HIGH AND LOW IMMUNE RESPONSIVENESS OF DAIRY CATTLE: MICROARRAY ANALYSIS OF GENE EXPRESSION ASSOCIATED WITH HIGH AND LOW IMMUNE RESPONSE PHENOTYPES

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INTRODUCTION

In the current climate of consumer concern for both improved food-safety and animal well-being an alternative approach for disease prevention of livestock is urgently needed. Selective breeding to enhance immune responsiveness should be considered a novel and prophylactic approach to improve animal health. Previous research indicates that it is possible to breed pigs for high (H) and low (L) immune responses using estimated breeding values (EBV) of antibody (AMIR) and cell-mediated immune responses (CMIR) (Mallard *et al*., 1998a). High responders had enhanced response to vaccination and increased rate of gain. There is substantial evidence in dairy cattle that innate and acquired defense mechanisms are impaired during the peripartum period when disease occurrence is maximal (Kehrli *et al*., 1989a ; 1989b ; Mallard *et al*., 1998b). Therefore identification of cows that do not exhibit lowered immune responses around calving may positively influence both cow and calf health. To test this hypothesis, a quantitative approach was devised to classify dairy cows based on phenotypic variation of peripartum serum antibody responses to an inert antigen, ovalbumin (OVA) (Wagter *et al*., 2000). Using an immune response (IR) index, cows could be identified as H, L or average responders. High responders had both higher serum and whey antibodies post immunization to OVA and to a J5 *E. coli* vaccine. High responders also had decreased mastitis in 2 out of 3 herds tested (Wagter *et al*., 2000).

To provide protection to a broad range of pathogens, the immune system is capable of invoking two types of responses, AMIR and CMIR. The initial IR-index (Wagter *et al*., 2000) did not contain indicators of CMIR; however, since the ultimate goal is to enhance broad-based disease resistance this is likely best achieved by including indicators of both antibody and CMIR in the index. To evaluate suitable measures of CMIR, various antigen/adjuvant combinations were tested as inducers of delayed-type hypersensitivity (DTH) to be added to the IR-index. The Bacillus Calmette Guèrin (BCG) induced/purified protein derivative (PPD) elicited tuberculin skin test is a reliable measure of DTH; however, its use to identify livestock with high CMIR may be confounded due to previous exposure to *Mycobacteria tuberculosis.* DTH to BCG/PPD was therefore compared with that induced by a non-pathogenic species, *Mycobacteria phlei* (a saprophyte), and its derivative phlein, as the test antigen. Adjuvants included Freund's Complete Adjuvant (FCA), non-ulcerative Freund's adjuvant (NUFA), and complete NUFA.

MATERIALS AND METHODS

Holstein cows (n=5 per treatment group) were assigned randomly to groups immunized with various antigen/adjuvant combinations to compare DTH to M*ycobacterium* and antibody responses to OVA. Briefly, on day 0 cows received OVA, and BCG or *M. phlei* cell wall extract, in FCA, NUFA, or *M. tuberculosis* plus NUFA (complete NUFA) depending on treatment group

assignment. Antigen and adjuvant volumes were determined so as to deliver equivalent amounts of OVA (1.0mg) and M*ycobacterium* (~0.65mg). Immunizations were given intramuscularly, except BCG which was injected intradermally. The negative control group for DTH did not receive M*ycobacterium*, or any of its components. Responses were boosted on day 14. On day 21, DTH was measured by the increase in double skin fold thickness at 6, 24 and 48 hours after intradermal injection of PPD, phlein, OVA, PHA mitogen, and phosphate buffered saline. Serum was collected on days 0, 14 and 21 to measure antibody.

To detect possible genetic differences between H and L, RNA from blood lymphocytes of cows with the highest and lowest antibody and DTH were reverse transcribed. Typically, 8-10µg of Qiagen purified total RNA was used in a 40µl RT reaction volume. RNA from H and L cows were fluorescently labeled with different colour fluors (Cyanine 3 and 5). Each 40µl labeling reaction generated sufficient cDNA to probe 1 array. In this preliminary investigation a human 1.7kD microarray (Ontario Cancer Institute, Princess Margaret Hospital, Toronto, Ca) containing 1700 known genes was probed with labeled cDNA from 2 H and 2 L classified cows following re-stimulation *in vitro* with OVA.

STATISTICAL ANALYSIS

The statistical analysis of the increase in double skin-fold thickness as an indicator of DTH response was performed using a mixed model (proc mix from SAS) and least square means (LSM) to compare the response to each antigen/adjuvant combination at 0, 6, 24 and 48 hours following injection of the test antigen.

Analysis of antibody response to OVA was performed using optical density values obtained by ELISA. A mixed model and LSM were performed to compare responses at day 0, 14 and 21 after immunization within each treatment group. A 95% confidence interval was applied in all analyses and probability values reported as $p<0.05$.

RESULTS AND DISCUSSION

Results indicated that BCG/PPD and *M. phlei*/phlein treatments induced similar DTH with peak reactions at ~ 48 hours after intradermal antigen injection, and that there was substantial phenotypic variation in DTH between cows. Antibody responses to OVA were also similar between treatments. Cows could be ranked for both antibody and CMIR. Only Freund's adjuvants, with or without *M. tuberculosis,* were compared in this study. Non-ulcerative Freund's adjuvant induced fewer granulomas than FCA or complete NUFA. It was generally concluded that BCG/PPD and *M. phlei/*phlein induced similar DTH, but that some cross reaction to PPD was evident following induction of DTH using *M. phlei,* making it a less than ideal alternative for testing CMIR of livestock.
Initial results from the microarrays indicate that the expression of over 200 genes, including; STAT1, natural killer cell enhancing factor A, cathepsin S precursor, leukocyte surface antigen CD53, immunoglobulin J chain, and zinc finger proteins, differ between H and L. Evaluation of a larger number of cattle classified as H or L immune responders is required to construct accurate genetic profiles.

CONCLUSION

Experiments in both pigs and cattle indicate that animals can be ranked based on antibody response and DTH, and that these are highly heritable traits amenable to genetic selection. High responders show enhanced response to vaccination and improved production characteristics (Mallard *et al*., 1998 ; Wagter *et al*., 2002). Livestock with improved immune responsiveness and subsequent inherently enhanced disease resistance will require less antibiotic and chemical intervention. Breeding for heightened immune responses will thereby reduce the risk of antibiotic resistance, enhance food quality and emend animal well-being.

The character and quantity of mRNA within cells, including lymphocytes, dictate to a large degree the biological capability of that cell. Given the ability to classify pigs and cattle based on immune response phenotype it is now feasible to determine which genes control H and L in terms of unique gene expression and quantity of message produced. Genomic methods, including use of micro-arrays, are expected to uncover factors which control immune response and influence resistance to complex infectious disease. Micro-arrays can also provide a genetic profile suitable for standard identification of livestock classified as H and L responders.

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