The immune system is a dynamic, robust, and complex system whose purpose is to rid a host organism of pathogenic organisms or cancerous cells. In addition, cells in this system form physical barriers that prevent entry of pathogens and can secrete molecules with antimicrobial actions. Together this network of cells and molecules is in a precarious balance between action and inaction. This system is comprised of numerous cells and molecules whose lethality must be potent enough to clear dangerous organisms or cancerous cells, and yet specific enough to kill without extensive collateral damage to the host. In cases when the immune system is suppressed the host may be overcome by disease. In contrast, when the immune system is hyper-reactive the result may be anaphylaxis or autoimmune disease with equally lethal results. Understanding the immune system has helped in the development of therapies to boost the weakened immune system and suppress an over-active system. Research into the immune system has yielded a constant stream of new regulatory molecules and new functions to known regulatory molecules that help control the immune system. In addition, the characterization of immune cells is continually being redefined and refined into more specific functional groups, each with a specific role to play in a response. Though the picture of the immune system is becoming more and more complicated, this research is filling important gaps in our knowledge of how the immune system functions. This knowledge has given us clues into how we may therapeutically manipulate this system during times when the immune system is suppressed or unable to adequately clear the host of an invading pathogen.

Nutrition, stress and reproduction are examples of generalized events that can have a dramatic impact on the immune system. Previously it was thought that the effects of these general health issues only had an ancillary affect on immune function. However, with the elucidation of more cellular and molecular immune pathways these general health issues and their affects on the immune system have started to be defined at the molecular level. Feed components, such as vitamins, directly affect gene expression in immune cells, stress causes the release of steroids that affect expression of molecules responsible to immune cell trafficking, and the reproductive system seems to need immune cells to maintain pregnancy. Understanding how all these factors interact with the immune system will help in developing disease control and management strategies that will aid in maintaining good health in dairy cattle, resulting in greater production.

NUTRIENTS AND THE IMMUNE SYSTEM

The impact of nutrition on health is the subject of a significant body of research. This research has shown that nutrition can affect the ability of an animal’s immune system to fight a disease. This connection between nutrition and immune function has
been described at the cellular and even the molecular levels. A significant amount of research is being focused on the cellular and molecular processes affected by calcium and vitamins A and D. Calcium and vitamins A and D have been shown to have a significant effect on the functionality of immune cells.

Hypocalcemia

Several epidemiological studies have shown an association between a diagnosed metabolic disease and subsequent development of mastitis. One metabolic disease that has been associated with immune system disorder is hypocalcemia or milk fever. A study of over 2000 cows showed that cows with hypocalcemia were 8 times more likely to develop mastitis than cows with normal blood calcium levels (Curtis et al., 1983). Severe hypocalcemia leads to the loss of proper skeletal muscle control. Clinical hypocalcemia occurs in 5-7% of transition dairy cows. Additionally, subclinical hypocalcemia occurs in 25% of heifers and greater than 50% of second lactation cows (Dr Ronald L. Horst, personal communication). Contraction rate and strength of smooth muscle tissue have been shown to be directly related to the level calcium in the blood (Daniel, 1983). A current hypothesis is that even a sub-clinical hypocalcemic cow would have decreased muscle tone in the smooth muscle that makes up the teat sphincter, and that this loss of muscle tone would cause the teat canal to remain partially open thus exposing the mammary gland to environmental pathogens.

In addition to calcium's critical role in muscle function, it also plays an essential role in intracellular signaling. In immune cells, intracellular calcium regulates many cellular functions including cytokine production, cytokine receptor expression and cell proliferation. Recently it has been shown that stimulated peripheral mononuclear cells from hypocalcemic cows have a muted intracellular calcium response compared to cows with normal blood calcium levels. Furthermore, when stimulated peripheral mononuclear cells from hypocalcemic cows were compared with stimulated peripheral mononuclear cells obtained from the same cows after intravenous treatment with a calcium solution, a muted intracellular calcium response was demonstrated only when the animals were hypocalcemic (Kimura et al., 2006). A muted intracellular calcium response would have a significant effect on the functional capacity of the cells of the immune system.

Maintenance of proper blood calcium levels is critical for an animal's health. There are two effective means of preventing periparturient hypocalcemia, both of which are diets used in the weeks prior to calving. The first diet reduces calcium intake prior to calving. The theory to this approach is that a mild hypocalcemia prior to lactation will cause the animal's calcium transport machineries to up regulate, making the absorption of calcium more efficient (Green et al., 1981). Then as lactation begins and the demand for calcium rapidly increases, the capacity for calcium transport has already been increased. The second means of preventing periparturient hypocalcemia is the use of the Dietary Cation-Anion Difference (DCAD) diet. Researchers in the early 1970's showed that the use of anionic salts in the diet could prevent hypocalcemia in dairy cows (Ender et al., 1971; Dishington, 1975). Since that time numerous studies have
shown that adjustment of the cation-anion balance can reduce the incidence of hypocalcemia seen in periparturient cows (Block, 1984; Goff et al., 1991).

Vitamins

T cells are generally divided into two general categories, cytotoxic and helper. In turn helper T cells (T\textsubscript{H}) are subcategorized by the different cytokines they express. Each of the T\textsubscript{H} cell types focuses the immune response towards a specific type of pathogenic challenge (Reiner, 2007). A recent study has shown that retinoic acid can affect which T\textsubscript{H} cell types are generated. In addition to responding to different types of pathogens, the various T\textsubscript{H} cell types are also associated with pathologies such as autoimmune and allergy responses. For example, the T\textsubscript{H}17 cell type is thought to be important for the immune response to extracellular bacterial infections. However, T\textsubscript{H}17 cells are also associated with autoimmune diseases, such as inflammatory bowel syndrome (Reiner, 2007). The bacterial flora of the gastrointestinal tract provides a unique challenge to the immune system to not react against normal gut bacteria. Inflammatory bowel disease is thought to be an immune response against the normal gut bacteria. Unknown is what mechanism redirects the immune systems away from a reaction against resident gut bacteria. Part of the answer may be answered by the action of retinoic acid on mesenteric lymph node dendritic cells. In the presence of cytokines that drive T\textsubscript{H}17 maturation, fewer T\textsubscript{H}17 cells were obtained when they were stimulated by mesenteric derived DC compared to stimulation by splenic derived DC (Mucida et al., 2007). When retinoic acid is added, both splenic and mesenteric DC stimulation of T\textsubscript{H}17 cells are equally inhibited. When an inhibitor of vitamin A signaling is added to both splenic and mesenteric DC, they equally stimulate a large number of T\textsubscript{H}17 cells. Thus, vitamin A may be a critical component in the control of helper T-cell maturation in the gastrointestinal tract. Immune system dysfunction caused by a vitamin A deficiency may be explained by this mechanism (Mucida et al., 2007). Retinoic acid has also been shown to augment the inhibition of IFN-\gamma secretion by bovine lymphocytes caused by the addition of vitamin D (Ametaj et al., 2000). Therefore, dietary levels of vitamins A and D are important, especially as they may exacerbate immune dysfunction during the typical immunosuppression in the dairy cow seen around the time of calving.

It has long been recognized that vitamin D deficiency causes decreased resistance to infection (Rook, 1986; Reinhardt and Hustmyer, 1987), but this action was generally thought to be secondary to endocrine effects of vitamin D on calcium metabolism. More recently, vitamin D has been shown to have a direct autocrine effect on human immune cell functions. Thus, vitamin D affects the immune system through two pathways. First, the endocrine pathway affects serum calcium homeostasis. Cows generally suffer a decline in plasma 25-hydroxyvitamin D\textsubscript{3} [25(OH)D\textsubscript{3}] around the time of calving as the calcium needs of the cow are in flux due to the demands of milk production (Horst et al., 2005). This periparturient period has been shown to be a time of general immune suppression and leaves the animals susceptible to various diseases (Kashiwazaki et al., 1985; Oliver and Sordillo, 1988; Kehrli et al., 1989; Kehrli et al., 1990; Cai et al., 1994). Through an autocrine pathway, vitamin D analogs directly affect DNA gene expression.
of immune cells. This is accomplished when the immune cells take up serum 25(OH)D3 and convert it to 1,25-dihydroxyvitamin D3 \([1,25(\text{OH})_2\text{D3}]\), which in combination with a nuclear transcription factor (vitamin D Receptor), can bind to specific DNA sequences and affects expression of multiple genes. The autocrine pathway for immune cell regulation requires sufficient circulating 25(OH)D3 such that activated immune cells can produce their own 1,25(\text{OH})_2\text{D3} in their local environment at cell concentrations that activate key pathways that would not be activated by circulating endocrine produced 1,25(\text{OH})_2\text{D3}. Screening of human and mouse genomes revealed over 3,000 genes with a vitamin D response element to which 1,25(01-103, in combination with the vitamin D binding protein, affects gene expression (Wang et al., 2005), some of which are involved in immune cell regulation. Additionally, it was shown that stimulation of the TLR induces the 1 \(\alpha\)-hydroxylase enzyme that catalyzes the conversion of 25(OH)D3 to the active 1,25(\text{OH})_2\text{D3}. The production of 1,25(\text{OH})_2\text{D3} was, in turn, necessary for the induction of antibacterial genes, such as cathelicidin (Liu et al., 2006). It was further demonstrated that lower serum concentrations of the precursor 25(OH)D3 were correlated with decreased ability of monocytes to kill bacteria (Liu et al., 2006). Thus, stimulation of immune cells with a TLR ligand in the presence of 25(OH)D3 resulted in the gene expression of additional products important for the antimicrobial response, and the lack of sufficient level of 25(OH)D3 had a negative impact on the immune response. Use of 1,25(\text{OH})_2\text{D3} as an adjuvant has also been reported, and it was shown that treatment of cows with 1,25(\text{OH})_2\text{D3} along with the \textit{E. coli} J5 vaccine resulted in greater levels of antibodies against \textit{E. coli} J5 in milk and serum compared with \textit{E. coli} J5 vaccine alone (Reinhardt et al., 1999).

STRESS AND THE IMMUNE SYSTEM

The causes of stress in animals are as varied as its manifestation. Types of stress include heat, negative energy balance, transportation, pregnancy, and mixing of unfamiliar animals. Some ways that an animal will manifest stress is in the form of sickness and failure to thrive. Recently, these very general manifestations have begun to be defined on a cellular and molecular level. Various immune cells, such as neutrophils, T-cells, and dendritic cells, are affected when an animal is stressed, and expression of specific molecules, such as CD62L (L-selectin), is affected during stress (Burton and Kehrli, 1995; Burton et al., 1995; Burton et al., 2005).

The initiation of a stress response involves the activation of the hypothalamus, pituitary gland and the adrenal gland to release hormones such as cortisol, epinephrine and norepinephrine. This response is known to have a dramatic effect on the immune system. For example, chronic stress in pigs caused by mixing unfamiliar animals resulted in subordinate pigs having significantly fewer white blood cells compared to the dominant animals (Sutherland et al., 2006). Furthermore, it has been established that animals subjected to restraint stress fail to mount a normal immune response that can result in failure to mount a protective immune response subsequent to pathogen challenge (Anglen et al., 2003).
The molecular mechanisms that explain the effects of stress are a subject of current research. Several groups have used gene expression microarray analysis to determine the genes affected by stresses, such as thermal stress (Collier et al., 2006), food deprivation (Ollier et al., 2007), and treatment with stress hormone, such as cortisol (Burton and Kehrli, 1995; Weber et al., 2001; Burton et al., 2005). One of the most well studied molecular effects of stress on the immune system is the effect of cortisol on the expression of the protein CD62L, which is expressed on the surface of immune cells, such as neutrophils, and is necessary for the transmigration of the cell from the vasculature into the tissue at the site of an infection. Cortisol causes the loss of CD62L expression on neutrophils and, thus, the loss of the ability to migrate through the vascular endothelium. This loss of neutrophil response is correlative with increased susceptibility of the animal to mastitis (Burton et al., 1995).

**REPRODUCTION AND THE IMMUNE SYSTEM**

The immune system is significantly affected during pregnancy. There are significant interactions between the immune system and cells and tissues of the reproductive system that are critical for the maintenance of pregnancy, but are responsible for immune suppression that is associated with increased risk of disease.

Numerous studies have demonstrated reduced immune competence in the dairy cow around the time of calving. Facets of both the innate and acquired immune response are compromised, beginning about 1-2 wks before calving and recovering between 2 and 4 wks after calving (Kashiwazaki et al., 1985; Kehrli and Goff, 1989; Kehrli et al., 1989; Kehrli et al., 1990; Cai et al., 1994). Impaired neutrophil and lymphocyte functions during this periparturient period contribute to new infections leading to such diseases as mastitis and metritis (Oliver and Sordillo, 1988). Cows that have silently carried pathogenic bacteria, such as *Mycobacterium avium* subspecies paratuberculosis and *Salmonella* for many years will suddenly break with clinical disease within a short time after calving (Radostits et al., 1994), attributable to decreased integrity of innate and/or acquired immune cell functions that had kept these bacteria in check.

The Periparturient Period

Factors such as pregnancy, parturition, blood calcium levels, initiation of lactation and feed intake all affect the ability of the cow's immune system to effectively combat infections. The periparturient period is the time where these complex physiological changes occur simultaneously, having a significant effect on the animal's health. Numerous studies have demonstrated that the immune system of a dairy cow around the time of calving is suppressed. Beginning about 1 to 2 weeks before calving and lasting until between 2 and 4 weeks after calving, numerous immune functions have been shown to be inhibited during this time period (Kashiwazaki et al., 1985; Kehrli and Goff, 1989; Kehrli et al., 1989; Kehrli et al., 1990; Cai et al., 1994). Impaired immune cell functions during this periparturient period contribute to new infections leading to such diseases as mastitis and metritis (Oliver and Sordillo, 1988).
The Affect of Lactation on the Immune System

To study the affect of lactation on the immune system, normal dairy cows were compared to cows that had undergone a mastectomy. Specific immune cell (lymphocytes or neutrophil) function was assessed in the mastectomized animals and compared to normal animals. Studies showed that lymphocyte function was significantly different in mastectomized animals compared to normal animals during transition. This demonstrated that the depression of lymphocyte function during the periparturient period could largely be attributed to the metabolic demands of milk production. These observations fit nicely with the molecular observations described above showing hypocalcemia's effect on lymphocyte intracellular calcium transport. In contrast to the lymphocytes, neutrophils showed a decrease in function starting about two week prior to calving and reaching the low point at the time of calving in both mastectomized and normal cows. However, mastectomized cows quickly recovered neutrophil function (7 days), whereas, normal animals had not recovered neutrophil function after 20 days (Kimura et al., 1999). These data suggest that lactation plays a significant role in the recovery phase of periparturient immunosuppression. Importantly, the absence of the mammary gland did not affect the manifestation of periparturient immunosuppression but only affected the duration of the suppression after calving. Therefore, the calcium imbalances that typically affect the immune system are not the only causes of suppression of neutrophil function.

The Affect of Pregnancy on Immune Cell Functions

Studies have shown that pregnancy alters immune cell functionality. In fact, immune suppression has been long thought to be necessary in maintenance of the pregnancy and that break down of the immune suppression is one factor that may be involved in spontaneous abortions. Pregnancy is known to exacerbate some human autoimmune diseases (e.g. Systemic Lupus Erythematosus) and ameliorate other diseases (e.g. rheumatoid arthritis). The mechanisms for these alterations of immune cell function are unknown.

Polymorphonuclear neutrophils are a type of immune cell that is of great importance to the health of the dairy cow. The neutrophil is the first line of defense in those pathogens that causes the majority of disease that effect production in the dairy cow. Research has shown that pregnancy affects the functional capacity of neutrophils. Stimulated neutrophils from pregnant women showed significantly less respiratory burst activity (a primary pathogen killing mechanism) compared to a control group (Crouch et al., 1995). Similarly, two enzymes in the hexose monophosphate shunt that is part of the pathway that produces NADPH required for respiratory burst activity, have been shown to be localized to different subcellular areas in pregnant versus non-pregnant women (Kindzelskii et al., 2004). Because pregnancy can effect important functions of immune cells and because of the reproduction schedule used with dairy cattle, future research into the effects of pregnancy in dairy cattle are critical to the understanding of the animal's immune system. Recently the intracellular location of myeloperoxidase, an
enzyme critical to respiratory burst, was shown to be altered when a women was pregnant (Kindzelskii et al., 2006). It is unknown why this occurs and what affect it has on the pregnancy.

One example of the immune system's importance to reproduction is illustrated by the interaction between leukocytes and the corpus luteum (Pate and Landis Keyes, 2001). The corpus luteum is the remnant of the ovulatory follicle. Its function is to produce progesterone, which is essential for the maintenance of pregnancy. In the absence of an embryo, the corpus luteum regresses and this regression is initiated by uterine release of prostaglandin $F_{2\alpha}$. Regression of the corpus luteum will allow a new follicle to ovulate. Interestingly, both macrophages and T-cells are found in the corpus luteum. During luteal regression, the number of lymphocytes and macrophages in the tissue increases by both recruitment of cells and proliferation of resident cells (Bauer et al., 2001). Cytokines thought to be expressed by these luteal immune cells have the ability to inhibit progesterone synthesis by the bovine luteal cells and cause apoptosis of these cells and thus regression of the corpus luteum (Pate and Landis Keyes, 2001). The exact mechanism by which the immune cells are signaled to actively work toward regression of the corpus luteum is the subject of much research. Understanding this mechanism may help in the generation of new methods to increase fertility in domestic animals.

During pregnancy, cells of the immune system undergo significant alterations that have yet to be thoroughly investigated. For example, stimulated neutrophils from pregnant women showed significantly less respiratory burst activity compared to a control group (Crouch et al., 1995). Similarly, two enzymes in the hexose monophosphate shunt that is part of the pathway that produces NADPH required for respiratory burst activity are localized to different subcellular areas in neutrophils from pregnant versus non-pregnant women (Kindzelskii et al., 2004). Finally, subcellular location of myeloperoxidase, an enzyme critical to oxidative burst, is altered in non-pregnant women (cytosol) compared to pregnant women (external to the cell and associated with the cell membrane) (Kindzelskii et al., 2006). These alterations in neutrophil functions associated with antimicrobial activities indicate significant perturbation of the neutrophil cellular functions as a result of pregnancy. These observations support the long held idea that immune suppression is an important mechanism in the maintenance of pregnancy, and a break down of the suppression is a factor in spontaneous abortions (Vince et al., 2001).

The periparturient period is a nexus of physiological events that combine to have a profound effect on the immune system. Periparturient immunosuppression is manifest in a wide range of immunological dysfunctions, including impaired neutrophil and lymphocyte functions (Kehrli et al., 1989; Shuster et al., 1996; Mehrzad et al., 2001). As part of the innate immune system, the neutrophil is an essential first responder to infection and is considered vital to effective clearance of bacteria from the mammary gland of the dairy cow (Mollinedo et al., 1999; Smith, 2000; Paape et al., 2003; Zychlinsky et al., 2003). Neutrophils have various killing mechanisms to destroy pathogens (Smith, 2000; Segal, 2005). Upon encountering invading bacteria
neutrophils will ingest the bacteria into phagosomes that are fused with lysosomes. This process stimulates neutrophils to produce large amounts of oxidizing agents in a process referred to as the respiratory burst, in which oxygen radicals are generated that serve as precursors to various antimicrobial oxidants. In addition to oxidizing agents, neutrophils contain numerous antimicrobial proteins, such as cathelicidins, hydrolases, proteases, lactoferrin, and lysozyme within granules. These proteins are either released into phagosomes to destroy ingested pathogens, or the granule contents are released out of the cell. These neutrophil functions are suppressed at and around the time of parturition (Kehrli et al., 1989; Shuster et al., 1996; Mehrzad et al., 2001). The molecular causes of periparturient neutrophil functional suppression are an area of intense research by this and other research groups.

SUMMARY

The immune system is a complex system that enables to host’s body to protect against or eliminate pathogens. This system is made up of numerous cell types whose functions are still matters of investigation. This system relies not only on cells defined as 'immune cells' but also relies on non-immune cells to detect and respond to various infectious agents. In fact, the initial signal that begins an immune response is likely a non-immune cell that detects pathogen through its pattern recognition receptors, such as the TLR. Stimulated non-immune cells of various types are known to be able to secrete cytokines that can initiate an immune response.

Understanding of an immune response must not only take into account the functions of the immune cells, but also the effects of various pathogen-stimulated non-immune cells on the immune response. Conversely, an immune response can have important effects on the cells, tissues and the whole host. The immune response can have negative impact such as those that are normally associated with uncontrolled inflammation (e.g., fever, edema, pain, tissue damage and potentially death). In addition, constant immune stimulation will lead to suppressed growth of an animal, as energy and nutrients go preferentially to immune and homeostatic pathways (Spurlock, 1997). This illustrates the important connection between general health and growth of an animal and the immune system. This connection has not only been shown at the whole animal level (e.g., growth) but also at the molecular level. Dietary components, such as vitamins, have been shown to affect gene expression of a number of immune cells. Thus, the molecular pathways that tie growth, nutrition and immune responses together are being elucidated.

The immune system is affected by various non-pathogenic stimuli and has an important role in processes other than disease control. For example, the immune system plays an important role in the maintenance of the corpus luteum. Therefore, the immune system plays an important role in reproduction. In addition, non-pathogenic stimuli such as stress can after prolonged exposure have a suppressive effect on the immune system and make the animal susceptible to infection.
To achieve the goal of generating therapeutics that prevent or cure diseases, we must not only have a better understanding of the mechanism and functions of the immune system but also how that system is integrated into the whole host. In order to have the greatest potential for a vaccine's success the animal's immune system must be working at optimal levels. Therefore, optimal diets must be given to ensure proper immune function, and stresses must be reduced to eliminate suppression of the immune response. There is likely no single treatment that will make animals disease-free, but a comprehensive plan to address the various aspects of the overall health of an animal will optimize the immune system and increase the likelihood of a successful immune response.

REFERENCES


