Frank Baker Essays

Understanding the genetic mechanisms that underlie variation in immune response and disease susceptibility

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Introduction

The beef industry is currently facing many challenges, from feed efficiency, to drought and costly feedstuff inputs, to the nutritional value of beef protein and nutritional benefits of beef to consumers, to disease and animal welfare. Animal health and disease has quickly moved to the front-line of issues that are currently facing the beef industry. Animal health and disease is an issue that affects production, quality, and the public perception of the beef and livestock industries. Disease is costly to the industry. Disease such as bovine respiratory disease (BRD) has been shown to cost the industry approximately $750 million dollars annually (HOLLAND *et al.* 2010). Disease outbreaks can devastate an industry and cost millions of dollar as was experienced with the bovine spongiform encephalopathy (BSE). The 2004 outbreak of BSE has been estimated to of cost the US 3.2 to 4.7 billion dollars in losses, though loss of export markets and recalls on retail products (PENDELL *et al.* 2007). Improving the understanding of the mechanisms that control disease susceptibility, can offer the potential of genetic selection to remove animals that are immunologically challenged and that have high susceptibility to diseases.

Disease prevention and treatment can be costly and in the case of some vaccines and treatments for some diseases, ineffective. The public consumer has developed many misconceptions about the use of antibiotics and hormones in food livestock production, which are beginning to play a role in management decisions of producers. In addition to misconceptions that have been created among consumers with regards to antibiotic and hormone use in cattle, legislature has tried to impose regulations to remove the use of sub-therapeutic antibiotic in livestock. Currently, disease prevention though vaccine use is one of the most commonly used methods for disease prevention. However, a large amount of variation in immune response and disease susceptibility has been observed among animals. The variation in the immune response and disease susceptibility phenotypes has been observed in all breeds and ages of cattle, contributing to the challenges associated with studying disease and immune response.

While, prevention methods such as vaccination serve to protect the animals from infectious pathogens, not all animals appear to respond equally to the same vaccine nor are

protected to the same degree (DUFF and GALYEAN 2007; SALAK-JOHNSON 2007). Improvement for feed efficiency, growth and performance, and other traits have began to use genetic selection to make improvement in quantitative traits. However, during this time of selection, little to no selection pressure has been implemented for animal health or disease resistance and susceptibility. This may be partially due to the complexity of phenotypes, complications and expense of collecting quality data, and limitations to understanding the genetic mechanisms that underlie immune response and disease resistance.

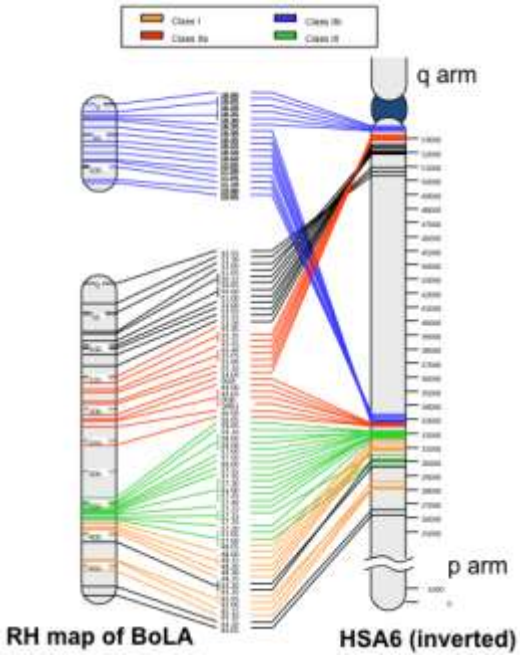
Variation in immune response and disease susceptibility has often been attributed to the highly polymorphic MHC and associated with serotypes, gene markers, and more recently single nucleotide polymorphism (SNP) defined haplotypes. However, evidence suggests that more variation exists in immune response than can be attributed to SNP-defined haplotypes. In addition to SNP variation, genomic structural variation, such as: insertion, deletions, inversions and copy number variant (CNV) regions, which may contribute to the variability in immune phenotypes that are observed. Approximately 2,600 copy number variant (CNV) regions have been identified in the cattle genome (BICKHART *et al.* 2012; HOU *et al.* 2012; LIU *et al.* 2011; LIU *et al.* 2010; SEROUSSI *et al.* 2010; STOTHARD *et al.* 2011). Evidence suggests there is an enrichment of CNVs in genes associated with immune function and metabolism pathways (BICKHART *et al.* 2012; FADISTA *et al.* 2010; HOU *et al.* 2012; STOTHARD *et al.* 2011; TENNESSEN *et al.* 2012). Enrichment of CNVs in genes involved in immune functions might account for the previously unexplained variation in immune response and disease susceptibility phenotypes. Improved understanding of the genetic structure of immune related genome regions might offer advancement in selection for animals with improved or optimal immune responses and improve the understanding of genetic mechanisms that underlie immune responses.

Literature review

Major Histocompatibility Complex Structure

The bovine major histocompatibility complex (MHC) is a gene dense region located on bovine chromosome 23, composed primarily of genes associated with immune response or disease susceptibility (MIYASAKA *et al.* 2011; TAKESHIMA and AIDA 2006). The bovine MHC, referred to as the bovine leukocyte antigen (BoLA), is divided into three regions, classes I, II, and III, which display conserved syntenic regions with the MHCs of other mammalian species (KELLEY *et al.* 2005), as illustrated in Figure 1. The organization of BoLA differs from that of other mammals at the class II region. The BoLA class II region has apparently been disrupted by

a large inversion to place a portion of the class II region, called IIb, near the centromere, separated from the main MHC region by approximately 20 Mb of non-MHC DNA (CHILDERS *et al.* 2006). BoLA class IIa region, composed of functionally expressed DQ and DR genes, is tightly linked to the class I region, creating a diverse cohort of MHC haplotypes (DAVIES *et al.* 1997). The classical BoLA class I and IIa are highly polymorphic and have been associated with various immune responses. The largest portion of variation in BoLA class I has been attributed to deletions and duplications, resulting in a number of different gene configurations (ELLIS *et al.* 1999). Altered gene configurations may result in varied immune responses that have not previously been detected by SNPs. Polymorphic BoLA genes and variation in BoLA haplotypes contribute to diversity in immune responses.



(BRINKMEYER-LANGFORD *et al.* 2009)

Figure 1. Map of the bovine major histocompatability complex.

BoLA genes encode five peptide-binding proteins: DQ, DR, DN, DO, and DY (BAKER *et al.* 2006; KELLEY *et al.* 2005). Class I and IIa genes have been the focus to understand the immune function of the MHC. MHC class IIa region, composed of functionally expressed DQ and DR genes, is tightly linked to the class I region creating a diverse cohort of MHC haplotypes (DAVIES *et al.* 1997). MHC class II alleles are redundant and highly polymorphic, enhancing the repertoire of epitopes that an individual can recognize (NORIMINE and BROWN 2005). Class II DQα and β and DRβ are the most polymorphic genes in the bovine MHC, similar to other species (ANDERSSON *et al.* 1986). There are five different DQα and β genes and three DRβ genes that have been identified. Most haplotypes express two DQα and β genes and one DRβ functionally expressed gene, the number of DQ genes is shown to vary with haplotype (ANDERSSON *et al.* 1988; GELHAUS *et al.* 1999; TAKESHIMA and AIDA 2006). To date, 106 DRB3, 46 DQA, and 52 DQB alleles have been reported (BAXTER *et al.* 2009; NORIMINE and BROWN 2005). Bovine MHC class I is composed of 10 genes and pseudogenes, four of these genes are transcribed but expression is highly variable among individuals (BABIUK *et al.* 2007). Twenty-eight distinct class I sequences have been identified (TAKESHIMA and AIDA 2006). Allelic polymorphisms are associated with the antigen-binding region which is used to define the specificity of the acquired immune response and for haplotype identification (BALLINGALL *et al.* 1998). Polymorphic MHC

genes and variation in the MHC haplotypes contributes to the diverse range of immune responses.

Haplotype Structure

The haplotype structure across the MHC is rather conserved in most mammals, however cattle and other ruminants are unique in the expression of multiple DQA and DQB loci which may be contributing to the variation in the immune phenotypes that are observed(SCOTT *et al.* 1987). While at least four DQB loci have been identified, and there is strong evidence for a fifth DQB loci, only two DQA and DQB loci have been identified in a single haplotype (GELHAUS *et al.* 1999). In haplotypes with single and duplicated versions of DQ loci, all loci appear to be functional (GELHAUS *et al.* 1999). The presence of a heterozygous DQ duplication may lead to the increase diversity of immune phenotypes, and may explain some of the variation that has not been previously identified by SNP-haplotypes in the event new loci have been identified and if duplications of the gene are not detected with classic SNP panels. To support this idea, Gelhaus et al. suggests that in the presence of DQA5 and DQB5 together in the same haplotype, DQA5 and DQB5 products are able to form a divergent DQ molecule suggesting a divergent immunological function (GELHAUS *et al.* 1999). Additionally, in the presence of a duplication of DQ, there is the potential to increase the variety of class II molecules at the cell surface to ensure inter- and intrahaplotype pairing of the alpha and beta chains (GLASS *et al.* 2000). Duplications of genes within given haplotypes might offer an advantage to the variation of the immune response that able to be mounted in cattle.

Approximately 80 BoLA haplotypes have been identified by SNPs across a diverse cohort of cattle breeds, largely influenced by taurine breeds. Haplotype differences have primarily been described by SNP differences rather than other structural variation differences. Creating identical genotypes with varying specificities, sequence to sequence variation or possible copy number influences on gene expression (USINGER *et al.* 1981). More than one copy of a gene can be expressed in some haplotypes but is unaccounted for in the current method of haplotype identification. Unaccounted for variation in copy number and expression might influence diversity in immune responses that are associated with a single haplotype (ELLIS *et al.* 1999). Absolute gene number has not been captured in the current haplotype identification system. Polymorphisms, some that are undetected yet, drive the variation that underlies the BoLA haplotypes.

Polymorphisms

The MHC contains some of the most polymorphic genes in mammalian genomes. High levels of polymorphisms expressed in the antigen presenting genes of the MHC contribute to the

diverse immune responses developed to host pathogens and individual variation of expressed immune response (BABIUK *et al.* 2007). Polymorphisms in BoLA class I and II genes influence immune response through peptide binding, antigen presentation, T-cell repertoire, humoral response, cytotoxic response, cytokine networks, vaccine response, and disease susceptibility (MIYASAKA *et al.* 2011; TAKESHIMA and AIDA 2006). SNPs in the HLA class II genes have been shown to determine the specificity of the immune response and play a role in conferring disease susceptibility (NAGAOKA *et al.* 1999). Similarly, SNPs and insertion/deletion polymorphisms have been identified and have been associated with individual variation in cattle (SCHRIDER and HAHN 2010). Allele specific polymorphisms have been shown to be different between breeds and might influence the duration of the immune response along with diversity of immune phenotypes (BAXTER *et al.* 2009; MIYASAKA *et al.* 2011).

Genome Structural Variation

The genome structure is constantly undergoing changes and rearrangements (STANKIEWICZ and LUPSKI 2010; ZHANG *et al.* 2009a), however the phenotypic contributions for many structural changes are unknown. Genomic structural variation includes: insertions, duplications, deletions, inversions and translocations of DNA (FADISTA *et al.* 2010; STANKIEWICZ and LUPSKI 2010). SNPs have been thought to be the major source of individual genetic variation, but undefined phenotypic diversity may be due to larger regions of variation such as CNVs (FADISTA *et al.* 2010; REDON *et al.* 2006). CNVs contain more total sequence than SNPs and are large enough to encompass whole genes. Therefore, CNVs have a potential for more significant effects on evolution, fitness, and genetic diversity (FADISTA *et al.* 2010; HOU *et al.* 2012; REDON *et al.* 2006; SCHRIDER and HAHN 2010; ZHANG *et al.* 2009b). Characterization of genetic variation in livestock species is an important step towards linking genes or genomic regions with phenotypes (STOTHARD *et al.* 2011). Detection of CNVs in the immune specified region of the genome might explain variation in immune response.

Copy Number Variation

Identified CNVs have included translatable genes, functional elements, and noncoding RNAs; many of which have not been associated with phenotypes (REDON *et al.* 2006). The GC content associated with CNV regions has been shown to be slightly higher than the GC content of the whole genome, suggesting CNVs arise more frequently in gene rich regions (FADISTA *et al.* 2010). Redon et al. (2006) defined a CNV as 1 Kb or larger, however other studies have identified CNVs of smaller sizes (CONRAD *et al.* 2010; DOAN *et al.* 2012; ZHANG *et al.* 2009a). The CNV detection resolution depends on the design of the array. This presents a challenge not only to detect CNVs but also to characterize expression and associate CNV changes with phenotypes.

Now that arrays with higher resolution have been designed, smaller CNVs have been reported to be as frequent as large (>1 Kb) CNVs (FADISTA *et al.* 2010). Due to the design of SNP arrays, the smaller CNVs would not have likely been detected and therefore may not be accounted for in haplotype characterization.

CNVs account for a significant source of variation in mammals (FADISTA *et al.* 2010). CNVs have been shown to be associated with quantitative phenotypes and to be known causative agents of genetic disorders (FADISTA *et al.* 2010). However, little is known about CNV variation in relation to bovine immune phenotypic diversity. The proportion of predicted CNVs per individual human varies between 2.3 and 4.2% (TENNESSEN *et al.* 2012), suggesting individual variation may be associated with differences in immune response expression (HOU *et al.* 2012; SCHRIDER and HAHN 2010; TENNESSEN *et al.* 2012). Pairwise comparisons of taurine and indicine cattle suggested that CNV differences between subspecies are greater than across breeds within a subspecies (BICKHART *et al.* 2012). Stothard *et al.* (2001) has shown that genomic regions enriched with CNVs are breed dependent, which may show selection pressure. Breed dependent CNVs may be relevant to unexplained haplotype variation that exists between breeds, and disease may be influencing selection pressure for CNVs enriched in immune function genes. CNV detection and variation may help interpret diverse expressed immune responses that have previously been associated with identical haplotypes.

Association with Disease

The MHC has been associated with immune response and disease susceptibility in many species (KELLEY *et al.* 2005). Disease association studies have shown large variability in allele association with immune response. In cattle, alleles of class II are associated with animal-to-animal variation in disease susceptibility to hoof-and-mouth disease, dematophilosis, mastitis, bovine leukemia virus, and tick resistance (FADISTA *et al.* 2010; LEWIN and BERNOCO 1986; MIYASAKA *et al.* 2011; SHARIF *et al.* 1998; UNTALAN *et al.* 2007; XU *et al.* 1993). An indirect relationship between health and production traits was shown based on which DRβ3 allele was present for mastitis resistance/susceptibility (OPRZADEK *et al.* 2012). Within some defined diseases, affected members share a common haplotype at a higher frequency than would be expected with independent segregations, thus suggesting that there is a haplotype association with disease susceptibility (TODD *et al.* 1988). BoLA haplotypes and CNVs have been associated with disease phenotypes, however the associations are not well characterized and need further investigation. BoLA heterozygotes have an advantage of enhanced resistance and increased diversity of antigens presented and recognized (TAKESHIMA *et al.* 2008). Multiple studies have demonstrated a strong link between copy number differences at a specific location and differ

-ences in phenotypic traits (SCHRIDER and HAHN 2010).

Conclusion

Immune response and disease resistance and susceptibility are not yet well characterized nor are the genetic mechanisms that control immune response and disease susceptibility well understood. More variation in immune phenotypes has been observed than can be accounted for in the current SNP-defined haplotypes and association studies. A limited about of research that is available on CNV and other structural arrangements shows strong evidence that structural rearrangements could contribute to the degree of variation in immune phenotypes. To increase the strength of the association tests, and to increase the likelihood of using genetic selection for health improvements, immune response and disease phenotypes need to be better characterized. This is one of the most restricting limiting factors to the use of genetic selection for improved health.

Genetic selection has now been used as a selection tool for many breeds and for quantitative traits. The literature has evidence for the possibility of using genetic selection for animal health improvement. The use of genetic selection to improve immune response and minimized disease susceptibility could serve as an alternative to minimize antibiotic use and improve the immune response to vaccines as disease prevention methods. The use genetic selection to improve animal health may minimize the financial costs that have previously been associated with disease and treatment, while boosting the benefits from disease prevention with improved immune response from vaccine treatments.

The MHC is a single region of the genome that is known to have immune related function, and has been the focus of this review. This multigene family constitutes the larges known region of the genome with immune function. There are studies that have showed associations between MHC genes and immune phenotypes that suggest that it might offer a region of selection to improve immune response and to decrease disease susceptibility. However, animal health improvement though genetic selection is not limited to the MHC, but offers a starting point for genetic selection and illustrates the complexity of immune mechanisms.

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