Genetic and Epigenetic Effects on Bovine Immune Responses and their Implications to Dairy Health

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Introduction

The immune system controls response to a wide range of pathogens and is subject to genetic and epigenetic regulation (Mallard (2007); Sordillo et al. (2009)). Adaptive cell-mediated (CMIR) and antibody-mediated (AMIR) immune responses tend to predominate in control of intra- and extracellular pathogens, respectively. Therefore a balance in CMIR (type 1 immune responses) and AMIR (type 2 immune responses) is central to selecting animals with improved IR to a diverse range of pathogens (Mallard (2007); Shaver-Weaver et al. (1999)). Previous emphasis on selecting livestock solely for production traits has inadvertently led to an increase in disease incidence, including mastitis in dairy cows (Simianer et al. (1991)). Improved knowledge of genetic and epigenetic regulation of IR traits should provide opportunities to improve disease resistance while still maintaining other performance goals.

Previously, IR testing by our group has been successful in identifying and selecting pigs with high (H), average (A) and low (L) immune responses (Wilkie and Mallard (2000)). More recently, a similar patented testing protocol and ranking system has been used to assess IR in dairy cattle. Generally, in both pigs and cattle H-IR is associated with health benefits (Wilkie and Mallard (2000); Mallard (2007)). The objectives of this paper are to summarize recent findings in regard to genetic and epigenetic regulation of the bovine adaptive immune system, particularly those traits that regulate type 1 and type 2 IR in the context of the peripartum period when IR is sub-optimal and disease risk is highest.

Material and methods

Animals. Purebred Holsteins (HO) in 2 University of Guelph research herds, and in a variety of commercial herds (87 Canadian herds and 1 large US herd) were used in these studies.

Immunization Protocol. A patented testing protocol was used to identify and classify cows and calves based on phenotype and on estimated breeding values (EBVs) as H, A or L responders. Briefly, animals were immunized using type 1 and type 2 antigens known to

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induce CMIR and AMIR, respectively. Animals were bled before and after immunization to collect sera and/or cells for various immunological or epigenetic assays as described below.

Evaluation of AMIR. Sera were obtained to assess AMIR by ELISA (Wagter et al. (2000)).

Evaluation of CMIR. Skin-fold measurements at the tail head were taken to assess delayed-type hypersensitivity (DTH) as an indicator of CMIR (Hernandez et al. (2005)).

Evaluating DNA Methylation Patterns. Bovine mononuclear cells were isolated at various time points (wk-4, day 4 or wk >4 relative to calving) using Histopaque (Sigma, ON). CD4+ T-lymphocytes isolated by MiniMACS (Miltnyi Biotech, CA) were cultured in a 96 well culture plate (37°C, 5% CO_2, 24-72 hours) at a concentration of 5.0×105cells/well in Phenol red free + Glutamine RPMI (Invitrogen, ON) and 10% Charcoal Stripped FCS (Invitrogen, ON). These cells were stimulated with 2.5ug/ml ConA, 10µM dexamethazone (DEX), or PBS as a negative control (n=3-6 cows/treatment).

To extract genomic DNA (gDNA), PBS was added (200ul) to the cells remaining in the culture plate following removal of culture supernatant for cytokine analyses. The cell suspension was mixed, washed (300g, 5min, rt) and then either stored at -80°C for future DNA extraction or used directly in DNA extraction performed using DNeasy® Tissue Kit (Qiagen, ON) as per manufacturer's instructions.

To evaluate DNA methylation, extracted gDNA was bisulphite treated using an EZ DNA methylation kit (Zymo Research, US) as per the manufacturer's instructions. Specific primers for both converted and unconverted promoter regions of bovine IFN-γ (GI:23821137, 6 CpG sites) and IL-4 (GI:1100926, 5 CpG sites) genes were designed using the BiSearch Software.

Cloning was performed using a TOPO TA Cloning Kit (Invitrogen, ON) and 2 LB plates of each treatment for each sample were prepared (20ul & 40ul cell suspension) and incubated at 37°C o/n. Individual colonies were selected between both plates from each treatment and cultured in 5ml LB liquid broth o/n. Plasmids were purified (GenElute Plasmid Miniprep Kit, Sigma, ON) and insertion of IFN-γ or IL-4 verified by PCR and gel electrophoresis (1.5% agarose). Plasmids containing inserts were sequenced (Robarts Research Institute, ON). Sequences (6-10 clones /cow) were annotated and edited in BioEdit (http://www.mbio.ncsu.edu/BioEdit/BioEdit.html) and analysed using BiQ Analyser software.

Cytokine Evaluation. Supernatant was collected from CD4+ T-lymphocyte cultures to evaluate cytokine (IFN-γ, IL-4) production (ConA or ConA + DEX or PBS treatments pre- or post-partum) by ELISA (Mabtech, Cincinnati, OH).

Statistical Analyses. Various SAS linear mixed models were used for analyses of AMIR or CMIR, and effects of animal, herd, year, season, provinces, age, and parity were included where appropriate depending on the study (Hernandez (2007), Thompson et al (2010)). Pedigree information was obtained by CDN (Guelph, ON) and multiple-trait animal models were used to estimate (co)variances: CMIR (DTH at 24 or 48hr), AMIR to antigen 1 or 2

(primary or secondary responses). Depending on the study VCE4.2 (Neumaier and Groeneveld (1998)) or DMU (Madsen and Jensen (2006)) software was utilized. The general model included the environmental effects of herd, IR testing technician, age and stage of lactation. All baseline measurements of immune response traits were accounted for by subtracting background or using appropriate covariates.

Significance of treatment effect in ELISA data were calculated with a two-tailed, paired t-test using Microsoft Excel. Analysis of methylation patterns in the IFN-γ and IL-4 promoters were conducted by comparison of the CpG sites within the promoter regions of cows (n=3-6 cows/treatment and 6-10 clones/cow). The average ratio of methylated to unmethylated DNA was determined by comparison at these sites. A p-value ≤ 0.05 was considered statistically significant for all analyses.

Results and discussion

Results of IR testing in 2 research herds, as well as a number of commercial herds (87 Canadian and 1 large US herd) indicated that this testing method can be successfully used to classify cows and calves as H, A or L responders, both phenotypically or based on EBVs, using a combination of CMIR and AMIR. Heritability estimates for CMIR and AMIR traits averaged 0.20-0.34 depending on test antigen and time relative to calving, indicating that selection for enhanced IR based on predicted breeding values that balance both traits is feasible. This is in agreement with previous studies in pigs (Wilkie et al. (2000)). High versus average responders had reduced odds ratio risk of mastitis (3.9x), metritis (7.9x), ketosis (2.8x), and retained fetal membranes (1.31x) when odds ratio benefits are added for CMIR and AMIR (DeLaPaz (2008)). This was in agreement with previous research from our group that showed reduced mastitis in 2 out of 3 herds tested (Wagter et al. (2000)). Important genetic and phenotypic correlations between IR and health traits were noted. These associations were generally positive but some negative associations with reproductive traits were seen. Genetic correlations between IR and production traits varied. Generally associations between H-CMIR and H-AMIR with milk production were positive and negative, respectively, again highlighting the need to include both traits in selection for enhanced health and productivity.

As might be expected, Holsteins with H-CMIR had more T-lymphocytes, whereas those animals with H-AMIR had more B-lymphocytes. Combining both CMIR and AMIR in selection is expected to capitalize on improvements in both traits which work together to control intra- and extracellular pathogens (Wilkie and Mallard (2000)). Microarray studies, confirmed by rtPCR, also indicated significant differences in gene expression between H, A and L responders, particularly within T-lymphocyte related genes (Nino-Soto et al. (2008)).

Epigenetic changes in bovine type I (IFN-γ) and type 2 (IL-4) cytokine promoter genes were seen between wk -4 and day 4 relative to calving (Fig 1). Epigenetic influences in these promoters in response to the immuno-suppressive hormone, DEX, indicated increased methylation of IFN-γ (18%) and decreased methylation of IL4 (31%). ELISAs indicated increased methylation associated with reduced cytokine. Sub-optimal IR, increased disease risk, and changes in the balance between type 1 and type 2 IR during peripartum have been previously reported (Shaver-Weaver et al. (1999); Wagter et al. (2000); Sordillo et al.

(2009)). However, epigenetic influences on bovine cytokine genes, known to steer type 1 and 2 IR, have not been previously reported and are expected to play a critical role in the IR bias that dictates the nature of immunity during the calving period.

Conclusion

These results demonstrate that Holsteins in research and commercial herds can be classified as H, A or L responders using indicators of the adaptive immune system (CMIR and AMIR). H-AMIR cows had more B-cells, whereas H-CMIR cows had more T-cells. High responders generally had lower disease occurrence. Combining AMIR and CMIR is important to enhance overall health benefits. Genetic and phenotypic correlations between IR traits with production and reproduction traits varied but generally there were inverse associations with AMIR and CMIR, again indicating the need to include both IR traits in selection to maximize health, production and reproductive performance. Epigenetic influences on key cytokines known to steer type 1 and 2 IR were also noted. Both genetic and epigenetic factors have been clearly shown here to influence the bovine immune system and should provide points of intervention to improve dairy health.

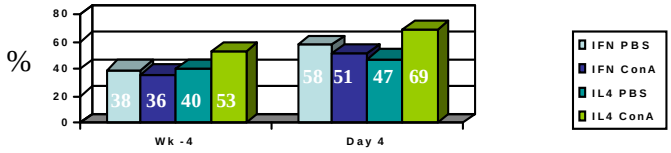

Figure 1: Proportion of CpG Methylation in IFN-γ and IL-4 Cytokine Promoter Genes before (wk -4) and after (day 4) Calving with and without ConA Stimulation of bovine CD4+ T-lymphocytes

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