Japanese Journal of Veterinary Research 63(Supplement 1): S37–S44, 2015

REVIEW

Genetic selection of cattle for improved immunity and health

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Received for publication, December 21, 2014

Abstract

The immune system is a sensing structure composed of tissues and molecules that are well integrated with the neuroendocrine system. This integrate system ensures non-self from self-discrimination. In this capacity the immune system provides detection and protection from a wide range of pathogens. In mammals, the immune system is regulated by several thousand genes (8–9% of the genome) which indicate its high genetic priority as a critical fitness trait providing survival of the species. Identifying and selectively breeding livestock with the inherent ability to make superior immune responses can reduce disease occurrence, improve milk quality and increase farm profitability. Healthier animals also may be expected to demonstrate improvements in other traits, including reproductive fitness. Using the University of Guelph's patented High Immune Response technology it is possible to classify animals as high, average, or low responders based on their genetic estimated breeding value for immune responsiveness. High responders have the inherent ability to produce more balanced and robust immune responses compared with average or low responders. High responders dairy cattle essentially have about one-half the disease occurrence of low responders, and can pass their superior immune response genes on to future generations thereby accumulating health benefits within the dairy herd.

Key Words: Breeding for Disease Resistance, Dairy Cattle, Immune Response

Introduction

The immune system is composed of molecules and cells that can distinguish self from non-self and in so doing can detect danger signals emanating from foreign pathogens. This system also has the capacity to rapidly diversify its response depending on the nature of the pathogen by utilizing a large number of immune response genes. In fact, the immune system has thousands of genes at its disposal (Breuer *et al.*, 2013). In this way, the immune system provides protection from a wide range of microbes as well as tumours. However, livestock species have been

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doi: 10.14943/jjvr.63.suppl.s37

largely selected for production traits, while until recently, paying little attention to health traits, including immune response. Recent studies have demonstrated that it is possible to identifying and selectively breeding livestock with an inherent ability to make superior immune responses that can reduce disease occurrence, improve milk quality and increase farm profitability (Thompson-Crispi *et al.*, 2014b). Healthier animals also may be expected to demonstrate improvements in other traits, including reproductive fitness and growth (Mallard and Wilkie, 2007; Thompson-Crispi *et al.*, 2012b; Hine *et al.*, 2014, Aleri *et al.*, 2015). In our research, using the University of Guelph's patented High Immune Response (HIR) technology it is possible to classify animals as high, average, or low responders based on their genetic estimated breeding value (EBV) for antibody and cell-mediated immune responses. High responders have the inherent ability to produce more balanced and robust immune responses compared with average or low responders. High responder dairy cattle essentially have about one-half the disease occurrence of low responders, and can pass their superior immune response genes on to future generations thereby accumulating health benefits within the dairy herd (Thompson-Crispi *et al.*, 2012b, 2014b). The Semex Alliance, Canada's largest dairy genetics company obtained an exclusive license from the University of Guelph to utilize the HIR procedure to identify sires with the high immune response classification. These sires are designated as *Immunity+*, marking their enhanced capacity to make protective immune responses. The immune response traits used in establishing HIR EBVs are moderately highly heritable having heritability estimates of approximately 0.25 to 0.35, which is in the same range as those for milk production traits, and well above those for most reproductive traits (Thompson-Crispi *et al.*, 2012a). To date, more than 1,000 Holstein sires and dams have been immune response phenotyped with many beneficial associations noted with health, production and reproduction parameters. In fact, several beneficial associations occur between immune response and reproductive traits such as calving ease and number of services to conception (Mallard *et al.*, 2014). Recent studies by our group have also evaluated genomic profiles of high and low immune responders using the *Illumina Bovine SNP50 BeadChip*. In these genome-wide association studies (GWAS) antibody-mediated immune responses, as well as cell-mediated immune responses, the two key components of the adaptive immune system, are associated with unique genomic profiles (Thomspon-Crispi *et al.*, 2014a). The significant genomic variation associated with these immune response traits is the first step toward a genomics test, to complement the currently available phenotypic test for immune response as an approach to improve inherent animal health.

Breeding for Livestock Health

The cost of disease to the agriculture industry is substantial and antibiotics treatment needs to be used judiciously in the livestock sector to help reduce the emergence of antibiotic-resistant pathogens. Therefore, alternate methodologies to manage animal health are needed. Genetic approaches to enhanced health are one way to meet this breeding objective. Hence, our laboratory has been motivated to develop genetic and genomic, as well as epigenetic methods to improve livestock health that capitalize on the animal's own inherent ability to make appropriate immune responses. Genetic approaches often also work well in combination with other preventive approaches, including vaccination, and may in fact enhance other traits, such as reproduction, feed efficiency and growth (Wilkie and Mallard, 1999; Wagter *et al.*, 2003; Mallard and Wilkie, 2007; Mallard *et al.*, 2014; Aleri *et al.*, 2015). Early studies in pigs noted that high responder pigs consistently reached market weight of 100 kg ten to twelve days before low responders (Mallard

and Wilkie 2007). Recent studies of Australian Holstein heifer calves showed that high antibody responders had greater average daily weight gains than low responders (Aleri *et al.*, 2015).

The immune system's ability to detect danger signals associated with pathogens initiates a protective cascade against infectious disease. Indeed, the immune system is unique in its ability to adapt the protective responses to match the nature of the pathogen and to modulate that response in reaction to rapidly shifting pathogens. These defense strategies are conveyed via complex genetically regulated mechanisms. It is estimated that 2,000 to 3,000 genes control host defense, providing mammals with a large repertoire of immune responses to combat foreign organisms (Breuer *et al.*, 2013). Some of these genes, such as those within the Major Histocompatibility Complex (MHC), are the most highly polymorphic in the entire mammalian genome enabling the immune system to detect a universal array of foreign epitopes. Although the genes within the MHC system perform a crucial role in recognizing and initiating adaptive immune responses, there are hundreds of non-MHC genes that also contribute to protective immune responses, adding a complex layer to genetic selection for disease resistance. Nevertheless, by taking advantage of well-established quantitative genetic methods, such as those used for decades to improve animal production traits, it is possible to identify and select animals with superior immunity (Mallard *et al.*, 1992; Wagter *et al.*, 2000; Thompson-Crispi *et al.*, 2014b). This approach formed the genetic foundation of the HIR technology. Importantly, the heritability of various immune response traits is sufficiently high to allow for improvement using genetic selection (Abdel-Azim *et al.*, 2005; Thompson-Crispi *et al.*, 2012a). In dairy cattle, utilizing this approach resulted in reduced mastitis in high immune responders, as well as improved response to vaccination and colostrum quality (Wagter *et al.*, 2000; Thompson-Crispi *et al.*, 2012b; Thompson-Crispi *et al.*, 2013). Both *E. coli* and *S. aureus* mastitis incidence, as well as other causative bacteria were lower in high responders. Likewise, daughters of HIR *Immunity+* sires have lower disease and higher productive indices than daughters of non-Immunity+ sires. For example, recent Semex sire proof data showed Immunity+ sires had a production index score of 186 points greater than non-Immunity+ bulls. They also demonstrated net merit scores that were $165 greater than non-Immunity+ (Mallard *et al.*, 2014).

The Impact of High Immune Response on Milk Production and Colostrum Quality

The term "high immune response" as denoted in the HIR technology means elevated and efficacious immunity. Numerous studies over two decades of research have shown that breeding for enhanced disease resistance based on breeding values of immune response improves livestock health while not negatively impacting production traits (Mallard *et al.*, 1992; Wagter *et al.*, 2003; Thompson-Crispi *et al.*, 2014b). In fact, in dairy cattle this approach appears to improve overall herd life, as well as certain reproductive traits, including first service to conception and non-return rate (Thompson-Crispi *et al.*, 2012a; Mallard *et al.*, 2014).

Of considerable importance to neonatal health and well-being is the enhanced quality of colostrum coming from high responder dams. Initial experiments showed that colostrum from high antibody responders contained more specific antibodies to test antigen compared with average and low responders (Wagter *et al.*, 2000). This finding has recently been verified in experiments that demonstrated a positive and significant correlation between serum antibody and antibody in colostrum or milk up to 280 days of lactation. Additionally, positive and significant correlations were seen between antibody in the dam's colostrum and antibody in their calves 2 days

after feeding colostrum from high or low responders (Wagter *et al.*, 2012). Wagter *et al.* (2000) also reported that cows with high antibody responses to a test antigen responded better to a J5 mastitis vaccine (O111: B4). Recent research has also shown that cows classified as high antibody responders had greater concentrations of total immunoglobulin (IgG) and β-lactoglobulin in colostrum compared with average and low responders (Fleming, 2014). It is well known that molecules such as IgG, and β-lactoglobulin exert anti-microbial activities against mastitis-causing pathogens (Butler, 1983; Chaneton *et al.*, 2011). The anti-microbial activity of molecules such as IgG, lactoferrin and β-lactoglobulin may explain, at least in part, the lower incidence of mastitis in high immune responders.

The Impact of High Immune Response on Reproductive Traits

Holstein cows with various immune response phenotypes show no substantial difference in production traits, including milk yield, milk fat, or milk protein (Wagter *et al.*, 2003; Thompson-Crispi *et al.*, 2012a). However, there are correlations with reproductive fitness. For example, a study by Thompson-Crispi *et al.*, (2012a) showed a negative correlation between high immune response and calving ease (−0.19), but beneficial correlations with 56-day non-return rate (0.16), numbers of services to conception (0.20), first service to conception (0.18) and gestation length (0.17). Many of these beneficial associations have been substantiated in daughters of Immunity+ sires where positive correlations with productive life, pregnancy rate and calving ease compared with non-Immunity+ sires have been reported (Mallard *et al.*, 2014). These studies indicate that dairy producers can selectively breed for improved immune response using the HIR methodology or semen from Immunity+ sires without the concern of reducing genetic gain in other important traits, such as production or reproductive efficiencies.

The Impact of Epigenetics on High Immune Response

Epigenetic effects include alterations to DNA, such as DNA methylation or histone modifications, and changes to DNA expression that occur through micro RNA that activate or repress target genes (Frésard *et al.*, 2013). Increases in DNA methylation generally are associated with gene silencing, while decreases in DNA methylation are associated with gene activation. In this way epigenetic changes can impact gene expression and account for individual differences, even between identical twins, that are not accounted for by genetic variation (Gudex *et al.*, 2014). Epigenetic marks on the DNA influence not only that individual but their offspring by overriding the removal of epigenetic marks which normally occurs early in embryonic development. In this way, these so called epimutations from the parent are passed on to the next generation affecting the phenotype. It is important to note that some epigenetic features are permanent, while others can be highly influenced by environmental factors. Environmental influences, such as stress and maternal nutrition, are particularly important in that they can rapidly alter phenotype and impact future generations of offspring (Skinner, 2011). A clear example of this kind of epigenetic influence is the well-known Dutch famine that occurred after World War II which left many pregnant women without adequate nutrition throughout gestation. This malnutrition was subsequently associated with chronic disease later in life for the infants born to those under nourished mothers (Veenendaal *et al.*, 2013). This demonstrated that early life experiences, both *in utero* and after delivery, can influence adult phenotype. In dairy cows the maternal *in utero* environment is critically important to the calf and is influenced by numerous characteristics, such as body condition score and nutrition. Specifically maternal effects of dairy dams can alter production and survival traits of their female offspring (Banos

et al., 2007).

Although the field of immuno-epigenetics is relatively new, accumulating research demonstrates that epigenetics does influence the immune response (Grogan *et al.*, 2001). However, very few studies have been conducted in cattle. To try to gain understanding of epigenetic effects on bovine immune responses our group has focused on key cytokines, such as interferon-γ and IL-4, that steer the immune response in a type 1 or type 2 direction, respectively (Paibomesai *et al.*, 2013). This study indicated that DNA methylation patterns of purified CD4+ T-cells correlate with bovine interleukin-4 and interferon-γ production and that treatment with the corticosteroid, dexamethasone, can substantially alter methylation status (Paibomesai *et al.*, 2013). Most recently, by comparing DNA methylation patterns of CD4+ T-cells from high antibody versus high cell-mediated immune responder cows, differences in DNA methylation at cytokine promoter regions were noted. Specifically, T-cells from the cows that produced more interferon-γ when stimulated with the T-cell mitogen, ConA, had significantly less DNA methylation at regulatory regions of the interferon-γ locus before and after stimulation. These data would suggest that the purified CD4+ T-cells from the cattle with heightened cell-mediated immune responses were epigenetically predisposed to produce more cytokine compared with the high antibody responder cows (Paibomesai *et al.*, 2014). It is important to note that cytokine responses of purified cells likely do not reflect the cytokine milieu of mixed cell populations found *in vivo*.

The Impact of High Immune Response on Genomics

The application of genomics in dairy cattle breeding has brought advancement in genetic accuracy and rapid improvement in production traits. However, genomic applications for improving fitness traits, such as reproduction and immunity, are still in their infancy. The objective of our research in this area is to identify genomic profiles associated with enhanced immune responsiveness using dairy cattle classified as having high, average or low EBVs for immune response. Specifically, the Illumina SNP 50K bovine BeadChip was used to evaluate differences in cows with either high or low antibody or cell-mediated immune responses. In initial studies, 186 genetic markers that are part of 11 genetic pathways were shown to differ between the low and high groups of cows based on their antibody responses, and 21 genetic markers were associated with cell-mediated responses (Thompson-Crispi *et al.*, 2014a). Genetic pathways of particular relevance included those within the bovine MHC, an important immune response gene cluster. Results of this work also were validated in a group of *Immunity*+ sires and indicate that it is likely possible to estimate genomic breeding values for immune response to improve dairy health (Thompson-Crispi *et al.*, 2014c). We currently are working to establish a large reference population of Holstein sires and dams with immune response phenotypes and genotypes. This is part of a larger Canada-wide 10,000 cow project that aims to obtain genomic information on various traits including milk spectral data and feed efficiency, as well as immune response and health traits.

Conclusions

The dairy industry continues to increase its focus on health traits (Koeck *et al.*, 2012; Parker Gaddis *et al.*, 2014). In Canada and elsewhere this focus includes distribution of sire proofs to improve mastitis based on physical udder characteristics and somatic cell score. Recently, information on clinical mastitis in Canada has been added to the sire proof (Miglior *et al.*, 2014). However, these indicators focus only on one disease, bovine mastitis, and while this is an

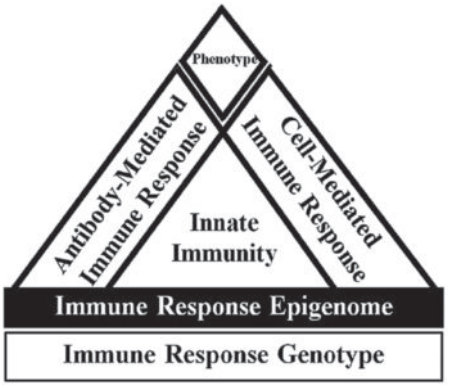

Fig. 1. Illustration of the Immune System Triad as Modified from: Wilkie, B. N. and B. A. Mallard. 1999. *Vet Immunol Immunopath* 72: 231–235

economically important trait there are many other diseases, including tuberculosis and Johne's disease, that plague the dairy industry worldwide. For this reason, the goal of our research and the HIR test is to enhance broad-based disease resistance by improving both antibody- and cell-mediated immune responses, the two aspects of the adaptive immune system that control response to extra-cellular and intra-cellular pathogens, respectively. In order to make robust and balance adaptive immune responses, initiating innate host defence mechanisms must also be operative. The immune response genotype, influenced by the enviroment and the epigenome are each components of the immune response phenotype of an individual (**Fig. 1**). When the overall immune response phenotype is accurately captured and immune response breeding values are estimated, it is possible to identify individuals with enhanced immune response genetics. This is in keeping with objective of our research which is to improve broad-based disease resistance of livestock species. Studies using this approach in both pigs and cattle have demonstated that high immune responders have improved host defense. In dairy cows high responders have increased resistance to a various economiclly important diseases, such as mastitis, metritis, pneumonia, and Johne's disease. These cows also produce colostrum with greater specific antibody, total immunoglobulin, and β-lactoglobulin. In addition, daughters of Immunity+ sires have improved pregnancy rates and daughter calving ease. No adverse associations with production traits have been noted indicating that it is possible to genetically improve animal health without compromising other economically important traits. It is important to keep in mind that in order to obtain maximal health benefits that both antibody- and cell-mediated immune responses should be included and kept in balance when selecting for enhanced disease resistance.

Acknowledgments

Contributions from all current and previous staff and students in the Mallard laboratory are gratefully acknowledged. Funding from NSERC, OMAFRA, DairyGen and Semex are highly appreciated.

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