# Are there Reproductive Implications when Dairy Cattle are Genetically Selected for Improved Immunity?

Bonnie A. Mallard*, Mehdi Emam, Kelly Fleming, Marlene Paibomesai,
Kathleen Thompson-Crispi, and Lauraine Wagter-Lesperance
Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph ON, N1G-2W1
*bmallard@ovc.uoguelph.ca

## Introduction

The immune system provides protection from a wide range of pathogens, and with reproduction, is considered an important fitness trait. Identifying dairy cows with the innate ability to make superior immune responses can reduce disease occurrence, improve milk quality, and 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 (**HIR**) technology it is possible to classify animals as high, average, or low responders based on their estimated breeding value (**EBV**) 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 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. 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. To date, we have tested more than 1,000 Holstein sires and dams, and have not seen any substantial negative impact of selecting for enhanced immune response on production traits and few negative associations with reproductive traits. This indicates that it possible to genetically improve immunity with minor if any impact on other economically important traits.

## The Immune System, Disease Occurrence and Breeding for Health

The cost of mastitis to dairy producers in Canada is about $200 per case, and 1 out of every 5 mammary gland quarters is infected with a mastitis-causing pathogen at any given point in time Canadian Bovine Mastitis Research Network, "What's New in the World of Mastitis Research?" http://www.medvet.umontreal.ca/rcrmb/dynamiques/PDF_AN/Results/NewspaperWhatsNew.pdf. 2009). Antibiotics may be used in treatment, but this can be problematic if the goal is to limit use of these therapeutics in order to reduce the emergence of antibiotic-resistant pathogens. Consequently, alternative approaches to manage animal health are needed. Genetic selection for enhanced disease resistance is 1 novel approach that may meet this goal. Therefore, our laboratory focuses on developing genetic and genomic (DNA sequence information), as well as epigenetic (DNA structural information) methods to improve animal health that take advantage of the animal's own inherent ability to make appropriate immune responses. Genetic approaches also can 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).

Function of the immune system is to detect danger signals emitted from foreign pathogens and provide protection from infectious disease. In fact, this is the only body system that has the unique ability to customize protective responses depending on the nature of the pathogen and to vary that response in reaction to rapidly changing sets of diverse pathogens. These host defenses are delivered via a complex compilation of genetically regulated mechanisms. It is estimated that 2,000 to 3,000 genes control host defense in mammals (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. The MHC genes are known to play a central role but thousands of genes are required to regulate this intricate system and this can complicate genetic approaches to improve animal health. Nonetheless, by using well established quantitative genetic methods, such as those used for decades to improve animal production traits, it is possible to identify and select individuals with enhanced immune response following exposure to a specified set of test antigens (Mallard et al., 1992). This is the basis for the HIR technology. Fortuitously, the heritability of many 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, applying this approach resulted in lesser occurrence of 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). This lesser occurrence Lower disease occurrence included the incidence of both *E. coli* and *S. aureus* mastitis, as well as other causative bacteria. In general, high responders have about one-half the disease occurrence of low responders (Figure 1). Similarly, daughters of *Immunity+* sires have lower disease and higher productive indices than non-Immunity+ sires. For example, data from recent Semex sire proofs 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+. (These data are courtesy of Mr. Jay Shannon, Sire Analyst, Semex Alliance).

## High Immune Response, Milk Production, and Colostrum Quality

Multiple studies over many years have shown that breeding for enhanced disease resistance based on breeding values of immune response does not negatively affect production traits, and indeed may improve overall herd life, as well as certain reproductive traits. In a Canada-wide study, no differences were detected in 305-day milk yield, protein yield, fat yield, or overall lifetime profitability in HIR cows compared with low or average responder cows. It is also worth noting that when relative immune response breeding values of sires were estimated from this study, beneficial associations were noted between immune response and herd life and some reproductive traits, as well as between high cell-mediated immune response and milk yield (Thompson-Crispi et al., 2012a), suggesting these traits may improve by breeding for enhanced immune response.

Of substantial relevance to animal health is the fact that high immune responders produce colostrum of enhanced quality. Initial experiments showed that colostrum from high responders contains more specific antibodies to test antigen compared with average and low responders (Wagter et al., 2000; Figure 1). This finding was recently substantiated in experiments showing that antibody to a test antigen in blood was positively and significantly correlated with antibody in colostrum or milk during early (calving to 5 days postpartum) and late lactation (around 280 days in milk on average). It is worth noting the positive and significant correlation detected between antibody in colostrum of the mother and in the blood of their calves 2 days after administration of colostrum (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). In addition, it recently has been shown that cows classified as high antibody-mediated immune responders have greater concentrations of total immunoglobulin (IgG) and β-lactoglobulin in colostrum compared with average and low responders (Fleming, 2014; Figure 1). 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). This The anti-microbial activity of molecules such as IgG, lactoferrin and beta-lactoglobulin may explain, at least in part, the lower incidence of mastitis in high immune responders and may provide a platform for future research into the plethora of immunologically-active components in colostrum from high immune responding cattle.

## Effects of Breeding for Immune Response on Reproductive Traits

Noteworthy is that high immune response animals show no substantial difference in production traits such as yield of milk, milk fat, or milk protein (Wagter et al., 2003; Thompson-Crispi et al., 2012a), but correlations of the antibody-mediated immune response and reproductive traits showed a negative correlation with calving ease (–0.19). On the other hand, beneficial correlations between the antibody-mediated immune response and 56-day non-return rate (0.16), numbers of services to conception (0.20) and first service to conception (0.18) were noted (Thompson-Crispi et al., 2012a). In addition, a positive beneficial association exists between cell-mediated immune responses and gestation length (0.17; Thompson-Crispi et al., 2012a). More important, daughters of Immunity+ sires have beneficial correlations with pregnancy rate and calving ease compared with non-Immunity+ sires (Table 1). As a result, dairy producers can selectively breed for improved immune response using the HIR technology without necessarily reducing genetic gain in other important traits, such as pregnancy rate and calving ease. 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 (Mallard et al., 1992; Thompson-Crispi et al., 2012a; Edwards, 2014).

## Epigenetic Aspects of Immune Regulation

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). For example, decreases in DNA methylation at gene regulatory regions, such as promoters, are associated with activation of that gene. Therefore, the epigenome can explain differences between individuals that are not accounted for by genetic variation, even though recent estimates of the epigenetic effects on heritability seems to be relatively small (Gudex et al., 2014). Epigenetic marks in mammals can influence subsequent generations through the formation of epimutations, which circumvent the epigenome erasure that occurs during early development. These epimutations are passed to the next generation and can effect phenotype of offspring, most of this evidence of transgenerational epigenetics have been determined in mice. Although some epigenetic features are permanent, such as those involved in lymphocyte receptor gene expression; environmental factors, such as stress, maternal nutrition, and various hormones can influence particular epigenetic modifications. Consequently, individual phenotypes can be rapidly altered, including effects on future generations of offspring (Skinner, 2011). A key example of this type of epigenetic influence is the Dutch famine at the end of World War II that left many pregnant women without adequate nutrition throughout pregnancy. This was associated with chronic disease later in life for infants born to those under nourished mothers (Veenendaal et al., 2013) demonstrating that early life experiences, both *in utero* and postnatally, can have significant effects on adult phenotypes. In the dairy cow the maternal environment, is influenced by body condition score, and nutritional status during gestation, and are associated with decreased survival to second lactation, decreased milk yield and increased somatic cell count of the progeny (Banos et al., 2007).

Mounting research to date demonstrates environmental effects on the epigenome, but relatively few studies have been conducted in cattle, particularly as they relate to immune regulation. To help regulate immune responses, cytokines which are the "hormones" of the immune system, are required to direct an appropriate immune response to a specific pathogen. Our group previously has shown that DNA methylation patterns correlate with bovine interleukin-4 and interferon-gamma cytokine production and that treatments such as application of the corticosteroid, dexamethasone, can alter methylation status (Paibomesai et al., 2013). Most recently, we have been examining the association of DNA methylation in high antibody vs. high cell-mediated immune responder dairy cows and shown differences in DNA methylation at cytokine gene control regions between these 2 phenotypes. Those individuals that produced more interferon-gamma when stimulated with a general stimulus had significantly less DNA methylation at regulatory regions of the interferon-gamma locus before and after stimulation. These data indicate that those cattle with heightened cell-mediated immune responses were epigenetically predisposed to produce more cytokine compared with the high antibody responder cows (Paibomesai et al., al., 2014).

## Genomic Profiles of Immune Response

The rapidly emerging application of genomics in dairy cattle breeding has brought advancement in genetic accuracy and improvement in production traits. Many novel applications for improving fitness traits using genomic information, however, still need to be explored. Our goal is to identify genomic profiles associated with enhanced immune responsiveness in order to improve dairy health. The Illumina SNP 50K bovine BeadChip currently .has been utilized to evaluate differences in cows with high or low antibody-mediated and cell-mediated immune responses. In one study, 186 genetic markers that are part of 11 genetic pathways were shown to differ between the low and high groups of cows based on antibody responses, and 21 genetic markers were associated with cellular responses (Thompson-Crispi et al., 2014). Genetic pathways included those within the bovine MHC, an important immune response gene cluster. Results of this work also were validated in the *Immunity+* sires and make it possible to estimate genomic breeding values for immune response to improve health in subsequent generations. 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, feed efficiency, health, and more. If successful, it may be possible in the future to identify high- or low-immune responders from a simple DNA sample.

## 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). These indicators focus, however, mostly on mastitis. 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. In keeping with this objective, high immune responding cows have improved resistance to a number of diseases including mastitis, metritis, pneumonia, and Johne's disease. These cows also produce colostrum with greater specific antibody, total immunoglobulin, and beta lactoglobulin. In addition, daughters of Immunity+ sires have improved pregnancy rates and daughter calving ease. No adverse associations with production traits have been noted, thus, demonstrating that it is possible to improve genetically animal health without compromising other economically important traits.

## 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.

## Literature Cited

Abdel-Azim, G. A., A. E. Freeman, M. E. Kehrli, Jr., S. C. Kelm, J. L. Burton, A. L. Kuck, and S. Schnell. 2005. Genetic basis and risk factors for infectious and noninfectious diseases in US Holsteins. I. Estimation of genetic parameters for single diseases and general health. J. Dairy Sci. 88:1199-1207.

Butler, J. E. 1983. Bovine immunoglobulins: An augmented review. Vet. Immunol. Immunopathol. 4:43-152.

Breuer, K., A. K. Foroushani, M. R. Laird, C. Chen, A., Sribnaia, R. Lo, G. L. Winsor, R. E. Hancock, F. S. Brinkman, and D. J. Lynn. 2013. InnateDB: systems biology of innate immunity and beyond. Nucleic Acid Res. 41:D1228-1233.

Chaneton, L., J. Pérez Sáez, and L. Bussmann. 2011. Anti-microbial activity of bovine β-lactoglobulin against mastitis-causing bacteria. J. Dairy Sci. 94:138-145.

Edwards, E. 2014. Preliminary investigation into mastitis rates as a predictor for bovine tuberculosis susceptibility and the possibility of implementation of high immune response dairy cows. Dissertation BSc (honors) in Agric. Royal Agric. Univ., Cirencester, UK.

Fleming, K. 2014. Bioactive components in colostrum and milk of dairy cattle classified as high, average and low immune responders. MSc thesis. Dept. Pathobiology, Univ. Guelph.

Frésard, L., M. Morisson, J. M. Brun, A. Collin, B. Pain, F. Minvielle, and F. Pitel. 2013. Epigenetics and phenotypic variability: some interesting insights from birds. Genet. Sel. Evol. 45:1-15

Gudex, B., D Johnson, and K. Singh. 2014. Prenatal maternal and possible transgenerational epigenetic effects on Milk Production. PLoS ONE. 9: e98928.

Koeck A, F. Miglior, D. F. Kelton, F. S. Schenkel. 2012. Health recording in Canadian Holsteins: data and genetic parameters. J. Dairy Sci.; 95:4099-4108.

Mallard, B. A., and B.N. Wilkie. 2007. Phenotypic, genetic and epigenetic variation of immune response and disease resistance traits of pigs. Adv. Pork Prod. 18:139-146.

Mallard, B. A, B. N. Wilkie, B. W. Kennedy and M. Quinton. 1992. Use of estimated breeding values in a selection index to breed Yorkshire pigs for high and low immune and innate resistance factors. Anim. Biotech. 3:257-280.

Miglior, F., A. Koeck, J. Jamrozik, F. S. Schenkel, D. F. Kelton, G. J. Kistemaker, and B. J. Van Doormaal. 2014. Index for mastitis resistance and use of BHBA for evaluation of health traits in Canadian Holsteins. Interbull Bull. No. 48. Berlin, Germany, Assessed May 20-21, 2014. https://journal.interbull.org/index.php/ib/article/view/1349/1420.

Paibomesai, M. A., and B. A. Mallard. 2014. Epigenetic differences in IFN and IL4 promoter regions of dairy cows identified with Type 1 and Type 2 immune response bias. Proc. 10th World Cong. Genet. Appl. Livest. Prod. August 17-22. Vancouver, B.C. (Abstr. 540).

Paibomesai, M. A., B. Hussey, M. Nino-Soto, and B. A. Mallard. 2013. Effects of parturition and dexamethasone on DNA methylation patterns of IFN-γ and IL-4 promoters in CD4+ T-lymphocytes of Holstein dairy cows. Can. J. Vet. Res. 77:54-62.

Parker Gaddis, K. L, J. B. Cole, J.S. Clay, and C. Maltecca. 2014. Genomic selection for producer-recorded health event data in US dairy cattle. J. Dairy Sci. 97: 3190-3199.

Skinner, M. K. 2011. Environmental epigenetics transgenerational inheritance and somatic epigenetic mitotic stability. Epigenetics 6:838-842.

Thompson-Crispi K. A., B. Hine, M. Quinton, F. Miglior, B. A. Mallard. 2012b. Short communication: Association of disease incidence and adaptive immune response in Holstein dairy cows. J. Dairy Sci. 95:3888-3893.

Thompson-Crispi K. A, F. Miglior, and B. A. Mallard. 2013. Incidence rates of clinical mastitis among Canadian Holsteins classified as high, average and low immune responders. Clin. Vaccine Immunol. 20:106-112.

Thompson-Crispi K. A., M. Sargolzaei, R. Ventura, M. Abo-Ismail, F. Miglior, F. Schenkel, B. A. Mallard. 2014. A genome-wide association study of immune response traits in Canadian Holstein cattle. BMC Genomics 15:559-569.

Thompson-Crispi K. A., A. Sewalem, F. Miglior F, B. A. Mallard. 2012a. Genetic parameters of adaptive immune response traits in Canadian Holsteins. J. Dairy Sci. 95:401-409.

Veenendaal, M. V., R. C. Painter, S. R. de Rooij, P. M. Bossuyt, J. A. van der Post, P. D. Gluckman, M. A. Hanson, and T.J. Roseboom. 2013. Transgenerational effects of prenatal exposure to the 1944-45 Dutch famine. BJOG. 120:548–553.

Wagter, L. C., B. A. Mallard, B. N. Wilkie, K. E. Leslie, P. J. Boettcher, and J. C. Dekkers. 2000. A quantitative approach to classifying Holstein cows based on antibody responsiveness and its relationship to peripartum mastitis occurrence. J. Dairy Sci. 83:488-498.

Wagter L.C., B. A. Mallard, B. N. Wilkie, K. E. Leslie, P. J. Boettcher, and J. C. Dekkers. 2003. The relationship between milk production and antibody response to ovalbumin during the peripartum period. J. Dairy Sci. 86:169-73.

Wagter, L. C., L. Cain, S. Cartwright, and B. A. Mallard. 2012. Feasibility of transferring High Immune Response (HIR) technology to the marketplace to improve the health and food quality of Canadian Dairy cattle. Proc. Graduate Student Research Symposium, Ontario Vet. College, Univ. Guelph. November 2012, p. 18 (Abstr.).

Wilkie, B. N., and B.A. Mallard. 1999. Genetic effects on vaccination. Academic Press. Adv. Vet. Med. 41:39-51.

**Figure 1.** Disease occurrence (%), colostral-specific antibody (ELISA Optical Density x 3), total immunoglobulin (mg/dl x 1000) and colostrum β-Lactoglobulin (mg/ml) in high, average, and low immune responding cows (Wagter et al., 2000; Thompson-Crispi et al., 2012b, 2013; Fleming, 2014)

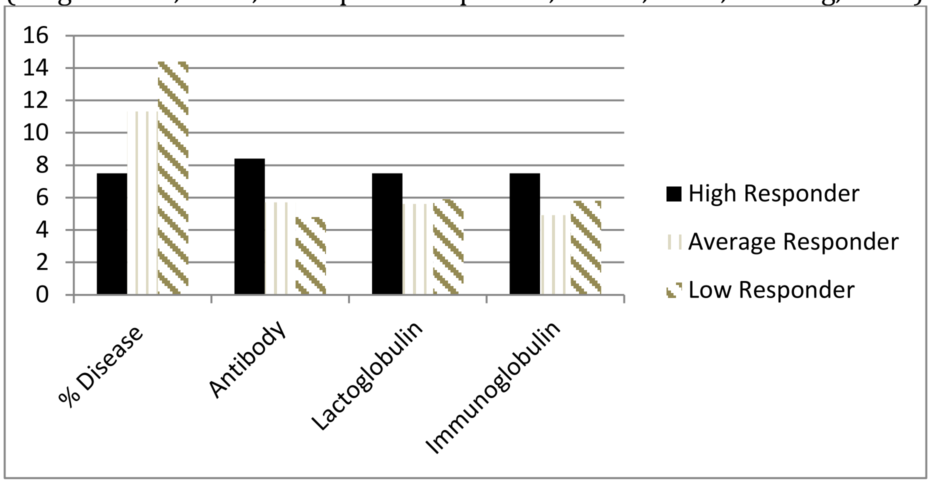


**Table 1.** Associations between Production Traits in Immunity+ and Non-Immunity+ Sires

| Trait | Immunity+ | Non-immunity+ | Difference |
|---|---|---|---|
| Productive life | +4.8 | +3.0 | +1.8 |
| Daughter pregnancy rate | +1.0 | +0.1 | +0.9 |
| Somatic cell score | 2.72 | 2.82 | –0.10 (favorable) |
| Daughter calving ease, % | 5.4 | 6.0 | –0.60 (favorable) |