Mastitis is a costly disease in dairy production. Coliforms such as *Escherichia coli* and *Klebsiella* that are ever-present in a cow’s environment can cause severe mastitis in fresh cows. Coliform infections normally cause a short-lived inflammation of the mammary gland that we observe as a temporary increase in milk somatic cell count. This increase reflects the beneficial movement of white blood cells, called neutrophils, into the udder to fight the infection. Within 12 to 24 hours, the inflammatory response ends and milk somatic cell count returns to normal because the neutrophils clear the infection. However, this beneficial neutrophil response may become harmful to the cow around the time of calving.

**The role of neutrophils**

In fresh cows, coliform mastitis can become so severe that the life of the animal is threatened. In this severe form of the disease, bacterial toxin (endotoxin) from the infected mammary gland escapes into the blood stream causing cows to go off feed and to stop milking. It also causes inflammation to occur throughout the cow’s body, leading to organ failure and shock. Because the onset of severe coliform mastitis is so rapid, there are no obvious clinical signs until after peak bacterial growth and endotoxin exposure have occurred. By that time, udder inflammation and shock are so massive as to leave few options for successful intervention. Thus, rapid death (or euthanasia) is a typical scenario for affected cows (8).

The question our research is trying to address is why fresh cows have an increased risk for the severe form of coliform mastitis. We designed experiments around two facts: neutrophils are always recruited from blood in response to coliform infection and there is a release of protein degrading enzymes called matrix metalloproteinases (MMPs) by the recruited neutrophils. Although the neutrophils are needed for clearing the infection, their MMPs can cause serious tissue damage. This is because MMPs degrade the blood-tissue barrier (Figure 1) and promote local tissue softening and fluidity so the neutrophils can move around easily to find and kill infecting pathogens.

There are 24 known MMPs. Together, this family of enzymes is responsible for the degradation and turnover of tissue extracellular matrix, which is the scaffolding of structural proteins (such as collagen) that hold tissues together and give them proper structure, strength, elasticity, and function.
Most MMPs are not expressed in normal healthy tissue. However, these proteases are always present in infected and inflamed tissues. Neutrophils contribute significantly to the MMP content of inflamed tissue by producing and releasing large amounts of two family members, MMP-8 and MMP-9. The activities of these neutrophil MMPs are normally kept in check by natural inhibitor molecules (called tissue inhibitors of metalloproteinases, or TIMPs) to avoid excessive tissue damage while neutrophils do their normal bacteria-fighting job. However, human research has shown that during certain inflammatory diseases in which neutrophils move into the damaged tissue, such as lung infections, arthritis, vascular disease, invasive cancers, the ratio of MMPs to TIMPs becomes dramatically elevated and, if left uncontrolled, leads to massive tissue damage with loss of function and even death. Given this, it is not surprising that medical researchers on the search for novel anti-inflammatory drugs are looking towards agents that inhibit overactive MMPs or stimulate the expression of TIMPs (5). Is it possible that this may be a good approach for controlling severe coliform mastitis in fresh cows?

Results

Results from our recent studies suggest that the answer might be yes! We employed functional genomics tools developed at the MSU Center for Animal Functional Genomics (1, 4, 7) to study the expression of hundreds of genes in blood neutrophils of cows transitioning from the dry period through calving and early lactation. We did this to get a broad view of what state the neutrophils are in during the time of highest risk for severe coliform mastitis. What we observed was a tremendous increase in neutrophil counts and expression of MMP-8 and MMP-9 during labor, delivery, and 1 to 2 days after calving (2). In addition, there was inhibited expression of TIMP genes and genes that normally keep TIMP expression at normal levels during this period. Furthermore, we could easily detect dramatically heightened MMP activity in the blood serum of these cows, suggesting that neutrophils become activated into a highly pro-inflammatory state with increased tissue destroying capacity around calving. But why does this occur and what could it mean for mammary gland health?

Continuing research

We are conducting further studies to address these questions with the current thinking that calving itself may be similar to a massive inflammatory response, requiring highly activated neutrophils to help prepare the reproductive tract tissues for delivery of the calf. For example, the cow’s cervix must dilate from less than one inch to greater than 24 inches in a matter of 2 to 4 hours during labor if a cow is to calve without difficulty. Neutrophils are excellent candidates to help carry out this process. For example, the cells can be massively recruited into the cervix from blood as their circulating numbers increase during labor. Also, these recruited neutrophils can produce and release their massive stores of MMP-8 and MMP-9 to help soften the cervical tissue for rapid dilation. So, neutrophils in an exaggerated pro-inflammatory state at calving could be considered beneficial for the process of calving itself. However, a caveat may be that mammary quarters becoming infected with coliform bacteria around the time of calving would recruit these same hyperactive neutrophils with increased potential for tissue damage. If our current studies show this to be true, it could explain why severe coliform mastitis has been so difficult to manage and may lead to the development of new anti-inflammatory compounds that effectively treat or prevent severe coliform mastitis in fresh cows.

Conclusion

Our functional genomics research has led us to view bovine calving as a necessary inflammatory process that shifts the role of neutrophils but, in doing so, creates opportunity for increased damage to mammary quarters that become infected with mastitis-causing coliforms.
References


