

## **Genetic Improvement of Immune Response and Disease Resistance: Past Experience and a Vision for the Future**

Bonnie A. Mallard and Bruce N. Wilkie

Email: [bmallard@ovc.uoguelph.ca](mailto:bmallard@ovc.uoguelph.ca)

Email: [bwilkie@uoguelph.ca](mailto:bwilkie@uoguelph.ca)

Department of Pathobiology

Ontario Veterinary College

University of Guelph

Guelph, Ontario

N1G 2W1

### **Summary**

The immune system is composed of integrated, genetically and environmentally regulated sets of cells and molecules that control the response to external and internal stimuli, including pathogenic microorganisms. In terms of infectious organisms, the response of the host largely reflects the relationship or adaptation between the host and agent. This response is influenced through genotype by environment interactions, both at the individual and population level. It may be that disease is largely the product of incompatible gene by environmental interaction. It is therefore particularly relevant to understand host-pathogen relationships and adaptations under various stress and management conditions. Improved understanding of the biology and genetic relationships between the host and pathogen, particularly those that effect the immune system during periods of production stress, should facilitate implementation of non-traditional approaches to improve the health of intensively reared livestock. Genetic regulation of immune response and selection for disease resistance in livestock is well documented and should be considered as an economical and prophylactic approach to improve animal health (reviewed by Stear et al., 2001). Diverse approaches however, have met with varied success and selection for resistance to one disease may result in susceptibility to others (reviewed by Wilkie and Mallard, 2000). To avoid this, recent studies have evaluated host defence mechanisms as indicators of broad-based inherent disease resistance. There is evidence that in rodents, poultry, sheep and swine selective breeding for high or low immune response influences resistance to infectious disease (reviewed by Kelm et al., 2001). In most species, including pigs and cattle, heritability estimates for antibody and cell-mediated immune responses are stable and moderate to high indicating that genetic selection is feasible. Given the diversity of pathogens in their ability to vary virulence, transmission route, and life cycle it is reasonable to improve control of infectious disease by manipulating the equally diverse and genetically pliable immune system which detects and inhibits infectious agents. In fact, genetic selection for high immune responses produced benefits in pig health and production (reviewed by Wilkie and Mallard, 2000). Genetically selected and immunologically-defined populations of livestock can also be utilized as a tool to understand the genes which govern these useful phenotypes. For example, as a means to discover the genes involved

in cell biology, genomic techniques to screen DNA or whole cell mRNA have been developed. These include various DNA mapping procedures and subtractive hybridization, differential display, and gene microarrays for RNA detection. Microarray analysis is one of the most recent advances and involves placing complete cDNAs, or oligonucleotides representing a large number of genes in an ordered array on a solid matrix. These arrays can then be probed using labelled cDNA derived from total RNA or mRNA. Microarrays commonly include known and/or anonymous genes and may contain several thousand genetic elements. Specialized microarrays, such as “lymphochips” which contain fewer genes of relevance to the immune system have also been produced. To indicate the power of this approach, a human lymphochip has been used to help define B and T-lymphocyte differentiation, and gene expression in certain immune disorders and malignancies (Staudt, 2001). Additionally, a bovine immune-endocrine chip that can hybridise bovine, ovine and porcine cDNA, has been produced by our group (Tao et al., 2003) and may be used to identify genes associated with high and low immune responsiveness or with disease resistance. Integrating phenotypic and genetic information on immune response, derived using a variety of genetic methods (molecular and quantitative), may enhance the understanding of immunity and provide novel means to improve disease resistance of livestock.

## **Introduction**

Infectious disease of livestock is the most costly and hazardous problem facing the Agri-food industry. Emerging and re-emerging diseases, many of which are zoonotic, the increasing restriction on antibiotic use in livestock and sizeable costs associated with new drug development are making it more difficult to manage animal health (Colditz, 2002; Woolhouse, 2002). Additionally, consumer concern for both improved food-safety and animal well-being, demands alternative approaches to disease prevention which do not rely on extensive use of anti-microbials (Kelm et al., 2001; Stear et al., 2001; Bishop and Mackenzie, 2003). Therefore, the goal must be to implement non-traditional prophylactic approaches to improve disease resistance of livestock, particularly during periods of production stress such as pregnancy, parturition, weaning, shipping and handling. This may best be accomplished through improved understanding of the phenotypes and genotypes that control host defence and regulate disease resistance or susceptibility.

The immune system is a genetically regulated and adaptable system with unique features to detect and control infectious agents that also have the ability to vary genetically in response to ecological change (Delves and Riott, 2000; Woolhouse, 2002). It may be that disease is largely the product of incompatible gene by environmental interactions and that a better understanding of host-parasite adaptation will lead to new approaches to health maintenance (Figure 1) (Brander and Walker, 2003). For these reasons, recent studies have evaluated host defence mechanisms as indicators of broad-based disease resistance (Edfors-Lilja et al., 1994; Detilleux et al., 1995; Mallard et al., 1998, Malek and Lamont, 2003). Under experimental conditions, pigs, cattle, sheep and poultry with inherently higher immune responses have been identified and demonstrate certain health and production attributes, such as increased growth and response to vaccination (Mallard et

al., 1992; O'Meara et al., 1992; Wilkie and Mallard, 1999; Wagter et al., 2000; Parmentier et al 2001; Malek and Lamont, 2003; Wagter et al., 2003). In some cases, traditional quantitative genetic methods have been employed to selectively breed pigs and other species for high antibody and/or cell-mediated immune responses (Mallard et al., 1992; Kelm et al., 2001; Bovenhuis et al., 2002). These methods do not require molecular genetic manipulation of the animal and therefore avoid current controversies surrounding production of genetically modified organisms (GMOs). However, the advantages of molecular genetic techniques can be employed in the laboratory to identify and study favourable animal genotypes (Houston et al., 2003; Zhou and Lamont, 2003; Tao et al 2003). Genetic approaches can be synergistic with other preventive approaches to animal health; for instance, pigs with the highest immune responses, based on a selection index, also had the highest responses to commercial vaccines (Wilkie and Mallard, 2000). Thus genetic approaches that use quantitative and/or molecular genetic information to identify animals with superior host defence should improve inherent broad-based disease resistance, enhance response to commercial vaccines, improve production and reduce the risks associated with antibiotics, chemical prophylaxis and other therapeutics. This is an evolving multidisciplinary approach to animal health that extends well beyond the traditional practice of veterinary medicine.

### **High Immune Response and Breeding Pigs for Enhanced Disease Resistance**

There are numerous diseases of pigs and other species that are controlled by one or a few genes (Nicholas, 2000; Kelm et al., 2001). Examples of these in pigs include, Rickets-vitamin D deficiency (deficiency in renal 25 hydroxy-cholecalciferol-1-hydroxylase), porcine atherosclerosis (a defect in alolipo-protein B gene), oedema disease (the receptor for the F18 fimbriated *E. coli* is expressed only in susceptible pigs), and cutaneous porcine malignant melanoma (susceptibility is governed by the interaction between the Major Histocompatibility Complex-(MHC) and other loci) (Edfors-Lilja and Wallgreen, 2000; Nicholas, 2000, Wilkie and Mallard, 2000). Examples in other animal species include, Mannosidosis, Chediak-Higashi Syndrome, vonWillbrand disease, Factor XI defect, and several immunodeficiencies, such as combined immunodeficiency of foals and immunoglobulin G2 deficiency of Danish Red cattle (Nicholas, 2000; Kelm, 2001). Many of these susceptibilities are due to single point mutations occurring in metabolic pathways. However, resistance to complex infectious diseases, such as pneumonia or mastitis, is often under the control of intricate interactions between many genes. For instance, upward of 2,000 genes regulate immune response. Therefore, complex infectious diseases are not effectively treated using simple genetic or management methods. Nonetheless, some individuals within the population are almost always naturally immune. Since the immune system predominantly controls the response to infectious disease and is itself under genetic control, it was hypothesized that optimal disease resistance should be a function of optimal innate and acquired host defence mechanisms. To test this hypothesis, 5 heritable traits relating to host defence (antibody response to hen egg white lysozyme, serum IgG, delayed-type hypersensitivity to a purified protein derivative of *Bacillus Calmette Guerin*, lymphocyte blastogenic response to the T-cell mitogen Concanavalin-A and the ability of macrophages to take up and kill

bacteria) were chosen to selectively breed Yorkshire pigs for high or low immune response (Mallard et al., 1992). The test of macrophage function showed unstable heritability and was removed after the first generation of selection, but otherwise pigs were selectively bred over 9 generations based on combined estimated breeding values of these traits using a selection index approach. The results of this selection experiment have been described elsewhere (Mallard et al., 1992; Mallard et al., 1998; Wilkie and Mallard, 2000) and will only be briefly reviewed here. Pigs of the high line not only had higher antibody responses to several test antigens but also to several commercial vaccines, including an *A. pleuropneumoniae* bacterin and an inactivated influenza vaccine (Wilkie and Mallard, 2000). Antibody of higher titre and avidity was noted in high line pigs and there were significantly fewer non-responders in the high line (Appleyard et al., 1992). Thus both antibody quantity and quality were affected as a result of selection (Wilkie and Mallard, 2000). Following experimental infection with *Mycoplasma hyorhinitis*, pigs of the high line had higher antibody and upon post-mortem had less peritonitis, pleuritis, and pericarditis. However, these pigs had higher arthritis lesion scores that related to differences in cytokine profiles between the lines (Magnusson et al., 1999). Given the generally lower post-mortem lesion scores in the high line, this result should not be taken to disqualify high immune response selection, particularly since traits within a selection index can easily be re-weighted to modify the emphasis on antibody or cell-mediated immune responses. Under natural challenge with porcine parvovirus, pigs of the high line also had significantly fewer mummified fetuses (Mallard et al., 1998).

High line pigs at all generations reached 100 kg, 10-12 days before low or control line pigs (Mallard et al., 1998). This was the case whether pigs were housed at the university experimental station or were transferred to sentinel herds with known high prevalence of disease. Differences in growth rate may relate to improved health and therefore appetite maintenance, variations in growth hormone profiles or behavioural differences between the lines. No differences in carcass traits were noted between the lines, except that high line pigs had slightly less back fat (Mallard et al., 1998).

Given the attributes of the high line Yorkshire pigs bred within a research station environment, it was decided to investigate whether high and low immune response phenotypes could be identified in commercial pig populations and whether production-breeding schemes could be modified to accommodate selection for enhanced immune response. Indeed, high and low immune response phenotypes were identified in commercial Yorkshire, Duroc and Landrace pigs, and there were breed variations in immune responses. Additionally, immune response traits could be accommodated within the current breeding programs used by commercial producers.

### **High and Low Immune Responses of Dairy Cattle and Molecular Genetic Methods to Investigate Gene Profiles**

Having found that pigs could be selectively bred for high and low immune responses it was decided to determine if high and low immune response phenotypes could be identified in other livestock species. Since there was evidence of sub-optimal host

defence during the peripartum period of dairy cows when disease occurrence is maximal (Kehrli et al., 1989a, b; Dettloux et al., 1995) this seemed a logical target species for further study. Comprehensive evaluation of antibody responses to ovalbumin (OVA) and *Escherichia coli* J5 during the peripartum period (3 wks before to 3 wks after calving) had not been previously reported and data generated here indicated that cows can be classified into 3 responder groups: 1. Cows that exhibit above average serum antibody, with no peripartum decline in response following systematic immunization at weeks -8, -3 and 0 relative to calving (High responders). 2. Cows with average antibody, but which exhibit a decline in response following immunization, beginning at, but not before, calving (Average responders). 3. Cows that exhibit below average antibody following immunization, with a lack of response beginning at 3 weeks prior to calving (Low responders). Colostral and milk antibody, which are important to calf immunity, correlated with serum antibody (Mallard et al., 1989; Wagter et al., 2000). High responders, had the highest immune response to the test antigen, OVA, and to the commercial *E. coli* J5 mastitis vaccine (Mallard et al., 1999). In 2 out of 3 herds, disease occurrence was lowest in this group. Serum growth hormone and IGF-1 concentrations were also highest for high responders (Mallard et al., 1989). There was no difference in glucocorticoid concentrations across the three response groups. Some production differences across parities were noted between groups in that first parity heifers in the low responder group tended to have higher milk yield but by parity 3 or greater the high responders had the highest milk yield (Wagter et al., 2003). Cell-mediated immune responses also showed phenotypic variation between groups (Hernandez et al., 2003). Phenotypic and genetic markers of resistance to complex infectious diseases have been difficult to identify, and accurate and practical methods of incorporating selection for disease resistance into existing production schemes are only now being realised (Van Doormaal, 2000; Maillard et al., 2003). To facilitate this process, the variation noted in peripartum antibody response was utilized to devise a mathematical index to rank cows based on serum antibody response profiles (Wagter et al., 2000). Indicators of cell-mediated immune responses are currently being added to this mathematical index and cows can now be classified based on measures of immune response in a manner similar to that used to classify pigs (Mallard et al., 1992; Hernandez et al., 2003).

MHC genes have often been implicated in disease resistance (Rothschild et al., 2000, Maillard et al., 2003). For example, studies here revealed associations between bovine MHC genes, BoLA class II DRB3.2 alleles \*23 (now 2701-08) and \*16 (now 1501/02), and mastitis susceptibility and resistance, respectively (Sharif et al., 1998). These alleles, as well as a high frequency control allele (DRB3.2\*8), were cloned, sequenced, and transfected into mouse L-cells to facilitate study of the biological mechanism(s) whereby they influence disease outcome (Sharif et al., 2000). For instance, peptides have been eluted from the binding grooves of these alleles and unique conserved sequences for each variant predicted (Sharif et al., 2003). This represents the first step toward investigating the possible roles that these molecules play in regulating peripartum immune response. Preliminary studies indicate that DRB3.2 alleles also differ in frequency between high and low immune response phenotypes. Several synthetic peptides which bind to allele \*23 with high and low binding affinities have also been identified and shown to influence T-cell responses (Alizadeh et al., 2003). In pigs, MHC alleles have similarly been shown

to influence immune response and disease outcome (Lunney et al., 1986; Mallard et al., 1989; Stear et al., 2001). Selective breeding of pigs for high and low immune response also appeared to alter the frequency of MHC class II gene expression; however, it was difficult to separate changes due to breeding from founder effects in those studies.

### *Gene Assessment using DNA Micro-Array Technology*

Remarkable effort has been expended to develop technologies for large-scale analysis of genes and proteins that influence complex traits, such as disease resistance (reviewed by Kiechle and Holland-Staley, 2003). Microarray is one of many new tools in biotechnology that facilitates analyses of hundreds to thousands of genes in the context of regulation of complex biological processes (White et al., 1999). A variety of large-scale commercial arrays are available for humans and other species; however, in some cases commercial arrays containing many thousand's of genes may be less efficient than smaller scale application-targeted arrays since many elements unrelated to the specific experiment are present (Rockett et al., 2001). From an economic point of view, arrays of distinct sets of genes may be more useful given that they can be produced to contain only genes that play pivotal roles in the biological processes of interest, thereby reducing the overall effort in analyzing data from causally associated genes. There is interest here in exploring gene expression profiles associated with the interactions within the immune and endocrine systems in cattle, pigs and sheep with high-low immune response or with distinct disease-related phenotypes. Therefore to monitor a network of genes that are involved in the immune-endocrine axis during periods of altered homeostasis provoked by physiological or environmental stressors, such as infection, vaccination or disease, a bovine immune-endocrine cDNA array was developed. Preliminary experiments using a large-scale human micro-array (OCI) (Hernandez et al., 2003), and available bovine annotated gene sequences published in the NCBI GenBank ([www.ncbi.nlm.nih.gov/](http://www.ncbi.nlm.nih.gov/)) plus expressed sequence tags (EST) obtained from The Institute for Genomic Research (TIGR, [www.tigr.org/tdb/tgi/btgi/](http://www.tigr.org/tdb/tgi/btgi/)) that contains 322,089 EST sequences (date to 2003, June) were used to identify candidate genes (Tao et al., 2003). Utilizing this information, a set of 167 well-characterized candidate genes were selected for placement on the array based on their function and evidence of expression during immune responses or activation of the immune-neuroendocrine axis. Array calibration and validation was carried out using total RNA extracted from un-stimulated and T-cell mitogen, Concanavalin-A (ConA)-stimulated bovine blood mononuclear cells (BMCs). This RNA was reverse transcribed and the cDNA labeled with two different cyanine dyes (Cy3 or Cy5) prior to array hybridization. Additionally, given the high level of genetic homology between bovine, ovine, and porcine genes, RNA similarly acquired from un-stimulated and ConA-stimulated BMCs was tested to determine the ability of the bovine array to detect cross hybridization of cDNA from related species. Generally, cytokine and chemokine genes such as interleukin (IL)-8, IL-1 $\alpha$ , MCP-1,2,3 and tumour necrosis factor- $\alpha$  were up-regulated in each species; whereas, antigen presenting molecules, including MHC-DR, DQ, DY and DM tended to be down-regulated at 6 and 24 hours following ConA stimulation, as compared to zero hour un-stimulated controls. Of the endocrine genes examined, only prolactin changed following ConA treatment with a slightly less than two-fold decrease in expression at 6 hours post-stimulation. This is a

first step toward improved understanding of gene expression during periods of altered homeostasis of livestock and these data demonstrate that a small-scale thematic array can be utilized to determine the relative gene expression profiles corresponding to immune-endocrine genes of cattle, as well as pigs and sheep. Experiments are currently underway to evaluate differential gene expression in livestock with high and low immune response phenotypes.

### **Vision for the Future**

The ultimate objective is to genetically enhance the health and well-being of agriculturally important species, particularly during periods of high production stress, and to increase fundamental knowledge of the composition and function of genes and their gene products which regulate immune response and disease resistance. This will be best achieved by continuing to apply quantitative genetic breeding strategies as the platform to produce populations of livestock with superior immune response and health traits and then to incorporate these traits into existing production indices. As specific data become available regarding favourable genes and genetic interactions in various environments this information can subsequently be added to the breeding platform. This may include information on single genes associated with disease susceptibility or multiple genes involved in disease resistance.

### **References**

- Alizadeh et al., 1998, *J Clin Immunol*, 18(6):373.  
Alizadeh et al., 2003, *Gen Sel Evol*, 35(1):51.  
Appleyard et al., 1992, *Vet Immunol Immunopathol*, 31:229.  
Bishop and Mackenzie, 2003, *Gen Sel Evol*, 35(1):3.  
Biozzi et al., 1979, *Immunol*, 36:427.  
Bovenhuis et al., 2002, *Poultry Sci*, 81(3):309.  
Brander and Walker, 2003, *Nature Med*, 9(11):1359.  
Colditz et al., 2002, *Livestock Production Sci*, 75:257.  
Delves and Roitt, 2000, *NEJM*, 343:37.  
Detilleux et al., 1995, *Vet Immunol Immunopathol*, 44:251.  
Edfors-Lilja et al., 1994, *Vet Immunol Immunopathol*, 40:1.  
Hernandez et al., 2003, *Gen Sel Evol*, 35(1):67.  
Houston et al., 2003, *Nature*, 423:498.  
Kehrli et al., 1989a, *Am J Vet Res*, 50(2):10.  
Kehrli et al., 1989b, *J Dairy Sci*, 73(8):2103.  
Kelm et al., 2001, *Immunology* 17(3):478.  
Kiechle and Holland-Staley, 1993, *Arch Pathol Lab Med*, 127(9):1089.  
Lunney et al., 1986, In: *Swine in Biomedical Research* (Ed. M.E. Tumbelson), Plenum Press, New York, pp 135.  
Magnusson et al., 1999, *Vet Immunol Immunopathol*, 68:131.  
Maillard et al., 2003. *Gen Sel Evol*, 35(1):193.  
Malek and Lamont, 2003, *Gen Sel Evol*, 35(1):99.

Mallard et al., 1989, *Anim Genet*, 20:167.

Mallard et al., 1998, *Proc 6<sup>th</sup> World Congress of Genetics Applied to Livestock Production*, 27:257.

Mallard et al., 1998, *J. Dairy Sci*, 81(2):585.

Mallard et al., 1992, *Anim Biotech*, 3(2):257.

Mallard, 1999, *Proc. North. American Coliform Mastitis Symposium*, Merial Inc., Denver, Colorado, pp 45.

Nicholas, 2000, *Veterinary Genetics*, Oxford Science Publications, Oxford, New York, Toronto.

O'Meara et al., 1992, *Res Vet Sci*, 52(2):205.

Owen et al., 2000, *Breeding for Disease Resistance in Farm Animals* (Eds. R.F.E. Axford et al), CABI Publishing, New York, New York, pp243.

Parmentier et al., 2001, *Poultry Sci*, 80(7):894.

Rockette et al., 2001, *Genome Biol*, 2(4):0014.1

Rothschild et al., 2000, *Breeding for Disease Resistance in Farm Animals* (Eds. R.F.E. Axford et al), CABI Publishing, New York, New York, pp 73.

Sharif et al., 1998, *Anim Genet*, 29:185.

Sharif et al., 2000, *Vet Immunol Immunopathol*, 76:231.

Sharif et al., 2003, *Anim Genet*, 34:116.

Staudt, 2001, *Trends Immunol*, 22(1):35.

Stear et al., 2001, *Res Vet Sci*, 71:1.

Tao et al., 2003, *Vet Immunol Immunopathol*, (In press).

VanDoormaal, 2000, *Holstein Journal* ([www.cdn.ca](http://www.cdn.ca)), February issue pp 34.

Wagter et al., 2000, *J Dairy Sci*, 83:488.

Wagter et al., 2003, *J Dairy Sci*, 86:169

White et al., 1999, *Science*, 286:2179.

Wilkie and Mallard, 1999, *Advances in Vet Med*, 41:39.

Wilkie and Mallard, 2000, *Breeding for Disease Resistance in Farm Animals* (Eds. R.F.E. Axford et al), CABI Publishing, New York, New York, pp 379.

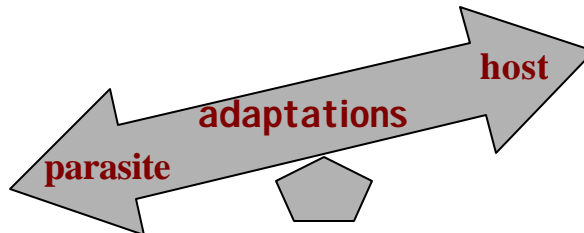
Woolhouse, 2002, *Trends in Micro*. 10(10):S3.

Zhou and Lamont, 2003, *Poultry Sci*, 82(7):1118.

Figure 1

**Host-parasite adaptations are governed by gene by environmental interactions.**

**Compatible interactions:** lead to resilience or resistance.



**Incompatible interactions:** lead to emergence or re-emergence of infectious diseases.