J. Dairy Sci. 99:1–8
http://dx.doi.org/10.3168/jds.2015-9449

Short communication: Cytokine profiles from blood mononuclear cells of dairy cows classified with divergent immune response phenotypes

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ABSTRACT

Genetic selection for enhanced immune response has been shown to decrease disease occurrence in dairy cattle. Cows can be classified as high (H), average, or low responders based on antibody-mediated immune response (AMIR), predominated by type-2 cytokine production, and cell-mediated immune response (CMIR) through estimated breeding values for these traits. The purpose of this study was to identify in vitro tests that correlate with in vivo immune response phenotyping in dairy cattle. Blood mononuclear cells (BMC) isolated from cows classified as H-AMIR and H-CMIR through estimated breeding values for immune response traits were stimulated with concanavalin A (ConA; Sigma Aldrich, St. Louis, MO) and gene expression, cytokine production, and cell proliferation was determined at multiple time points. A repeated measures model, which included the effects of immune response group, parity, and stage of lactation, was used to compare differences between immune response phenotype groups. The H-AMIR cows produced more IL-4 protein than H-CMIR cows at 48 h; however, no difference in gene expression of type-2 transcription factor *GATA3* or *IL4* was noted. The BMC from H-CMIR cows had increased production of IFN-γ protein at 48, 72, and 96 h compared with H-AMIR animals. Further, H-CMIR cows had increased expression of the *IFNG* gene at 16, 24, and 48 h post-treatment with ConA, although expression of the type-1 transcription factor gene *TBX21* did not differ between immune response groups. Although proliferation of BMC increased from 24 to 72 h after ConA stimulation, no differences were found between the immune response groups. Overall, stimulation of H-AMIR and H-CMIR bovine BMC with ConA resulted in distinct cytokine production profiles according to genetically defined groups. These distinct cytokine profiles could be used to define disease resistance phenotypes in dairy cows according to stimulation in vitro; however, other immune response phenotypes should be assessed.

Key words: blood mononuclear cells, dairy cows, type-1 and type-2 cytokines, immune response

Short Communication

Genetic selection using measurable phenotypic immune response (**IR**) traits has been proposed as a candidate for improving the overall health of livestock (Mallard et al., 2011, 2015; Thompson-Crispi et al., 2012a). In dairy cattle, the selection for increased milk production traits has been associated with increased occurrence of both metabolic and infectious diseases (Van Dorp et al., 1998; Fleischer et al., 2001; Koeck et al., 2013; Pritchard et al. 2013; Hagiya et al., 2014) and a negative effect on reproduction (Pryce et al., 2004). Recent research indicates that dairy cattle can be classified as high (**H**), average, or low responders based on their type-1 cell-mediated immune response (**CMIR**) and type-2 antibody-mediated immune response (**AMIR**) through EBV (Heriazon et al., 2011; Hine et al., 2012; Thompson-Crispi et al., 2013). Both AMIR and CMIR have been demonstrated to be heritable, indicating it is possible to select for improved immune responsiveness (Thompson-Crispi et al., 2012b; Heriazon et al., 2013). Cows possessing a robust or H immune response have a decrease in disease occurrences for both metabolic and infectious diseases (Thompson-Crispi et al., 2012a, 2013). Therefore, as these branches of the IR provide protection against diverse pathogens, selection for robust and balanced CMIR and AMIR is expected to confer broad-based disease resistance to a range of pathogens (Abdel-Azim et al., 2005; Pinedo et al., 2009b; Thompson-Crispi et al., 2012a). In mam-

Received February 10, 2015.
Accepted October 23, 2015.
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mals, AMIR and CMIR are both genetically (Chaudhri et al., 2004; de Crean et al., 2005; Thompson-Crispi et al., 2012b; Filbey et al., 2014) and epigenetically regulated (Martino et al., 2011; Paibomesai et al., 2013; Scharer et al., 2013).

The CMIR are typically mounted to protect against intracellular pathogens. Representing the cellular responses, CMIR is predominated by the expression of inflammatory cytokine, such as IFN-γ and IL-12, which is controlled by the transcription factor Tbox 21 (TBX21). The AMIR typically control and protect the host from extracellular pathogens (Estes and Brown, 2002; Zhu et al., 2010). The GATA binding protein 3 (GATA3) is a T_H2 transcription factor which promotes production of AMIR cytokines, such as IL-4, IL-5, and IL-13. Both AMIR and CMIR are negatively correlated and evidence exists that these mechanisms are antagonistic to one another (Edwards, 2011; Kanno et al., 2012). Thus, the mechanisms of disease susceptibility of an individual could be explained, at least in part, through differences in cytokine profiles and transcription factor expression of T_H cells and blood mononuclear cells (**BMC**).

The purpose of our study was to investigate whether a correlation between genetic parameters for immune response exists using in vitro testing methods to develop a rapid IR test. In the current study, cows are classified by assessment of overall AMIR and CMIR to a representative of an immune challenge and ranked based on IR-EBV. The groups are defined as having H-AMIR and low-CMIR (**H-AMIR**) or H-CMIR and low-AMIR (**H-CMIR**) as contrasting phenotypes. Individuals expressing these phenotypes were then used to investigate potential of using in vitro challenge of BMC to determine adaptive IR differences based on genetically defined groups. Immune responses were assessed by evaluating cytokine production by BMC and ability of BMC to proliferate.

Dairy cattle used in our study were previously categorized as H, average, or low for AMIR and CMIR by assessing responses to type-1 and type-2 test antigens using the high immune response (HIR) technology (US Pat. No. 7258858; Wagter and Mallard, 2007; Hine et al., 2012). Briefly, 128 cows were immunized intramuscularly at d 0 with both a type-1 and a type-2 test antigen. Blood samples were collected at d 0 and 14 to evaluate antibody response to the type-2 test antigen by ELISA. To assess CMIR, a delayed-type hypersensitivity test using the type-1 test antigen was performed in the caudal fold of the tail. Estimated breeding values were determined for both AMIR and CMIR. Cows were ranked based on distance from the population mean; cows are considered H-IR at 1 standard deviation above the population mean, whereas L-IR are 1 standard deviation below (see Figure 1). All experimental procedures used in our study were approved by the University of Guelph Animal Care Committee under the guidelines of the Canadian Council on Animal Care (CCAC, 1993). All animals showed no signs of clinical disease at the time of classification and at the time of sampling. Blood (100 mL) was collected from the coccygeal vein into EDTA-containing tubes (BD Vacutainer, Becton Dickinson, Franklin Lakes, NJ) and overlaid on Histopaque 1077 (Sigma Aldrich, St. Louis, MO) as per the manufacturer's instructions. Following centrifugation (400 × *g*, 30 min at room temperature), BMC were collected, washed twice with PBS, and centrifuged again (300 × *g*, 13 min at room temperature). The BMC pellets were then resuspended in supplemented RPMI 1640 (Pathobiology Media Supply, Guelph, ON, Canada) containing 1% penicillin streptomycin (Pathobiology Media Supply), 10% fetal bovine serum (Gibco, Burlington, ON, Canada), 2 m*M* L-glutamine, and 50 μ*M* B-mercaptoethanol (Sigma Aldrich). Viable cells were stained with trypan blue (Sigma Aldrich) and quantified using a hemocytometer. Cells were diluted in supplemented RPMI to a final concentration of 2.5×10^6 cells/mL. For mRNA and cytokine protein quantification, 1 mL of BMC were seeded onto Costar 24-well flat-bottom plates (Corning Inc., Corning, NY) in quadruplicate for each time point (pooled after harvesting to show average gene expression and protein production). For BMC proliferation, 200 μL of cells were plated onto Costar 96-well flat-bottomed plates (Corning Inc.) in sextet. The BMC were either treated with 5 μg/mL of concanavalin A (**ConA**; Sigma Aldrich) or remained untreated to act as controls. Cell culture supernatants were collected at 24, 48, 72, and 96 h poststimulation and stored at −20°C until analyzed for cytokine production. Cell proliferation was measured at 24, 48, 72, and 96 h poststimulation.

Interferon-γ and IL-4 protein concentrations in cell culture supernatants were determined using the bovine IFN-γ ELISA (Mabtech, Cincinnati, OH) and bovine IL-4 ELISA (Thermo Fisher Scientific, Nepean, ON, Canada) kits according to the manufacturer's instructions. The IFN-γ ELISA (Mabtech) detection range was 5 to 500 pg/mL and the IL-4 ELISA detection range was 16 to 1,000 pg/mL. Triplicates from all time points for each individual animal were run on the same plate. Numbers of samples from H-AMIR and H-CMIR animals were balanced on each plate. Plates were read using a scanning multiwell plate reader (Powerwave XS2, Biotek, Winooski, VT). Samples were repeated if the coefficient of variation between replicates were greater than 10%. Cellular RNA was extracted from freshly cultured BMC at 4, 16, 24, and 48 h poststimulation using 1 mL of TRIzol per well