IMMUNE DEVELOPMENT OF THE RUMINANT NEONATE

A. R. Woolums, DVM MVSc PhD
Department of Large Animal Medicine
University of Georgia

INTRODUCTION

The neonatal calf is born into an environment populated by bacteria, viruses, and parasites with the capacity to overtake the calf's body and end the calf's life. In order to resist invasion by these infectious agents, the calf has a system of immunity that is capable of recognizing and eliminating thousands of different pathogens, while limiting damage to the host. However, the immune response is immature and "uneducated" in early life, and must learn though interaction with infectious agents. Assistance during this naïve phase of immunity comes from factors absorbed from the dam's colostrum. As the calf grows, its interactions with pathogens through vaccination or natural infection lead to a mature immune response capable of protecting the adult cow from numerous diseases, and allowing her to produce colostrum that protects her calves from disease.

BRIEF REVIEW OF THE IMMUNE RESPONSE

As for many other species, the bovine immune response has two major branches; the innate immune response and the acquired immune response. The innate immune response is immediately effective, and does not change over time. Innate immunity is mediated by soluble factors in body fluids, as well as several types of cells. Acquired immunity takes days to weeks to develop, and has "memory"—that is, the acquired immune response is more effective when it encounters pathogens it has seen before. Vaccines are administered to animals (and people) with the goal of stimulating memory in the acquired immune response, so the vaccinated individual has a more protective immune response when they are later infected by the pathogen in the vaccine. Like innate immunity, acquired immunity is also mediated by both soluble factors and certain cells. These two branches of the immune response function in a complementary way. Innate immune mechanisms help the host immediately, but they never improve; acquired immune mechanisms take some time to develop, but once they have developed they are more specific and, in some ways, more effective than innate responses.

Innate immunity. Soluble factors that feature in the innate immune response include the many proteins of the complement system, enzymes such as lysozyme, and proteins such as lectins and defensins. These soluble factors bind rather nonspecifically to various classes of molecules typical of infectious agents. Once bound, the soluble factors may directly impair or kill the infectious agent, or they may serve as a signal to cells of the immune system so that these cells can quickly identify and destroy the agent.

Cells of the innate immune response include granulocytes (neutrophils, eosinophils, and basophils), macrophages, natural killer cells, and gamma delta ($\gamma\delta$) T lymphocytes. Neutrophils

and macrophages are particularly important because of their ability to ingest (phagocytose) or otherwise destroy infectious organisms. These cells are the "front line" of the immune response, and when they identify infectious agents, they secrete chemicals (cytokines) that alert other cells in the host that a response to an infection is required. Natural killer cells are especially important in fighting viral infection and cancer. Gamma delta T lymphocytes have functions similar to the T lymphocytes of the acquired immune response, in that they secrete cytokines that modify the function of other immune cells, and they can directly kill host cells infected with viruses, bacteria, or parasites.

Acquired immunity. The main soluble factor that features in the acquired immune response is antibody (also known as immunoglobulin), which is produced by B lymphocytes (also known as B cells) and which is found on the surface of the respiratory, gastrointestinal, and urogenital tracts, and in plasma and other body fluids. Antibody molecules bind to molecules on pathogens and prevent them from infecting the host (neutralizing antibody), or target them for destruction by immune cells (opsonizing antibody). Different types of antibody molecules are produced by B cells, including immunoglobulin M (IgM), IgG, and IgA. These different molecules have different functional characteristics and exist in variable concentrations in different parts of the body. For example, IgM is able to bind to many infectious agents simultaneously, and it is the first antibody produced when the acquired immune response is activated. IgA is present in high concentrations on body surfaces, and is particularly important for preventing infection by pathogens at those surfaces (neutralization). A subtype of IgG, IgG1, is the antibody present in the highest concentration in bovine colostrum. Levels of antibody in host fluids increase slowly the first time a pathogen is encountered, but in subsequent encounters with the same or similar pathogens, antibody levels can increase very rapidly.

In addition to B cells, which produce antibody, other cells of the acquired immune response are T lymphocytes (or T cells), which are subdivided into "helper" T cells (usually identified as CD4 T cells, because they express a molecule on their surface which has been designated as "CD4"), and "cytotoxic" T cells (usually identified as CD8 T cells, or "cytotoxic lymphocytes" [CTL]). Helper T cells are well named, as they help a wide variety of other cells respond optimally to infection; they do this through production of cytokines and expression of surface molecules that can stimulate other cells to improved activity (costimulatory molecules). Helper T cells have been further subdivided into groups designated as "T helper 1 cells" (TH1), TH2 cells, TH0 cells, and the recently identified TH17 cells. These groups are based on the combination of cytokines expressed by a given TH cell, and they have practical relevance: TH1 cells are particularly important in the development of effective responses to viruses and other pathogens that live inside of host cells, while TH2 cells are particularly important in the induction of antibody production, especially on mucosal surfaces. TH0 cells are an "in-between" cell type that produces some cytokines typical of TH1 cells, and some cytokines typical of TH2 cells; TH0 cells are commonly found in cattle. TH17 cells appear to be important in chronic inflammatory responses (such as tuberculosis), and in modulating responses of other TH cells.

It should be noted that TH cells receive their first stimulus to respond to infection by "antigen presenting cells" (APCs). Antigen presenting cells, such as dendritic cells which live in many tissues, constantly sample the environment to identify infectious agents when they first enter the host. When infectious agents are identified, APCs pick them up and break them into pieces

called “antigens”, which they “present” to young TH cells. When APCs present antigen to young TH cells, they also secrete a mixture of cytokines that modify the response of young TH cells and determine whether they will become TH1, TH2, TH0, or TH17 cells. Because APCs influence the first steps of activation of TH cells, which then influence the host response for the rest of the host’s life, much research is currently focused on developing vaccines designed to optimally induce APC responses that lead to the most helpful TH responses in vaccinated individuals.

Cytotoxic T cells are of particular importance in killing host cells that have become infected; since viruses spend most of their life cycle inside host cells, cytotoxic T cells are particularly important in the host response to viral infection. They also kill tumor cells, and so are important in controlling cancer.

IMMUNE DEVELOPMENT IN THE CALF

Prenatal immunity

The immune response begins developing while the calf is *in utero*. For example, the thymus, which is the site of T lymphocyte development, is evident by 40 days of gestation, and fetal calves are able to mount an immune response to certain viruses as early as 73 days of gestation. The ability of the immune system to respond to infection gradually broadens as gestation progresses, although in the days immediately preceding birth many functions diminish because of the immunosuppressive effect of steroid hormones in the fetus and dam. If a calf is infected *in utero* by an agent which does not kill the fetus and/or cause abortion, measurable levels of antibody to the agent can be found in the serum of the newborn calf at birth, before it nurses colostrum.

Immunity in the neonatal calf

Passive antibody acquisition from colostrums. Unless a calf is infected *in utero*, at birth the calf has no significant levels of antibody in plasma or on body surfaces. Levels of soluble factors involved in the innate immune response, such as proteins of the complement system, are also lower in the neonatal calf than in adult cattle. Moreover, cells of both the innate and acquired arms of the immune response have diminished function in the newborn calf, and cells of the acquired immune response have not yet been “educated” by exposure to vaccines or infections. Thus, the newborn calf is exquisitely sensitive to infection. A solution to this problem is provided in the form of colostrum from the dam. Colostrum, which is produced in the cow’s mammary gland in the final weeks of gestation, contains a very high level of antibodies to any pathogen the cow has previously been exposed to. In the first few days of life, the calf’s intestinal cells take up proteins whole and intact (rather than digesting them into component amino acids, as will be the case after the calf is a few days old). Thus, antibody molecules in colostrum are taken up intact into the plasma, where they circulate for months, providing the calf with protection against infectious agents it encounters. Moreover, colostral antibody also provides protection on the surface of the intestine (and can be secreted from the plasma back into the intestine), providing the calf with protection against intestinal infections that can cause diarrhea and death. The critical nature of colostrum in the health of the neonatal calf has been

proven by repeated studies which show that calves that fail to obtain adequate passive transfer of antibody from maternal colostrum are much more likely to develop disease and to die, or to fail to grow and produce at their expected rate, as compared to calves that obtain adequate levels of antibody from colostrum.

In addition to antibodies, colostrum provides calves with a large dose of fat soluble vitamins, which are otherwise low in newborn calves. Fat soluble vitamins, such as vitamin A, D, and E, are necessary for a variety of responses by immune cells. Soluble factors such as lactoferrin in colostrum also appear to influence the neonatal immune response.

Importance of maternal cells in colostrums. While the importance of colostrum in transferring antibody to calves is well known, recent research has shown that maternal lymphocytes which are present in colostrum are also absorbed (or migrate) across the intestine of calves and enter the tissues, where they influence immune development. Researchers have shown that calves fed colostrum containing maternal cells developed the ability to stimulate an immune response faster than calves fed colostrum with maternal cells removed (Reber et al, 2005). Moreover, immune responses to the viral pathogen bovine viral diarrhea virus (BVDV) could be identified in calves at one day of age when they were fed colostrum containing maternal cells, but not in calves fed colostrum without maternal cells (Donovan et al, 2007). This and related research indicates that the cells in colostrum, as well as antibody, have an effect on calf immune development. The significance of this finding is not yet entirely clear, but it may indicate that efforts to provide calves with fresh (cell containing) colostrum, as opposed to frozen or fermented colostrum, may be worthwhile in terms of optimizing calf immune development.

Development of acquired immune responses in neonatal calves. Significant changes in the components of the acquired immune response occur in the first few weeks of calf life. At birth, calves have higher numbers of γδ T cells and neutrophils circulating in the blood, as compared to adults, and lower numbers of B cells (reviewed in Chase et al, 2008). In spite of these differences, calves can mount an immune response to an infection in the first few days of life, although these responses will not be as strong or effective as those in an adult animal. It is most accurate to think of the immune response of the newborn calf as functional, but immature and naïve. If calves have high levels of antibody passively acquired through colostrum ingestion, acquired immune responses in the first month or more of life will be blunted, because maternal antibodies provide negative feedback to the calf's immune response, suppressing further antibody production. Interestingly, recent research has shown that, while vaccination of calves with moderate levels of antibody from colostrum will not induce calves to produce more antibody, T cells in vaccinated calves are nonetheless stimulated, and able to mount a memory-type response when they are re-exposed at a later date to the infectious agent contained in the vaccine.

Immune responses in neonatal calves are noteworthy for being relatively biased toward a TH2 type response. As discussed above, this means that the neonatal calf immune response is more effective at producing antibody than it is at activating responses effective against intracellular infections, such as those by viruses and certain bacteria and parasites. Thus calves have relatively diminished capability to develop protective immunity to these agents, and vaccines

against these agents may be less effective in neonatal calves than they are in older calves or adults.

Effect of nutrition on neonatal immunity. Adequate nutrition is well known to be necessary for adequate immune responses to occur in cattle. In general, supplementing diets that are deficient in protein, energy, vitamins, or minerals has been shown to improve immune responses to vaccination and other stimulation. However, treatment of cattle with vitamins and minerals in excess of required levels has not reliably led to important improvements in immune response (reviewed in Galyean et al, 1999). With specific reference to neonatal calves, newborn Holstein calves fed at 50% of maintenance requirements for protein and energy for the first month of life had decreased lymphocyte responses and decreased ability to produce antibody following vaccination, as compared to calves fed maintenance requirements (Griebel et al, 1987). Because these investigators fed calves 50% of their requirement of milk replacer but did not correct for deficiencies of vitamins and minerals that may have occurred in underfed calves, vitamin and mineral deficiency may have also impacted immune responses in the calves in this study. In other research, lymphocytes from calves fed an intensified milk replacer (30% crude protein and 20% fat, fed at 2.5% of body weight on a dry matter basis per day) produced less interferon gamma and more nitric oxide following stimulation, as compared to calves fed a standard milk replacer diet (20% crude protein and 20% fat fed at 1.4% body weight of dry matter per day) (Nonnecke et al, 2003). As calves aged, lymphocytes from calves fed an intensified milk replacer diet did not respond to stimulation to the same degree as calves fed a standard milk replacer (Foote et al, 2005). The importance in these differences is not yet clear, but the research does indicate that intensified milk replacer feeding has an effect on immune responses in calves on these diets.

Vaccination of calves. For decades, veterinary students and cattle producers have been taught that neonatal calves cannot respond to vaccination because of the blocking effects of maternal antibody. However, research in the past 10-15 years has shown that, in at least some cases, young animals vaccinated in the face of maternal antibody, while not showing evidence of an increase in serum antibody levels typically seen in older animals responding to vaccination, will show evidence of T cell activation or, more importantly, protection from disease when they are exposed to infection after maternal antibodies have disappeared. In general, successful vaccination of calves with moderate levels of maternal antibody requires two doses of modified live vaccine given at least 2-4 weeks apart, but exceptions to this rule have been identified. However, these findings are not consistent; occasionally young animals vaccinated in the face of maternal antibody fail to develop a protective immune response to later challenge. The reasons that calves are often but not always successfully protected when vaccinated in the face of maternal antibody are not completely defined, but are likely related to the age of the animal at vaccination, the amount of maternal antibody present, the type of vaccine the calf receives, the virulence of the challenging pathogen, and the outcome used to define success of vaccination. While more research is needed before perfect recommendations for successful vaccination of calves with maternal antibody can be made, ample evidence suggests that vaccination of such calves can protect them from disease when they are exposed to infectious agents after maternal antibodies have disappeared in at least some cases (reviewed in Woolums, 2007).

TAKE HOME MESSAGE

1. The immune system protects the bovine host from attack by a variety of infectious agents. While the neonatal calf has an immune system capable of responding to infection, it is naïve and immature, compared to the adult immune system. Intake of adequate amounts of colostrum at birth is essential to protect calves from infection while their immune response is maturing and developing the capacity to respond to infection.

2. New research indicates that maternal cells in colostrum move across the intestinal wall of calves and enter their tissues, where they influence the development of neonatal immune responses. The importance of this finding is that frozen colostrum and colostrum replacers do not contain whole functional cells and thus may not stimulate the same type of response as that stimulated by fresh colostrum containing whole cells; more research is necessary to determine the practical importance of this difference.

3. Feeding intensified milk replacer to calves increases some immune responses and decreases others, as compared to responses in calves fed traditional milk replacer. More research is necessary to determine how these effects impact calf health.

4. While veterinarians and producers have traditionally understood that calves cannot be vaccinated effectively while they have circulating levels of maternal antibodies from colostrum, recent research indicates that calves vaccinated in the face of maternal antibody can sometimes mount T cell responses to vaccination, and may have improved protection against disease when maternal antibodies have disappeared. This is true even when calves do not develop elevated levels of serum antibodies following vaccination in the face of maternal antibodies.

LITERATURE CITED

Chase, C.C.L., D.J. Hurley, A.J. Reber. 2008. Neonatal immune development in the calf and its impact on vaccine response. Vet. Clin. Food Anim. 24:87-104.

Donovan, D.C., A.J. Reber, J. Gabbard, M. Aceves-Avila, K.L. Galland, K. Holbert, L.O. Ely, D.J. Hurley. 2007. Effect of maternal cells transferred with colostrum on cellular responses to pathogen antigens in neonatal calves. Am. J. Vet. Res. 68:778-782.

Foote, M.R., B.J. Nonnecke, M.A. Fowler, B.L. Miller, D.C. Beitz, W.R. Waters. 2005. Effects of age and nutrition on expression of CD25, CD44, and L-selectin (CD62L) on T-cells from neonatal calves. J. Dairy Sci. 88:2718-2729.

Galyean, M.L., L.J. Perino, G.C. Duff. 1999. Interaction of cattle health/immunity and nutrition. J. Anim. Sci. 77:1120-1134.

Griebel, P.J., M. Schoonderwoerd, L.A. Babiuk. 1987. Ontogeny of the immune response: effect of protein energy malnutrition in neonatal calves. Can. J. Vet. Res. 51:428-435.

Nonnecke, B.J., M.R. Foote, J.M. Smith, B.A. Pesch, M.E. Van Amburgh. 2003. Composition and functional capacity of blood mononuclear leukocyte populations from neonatal calves on standard and intensified milk replacer diets. J. Dairy Sci. 86:3592-3604.

Reber, A. J., A.R. Hippen, D.J. Hurley. 2005. Effects of the ingestion of whole colostrum or cell-free colostrum on the capacity of leukocytes in newborn calves to stimulate or respond in one-way mixed leukocyte cultures. Am. J. Vet. Res. 66:1854-1859.

Woolums, A.R. 2007. Vaccinating calves: new information on the effects of maternal immunity. Proc. Am. Assoc. Bov. Pract. 40:10-17.