

Outsmarting Johne's Disease

PROJECT TITLE Pathogenesis and Control of *Mycobacterium avium* subspecies paratuberculosis (6.21)



PROJECT LEADER *Dr. Andy Potter; Vaccine and Infectious Disease Organization (VIDO)*

Background

Mycobacterium avium subspecies paratuberculosis (MAP) causes Johne's disease in cattle. Some researchers think that MAP may also play a role in human Crohn's disease.

Johne's disease is difficult to accurately diagnose, current vaccines are unreliable and no effective treatments have been developed. One major challenge to developing good diagnostic, vaccination or treatment strategies is that MAP somehow prevents the animal's immune system from fully responding to the MAP infection. A better understanding of how MAP tricks the immune system when it infects cattle will help develop better vaccine, diagnostic and treatment methods for Johne's disease.

This study focused on "protein kinases", which are enzymes that add a phosphate group to other proteins. Phosphorylation changes the activity of the target protein, so kinases have very important roles in regulating cellular pathways involved in animal health and disease. Kinases are found in all living things. For example, MAP releases cattle-like kinases when it infects white blood cells. This may play a role in helping MAP undermine the bovine immune response. All the different kinases are collectively called the "kinome". Examining changes in the kinome that occur when animals are first infected with MAP may help to determine how MAP tricks the immune system.

Objectives

1. To determine the kinomic response of white blood cells in the small intestine and blood to infection with MAP;
2. the kinomic response of calves to infection with MAP using an intestinal loop;
3. the ability of various immunomodulators to activate macrophages for MAP killing; and
4. if local immunization results in protection against MAP.

What they did

These researchers used an innovative "gut loop" technique that allows them to isolate small segments of the intestine. As a result, the same experimental animal can be both infected and uninfected at the same time. This removes animal-to-animal variability so that any differences



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in the kinomes between infected and uninfected loops are due to the MAP infection.

Numerous gut loops were prepared in eight calves; in each calf, some gut loops were infected with MAP and others were not infected. Samples were collected at 30 minutes and 1, 3 and 24 hours after infection. These samples were examined to compare changes in the phosphorylation activities and patterns in the enzymes and proteins produced by white blood cells in infected and uninfected loops.

Understanding how MAP disguises itself from the immune system will help develop effective vaccines, diagnostic and treatment methods against Johne's disease in cattle.

What they learned

The researchers discovered at least 15 amino acids in at least nine proteins that had a different phosphorylation status between infected and uninfected gut loops. These differences appeared less than a day after the gut loops were infected with MAP. The researchers then identified six kinase genes and three phosphatase genes in the MAP DNA that may have caused these differences. The kinase and phosphatase proteins encoded by these MAP genes are being studied further to determine whether they may make good targets for a MAP-specific vaccine.

A MAP vaccine will only be effective if MAP's ability to hide from the immune system is overcome. Tiny pieces of DNA known as CpG oligodeoxynucleotides (CpG ODNs) and small proteins known as host defense peptides (HDP) are two types of "immunomodulator" molecules that can greatly amplify an animal's immune response. These researchers formulated and patented an HDP/CpG ODN mixture that promoted effective clearance of MAP from infected cattle white blood cells. Ongoing research is studying potential vaccine formulations and will determine whether these compounds may also help eliminate MAP from cattle, opening the door for potential treatments for Johne's disease.

What it means

This knowledge about what happens when MAP infects cattle brings us closer to understanding how MAP tricks the immune system and will aid efforts to develop better Johne's disease vaccine, diagnostic and treatment methods.

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CANADIAN CATTLEMEN'S ASSOCIATION

#310, 6715 - 8th St. NE, Calgary, AB T2E 7H7

Tel: (403) 275-8558 Fax: (403) 274-5686