Building a Healthier Dairy Cow Using Functional Genomics

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New knowledge is being revealed that may provide additional strategies for the dairy producers to fight mastitis. Many livestock producers would say that one of the most important events on the farm is the birth of the next generation. A profitable and viable operation depends on the success of this event both for the cow and the calf. In the case of the dairy cow, a successful parturition, or calving, is linked to a healthy and productive lactation. At calving, cows often face health challenges, including mastitis. The question is, "Why?" Why are cows more susceptible to mastitis and other such inflammatory diseases at this critical time in the production cycle? Dr. Jeanne Burton and the research group in the Immunogenetics Laboratory at Michigan State University set out to answer this question using an exciting new tool for scientific investigation called functional genomics. Functional genomics is a way to explore production and health traits at the gene level. It links changes in the way genes are expressed under different environmental conditions to the actual cell functions such as milk production, muscle growth, or immune response, for example. Outside circumstances, like parturition and stress, can send signals to cells to turn genes on or off and change functions or processes within cells. Functional genomics promises to solve some of the puzzles surrounding mastitis through a better understanding of how parturition impacts a cow's immune cells.

Why more mastitis at calving?

Years ago, scientists began to hypothesize that cows were getting mastitis around calving because something in the parturition process was interfering with the way the cow's immune system worked. This interference was potentially allowing opportunistic bacteria to take over without a fight. Many processes in the cow's body change to support a growing calf and safely expel it from the body at birth. As calving approaches, blood levels of the steroid hormones progesterone, estrogen, and cortisol rapidly shift up or down. Dr. Burton and others have shown that some cells of the immune system complex respond to these hormonal changes, altering how they function and then failing to protect the cow against mastitis-causing bacteria.

A cow's immune system and how it responds.

The cow's immune system is made up of two components that continually work together to ward off invading pathogens: the innate and the acquired. Innate immunity exists from birth and is comprised of white blood cells called **phagocytes** that continually move throughout the body, searching for invading pathogens to kill. However, the innate system has only a limited ability. If an infection becomes established, additional help from the acquired immune system is needed. Acquired immunity is developed throughout the life of the cow in response to immune challenges from various pathogens present in the animal's environment. The acquired component becomes very specific to each pathogen encountered so that the body can easily recognize that same pathogen if it shows up in the future. It is comprised of other white blood cells called **lymphocytes**. These cells respond to new pathogens by forming antibodies against them or by signaling phagocytes of the innate system to locate and kill the invaders. Some lymphocytes can also specifically target and kill virus-infected cells.

The phagocytes of the innate immune system that form the first line of defense in response to invading bacteria are the **neutrophils**. Billions of neutrophils are continually being produced and released by the bone marrow each day. They roll along the walls of blood vessels, searching for signals of infection that tissues will send out if bacteria or other pathogens are present. Once neutrophils detect these signals, they migrate from the blood vessel into an infected tissue to seek out and kill the bacteria (Figure 1). In fact, the increased somatic cell count (SCC) witnessed during a mastitis challenge is the increase of neutrophils migrating from the bloodstream into milk to fight off the bacteria.

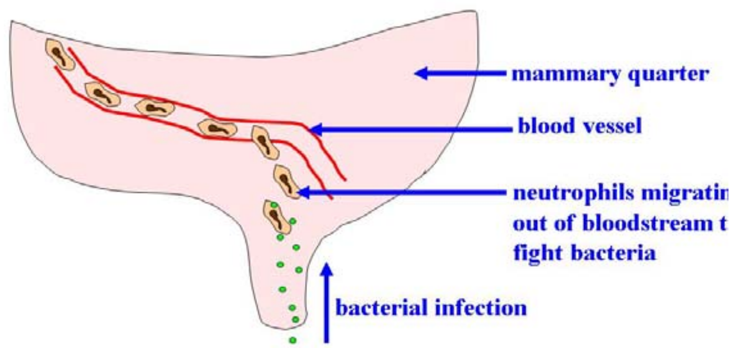

Figure 1. Normal functions of neutrophils. Neutrophils roll along blood vessel walls until they meet a signal of infection. They then migrate into the mammary gland to phagocytose and kill bacteria. Neutrophils die by apoptosis in a few hours after being released from the bone marrow.

Neutrophils actually "eat" bacteria, engulfing them by a process called phagocytosis and then breaking them into pieces using enzymes and other chemicals. Since neutrophils contain harmful enzymes and chemicals used to kill bacteria, they live a short life of 6 to 12 hours. Then they undergo a programmed cell death called apoptosis. Apoptosis prevents the release of the dangerous chemicals into the mammary gland or other tissue, thus preventing further damage in addition to what the original infection has already caused.

What's changing at calving?

Many scientists have previously shown that several of these normal neutrophil functions do not work properly at parturition. For example, surface proteins that help neutrophils migrate into infected tissues disappear. As a result, the neutrophils cannot move from the bloodstream into the infected tissue. In addition, neutrophils do not produce as much of the chemicals that kill bacteria after they have engulfed them. Scientists have also shown that the genes controlling these functions are being regulated by signals in the body and are being turned on or off to create this depressed immune function.

Dr. Burton's group has previously shown that several of these cell functions are altered by the extreme spike in cortisol that occurs right at calving. Cortisol is the main stress hormone that increases during the "fight or flight" response and is also necessary to initiate parturition. Neutrophils possess receptors that attach to this hormone and directly respond to it by changing their gene expression and behavior when it is present in high levels in the blood. This appears to result in a down regulation of some of the neutrophil's bacteria-fighting behaviors. So why would a cow's body want to shut down its innate immune system at a time of high stress, by making its first responder cell, the neutrophil, dysfunctional?

A broad method that could provide many clues or answers simultaneously was needed to investigate what is going on in the immune system at calving. This is where functional genomics comes in. Technology such as microarray analysis used in functional genomics allows researchers to look at the expression of thousands of genes at once, comparing genes in cells or tissues between certain times or treatments to see what genes are turned on or off. For example, liver cells from cows on two different types of feed can be compared, or genes expressed in cells from a diseased cow can be compared to those of a healthy cow. Most often, these microarray experiments are referred to as "fishing missions," since it is rarely known exactly what will turn up. But researchers always hope to catch something big by identifying genes controlling the function of pathways of interest. During the course of their efforts on parturient cow neutrophils, Dr. Burton's group hooked a number of big fish.

In their experiment, neutrophils were first isolated from blood collected from cows before, during, and after parturition. **RNA** (ribonucleic acid) extracted from samples of these cells were then compared for the three situations on microarrays. Microarrays are glass slides spotted with **DNA** (deoxyribonucleic acid) sequences representing thousands of genes expressed in cells of cows. Many of the genes spotted on the microarrays are needed by white blood cells to survive and respond to infection. Dr. Burton's group detected hundreds of genes that had been significantly affected by parturition, i.e., turned on or off. Changes were happening in the immune system all the way down to the DNA level, causing functional changes in cells. Once Dr. Burton's team had searched existing literature and studied the affected genes that were changing, some pieces of the puzzle began to come together. Genes that controlled apoptosis, or cell death, were shifting their expression so that the neutrophils lived much longer than normal, up to 24 to 48 hours. Also, many genes needed for migration of neutrophils from the blood into infected tissue and for killing bacteria were down-regulated at and after parturition. However, genes that create the powerful enzymes that degrade and break up tissue proteins were up-regulated at parturition. Dr. Burton's group began to hypothesize that neutrophils must have other functions besides their traditional immune function of killing bacteria. They could be needed in high numbers in a cow's reproductive system to produce enzymes that break up tough proteins

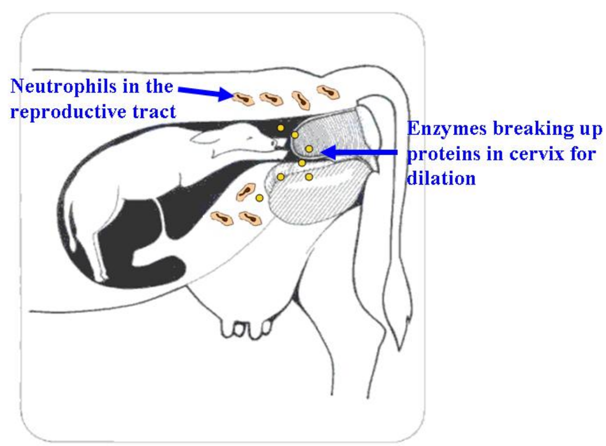

Figure 2. Neutrophils migrate to the cow's reproductive tract at the onset of parturition to secrete enzymes that break the water bag, help dilate the cervix, and release the placenta. They also help the uterus to return to its normal size and shape after labor.

to help in cervical dilation, breaking of the water bag, and release of the placenta after calving (Figure 2). Neutrophils may also help break up and clear extra cells in the expanded uterus so that it can return to its normal shape and size after the calf is born. These needs of reproduction may demand that the neutrophils live longer than normal to complete their new tasks of tissue remodeling. The cow's body tells the cells of the immune system that these jobs are more important right now than protecting it from invaders. However, this puts the cow more at risk for infection, especially by opportunistic bacteria that cause mastitis. While the neutrophils are distracted by the reproductive tract, the bacteria in the udder would have little competition and can easily multiply and take over, causing serious infections.

While this may sound like a negative scenario, it holds many promising opportunities for future development of strategies to control mastitis at calving. With this new understanding of changes in gene expression in neutrophils at parturition, researchers can begin to target affected genes through development of new drugs, feed supplements, hormone regulation, and even genetic selection to improve cows' health around parturition. Dr. Burton's collaborators at USDA laboratories in Beltsville, Maryland and Clay Center, Nebraska are already investigating many of these known genes to identify genetic markers that will enable the selection of cows with superior genotypes for mammary health and reproduction.

Dr. Burton's group has concluded that depressed immune function at calving may be necessary for the successful birth of the calf and remodeling of the reproductive tract. However, their research has also provided excellent therapeutic drug targets for further studies. Producers should have real options in the future to help cows recover quickly after calving and overcome this brief period of increased susceptibility to mastitis and other diseases. This will lead to less disease and stress at calving and more productive lactations.

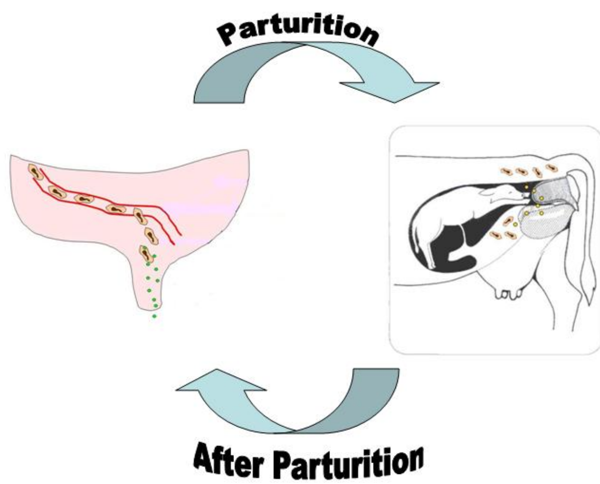

Figure 3. A shift occurs in neutrophil function in parturient cows as neutrophils move away from killing bacteria and toward remodeling the reproductive tract for the birth of the calf. This scenario eventually shifts back toward fighting against infection but leaves a window of depressed immune function and susceptibility to disease.

In the mean time, producers should acknowledge this window of disease susceptibility and minimize the exposure of cows to pathogens as much as possible by using appropriate management strategies. Excellent nutrition, clean bedding, proper udder care, and a good vaccination program are practices that most producers have probably already considered but should stress even more at calving time.