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Genetic study of immunological parameters in periparturient Holstein cows

Johann Clara Detilleux
Iowa State University

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Detilleux, Johann Clara, Ph.D.

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U·M·I
300 N. Zeeb Rd.
Ann Arbor, MI 48106

Genetic study of immunological parameters in periparturient Holstein cows

by

Johann Clara Detilleux

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ABBREVIATIONS USED

INTRODUCTION

AI	Artificial insemination
RFLP	Restriction fragment length polymorphism
VNTR	Variable tandem repeat
STR	Single tandem repeat
DNA	Deoxyribonucleic acid

LITERATURE REVIEW

PMN	Neutrophils
MHC	Major histocompatibility complex
IMI	Intramammary infection
SCC	Somatic cell counts
BLV	Bovine leukosis virus
PL	Persistent lymphocytosis
EBL	Enzootic bovine leukosis
BIV	Bovine immunodeficiency virus
Ig	Immunoglobulin
BLAD	Bovine leukocyte adhesion deficiency
T_H	Helper T cell
CTL	Cytotoxic T cell
NK	Natural killer

LAK	Lymphokine activated killer
TcR	T cell receptor
h^2	Heritability
BoLA	Bovine lymphocyte antigen
CNS	Coagulase negative staphylococci
RFLP	Restriction fragment length polymorphism
Fab	Antigen recognition portion of immunoglobulin
Fc	Constant portion of immunoglobulin
TNF	Tumor necrosis factor
IFN	Interferon
CSF	Colony-stimulating factor

PAPER I

PMN	Neutrophil
Ig	Immunoglobulin
cpm	count per minute
log	logarithm
PWM	Pokeweed mitogen
PHAP	Phytohemaglutinin P
ConA	Concanavalin A
PME	Eosinophil
ADNC	Antibody-dependent neutrophil cytotoxicity
AINC	Antibody-independent neutrophil cytotoxicity
IL	Interleukin

PAPER II

PMN	Neutrophil
PME	Eosinophil
Ig	Immunoglobulin
PDM	Predicted difference in milk
PTA	Predicted transmitting ability
REML	Restricted maximum likelihood
R^2	Coefficient of determination or R square
h^2	Heritability
ADNC	Antibody-dependent neutrophil cytotoxicity
ConA	Concanavalin A
PWM	Pokeweed mitogen
PHAP	Phytohemaglutinin P
HSP	Heat shock protein

PAPER III

REML	Restricted Maximum Likelihood
SCC	Somatic cell counts
BIV	Bovine immunodeficiency virus
BLV	Bovine leukosis virus
PTA	Predicted transmitting ability
PDM	Predicted difference in milk
CNS	Coagulase negative staphylococci

CPS	Coagulase positive staphylococci
THRE	Threshold model
BLUP	Best linear unbiased prediction
MHC	Major histocompatibility complex

PAPER IV

BoLA	Bovine lymphocyte antigen
MHC	Major histocompatibility complex
BLAD	Bovine leukocyte adhesion deficiency
PCR	Polymerase chain reaction
RFLP	Restriction fragment length polymorphism
SCS	Somatic cell score
BIV	Bovine immunodeficiency virus
BLV	Bovine leukosis virus
BLUP	Best linear unbiased prediction
REML	Restricted Maximum Likelihood
PMN	Neutrophil

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GENERAL INTRODUCTION

Modern methods of animal agriculture and intensification of livestock production would be enhanced with improved resistance to diseases. The chief methods used to control spread of diseases among domestic animals are eradication of infected stock, isolation and quarantine of susceptible animals, sanitation, vaccination, and medication. These methods carry several disadvantages: high cost, potential environmental damage due to drug residues, development of drug-resistant microbial strains, reduction of selective advantage of natural disease resistance, and inefficiency in preventing production diseases (4).

Selection for disease resistance may be done either directly or indirectly. Direct selection includes observation and choice of the most resistant breeding stock under normal environment, and challenge of the breeding stock with specific pathogens, or challenge of its progeny and sibs. Indirect selection is based upon detection of indicators of disease resistance. Such parameters are linked to resistance to diseases. They can be classified into five categories: (1) morphological markers such as eye margin pigmentation in bovine infectious keratoconjunctivitis (pink eye), (2) physiological markers such as hemoglobin type in malaria, (3) immunological parameters such as innate or acquired immune

responses, (4) genes of the immune system such as major histocompatibility complex (MHC) genes, immunoglobulin (Ig) genes, or T cell receptor (TcR) genes, and (5) molecular genetic markers such as restriction fragment length polymorphisms (RFLPs), variable number of tandem repeats loci (VNTRs), single tandem repeats loci (STRs) and microsatellite loci (3,106).

Indirect selection is advantageous because there is no risk to spread disease. We decided to use immunological markers as indicators of disease resistance because in vitro immune assays are relatively easy to measure, at low cost, early in life, in both sexes, and are repeatable. They also reflect more closely the effector stage of the immune response than molecular genetic markers. Indeed, it has been shown that MHC haplotype may confer resistance to one disease challenge while being associated with susceptibility to another (143). Thus, it can be argued that polymorphisms at the level of the DNA protects the species whereas an effective immune response to infection protects in general the individual.

This research is part of a project whose long-term objective is to select bulls entering artificial insemination (A.I.) for health of their daughters. Previous research from this project showed evidence of sire progeny group differences for assays measuring various aspects of the innate immune system in Holstein cows in mid-lactation (72). In the

research reported here, cows are sampled around parturition when most health problems are known to occur. Particular attention is placed upon the study of mastitis which is the single most costly disease on dairy farms, and upon the study of two important retroviruses.

The objectives of the research reported in this dissertation are threefold. The first objective was to obtain estimates of genetic parameters for various immune cell functions in Holstein cattle. The second objective was to obtain estimates of genetic parameters for disease resistance. A final objective was to obtain correlations between health, immunological profiles, and various DNA molecular markers.

Explanation of Thesis Format

This dissertation is presented as an introduction and review of the literature followed by four papers that report the author's work. Each of these papers are in journal format and intended for publication in scientific journals. The organization of the papers vary because they are written for publication in different scientific journals. Computational details are shown in the appendices following each articles but are not intended for publication. A general summary intended to list major conclusions and implications and to suggest further areas of study follows the last article.

LITERATURE REVIEW

Introduction to Epidemiology and Genetics of Infectious Diseases

Heritability (h^2) is a measure of the proportion of the variation in a population which is of additive genetic origin, is a measure of resemblance between relatives, and is the basis for animal selection (34). In dairy cattle, heritability estimates for mastitis vary from less than 1% up to 48% (31,82,85,92,120). Heritability estimates for dystocia decrease from 15% in primiparous calvings to 5% in cows third parity and over (82). Heritability estimates for retained placenta vary from 5% up to 38% (82). Digestive problems are more heritable (h^2 = 21%) than reproductive and respiratory problems (h^2 < 10%) (85). Heritabilities of locomotive problems are less than 20% (85). Heritability of resistance to nematode infection, based on fecal nematode egg counts, is around 50% (131,132). Heritability estimates for ketosis vary from 2 to 31% (89,120). Milk fever has been shown to be influenced by hereditary factors, although most estimates of heritability are close to zero (82). It has also been shown that the three major features of the pathogenesis of bovine leukosis virus (BLV: seroconversion to the major viral envelop protein gp51, proliferation of virus-infected B cells, and

lymphosarcoma) are associated with particular bovine lymphocyte antigen (BoLA) antigenic type (76,77). Given the genetic parameters reviewed here, breeding for disease resistance appears justified.

Besides genetic background, environmental and physiological factors are known to affect health problems. They include management (92), herd (85,119), year (85,110), season (36,110), calving season (119), productivity level (110,146,154), nutritional status (74), sex (74), age (30), stage of lactation (110), and parity effects (85,110). Associations have been found between high milk production and increased susceptibility to mastitis (119); increased prevalence of silent heat, of cystic ovaries, and of metritis (121); and greater health costs (109,154). Diets for dairy cattle have been associated with reproductive diseases (116). The importance of aging in disease resistance is well known. In Holsteins, it has been shown that the risk of milk fever, mastitis, cystic ovaries, dystocia, respiratory diseases, traumatic reticuloperitonitis and foot and leg problems increases with age (30,119). In Jerseys, it has been shown that health costs were significantly greater for third and later parities than in earlier parities (110). In Norwegian cattle, frequencies of cystic ovaries and retained placenta increased with lactation number (121). In Holsteins, seasonal patterns were found for ketosis, cystic ovaries, mastitis,

teat injuries, abortions and traumatic reticuloperitonitis (30).

Mastitis

Mastitis is defined as inflammation of the mammary gland. Worldwide, mastitis is the single most costly disease on the dairy farm. Annual losses associated with mastitis have been estimated at about $1.3 billion for the entire U.S. dairy population, or about $140 to $300 per cow (39,154). Approximately 70% to 80% of the loss is due to lost milk production due to subclinical mastitis (15).

Intramammary infections (IMI) occur when microorganisms gain entrance to the gland via the teat canal, and colonize the duct system or alveoli (27). Pathogens causing mastitis may be put into two categories: major and minor pathogens. Major pathogens include environmental pathogens which inhabit the environment present on dairy farms and transmit the infection by teat end contamination (such as Escherichia coli and esculin hydrolysing streptococci) and contagious pathogens that cause IMI by cow to cow transmission, principally at milking time (such as Staphyloccus aureus and Streptococcus agalactiae). Minor pathogens, such as the coagulase negative staphylococci (CNS) and Corynebacterium bovis, cause IMI mainly through contaminated milking machines (117,118). Infections of the mammary gland caused by E. coli bacteria

occur when E. coli penetrates the teat canal into the teat cistern. If the mammary environment is hospitable, the organism multiplies rapidly and produces large amounts of endotoxin (1). Coliform mastitis occur at any stage of lactation but peak rates of clinical mastitis occur shortly after parturition (1,60,117,118). The best producers and the easiest milkers are more susceptible (1,60,148). The acute symptoms associated with coliform mastitis during the periparturient period are due to the rapid and unrestricted growth of the organism, the release of endotoxin, and the subsequent development of an unlimited inflammatory reaction in the gland (27,53). The S. aureus also causes clinical mastitis in early lactation. If the infection is not cleared by the cow's immune response, a subclinical chronic infection with occasional acute flareups tends to develop (60). A major obstacle to producing a protective immune response to S. aureus is the presence of an exopolysaccharide capsule which masks recognition of cell wall antibodies to neutrophils (PMNs) (44). In addition, these polysaccharides are low in immunogenicity, T-cell-independent, and do not induce protective immunological memory (44). Mastitis due to other staphylococci usually show mild leukocytosis in milk without clinical signs. Mastitis due to streptococci spp. are usually subclinical or chronic with periodic acute flareups (60).

Mechanisms of defense of the udder are classified as non-

phagocytic and phagocytic defenses (25,60). Non-phagocytic defenses are teat duct defenses (keratin plug, teat sphincter, stratified squamous epithelium), non-specific humoral defenses (lactoferrin, lactoperoxidase, lysozyme, cationic proteins, complement), lymphocyte activity of gland, and immunoglobulins. Phagocytic defenses include phagocytic cells, primarily PMNs (139). During mammary inflammation, more than 90% of leukocytes present are PMNs that migrate from blood. However, phagocytic and bactericidal activities of milk neutrophils are depressed when compared with peripheral blood leukocytes (104,139). This could be due to the composition of the milk which lacks energy resource, has low concentration of opsonins, and contains casein micelles and fat globules which are ingested by PMNs and interfere with their ability to phagocyte bacteria (103,139). Similarly, mammary gland lymphocytes respond less to mitogenic stimulation than do peripheral blood lymphocytes (6,7). Possible reasons for this hyporesponsiveness are suppression by factors in gland secretions, low ratio of helper/suppressor T cells, and more likely, suppressive soluble factors produced by bovine mammary leukocytes (7). Bovine lactoferrin is an iron-binding protein occurring among others in milk and in PMNs. During acute bovine mastitis, milk lactoferrin increases by 30-fold corresponding to severity of infection (37,97). The biological roles of lactoferrin include

promoting the adhesion and aggregation of PMNs to the endothelial surfaces, causing a feedback inhibition of granulocyte-monocyte colony-stimulating factor, contributing to PMN bacterial killing (hydrogen peroxide dependent or independent mechanisms), and producing an iron-deficient environment that limits microbicidal growth (37,138)

Genetic resistance to mastitis is likely conferred by a large number of genes each having a small effect. The range of heritability estimates for mastitis based on bacteriological counts in milk is 0 to 20% with most estimates falling below 5% (30,92). For estimates based on clinical mastitis cases, the range is 0 to 50% with most estimates being around 5% (30,92,149). Finally, estimates based on somatic cell counts (SCC), which is often considered as an indicator of mastitis, range from 0 to 38% with most estimates falling between 5 to 10% (16,23,26,31,62,149). Estimates of the genetic correlation between SCC and prevalence of mastitis range from 0.1 to 0.8 (23,31). Various authors found a relationship between MHC Class I alleles and occurrence of mastitis (90,146), and with SCC (99). A positive relationship between bovine immune response to human serum albumin and mastitis susceptibility was also reported (79). Heritability for lactoferrin concentration during lactation has been estimated at 0.35 - 0.44 (37).

Studies showed that incidence of mastitis increased as

parity increased (36,107) and that seasonal differences affect mastitis incidence (36,119). Dietary supplementation with selenium and vitamin E reduces the incidence of mastitis in cows deficient in these nutrients (116). Various authors have found increased mastitis frequency with increasing milk yield (119). In the literature, estimates of genetic correlations between milk yield and mastitis ranged from -0.30 to +0.66 (31). It is well known that cows are highly susceptible to mastitis during the periparturient period. Many intramammary infections (IMI) occur late (last two weeks) in the dry period (117,118) and are associated with clinical mastitis during early lactation (1,117,118). This increased susceptibility of the bovine mammary gland to mastitis is related partially to the ease with which pathogens penetrate and colonize the teat canal (dilatation and shortening of the streak canal, no flushing action of the milking process) and to the diminished capacity of local mammary gland immunity (117,118).

Retroviral infections

Bovine leukosis virus (BLV) and bovine immunodeficiency virus (BIV) are both retroviruses. The total annual cost associated with BLV infection has been estimated at about $44 million for the U.S.A. (137). Bovine immunodeficiency virus has been related to the human immunodeficiency virus (56).

Retroviruses are spherical enveloped particles possessing

glycoprotein surface projections. The internal structure is composed of a core containing two copies of single-stranded RNA. The genetic information is contained in the genes gag (coding for the internal proteins), pol (coding for the RNA polymerase) and env (coding for the envelope proteins). Upon entry in the cell and uncoating, replication starts with the reverse transcription of virus RNA into DNA, which is then made double-stranded. This viral DNA is integrated into the chromosomal DNA of the host cell at non-specified sites. Cellular RNA polymerase II transcribes the integrated provirus into virion mRNA. Virus is released from the cells by budding (32).

Bovine leukosis virus has been shown to be the etiologic agent of enzootic bovine leukosis (EBL) (91). The virus BLV is a C-type oncornavirus that preferentially infects B cells in cattle but also T cells (133). Natural spread of BLV infection among cattle occurs predominantly by horizontal transmission through transfer of infected blood lymphocytes (33). Between 30% to 70% (57) of cows infected with BLV develop persistent lymphocytosis (PL) attributable to polyclonal expansion of the immature B lymphocyte population (18). After a variable period of lymphocytosis, 0.1 to 5% of seropositive cattle and 10 to 50% of lymphocytotic cattle develop lymphosarcoma (18). The humoral and cellular immune response are impaired in BLV-infected cows. Cows with EBL

show a reduction of IgM-producing cells, cows naturally infected with BLV produce immunoglobulins with impaired structural and biological reactivity, and cows experimentally infected with BLV have lower levels of surface IgM and a decrease of T-lymphocytes in the peripheral blood (18).

Bovine immunodeficiency virus (BIV) was first discovered in 1972 (140). This lentivirus causes persistent lymphocytosis, lymphadenopathy, central nervous system lesions, weakness, and emaciation (17,56). The virus infects T helper lymphocytes and monocyte/macrophages and can be transmitted by blood (150). Specific antibodies to BIV are detected two to three weeks after inoculation (17,150). Lentiviruses persist indefinitely in infected hosts and cause disease after prolonged incubation periods (months-years) (141). Natural prevalence of BIV has been estimated at around 4% (150). In one study, 5% of cattle were infected with both BIV and BLV (2) while in another, no correlation between BIV and BLV infection was found (150). It seems that BLV superinfection of cattle previously infected with BIV does not influence lymphocyte replication (141).

Bovine leukocyte adhesion deficiency (BLAD)

This genetic disorder is characterized clinically by recurrent respiratory and gastrointestinal tract infections, periodontitis, delayed wound healing, persistent neutrophilia,

and death at an early age (64,71). Leukocytes of cattle with BLAD show abnormality in the ß-subunit (CD18) of β_2 integrin adhesion molecules (111). This abnormality is due to a mutation of an aspartic acid to glycine substitution at amino acid 128 of the adhesion protein (111). In patients homozygous for the D128G allele, chronic progressive neutrophilia (no left shift), diminished neutrophil ingestion, and impaired C3b-receptor function were observed (71).

Introduction to Genetics of the Immune System

The prerequisite of genetic selection for immunocompetence is the presence of genetic variation in the immune system. Many individual components of the immune system are under polygenic control. In dairy cattle, significant genetic influence has been found for the following immunologic traits: neutrophil phagocytosis (72,147), lymphocyte responses to mitogens (72,147), serum Ig levels (79), serum and milk lysozyme levels (80), and serum hemolytic complement levels (81). In dairy cattle, heritability for IgG and IgM isotypes has been estimated in calf and in mature cows at around 0.07 (21,88). In Angus and Hereford cattle, heritability for serum IgG_1 has been estimated at 0.56 for 36 hr-old calf and at 0.05 for calves at weaning (38). Genetic control of antibody response to various antigens has also been

confirmed in other species (11,87,112).

Some of the most important genes associated with the immune system are MHC genes, genes determining the structure of immunoglobulins, and T cell receptor genes. All those genes belong to the immunoglobulin gene superfamily (12) and their expression is regulated, among others, by some proteins of the immune system, called cytokines.

Bovine Major Histocompatibility complex

Traditionally, three classes of genes have been defined within the MHC. Two of these families encode the class I and class II antigen presenting molecules recognized by T cell receptors. The so-called "class III" gene cluster includes sequences encoding components of the complement system. Class III genes exhibit less polymorphism than members of either class I or class II gene families. Several other unrelated genes, dispersed among the classically defined members of the MHC, encode known proteins such as neuraminidase, steroid 21-hydroxylase, tumor necrosis factor, major heat shock proteins, peptide transporter, collagen, B-associated transcripts, and other proteins with unknown functions (61).

The bovine MHC is referred to as BoLA (Bovine Lymphocyte Antigen) and is located on chromosome 23 (124). Class I molecules consist of a heterodimer of a single α chain associated with a β2 microglobulin which is not encoded within

the MHC. Digestion of the DNA of MHC homozygous cattle with rare cutter restriction enzymes suggests the presence of at least six class I genes (100). So far, only two class I genes (A and B loci) with a functional product have been identified (9). Fifty alloantigenic specificities are recognized internationally (144). Class II molecules consist of an heterodimer of a single α chain non-covalently associated with a single ß chain (20). Studies indicated the existence of a single DRα gene, at least three DRß genes (one of which is a pseudogene), two DQα and DQß genes, one DNα gene, one DOß gene, one DIß gene, one DYα gene and one DYß gene (20,43,114,142). Extensive polymorphism was found by RFLP analysis, with as many as 20 DQα, 17 DQß and 25 DRß RFLP types (113). Southern blot analyses showed close linkage between BoLA-DQß locus and BoLA-A locus (144) and between DQ and DR RFLP polymorphisms (113). Recently, it has been possible to link the C4 complement gene with the DR and DQ genes (42). Other genes located on bovine chromosome 23 are genes for steroid 21-hydroxylase, prolactin, glyoxalase 1, heat shock 70kDa protein 1, and T complex protein (153). There is also evidence that the M blood group locus (M′) is within the class I region and that M′ may be linked to BoLA-A W16 serotype (58).

Class I molecules are present on most nucleated cell types, highest levels are found on lymphoid cells, with lower

levels on liver, intestine, lung, and heart cells. Neither brain nor sperm cells express detectable levels of class I molecules (115). Class II molecules are found primarily on B cells, macrophages, thymic epithelial cells, human activated T-cells and Langerhans cells in the skin. Class II molecules receive their target peptides predominantly from extracellular sources by endocytosis, with subsequent degradation in endosomes or lysosomes, while class I molecules derive their target peptides from intracellular sources with degradation in the cytosol and/or the endoplasmic reticulum (93). Recently, genes encoding molecules involved in the degradation and transport of cytoplasmic proteins binding to class I molecules were located in the BoLA region (28).

Immunoglobulin genes

Antibodies are composed of immunoglobulin (Ig) heavy (H) and light (L) polypeptide chains. The amino-terminal domain (Fab) of both H and L chain comprises the variable (V) region involved in antigen binding. The remainder of the H-chain comprises the hinge region that gives flexibility to the molecule and the constant region (Fc) that mediates immunologic effector functions (complement fixation, placental transfer, and binding to cell surface Fc receptors) (12). Amino acid differences in the carboxyterminal portion of the H chains identify antigenically distinct H chain isotypes which

define the classes and subclasses of Igs. The allelic variants of the isotypes are called allotypes. Individual B cells produce Ig with identical antigen-binding specificities because B cells express the products of only a single H and a single L chain gene (allelic exclusion). The theory of clonal selection of B lymphocytes postulates that each B cell is capable of responding to only a single antigen. When stimulated by this antigen, the lymphocyte proliferates as a clone, producing more antigen-specific antibody. Later in the immune response, B lymphocytes can express a different H chain isotype (class switching). Some antigen-specific B lymphocyte clones differentiate into "memory" cells, which are retained in the animal to allow it to respond rapidly with high-affinity antibodies if reexposed to the antigen (secondary response). The antibodies produced during an immune response to a particular antigen are often encoded by H and L chain genes in which individual nucleotides within the V region are altered (somatic mutation) and B lymphocyte clones producing higher affinity antibodies are selected to proliferate (12).

Thus, the immense diversity of antibody specificities that can be produced by mammals in response to immunization is generated by the combined effects of the heterogeneity of the germ-line encoded antibody, the process of somatic mutation, and the selection of various lymphocyte clones.

T cell receptor genes

T cells recognize antigen via a heterodimeric receptor (TcR). T cells express one of two different types of receptor (TcR1 and TcR2), both complexed to CD3 molecules. The TcR2 consists of an α and ß peptide chain while the TcR1 consists of a γ and δ peptide chain. Two subpopulations of α/ß T lymphocytes, the helper/inducer (T_H) and the cytotoxic/suppressor (CTL) T cells, are identified by antigenically distinct CD4 and CD8 accessory molecules. These molecules determine how the reactivity of the receptors on T_H and CTL subpopulations is directed, respectively, towards class II and class I MHC molecules (41). Most γ/δ T cells do not express CD4 or CD8 molecules. Those γ/δ T cells are relatively more frequent in the ruminant than in humans and mice (49). Ten to 40% of bovine peripheral blood T cells bear TcR1 (134). They are more frequent in young animals (50,134). They constitute 60% of blood-borne T cells in lambs 1-2 weeks after birth, 30% in animals of one year of age, and 5-10% in adult animals of 5-8 years (50). In adult cattle, they are located in the dermal layer of the skin, intestinal epithelium, lamina propria, and in the circulation (49). The localization within the epithelia and the recognition of highly conserved antigens such as heat shock proteins suggests that γ/δ T cells may present the first line of epithelial defenses by eliminating microbial invaders and damaged cells

within the host (47). The question of whether the recognition of foreign antigen by γ/δ T cells is restricted to classical or non-classical MHC molecules remains to be clarified (47)

In addition to the carboxyterminal constant domain, TcR peptide genes contain variable (V), joining (J), and in the case of β and δ chains, diversity (D) regions, which code for the variable aminoterminal portion of TcR peptides. By a process of gene rearrangement that occurs during maturation of T cells in the thymus, V-region and J-region elements combine randomly to form different TcR chain genes. When gene rearrangement events are completed, the T cell repertoire is shaped through positive and negative selection by self antigens in association with MHC molecules. The T cells undergo positive selection to produce a repertoire that recognizes foreign antigen only in the context of self MHC, and negative selection to eliminate from the repertoire those T cells with high affinity to self antigens (98).

Cytokines

Cytokines are proteins involved in regulating the immune response. Lymphokines are produced by lymphocytes and monokines by monocytes or macrophages. Some cytokines are both monokines and lymphokines. Interleukins (IL) are proteins who function as communicators between leukocytes (13). In the bovine, the gene coding for IL-2 has been

located in synthenic group U23 (153).

Generally, following immune recognition, an antigen is processed by an antigen presenting cell (APC), mainly of the monocyte/macrophage lineage. Following this, the first lymphokine (IL-1) is secreted. Interleukin-1 induces T_H cells to secrete IL-2, which is the main growth and differentiation factor for T cells. Other immune activities of IL-1 include maturation of B lymphocytes and natural killer (NK) cells, stimulation of production of bone marrow hematopoietic cells, and chemotaxis (86). Upon its secretion, IL-2 activates B cells to differentiate and divide, activates CTL and NK cells, induces lymphokine activated killer (LAK) cells, and enhances the expression of its own receptor (72). Another lymphokine secreted with IL-2 is IFNγ which induces secretion of Ig by B cells, enhances expression of Fc receptor on macrophage/monocytes and enhances NK cell activity (5). Helper T cells also secrete IL-4 and IL-5. Interleukin-4 is a multifunctional lymphokine responsible for various B cells functions such as proliferation, expression of class II MHC antigen and Fc receptors, and secretion of Igs. Interleukin-4 serves also as a growth factor for T lymphocytes, mast cells, and hematopoietic progenitor cells. Interleukin-5 enhances B cell proliferation and secretion of various Igs (14). In addition, T_H cells participate in the secretion of colony-stimulating factors (CSFs) and IL-3 (65). Colony-stimulating

factors are also produced by other cell types of different embryonic origin and stimulate proliferation and growth of many different cell lineages (13,70,86). Interferon-α (leukocyte IFN), IFN-ß (fibroblast IFN), and IFN-γ (immune IFN), are proteins elaborated by virally infected leukocytes and fibroblasts (IFN-α and -ß) or by immune stimulated leukocytes (IFN-γ) (10). In cattle, the gene coding for IFN-γ has been located on chromosome 5 and the genes for IFN-α and IFN-ß are in synthenic group U18 (153). Antibody production and many neutrophil functions are modulated by IFNs whose effects are up- or down-regulation depending upon the cellular environment, IFN type, IFN dose and duration of exposure, and especially the health status of the recipient animal (70). Tumor necrosis factor α (TNF-α) is produced primarily by activated macrophages. In addition to antitumor effects, TNF-α shares many of the biological activities of IL-1 (13). Interferons, TNF, and lymphotoxin increase Class I expression (145). For an extensive review of the effects of these cytokines in cattle, see Kehrli et al. (70)

Non-genetic effects on the immune system

Some of the environmental and physiological factors found to affect cells and tissues of the immune system include such factors as reproductive stage, age, nutritional status, and season. Deficiencies in protein, energy, zinc, iron, vitamins

B6, E or D have been shown to impair selected phagocytic functions (55).

In the dairy cow, a marked impairment has been observed at parturition for various in vitro immunological tests measuring PMN functions (69,96,108), lymphocyte blastogenesis (59,68,108), and in vitro IgM production (125,126). This periparturient immunosuppression is not due to parturient hypocalcemia, nor to increase in hormone 1, 25-dihydroxy-vitamin D3 [1, 25-(OH_2)D] (67). Some immunosuppression can be prevented by injection of recombinant bovine granulocyte colony-stimulating factor (66,125).

Studies in mice and dogs showed involution of the thymus after sexual maturity (40,94), attenuation of immune function of T and B cells and a decline in phagocytic activity (40,102).

Several of the antibiotics and anti-inflammatory drugs used for the treatment of infections in dairy cattle have effects on phagocytic activity of bovine PMN. In cattle, it has been shown that tetracycline and aminoglycosides decrease PMN phagocytosis (101,155), acetylsalycilic acid increases mammary PMN phagocytosis while macrolides, and antibacterial quinolones do not interfere with PMN phagocytic functions (101). In another study, cloxacillin, cephapirin (ß-lactam antibiotics), novobiocin, and dihydrostreptomycin with procaine penicillin G reduced bovine PMN ingestion of S.

aureus and reduced PMN killing of ingested S. aureus (83). Dexamethazone decreases luminol-dependent chemiluminescence, phagocytosis of Pasteurella multocida and S. aureus, and antibody-dependent cell cytotoxicity (ADCC) against bovine herpes virus 1 infected fibroblasts (105)

Association between Innate Immune Response and Infectious Diseases

Cells and proteins of the immune system

Susceptibility to infectious diseases has been associated with altered immune functions in virtually all animal species. However, we will restrict our review to the bovine species.

Increased prevalence of clinical mastitis has been shown in cows whose neutrophils had a low capacity for phagocytosis (52,151). In an experiment in which the respiratory burst competence of blood PMNs was measured in cows subsequently infected with E. coli, Heyneman et al. (52) showed that elevated blood PMN numbers in combination with high ability to produce oxygen metabolites limited bacterial replication in the mammary gland. Chemotactic activity of peripheral leukocytes was decreased in cows with retained placenta (45,46). Decreased random migration, ingestion of S. aureus, cytochrome C reduction, iodination, antibody-independent cell-mediated cytotoxicity, and cytoplasmic calcium flux were

observed in cattle persistently infected with Bovine Viral Diarrhea (BVD) virus (19). This impairment is thought to contribute to secondary viral and bacterial diseases of cattle.

Complement activation products attract phagocytic cells to the site of injury causing leukocytes to localize at the site of tissue injury. Complement activation products can also neutralize invading pathogens by causing direct cell lysis or by stimulating phagocytes to release toxic metabolites. Complement has also roles in antigen clearance, immune complex solubilization and immune responsiveness to antigens (35,95). Complement may play a significant role in the host response to gram-negative bacterial infections (54).

Roles of immunoglobulins (Ig) include agglutination of antigenic determinants, neutralization of bacterial exotoxins, complement activation, activation of immnune cells (phagocytosis, cell degranulation, antibody-dependent cell cytotoxicity or ADCC, and cell chemotaxis) (22). Subclass IgG_1 constitutes more than 90% of all colostral Ig which are transferred to the calf and protect it against diseases during early life (24). Immunoglobulin G_1 is the main IgG subclass in the milk and is derived mainly by active transport from serum (22). Allotype IgG_{2a} has been shown to be most important in neutrophil opsonization and ADCC which could be due to the presence of an unique IgG_{2a} Fc receptor (51). Suboptimal serum

levels in subclass IgG_2 have been associated with increased incidence of pyogenic mastitis (78). Immunoglobulin M (IgM) plays a crucial role in resistance to bacterial and protozoal parasites because of its good complement-binding activity and efficient agglutination (22). After activation of the complement system, IgM shows more bactericidal activity against coliforms than IgG_1 (60).

Lymphocyte functions are also altered by infectious diseases. Clinical mastitis was associated with impaired blastogenic response of mammary gland lymphocytes to different mitogens (6). Blast cell proliferation is reduced in animals infected with Mycobacterium bovis (75) and in cattle infected with BVD (19). The expression of BoLA-DR is reduced on B cells of approximately 25% of cows with BLV-derived persistent lymphocytosis while level of surface IgM is increased (29). Lymphocytes from cattle with EBL bound more IgG than normal lymphocytes but the binding capacity of Fc-receptors towards IgG is lowered (29).

Cytokines are another family of proteins involved with immune regulation. Cytokines are diverse in their chemical nature and in their effects on the immune system. Recombinant bovine interferons α and γ (rBoIFN-α and rBoIFN-γ) reduce the severity of bovine respiratory disease in bovine herpes virus (BHV-1) infected calves. Recombinant bovine rBoIFN-α reduces also the incidence of enzootic calf pneumonia and decreased

the septicemia induced by Salmonella typhimurium (70). Recombinant human granulocyte-colony stimulating factor (rhG-CSF) appears to reduce the incidence of experimentally induced intramammary (IMI) with S. aureus and Klebsiella pneumoniae (86) while recombinant bovine IL-2 (rBoIL-2) increases the cure rate of mastitis due to S. aureus (152).

Genes of the immune system

Because Ig genes and the TcR genes undergo rearrangement and diversification during the ontogeny of an immune response, the haplotypes of an animal for those genes have much less influence on their immune response than their MHC haplotypes which remains fixed. Although the MHC molecules are fixed within an individual, they are extremely polymorphic within the population as a whole. It should be noted that the degree of polymorphism within the MHC is greater at the level of the DNA than at the level of the cell surface (143). Mechanisms by which MHC molecules could facilitate the development of some diseases is by presenting foreign peptides to TcR with different efficiency (e.g., pemphigus vulgaris), by combinatorial pairing of different α and ß chains (e.g., Sjögren's syndrom), by abnormal cell expression (e.g., autoimmune thyroid disease) and by molecular mimicry (e.g., rheumatoid arthritis) (48,136).

There have been numerous reports of BoLA association with

various diseases (156) and health costs (8,146). Previous studies found association of W6 class I serotype with higher milk cell counts and antitrypsin levels (99), with higher levels of artificial tick infestation (Boophilus microplus) (128,129), with higher incidence of ocular squamous cell carcinoma (cancer-eye) (130), and with higher prevalence of persistent lymphocytosis due to BLV (127), when compared to the other class I serotypes. There are some indications that class I serotype W2 is associated with resistance to clinical mastitis and W16 with susceptibility (122,123). Allele W14(W18) has been associated with a decrease in California and Wisconsin mastitis tests while allele W11 was associated with decreased clinical mastitis (146). Class II DQ1α genotype has been associated with low breeding value for clinical mastitis (84).

BoLA class I serotypes have also been associated with incidence of BLV-dependent persistent lymphocytosis (73,76,127). Cows with W12(W30) serotype seroconvert at a younger age and have an higher incidence of persistent lymphocytosis (PL), whereas cows with W14 seroconvert at a older age and have a lower incidence of PL, than cows with other BoLA-A alleles (76,77). Reports showed also weak association of W9 and CA45 class I serotype with high and low faecal worm egg counts, respectively (131). No association of BoLA class I serotypes with the frequency of treatment for

respiratory diseases has been found (156). It has also been suggested that MHC Class I compatibility between dam and calf increased the risk of retained placenta in healthy, normally calving cattle (63).

So far, no work has been done in cattle on the genes controlling macrophage activity. In the mouse, however, the role of macrophages in resisting infectious diseases has been shown to be controlled by a single gene or gene complex for four infectious diseases Bcg gene (Mycobacterium lepraemurium, Mycobacterium bovis), Ity gene (Salmonella Typhymurium), and Lsh gene (Leishmania donovani). The gene(s) controlling resistance to all four of these infectious organisms has(have) been mapped to chromosome 1 and could possibly be the same gene (135).

PAPER I. STUDY OF IMMUNOLOGICAL PARAMETERS IN PERIPARTURIENT HOLSTEIN CATTLE SELECTED FOR HIGH AND AVERAGE MILK PRODUCTIVITY

Study of Immunological Parameters in Periparturient Holstein Cattle Selected for High and Average Milk Productivity

J. C. Detilleux[1], M. E. Kehrli, Jr.[2], J. R. Stabel[3], A. E. Freeman[1], D. H. Kelley[1]

[1]Animal Science Department, Iowa State University, Ames, IA 50011

[2]US Department of Agriculture, Agricultural Research Service, National Animal Disease Center, Metabolic Diseases and Immunology Research Unit, Ames, IA 50010

[3]US Department of Agriculture, Agricultural Research Service, National Animal Disease Center, Leptospirosis/Mycobacteriosis Research Unit, Ames, IA 50010

ABSTRACT

Immunological profiles were evaluated in 137 periparturient Holstein cows selected for different levels of milk productivity. The total number of white blood cells decreased during the periparturient period. With the exception of neutrophil ingestion of Staphylococcus aureus, all assays measuring neutrophil functions were depressed beginning two to three weeks before calving through three weeks after calving. Serum concentration in immunoglobulin G_1 decreased while serum concentration in immunoglobulin G_2 increased around calving time. Serum complement and conglutinin activities decreased before calving and reached a minimum around calving time.

High producers for milk pounds of fat plus proteins and for milk production had significantly higher numbers of circulating neutrophils and mononuclear cells, had higher neutrophil resting chemiluminescence and higher neutrophil directed migration than average producers. There were significant sire progeny group differences for most immunological parameters tested.

INTRODUCTION

Infectious disease in livestock is a prominent factor reducing the efficiency of cheap and quality food production. Annual losses associated with mastitis alone have been estimated at $140 to $300 per cow (Gill et al., 1990; Shanks et al., 1981). Researchers have proposed to improve disease resistance by genetic selection. Although improved general resistance to diseases is probably difficult to attain, it is possible to select for animals resistant to economically important diseases, such as mastitis (Axford and Owen, 1991). The bovine mammary gland is more susceptible to infection and clinical disease during the periparturient period than the remainder of lactation or the dry period (Smith et al., 1985). The immune system plays a crucial role in response to invading organisms and disease may result when the immune system is unable to efficaciously respond to the presence of infectious agents in the host. On the other hand, the protection of the fetus from maternal rejection and its survival to term suggest some alterations in the immune capabilities of a female during pregnancy and parturition. Indeed, several authors observed marked impairment in bovine neutrophil and lymphocyte functions at parturition (Kehrli et al., 1989a; Kehrli et al., 1989b; Saad et al., 1989; Guidry et al., 1976). Therefore, this immunosuppression may compromise the ability of the cow

to combat infection occurring around parturition.

Our hypothesis is that there are genetic differences in immune function in periparturient cows. To test this hypothesis, it is necessary to first describe the immunological dysfunction observed in dairy cows in our herd. Reported here are functional alterations in activities of bovine neutrophils and lymphocytes, as well as changes in serum protein levels (immunoglobulin, complement, and conglutinin) during the periparturient period in cows selected for different levels of milk productivity.

MATERIALS AND METHODS

Animals and Experimental Design

Periparturient Holstein cows (n=137) were used in this study. For up to seven generations (1968-1986), cows were selected for milk production using artificial insemination (A.I.) sires selected for highest and average Predicted Difference for Milk (PDM) from all sires used in the USA. Since 1986, sires are selected for highest and average Predicted Transmitting Ability for combined pounds milk fat plus protein (PTA-FP) (Bertrand et al., 1985). All animals are managed the same and fed for high production. Blood samples were collected weekly starting 5 weeks before calving until 5 weeks after calving. Approximately 20 cows were sampled each week. After excluding twins, cows were randomly sampled and balanced by genetic lines. Five Holstein steers were used as laboratory standards to reduce the day-to-day variability typically seen in immune cell function assays.

Immune Assays

Leukocyte preparation

Granulocytes were separated by hypotonic lysis from packed red blood cells (Kehrli et al., 1989a). Remaining

cells, usually more than 95% granulocytes (neutrophils and eosinophils), were resuspended to $5X10^7$/ml in physiologic buffered saline (PBS). Lymphocytes were isolated from buffy coat as previously described (Kehrli et al., 1989b).

Hematologic studies

Leukocyte counts (cells/μl) in blood were obtained by electronic counting (CellTrack, Angel Engineering Corp., Trumbull, CT). Cytocentrifuge films were stained (StatStain, Volu-Sol Corp., Henderson, NV) and 200 cells or more were differentiated into neutrophils, eosinophils, or mononuclear cells. Relative proportions and numbers of cells per microliter of blood were calculated for each cell type.

Neutrophil function assays

Procedures for evaluating blood neutrophil (PMN) functions were performed as previously described and included the following assays. Random (area mm^2) and directed (directed mm/random mm) migration under agarose were performed as described (Kehrli et al., 1989a). These assays are in vitro measures of the ability of neutrophils to move through body tissues. Opsonized zymosan phagocytosis-associated native (nonluminol-dependent) chemiluminescence activity (log_{10} photons detected over a 2-h period) detects light emission during the metabolic burst and is dependent upon the

generation of free radicals (Roth and Kaeberlee, 1981a). Cytochrome C reduction (nmoles O_2 reduced/10^7 PMN/h) specifically measures production of superoxide anion (Canning et al., 1986). Iodination (nmoles NaI/10^7 PMN/h) evaluates degranulation of primary granules by measuring the peroxidase-H_2O_2-iodide system which results in protein iodination and bacterial killing (Roth and Kaeberlee, 1981a). Ingestion (% of 60 Staphylococcus aureus ingested/PMN) evaluates the percent engulfment of a mastitis pathogen in 10 minutes (Roth and Kaeberlee, 1981a). Antibody-dependent and antibody-independent neutrophil cytotoxicity (% release of ^{51}Cr) are measures of the extracellular killing ability of the neutrophils. Radiolabelled target cells are used in both the presence and absence of a specific antibody against these target cells (Roth and Kaeberlee, 1981c).

Lymphocyte blastogenesis

Blastogenic transformation (log_{10} cpm) of lymphocytes was measured as described (Kehrli et al., 1989b). This test detects the amount of radiolabelled thymidine incorporated into cultured cells. Media for mitogenic stimulation contained either 5 μg of concanavalin A (ConA, C-2010, Sigma Chemical Company, St Louis, MO), 5 μg of phytohemagglutinin P (PHAP, L-9132, Sigma Chemical Company, St Louis, MO) or 5 μg of pokeweed mitogen (PWM, L-9379, Sigma Chemical Company, St

Louis, MO) per ml of medium. The binding of plant lectins to the surface of lymphocytes mimics physiologic interaction between antigen presenting cells and lymphocytes which leads to blast transformation and clonal expansion of lymphocytes.

In vitro antibody production

Pokeweed mitogen-driven production of polyclonal immunoglobulin M (ng IgM/ml) by cultures of B lymphocytes was determined as described (Stabel et al., 1991).

Serum protein assays

Immunoglobulin isotypes (IgM, IgG_1, IgG_2) were determined by radial immunodiffusion using commercially available kits (mg/dl; VET-RID, Bethyl Labs, Inc., Montgomery, TX). Serum complement activity (hemolysis area in mm^2) was determined by a hemolysis in gel (HIG) assay in which hemolysis of guinea pig erythrocytes opsonized with specific bovine antisera is induced by bovine sera (Thurston et al., 1986). Serum conglutinin activity (ratio to serum standard) was determined by agglutination of E. coli (Thurston et al., 1989).

Statistical Analyses

To account for the variability inherent to neutrophil function and lymphocyte blastogenesis assays, daily ratios

were computed for each cow by dividing each daily individual cow result by the daily mean of the results for the five steers. A logarithmic (log_{10}) transformation was then applied to each ratio. A square root transformation was applied to the number of white blood cells. Both transformations provide measures that are more normally distributed than the raw data. The ratios were computed for each week the cow was sampled and expressed in relation to each cow's calving date.

It is known that eosinophils (PMEs), which are co-isolated with PMNs in our laboratory procedures, alter PMN assays (Roth and Kaeberlee, 1981c). Therefore, the effect of percentage of PME (%PME) on the PMN assays (PMN ratio) was estimated by linear regression. For each individual PMN assay, ordinary least squares estimation was applied with the model [1], and the least squares estimates of ß were used to adjust the values obtained for each PMN assay to a common level of PME contamination. The model [1] is:

$$y = \mu + \beta x + e \qquad [1]$$

where:

- y = log_{10} of the lab standards ratio for one particular PMN assay,
- x = log_{10} of the % of PME contamination in the PMN preparation,
- β = regression coefficient of x on y, and

e = error which is assumed N (0, σ_e^2).

To study the effects of genetic lines on immune assays, the following model [2] was applied to the adjusted PMN values from the entire set of immune assays:

$$y_{ijklmn} = \mu + \lambda_i + s_{ij} + \zeta_k + \psi_l + \pi_m + \epsilon_{ijklmn} \qquad [2]$$

where:

y_{ijklmn} = adjusted value for one immune assay,

μ = overall mean,

λ_i = genetic line (i=1, 2, 3, 4),

s_{ij} = sire effect (j=1, 2, ..n_i; n_i= number of sires per genetic line),

ζ_k = season of calving (k=1, 2),

ψ_l = time when the sample was taken (week with respect to calving date),

π_m = parity number (m=1, 2, 3, 4), and

ϵ_{ijklmn} = error which is assumed N (0, σ_ϵ^2).

Least squares analysis of variance computed with PROC GLM in SAS (380) was used to determine which effects significantly

affected the immune assay. The sires within line mean square was used to test for line effects. Two seasons of calving were used. The first season included cows calving from May 1, 1990 through October 1, 1990 and from May 1, 1991 until the end of the study in September 1991 (= summer). Cows calving outside the summer season were included in the second season effect (= winter). Cows were also classified as first parity cows, and second parity cows or over.

RESULTS

Effects of Eosinophil Contamination on Neutrophil Assays

Least squares estimates for the effects of PME contamination on PMN assays are shown on Table 1. These estimates demonstrate that bovine PMEs tended to be less active than PMNs in ingesting S. aureus and in reducing cytochrome C, and more active in performing iodination and native chemiluminescence. No significant differences were found between the two cell types in their ability to perform ADNC and AINC and to migrate under agarose.

Periparturient Changes in Leukograms and Immune Assays

The number of blood mononuclear cells, neutrophils, and eosinophils had tendency to decrease while the number of band cells had tendency to increase during the periparturient period (Fig. 1).

Random migration (Fig. 2) by neutrophils increased up to two weeks before parturition, and decreased rapidly the first week after calving. No significant changes were observed for PMN directed migration.

The burst of oxidative metabolism associated with phagocytosis was clearly impaired the first week after calving

(Fig. 3). Compared to standardized values obtained before calving, values on the first week post-partum decreased by 25%, 5%, and 10% for iodination, cytochrome C, and stimulated chemiluminescence assays, respectively. Neutrophil bacterial ingestion was enhanced at calving time, reaching a plateau 20% higher than initial prepartum levels.

Antibody-dependent neutrophil cytotoxicity (ADNC) was also altered around parturition (Fig. 4). Standardized ADNC values increased gradually from a value of 67%, five weeks before calving; then reached a maximum at 72%, three weeks before calving; and then decreased in the week following calving.

Proliferative responses of lymphocytes stimulated by polyclonal mitogens (inducing proliferation of T and B cells) were impaired at calving time (Fig. 5). The general response induced by all three mitogens peaked 3 weeks before calving and then decreased gradually to reach a minimum one week after calving. One week after calving, lymphocyte response to CONA was 48% lower, lymphocyte response to PWM was 24% lower, and lymphocyte response to PHAP was 42% lower than responses observed three weeks before calving. Five weeks after calving, lymphocyte responses to CONA and PWM were higher than responses observed three weeks before calving. Responses to PHAP returned to initial prepartum levels three to four weeks after calving.

In vitro IgM production by B lymphocytes decreased as calving approached, reached a minimum at calving time, and increased after parturition (Fig. 6). Compared to levels observed four weeks before calving, IgM production decreased by 18.5%. Serum IgG_1 concentration one week before calving were 62% lower than levels observed five weeks before calving (Fig. 7). Five weeks after calving time, serum concentration in IgG_1 were 30% higher than levels observed five weeks before calving. Serum IgG_2 concentration was 20% higher two to three weeks after calving than five weeks before calving. On the other hand, serum IgM concentration decreased slightly by 7% over the 10 weeks of sampling.

Serum conglutinin reached a minimum one week after calving and returned to initial prepartum levels in the second week after calving (Fig. 8). Serum complement was 6% higher two weeks after calving than five weeks before calving.

Effects of Genetic Lines on the Immune Assays

The effects of genetic lines on the immune assays are shown in Tables 2 and 3. Statistically significant effects of lines were found for resting chemiluminescence ($p<0.10$), directed migration ($p<0.10$), number of circulating neutrophils ($p<0.10$) and number of circulating mononuclear cells ($p<0.10$). Directed migration was highest in cows selected for high PTA-

FP (Table 2). Cows selected for high PTA-FP or high PDM had higher number of circulating leukocytes, higher values for three PMN assays (native chemiluminescence, iodination, and ADNC), lower values for S. aureus ingestion, lower serum IgG_1 concentration, and lower lymphoblastic response to PHAP and PWM than cows with average PTA-FP or average PDM, respectively. With the only exception of PMN directed migration, sire effect (not shown in tables) was statistically significant for all the assays shown in Tables 2 and 3 ($p<0.01$).

DISCUSSION

Periparturient Changes in Leukograms and Immune Assays

Impaired immune cell functions at parturition has been shown in Holsteins (Kehrli et al., 1989b; Kehrli et al., 1989a). The hormonal and metabolic changes that prepare the mammary gland for lactation and the reproductive tract for parturition may contribute to impaired immune function. In dairy cattle, negative energy and protein balance, and various degrees of hypocalcemia are associated with the onset of lactation (Kehrli and Goff, 1989). High concentration of estrogens are reached during the final days of gestation in cows and decrease rapidly after parturition. Plasma progesterone concentrations decrease rapidly about one day before parturition. Prolactin level peaks at calving time and serum concentration of cortisol elevates sharply with parturition and decreases within days (hours) after parturition (Wetteman, 1980).

Increased concentrations of progesterone have been associated with depressed iodination, enhanced ADNC, and PMN random migration (Roth et al., 1982a). Administration of adrenocorticotrophic hormone (ACTH) to yearling steers has been shown to cause neutrophilia (left shift), eosinopenia, a decrease in lymphocytes blastogenic response to mitogens, a

decrease in PMN iodination, and an increase in the ability of PMNs to migrate under agarose (Roth et al., 1982b). Dexamethasone injections inhibit ingestion of S. aureus, nitroblue tetrazolium reduction, chemiluminescence, and ADNC by bovine PMNs (Roth and Kaeberlee, 1981b).

Although we did not explicitly compare the immune functions of PMEs and PMNs, the estimates shown in Table 1 agree with previous reports showing that bovine PMEs are less active than PMNs in ingesting S. aureus, more active in performing iodination and resting or stimulated chemiluminescence, and as effective as PMNs in migrating (Roth and Kaeberlee, 1981c).

As shown in previous studies (Saad et al., 1989; Nagahata et al., 1988; Kehrli et al., 1989b), all cows were leukopenic around calving time (Fig. 1). It is thought that the extensive influx of PMNs into the reproductive tract is the cause of the transient neutropenia after calving (Gunnink, 1984). In this study and in others, the number of immature PMNs (band cells) was found to increase at calving (Saad et al., 1989).

We confirmed in this study that neutrophil functions are impaired in periparturient cows (Kehrli et al., 1989a; Saad et al., 1989; Guidry et al., 1976). The burst of oxidative metabolism associated with phagocytosis and the neutrophil bacterial ingestion were altered in reversed pattern around

calving time (Fig. 3). One interpretation for this reversed relationship is that less energy is consumed by oxidative reactions and more energy is available to perform ingestion (Kehrli et al., 1989b). It should also be noted that the number of band cells increased at calving (Saad et al., 1989) and that no oxidative metabolic stimulation was seen in immature bovine neutrophils while phagocytosis of E. coli was seen in all maturation stages (Silva et al., 1989).

It was shown in the study reported here and in previous studies (Ishikawa, 1987; Kehrli et al., 1989b; Saad et al., 1989; Kashiwazaki et al., 1985) that proliferative responses of blood lymphocytes to mitogens are impaired at calving time (Fig. 5). Periparturient alterations of in vitro lymphocyte activities may reflect in vivo changes: different blood lymphocyte subpopulations (Saad et al., 1989) or occurrence of inhibitor factors or suppressive cells which may reduce cell metabolism or induce lymphotoxicity (Birkeland and Kristoffersen, 1980). In Holsteins, the ratio of helper to suppressor T cells do not change during the periparturient period, but a higher percent of T helper cells was observed after calving than before calving (Harp et al., 1991).

As previously shown (Stabel et al., 1991), in vitro IgM production by B lymphocytes is altered around calving (Fig.6).

Serum immunoglobulin concentration varied around calving time (Fig. 7). In bovine and sheep, it has been reported that

serum IgG levels decrease rapidly from the 3rd trimester to a minimum at parturition (Ishikawa, 1987). Therefore, it is possible that IgG_2 concentration started to decrease more than five weeks before calving, at the time when we started to sample our cows. The observed decline in serum IgG_1 could be due to compartimentalization of IgG_1 into lacteal secretion during colostrogenesis and/or follow impairment of plasma cell production of IgG_1 (Ishikawa, 1987), also shown for the production of IgM by our cows in Fig. 6.

Effects of Genetic Lines on Immune Assays

High producers had high values for numerous immune assays (Tables 3 and 4). This result is promising in that selection for improved immune function could be achieved without detriment to milk productivity. The findings that high PTA-FP and high PDM cows had significantly higher number of PMNs and higher PMN ability to perform the metabolic burst is important because PMNs provide front line defenses that can be rapidly mobilized and activated against infectious or toxic agents (van der Valk and Herman, 1987). However, additional work is needed to determine precisely the genetic effects of innate immune response on resistance to diseases.

The highly significant sire effects for all the immune assays confirmed that general immune function is subject to

some genetic control. Indeed, in dairy cattle, significant genetic influence has been found for the following immunologic traits: neutrophil phagocytosis and lymphocyte responses to mitogens (Kehrli et al., 1991b; Weigel et al., 1991), serum Ig and lysozyme levels (Lie et al., 1986), and serum hemolytic complement levels (Lie et al., 1983)

CONCLUSIONS

In conclusion, we have demonstrated immunologic decreased function in 137 periparturient dairy cows under different management conditions than our previous reports on 39 cows (Stabel et al., 1991; Kehrli et al., 1989b; Kehrli et al., 1989a). Other reports (Ishikawa, 1987; Nagahata et al., 1988; Saad et al., 1989; Nagahata et al., 1991) have confirmed immunosuppression in periparturient dairy cows. This period coincides with increased incidence of subclinical and clinical infectious disease. So far, attempts to prevent the immunosuppression by preventing hypocalcemia (Kehrli et al., 1990; Kehrli and Goff, 1989), by administrating cytokines (Daley et al., 1991; Nickerson et al., 1989; Kehrli et al., 1991a), or by nutritional manipulations (Jain, 1979) have had limited success.

We have shown genetic differences among periparturient cows and sire progeny groups. If sufficient genetic variation can be demonstrated in the immune response of periparturient cows, genetic selection for dairy cows who experience less severe periparturient immunosuppression would appear to be both achievable and desirable in the light of the published research over the past six years.

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Table 1. Effects of eosinophil contamination in neutrophil preparations on values obtained for neutrophil functions. Ordinary least squares estimates of θ and standards errors obtained from model (1) explained in the text

PMN assay	Least squares estimates	Standard error
Migration		
Random	-0.006	0.009
Directed	-0.012	0.005
Chemiluminescence		
Resting	0.090	0.007
Directed	0.027	0.006
Cytochrome C reduction	-0.020	0.005
Iodination	0.117	0.009
Ingestion	-0.031	0.007
AINC	-0.010	0.030
ADNC	0.004	0.009

Table 2. Genetic line least-squares means for leukograms and neutrophil functions obtained from model (2) explained in the text

			Least-squares means			
			PTA-FP[a]		PDM[b]	
Immune assays	R^2	p[c]	High	Avg.[d]	High	Avg.
Leukograms (number/μl)						
Neutrophils	0.21	0.05	2997	2958	2668	2205
Eosinophils	0.35	0.29	332	264	274	222
Mononuclear cells	0.34	0.06	4806	4761	3722	3152
PMN assays (% lab standards)						
Migration						
Random	0.16	0.83	91	94	95	95
Directed	0.05	0.05	102	96	99	101
Chemiluminescence						
Resting	0.24	0.06	68	64	84	78
Stimulated	0.19	0.11	97	91	101	95
Cytochrome C reduction	0.07	0.65	104	104	100	104
Iodination	0.20	0.93	62	61	63	62
Ingestion	0.13	0.72	116	116	115	121
ADNC[e]	0.10	0.18	63	60	73	72

[a]PTA-FP= Predicted transmitting ability for pounds of milk fat plus protein; [b]PDM= predicted difference for milk; [c]p =probability of F test for significance of line effect; [d]Avg. = average; [e]Antibody-dependent neutrophil cytotoxicity

Table 3. Genetic line least-squares means for lymphocytes and serum protein functions obtained from model (2) explained in the text

			Least-squares means			
			PTA-FP[a]		PDM[b]	
Immune assays	R^2	p[c]	HFP[a]	AFP[b]	HM[c]	AM[d]
Lymphoblastogenesis assays (% lab standards)						
Concanavalin A	0.18	0.27	105	133	73	70
Phytohemagglutinin P	0.20	0.75	78	81	49	49
Pokeweed mitogen	0.18	0.42	98	128	73	85
In vivo IgM production (ng/dl)	0.18	0.52	99	111	75	73
Serum protein assays						
IgG_1 concentration (mg/dl)	0.56	0.23	411	458	459	548
IgG_2 concentration (mg/dl)	0.37	0.23	1597	1335	1607	1584
IgM concentration (mg/dl)	0.39	0.20	238	282	233	209
Serum complement (mm of hemolysis)	0.28	0.42	8.6	8.6	8.3	8.1
Serum conglutinin (reciprocal titer)	0.14	0.27	7.5	7.2	6.8	6.9

[a]PTA-FP= Predicted transmitting ability for pounds of milk fat plus protein; [b]PDM= predicted difference for milk; [c]p= p-value for significance of line effect

Figure 1. Total number of leukocytes per μl of blood

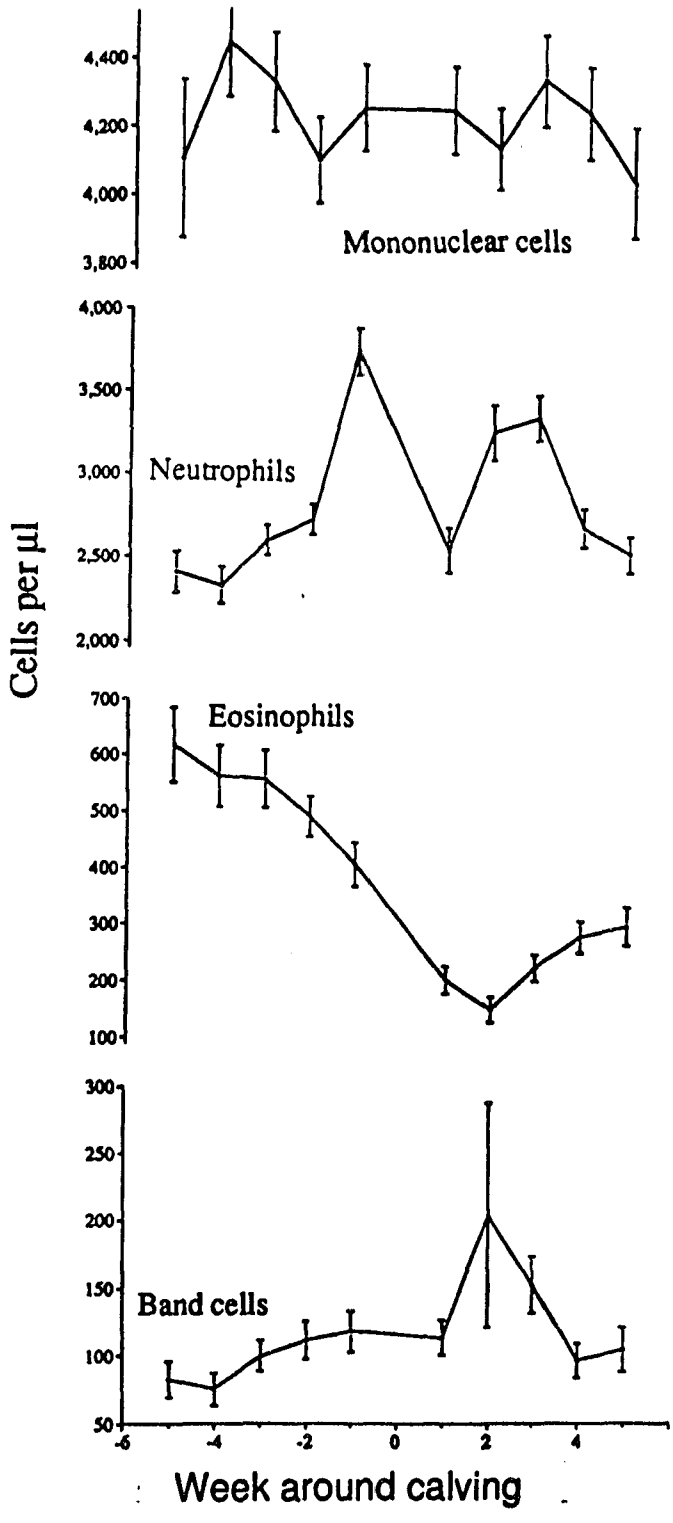

Mononuclear cells
Neutrophils
Eosinophils
Band cells
Cells per µl
Week around calving
4,400
4,200
4,000
3,800
3,500
3,000
2,500
2,000
700
600
500
400
300
200
100
250
150
50
-6
-4
-2
0
2
4

Figure 2. Neutrophil random migration and directed migration under agarose toward chemotactic factor

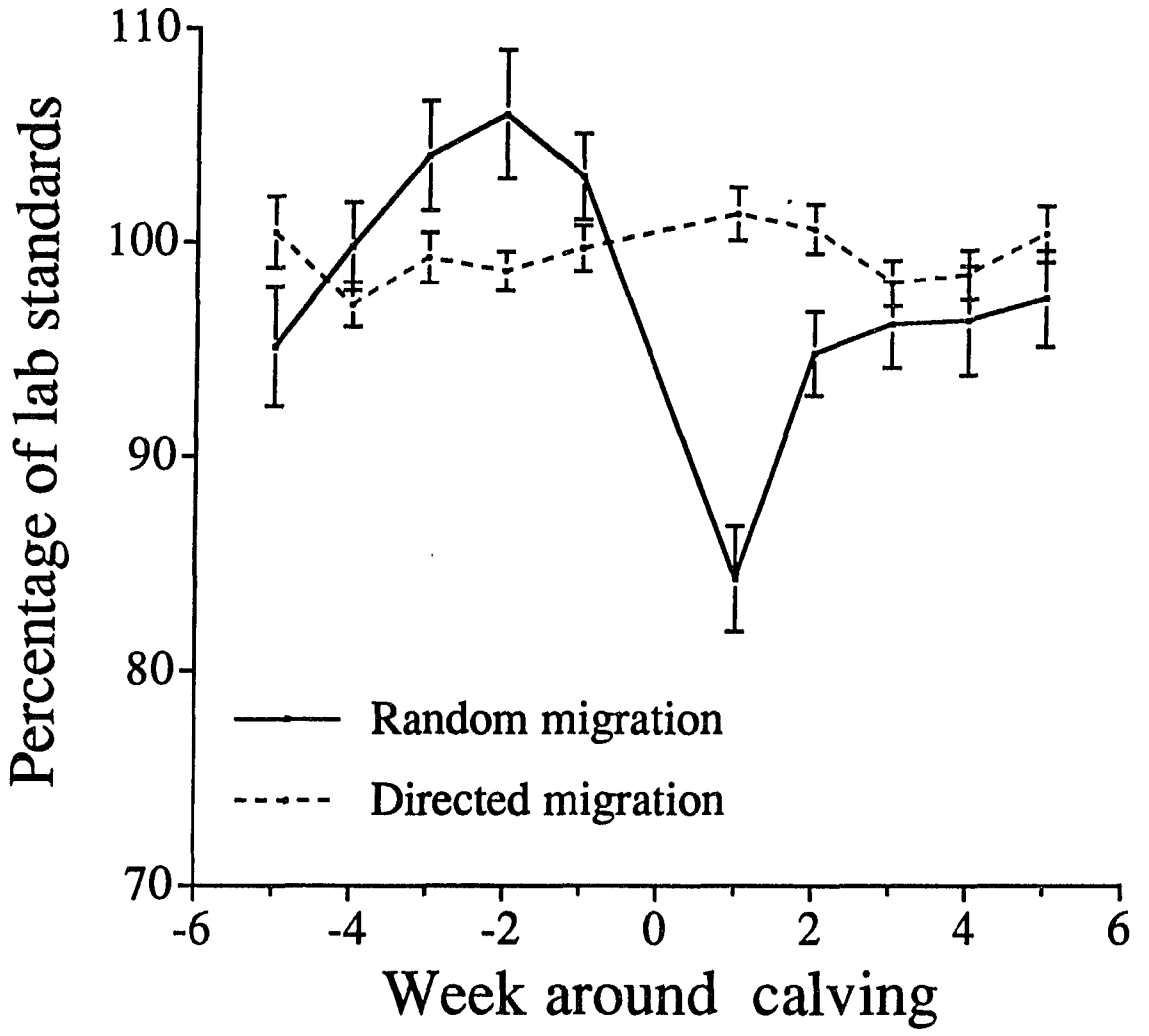

110
100
90
80
70
Percentage of lab standards
Random migration
Directed migration
-6
-4
-2
0
2
4
6
Week around calving

Figure 3. Neutrophil Fc receptor-mediated ingestion of antibody-opsonized ^{125}I-labeled S.aureus. Neutrophil stimulated chemiluminescence assay using zymosan particles preopsonized with serum C3-derived ligands. Neutrophil superoxide anion production initiated by phagocytosed C3b-coated zymosan particles. Neutrophil myeloperoxidase-catalyzed reaction initiated by phagocytosed C3b-coated particles

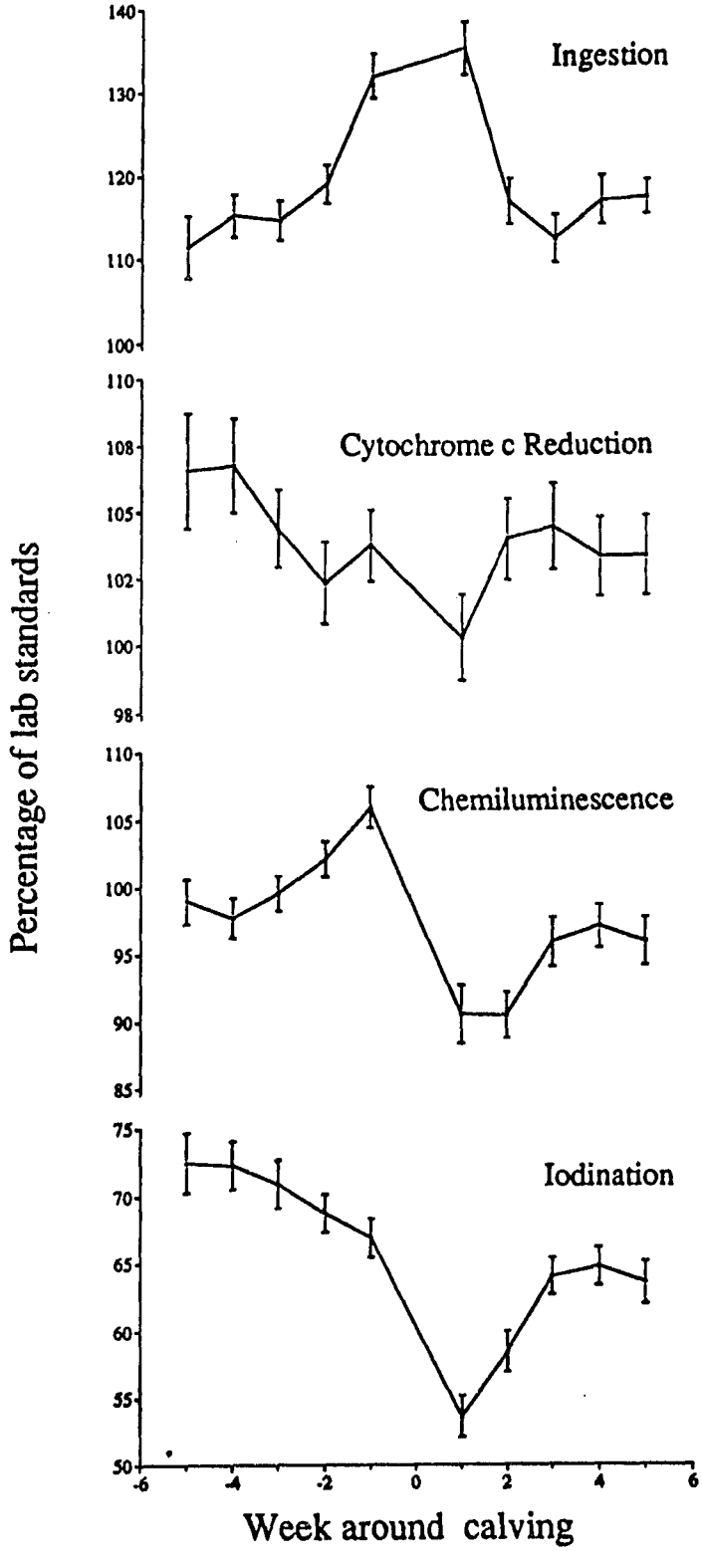

Ingestion
Cytochrome c Reduction
Chemiluminescence
Iodination
Percentage of lab standards
Week around calving
140
130
120
110
100
108
105
102
98
95
90
85
75
70
65
60
55
50
-6
-4
-2
0
2
4
6

Figure 4. Antibody-dependent neutrophil-mediated cytotoxicity toward ^{51}Cr-labeled chicken erythrocyte target cells

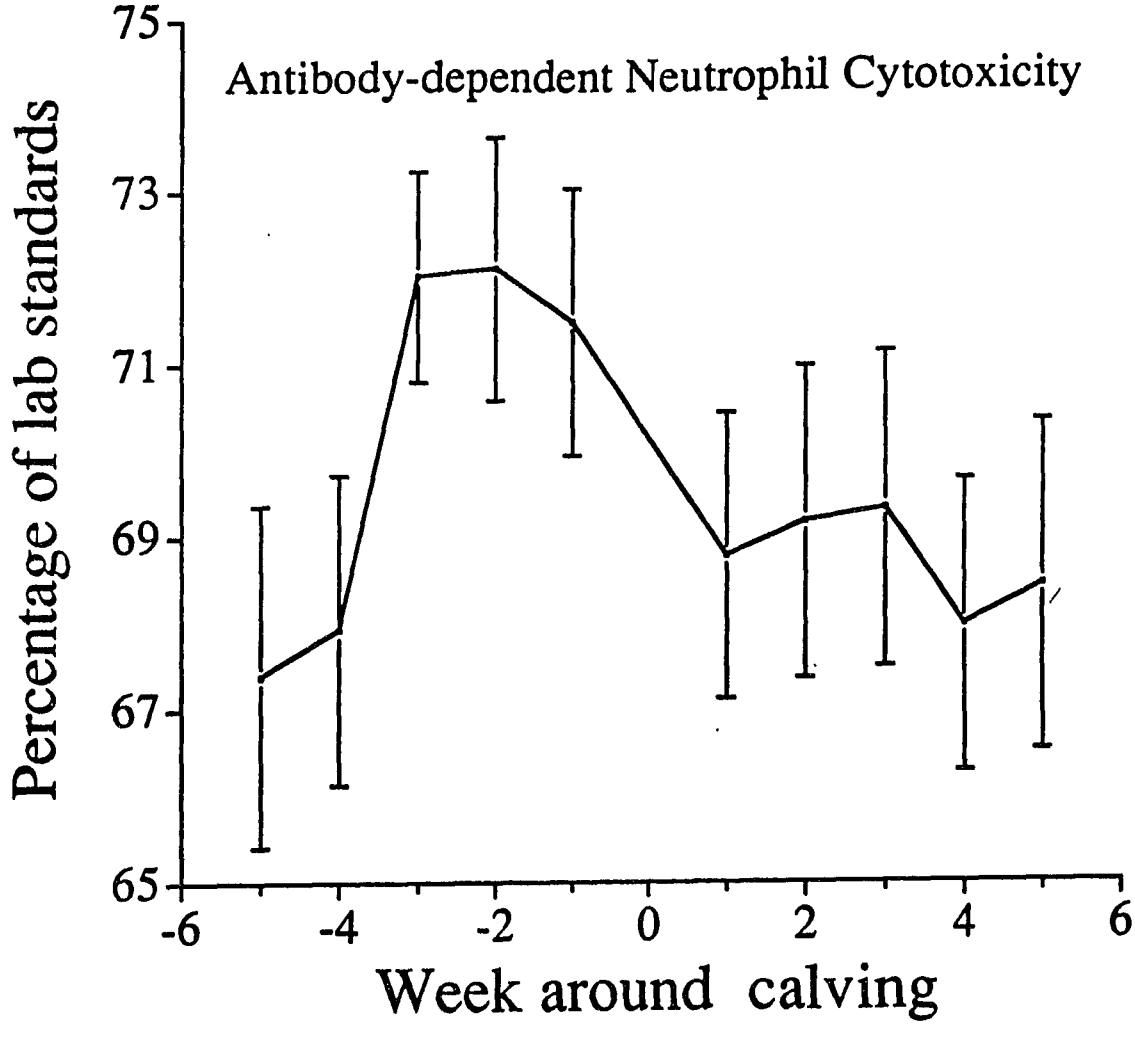

Antibody-dependent Neutrophil Cytotoxicity
Percentage of lab standards
75
73
71
69
67
65
-6
-4
-2
0
2
4
6
Week around calving

Figure 5. Lymphocyte blastogenesis assays: Concanavalin A-stimulated lymphocyte blastogenesis, Phytohemagglutin P-stimulated lymphocyte blastogenesis, Pokeweed mitogen-stimulated lymphocyte blastogenesis

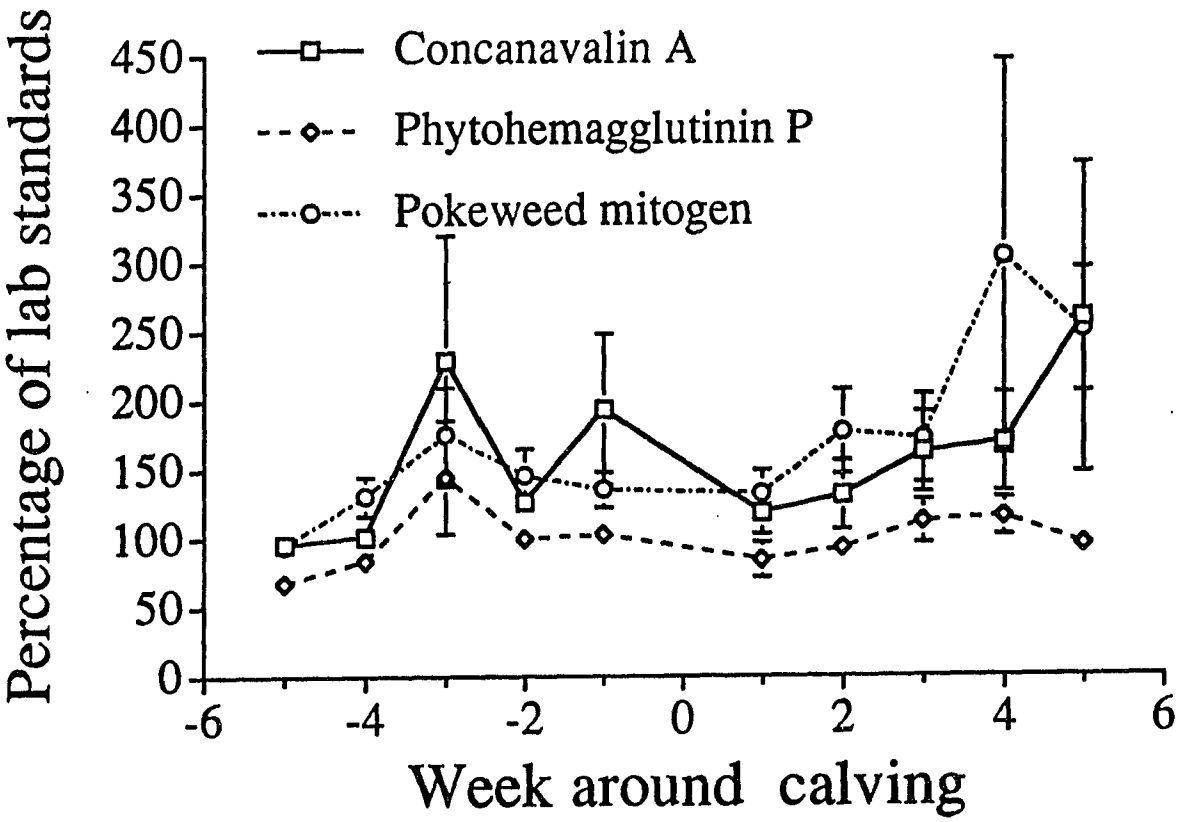

Concanavalin A
Phytohemagglutinin P
Pokeweed mitogen
Percentage of lab standards
450
400
350
300
250
200
150
100
50
0
-6
-4
-2
0
2
4
6
Week around calving

Figure 6. In vitro IgM production by B cells in mixed mononuclear cell cultures

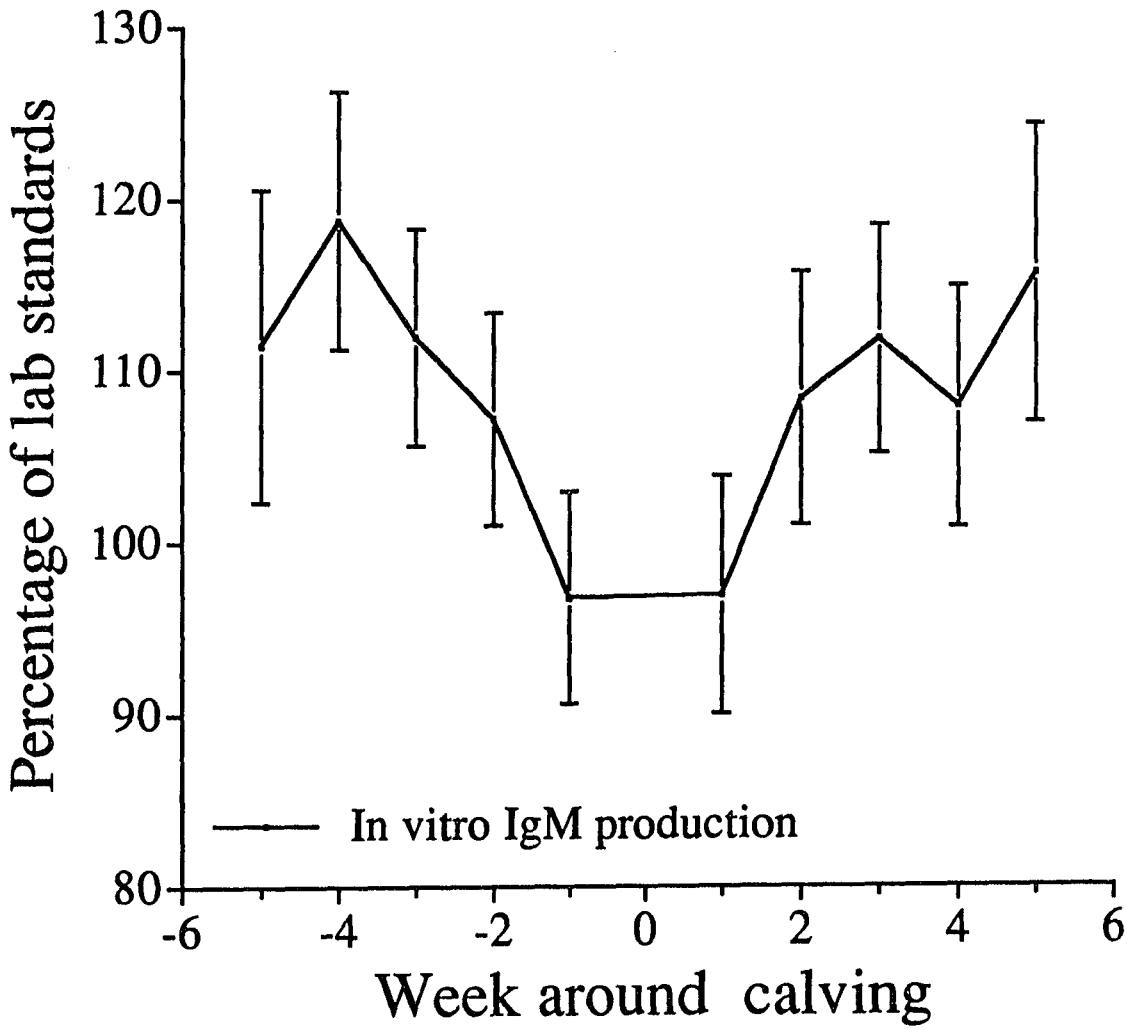

130
120
110
100
90
80
Percentage of lab standards
In vitro IgM production
-6
-4
-2
0
2
4
6
Week around calving

Figure 7. Serum immunoglobulin concentration: IgG_1, IgG_2, IgM isotypes

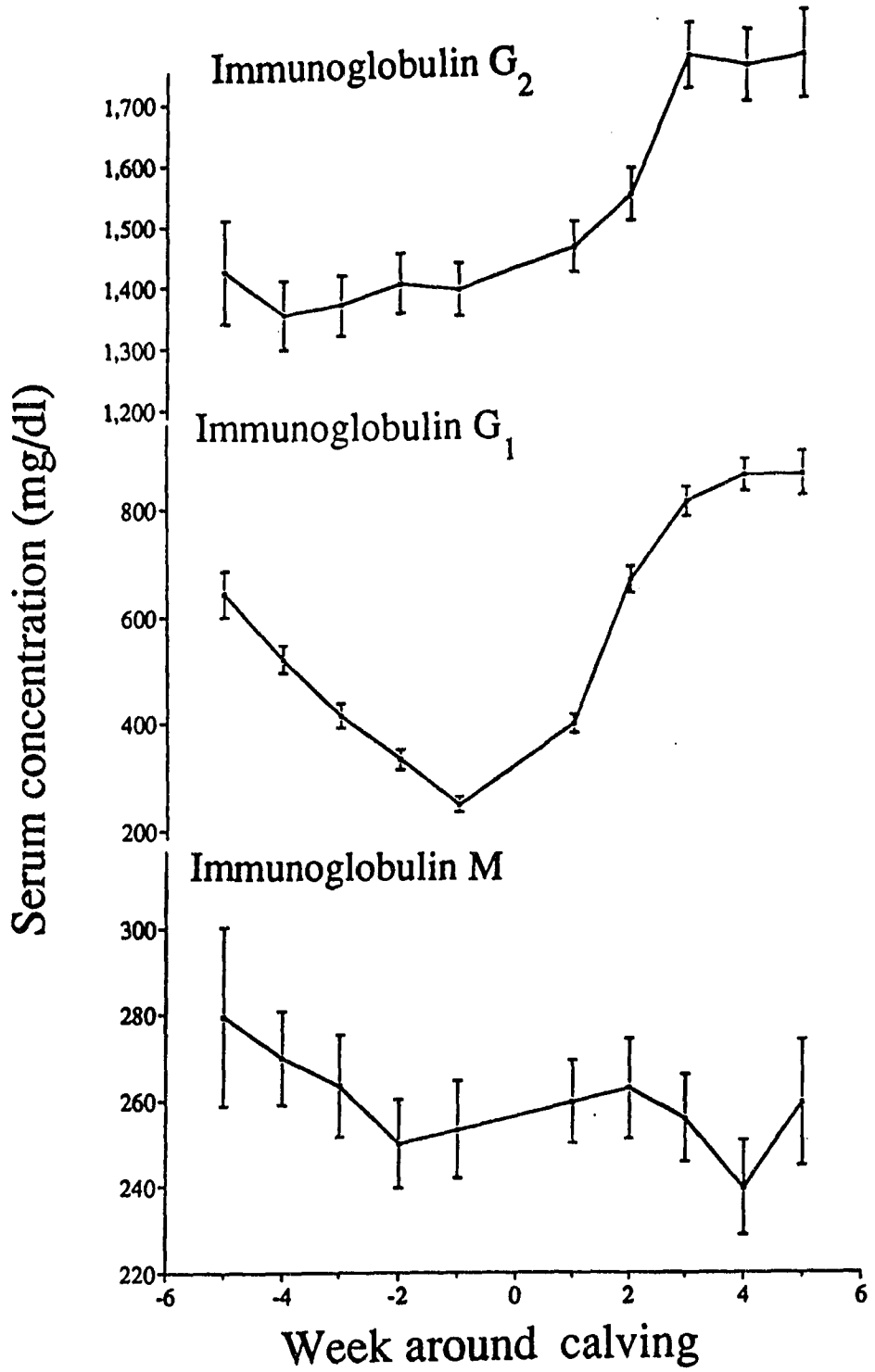
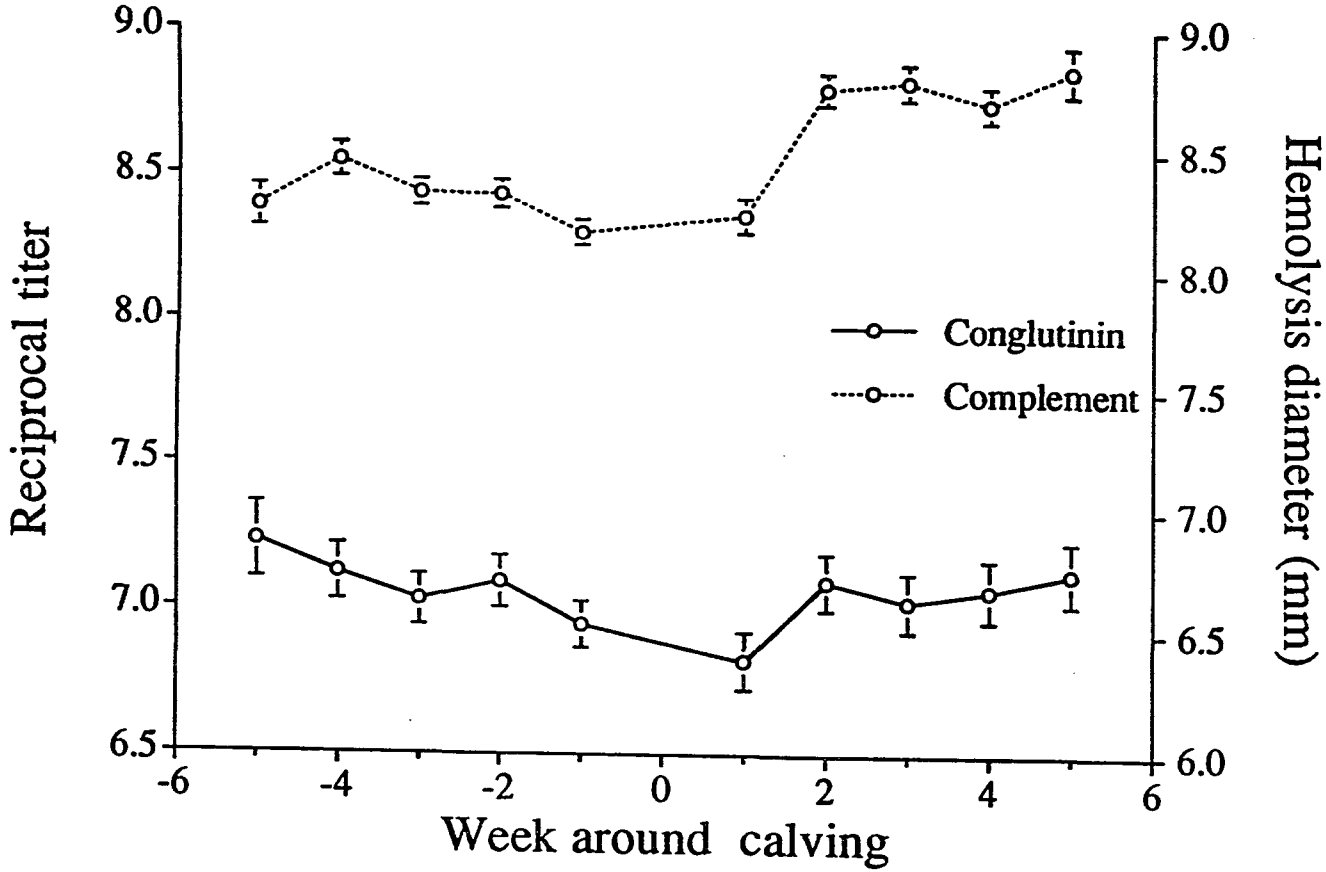

Immunoglobulin G2
1,700
1,600
1,500
1,400
1,300
1,200
Immunoglobulin G1
800
600
400
200
Immunoglobulin M
300
280
260
240
220
-6
-4
-2
0
2
4
6
Serum concentration (mg/dl)
Week around calving

Figure 8. Serum complement activity measured by hemolysis in gel of guinea pig erythrocytes opsonized with specific bovine antisera. Conglutinin activity determined by agglutination of E. coli


Reciprocal titer
Hemolysis diameter (mm)
Week around calving
Conglutinin
Complement
9.0
8.5
8.0
7.5
7.0
6.5
6.0
-6
-4
-2
0
2
4
6

PAPER II. IMMUNOLOGICAL PARAMETERS IN PERIPARTURIENT HOLSTEIN CATTLE: GENETIC VARIATION

Immunological Parameters in Periparturient Holstein Cattle:Genetic Variation

J. C. Detilleux[1], K. J. Koehler[2], A. E. Freeman[1], M. E. Kehrli, Jr.[3], D. H. Kelley[1]

[1]Animal Science Department, Iowa State University, Ames, IA 50011

[2]Statistics Department, Iowa State University, Ames, IA 50011

[3]US Department of Agriculture, Agricultural Research Service, National Animal Disease Center, Ames, IA 50010

ABSTRACT

The genetic variability of blood neutrophil functions, lymphocyte blastogenic responses, serum immunoglobulin concentration, serum complement, and serum conglutinin activities were investigated from 35 days prepartum to 35 days postpartum in 137 Holstein cows. Periparturient cows undergo an immunosuppression of various immunologic parameters at calving time. Heritability estimates were obtained before, during, and after these episodes of immunosuppression. Significant genetic variability was found in the periparturient changes for total number of PMNs, PMN chemokinesis, assays measuring PMN respiratory burst (cytochrome C reduction, chemiluminescence, and iodination), serum concentration of IgG_1, IgG_2, and IGM, and serum hemolytic complement activity. Two sires with relatively large progeny groups had a tendency to rank high consistently for all assays associated with PMN respiratory burst. Those sires had also high breeding values for pounds of milk fat and protein. This implies that immune profiles could be used for the selection of cattle with improved immunity without adverse effects on milk productivity. Non-genetic effects on immune assays were also found.

INTRODUCTION

Modern methods in animal agriculture and intensification of livestock production would be enhanced with improved resistance to diseases. The chief methods used to control spread of diseases among domestic animals are eradication of infected stock, isolation and quarantine of susceptible animals, sanitation, vaccination, and medication. These methods carry several disadvantages: high cost, potential environmental damage due to drug residues, development of drug-resistant microbial strains, reduction of selective advantage of natural disease resistance, and inefficiency in preventing production diseases. Therefore, research on genetic resistance to disease is highly desirable, especially in the developing world, where it is difficult to obtain expensive and often ineffective drugs and vaccines.

Direct selection for disease resistance may be done either by observing and selecting the most resistant breeding stock under normal environmental conditions, or by challenging with specific pathogens the breeding stock, its progeny or its sibs. Indirect selection is based upon identification of reliable indirect indicators of disease resistance. Such indicators can be classified into five categories: morphological markers such as eye margin pigmentation in bovine infectious keratoconjunctivitis (pink eye),

physiological markers such as hemoglobin type in malaria, immunological parameters such as innate or acquired immune response traits, genes of the immune system such as major histocompatibility complex (MHC) genes, immunoglobulin genes, or T cell receptor genes, and molecular genetic markers such as restriction fragment length polymorphisms (RFLPs), variable number of tandem repeats loci (VNTRs), single tandem repeats loci (STRs) and microsatellite loci (1).

Indirect selection is advantageous because there is no risk of spreading disease. Among other alternatives, one possibility is to use immunological markers. In vitro immune assays are easy to measure, low in cost, can be done early in life for both sexes, and are repeatable. They also reflect more closely the effector stage of the immune response than molecular genetic methods. Indeed, it has been shown that MHC haplotype may confer resistance to one disease while being associated with susceptibility to another (33). Thus, it can be argued that polymorphism at the level of the DNA protects the species whereas in general an effective immune response to infection protects the individual.

Susceptibility to infectious diseases has been shown in dairy cattle with depressed immune functions. Increased prevalence of clinical mastitis has been shown in cows whose neutrophils had a low capacity for phagocytosis (14,34). Chemotactic activity of peripheral leukocytes has tendency to

be lower in cows with retained placenta (11).

To improve resistance to infectious diseases (such as mastitis) characteristically under the influence of various epidemiological determinants, it is necessary to study various aspects of the immune response. The goal of this study was to obtain estimates of genetic parameters for various immune cell functions in periparturient Holstein cattle. Periparturient cows were used because bovine neutrophil and lymphocyte functions are altered (6) and susceptibility to mastitis and other diseases (28,36) is increased during the periparturient period.

MATERIALS AND METHODS

Animals and Experimental Design

Periparturient Holstein cows (n=137) from the I-O-State long-term milk selection experiment were used in this study. For up to seven generations (1968-1986), selection has been only for milk production using artificial insemination (A.I.) sires selected for highest and average Predicted Difference for Milk (PDM) from all sires used in the USA. Since 1986, sires were also selected for highest and average Predicted Transmitting Ability for pounds of fat plus protein (PTA-FP) (2). All animals were managed the same and fed for high production. Weekly blood samples were collected starting 35 days before calving until 35 days after calving. Around 20 cows were sampled per week. Twins have been excluded from the analysis. Five steers were used as laboratory standards to reduce the day-to-day variability typically seen with immune cell function assays.

Immune Assays

Blood leukocyte counts (cells/μl blood) were obtained and cells were differentiated into neutrophils (PMNs), eosinophils (PMEs), or mononuclear cells. Various PMN functions were

assessed as described (16): random (area mm^2) and directed (directed mm/random mm) migration under agarose, zymosan phagocytosis-associated native chemiluminescence activity (log_{10} photons detected over a 2-h period), cytochrome C reduction (nmoles O_2 reduced/10^7 PMN/h), iodination (nmoles NaI/10^7 PMN/h), ingestion (% of 60 Staphylococcus aureus ingested/PMN), antibody-dependent neutrophil cytotoxicity (ADNC; % release of ^{51}Cr).

Blastogenic transformation (log_{10} cpm) of lymphocytes stimulated either with concanavalin A (ConA; C-2010, Sigma Chemical Company, St Louis, MO), phytohemagglutinin P (PHAP; L-9132, Sigma Chemical Company, St Louis, MO) or pokeweed mitogen (PWM; L-9379, Sigma Chemical Company, St Louis, MO) was measured as described (17). Serum concentrations (mg/dl) of three immunoglobulin isotypes (IgM, IgG_1, IgG_2) were determined. Serum complement (hemolysis area in mm^2) activity was determined by a hemolysis in gel (HIG) assay (30). Serum conglutinin activity (ratio to serum standard) was determined by agglutination of E. coli (31).

Statistical Analyses

The statistical analysis was performed in three stages. In the first stage, immune assay results were adjusted for laboratory variability and PME contamination in PMN cultures.

In the second stage, a non-linear regression model was used to describe the immunosuppression typically observed in cows during the periparturient period. We derived three extremes from the estimated model: two maxima and one minimum (Figure 1). Each extreme has two coordinates: one ordinate described the value of the assay (E_Y_1, E_Y_2, E_Y_3) while the other described the time in week when E_Y_1, E_Y_2, E_Y_3 were reached (E_X_1, E_X_2, E_X_3). Third, an animal model was applied to the six coordinates (E_Y_1, E_Y_2, E_Y_3; E_X_1, E_X_2, E_X_3) to obtain estimates of genetic parameters.

Data adjustment

To account for the variability inherent to neutrophil and lymphocyte blastogenesis assays, daily individual cow results were expressed as ratios of the corresponding daily means for the steers (i.e. ratios of laboratory standards). Tests of kurtosis and skewness indicated that applying a logarithmic ($\log_{10}$) transformation to the ratios and apllying a square root transformation to the number of white blood cells provided measures that are more nearly normally distributed and have more stable variances than the original daily values.

It is known that eosinophils (PMEs), which are co-isolated with PMNs in our laboratory procedure, alter PMN assays (25). Therefore, PMN assays were adjusted to a common level of PME contamination. These adjusted values were used

in the following statistical analyses.

Non-linear model for repeated measurements

As an alternative to standard growth curve models that require balanced and complete data, segmented polynomial regression models have been proposed for the analysis of repeated measures data (7). We fit a separate model to the adjusted daily results for each individual cow and each test. We determined that two polynomials of order three would adequately describe the immunosuppression typically seen around parturition. One polynomial describes the periparturient changes before immunosuppression and the other describes the periparturient changes after immunosuppression. The two polynomials joined at an unknown (estimated) join point (near immunosuppression) by forcing the first derivatives to be equal at the join point. For the jth observation on ith cow, the models are:

$$y_{ij} = f(\delta'_i, t_i) + \nu'_{ij} \quad \text{if } t_{ij} <= \zeta_i$$

with:

$$f(\delta'_i, t_i) = \delta'_{i0} + \delta'_{i1} t_{ij} + \delta'_{i2} t_{ij}^2 + \delta'_{i3} t_{ij}^3 \quad [1]$$

and:

$$y_{ij} = f(\delta_i, t_i) + \nu_{ij} \quad \text{if } t_{ij} > \zeta_i$$

with:

$$f(\delta_i, t_i) = \delta_{i0} + \delta_{i1} t_{ij} + \delta_{i2} t_{ij}^2 + \delta_{i3} t_{ij}^3 \quad [2]$$

where:

y_{ij} = value of the jth observation on the ith cow (i=1, 2, ..137; j=1, 2, ...10),

ζ_i = join point,

δ'_{i0}, δ'_{i1}, δ'_{i2}, δ'_{i3} = polynomial parameters before or at ζ_i,

δ_{i0}, δ_{i1}, δ_{i2}, δ_{i3} = polynomial parameters after ζ_i

t_{ij} = time effects (week before an after calving),

v'_{ij} = error for y_{ij} before or at ζ_i, and

v_{ij} = error for y_{ij} after ζ_i.

It is assumed that v_{ij} and v'_{ij} are i.i.d. $N(0, I\,\sigma_i^2)$.

To obtain a continuous and smooth line, the values of the two polynomials and the values of their first derivatives were set equal at the unknown join point ζ_i. This means that the curves join with equal slope and with a smooth curve. Straighforward computations (see appendix for more details) show that models (1) and (2) are reparameterized as models (1′) and (2′), respectively:

$$f(\delta'_i, t_i) = \delta_{i0} + \delta_{i1} t_{ij} + \delta_{i2} t_{ij}^2 + \delta_{i3} t_{ij}^3 + \delta_{i4} w_{ij} + \delta_{i5} v_{ij} \quad [1']$$

where: $w_{ij} = (\zeta_i - t_{ij})^2$, and

$$v_{ij} = (t_{ij} - \zeta_i)^3 + 3\,\zeta_i\,(t_{ij} - \zeta_i)^2$$

$$f(\delta_i, t_i) = \delta_{i0} + \delta_{i1} t_{ij} + \delta_{i2} t_{ij}^2 + \delta_{i3} t_{ij}^3 \quad [2']$$

where:

ζ_i = join point,

δ_{i0}, δ_{i1}, δ_{i2}, δ_{i3}, δ_{i4}, δ_{i5} = polynomial parameters, and

t_{ij} = time effects (week before an after calving).

Because join points ζ_i are estimated from the data, the model is nonlinear in the parameters (δ_{i0}, δ_{i1}, δ_{i2}, δ_{i3}, δ_{i4}, δ_{i5}, and ζ_i). The nonlinear ordinary least squares (OLS) estimates of the unknown parameters are the values that minimize the error sum of squares. The estimates of $[\delta_{i0}, \delta_{i1}, \ldots, \delta_{i5}]$ are $[d_{i0}, d_{i1}, \ldots, d_{i5}]$, and z_i is the OLS of ζ_i. The NLIN procedure on SAS was used to obtain the non-linear OLS estimates (27). No closed form solution for the estimators exists, and iterative methods (here: Gauss Newton) must be used.

Because poor starting values may result in convergence to local minima, starting values for the nonlinear regression were obtained by using the same model with an arbitrary join point chosen from the plot of the values obtained for each assay and for each cow. Also, for cows with less than 8 repeated measurements, polynomials of order lower than three were used in the nonlinear regression.

Data reduction

Because we are primarily concerbed with describing immunosuppression at parturition and because the nonlinear estimators are difficult to interpret, extremes were obtained by computing roots of the first partial derivatives for each polynomial, before and after the join point. On the X-axis, the abscissae of the four extremes are denoted E_X_{ik} (k=1, 2, 3, 4) and on the Y-axis, the ordinates of the four extremes are denoted E_Y_{ik} (k=1, 2, 3, 4). For more details, see the appendix.

For the two polynomials of order three, we have:

$$E\_X_{ik} = [-2u_{i2} \pm (4u_{i2}^2 - 12u_{i2}u_{i1})^{0.5}]/6u_{i3} \quad \text{if } t_{ij} \leq z_i \quad [3]$$

$$E\_X_{ik} = [-2d_{i2} \pm (4d_{i2}^2 - 12d_{i3}d_{i1})^{0.5}]/6d_{i3} \quad \text{if } t_{ij} > z_i \quad [4]$$

where:

E_X_{ik} = kth extreme obtained from cow i (k=1, 2, 3, 4),

d_{i0}, d_{i1}, d_{i2}, d_{i3}, d_{i4}, d_{i5} = nonlinear least squares estimators for parameters describing time effects (week before and after calving), and

$u_{1i} = d_{1i} - 2\, d_{4i}\, d_{6i} - 3\, d_{5i}\, d_{6i}^2$, $u_{2i} = d_{2i} + d_{4i}$, and $u_{3i} = d_{3i} + d_{5i}$.

By evaluating each polynomials at E_X_{ik}, we obtained E_Y_{ik} before and after z_i. Among the four extremes obtained for both polynomials together, we retained only one minimum with coordinates (E_X_2, E_Y_2) and two maxima with coordinates (E_X_1,

E_Y_1) and (E_X_3, E_Y_3), respectively (Figure 1). The ordinate of minimum (E_Y_2) described the immune assay level at immunosuppression while the ordinates of both maxima (E_Y_1, E_Y_3) described the immune assay level before and after immunosuppression, respectively. The abscissae (E_X_2, E_X_1, E_X_3) described the time in week when the corresponding periparturient changes occurred.

Animal model

An animal model was applied to the extremes of each immune assay. The value of the abscissa (E_X_l for l=1, 2, 3) and the value of the ordinate (E_Y_l for l=1, 2, 3) for the minimum and the two maxima were analyzed separately. Two seasons of calving were used. The first season included cows calving from May 1, 1990 through October 1, 1990 and from May 1, 1991 until the end of the study in September 1991 (= summer). Cows calving outside the summer season were included in the second season effect (= winter). Cows were also classified as first parity cows, and second or greater parity cows. For each extreme separately, the model is:

$$\mathbf{E}_l = \mathbf{X}_l \boldsymbol{\beta} + \mathbf{Z}_l \mathbf{a}_l + \epsilon_l \qquad [5]$$

where:

$\mathbf{E}_l$ = n_lX1 vector of values for abscissae and ordinate of the minimum and two maxima (E_X_l or E_Y_l; l=1, 2, 3),

n_1 = number of cows for extreme 1 (E_X_1 or E_Y_1),

$\mathbf{X}_1$ = incidence matrix for the fixed effects ,

$\mathbf{Z}_1$ = incidence matrix for the animal effects,

β = 5X1 vector of unknown parameters for fixed effects of parity and season of calving,

a_1 = n_1X1 vector of unknown parameters for random animal effects, and

ϵ_1 = n_1X1 vector of random error.

The ϵ_1 are assumed i.i.d $N(0, I\ \sigma_{e1}^2)$ and a_1 are assumed i.i.d. $N(G_1, A_1\ \sigma_{a1}^2)$, where G_1 is the additive genetic value for the extreme 1, A_1 is the relationship matrix between animals and their known ancestors for extreme 1, σ_{e1}^2 is the residual variance for the extreme 1, and σ_{a1}^2 is the additive genetic variance for extreme 1. Variances and heritability were estimated using DF-REML (21). Heritability estimates were derived from additive and error variance components. Standard errors were obtained from an approximation of the matrix of second derivatives of the log-likelihood function with respect to additive genetic and error variances (21). Because the algorithm used to compute the REML estimates of variance components does not guarantee convergence to the global maximum, several priors for the variance ratio were used. At convergence, all priors gave the same solutions. Based on the REML estimates of variance components, breeding values were computed with the PEST program (10).

RESULTS AND DISCUSSION

Nonlinear Model for Repeated Measurements

The coefficients of determination (R^2) obtained for the nonlinear regression (Models 1′ and 2′) are shown on Table 1 for each immune assays. The mean of the R^2 values was close to 0.8 for each assay, with more than half of the R^2 values greater than 0.85. From these R^2 values, it can be concluded that our model explained variation between repeated measurements within individual cow quite well.

Heritability Estimates

Heritability estimates obtained for extremes (minimum and maxima) and immune assays are shown on Tables 2 to 7. With few exceptions, the periparturient changes in all our immune assays were found to have an additive genetic component upon which selection and genetic gain are based. Heritability estimates were small with large standard errors. The method used to approximate sampling variances has been shown to work well for animal models with only additive genetic and error variances (21). The large standard errors are probably due to the small sample size, small progeny groups, and the importance of non-genetic effects, such as disease, on the

immune response. The effect of disease was not included in the animal model (Model [5]) because it was not possible to differentiate if a pathologic process affected the immune assay or whether the immune status influenced the disease outcome.

Heritability estimates had a tendency to be higher for test results than for time effects (week with respect to calving). As an example, the heritability estimates for serum IgM concentration before, during, and after immunosuppression were relatively high (Table 6) while heritability estimates for the times when these changes occurred were null.

Due to the multiplicity of the assays, the probability of obtaining high heritability estimates just by chance is dramatically increased (8). This problem is difficult to resolve due to the dependency between assays. Therefore, at the risk of being too conservative, we will discuss only assays with high heritability estimates when compared to their standard errors.

For each of the following paragraphs, a small introduction is included which summarizes the changes in the immune assays observed during the periparturient period (6).

Leukograms

Number of white blood cells decreased during the first two weeks after calving. This decrease was primarily due to a decrease in the number of circulating neutrophils. Number of white blood cells returned to initial prepartum levels two to three weeks after calving (6).

Heritability estimates for the number of blood PMNs were null before and at the peak of immunosuppression (Table 2), but they were relatively high two to three weeks after calving ($h^2 = 0.87$) when they returned to normal values. When compared to the approximate standard errors, heritability estimates for the two other white blood cell types were close to zero before, at, and after immunosuppression.

Neutrophil function assays

With the exception of ingestion, all assays measuring PMN functions were clearly impaired on the first week after calving. Values started to decrease one to two weeks before calving and returned to initial preparturient levels two to three weeks after calving. The pattern was reversed for ingestion (6).

At the peak of immunosuppression, heritability estimates obtained for the cytochrome C reduction assay and for stimulated chemiluminescence were highest when compared to the other assays associated with PMN metabolic burst (Table 3).

After immunosuppression, heritability observed for cytochrome C reduction assay was also very high (h^2=0.99). Smaller genetic variation was found for the iodination assay before and at immunosuppression, and for the resting chemiluminescence assay before immunosuppression. The early post-partum period corresponds to the time when the incidence of environmental mastitis is very high (28). Therefore, the genetic variability found in PMN ability to destroy ingested microorganisms and the observed genetic influence on the number of blood PMNs after calving is likely very important in the control of mastitis caused by environmental pathogens (e.g., E. coli). Indeed, actual mastitis control practices are not very effective against environmental pathogens (28,36) and selection of animals for resistance to mastitis could become an alternative method of control. Also, a steady influx of highly functional blood PMNs in the mammary gland is required for effective local defense against coliforms. The acute symptoms associated with coliform mastitis during the periparturient period are due to the rapid and unrestricted growth of the organism, the release of endotoxin, and the subsequent development of an unlimited inflammatory reaction in the gland (28). Phagocytic and bactericidal activities of blood PMNs migrating from the blood to the inflamed udder are also compromised. This could be due to the composition of the milk which lacks energy resource, has low concentration of

opsonins, and contains casein micelles and fat globules which are ingested by PMNs and interfere with their ability to phagocyte bacteria (24,32). Therefore, animals with high numbers of circulating PMNs with effective killing ability should be able to better resist coliform infection. In support of this hypothesis, Heyneman et al. (14) showed in an experiment in which the respiratory burst competence of blood PMNs was measured in cows subsequently infected with Escherichia coli that elevated blood PMN number in combination with high ability to produce reactive oxygen metabolites limited bacterial replication in the mammary gland.

Heritability estimates for neutrophil ingestion and ADNC changes around calving time were null or close to zero (Table 4). Random and directed migration showed little or no genetic variability (Table 4). Using a fixed sire model, significant differences in sire progeny groups were detected for all PMN assays except for cytochrome C reduction in non-parturient cows belonging to the herd used in this study (19). These results are different from our observations. These discrepancies could be explained by the different statistical methodologies and by the difference in cows hormonal status.

Lymphocyte blastogenesis

Responses of lymphocytes induced by mitogens peaked 3 weeks before calving, decreased gradually to reach a minimum one week after calving, and increased two to three weeks after calving (6).

Heritability estimates for lymphocyte blastogenesis were very low (Table 5). It is possible that the procedure was not precise enough to detect genetic influence on lymphocyte blastogenesis. Furthermore, the lymphocyte population is itself a mixed cell population, composed of α/β T cells, of γ/δ T cells, of B cells, and killer cells (12). Each of those cells produce lymphokines that affect positively and/or negatively the proliferation of the other cells (18).

Some genetic variability was observed before, at, and after the peak of immunosuppression for the response of lymphocytes to ConA and at immunosuppression for the response of lymphocytes to PHAP. Using a fixed effect model, a significant sire effect was found for the response of lymphocytes to ConA for non-parturient cows belonging to the herd used in this study (19). A significant percentage of peripheral blood T cells (15% in adult cows up to 50% in young animals) are γ/δ T cells (12) whose receptors recognize heat shock proteins which are major antigens of many pathogens (37). The majority of lymphocytes in the mammary gland are T lymphocytes (13,35). Therefore, genetic variability in T cell

blastogenesis might become relevant in the selection of cows for resistance against infections of epithelial surfaces where γ/δ T cells are found in high number (12).

Serum immunoglobulin concentration

Serum IgG_1 concentration decreased before calving, reached a minimum the week before calving, and returned to prepartum levels after calving time. Conversely, serum concentration in IgG_2 reached higher values two to three weeks after calving than at calving. Serum concentration in IgM decreased slightly during the periparturient period (6).

Heritability estimates for periparturient changes in serum immunoglobulin (Ig) concentrations were higher than heritability estimates for changes in neutrophil and lymphocyte assays (Table 6). Among immunoglobulin isotypes, IgM had the highest heritability estimates before and after immunosuppression while serum concentration in IgG_2 had the highest heritability estimates at immunosuppression. In dairy cattle, heritability for IgG and IgM isotypes has been estimated in calf and in mature cows at around 0.07 (3). Our estimates are much higher but the age, breed and physiologic status of the cows differed among studies.

Around calving time, heritability estimates for IgG_1 and IgG_2 were relatively high. This is important because both molecules constitute nearly 90% of all colostral

immunoglobulins which are transferred to the calves and because disease incidence in young calves has been correlated with calf serum immunoglobulin concentration. Isotype IgG_2 has been shown to be very important in neutrophil opsonization and antibody-dependent neutrophil cytotoxicity (5). Suboptimal levels of serum IgG_2 have been associated with increased incidence of pyogenic mastitis (29). Immunoglobulin M levels demonstrated heritable changes before, at, and after immunosuppression. The molecules of IgM play a crucial role in resistance to bacterial and protozoal parasites because of their good complement-binding activity and efficient agglutination (5).

Serum protein assays

Serum complement and conglutinin activities decreased before calving and reached a minimum one week after calving time (6).

It can be seen from Table 7 that heritability estimates for changes in serum hemolytic complement levels at, and after immunosuppression were high while heritability estimates for changes in conglutinin activity were null or close to zero. In Norwegian Red Cattle, heritability estimate (h^2= 0.75) for hemolytic complement activity were similar to our results (20). Among other functions, the complement cascade is important in serum bactericidal activity against gram-negative

bacterial infections (15).

Rank of Sires

When possible (h^2 estimates different from zero), sires transmitting abilities were obtained. Ranking of top sires are shown in tables 8-12. A low number (high rank) indicates a high level for the immune assay. A general finding was that sires ranking high before immunosuppression for one particular assay, ranked also high at, and after immunosuppression for the same assay. As an example, the sire rank correlation varied from 0.4 to 0.8 for serum immunoglobulin concentration measured before, at, and after immunosuppression. It averaged 0.8 for the number of circulating mononuclear cells found before, at, and after immunosuppression. Sires 8H1280 and 7H241 had daughters with high number of mononuclear cells before, at, and after immunosuppression (Table 8). Both sires belonged to the genetic line selected for average PTA for milk fat and protein.

With few exceptions (sires 29H3906, 8H1280), top sires for one assay were not top sires for another assay. As an example, sire 9H888 (Table 10) ranked highest for serum concentration in IgM but ranked very low for concentration in IgG_2 and IgG_1. Therefore, efforts to improve the quality of the innate immune system by selecting animals ranking high for

one particular immune assay seems unlikely to be immediately rewarded by a generalized enhancement of all aspects of the immune status. Conversely, both sires 29H3906 and 29H5100 had a tendency to rank similarly for all assays associated with PMN oxygen-dependent bacterial killing (Table 9). Those sires had 12 and 9 daughters in the experiment, respectively. Sire 29H3906 was also associated with high values in serum IgG_1 concentration (Table 10). Both sires belonged to the genetic line selected for high PTA for milk fat and protein. This result is promising in that selection of such sires could improve the immune status of their daughters without detriment to milk fat and protein production. Also, at immunosuppression, the rank correlation between PMN native chemiluminescence and PMN ingestion was statistically significant ($r=0.5$ for stimulated chemiluminescence and $r=0.3$ for resting chemiluminescence). Similarly, the rank correlation between PMN cytochrome C assay and PMN resting chemiluminescence was also statistically significant ($r=0.4$). More similar results would have been found if a greater number of sires with large progeny size had been available for study.

Non-Genetic Effects on Immune Assays

Only statistically significant solutions obtained for season and parity effects are shown on Tables 13 and 14, respectively. There were no significant effects of season and parity on any of the assays not shown in the tables (lymphocyte blastogenesis, ingestion, and ADNC).

Two general observations can be made. There was no effect of season and parity on the time (week before and after calving) at which the changes in immune assays occurred. On the other hand, the effects of season and parity on the assay results were consistent before, at, and after immunosuppression.

As shown before (4), serum Ig concentrations in cows calving during summer were higher than in cows calving in winter. Neutrophil random migration was enhanced in cows calving in summer when compared to cows calving in winter while assays measuring the changes in PMN metabolic burst and in serum complement concentration had tendency to have higher values in cows calving in winter than in cows calving in summer. One possible explanation for the observed seasonal differences is related to stress due to high temperature during summer in Iowa. The body responds to stress by secreting corticoids which have been shown to inhibit bovine PMN oxidative metabolism, to enhance the ability of bovine PMN

to migrate under agarose, and to decrease serum complement levels (26). The seasonal incidence of diseases could also affect differently the immune response of cows calving in summer or in winter.

Effects of parity on impairment of immune function were mixed. The causes of the observed differences could not be determined. Studies in mice and dogs showed involution of the thymus after sexual maturity (9,22), attenuation of immune function of T and B cells and a decline in phagocytic activity (9,23).

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Figure 1. Example of the methodology used in the text. Coordinates for the extremes derived from the nonlinear regression explained in the text (models 1 to 4)

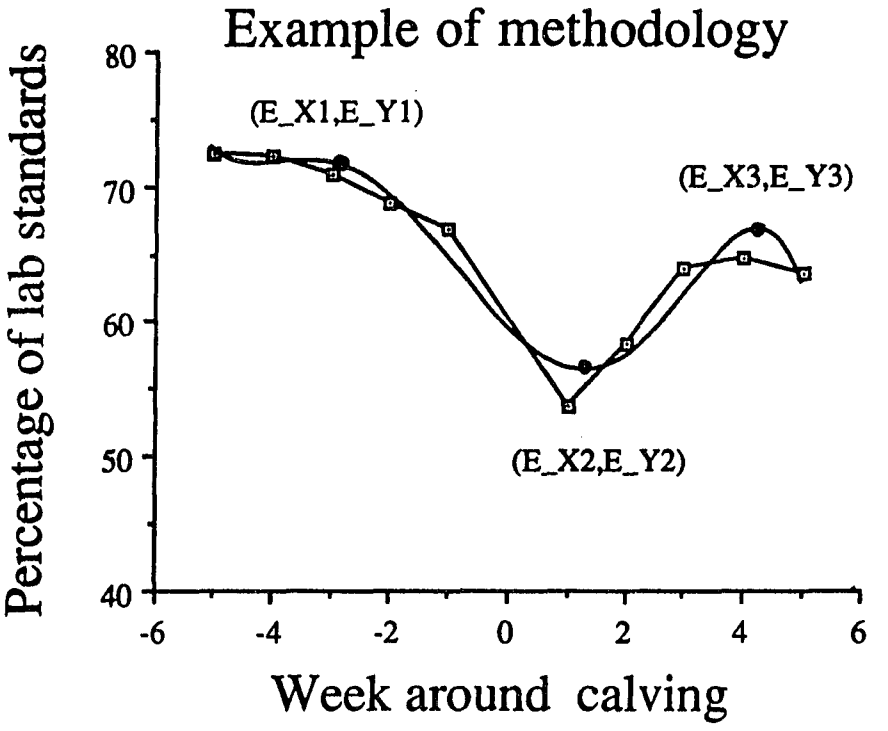
Example of methodology
Percentage of lab standards
80
70
60
50
40
(E_X1,E_Y1)
(E_X3,E_Y3)
(E_X2,E_Y2)
-6
-4
-2
0
2
4
6
Week around calving

Table 1. Coefficients of determination (R^2) for nonlinear regression on leukograms and immune assays: mean, range, and % of R square values greater than 0.85

Immune assays	Mean	Range	$R^2 \geq 0.85$
Leukograms			
Neutrophils	0.84	0.41 - 1.00	63%
Eosinophils	0.88	0.36 - 1.00	70%
Mononuclear cells	0.80	0.34 - 1.00	52%
PMN assays			
Migration			
random	0.79	0.31 - 1.00	49%
directed	0.76	0.25 - 1.00	46%
Chemiluminescence			
resting	0.85	0.42 - 1.00	61%
stimulated	0.85	0.40 - 1.00	63%
Cytochrome C reduction	0.79	0.35 - 1.00	48%
Iodination	0.80	0.38 - 1.00	47%
Ingestion	0.83	0.33 - 1.00	62%
AINC	0.85	0.54 - 1.00	63%
ADNC	0.84	0.45 - 1.00	60%
Lymphoblastogenesis assays			
Concanavalin A	0.80	0.36 - 1.00	50%
Phytohemagglutin P	0.79	0.30 - 1.00	48%
Pokeweed mitogen	0.81	0.33 - 1.00	52%
Serum protein assays			
Immunoglobulin isotypes			
IgM	0.83	0.26 - 1.00	55%
IgG1	0.94	0.62 - 1.00	93%
IgG2	0.87	0.47 - 1.00	67%
Serum complement	0.86	0.35 - 1.00	62%
Serum conglutinin activity	0.82	0.28 - 1.00	58%

Table 2. Extremes obtained for total number of white blood cells: Heritability estimates and (approximate standard errors)

	Neutrophils	Eosinophils	Mononuclear cells
Before I.S.[a]			
Week	0	0.42 (0.35)	0.14 (0.64)
Test result	0	0.25 (0.60)	0.10 (0.27)
At I.S.[b]			
Week	0.18 (0.29)	0	0.40 (0.39)
Test result	0	0	0.40 (0.38)
After I.S.[c]			
Week	0	0	0
Test result	0.87 (0.18)	0	0.07 (0.36)

[a] Time (week with respect to calving) and values of extremes when cows started to become immunosuppressed (I.S.)

[b] Time (week with respect to calving) and values of extremes when cows were immunosuppressed (I.S.)

[c] Time (week with respect to calving) and values of extremes when cows returned to normal values.

Table 3. Extremes obtained for neutrophil assays measuring oxygen-dependent killing ability: Heritability estimates and (approximate standard errors)

	Chemiluminescence			Cytochrome C
	Resting	Stimulated	Iodination	reduction
Before I.S.[a]				
Week	0.39 (0.37)	0	0	0.19 (0.36)
Test result	0.53 (0.36)	0	0.51 (0.38)	0.22 (0.31)
At I.S.[b]				
Week	0	0	0.23 (0.31)	0.41 (0.33)
Test result	0.19 (0.35)	0.71 (0.37)	0.25 (0.20)	0.88 (0.35)
After I.S.[c]				
Week	0	0.68 (0.63)	0	0
Test result	0	0.30 (0.56)	0	0.99 (0.22)

[a] Time (week with respect to calving) and values of extremes when cows started to become immunosuppressed (I.S.); [b] Time (week with respect to calving) and values of extremes when cows were immunosuppressed (I.S.); [c] Time (week with respect to calving) and values of extremes when cows returned to normal values.

Table 4. Extremes obtained for neutrophil assays associated with ingestion, migration, and antibody-dependent neutrophil cytotoxicity (ADNC): Heritability estimates and (approximate standard errors)

		Migration		
	Ingestion	Random	Directed	ADNC
Before I.S.[a]				
Week	0	0	0	0
Test result	0.26 (0.32)	0.30 (0.30)	0.03 (0.37)	0
At I.S.[b]				
Week	0	0	0.14 (0.30)	0
Test result	0	0	0.23 (0.24)	0.11 (0.25)
After I.S.[c]				
Week	0	0.19 (0.47)	0	0
Test result	0.27 (0.42)	0.47 (0.47)	0	0

[a] Time (week with respect to calving) and values of extremes when cows started to become immunosuppressed (I.S.)

[b] Time (week with respect to calving) and values of extremes when cows were immunosuppressed (I.S.)

[c] Time (week with respect to calving) and values of extremes when cows returned to normal values.

Table 5. Extremes obtained for lymphocyte responses to three mitogens: Heritability estimates and (approximate standard errors)

	ConA	PHAP	PWM
Before I.S.[a]			
Week	0	0	0.49 (0.31)
Test result	0.16 (0.30)	0	0
At I.S.[b]			
Week	0.04 (0.18)	0	0
Test result	0.40 (0.31)	0.35 (0.37)	0
After I.S.[c]			
Week	0	0	0
Test result	0.01 (0.32)	0	0

[a] Time (week with respect to calving) and values of extremes when cows started to become immunosuppressed (I.S.)

[b] Time (week with respect to calving) and values of extremes when cows were immunosuppressed (I.S.)

[c] Time (week with respect to calving) and values of extremes when cows returned to normal values.

Table 6. Extremes obtained for serum immunoglobulin concentration: Heritability estimates and (approximate standard errors)

	Serum Immunoglobulin		
	IgG_1	IgG_2	IgM
Before I.S.[a]			
Week	0	0.02 (0.31)	0
Test result	0	0.25 (0.26)	0.74 (0.30)
At I.S.[b]			
Week	0	0.24 (0.23)	0
Test result	0.29 (0.23)	0.63 (0.23)	0.55 (0.29)
After I.S.[c]			
Week	0.55 (0.41)	0.16 (0.30)	0
Test result	0.43 (0.27)	0.24 (0.26)	0.84 (0.33)

[a] Time (week with respect to calving) and values of extremes when cows started to become immunosuppressed (I.S.)

[b] Time (week with respect to calving) and values of extremes when cows were immunosuppressed (I.S.)

[c] Time (week with respect to calving) and values of extremes when cows returned to normal values.

Table 7. Extremes obtained for serum protein assays: Heritability estimates and (approximate standard errors)

	Complement	Conglutinin
Before I.S.[a]		
Week	0	0
Test result	0	0
At I.S.[b]		
Week	0	0
Test result	0.92 (0.28)	0
After I.S.[c]		
Week	0.47 (0.61)	0.31 (0.44)
Test result	0.41 (0.11)	0

[a] Time (week with respect to calving) and values of extremes when cows started to become immunosuppressed (I.S.)

[b] Time (week with respect to calving) and values of extremes when cows were immunosuppressed (I.S.)

[c] Time (week with respect to calving) and values of extremes when cows returned to normal values.

Table 8. Rank of sires for number of white blood cells: Top sires for results before, at, and after immunosuppression

	Mononuclear cells			Neutrophils	Eosinophils
	Pre	At	Post	Post	Pre
n sires	36	42	34	37	37
h^2	0.10	0.40	0.07	0.88	0.25
Sires ID					
9H584	-	1	3	-	8
8H1280	3	2	8	12	5
7H241	1	4	1	11	28
29H5100	12	19	16	5	2
29H3906	36	34	33	19	9

Table 9. Rank of sires for neutrophil assays associated with oxygen-dependent killing ability: Top sires for results before, at, and after immunosuppression

	Iodination		Cytochrome c Reduction			Chemiluminescence Resting		Chemiluminescence Stimulated	
	Pre	At	Pre	At	Post	Pre	At	At	Post
n sires	42	46	41	44	41	43	44	44	41
h^2	0.52	0.25	0.22	0.88	0.99	0.53	0.19	0.71	0.30
Sires ID									
29H3906	1	3	13	6	9	1	1	20	12
29H5100	3	1	1	20	13	43	6	1	36
7H241	10	5	37	1	5	40	2	35	37
29H3646	4	46	7	3	35	34	40	29	-
4H271	19	31	17	43	1	4	39	38	1
3H806	42	45	23	35	30	11	36	43	2

Table 10. Rank of sires for serum concentration in immunoglobulins: Top sires for results before, at, and after immunosuppression

	IgG_1		IgG_2			IgM		
	At	Post	Pre	At	Post	Pre	At	Post
n sires	45	44	43	44	41	40	44	42
h^2	0.29	0.43	0.25	0.63	0.24	0.74	0.55	0.85
Sires ID								
7H587	2	3	5	6	7	9	7	10
29H3906	1	1	35	36	23	18	37	40
29H3869	30	31	4	2	2	34	42	33
8H1280	18	26	1	1	5	17	35	35
9H888	10	17	30	18	39	1	1	1
8H474	21	41	42	42	37	6	2	27
7H241	20	21	43	44	41	15	4	11
29H5100	45	44	27	35	18	7	29	14

Table 11. Rank of sires for serum concentration in serum hemolytic complement activity: Top sires for results before, at, and after immunosuppression

	Complement	
	At	Post
n sires	46	41
h^2	0.92	0.41
Sires ID		
29H3906	2	1
21H1567	1	3
7H528	5	5
8H1280	6	6
8H474	9	8

Table 12. Rank of sires for PMN random and directed migration: Top sires for results before, at, and after immunosuppression

	Directed migration		Random migration	
	Pre	At	Pre	Post
n sires	38	43	43	39
h^2	0.03	0.23	0.30	0.47
Sires ID				
7H241	34	43	1	3
3H806	24	1	2	20
21H805	6	39	14	1
8H1122	1	36	39	31
3H1221	2	41	16	4

Table 13. Extremes obtained for immune assays: Estimable differences between seasons[1]

	Before I.S.[2]		At I.S.[3]		After I.S.[4]	
	Week	Test	Week	Test	Week	Test
PMN assays						
Migration						
Random	-	.02	-	-	.03	.05*
Directed	-	.01	.16			
Chemiluminescence						
resting	-.20	-.01	-	-.04†	-	-
stimulated	-	-	-	-.01	-.03	.04†
Cytochrome C reduction	.28	.00	.14	-.02†	-	-.01
Iodination	-	-.03	.13	-.08†	-	-
Serum protein assays						
Immunoglobulin isotypes						
IgG_1	-	-	-	.17**	.32	.10*
IgG_2	.28	-.01	-.17	.02	.39	.03
IgM	-	.13**	-	.12**	-	.11**
Complement	-	-	-	-.50**	-.19	-.49**
Conglutinin	-	-	-	-	-.22	-

†$p \leq .10$; *$p \leq .05$; **$p \leq .01$

[1]Difference between first and second season

[2]Time (week with respect to calving) and values of extremes when cows started to become immunosuppressed (I.S.)

[3]Time (week with respect to calving) and values of extremes when cows were immunosuppressed (I.S.)

[4]Time (week with respect to calving) and values of extremes when cows returned to normal values.

Table 14. Extremes obtained for immune assays: Estimable differences between parities[1]

	Before I.S.[2]		At I.S.[3]		After I.S.[4]	
	Week	Test	Week	Test	Week	Test
PMN assays						
Migration						
Random	-	-.001	-	-	.26	-.03*
Directed	-	-.052	.94	-	-	-
Chemiluminescence						
resting	-.33	.06†	-	.01	-	-
stimulated	-	-	-	.04	-.36	.01
Cytochrome C reduction	.28	-.01	-.19	-.03†	-	-.001
Iodination	-	.02	.02	.03	-	-
Serum protein assays						
Immunoglobulin isotypes						
IgG_1	-	-	-	.03	-.19	.03**
IgG_2	-.01	-.10	-.90*	-.08†	-.04	-.07†
IgM	-	-.03*	-	.04*	-	.03**
Complement	-	-	-	.08	-.13	.10
Conglutinin	-	-	-	-	-.40†	-

†p≤.10; *p≤.05; **p≤.01

[1]Difference between first parity and others

[2]Time (week with respect to calving) and values of extremes when cows started to become immunosuppressed (I.S.)

[3]Time (week with respect to calving) and values of extremes when cows were immunosuppressed (I.S.)

[4]Time (week with respect to calving) and values of extremes when cows returned to normal values.

APPENDIX

This appendix describes the derivation of the extremes of the joined polynomials describing the immunological changes observed during the periparturient period. For the jth observation occurring before the join point (ζ), the model is:

$$y_j = f(\delta', t_j) + v'_j,$$

or, equivalently:

$$f(\delta', t_j) = \delta'_0 + \delta'_1 t_j + \delta'_2 t_j^2 + \delta'_3 t_j^3 \qquad (1)$$

where:

y_j = jth value for one assay on one cow occurring before or at the join point,

ζ = join point,

δ'_0, δ'_1, δ'_2, δ'_3 = polynomial parameters before or at ζ,

t_j = time effects (weeks before or after calving), and

v'_j = error for y'_j.

For a particular observation occurring after the join point, the model is:

$$y_j = f(\delta, t_j) + v_j,$$

or, equivalently:

$$f(\delta, t_j) = \delta_0 + \delta_1 t_j + \delta_2 t_j^2 + \delta_3 t_j^3 \qquad (2)$$

where:

y_j = jth value for one assay on one cow occurring after the join point,

ζ = join point,

δ_0, δ_1, δ_2, δ_3 = polynomial parameters after ζ,

t_j = time effects (weeks before or after calving), and

v_j = error for y_j.

It is assumed that v_j and v'_j are i.i.d. $N(0, I\,\sigma^2)$.

As an example, take a cow with the following assay results:

Week with respect to calving (t)	Assay result
-5	72.5
-4	72.3
-3	70.9
-2	68.7
-1	66.9
1	53.6
2	58.4
3	64.2
4	64.7
5	63.6

Let assume that the join point is 0, then before the join point, the model (1) is:

$$72.5 = \delta'_0 - 5\,\delta'_1 + 25\,\delta'_2 - 125\,\delta'_3 + v'_1,$$

$$72.3 = \delta'_0 - 4\,\delta'_1 + 16\,\delta'_2 - 64\,\delta'_3 + v'_2,$$

$$70.9 = \delta'_0 - 3\,\delta'_1 + 9\,\delta'_2 - 27\,\delta'_3 + v'_3,$$

$$68.7 = \delta'_0 - 2\,\delta'_1 + 4\,\delta'_2 - 8\,\delta'_3 + v'_4, \text{ and}$$

$$66.9 = \delta'_0 - 1\,\delta'_1 + 1\,\delta'_2 - 1\,\delta'_3 + v'_5.$$

After the join point, the model (2) is:

$$53.6 = \delta_0 + 1\ \delta_1 + 1\ \delta_2 + 1\ \delta_3 + v_6,$$

$$58.4 = \delta_0 + 2\ \delta_1 + 4\ \delta_2 + 8\ \delta_3 + v_7,$$

$$64.2 = \delta_0 + 3\ \delta_1 + 9\ \delta_2 + 27\ \delta_3 + v_8,$$

$$64.7 = \delta_0 + 4\ \delta_1 + 16\ \delta_2 + 64\ \delta_3 + v_9, \text{ and}$$

$$63.6 = \delta_0 + 5\ \delta_1 + 25\ \delta_2 + 125\ \delta_3 + v_{10}.$$

To obtain a continuous and smooth model, the values of the two polynomials were set equal at the unknown join point ζ:

$$f(\delta', \zeta) = f(\delta, \zeta),$$

$$\delta'_0 + \delta'_1\ \zeta + \delta'_2\ \zeta^2 + \delta'_3\ \zeta^3 = \delta_0 + \delta_1\ \zeta + \delta_2\ \zeta^2 + \delta_3\ \zeta^3.$$

The values of the first derivatives of the two polynomials with respect ζ were also set to zero.

$$\delta'_1 + 2\ \delta'_2\ \zeta + 3\ \delta'_3\ \zeta^2 = \delta_1 + 2\ \delta_2\ \zeta + 3\ \delta_3\ \zeta^2.$$

Expressing $f(\delta', t_j)$ as a function of $f(\delta, t_j)$ and after simplifications, model (1) is reparameterized as model (1′):

$$f(\delta', t_j) = \delta_0 + \delta_1\ t_j + \delta_2\ t_j^2 + \delta_3\ t_j^3 + \delta_4\ w_j + \delta_5\ v_j, \qquad (1')$$

where $w_j = (\zeta - t_j)^2$, and

$$v_j = (t_j - \zeta)^3 + 3\ \zeta\ (t - \zeta)^2$$

Model (2) remains as before:

$$f(\delta, t_j) = \delta_0 + \delta_1\ t_j + \delta_2\ t_j^2 + \delta_3\ t_j^3. \qquad (2')$$

For our example, the reparameterized model (1′) is:

$$72.5 = \delta_0 - 5\ \delta_1 + 25\ \delta_2 - 125\ \delta_3 + 25\ \delta_4 - 125\ \delta_5 + v_1,$$

$$72.3 = \delta_0 - 4\ \delta_1 + 16\ \delta_2 - 64\ \delta_3 + 16\ \delta_4 - 64\ \delta_5 + v_2,$$

$$70.9 = \delta_0 - 3\,\delta_1 + 9\,\delta_2 - 27\,\delta_3 + 9\,\delta_4 - 27\,\delta_5 + \nu_3,$$

$$68.7 = \delta_0 - 2\,\delta_1 + 4\,\delta_2 - 8\,\delta_3 + 4\,\delta_4 - 8\,\delta_5 + \nu_4, \text{ and}$$

$$66.9 = \delta_0 - 1\,\delta_1 + 1\,\delta_2 - 1\,\delta_3 + 1\,\delta_4 - 1\,\delta_5 + \nu_5.$$

The reparameterized model (2′) is:

$$53.6 = \delta_0 + 1\,\delta_1 + 1\,\delta_2 + 1\,\delta_3 + \nu_6,$$

$$58.4 = \delta_0 + 2\,\delta_1 + 4\,\delta_2 + 8\,\delta_3 + \nu_7,$$

$$64.2 = \delta_0 + 3\,\delta_1 + 9\,\delta_2 + 27\,\delta_3 + \nu_8,$$

$$64.7 = \delta_0 + 4\,\delta_1 + 16\,\delta_2 + 64\,\delta_3 + \nu_9, \text{ and}$$

$$63.6 = \delta_0 + 5\,\delta_1 + 25\,\delta_2 + 125\,\delta_3 + \nu_{10}.$$

It can be seen from these equations that if we assume that the join point is really 0, the model is linear in the parameters and we can use linear regression methodology to obtain least squares estimates of the regression coefficients. However, the join point is unknown and must be estimated from the data. Non linear methodology is used to obtain non-linear ordinary leasts squares estimates $d_0, \ldots d_5$ for $\delta_0, \ldots \delta_5$, and to obtain least squares estimate z of the join point ζ.

Having obtained the non-linear least squares estimates, we can derive the extremes of each polynomial, before and after the estimated join point (z). The extremes are obtained by finding the roots of the first derivatives for each polynomial.

In our example, for $t_j > z$ we have (Model 2′):

$$f(d, t) = d_0 + d_1\,t_j + d_2\,t_j^2 + d_3\,t_j^3.$$

The first derivative is set to zero:

$$df/d(t) = d_1 + 2\ d_2\ t_j + 3\ d_3\ t_j^2 = 0.$$

Then, we solve for t_j to obtain the values for the extremes on the X axis:

$$E\_X = [-2d_2 \pm (4d_2^2 - 12d_3d_1)^{0.5}]/6d_3.$$

To obtain the values of the extremes on the Y axis, we evaluate the polynomial at E_X to obtain E_Y as follows:

$$E\_Y = d_0 + d_1\ E\_X + d_2\ E\_X^2 + d_3\ E\_X^3.$$

The same procedure is applied if $t_j \leq z$ (model 1′):

$$f(d,t) = d_0 + d_1\ t_j + d_2\ t_j^2 + d_3\ t_j^3 + d_4\ w_j + d_5\ v_j$$

where: $w_j = (z - t_j)^2$,and

$$v_j = (t_j - z)^3 + 3\ z\ (t - z)^2$$

The first derivative is:

$$df/d(t) = d_1 - 3\ d_5\ z^2 - 2\ d_4\ z + 2\ t_j\ (d_2 + d_4) + 3\ t_j^2\ (d_3 + d_5).$$

Solving for t_j, we obtain the values for the extremes on the X axis:

$$E\_X = [-2u_2 \pm (4u_2^2 - 12u_3u_1)^{0.5}]/6u_3,$$

where: $u_1 = d_1 - 2\ d_4\ z - 3\ d_5\ z^2$, $u_2 = d_2 + d_4$, and $u_3 = d_3 + d_5$.

To obtain the values of the extremes on the Y axis, we evaluate the polynomial at E_X and obtain E_Y:

$$E\_Y = d_0 + d_1\ E\_X + d_2\ E\_X^2 + d_3\ E\_X^3 + d_4\ (z - E\_X)^2$$
$$+ d_5\ (2z^3 - 3\ z^2\ E\_X + E\_X^3).$$

Among the four extremes obtained for both polynomials

together, we retained only one minimum and two maxima which characterize the immune suppression seen at calving time

PAPER III. AN EPIDEMIOLOGICAL AND GENETIC STUDY OF DISEASES IN PERIPARTURIENT HOLSTEIN CATTLE

An Epidemiological and Genetic Study of Diseases in Periparturient Holstein Cattle

J. C. Detilleux[1], A. E. Freeman[1], M. E. Kehrli, Jr.[2], C. A. Whetstone[3], L. K. Fox[4], L. D. Miller[5], D. H. Kelley[1]

[1]Animal Science Department, Iowa State University, Ames, IA 50011

[2]US Department of Agriculture, Agricultural Research Service, National Animal Disease Center, Metabolic Diseases and Immunology Research Unit, Ames, IA 50010

[3]US Department of Agriculture, Agricultural Research Service, National Animal Disease Center, Virology of Cattle Research Unit, Ames, IA 50010

[4]Department of Veterinary Clinical Medicine, Washington State University, Pullman, WA 99164

[5]College of Veterinary Medicine, Iowa State University Ames, IA 50011

ABSTRACT

Environmental and genetic factors affecting mastitis indicators (somatic cell counts, clinical mastitis cases, bacteriological infection status) and retroviral infections (bovine leukosis virus, bovine immunodeficiency virus) were studied on 137 periparturient Holstein cows selected for different levels of milk productivity. Environmental effects were obtained by generalized least squares and by logistic regression. Genetic parameters were obtained from animal models and threshold models using variation among animals. Lactation number affected both the number of mastitic quarters and the number of quarters infected with minor pathogens. Prevalence of bovine leukosis virus was lowest and the prevalence of bovine immunodeficiency-like virus was highest in cows selected for high predicted transmitting ability for pounds of milk fat plus proteins. Heritabilities for all mastitis indicators averaged 0.10, but differences were seen among mastitis indicators. Heritability estimates for susceptibility to retroviral infections were close to zero. A statistically significant negative genetic correlation (r_g = -0.26) was observed between the number of quarters infected with minor pathogens and the number of quarters infected with major pathogens.

INTRODUCTION

Selection against disease depends mainly whether indicators of disease susceptibility are measurable in an accurate and economic manner, and whether there is sufficient genetic variation in the animal population to make rapid genetic progress. Diseases heritability estimates reported in the literature vary with the etiologic agent, the environment and host species, with the method of evaluation, the criteria used to define the disease, and the data sampling method. In dairy cattle, heritability estimates for mastitis vary from less than 1% up to 48% (7,14,15,27). Heritability estimates for dystocia decrease from 15% in first calf heifers to 5% in cows third and greater parities (14). Heritability estimates for retained placenta vary from 5% up to 38% (14). Digestive problems are more heritable (h^2 = 21%) than reproductive and respiratory problems (h^2 < 10%) (15). Heritabilities of locomotive problems are less than 20% (15). Heritability of resistance to nematode infection, based on fecal nematode egg counts, was around 50% (28). Heritability estimates for ketosis varied from 2 to 31% (16,27). Milk fever has been shown to be influenced by hereditary factors, although most estimates of heritability are close to zero (14). It has been shown that the three major features in the pathogenesis of enzootic bovine leukosis following Bovine Leukosis Virus (BLV)

infection (seroconversion to the major viral envelop protein gp51, proliferation of virus-infected B cells, and lymphosarcoma) are associated with particular BoLA antigenic types (12,13).

Given the genetic parameters reviewed here, breeding for disease resistance appears justified. The purpose of this study was to obtain estimates of genetic parameters for three classes of disorders: mastitis, viral infections with bovine leukemia virus and/or bovine immunodeficiency-like virus, and all other clinical disease.

MATERIALS AND METHODS

Animals and Experimental Design

Periparturient Holstein cows (n=137) from the I-O-State long-term milk selection experiment were used in this study. For up to seven generations, selection has been only for milk production using artificial insemination (A.I.) sires selected for highest and average Predicted Difference for Milk (PDM) from all sires used in the USA. In 1986, the objectives of the experiment changed (2). All animals selected for high and average milk were randomized into two groups. One group and their progeny were bred to sires selected for high combined pounds fat plus protein. The other group and their progeny were bred for average combined pounds fat plus protein. Sires were randomly selected from the active sires that were currently available each year. Selection was based on Predicted Transmitting Ability (PTA). In this experiment, 84 cows belonged to the PTA lines while 53 cows belonged to the PDM lines. All animals are managed the same and fed for the same level of high production.

Information on calving and lactations were recorded routinely throughout the study. For herd life data, records were used that started in 1985 and finished in 1991. Seasonal effects were accounted for by defining two seasons of calving.

The first season (summer) included cows calving from May, 1 through October, 31 and the second season (winter) included cows calving from November, 1 through April, 31. Lactation numbers were defined as first, second, third, and fourth and over.

Diseases Indicators

Mastitis indicators

Indicators of mastitis were somatic cell counts (SCC) bacteriological culture results, and clinical cases of mastitis. When possible, records on SCC and clinical cases of mastitis were obtained for complete lactations (ended with dry period). To minimize the bias due to culling cows with incomplete lactations (cow left the herd or termination of study), data collected 250 days after calving were not used in the study. Using only 250 days for incomplete lactations included the majority of mastitis cases reported (93.5 %) and decreased the risk of culling for reasons other than mastitis. Variation in culling may cause bias because a culled cow has a lesser chance of getting mastitis than a surviving cow. Cows culled because of a mastitis were included in the mastitis category.

To establish the presence of intramammary infections (IMI) among periparturient cows calving during our study

period (1990 and 1991), duplicate quarter foremilk samples were taken about 30 days before expected calving date, at parturition, and about 30 days after calving. In addition, if a cow developed mastitis between scheduled sample times, duplicate quarter samples were additionally collected on all four quarters. Samples were frozen immediately after collection. Bacteria were categorized as major (all streptococci, Staphylococcus aureus, coagulase-positive staphylococci other than S. aureus, and coliforms) and minor (coagulase-negative staphylococci and Corynebacterium bovis) pathogens. When a pathogen was isolated from one duplicate sample and the other sample was bacteriologically negative, the quarter was considered infected by the pathogen isolated. Contaminated samples were categorized as uninfected and accounted for 6% of all samples.

A minor pathogen IMI and a major pathogen IMI score was obtained separately by applying the following method. For each cow and each parity, we computed over the three periparturient samples, the number (N_{ij}: i= 1, .., n cow-parity units; j=1, ..4 quarters) of times a pathogen was detected in a particular quarter. We computed N_{ij} separately for minor and major pathogens and we took the sum over the four quarters of these N_{ij} for each cow-parity ($N_i = \Sigma_j N_{ij}$). The totals for each cow (N_i) represented the minor pathogen IMI or major pathogen IMI score depending upon the nature of the pathogen

detected. By this method, a cow with three quarters infected with a major pathogen (or minor pathogen) during our sample period or a cow with one quarter infected with a major pathogen (or minor pathogen) that remained infected over the three samples received the same major pathogen IMI (or minor pathogen IMI) score of 3.

The number of cases of clinical disease per lactation was determined during the herd life of the cow. Cases of mastitis were counted as new cases if 30 days had expired since the last clinical symptoms of a previous case for the same quarter. The number of clinically mastitic quarters per lactation was determined to give the clinical mastitis score.

Measurements of somatic cell counts were from monthly Dairy Herd Improvement sampling, starting in January 1986. Scores were averaged per lactation to give the lactation somatic-cell score.

Retroviral infections

Bovine leukosis virus (BLV) and bovine immunodeficiency-like virus (BIV) infections were determined for the parity when bacteriological cultures for mastitis were collected. The BLV status of the cows was determined on blood serum samples using a commercial kit (Leuk-Assay B, Pitman Moore Inc., Mundelein, IL). Antibody to BIV-p26 protein antigen was detected by western blot assay (34). Data were coded as

positive or negative for presence of antibodies against these antigens.

Clinical disease other than mastitis

This category included all clinical diseases other than treatments for mastitis. Clinical disease cases represent all health disorders observed in the herd. For an incomplete lactation, cases were recorded from 10 days before to 250 days after calving. Because of low disease incidence, clinical diseases were grouped into five categories: foot problems (foot rot, foot abscess and others), metabolic disorders (ketosis, milk fever), digestive disorders (mainly diarrhea), respiratory problems (mainly pneumonia), dysfunctions of reproductive system (metritis, retained placenta, and others), and death for all causes. Cows culled because of a particular disease were included in the corresponding disease category. Cases were counted as new cases if a minimum period without symptoms (determined appropriate for each health disorder separately) had expired since the last clinical symptoms of a previous case for the same health disorder.

Statistical Analyses

To explore all effects thought to influence disease prevalence, we examined the effects of season and year of calving, parity, lactation length, and genetic line on the outcome of mastitis, viral infections, and other health problems. Next, we computed breeding values for the disease indicators by using animal and threshold (THRE) models. The structure of the models used in the statistical analyses are summarized in Table 1.

Exploratory models

The following model was used to study lactation somatic-cell score:

$$y_{ijklmn} = \mu + \lambda_i + \delta_{ij} + \zeta_k + \psi_l + \pi_m + \beta\delta_{ijklmn} + \epsilon_{ijklmn} \quad [1]$$

y_{ijklmn} = lactation somatic cell score,

λ_i = genetic line (i=1, 2, 3, 4),

δ_{ij} = random cow effect (j=1, 2, ..., 122),

ζ_k = season of calving (k=1, 2),

ψ_l = year of calving (l=1, 2, .., 7),

π_m = parity number (m=1, 2, 3, 4),

δ_{ijklmn} = days in milk (covariate), and

ϵ_{ijklmn} = error which is assumed N (0, σ_e^2).

An analysis of variance table (25) was used to determine whether these effects were significantly associated with lactation somatic-cell score. All effects were tested against the error term with the exception of genetic line which was tested against the cow term.

Categorical data were analyzed with a multivariate logistic regression technique. In logistic regression, the regression coefficients, ß, are interpreted as the rate of change in one category of the response variable Y per unit of change in independent variables **X** with respect to a arbitrary reference category (11). The following model (30) was applied separately to prevalence (π) of clinical mastitis, minor pathogen IMI, and major pathogen IMI scores:

$$Y = \pi + \epsilon$$

$$\pi = P(Y=y|\mathbf{X}) = [1 + e^{g(X)}]^{-1} e^{g(X)}$$

$$g(\mathbf{X}) = \beta_0 + \beta_1 \mathbf{X}_1 + \beta_2 \mathbf{X}_2 + \beta_3 \mathbf{X}_3 + \beta_4 \mathbf{X}_4 + \beta_5 \mathbf{X}_5 \quad [2]$$

where:

Y = one of the three scores (clinical mastitis, minor pathogen IMI, or major pathogen IMI),

y = 1 if the cow belongs to one score category,

= 0 otherwise,

β_0 = constant terms for each categories,

β_1, β_2, β_3, β_4, β_5 = regression coefficients,

$\mathbf{X}$ = $[\mathbf{X}_1, \ldots\ldots, \mathbf{X}_5]$ = independent variables, and

ϵ = error which is distributed multinomially with parameter π.

As described in the experimental design, the independent variables were four genetic lines, six years of calving, two seasons of calving per year, four classes for lactation number, and days in milk as covariate. Years of calving were grouped as: cows calving before 1986, in 1986, in 1987, in 1988, in 1989, or after 1989. Dummy variables were used for all variables with the exception of days in milk. The model used to study the prevalence of BIV and BLV infections was similar to (2) with two categories (y=0 if cow is uninfected, y=1 if cow is infected) for the binomially distributed response variable Y and did not include year of calving and days in milk.

To find the most parsimonious model which best fitted the data, a stepwise procedure was used to select which variables explained significantly the variation seen in the prevalence of mastitis scores and retroviral infections. The maximum likelihood estimates of the parameters ß were obtained using the procedure PROC LOGISTIC on SAS (25). In SAS, the reference category is the score of the highest category of the variable which is set to zero. See Table 1 for the value of the highest category for each score. The assumption of identical regression coefficients or proportional odds

assumption was checked.

The procedure PROC FREQ on SAS (25) was used to study the frequency of clinical disease other than mastitis, diagnosed during the herd life of the cows. Pearson's chi-square was used to test association among genetic lines, year and season of calving while Mantel-Haenzel's chi-square was used to test association among parities (25).

Genetic models

To obtain breeding values for lactation somatic-cell score, two categories of additive animal models were used: animal model with repeated measurements for records on all parities and simple animal models for records on each parity separately. Years of calving were grouped into three categories: cows calving before 1988, cows calving in 1988 and 1989, and cows calving after 1989. For each model, two seasons of calving were used as described in the experimental design, and days in milk was included as covariate. For data on each parity, the model was:

$$\mathbf{Y} = \mathbf{X}_1 \boldsymbol{\beta}_1 + \mathbf{X}_2 \boldsymbol{\beta}_2 + \mathbf{Z}\mathbf{u} + \mathbf{e} \qquad [3]$$

where:

$\mathbf{Y}$ = vector of lactation somatic-cell scores,

$\mathbf{X}_1$, $\mathbf{X}_2$ = incidence matrices for fixed effect,

$\mathbf{Z}$ = incidence matrix for animal effect,

β_1 = vector of fixed effects for year-season of calving,

β_2 = vector of fixed effects for days in milk,

$\mathbf{u}$ = vector of random animal effect, and

$\mathbf{e}$ = vector of error terms.

The $\mathbf{e}$ were assumed $N(0, I\ \sigma_e^2)$, and $\mathbf{u}$ were assumed $N(G, A\ \sigma_a^2)$, where $\mathbf{G}$ is the additive genetic value, $\mathbf{A}$ is the relationship matrix between animals and their known ancestors, σ_e^2 is the residual variance, and σ_a^2 is the additive genetic variance. A unitrait repeated records animal model was used for genetic evaluation of data on all parities. The model was similar to [3] but included a fixed effect for parity and a permanent environment effect $\mathbf{p}$ which was assumed $N(0, I\ \sigma_p^2)$. The fixed effects included in the models were deduced from the results of the exploratory models (Table 1). Variances in both animal models were estimated using DF-REML (19). Standard errors were obtained from an approximation of the matrix of second derivatives of the log-likelihood function with respect to additive genetic and error variances (19). Heritability estimates were obtained from genetic additive and error variance components.

Threshold (THRE) models for ordered categories postulate that the mutually exclusive, and exhaustive phenotypic categories (disease categories) are related to an underlying

normally distributed variable called liability. When the liability is below some threshold level, one phenotypic category is observed; when it is above, another phenotypic category is observed. The liability is determined by environmental and genetic factors acting homogeneously, independently, and additively (8,9). In our analysis of mastitis scores and retroviral infections, the following model was assumed:

$$\mathbf{Y} = \mathbf{X}\,\boldsymbol{\beta} + \mathbf{Z}\mathbf{u} + \mathbf{e} \qquad (4)$$

where:

$\mathbf{Y}$ = liability for one of the mastitis scores (clinical mastitis, major pathogens IMI, minor pathogens IMI) or retroviral infections,

$\mathbf{X}$ = incidence matrix for fixed effects,

$\mathbf{Z}$ = incidence matrix for the animal effects,

$\boldsymbol{\beta}$ = vector of fixed effects, and

$\mathbf{u}$ = vector of unknown parameters for random animal effects.

Table 1 contains the list of fixed effects ($\boldsymbol{\beta}$) included in the threshold models. The $\mathbf{e}$ were assumed $N(0, I\,\sigma_e^2)$, and $\mathbf{u}$ were assumed $N(G, A\,\sigma_a^2)$, where G is the additive genetic value, A is the relationship matrix between animals and their known ancestors, σ_e^2 is the residual variance, and σ_a^2 is the additive

genetic variance. The fixed effects that were included in THRE models were deduced from the results of the exploratory models (Table 1). Finally, categories for the categorical trait were grouped to decrease the extreme category problem (21). Variance components were estimated by the methods of Misztal et al. (21). Heritability were obtained as the ratio of animal variance to total variance.

In Table 1, the structure of the different models used for analyzing the disease indicators is summarized.

RESULTS AND DISCUSSION

Basic Statistics

Mastitis indicators

For bacteriological data, the agreement rate between cultures of duplicate quarter milk samples was 98%. The results of examination of bacteriological samples collected 30 days before, at, and 30 days after calving are summarized in Table 2. Around 72% of bacteriological cultures were negative. Minor pathogens and major pathogens constituted 86% and 14% of all bacteriologically positive cultures, respectively. The minor pathogen IMI score varied from 0 to 10 (mean=2.4) and the major pathogen IMI score from 0 to 4 (mean=0.4). Therefore, over the three samples taken per lactation, a cow had 1 chance over 5 (2.4/12=0.2) to have one quarter infected with a minor pathogen or 0.8 (0.2X4) quarter infected with minor pathogens that did not spontaneously cure. Mastitis caused by minor pathogens is an important problem in primigravid heifers (22). This explains the high frequency of intramammary infections caused by coagulase negative staphyloccoci observed in our herd (Table 2). Around 55% of our bacteriological samples originated from first parity cows.

The lactation somatic cell scores varied from 1.20 to 8.90 (overall mean=3.46). The lactation somatic cell score

increased significantly as bacterial status changed from uninfected to major pathogens. On average, the lactation somatic-cell score was 3.2 for uninfected cows and for minor pathogens, and 3.5 for major pathogens. The score obtained for cows infected with major and minor pathogens was lower than the values published in the literature (22,24).

Among cows receiving treatment for clinical mastitis, the average cow had 0.63 quarters infected per lactation, for a period of around 31 days. Approximately 27% of total clinical cases of mastitis occurred in the first month of lactation. Cows with clinical cases of mastitis had an average lactation somatic cell score of 4.1.

Retroviral infections

Overall, prevalence of BIV-infected cattle was 10.2% and prevalence of BLV-infected cattle was 11.3 %. Prevalence of BIV and BLV infection per parity are shown in Table 3.

The prevalence for BLV seems to be decreasing in the present herd. Indeed, from 1982 to 1987, the herd was characterized with an average prevalence rate of 20% (5). On the other hand, the prevalence of BIV infection was high compared to USA national surveys in which natural prevalence of BIV had been estimated at around 4% (10). In some herds, there have been reports of natural mixed infections with both BLV and BIV (1) while in others, like in our herd, no

relationship between BIV and BLV infection was found (32).

Clinical disease other than mastitis

Prevalence of clinical disease others than mastitis is shown in Table 10. The most frequent health problems were metabolic disorders (ketosis), digestive problems (diarrhea) and foot problems (foot rot and foot abscesses).

Exploratory Models

The main goal of the exploratory models was to determine which environmental determinants significantly affected our measures of disease prevalence.

Mastitis indicators

The results of the analysis of covariance for lactation somatic cell score are shown on Table 4. The only significant effects on lactation somatic cell score were cow and days in milk. The cow effect represents both genetic and non-genetic effects of the cow on lactation somatic cell score. This study confirmed the important role of the status of the gland on SCC in milk (3,24). It has been shown that milk SCC in uninfected cows is high at calving and lowest from peak to middle lactation (24). This is one explanation for our observation that the lactation somatic cell score decreased by

0.008 (st.error= 0.001) per day in milk.

The results of the logistic regression for clinical mastitis score are shown on Table 5. Parity was the only factor significantly affecting clinical mastitis score. The odds of having one quarter clinically infected per lactation in third parity was 3.4 ($e^{1.22}$) times greater than in first parity. This was expected since older cows have more opportunity of exposure to pathogens. The proportional odds assumption holds ($p=0.20$) meaning that the effect of parity was the same for each clinical mastitis score category.

Only days in milk induced statistically significant changes in the major pathogen IMI score ($p<0.10$). The odds of having one quarter infected with a major pathogen (or, by definition of the score, the odds of having an infected quarter that did not spontaneously cure) increased by 1% per day of lactation.

The stepwise selection procedure for minor pathogen IMI scores (Table 6) retained only parity as a significant infection determinant. The odds of having one quarter infected with a minor pathogen (or the odds of having an infected quarter that did not cure) was 4.6 ($=e^{1.52}$) times greater in first parity than in second or third parity cows. This observation confirmed previous reports showing that the frequency of minor pathogens is higher in primigravid heifers than in older cows (22).

The proportional odds assumption for major pathogen IMI and minor pathogen IMI scores did not hold. This implies that the effect of independent variables was not similar for each score category (11)

Retroviral infections

Only genetic line had a significant ($p<0.05$) effect on probability of being infected with BLV or BIV. The odds of being BLV seropositive was lower in high producers when compared to average producers (Table 7). The odds of being seropositive was higher in average than in high producers. A possible explanation for this difference is that high producers are more able to resist BLV infection than average producers and/or that more BLV seropositive cows were culled among high producers than among average producers. Indeed, it has been shown that resistance to BLV infection is under genetic control (12,13) and that cows infected with BLV do not produce the amount of milk and percentage of fat expected from their genetic potential (33).

The odds of being BIV seropositive (Table 8) was significantly higher in the high PTA line than in the average PTA line for milk fat and protein. Similarly, it has been shown that BIV-infected cattle tend to be more productive than their uninfected contemporaries (Dunklee, Personal communication). No difference was found among cows selected

for high and average PDM.

Clinical disease other than mastitis

Analyses of the effects of parity, year and season of calving, and genetic line on the prevalence of clinical disease other than mastitis are shown on Table 9. As expected, most deaths and metabolic problems occurred in older cows (16) while most digestive (mainly diarrhea) problems occurred in younger cows. Because of an outbreak of salmonellosis or bovine viral diarrhea (not determined) occurring in the herd in 1990, cows calving in 1990 had more health problems than cows calving the other years. Cows calving in summer suffered more from foot and reproductive problems than cows calving in winter. Cows with high PDM significantly had more metabolic (mainly ketosis and milk fever) problems than cows of the other genetic categories. The genetic correlation between ketosis and milk yield has been estimated at 0.17 (16).

Genetic Models

With categorical traits, the assumptions of normality and homoscedasticity of variances used in linear models are violated. Also, important properties of BLUP, i.e. maximum likelihood estimation of the best predictor and maximization

of the probability of correct pairwise ranking, are only derived under the assumptions of multivariate normality of data and genetic merit to be predicted (17). In a simulation study of THRE and BLUP models, Meijering and Gianola (18) concluded that THRE models are most advantageous when the heritability is high to moderate, the trait has two to four categories, the data are unbalanced, and the model has several fixed factors with large effects on the underlying liability. Our data fit this situation with the exception that heritabilities were expected to be low.

The effect of genetic line was not included in any of the genetic models because it was expected to reduce the genetic variability among cows. Because too few data were available, genetic models were not applied to clinical disease other than mastitis.

Mastitis indicators

Table 10 gives estimates of genetic parameters obtained for the lactation somatic cell score. Our heritability estimates (0.07) are close to estimates found in the literature (3,7,31). Repeatability and permanent error variances were computed only for data with repeated measurements. The high repeatability (0.24) was due to the large permanent environment variance. It is important to remember that elevated SCC is an indirect measure of host

response to infection. The cellular infiltration of the mastitic gland is a normal response to bacterial infection and the development of mammary infections depends upon the dynamic interaction between somatic cells and invading bacteria. Because IMI due to minor and environmental pathogens are of short duration and because percent of quarters infected at any time is generally low, somatic cell counts at monthly interval are of questionable value (26). Also, somatic cells may act as a reservoir of bacteria, especially if they are dysfunctional. Therefore, some reservation remains regarding the use of SCC as a selection criteria for mastitis resistance.

Heritability estimates for liability of clinical cases of mastitis and bacteriological cultures are shown in Table 11. The threshold values correspond to the values of the liability variables separating the observed phenotypic categories. As shown previously (31), heritability for liability of clinical cases of mastitis was around 10% in first and second parity cows. Because no permanent effect was included in the model to take account of the repeatability of clinical cases of mastitis across parities, high heritability value was obtained for clinical cases of mastitis when combining all parities.

Heritability for liabilities of major pathogen IMI and minor pathogen IMI scores were estimated at around 10% (Table 11). In the literature, heritability estimates for IMI range

from 0 to 20% with most estimates falling below 5% (6,20). In a large study of first parity cows, heritability of incidence of bacterial infection was estimated by THRE models to be 4.5% (31). This discrepancy may be due to differences in sample sizes, definition of bacteriological status, and genetic model.

The phenotypic and genetic correlations between mastitis scores are shown on Table 12. Because the permanent environment effect was not included in the THRE model for clinical mastitis score, only results at first and second parities are reported. The genetic correlations of lactation somatic cell score with clinical mastitis and major pathogen IMI scores were not statistically significant. In the literature, estimates of genetic correlations between SCC and various measures of clinical mastitis ranged from 0.01 to 0.8 (4,7) indicating that SCC and clinical mastitis do not measure the same trait. The phenotypic correlation of clinical mastitis score in first parity with major pathogen IMI and minor pathogen IMI scores were negative while the genetic correlations were positive. The pattern was reversed for second parity cows. This may be due to the nature of the pathogens infecting cows in first (mainly minor pathogens) and second parities. Finally, the genetic correlation between major pathogen IMI and minor pathogen IMI scores was negative. Therefore, a quarter infected with minor pathogens will be

more resistant to infection by major pathogens. It has been shown that mammary infections by minor (or major) pathogens augment the resistance of quarters to experimentally induced infections with major (or minor) pathogens (23).

Retroviral infections

Heritability estimates for infections with BIV and BLV were close to zero (Table 13) confirming the important role of the environment in the transmission of the viruses. Previous studies found association of various MHC class I serotypes (W6, W12, W14) of the bovine lymphocyte antigen complex with persistent lymphocytosis due to BLV (12,13,29).

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Table 1. Structure of the models explained in the text

Trait under study	Categories for trait	Effects in the model[a]						Model in text
		Line	Cow	Calving season	Calving year	Parity	Days in milk	
Exploratory models								
Somatic cell score		X	X	X	X	X	X	1
Clinical mastitis	0,1,2,3,>=4	X		X	X	X	X	2
Major pathogens IMI	0,1,2,3,4	X		X	X	X	X	2
Minor pathogens IMI	0,1,2,3,>=4	X		X	X	X	X	2
Retroviral infections	0,1	X		X		X		2
Genetic models								
Somatic cell score			X		X	X	X	3
Clinical mastitis	0,1,>1[b]		X			X[c]		4
Major pathogens IMI	0,>=1		X				X	4
Minor pathogens IMI	0,>=1		X			X		4
Retroviral infections	0,1	X	X					4

[a] Intercept was included in all models. [b] Only two categories for first parity cows (0,>=1). [c] Only for clinical cases over all parities

Table 2. Frequencies of pathogens (n=1084 bacteriological cultures)

Quarter status	Frequency (%)	
Major pathogens	3.9	
Coagulase positive staphylococci		0.9
Escherichia coli		1.6
Minor pathogens	23.8	
Coagulase negative staphylococci		23.7
Non pathogens	72.3	
Contaminants		6.0
Negative (No growth)		66.3

Table 3. Prevalence (%) per parity of bovine leukosis and bovine immunodeficiency virus carriers among n cows tested

	BLV		BIV	
	%	n	%	n
First parity	9.26	54	10.17	59
Second parity	3.13	32	11.43	35
Third parity	23.81	21	4.35	23
Fourth parity and greater	17.65	17	15.00	20

Table 4. Study of effects of genetic line, parity, season and year of calving, and days in milk on somatic-cell score by model 1 in Table 1: R^2 model =0.75

Effects in model	Degree of freedom	Mean square error	Significance level[a]
Genetic line[b]	4	2.493	<0.10
Cow:Genetic line	125	1.362	<0.05
Season of calving	1	0.024	>0.10
Year of calving	6	1.290	>0.10
Days in milk	1	34.222	<0.05
Parity	3	0.293	>0.10

[a]Significance level for testing effects in model

[b]Error term for testing genetic line is cow:genetic line.

Table 5. Results of logistic regression analysis of clinical mastitis score: estimated coefficients and standard errors (model 2 in Table 1)

Variables in the model	Estimated coefficient	Standard error
Parity 1 - parity 2	-0.59*	0.32
Parity 1 - parity 3	-1.22**	0.37
Parity 1 - parity 4 and over	-1.01**	0.38

**p<0.01; *p<0.10

Table 6. Results of logistic regression analysis of minor pathogen IMI score: estimated coefficients and standard errors (model 2 in Table 1)

Variables in the model	Estimated coefficient	Standard error
Parity 1 - parity 2	1.52**	0.44
Parity 1 - parity 3	1.52*	0.67
Parity 1 - parity 4	0.07	0.84

** $p<0.01$; * $p<0.10$

Table 7. Results of logistic regression analysis of bovine leukosis virus infection: estimated coefficients and standard errors (model 2 in Table 1)

Variables in the model	Estimated coefficient	Standard error
High (F+P) vs average (F+P)	-0.96	0.90
High (F+P) vs high milk	-0.69	1.03
High (F+P) vs average milk	-2.13*	0.87

* $p<0.05$

Table 8. Results of logistic regression analysis of bovine immunodeficiency virus infection: estimated coefficients and standard errors (model 2 in Table 1)

Variables in the model	Estimated coefficient	Standard error
High (F+P) vs average (F+P)	2.20*	1.08
High (F+P) vs high milk	1.84	1.08
High (F+P) vs average milk	0.62	0.72

* $p<0.05$

Table 9. Prevalence (%) of clinical disorders per category and chi-squares[a] (χ^2)

	Metabolic	Foot	Digestive	Respiratory	Death	Reproduction
Genetic line	χ^2=14.7**	χ^2=3.6	χ^2=29.4***	χ^2=6.2	χ^2=0.2	χ^2=4.9
High (F+P)	4.3	6.45	10.75	0.00	4.30	3.22
Aver. (F+P)	1.44	10.14	18.84	4.34	4.34	0.00
High milk	16.51	10.09	1.83	0.92	3.67	0.00
Aver. milk	3.00	4.00	2.00	1.00	5.00	1.00
Calving year	χ^2=10.1	χ^2=8.9*	χ^2=31.6***	χ^2=7.7*	χ^2=13.2*	χ^2=6.1
<=1989	5.19	7.79	1.95	3.90	0.00	0.65
1990	10.09	11.01	15.60	10.09	7.34	0.00
>=1991	7.83	3.48	5.22	7.83	8.67	2.61
Calving season	χ^2=1.5	χ^2=9.4***	χ^2=0.2	χ^2=0.6	χ^2=0.1	χ^2=5.1*
Summer	8.51	12.77	7.80	0.71	4.25	2.84
Winter	5.91	4.22	6.33	1.69	5.06	0.00
Parity	χ^2=14.7***	χ^2=0.1	χ^2=24.6***	χ^2=2.2	χ^2=3.1*	χ^2=0.3
First	1.40	3.50	15.38	2.80	3.50	0.70
Second	6.36	2.73	1.82	0.00	2.73	2.73
Third	9.52	7.94	1.59	1.59	7.94	0.00
>=fourth	6.79	8.06	1.61	0.00	8.06	0.00

* p<0.1; ** p<0.01; *** p<0.005; [a]Chi-squares tested the alternative hypothesis of association between row and column variable

Table 10. Estimates of genetic parameters for lactation somatic-cell score: REML estimates (model 3 in Table 1)

Parity number	All	First	Second	Third	Fourth
Heritability	0.07 (0.21)[a]	0.07 (0.23)	0.50 (0.33	0.00	0.12 (0.86)
Additive genetic variance (σ^2_a)	0.075	0.075	0.591	n.a.[b]	0.213
Repeatability	0.24 (0.22)	n.a.	n.a.	n.a.	n.a.
Permanent error variance (σ^2_p)	0.233	n.a.	n.a.	n.a.	n.a.
Error variance (σ^2_e)	0.660	0.948	0.591	n.a.	1.555
Number of animals in pedigree	262	253	217	133	81

[a]standard error; [b]n.a.= not available

Table 11. Estimates of genetic parameters for liability[a] of clinical-mastitis, major pathogens IMI, and minor pathogens IMI and solutions for fixed effects (model 4 in Table 1)

Mastitis score	Clinical mastitis				Major pathogens IMI	Minor pathogens IMI
Parity	All	First	Second	Third	All	All
Heritability	0.27	0.06	0.08	0.00	0.10	0.10
Threshold[b]						
No case vs one case	0	0	0	0	0	0
One case vs more than one cases	0.74	n.a.	0.81	0.51	n.a.	n.a.
Parity[c]						
First	-0.78	n.a.	n.a.	n.a.	n.a.	1.25
Second	-0.38	n.a.	n.a.	n.a.	n.a.	-0.04
Third	-0.01	n.a.	n.a.	n.a.	n.a.	0.13
Fourth	0	n.a.	n.a.	n.a.	n.a.	0.69
Days in milk[d]						
Less than 300	n.a.	n.a.	n.a.	n.a.	-1.21	n.a.
More than 300	n.a.	n.a.	n.a.	n.a.	-0.69	n.a.

[a] Liability is defined in the text; [b]Values of the liability variable when mastitis scores change of category; [c]Solutions for the parity effect on liability of mastitis score; [d]Solutions for the effect of day-in-milk on liability of mastitis score

Table 12. Phenotypic and genetic correlations between mastitis scores

Mastitis scores	SCC	CLI1	CLI2	Major	Minor
Somatic cell score (SCC)	0.07	-0.18	-0.10	-0.08	0.06
Clinical mastitis					
First parity (CLI1)	-0.04	0.07	0.04	0.22*	0.17
Second parity (CLI2)	0.09	0.08	0.09	-0.15	-0.07
Major pathogens IMI	0.11	-0.01	0.06	0.11	-0.26**
Minor pathogens IMI	0.06	-0.02	0.18	0.00	0.12

* $p<0.10$; ** $p<0.05$
Heritabilities are diagonal elements, genetic correlations are upper off-diagonal elements, and phenotypic correlations are lower off-diagonal elements

Table 13. Estimates of genetic parameters for liability of retroviral infections and solutions for fixed effects (model 4 in Table 1)

Viral infections	Bovine leukosis virus	Bovine immunodeficiency virus
Heritability	0.00	0.04
Parity[a]		
First	-1.32	-1.28
Second	-1.88	-1.21
Third	-0.71	-1.72
Fourth and over	0	0
Number of animals in pedigree	264	279

[a]Solutions for the parity effect on liability of mastitis score

APPENDIX

EXAMPLE OF THE STRUCTURE OF DATA

COWID	Parity	MASTITIS SCORES			
		Somatic cell	Clinical mastitis	Major pathogen IMI	Minor pathogen IMI
1878	4	2.72	2	n.a.	n.a.
	5	3.54	1	n.a.	n.a.
	6	3.20	0	n.a.	n.a.
	7	4.16	0	n.a.	n.a.
	8	4.41	0	n.a.	n.a.
1899	3	2.49	0	n.a.	n.a.
	4	2.52	0	n.a.	n.a.
	5	2.59	0	n.a.	n.a.
	6	2.78	0	n.a.	n.a.
2393	2	3.57	0	n.a.	n.a.
	3	4.73	1	n.a.	n.a.
	4	5.76	1	0	3
2459	2	2.06	0	n.a.	n.a.
	3	4.12	2	n.a.	n.a.
	4	4.34	1	n.a.	n.a.
	5	2.07	0	4	2
2528	1	2.90	0	n.a.	n.a.
	2	4.85	0	n.a.	n.a.
2610	2	3.05	0	n.a.	n.a.
	3	3.00	0	0	0
2631	2	3.81	0	n.a.	n.a.
	3	2.81	0	n.a.	n.a.
	4	1.34	0	0	3

n.a.= information not available

COWID	Parity	MASTITIS SCORES			
		Somatic cell	Clinical mastitis	Major pathogen IMI	Minor pathogen IMI
2764	2	3.30	0	n.a.	n.a.
	3	3.18	0	n.a.	n.a.
	4	2.55	0	0	0
2793	1	2.67	0	n.a.	n.a.
	2	3.63	0	0	3
	3	3.86	0	n.a.	n.a.
2874	1	2.89	0	n.a.	n.a.
	2	3.14	0	n.a.	n.a.
	3	4.84	2	0	0
3042	2	3.11	0	0	0
3046	2	2.15	0	0	0
3064	2	2.89	0	0	0
3164	1	1.48	0	0	6
3167	1	2.87	0	0	7
3004	1	3.38	0	0	2
	2	6.70	3	0	2
3010	1	4.00	0	0	4
	2	3.28	1	0	4
3049	1	2.34	0	n.a.	n.a.
	2	3.83	1	0	0
3090	1	2.45	0	2	0
2964	1	3.51	0	n.a.	n.a.
	2	2.16	0	0	3
2972	2	2.94	0	0	1
	3	3.10	0	0	2

n.a.= information not available

PAPER IV. GENETIC ASSOCIATION BETWEEN PARAMETERS OF INNATE IMMUNE RESPONSE TO INFECTIOUS DISEASES IN PERIPARTURIENT HOLSTEIN CATTLE

Genetic Association Between Parameters of Innate Immune Response and Resistance to Infectious Diseases in Periparturient Holstein Cattle

J. C. Detilleux[1], A. E. Freeman[1], M. E. Kehrli, Jr.[2], A. B. Dietz[2], J. E. Butler[3], L. K. Fox[4], L. D. Miller[5], D. H. Kelley[1]

[1]Animal Science Department, Iowa State University, Ames, IA 50011

[2]US Department of Agriculture, Agricultural Research Service, National Animal Disease Center, Ames, IA 50010

[3]Department of Microbiology, University of Iowa Medical School, Iowa City, IA 52242

[4]Department of Veterinary Clinical Medicine, Washington State University, Pullman, WA 99164

[5]College of Veterinary Medicine, Iowa State University, Ames, IA 50011

ABSTRACT

The genetic relationships between indicators of disease resistance (somatic cell score, number of quarters with clinical mastitis, number of quarters infected with minor pathogens or major pathogens, serological status to bovine leukosis or to bovine immunodeficiency viruses) and various immunological parameters (eleven in vitro immunological assays, BoLA-DRB3 locus, IgG_{2a} isotype, and CD18 genotype) were examined in 137 periparturient Holstein cows. The additive genetic effects of the parameters were analyzed with a gene substitution effect model. The genetic relationship between animals was included in the computation of the solutions. Significant genetic associations with disease indicators were found only for alleles of the BoLA-DRB3 locus. Negative genetic associations were found between all mastitis indicators and presence of BoLA-DRB3 alleles b12 and b11, various neutrophil assays, number of blood mononuclear cells, and presence of IgG_{2a} allele A1. Cows with allele b27 were significantly more resistant to infection by mastitis pathogens but significantly less resistant to their pathogenic effects. There are some indication that allele b27 belongs to the haplotype containing class I W16 serotype and class II DQ^{1B} RFLP, both associated with susceptibility to mastitis. The contribution of all immunological parameters to the total

genetic variation in mastitis indicators was around 40%. Absence of bovine immunodeficiency virus infection was significantly associated with BoLA-DRB3 allele b3. No significant genetic effects of any of the immunological parameters on infection with bovine leukosis virus could be demonstrated.

INTRODUCTION

Animal health is one of the many factors that affect the economic efficiency of a dairy herd (Shanks, Freeman & Dickinson, 1981) and selection of animal resistant to diseases is one alternative to improve animal health. To protect its integrity, the body has developed a large variety of defense reactions among which the immune response plays an important role. Innate resistance to infectious diseases reflects those fixed physiological attributes of an animal which make it more or less susceptible to the development of disease caused by a particular pathogen. Two sets of genes which govern the innate immune response to infection can be distinguished: those that control the quality and quantity of the immune response (such as genes involved in phagocytosis) and those which determine the specificity of the immune response (such as major histocompatibility complex (MHC), T cell receptor, and immunoglobulin molecule genes).

To measure aspects of the quality/quantity of innate immune response, in vitro immune assays can be used. These assays are correlates of in vivo immune functions. They are relatively easy to measure, at low cost, early in life, in both sexes, and are repeatable. The additive genetic values for these assays represent the additive effects of all genes involved in the effector functions of the immune cells.

To measure aspects of the specificity of the innate immune response, DNA molecular markers may be used. Bovine lymphocyte antigen (BoLA) has been associated with various diseases (østergård, Kristensen & Andersen, 1989) and with total health costs (Weigel et al., 1990). Among others, earlier studies found association of class I serotypes with resistance to clinical mastitis (Weigel et al., 1990) and with incidence of bovine leukosis virus-dependent persistent lymphocytosis (Lewin & Bernoco, 1986; Lewin et al., 1988). Immunoglobulin allotypes have been characterized in cattle for most of the known isotypes. The IgG_{2a} isotype has been defined on the basis of its expression of either the A1 or A2 allotypic determinants on the Fc portion of the molecule (Butler, 1983). In the bovine, IgG_{2a} constitutes half of the total serum IgG and is an important opsonins in phagocytosis (Heyermann, Butler & Frangione, 1992).

Cells of the immune system depend on regulated interactions with other cells to activate the response to infection. Three family of adhesion receptors mediate these interactions: the immunoglobulin superfamily, the integrin family, and the selectins (Springer, 1990). In the bovine, a genetic disorder of one integrin has recently been described. Bovine leukocyte adhesion immunodeficiency (BLAD) is characterized clinically by recurrent respiratory and gastrointestinal tract infections, periodontitis, delayed

wound healing, persistent neutrophilia, and death at an early age (Kehrli et al., 1990; Shuster et al., 1992). This genetic disorder is due to a mutation (D128G) of an aspartic acid to glycine substitution at amino acid 128 of the ß-subunit of β_2 integrin adhesion molecules (Shuster et al., 1992).

The objective of this study was to obtain genetic correlations between infectious disease indicators and immunological markers. Particular emphasis was placed upon the study of mastitis and upon two bovine retroviruses. The immunological markers consisted of eleven in vitro assays of immune functions and of the genotypes for loci involved with the immune system (BoLA class II DRB3 locus, IgG_{2a} locus, and CD18 locus).

MATERIALS AND METHODS

Animals and Experimental Design

Periparturient Holstein cows (n=137) from the I-O-State herd were used in this study. All animals are managed the same and fed for high production. For immune assays, weekly blood samples were collected starting 35 days before calving until 35 days after calving. Around 20 cows were sampled per week. Twins have been excluded from the study. Five steers were used as laboratory standards to reduce the day-to-day variability typically seen with immune cell function assays.

Genotypes Typing

BoLA-DRB3 Typing

BoLA class II alleles were assigned by a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method (van Eijk, Stewart-Haynes & Lewin, 1992) with some modifications (Dietz et al., 1988).

Immunoglobulin G_{2a} isotype

Immunodiffusion analysis was conducted to determine the IgG_{2a} isotype of the cows, using antisera against the whole $IgG2_{2a}$ molecule or its Fc fragment (Heyermann, Butler &

Frangione, 1992).

CD18 genotype

Animals were genotyped for the mutation responsible for the bovine leukosis adhesion deficiency (BLAD) syndrome (Kehrli et al., 1990). In summary, genomic DNA was isolated from peripheral blood leukocytes and amplified. Amplification products were subjected to restriction endonuclease digestion separately by direct addition of Taq I or Hae III, respectively (Shuster et al., 1992). The CD18 mutation was detected by the different restriction fragment lengths (Shuster et al., 1992).

Disease and Immunological Genotypes

Disease genotypes

Disease recording was carried out as previously described (Detilleux et al., 1993b). For each cow and each lactation, indicators of mastitis were DHIA somatic cell score (SCS), number of quarters with clinical mastitis, number of quarters infected with major pathogens, and number of quarters infected with minor pathogens. The serological status of the cows to bovine leukemia virus (BLV) and to bovine immunodeficiency virus (BIV) were also determined. Categorical traits (number quarters with clinical mastitis or infected with major or

minor pathogens, and retroviral serological status) were assumed to have an underlying continuous variable (called liability) with a threshold which imposes a discontinuity on their phenotypic expression (Harville & Mee, 1984). When the liability is below some threshold level, one observes one phenotypic category; when it is above the threshold level, one observes another phenotypic category. Estimates of the additive genetic values (called breeding values) for somatic cell score and for liabilities were obtained using the estimates of heritability shown in Table 1. Heritability estimates for somatic cell score and for clinical mastitis were obtained from the literature (Boettcher et al., 1992) and used to compute best linear unbiased predictor (BLUP) of the additive genetic values (Henderson, 1988). When no published heritability estimates were available (retroviral and intramammary infections), they were estimated by restricted maximum likelihood (REML) from an animal model including parity, season of calving, and days-in-milk as fixed effects (Detilleux et al., 1993b).

Immune assays

In vitro immune assays were performed as described (Kehrli, Nonnecke & Roth, 1989a; Kehrli, Nonnecke & Roth, 1989b; Detilleux et al., 1993c): neutrophil (PMN) migration under agarose, PMN zymosan phagocytosis-associated native

chemiluminescence, PMN cytochrome C reduction, PMN iodination, PMN ingestion, lymphocyte blastogenic response to mitogen concanavalin A, serum immunoglobulin levels (IgG_1, IgG_2, IgM), and leukograms. To account for the variability inherent to neutrophil and lymphocyte blastogenesis assays, daily individual cow results were expressed as a percentage of the steers daily mean (=ratio of laboratory standards). A logarithmic (log_{10}) transformation was applied to the ratio and a square root transformation was applied to the number of mononuclear cells to have measures that were more normally distributed than the original data. Because of the immunosuppression observed in periparturient cows (Kehrli, Nonnecke & Roth, 1989a; Kehrli, Nonnecke & Roth, 1989b), animal model models were applied to each immune assay before, at, and after immunosuppression to obtain REML estimates of heritability (Detilleux et al., 1993a). The animal models included parity and season of calving as fixed effects. When heritability estimates were different from zero (Table 2), the additive genotypic values for each immune assays were derived (Detilleux et al., 1993a).

Statistical Analyses

For each disease trait and each immune assay separately, we used the following "gene substitution" model to study the effects of the estimated additive genotypic values (breeding values) for our immune assays, the effects of DRB3 class II genotype, the effects of isotype IgG_{2a} genotype, and the effects of the genotype at locus CD18 on the breeding values for each disease indicator.

$$G_{dr} = \mathbf{MHC} + \mathbf{IgG} + \mathbf{CD18} + b_{ir}\, G_{ir} + e_{dr} \qquad [1]$$

where:

G_{dr} = nX1 vector of breeding values for one disease trait (mastitis indicators or retroviral infections),

$\mathbf{MHC} = \Sigma_m\, b_m\, n_m$ for m=1, 2, ...22,

where:

b_m = gene substitution effect of each allele m in the BoLA-DRB3 genotype,

n_m = number of copies of allele m in the genotype (n_m=0, 1, 2),

constraint: $\Sigma_m\, b_m = 0$,

$\mathbf{IgG} = \Sigma_g\, b_g\, n_g$ for g=1, 2,

where:

b_g = gene substitution effect of each

allele g in the isotype IgG_{2a},

n_g = number of copies of allele g in the isotype (n_g=0, 1, 2),

constraint: $\Sigma_g\ b_g = 0$,

CD18 = $\Sigma_c\ b_c\ n_c$ for c=1, 2,

where:

b_c = gene substitution effect of each allele c in the genotype CD18,

n_c = number of copies of allele c in the genotype (n_c=0, 1, 2),

constraint: $\Sigma_c\ b_c = 0$,

$\mathbf{G}_{ir}$ = nX1 vector of random effects for breeding values for one of the immune assay (before, at, or after immunosuppression),

b_{ir} = regression coefficient of estimated genotypic value of immune response on the breeding value for disease trait, and

$\mathbf{e}_{dr}$ = nX1 vector of random error affecting breeding values for disease.

In the model, $\mathbf{G}_{dr}$ were assumed N(0, $\mathbf{A}\ \sigma^2_{adr}$) and $\mathbf{G}_{ir}$ were assumed N(0, $\mathbf{A}\ \sigma^2_{air}$) where **A** is the known genetic relationship matrix between animals and their known ancestors, σ^2_{air} is the estimated additive genetic variance for one particular immune assay (before, at, or after periparturient immunosuppression),

and σ^2_{adr} is the estimated additive genetic variance for the disease trait under study. Under those assumptions, the vector e_{dr} is, from model [1]:

$$e_{dr} = G_{dr} - \mathbf{MHC} - \mathbf{IgG} - \mathbf{CD18} - b_{ir}\, G_{ir}$$

and is distributed $N(0, \mathbf{A}\, \sigma^2_{adr} - b_{ir}^2\, \mathbf{A}\, \sigma^2_{air})$. The solutions for the unknown parameters (b_m, b_c, b_g, b_{ir}) were obtained using the procedure IML on SAS (SAS Institute, 1988), and test of significance were conducted against the error term (e_{dr}).

In gene substitution effect models (østergård, Kristensen & Andersen, 1989; østergård & Jensen, 1989), animals heterozygous at a locus for a particular allele are assigned one copy of that allele, while homozygotes are assigned two copies of that allele. Because the sum of additive effects over all alleles at one locus is zero (constraints in model [1]), an estimable gene substitution effect for each allele was expressed as the difference from the mean of all alleles for each locus (Kehrli et al., 1991). Therefore, for each locus separately, the gene substitution effect referred to the difference in the disease trait associated with exchanging one copy of a particular allele for a copy of a hypothetical allele expressing the mean effect of all alleles tested. To decrease type I errors when comparing the 22 alleles at DRB3 locus, each individual comparison out of the 22 available was

performed at level $\alpha/22$ to ensure a significance level less or equal to α (Steel & Torrie, 1980). Our model has only additive components; no component for epistaxis between loci or dominance between alleles at one locus were included.

The phenotypic and genetic correlations between immune assays were computed before, at, and after immunosuppression and averaged over the three time periods.

RESULTS

Heritability Estimates

Table 1 gives estimates of heritability for the disease indicators. Heritability for all three mastitis indicators was estimated at around 10%. Heritability estimates for infections with BIV and BLV were close to zero.

Heritability estimates obtained for each immune assays are shown before, at, and after the parturient immunosuppression in Table 2. With few exceptions, the periparturient changes were found to have some additive genetic component. Periparturient changes in serum immunoglobulin concentrations were more heritable than changes in neutrophil and lymphocyte assays. We observed some genetic influence on the ability of PMN to perform the oxidative burst associated with phagocytosis (cytochrome c reduction, iodination, and native chemiluminescence).

Observed Allelic Frequencies

Tables 3 and 4 summarize the number and relative allelic frequencies for BoLA-DRB3 locus, for IgG_{2a} isotype, and for CD18 genotype.

Mastitis Indicators and Immune Assays

The regression coefficients of the breeding values for immune assays on the breeding values for mastitis indicators are shown in Tables 5 and 6. Although none of the regression coefficients were statistically significant, some trend can be seen from the tables.

Overall, a negative association was found between PMN assays and mastitis indicators (Table 5). High breeding values for PMN random migration assay were associated with low breeding values for somatic cell score and for the liability of number of quarters infected with major pathogens. Similarly, high breeding values for PMN ingestion assay had a tendency to be associated with low breeding values for mastitis indicators. At immunosuppression, the coefficients of regression of breeding values for PMN iodination assay on the liability of number of quarters with clinical mastitis or infected with major or minor pathogens were all negative. High breeding values for somatic cell score were associated with low breeding values for PMN cytochrome C reduction assay. At immunosuppression, breeding values for PMN chemiluminescence assays were negatively associated with breeding values for somatic cell score and liability of number of quarters infected with minor pathogens. Finally, more negative gene substitution effects were observed at the nadir

of parturient immunosuppression than before or after immunosuppression.

High breeding values for the number of blood mononuclear cells were associated with low breeding values for almost all mastitis indicators (Table 6). Gene substitution effects of breeding values for number of monocytes were lower after parturient immunosuppression than before or at immunosuppression. Overall, high breeding values for serum concentration of IgG_1, IgG_2, and IgM were associated with high breeding values for the majority of mastitis indicators. High breeding values for somatic cell scores were associated with high breeding values for serum concentrations of all Ig isotypes. High breeding values for serum concentration of IgG_2 were generally associated with high breeding values for mastitis indicators. At the nadir of parturient immunosuppression, a positive association was found between breeding values for all mastitis indicators and breeding values for serum concentration of IgM.

Mastitis Indicators and BoLA-DRB3 Genotype

Gene substitution effects for BoLA-DRB3 locus, for IgG_{2a} isotype, and for CD18 genotype on mastitis indicators are shown on Table 7. Most gene substitution effects of alleles at BoLA-DRB3 were statistically significant. Additive effects

of the BoLA-DRB3 alleles accounted for 30% of the total genetic variance in mastitis indicators compared to the 40% of the total genetic variance in mastitis indicators explained by all immunological parameters (MHC, IgG, CD18, and G_{ir} in model 1). A decrease in all mastitis indicators was found in cows carrying the alleles b12 or b11. The presence of allele b23 was associated with low breeding values for all mastitis indicators with the exception of the breeding value for liability of number of quarters infected with minor pathogens. Presence of allele b27 was associated with the largest increase in breeding values for liability for number of quarters with clinical mastitis and with the largest decrease in number of quarters infected with major pathogens, when compared to the other DRB3 alleles (with the exception of alleles with frequencies below 2%).

Although the effects of IgG_{2a} allotypes on breeding values of mastitis indicators were not statistically significant, the presence of allele A1 was associated with a decrease in all mastitis indicators. In animals heterozygous for the D128G mutation (animal with the A2 allele), a decrease in somatic cell score and in liability for number of quarters with clinical mastitis was found while an increase was observed for the liability for number of quarters infected with minor or major pathogens, but none of the gene substitution effects on any mastitis indicators were statistically significant.

Regression coefficients of the breeding values for immune assays on the breeding values for BLV and BIV serological status are shown in Tables 8 and 9. Although not significant, negative genetic associations were found (before, at, and after immunosuppression) between BIV serological status liability and the number of blood mononuclear cells and the ability of lymphocytes to respond to concanavalin A stimulation (Table 8). We found also a positive association between BLV serological status liability and the number of blood mononuclear cells. No statistically significant effects of PMN assays were found on liabilities for BIV and BLV serological status (Table 9).

The only statistically significant gene substitution effects of BoLA-DRB3 alleles on liability for BIV infection was estimated at 0.026 for allele b3 ($p<0.01$). Liability for BLV serological status was not influenced by any of the BoLA-DRB3 alleles. Finally, no statistically significant gene substitution effect was found on BIV or BLV liabilities for alleles of IgG_{2a} and CD18 genotypes. Tables are not presented for these associations.

Correlation Between Immune Assays

Phenotypic and genetic correlations between the immune function assays are shown in Tables 10 and 11. At the phenotypic level, serum concentrations of IgG_1, IgG_2, and IgM were positively correlated with each other and with PMN cytochrome C reduction assay. Both phenotypic and genotypic correlations of serum concentrations of IgG_1 and IgG_2 with number of circulating mononuclear cells were negative (Table 10).

Assays measuring PMN metabolic burst (chemiluminescence, cytochrome C reduction, and iodination) were positively correlated to each other, and were negatively correlated with random migration assay (Table 11). Phenotypic and genetic correlations between assays from Table 10 and assays from Table 11 were statistically not significant.

DISCUSSION

Although a larger number of data would allow more conclusive statements, additive effects of the immunological and genetic markers accounted for more than 40% of the total genetic variation in mastitis indicators. Therefore, the direct effects found in this study have enough impact to be included in a selection program for resistance to mastitis.

The cause-effect relationship between immune assays and disease markers was not assessed in this study. Although low gene substitution effects for any of our immune assays suggested reduced innate resistance to mastitis, it is not known whether presence of intramammary infections or clinical mastitis altered the immune response or whether different levels for the immune assays altered resistance to mastitis. Indeed, clear cause/effect associations must be proven by experimentation.

Because somatic cell score is an indirect measure of host response to infection by mammary pathogens, high breeding values for somatic cell score and for number of quarters infected with major or minor pathogens indicate greater genetic susceptibility to intramammary infections. On the other hand, high breeding values for number of quarters with clinical mastitis is an indicator of high genetic susceptibility to the pathological effects of the mastitis

pathogens. High values for the immune assays should be interpreted as indicative of a highly functional immune system.

Mastitis Indicators and Immune Assays

A negative genetic association was found between PMN assays (random migration, ingestion, cytochrome C reduction, iodination, and chemiluminescence) and mastitis indicators (Table 5). The negative association was more important at than before or after immunosuppression. Those assays are correlates of PMN activities in response to invasion by foreign pathogens. At the phenotypic level, earlier studies showed that resistance to mastitis pathogens is lowered in cows whose PMNs had a low capacity for the oxidative burst associated with phagocytosis (Heyneman, Burvenich & Vercauteren, 1990; Williams et al., 1984).

A negative association was found between breeding values for the number of blood mononuclear cells and breeding values for mastitis indicators, especially after parturient immunosuppression (Table 6). Because of the central role of the lymphocytes in the immune response, it seems biologically sound to select cows with higher number of mononuclear cells. However, the functional capability of those cells is even more important. In our herd, high breeding values for serum

concentration of Igs were associated with high breeding values for mastitis indicators. However, this does not necessarily mean that we should select for resistance to mastitis by selecting cows with low Ig serum levels. Indeed, Igs are produced by B lymphocytes in response to exposure to an antigen. Isotype IgM is the first antibody that an immunologically committed B lymphocytes will produce and is very efficient at activating the classical pathway of the complement (Butler, 1983). Isotype IgG is the major antibody produced in the secondary immune response. In milk, subclass IgG_1 is present in by far the highest concentrations and is derived mainly by active transport from serum (Butler, 1983). Isotype IgG_{2a} has been shown to be very important in bacterial opsonization for phagocytosis by neutrophils and antibody-dependent neutrophil cytotoxicity (Heyermann, Butler & Frangione, 1992). Suboptimal levels of serum IgG_2 have been associated with increased incidence of pyogenic mastitis (Lie, 1985).

The genetic association between mastitis indicators and lymphocyte functions was low. One reason is that MHC class II molecules affected not only breeding values for mastitis indicators (as shown in the next section) but also lymphocyte functions. Indeed, MHC molecules have two important roles in the immune response: presenting foreign peptides to T cell receptor and shaping the T cell receptor repertoire (Charron,

1990). In our herd, significant effects of BoLA-DRB3 alleles have been found on all assays measuring lymphocyte functions (serum concentration of Ig, lymphocyte response to mitogens, and number of circulating mononuclear cells) (Dietz et al., 1988)).

Mastitis Indicators and BoLA-DRB3 Genotype

This study confirmed that the BoLA class II region or loci linked to BoLA class II region may serve as markers for mastitis resistance. Presence of alleles b12 and b11 decreased breeding values for all mastitis indicators (Table 7). The gene substitution effects of allele b27 was very large but not consistent for all mastitis indicators. Cows with allele b27 were more resistant to infection with minor and major pathogens but once they were infected, they were less able to resist the pathogenic effects of the bacteria and developed clinical mastitis. There are some indication that allele b27 belongs to the haplotype containing class I W16 serotype and class II DQ^{1B} RFLP (H. Lewin, personal communication).

The gene DRB3 was originally chosen for genetic characterization because it is the most actively transcribed gene in the Class II DRB region (van Eijk, Stewart-Haynes &

Lewin, 1992). Class II DQ^{1A} RFLP has previously been associated with a low breeding value for clinical mastitis (Lundén et al., 1990). Class I serotypes W2, W14(W18), and W11 are associated with resistance to mastitis and W16, and W6 with susceptibility to mastitis (Spooner et al., 1988; Weigel et al., 1990).

Because of the biological constraints on model 1 (all gene substitution effects sum to zero), the question remains as to whether negative gene substitution effects of some allele indicates that those alleles provide some protection against mastitis or whether the negative effects are the consequences of the positive effects of other alleles at DRB3 locus.

Retroviral Infections

To our knowledge, this is the first report of a genetic association between BoLA-DRB3 and BIV serological status. In our herd, allele b3 at BoLA-DRB3 was positively associated with liability for BIV serological status. No significant association between BoLA-DRB3 alleles and breeding values for BLV liability were found. However, BoLA class I serotypes have been associated with incidence of BLV-dependent persistent lymphocytosis (Lewin et al., 1988). Cows with W12(W30) serotype seroconvert at a younger age and have an

higher incidence of persistent lymphocytosis, whereas cows with W14 seroconvert at a older age and have a lower incidence of persistent lymphocytosis, than cows with other BoLA-A alleles (Lewin et al., 1988).

Because heritability estimates for liability of serological status to BIV and BLV were very low (Table 1), the genetic associations found in this study were expected to reflect phenotypic associations. Similar to our findings (Tables 8 and 9), it has been shown that PMN functions were not altered by BIV inoculation (Flaming K., personal communication). It is also known that 30% to 70% of cows infected with BLV develop persistent lymphocytosis (Lewin & Bernoco, 1986).

Correlation Between Immune Assays

Phenotypic and genetic correlations between our immune assays are shown on Tables 10 and 11. Genetic correlations were statistically significant for a variety of immune assays and should therefore be incorporated in selection indexes that maximize innate immune function.

The negative genetic correlations between number of blood mononuclear cells and serum concentration of IgG_1 and IgG_2 indicated that selecting cows for high number of mononuclear cells will decrease the quantity of IgG_1 and IgG_2 produced by

the B lymphocytes. Similarly, selecting cows with high PMN ability to perform metabolic burst will decrease the ability of those PMNs to migrate randomly. The reasons for those negative correlations are unknown. Notice however that in the genesis of an immune response, secretion of Igs follows clonal expansion of B lymphocytes. Similarly, in response to invasion by foreign pathogens, PMNs first exit the blood stream, migrate towards the invading pathogen and then phagocytose it. Therefore, these temporal variations may have biased our estimates of correlations.

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Table 1. Heritability estimates for disease indicators and method used to compute them, as explained in the text

Disease indicators	Method of estimation	Heritability estimates
Somatic cell score[a]	BLUP[e]	0.10
Clinical mastitis score[b]	BLUP	0.10
Major pathogens IMI score[c]	REML[f]	0.10
Minor pathogens IMI score[c]	REML	0.10
Bovine leukosis virus[d]	REML	0.001
Bovine immunodeficiency virus[d]	REML	0.036

[a]DHIA somatic cell score.
[b]Liability of number of quarters clinically infected.
[c]Liability of number of quarters infected with major (minor) pathogens.
[d]Liability of BLV (BIV) serological status.
[e]BLUP=best linear unbiased prediction.
[f]REML=restricted maximum likelihood.

Table 2. Restricted maximum likelihood estimates of heritability for immune assays: before, at, and after immunosuppression (I.S.)

Immune assays	Before I.S.	At I.S.	After I.S.
Serum concentration in IgM[a]	0.74	0.55	0.85
Serum concentration in IgG_1[a]	0.00	0.29	0.43
Serum concentration in IgG_2[a]	0.25	0.63	0.24
PMN[b] random migration[c]	0.30	0.00	0.47
PMN ingestion[c]	0.26	0.00	0.27
PMN cytochrome C reduction[c]	0.22	0.88	0.99
PMN iodination[c]	0.51	0.25	0.00
PMN resting chemiluminescence[c]	0.53	0.19	0.00
PMN stimulated chemiluminescence[c]	0.00	0.71	0.30
Lymphocyte response to CONA[c]	0.16	0.40	0.01
Number of blood monocytes[d]	0.10	0.40	0.07

[a]Log_{10} of concentration in ng/ml.
[b]PMN = neutrophil
[c]Log_{10} of ratio of laboratory standards.
[d]Square root of number of cells per μl of blood.

Table 3. Number and phenotypic frequencies for alleles at IgG_{2a} isotype and at mutation responsible of the bovine leukocyte adhesion deficiency (BLAD) syndrome

	Number observed	Phenotypic frequencies
IgG_{2a} allotype		
A1A1	76	50.33
A1A2	58	38.41
A2A2	17	11.26
BLAD mutation		
A1A1	81	86.17
A1A2[a]	13	13.83
A2A2	0	0.00

[a]Cows heterozygous for the mutation responsive for the bovine leukocyte adhesion deficiency syndrome.

Table 4. Number and phenoytypic frequencies for alleles at DRB3 locus of bovine major histocompatibility complex (BoLA-DRB3). Number of homozygous are in parentheses

BoLA-RB3 allele	Number observed	Phenotypic frequency
b8	54(5)	21.34
b11	46(6)	18.18
b23	22(0)	8.69
b24	21(0)	8.30
b22	20(1)	7.90
b16	17(1)	6.72
b27	13(1)	5.11
b12	11(0)	4.35
b26	7(0)	2.77
b3	7(1)	2.77
b28	7(0)	2.77
b15	5(0)	1.98
b9	2(0)	0.34
b10	4(0)	1.58
b7	3(0)	1.18
b13	3(0)	1.18
b25	3(0)	1.18
b33	3(0)	1.11
b9	2(0)	0.79
b1	1(0)	0.39
b6	1(0)	0.39
b21	1(0)	0.39

Table 5. Regression coefficients of breeding values for neutrophils assays (log_{10} of ratio of laboratory standards) on breeding values for mastitis indicators: before, at, and after immunosuppression (model [1] explained in the text)

	RANG[e]	ING[f]	CYTO[g]	IOD[h]	REST[i]	STIM[j]
Before immunosuppression						
SCS[a]	-0.002	-0.005	-0.452	-0.036	-0.002	n.a.[k]
Clinical[b]	0.390	-0.089	0.989	0.457	0.390	n.a.
Major[c]	-0.175	0.089	-1.283	0.145	0.057	n.a.
Minor[d]	0.548	-0.065	1.362	-0.138	0.050	n.a.
At immunosuppression						
SCS	-0.790	n.a.	-0.223	0.010	-0.790	-0.199
Clinical	0.659	n.a.	-0.024	-0.295	0.659	-0.169
Major	-0.569	n.a.	0.034	-0.223	-0.083	0.044
Minor	-0.582	n.a.	0.003	-0.870	-0.804	-0.168
After immunosuppression						
SCS	n.a.	-0.342	-0.029	n.a.	n.a.	0.164
Clinical	n.a.	-0.054	0.008	n.a.	n.a.	0.923
Major	n.a.	-0.019	0.176	n.a.	n.a.	0.105
Minor	n.a.	0.192	-0.001	n.a.	n.a.	0.016

[a]DHIA somatic cell score.
[b]Liability for number of quarters with clinical mastitis per lactation.
[c]Liability for number of quarters infected with major pathogens per lactation.
[d]Liability for number of quarters infected with minor pathogens per lactation.
[e] Random migration.
[f]Ingestion.
[g]Cytochrome C reduction.
[h]Iodination.
[i]Resting chemiluminescence.
[j]Stimulated chemiluminescence.
[k]not available ($h^2=0$).

Table 6. Regression coefficients of breeding values for serum concentration in immunoglobulins (IgG_1, IgG_2, IgM; log_{10} of concentration), lymphocyte response to concanavalin A (CONA; log_{10} of ratio of lab standards), and square root of number of blood mononuclear cells (MONO) on breeding values for mastitis indicators: before, at, and after immunosuppression (model [1] explained in the text)

	IgG_1	IgG_2	IgM	CONA	MONO
Before immunosuppression					
SCS[a]	n.a.[e]	0.356	0.112	0.104	-0.097
Clinical[b]	n.a.	0.133	-0.189	-0.883	-0.090
Major[c]	n.a.	0.349	-0.053	0.163	-0.084
Minor[d]	n.a.	0.105	0.036	0.144	-0.034
At immunosuppression					
SCS	0.228	0.071	0.133	-0.028	-0.019
Clinical	0.107	-0.132	0.044	-0.053	-0.012
Major	-0.074	0.182	0.072	0.031	0.010
Minor	-0.048	0.180	0.097	0.029	-0.009
After immunosuppression					
SCS	0.397	0.397	0.084	0.140	-0.215
Clinical	0.379	-0.195	-0.060	-0.836	-0.146
Major	0.381	0.164	-0.138	-0.424	-0.095
Minor	0.198	0.023	0.184	-0.278	-0.123

[a]DHIA somatic cell score.
[b]Liability for number of quarters with clinical mastitis per lactation.
[c]Liability for number of quarters infected with major pathogens per lactation.
[d]Liability for number of quarters infected with minor pathogens per lactationn
[e]not available (h^2=0).

Table 7. Gene substitution effects for alleles at locus DRB3 of the bovine major histocompatibility complex (BOLA-DRB3), for alleles at IgG_{2a} isotype and for alleles at mutation responsible of the bovine leukocyte adhesion deficiency (BLAD) syndrome on breeding values for mastitis indicators (model explained in the text)

	Somatic cell score	Clinical mastitis score[a]	Major pathogen IMI score[a]	Minor pathogen IMI score[a]
BoLA-DRB3 alleles				
b8	0.005*	0.044**	-0.018*	-0.025*
b11	-0.032**	-0.039*	-0.012*	-0.024*
b23	-0.087**	-0.085**	-0.036*	0.013*
b24	-0.065**	-0.002	0.086**	0.004
b22	0.039**	0.023*	-0.051**	0.025*
b16	0.063**	-0.034*	0.014*	-0.020*
b27	-0.020*	0.110**	-0.135**	-0.004
b12	-0.036*	-0.037*	-0.016*	-0.011
b26	-0.096**	0.020*	-0.016*	0.043*
b3	-0.001	0.038*	-0.042*	0.053**
b28	0.025*	0.002	-0.060**	0.016*
others[b]	0.205	-0.040	0.286	-0.070
IgG_{2a} alleles				
A1	-0.006	-0.011	-0.005	-0.017
A2	0.006	0.011	0.005	0.017
BLAD alleles				
A1	0.030	0.041	-0.045	-0.011
A2[c]	-0.030	-0.041	0.045	0.011

* $p<0.05$ ** $p<0.01$
[a]Breeding values for liability of the mastitis indicator. IMI=intramammary infection.
[b]DRB3 alleles designed "others" have frequencies below 2%.
[c]Recessive allele responsive for BLAD.

Table 8. Regression coefficients of breeding values for serum concentration in immunoglobulins (IgG_1, IgG_2, IgM; log_{10} of concentration), lymphocyte response to concanavalin A (CONA; log_{10} of ratio of lab standards), and square root of number of blood mononuclear cells (MONO) on breeding values for retroviral infections: before, at, and after immunosuppression (model explained in the text)

	IgG_1	IgG_2	IgM	CONA	MONO
Before immunosuppression					
BIV[a]	n.a.[c]	.017	.0426	-.012	-.010
BLV[b]	n.a.	.0003	.0000	.0005	.0007
At immunosuppression					
BIV	-.0091	.0309	.0067	-.005	-.001
BLV	.0014	-.0003	-.0000	.0003	.0000
After immunosuppression					
BIV	-.0727	.0331	.0258	-.012	-.004
BLV	.0000	-.0008	.0000	.0001	.0006

[a]Bovine immunodeficeincy virus.
[b]Bovine leukosis virus.
[c]not available ($h^2=0$).

Table 9. Regression coefficients of breeding values for neutrophils assays (log_{10} of ratio of laboratory standards) on breeding values for retroviral infections: before, at, and after immunosuppression (model explained in the text)

	RANG[c]	ING[d]	CYTO[e]	IOD[f]	REST[g]	STIM[h]
Before immunosuppression						
BIV[a]	.005	.101	.204	-.108	.028	n.a.[i]
BLV[b]	.003	-.001	-.001	-.000	-.001	n.a.
At immunosuppression						
BIV	.006	n.a.	.001	-.021	-.008	-.003
BLV	.004	n.a.	.001	-.003	.000	-.001
After immunosuppression						
BIV	n.a.	.047	.017	n.a.	n.a.	-.006
BLV	n.a.	.001	-.000	n.a.	n.a.	.001

[a]Bovine immunodeficiency virus.
[b]Bovine leukosis virus.
[c]Random migration.
[d]Ingestion.
[e]Cytochrome C reduction.
[f]Iodination.
[g]Resting chemiluminescence.
[h]Stimulated chemiluminescence.
[i]not available ($h^2=0$).

Table 10. Phenotypic (below the diagonal) and genotypic (above the diagonal) correlations between serum concentration in immunoglobulins (IgG_1, IgG_2, IgM), lymphocyte response to concanavalin A (CONA), neutrophil cytochrome C reduction (CYTO), and number of blood monocytes (MONO)

	IgG1	IgG2	IgM	CONA	CYTO	MONO
IgG1[a]		0.09	0.02	0.10	-0.01	-0.16**
IgG2[a]	0.19†		0.11	0.05	0.12†	-0.16**
IgM[a]	0.20†	0.36†		0.09	0.05	0.05
CONA[b]	0.03	0.06	0.14			0.23**
CYTO[b]	0.15	0.39†	0.35†	-0.03		-0.03
MONO[c]	-0.18†	-0.21†	-0.06	0.12	-0.05	

† $p<0.1$ ** $p<0.01$
[a]$\log_{10}$ of serum concentration (ng/ml).
[b]$\log_{10}$ of ratio of lab standards.
[c]square root of number of blood monocytes.

Table 11. Phenotypic (above the diagonal) and genotypic (below the diagonal) correlations between neutrophils assays (log_{10} of ratio of laboratory standards)

	CYTO	REST	STIM	IOD	ING	RANG
CYTO[a]		0.17*	0.22*	0.18	0.11*	-0.10
REST[b]	0.20**		0.33**	0.37**	n.a.	n.a.
STIM[c]	0.12	0.35**		0.30**	0.17	-0.07
IOD[d]	0.09	0.01	0.04		-0.15	-0.23*
ING[e]	-0.05	-0.05	0.11	0.05		-0.19*
RANG[f]	0.15*	0.10	-0.02	-0.07	0.05	

* $p<0.1$ ** $p<0.01$
[a]Cytochrome C reduction.
[b]Resting chemiluminescence.
[c]Stimulated chemiluminescence.
[d]Iodination.
[e]Ingestion.
[f]Random migration.

GENERAL SUMMARY

In this study, we have demonstrated that immunological parameters could be used as indirect indicators of resistance to mastitis in periparturient Holstein cows.

The cost effectiveness of selection against any particular disease depends upon the economic importance of the disease considered, whether there is sufficient genetic variation in the animal population to make rapid genetic progress, and whether genes with major effects on disease incidence are identifiable.

Because mastitis is by far the most costly disease on the dairy farms, selection for resistance to mastitis is of primary interest to the dairy farmer. At calving time, cows undergo an immunosuppression of various immunologic parameters. Also, clinical mastitis are high during the periparturient period. Therefore, parturient immunosuppression is thought to compromise the ability of the cow to combat infection occurring around parturition.

Indirect selection for resistance to mastitis is based upon detection of indirect indicators of disease resistance. Among other alternatives, one possibility is to use immunological markers. In vitro immune assays are easy to measure, at low cost, early in life, in both sexes, and are repeatable. We found relatively high additive genetic

variation for the periparturient changes observed in the immune assays. These findings need however to be corroborated in commercial herds using field data. Breeding selection experiments are also necessary to quantify more precisely the additive genetic component of the immune response.

Significant genetic variability was also observed in all direct indicators of clinical mastitis or intramammary infections. In this study, data on mastitis incidence were collected in a research herd and the definition of mastitis was clearly stated. In commercial herds, however, it is more difficult to obtain a clear and consistent definition of mastitis (such as clinical cases versus intramammary infections or infection with major versus minor pathogen) across herds. This lacks of precise measurement of mastitis makes conclusive statements on genetic variability in mastitis resistance not repeatable across studies.

We found significant genetic associations between alleles of the BoLA-DRB3 locus and mastitis incidence. Associations were also found between mastitis and various immune assays. Therefore, immunological parameters could serve as basis for selection for resistance to mastitis. However, because of the interrelations between all components of the immune response to infectious pathogens, interpretation of the associations between mastitis and immunological parameters should be done with care. The controversy on the use of somatic cell counts

as selection criteria for resistance to mastitis illustrates this concept. Is high milk somatic cell score indicative of a cow with mastitis or does it help the cow combat or resist infection. Conclusive cause/effect relationship between an immune parameter and resistance to particular infection must be prooven by experimentation. In this type of experimentation, animal free of mastitis are selected for some particular component of the immune response and then challenged with the mastitic pathogen. Although difficult to implement practically, those "prospective breeding studies" will be helpful in determining if selection of the particular component of the immune response really affect genetic resistance to the mastitic pathogen.

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