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Genetic parameters for natural antibodies and associations with specific antibody and mastitis in Canadian Holsteins

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ABSTRACT

The objectives of this study were to evaluate (1) natural antibodies (NAb) in Canadian Holstein cows, (2) genetic parameters and associations between NAb and specific antibody (SpAb), and (3) the association of NAb with clinical mastitis and differences in incidence rates of clinical mastitis (IRCM) among cows classified as high, average, or low responders for NAb. Natural antibodies (IgG and IgM) to keyhole limpet hemocyanin and SpAb to a type 2 test antigen were measured on 451 Holsteins from 41 herds across Canada. A series of uni- and tri-variate linear animal models were used to estimate genetic parameters and breeding values for NAb and SpAb. The models included the fixed effects of parity and stage of lactation and the random effects of herd-technician, animal, and residual. Using estimated breeding values for NAb, cows were classified as high, average, or low responders and phenotypic associations with the IRCM were investigated and a logistic regression performed. The estimated heritability was 0.27 for SpAb, and was 0.32 and 0.18 for NAb of the IgG and IgM isotypes, respectively. No significant genetic correlations were found between SpAb and NAb. Although no significant differences in the IRCM were found when cows were classified based on NAb IgG, cows classified as high responders for NAb IgM tended to have a lower IRCM compared with other cows. Immunoglobulin-M was associated with a decreased risk of clinical mastitis (odds ratio = 0.958). Results of this study suggest the potential to use NAb IgM as an additional tool to select for disease resistance in cattle, but results need to be validated with a larger sample size.

Key words: natural antibodies, immune response, mastitis, genetic parameter

INTRODUCTION

A robust and balanced immune system of the dairy cow is vital for protection against economically important diseases. Holstein cows with superior or high antibody- (**AMIR**) and cell-mediated immune responses (**CMIR**) have been shown to have a lower occurrence of diseases, including mastitis, metritis, retained placenta, and displaced abomasum, and are less likely to be seropositive for *Mycobacterium avium* ssp. *paratuberculosis* compared with average- and low-immune responding cows (Wagter et al., 2000; Pinedo et al., 2009; Thompson-Crispi et al., 2012a). The adaptive immune response traits, AMIR and CMIR, predominate in protection against extracellular and intracellular pathogens, respectively, and both are therefore required for broad based disease resistance. Both AMIR and CMIR have been demonstrated to be heritable (0.29 and 0.19, respectively), and their inclusion in breeding indices has been suggested to improve inherent animal health and decrease the incidence of disease in the dairy industry (Mallard et al., 2011; Thompson-Crispi et al., 2012b). Previous studies in numerous herds have shown specific antibody (**SpAb**) to be a reliable predictor of enhanced dairy cattle health (Wagter et al., 2000; Mallard et al., 2011; Thompson-Crispi et al., 2012a), and this association has recently been confirmed in a national immune response study across Canada. Cows classified as high for SpAb had a lower incidence rate and less severe clinical mastitis compared with other cows in the herd (Thompson-Crispi et al., 2013).

Natural antibodies (**NAb**), on the other hand, are antibodies that circulate in normal individuals in the absence of exogenous antigenic stimulation, and have been considered as a humoral component of the innate immune system (Baumgarth et al., 2005). Natural antibodies are predominantly of the IgM isotype, and to a lesser extent IgG and IgA (Kohler et al., 2003), and are typically polyreactive to conserved structures such as nucleic acid, carbohydrates, and phospholipids (Boes, 2000). Natural antibodies are produced by B-1 cells, a long-lived, self-renewing B cell subset that differs from

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conventional B-2 cells in their ontogeny and localization in peritoneal and pleural cavities (Baumgarth, 2011). The B-1 cells lack terminal deoxynucleotidyl transferase activity early in ontogeny contributing to a more limited set of gene rearrangements and restricted NAb repertoire than antibodies produced by conventional B cells (Boes, 2000). Natural antibodies provide protection against infection by direct neutralization, activation of the complement system, and formation of antigen-antibody complexes leading to pathogen elimination in the spleen, thereby enhancing specific immune responses in secondary lymphoid organs (Ochsenbein and Zinkernagel, 2000).

Keyhole limpet hemocyanin (**KLH**) is a high-molecular mass metalloprotein derived from the giant keyhole limpet *Megathura crenulata* (Harris and Markl, 1999) that has remarkable immunostimulatory properties. As it is derived from the ocean, livestock species are naive to KLH, making it a good antigen to measure NAb. In dairy cattle, NAb-binding KLH have been found in both milk and serum, and their measurements have been shown to be repeatable over time (Ploegaert et al., 2011). The across-herd heritability of bovine NAb to KLH has been estimated to be 0.36 (SE = 0.08), and NAb have been suggested as potential immune parameters for disease resistance in dairy cows (Ploegaert et al., 2010). Previous studies in poultry have found NAb to be positively correlated with survival and health status (Star et al., 2007). However, a direct correlation of NAb and disease in dairy cattle has yet to be demonstrated, as has been shown in multiple studies for SpAb (Mallard et al., 1997; Wagter et al., 2000; Thompson-Crispi et al., 2012a). Therefore, the objectives of this study were to (1) evaluate NAb of the IgG and IgM isotypes in serum to KLH of 451 Holstein cows across Canada that have previously been immune response phenotyped for SpAb, (2) estimate the genetic parameters and associations between NAb and SpAb, and (3) determine the association with NAb and clinical mastitis and evaluate phenotypic differences in the incidence rate of clinical mastitis between cows classified as high, average, or low immune responders for NAb.

MATERIALS AND METHODS

Animals

Immune response profiles of 451 lactating Holsteins, outside the peripartum period, from 41 herds across Canada were evaluated (Thompson-Crispi and Mallard, 2012) in collaboration with the Canadian Bovine Mastitis Research Network (**CBMRN**). Lactating cows enrolled in the longitudinal incidence-density mastitis sampling for the CBMRN were also immune response tested (Reyher et al., 2011). Ten cows expected to remain in the herd for at least 2 mo were chosen at random along with the 5 cows that had most recently calved. Immune responses were tested between July 2007 and August 2008. Distribution by parity was as follows: n = 141 in parity 1, n = 134 in parity 2, n = 77 in parity 3, and n = 99 in parity 4 or higher. All experimental procedures were approved by the Animal Care Committee of the University of Guelph under guidelines of the Canadian Council of Animal Care.

Immunization Protocol for Specific Antibody

Cows at least 28 DIM were immunized to stimulate AMIR, as described and reported previously (Thompson-Crispi et al., 2012b). Briefly, the primary antibody to a type 2 test antigen, hen egg white lysozyme, was used as an indicator of AMIR. On d 0 cows received an intramuscular injection of 0.5 mg of hen egg white lysozyme (Sigma-Aldrich Canada Ltd., Oakville, Canada) and 0.5 mg of Quil-A adjuvant (Cedarlane Laboratories, Hornby, Canada) dissolved in 1 mL of PBS (pH 7.4).

Specific Antibody

Blood was collected on d 0 and 14 of the immunization protocol to evaluate the specific serum antibody of the IgG1 isotype to the type 2 test antigen by a modified ELISA as described and reported previously (Thompson-Crispi et al., 2012b).

Natural Antibody Positive Control

Three cows were immunized with 1.0 mg of *Megathura crenulata*-derived KLH from MP Biomedicals (Solon, OH) and 0.5 mg of Quil-A adjuvant (Cedarlane Laboratories Ltd.) dissolved in 1 mL of PBS (pH 7.4). Using a 22-gauge needle, a 1.0-mL injection was administered intramuscularly in the rump. Cows were boosted 14 d later to achieve maximum antibody response to KLH on d 21. Serum was collected at d 0, 14, and 21, and the cow with the highest serum IgG and IgM to KLH on d 21 was used as the positive control.

Natural Antibodies

Serum from d 0 of the immunization protocol was used to evaluate natural antibody to KLH by a modified ELISA. The negative control was fetal calf serum. Flat bottomed 96-well polystyrene plates were coated with 100 μL/well of 1 μg of KLH dissolved in 1 mL of carbonate-bicarbonate buffer (pH 9.6) and incubated at 4°C for 24 h. Plates were washed with PBS and 0.05%