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Clinic to Bench

Optimal phosphorus management technologies on dairy farms

Phosphorus (P) management is a national issue with respect to water quality and the sustainability of animal agriculture. A major problem facing dairy farms is the surplus of phosphorus resulting from the excess quantity of the element in feeds and minerals. The cow can only utilize a small fraction of the nutrient, excess phosphorus is excreted in manure. It accumulates in soils, increasing the potential for phosphorus losses in runoff, which in turn contributes to accelerated water quality deterioration.

The traditional approach to reduce phosphorus problems is focused on the management of manure at the end of the production cycle, i.e. after manure is excreted. A more efficient and cost-effective approach of managing phosphorus on dairy farms is to eliminate excess of it in diets with optimal nutrient balances in the rations. This front-end approach minimizes phosphorus excretion in manure without impairing cow performance and farm profitability. This approach saves feed cost for the farmer.

In fact, phosphorus is an essential nutrient, needed by all plants and living species. The problem is that in many areas soils already have phosphorus buildup to levels far exceeding crop needs. Manure added as fertilizer compounds the problem. Run-offs from these high-phosphorus soils to rivers, streams, and the Chesapeake Bay impede the water quality and interfere with balance of plant and marine life in the watershed.

To help dairy farmers better manage phosphorus for enhanced farm profitability and environmental quality, a multi-state, multi-disciplinary project ($1,797,000 for year 2002-2005) supported by the USDA-IFAFS Program was recently initiated to develop optimal phosphorus management technologies on dairy farms. The long-term goal is to develop and deliver practical, scientifically sound, and economically viable phosphorus source control measures and management tools that sustain dairy farming and protect the environment.

Penn is the lead institution with **Dr. Zhengxia Dou** as the project director and **Dr. James Ferguson** the co-director. Researchers from five other institutions are collaborating on the project. They visit farms in their areas and collect samples, then send farm information and samples to Penn for processing and analyses. Drs. Dou and Ferguson are in charge of project planning, monitoring, implementation, evaluation, supervising, and reporting findings to USDA. They are also working with 20 dairy farms in Southeast Pennsylvania and Delaware to collect farm information and samples, just like the collaborators do for this project.

The specific objectives of the project are:

(A) Determine the dietary P range adequate for optimal cow performance but not in excess of animal needs by combining farm data across five states (PA, NY, DE, MD, VA) with research findings.

(B) Develop easy-to-use management tools including a fecal P testing procedure for assessing diet P adequacy vs. overfeeding and a modified ration formulation software program for balancing diet P and nitrogen.

(C) Establish quantitative relationships between dairy diets, fecal P, and P loss in runoff through laboratory and field-scale experiments.

(D) Provide training and education to veterinarians, nutritionists, producers, nutrient management personnel.

(E) Prepare a "white paper" on best management practices for P in dairy operations and present it to state/regional nutrient management commissions.

The five states participating in the project make up much of the Chesapeake Bay watershed.

The results of the study will be disseminated rapidly through existing multiple outreach channels. Project findings will be equally applicable on small, medium, or large farms. Project impact will be large-scale and long-lasting beyond the project period.

The principal investigators are **Dr. Zhengzia Dou** and **Dr. James D. Ferguson** at the School. The collaborating scientists are: Dr. L.E. Chase, Cornell University, Dr. K.F. Knowlton, Virginia Polytechnic Institute, Dr. R.A. Kohn, University of Maryland, Dr. J.T. Sims, University of Delaware, Dr. Z. Wu, Penn State University.

Clinic to bench

Christopher Hunter's lab on the second floor of Rosenthal is overflowing with equipment and people. In the broadest sense, the work here focuses on the role of cytokines in triggering immune responses. Cytokines, soluble messenger proteins, tell cells when to mount an immune response or when to stop such response. Cytokines are indispensable for the ability to fight diseases.

A major aspect of the basic research work here involves *Toxoplasma gondii*, a protozoan parasite that infects most warmblooded animals, including birds and man. Cats, domestic and wild, are the only known definitive host of the organism and serve as the main reservoir. Toxoplasmosis occurs world-wide. It is a major concern for pregnant women because the disease causes birth defects in fetuses. The disease is also a major concern for people with immune system dysfunction; here it causes meningoencephalitis.

Hunter's laboratory is home not only to bench scientists but also to clinicians who are pursuing basic research to enhance their clinical work. **Dr. Lillian Aronson, V'92**, assistant professor of surgery and head of the feline kidney transplant program at VHUP, and **Dr. Nicola Mason**, on leave from the section of medicine, and in the process of earning a Ph.D., are both working with Dr. Hunter's group to learn more about the immune complex.

Dr. Aronson came to the lab to evaluate the effects of a drug CTLA4-Ig on feline lymphocyte function. The theory behind this drug is that it is more specific in its mechanism of action, i.e. it can hopefully still prevent rejection (suppress T lymphocytes that are specifically involved with rejection), but also allow a patient to fight off an infection (not have an affect on memory T cells).

Some of her patients, after a kidney transplant when they received regular doses of cyclosporine and prednisolone as immunosuppressants to prevent rejection of the new kidney, suddenly developed acute generalized toxoplasmosis (because of a reactivation of a latent

How are viruses released from a cell?

Basic research projects often involve narrow, focused aspects of basic processes. One such piece is the process by which certain viruses move from one cell to then spread and infect additional cells.

To replicate, viruses turn host cells into "virus factories." The new viruses then move on to infect other cells and the whole process begins again. The mechanism that allows a virus to be released efficiently from one cell and move to another is the main interest of **Ronald Harty's** laboratory at the School.

Viruses are released from a cell though a process called budding. The virus pushes the lipid membrane of the cell outward so that it enfolds the virus. This budding virus separates from the cell and the new virus particle is ready to infect the next cell.

The mechanism that governs this process is not well understood and scientists have been trying to understand this process of budding as it could help in combating viral diseases. Dr. Harty, assistant professor of microbiology, and his colleagues, two years ago identified a sequence of amino acids that is instrumental in the movement of Ebola virus out of a cell. "We discovered a short sequence of four amino acids contained in the matrix protein of Ebola virus that is instrumental to budding," says Dr. Harty. "If this sequence or motif, as it is called, is changed, the budding process slows down significantly."

This motif not only is present in the matrix protein of the Ebola virus, but also in the matrix proteins of vesicular stomatitis virus (VSV) and HIV. All three viruses are RNA viruses with different characteristics and they represent three different virus families: Ebola is a filovirus, one of the biggest viruses; VSV is rhabdovirus, this family includes rabies; HIV is a retrovirus. The motif is interchangeable between these different virus groups, such that the motif from one initiates budding in another group when implanted in that matrix protein.

"We have found that budding occurs if we move the motif from one place to another on the matrix protein," says Harty. "The protein by itself also causes budding. It is thought that the motif interacts with specific cellular proteins for budding to occur. We are trying to identify these cellular proteins. Because budding doesn't completely cease if the amino acid sequence of the motif is changed, we know other interactions are involved in the process."

Most of the work in the laboratory occurs with VSV, a RNA virus with an RNA genome of about 11,000 bases, considerably smaller than the Ebola virus genome of 19,000 bases. VSV, a reportable disease, afflicts cattle and equines. Symptoms in cattle resemble foot and mouth disease and animals must be quarantined until an accurate diagnosis is made. "Although we work with VSV, we do not work with actual Ebola virus, just with DNA plasmids that make the proteins we are studying," says Harty. "These plasmids are just pieces of DNA that are not infectious. We could not work with Ebola virus or any other lethal virus as that requires the highest grade secure laboratory which we do not have."

The members of Dr. Harty's laboratory working on the Ebola virus project are **Jill Licata**, Ph.D. student and **Dr. Ziying Han**, a post doc. Recently, Dr. Harty and **Dr. Bruce Freedman, V'87**, assistant professor of pathobiology, received a NIH grant to examine Ebola virus proteins that may have channel activities in the cell during budding. These may well be another piece needed in the budding process. It may bring scientists closer to a means that can prevent the spread of virus from cell to cell. Dr. Harty's research on the amino acid motifs is also supported by a second NIH grant.

infection; 30-40% of cats in the NE are carriers for the infection) that they could not overcome because they were immunocompromised.

"Right now we give cyclosporine and prednisolone to our transplant patients to prevent rejection of the new kidney," explains Aronson. "Unfortunately, long term use of these immune system suppressing drugs can make a patient more susceptible to infection and cancer. This has been seen in people and we have also seen it in our transplant patients."

The new drug, CTLA4-Ig, is being used in clinical trials in human transplant patients. It is hoped that it does not have the long-term effects of the drugs currently used. "The drug has been shown to prevent rejection in many research models. Hopefully, its more specific mechanism of action will allow a patient to respond to an infection as well." says Aronson. "We are now investigating the drug in vitro to determine its effect on feline lymphocytes, a group of infection-fighting cells. It appears that the drug suppresses feline lymphocyte proliferation, but allows some cells to remain intact. Some of the cells that remain intact appear to be cells (memory cells) that have previously seen the infectious agent and are present to respond to the infectious agent again. Eventually, we have to determine if the degree of immunosupression seen in vitro using CTLA4-Ig is enough to prevent rejection in an in vivo model."

Aronson hopes the drug will provide an alternative to cyclosporine and prednisolone so that her feline transplant recipients remain able to suppress a latent toxoplasmosis infection and fight of any other infections.

Mason is looking at another factor involved in the immune response to *Toxoplasma gondii*. In Hunter's lab, she is studying the role of c-Rel, a gene that controls the production of the cytokines interferon gamma (IFN-g) and Interleukin 12 (IL-12), both essential for resistance to toxoplasma infections.

"The cytokines are important in resistance to toxoplasmosis," says Mason. "We are studying the immune response to toxoplasma in mice that lack the c-Rel gene. When these mice are infected with *Toxoplasma gondii*, their resistance to the disease is very low. They have normal levels of IL-12 and reduced levels of IFN-g early on during infection. This may allow the parasite to establish itself in the mouse and result in a reduced survival time compared to wild type mice."

To know the role the gene c-Rel plays in the immune response is important as this gene is also involved in producing resistance to Leishmania, another protozoan disease that is devasting to people and animals, and to viral infections. Once this role is completely understood, scientists may be able to develop treatments that reinforce c-Rel and help people and animals overcome these infectious diseases.

Aronson and Mason are just two clinicians at the School who are taking advantage of the proximity of the basic scientists to the clinical facilities; there are many more clinicians, at VHUP and at New Bolton Center, who work closely with bench scientists, incorporating basic science findings into clinical applications.