant member of the gastric microbiota of nearly half of the world's population. *H. pylori* phylogenetic tree reflects the major human migration out of Africa, across Europe, through Asia, and into the New World. Gastric infection with *H. pylori* has been associated with various gastric diseases, including gastritis, peptic ulcer disease, gastric adenocarcinoma, and gastric mucosa-associated lymphoma.

While *H. pylori* infection is associated with severe disease, mounting evidence suggests a beneficial role of this bug in chronic inflammatory, allergic or autoimmune diseases. Specifically, there also is increasing evidence of *H. pylori* protection against esophageal and cardial pathologies, childhood asthma, childhood allergies, obesity and diabetes. The mechanisms underlying this protective effect of *H. pylori* acting as a commensal bacterium or a pathogen are largely unknown.

Interestingly, there is an inverse secular trend between the incidence of obesity and gastric colonization with *H. pylori*, a bacterium that can affect the secretion of gastric hormones that relate to energy homeostasis. Previously, the NIMML team characterized the whole genome and function of *H. pylori* V225d, cultured from a Venezuelan Piaroa Amerindian, an atypical strain found in areas with low incidence of *H. pylori*-associated cancer. During this reporting period, we demonstrate that colonization with *H. pylori* exerts beneficial effects in obesity and diabetes. Specifically, we examined metabolic and inflammatory markers in genetically obese/diabetic (db/db) mice and mice with diet-induced obesity experimentally infected with isogenic forms of *H. pylori* strain 26695: the cag PAI wild-type and its cag PAI mutant strain 99-305. *H. pylori* colonization decreased fasting blood glucose levels, increased levels of leptin, improved glucose tolerance, and suppressed weight gain. A response found in both wild-type and mutant *H. pylori* strain-infected mice included decreased white adipose tissue macrophages (ATM) and increased adipose tissue regulatory T cells (Treg) cells. Gene expression analyses demonstrated upregulation of gastric PPARγ-responsive genes (i.e., CD36 and FABP4) in *H. pylori*-infected mice. The loss of PPARγ in immune and epithelial cells in mice impaired the ability of *H. pylori* to favorably modulate glucose homeostasis and ATM infiltration during high fat feeding. Thus, gastric colonization with some commensal strains of *H. pylori* ameliorates glucose homeostasis in mice through a PPARγ-dependent mechanism and modulates macrophage and Treg cell infiltration into the abdominal white adipose tissue.

These data suggest that colonization by *H. pylori* strains lacking the cag PAI could provide partial protection against some metabolic disorders. Thus, if this theory holds true, the disappearance of *H. pylori* in developed countries may be a contributing factor to the epidemics of obesity and diabetes. Future studies will examine the mechanisms by which specific *H. pylori* strains modulate regulatory and effector pathways in the gastric mucosa, and their correlation with improvements of chronic inflammatory diseases. Ongoing efforts under the MIEP program involve creating mathematical and computational models of the effector and regulatory pathways triggered by *H. pylori* at the gastric mucosa. The better understanding of such pathways will shed new light on the dual role of *H. pylori* as a pathogen and a commensal organism.

ABA

Abscisic Acid & Influenza

NIMML researchers have discovered that abscisic acid has anti-inflammatory effects in the lungs as well as in the gut. While the immune effects of abscisic acid are well understood in the gut, less was known about its effects in the respiratory tract. Recent evidence from the NIMML demonstrates that not only does abscisic acid ameliorate disease activity and lung inflammatory pathology, it also aids recovery and survival in influenza-infected mice.

Influenza accounts for anywhere from 3,000 to 49,000 deaths per year in the United States alone, according to the Centers for Disease Control. It is difficult to treat if not caught immediately; antivirals usually become ineffective after the virus incubation period has passed and resistance to antiviral drugs poses a serious public health problem in the face of outbreaks. Abscisic acid, however, has been shown to be most effective at about seven to ten days into the infection, targeting the immune response rather than the virus itself, which many researchers feel is a safer way to reduce flu-associated fatalities.

Respiratory pathogens causing pneumonia are the leading cause of infectious disease-related death in industrialized countries. Although bacterial infections are more common in adults, respiratory viruses are the main etiologic agents of pneumonia in immunocompromised population groups, namely children and the elderly. Influenza, parainfluenza, respiratory syncytial virus (RSV), and rhinoviruses are most frequently associated with respiratory infections in young infants and children. Influenza virus infections alone or complicated by secondary bacterial superinfections kill more than 35,000 people in the United States every year. In addition, the yearly seasonal flu epidemics are an economic burden to the public health system, due to days of restricted activity, hospitalizations and visits to physicians.

Under the grant entitled “Mechanisms of Immune Modulation by Abscisic Acid” awarded by the NIH/NCCAM (Grant No R01AT004308) to Raquel Hontecillas and Josep Bassaganya-Riera the NIMML investigated the mechanisms of immune modulation by abscisic acid (ABA) and discovered an alternative mechanism of activation of peroxisome proliferator-activated receptor (PPAR) γ and anti-inflammatory activity by ABA. This and other discoveries resulted in 20 published papers, 10 patents filed or issued and 10 invited presentations.

Traditional antiviral therapeutics may offer limited efficacy due to the rapid evolution of emerging influenza virus strains, and the emergence of strains resistant to antiviral treatment. Since excessive inflammation has been linked to influenza virus-related mortality, immune modulation may be required in addition to virus clearance to limit or prevent severe disease. Exogenous administration of PPAR ligands can broadly target the majority of cells involved in virus induced pulmonary pathology (Fig. 18), and it represents an effective means of immune modulation.

In previous work, the NIMML team demonstrated that ABA, a naturally occurring compound derived from plants, has beneficial effects on several conditions and diseases including obesity-related inflammation, diabetes, atherosclerosis, and inflammatory bowel disease. One idea for how ABA reduces inflammation in these instances is that it binds to a special region of peroxisome proliferator-activated receptor-gamma, a binding site known as the ligand-binding domain where the drug would be expected to latch on to and exert its effect. Our results show that this is not the case and, for the first time, we have demonstrated that ABA acid works independently of this ligand-binding domain of the receptor. The outcomes of this research illustrate the synergism that can result from combining computational and experimental approaches to characterize therapeutic targets. By using molecular modeling approaches we were able to identify potential binding sites for ABA on the lanthionine synthetase C-like 2 (LANCL2), a protein required for the beneficial health effects of ABA (Fig. 19).

Furthermore, our results showed for the first time that dietary ABA ameliorates influenza-virus-induced pulmonary pathology through a mechanism that depends on the full expression of PPARγ in epithelial and immune cells of the lung, designated as PPARγ cKO.

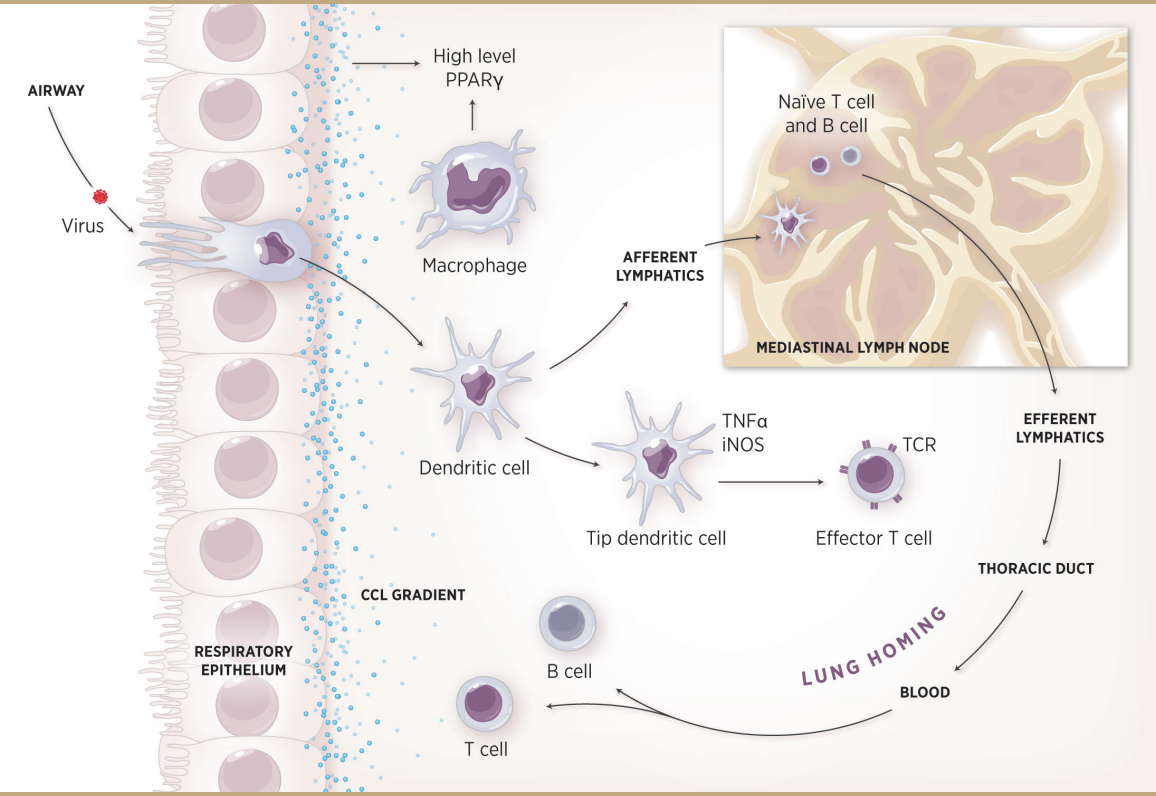


Figure 18. Schematic representation of lung infection in mice

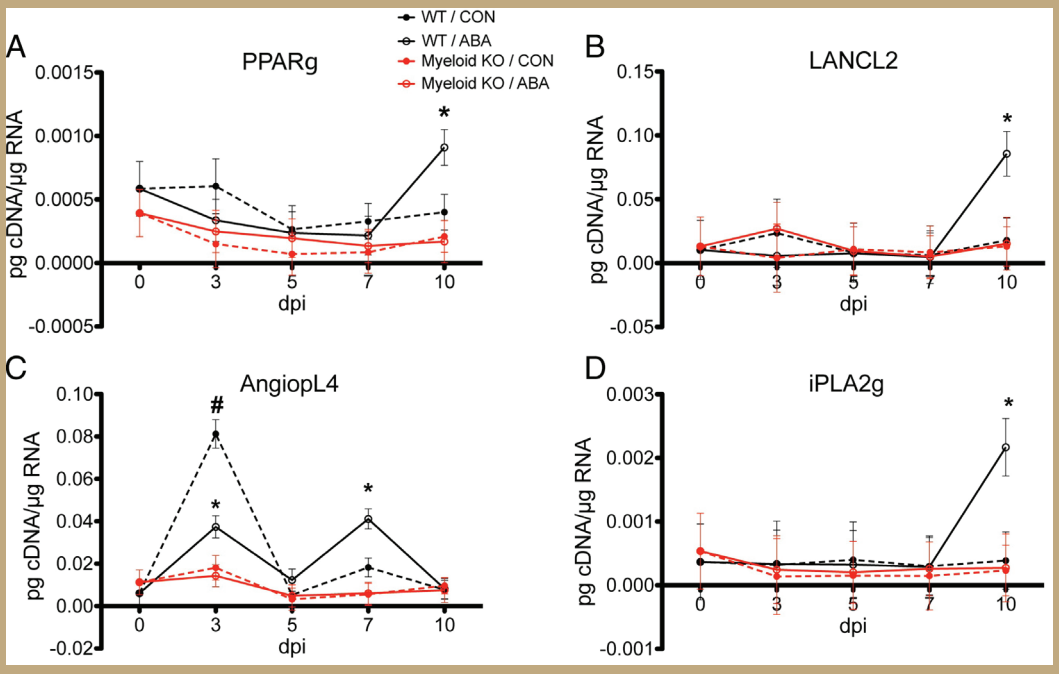


Figure 20. Effect of dietary ABA-supplementation on pulmonary expression of PPARγ and MCP-1

To unveil molecular targets modulated by ABA, we conducted targeted gene expression analysis in lung specimens collected from the WT and myeloid KO mice. Unexpectedly, we found that the major impact of ABA on gene expression occurred between days 7 and 10 postinfection. Our results show that there was a significant effect of ABA treatment in WT mice. Specifically, both PPARγ and LANCL2 were significantly up-regulated on day 10 (Fig. 20A and B). The PPARγ target gene angiopoietin-like 2 showed two peaks on days 3 and 7. On day 3, the expression was higher in WT mice that received vehicle, while on day 7, angiopoietin-like 2 expression was significantly higher in WT mice that received ABA (Fig. 20C). iPhospholipase A2 (iPLA2), which acts in proximal steps of the lipoxygenase and cyclooxygenase pathways by releasing arachidonic acid from cell membranes, was also significantly up-regulated in WT mice treated with ABA (Fig. 20D).

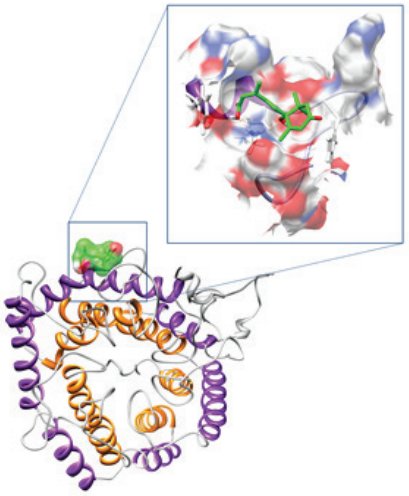


Figure 19. Identification of potential binding sites for ABA on the lanthionine synthetase C-like 2

In addition, dietary ABA supplementation was effective in down-regulating lung MCP-1 mRNA expression. This chemokine is required for homing of CCR2+ monocytes, which then differentiate into exudate macrophages and monocyte-derived dendritic cells, two major contributors to the pathogenesis of influenza by producing large amounts of TNFα and iNOS.

ABA treatment diminished the extent of vascular infiltrates as well as the infiltration of respiratory airways mucosa and submucosa, and alveolar space in WT mice when compared to infected WT mice fed the control diet. Moreover, the effect of ABA in decreasing pulmonary damage was PPARγ dependent since the beneficial effect of ABA on lung inflammatory cell infiltration observed in WT mice was abrogated in cKO mice fed ABA. These results suggest that the mechanism by which ABA protects from influenza-induced lung pathology is related to diminished recruitment of inflammatory cells into the lung and requires full expression of PPARγ in the lungs.

The results of these studies support the use of ABA as a novel adjunct for influenza that dampens the host inflammatory response in the lungs, ameliorates clinical disease and lung pathology, and improves resolution of lung pathology and survival. We also provided definitive molecular evidence in vivo demonstrating that the beneficial effects of ABA on influenza are mediated through a mechanism involving immune cell PPARγ at both the initiation and resolution stages of disease. Thus, ABA has the potential to become an effective immune modulatory treatment for respiratory infections that targets the host and not the pathogen and has no known adverse side effects. Given these results, we have also shown that ABA affects the expression of several genes involved in inflammation, metabolism and cell signaling, which provides further clues for possible intervention points in the treatment of inflammatory, infectious and immune-mediated diseases.

Most drugs for respiratory infections target the virus rather than the inflammatory responses caused by the virus. ABA suppresses lung inflammation and damage through activation of LANCL2, a newly identified pathway. This research led by Dr. Hontecillas was published in the Journal of Nutritional Biochemistry (dx.doi.org/10.1016/j.jnutbio.2012.07.010).

IBD

Conjugated Linoleic Acid

The NIMML first reported the anti-inflammatory efficacy of conjugated linoleic acid (CLA) in a pig model of colitis in 2002. We next characterized the mechanism of action of CLA *in vivo* in mice. Under a contract from Cognis (now BASF) awarded to the NIMML we discovered important new information on the efficacy of CLA in treating human Crohn's disease (CD), a form of inflammatory bowel disease (IBD).

Despite evidence that Crohn's Disease therapies have improved, they are modestly successful for the long-term management of the disease and result in significant side effects such as immune suppression, enhanced susceptibility to malignancies, and suppressed resistance against infectious diseases. Moreover, two-thirds to three-quarters of patients with Crohn's disease will require surgery at some point during their lives, which becomes necessary in Crohn's disease when medications can no longer control the symptoms. Therefore, there is a need to identify novel and safer therapies for the treatment of IBD.

Many polyunsaturated fatty acids (PUFA) and their metabolites are natural ligands for PPARγ and have been proposed as a promising avenue for developing safer nutritional interventions against gut inflammation without adverse side effects. One of these PUFA is CLA, which is naturally present in milk, cheese and ruminant products, and has been considered for the prevention and treatment of gut inflammation since 2002. By using a bacterial-induced colitis pig model, we found that dietary CLA supplementation suppresses colonic inflammation and up-regulates colonic PPARγ expression. Specifically, CLA ameliorated tissue inflammation and weight loss associated with *B. hyodysenteriae*-induced colitis. In this publication we also provided molecular in vivo evidence demonstrating that the loss of the PPARγ gene in the colon abrogates the beneficial effects of CLA in DSS-induced colitis, suggesting that CLA ameliorates colitis through a PPARγ-dependent mechanism. PPARγ is mainly expressed in the colon by epithelial cells and immune cells in the lamina propria including macrophages and lymphocytes. Although some studies show the importance of PPARγ in each cell type, additional investigations in animals with cell type-specific expression of PPARγ are required to determine the main cellular source responsible for the therapeutic effect of PPARγ.

In light of the encouraging CLA anti-inflammatory effects reported in animal models of colitis, and consistent with the concept from bench to bedside, our group has translated the basic scientific understanding of cellular and molecular processes to the clinic. Under a contract from Cognis (now BASF) awarded to the NIMML, we discovered important new information on the efficacy of CLA in treating human Crohn's disease (CD), a form of inflammatory bowel disease (IBD). The clinical trial, which was managed as an Investigational New Drug (IND) trial and was done in collaboration with the Division of Gastroenterology and Hepathology at University of North Carolina School of Medicine and the Wake Forest Medical Center, found that CD patients who took supplementary CLA showed noticeable improvement. CLA was administered as a supplement (6 g/day orally) in thirteen study subjects with mild to moderate CD for 12 weeks and we found a marked improvement in disease activity and quality of life, and importantly, no adverse side effects since CLA was well tolerated by all of the study subjects. Specifically, there was a statistically significant drop in Crohn's Disease Activity Index (CDAI) from 245 to 187 (p=0.013) (Fig.21) and

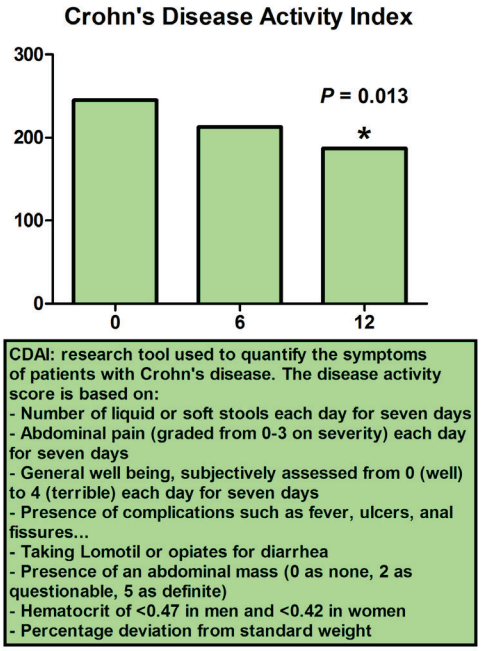
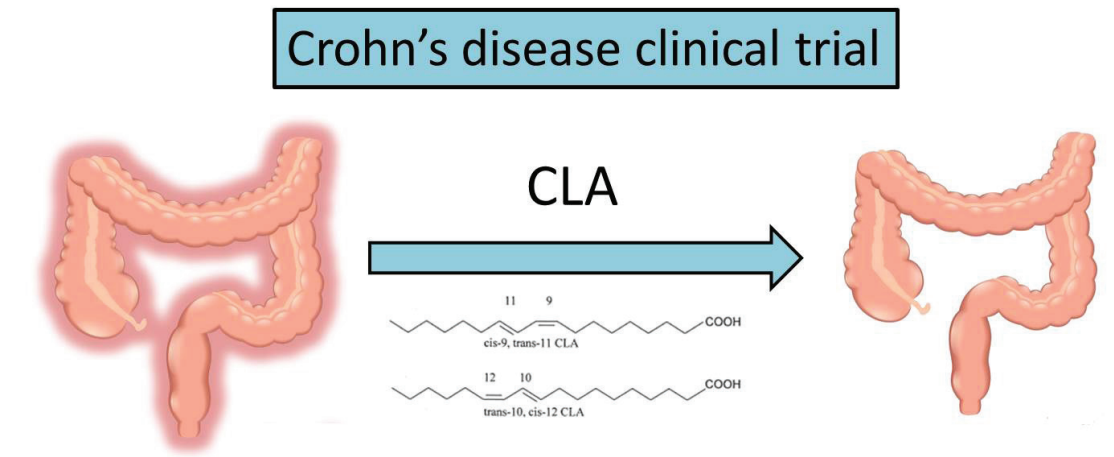


Figure 21. Crohn's Disease Activity Index (CDAI) at baseline, 6 and 12 weeks



Conjugated linoleic acid

CLA is a naturally occurring fatty acid found in meat and dairy products known for its anti-cancer, immune modulatory properties and its demonstrated anti-inflammatory efficacy in mice and pig models of colitis. The aim of this project was to investigate the ability of CLA to ameliorate inflammation and gut health in patients with Crohn's Disease.

Figure 23. Oral administration of CLA ameliorates disease symptoms

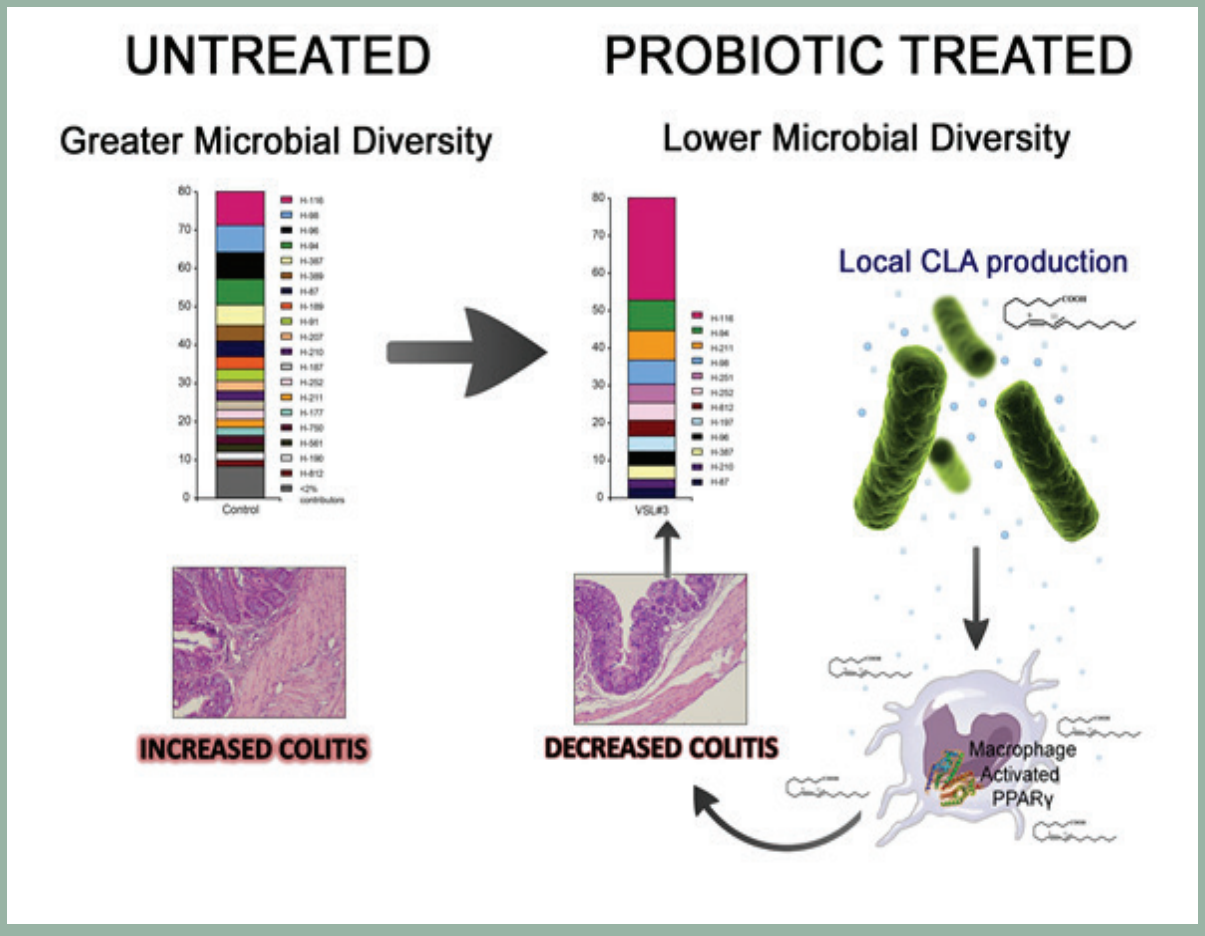


Figure 24. Mechanisms of modulation of microbial diversity by probiotics

increase in Inflammatory Bowel Disease Questionnaire (IBDQ) regarding quality of life from 141 to 165 (p=0.017) on week 12 (Fig. 22). Moreover, CLA significantly suppressed the ability of peripheral blood CD4+ and CD8+ T cell subsets to produce pro-inflammatory cytokines including IFN-γ, TNF-α and IL-17 and to proliferate at week 12.

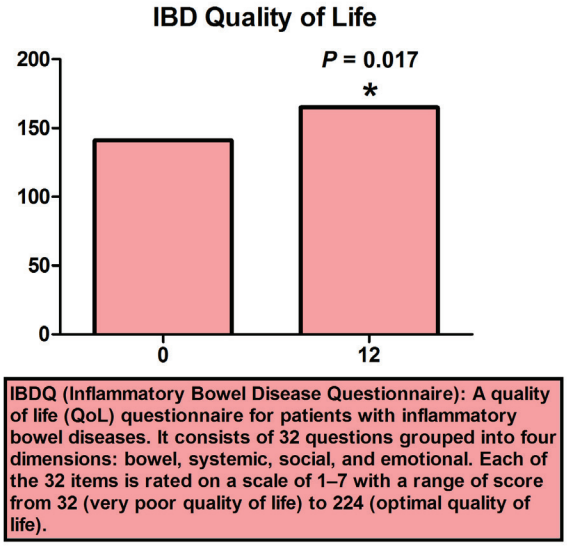


Figure 22. Quality of life scores at baseline and week 12 of CLA treatment

In summary, CLA represents a promising new supportive intervention for gut inflammation (Fig. 23). This is in contrast with the results of human clinical studies using n-3 polyunsaturated fatty acids in IBD that remain largely unimpressive. The present study has shed new light on the clinical potential of this compound and provided insights on the possible mechanisms of immune modulation targeted by CLA in the human system. Based on these results, a larger Phase II double-blind, placebo-controlled, randomized trial with several doses of CLA is warranted.

In a related project we investigated the beneficial effects of probiotic administration in DSS-induced colitis in mice. Probiotics are live microbial supplements which beneficially impact on host health. These products rely on introducing particular exogenous bacterial strains into the intestinal microflora. Several probiotics have been shown to be efficacious in the treatment of IBD, specially the commercially available mixture VSL#3, the *Escherichia coli* strain Nissle 1917 and several *Lactobacillus* species. VSL#3 mixture has demonstrated efficacy in patients with ulcerative colitis and in animal models of colitis and is claimed to regulate intestinal microbial balance as well as suppress gut inflammation by up-regulating anti-inflammatory cytokines. However, there is a lack of comprehensive understanding of the mechanisms of action underlying the protective effects of VSL#3 and probiotics in general. In this regard, we aimed to investigate the mechanisms of immunoregulation of gut probiotic bacteria in mice by focusing on their ability to produce anti-inflammatory metabolites and influence mucosal immune responses. As a result, we demonstrated that gut probiotic bacteria contained in the VSL#3 mixture can produce CLA locally in the gut where it contributes to modulating the composition of gut bacteria and decrease infiltration of inflammatory cells such as macrophages in the intestinal wall of mice with IBD and inflammation-associated colorectal cancer. Thus, changes in microbial diversity and local CLA production are implicated in PPARγ-dependent mechanisms of action underlying the anti-inflammatory and anti-carcinogenic effects of probiotic bacteria (Fig. 24).

This novel mechanistic model is supported by: results of loss-of-function analyses illustrating the requirement of macrophage PPARγ in mediating the full spectrum of anti-inflammatory effects of probiotic bacteria in the gut; *in vivo* evidence indicating a reduction of colonic bacterial diversity with a marked predominance of certain commensal strains and local CLA production in colons of probiotic-treated mice; and remarkable similarities in the ability of probiotic bacteria and CLA to modulate macrophage function at the gut mucosa.

Financial Objectives and Performance

NIMML Nutritional Immunology & Molecular Medicine Laboratory

HIGHLIGHTS

Over $12M in extramural funding over the last 5 years

Generating over $1M of overhead annually

Center for Modeling Immunity to Enteric Pathogens is a $10.6M NIAID-funded program

Received $1.2M RO1 funding from NCCAM to study immune modulation by abscisic acid

Over 80 publications and 7 patents

Established a joint Gastroenterology research program in collaboration with Carillion Medical School

Pending grants exceeding $100M

NIMML

Education

Outreach

The NIMML is training 6 PhD students in the Genetics, Bioinformatics and Computational Biology program. The GBCB students in the NIMML receive training at the interface of immunology, bioinformatics and computational modeling and simulation of biological processes. The NIMML has over the last 5 years trained 10 PhD students, 40 undergraduate students and 9 post-doctoral associates.

The Interdisciplinary PhD Program in Genetics, Bioinformatics, and Computational Biology (GBCB) at Virginia Tech was established in 2003 witht he goal of providing an educational environment that enables students to apply quantitative methods in computer science, mathematics, and statistics to all areas of the life sciences. Course requirements enable students to take advanced courses in their primary area as well as gain a deeper understanding of the complementary fields of study providing the foundation from which students can combine these disciplines in their interdisciplinary research projects.

Nikki Lewis

Nikki Lewis, one of the PhD students that is being co-advised with Dr. Bevan in NIMML, received a NIH F31 award.

Adria Carbo

Adria Carbo, a second year PhD student in GBCB, received a first poster award in the MIB immune modeling Symposium and a travel award by University of Rochester School of Medicine for his modeling studies related to CD4+ T cell differentiation. He also received a travel award from the American Association of Immunologists (AAI) to present his research related to the role of CD4+ T cells during *H. pylori* infection.

Monica Viladomiu

Monica Viladomiu, a first year PhD student in GBCB, received a travel award from the American Association of Immunologists to present her research related to host responses to *Clostridium difficile* and new therapeutic development.

Casandra W. Philipson

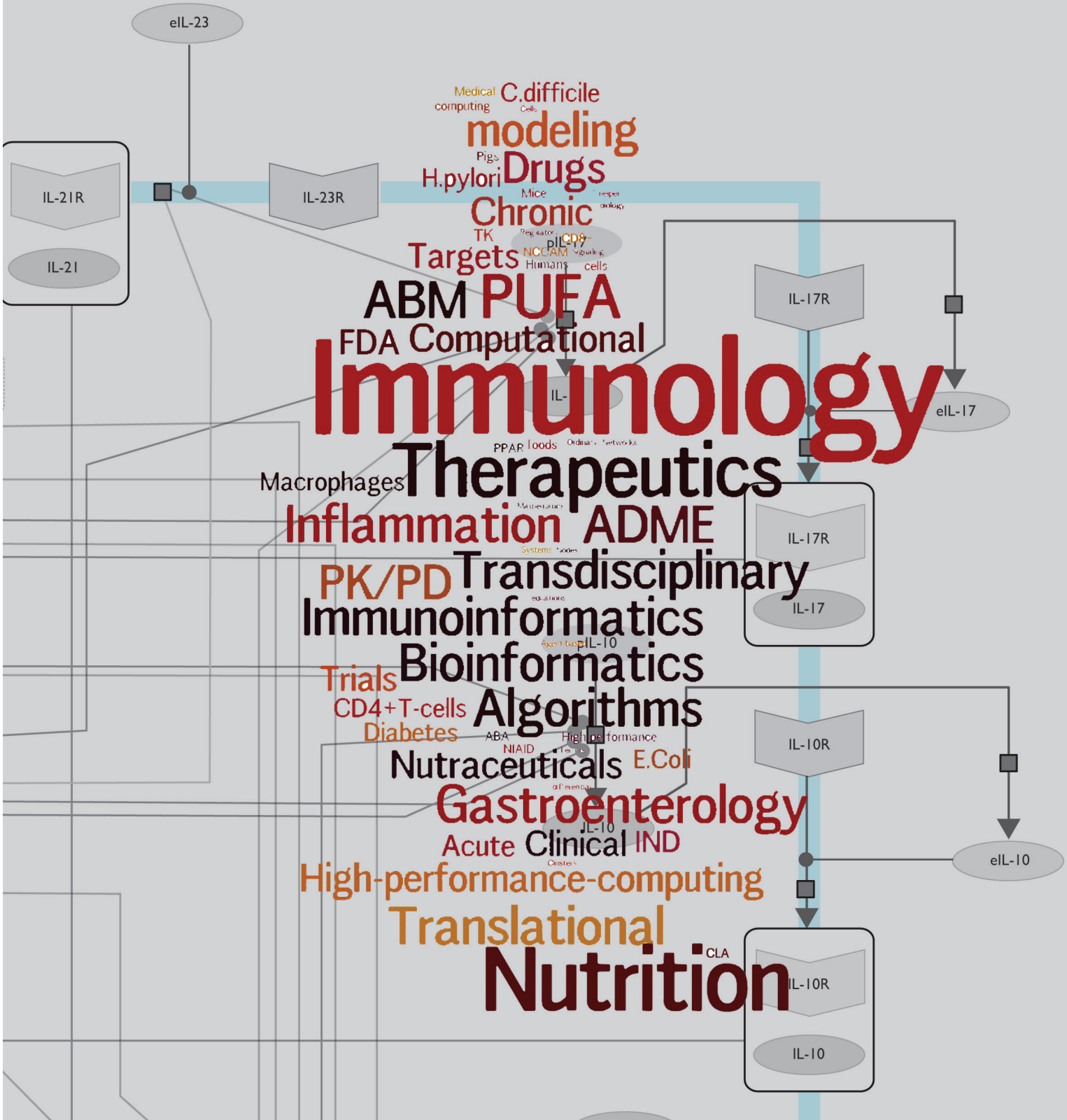
Casandra W. Philipson, a first year PhD student in GBCB, received a travel award from the American Association of Immunologists to present her research related to host responses to *E. coli* and new therapeutic development.

Pinyi Lu

Pinyi Lu, a third year PhD student in GBCB, has succesfully developed methods that discovered novel classes of anti-inflammatory drugs targeting LANCL2. He is also pursuing a master in Computer Science as well as the doctoral degree.

Mireia Pedragosa

Mireia Pedragosa, a first year PhD student in GBCB, is actively working on deciphering the contribution of different cell subsets activated after *H. pylori* infection and how anti-inflammatory mediators could ameliorate the process of infection.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Trials
CD4+T-cells
Algorithms
Diabetes
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Nutraceuticals
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