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Genetic variation and responses to vaccines

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Abstract

Disease is a major source of economic loss to the livestock industry. Understanding the role of genetic factors in immune responsiveness and disease resistance should provide new approaches to the control of disease through development of safe synthetic subunit vaccines and breeding for disease resistance. The major histocompatibility complex (MHC) has been an important candidate locus for immune responsiveness studies. However, it is clear that other loci play an important role. Identifying these and quantifying the relative importance of MHC and non-MHC genes should result in new insights into host–pathogen interactions, and information that can be exploited by vaccine designers. The rapidly increasing information available about the bovine genome and the identification of polymorphisms in immune-related genes will offer potential candidates that control immune responses to vaccines. The bovine MHC, *BoLA*, encodes two distinct isotypes of class II molecules, DR and DQ, and in about half the common haplotypes the *DQ* genes are duplicated and expressed. DQ molecules are composed of two polymorphic chains whereas DR consists of one polymorphic and one non-polymorphic chain. Although, it is clear that MHC polymorphism is related to immune responsiveness, it is less clear how different allelic and locus products influence the outcome of an immune response in terms of generating protective immunity in outbred animals. A peptide derived from foot-and-mouth disease virus (FMDV) was used as a probe for BoLA class II function. Both DR and DQ are involved in antigen presentation. In an analysis of T-cell clones specific for the peptide, distinct biases to particular restriction elements were observed. In addition inter-haplotype pairings of DQA and DQB molecules produced functional molecules, which greatly increases the numbers of possible restriction elements, compared with the number of genes, particularly in cattle with duplicated *DQ* genes. In a vaccine trial with several peptides derived from FMDV, *BoLA* class II *DRB3* polymorphisms were correlated with both protection and non-protection. Although variation in immune responsiveness to the FMDV peptide between different individuals is partly explainable by *BoLA* class II alleles, other genetic factors play an important role. In a quantitative trait locus project, employing a second-generation cross between Charolais and Holstein cattle, significant sire and breed effects were also observed in T-cell, cytokine and antibody responses to the FMDV peptide. These results suggest that both MHC and non-MHC genes play a role in regulating bovine immune traits of relevance to vaccine design. Identifying these genes and quantifying their relative contributions is the subject of further studies.

Keywords: vaccines; immune response; genetic variation; MHC genes; non-MHC genes

Introduction and background

Infectious diseases are a continuing threat to the health and welfare of farm animals, and represent a major source of economic loss in both temperate and tropical climates. For example, costs due to disease constitute 17% of turnover within the livestock sector of the developed world (Office International des Epizooties, 1998). There is growing consumer demand for animal products with lower chemical inputs. Allied to this, there is growing pathogen resistance to control measures such as antibiotics, anthelmintics and ascaricides, limiting the

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possibilities for long-term pharmacological intervention. Moreover, antibiotic resistance in animal populations is perceived as a threat to human health.

In the UK, one has only to think about the foot-and-mouth outbreak (Knowles *et al*., 2001; Royal Society, 2002;), or the less publicized classical swine fever outbreak (Anon, 2000) to realize that, with increased globalization, the issues of biosecurity and readiness to deal with exotic disease outbreaks have become paramount. In addition, endemic diseases continue to affect the health and welfare of livestock, while those that are also zoonoses pose human health risks.

Thus, new approaches to disease control are essential. The most cost-effective solutions could be the development of rationally designed vaccines allied to long-term programs of genetic selection to enhance natural disease resistance. Exactly which paths to follow depends on both the host and the pathogen. This review aims to concentrate on the role that genetics and genomics might play in improving our understanding of how to elicit a protective immune response with vaccines that are safe, do not lead to an infectious status, do not induce a carrier state, do not induce pathology, and yet deliver long-lasting protection in all susceptible animals. The review concentrates mainly on cattle, but many of the points are relevant to other livestock species. Identifying genetic variants that control responsiveness to vaccines and disease resistance and investigating gene expression differences could together identify new pathways to target for vaccines with improved efficacy. In addition, it may be possible to select animals for improved responsiveness to vaccination, an approach advocated by Wilkie and Mallard (1999).

Although there are many vaccines for livestock currently available or at various stages of development, most could not be considered to meet all of the above criteria. For example an 'emergency' foot-and-mouth disease virus (FMDV) vaccine was not used in the UK during the 2001 FMDV outbreak to ring-fence areas with infected livestock, because of fears of concealing infectious animals and prolonging the period when exports would be banned (even though it is debatable whether carrier animals pose an infectious risk) (Kitching, 2002; Alexandersen *et al*., 2002; Sutmoller *et al*., 2003; Woolhouse, 2003). An ideal vaccine would be a synthetic one that induced long-lasting protection in all animals (see above). However, incomplete understanding of the protective mechanisms induced by replicating pathogens, particularly for bovine parasites and intracellular pathogens, and partial knowledge of how to stimulate immune cell-mediated pathways with 'molecular' vaccines, still hamper progress towards safe, effective vaccines for livestock (e.g. Brown, 2001; Dalton and Mulcahy, 2001; Glass, 2001; Norimatsu *et al*., 2003).

The relationship of host genotype to responsiveness to vaccines has not been greatly explored but is likely to involve the major histocompatibility complex MHC (e.g. processed peptides may not bind or only bind at low affinity to particular alleles) and genes controlling cytokine profiles (e.g. Th1 versus Th2) or levels of proinflammatory cytokines. Such genes could account for low or non-responsiveness through to adverse reactions to vaccines and their components. In addition, individual variation in responsiveness to vaccine candidates, such as recombinant antigens and peptides, is likely to become more of an issue, particularly with non-responders, as has been observed in humans vaccinated with the recombinant hepatitis B vaccine (De Silvestri *et al*., 2001).

Genetics

Most immunogenetic studies in humans and ruminants have concentrated on disease resistance and susceptibility (for recent reviews see Teale, 1999; Hill, 2001; Kelm *et al*., 2001) and to a lesser extent on responsiveness to vaccination (Outteridge, 1993; Newman *et al*., 1996; Raadsma *et al*., 1999; Wagter *et al*., 2000; Tan *et al*., 2001; Sitte *et al*., 2002; Hohler *et al*., 2002; Moreno *et al*., 2003). These studies indicate that a significant part of individual variation in these traits has a genetic component, with varying degrees of heritability. Thus, for cattle such traits could be selected for in targeted breeding schemes.

What evidence there is for the underlying genetic control of responsiveness to vaccines indicates that it is unlikely that many examples of single genes with great effect will be discovered, although the effects of a defect in the bovine integrin Mac-1 [bovine leukocyte adhesion deficiency (BLAD)] in a single sire used extensively in US cattle herds had a considerable detrimental effect on the health of these herds, at least in BLAD homozygous recessive cattle (Shuster *et al*., 1992). Instead, it is more likely that many genes, each with smaller and interacting effects, play a role in determining an individual's response to vaccination.

Because of host–pathogen dynamics, it is likely that many genes controlling immune responsiveness will themselves be highly variable, and, contrary to some opinions, most populations will probably harbor immune response-associated polymorphisms. From these studies and current knowledge of the mechanisms/pathways involved in protective immunity, a variety of candidates that are likely to influence vaccine responsiveness can be proposed, including the MHC and other candidate regions containing clusters of immune function genes, such as the T-cell receptor, killer immunoglobulin-like receptor, and immunoglobulin heavy-chain loci. The pathways that vaccine designers want to stimulate include innate as well as specific acquired cellular and humoral immunity, and variation in genes involved in these pathways, particularly those whose products control rate-limiting steps in

pathways, are likely to be a fruitful source of gene candidates. However, the relative importance even of known candidates still remains to be quantified.

Interaction with performance traits

Concerns are sometimes expressed that selecting for improved disease resistance or vaccine responsiveness could have adverse effects on performance. For example, in dairy cattle an unfavorable genetic relationship between milk production and mastitis has been reported (e.g. Fleischer *et al*., 2001; Hansen *et al*., 2002). It is unclear, however, if this is a causal relationship, and it may not relate to immunity. Even in the case of the BLAD mutation (D128G), carriers of this homozygous recessive allele were no more susceptible to mastitis than non-carriers (Wanner *et al*., 1999). Relatively few studies of quantitative trait loci (QTL) have investigated disease resistance traits and performance traits together. However, some QTL for clinical mastitis and somatic cell count do not overlap with QTL for performance traits (Klungland *et al*., 2001; Boichard *et al*., 2003; Kuhn *et al*., 2003). Certain alleles of the bovine MHC (*BoLA*) *DRB3* genes, which are associated with increased resistance to mastitis, do not appear to be linked to milk production traits (Sharif *et al*., 1999). In another study, similar production traits did not correlate with antibody response to a protein antigen, although high antibody response was associated with increased resistance to mastitis (Wagter *et al*., 2000).

Although tropical breeds of cattle may be both more resistant to infection and less productive than highly selected Western breeds, this does not mean that the phenotypic traits of disease resistance and productivity are necessarily linked at the genetic level. Indeed, Hanotte *et al*. (2003) reported that, of 19 QTL for trypanotolerance detected in a cross between two indigenous African breeds of cattle, most did not overlie QTL affecting body weight. In general, relationships of performance with disease resistance and/or vaccine responsiveness will be a balance between the 'costs' of mounting an effective immune response versus the beneficial consequences of an ability to minimize the impact of infection and disease (Bishop and Stear, 2003). This relative balance will depend on genetic as well as non-genetic factors, such as the severity of the infectious challenge and the nutritional status of the host animal. The genetic factors may or may not include genes directly affecting immune responses.

Genomics and bioinformatics

The acceleration in genomics research and information should provide new opportunities for controlling disease by identifying genetic factors that confer disease resistance, which could be used in selected breeding, and by improvement in vaccine responsiveness, particularly identifying pathways that lead to protection. Sequencing of the human genome (Lander *et al*., 2001) has been a spur to genome mappers in livestock species. The bovine genome has been mapped using microsatellite markers, *in situ* hybridization, linkage mapping and radiation-hybrid mapping (Barendse *et al*., 1997; Kappes *et al*., 1997; Band *et al*., 2000; Williams *et al*., 2002; Williams, 2004). There are now over 2400 microsatellite markers mapped onto the bovine genome (see http://locus.jouy.inra.fr/cgi-bin/lgbc/mapping/common/intro2.pl/BASE=cattle; http://sol.marc.usda.gov/genome/cattle/cattl.htlm; http://spinal.tag.csiro.au/cgd.html). Assembly of a whole-genome 'sequence ready' bovine artificial chromosome (BAC) contig for the bovine genome is under way, sequencing of the bovine genome and a single nucleotide polymorphism (SNP) map are currently being discussed for cattle, and there are over 200 000 expressed sequence tags (ESTs) available for cattle (Smith *et al*., 2001; Sonstegard *et al*., 2002). All of this will increase the information available (see http://hgsc.bcm.tmc.edu/projects/bovine/).

SNPs are reported to occur every 300–1000 DNA bases, but their frequency in particular regions of the genome probably varies and many variant alleles may not have biological relevance (for a review of SNPs in livestock genetics see Vignal *et al*., 2002). There are limited data available for cattle; however, the diversity of SNPs in some cytokine genes is high (Heaton *et al*., 2001a). A draft sequence and annotation of the bovine MHC (*BoLA*) is currently under way (Gibbs *et al*., 2003). Cataloguing of genotype–phenotype correlations and databases of SNPs for immune genes have been proposed for human studies (Foster and Chanock, 2000; Geraghty *et al*., 2002). Similar compilations would be of immense value for livestock. However, there is limited information on the extent of polymorphism of candidates, such as cytokine genes and their receptors, in cattle (Agaba *et al*., 1996; Grosse *et al*., 1999; Heaton *et al*., 2001a, b; Schmidt *et al*., 2002). Coussens and Nobis (2002) have reported a web-accessible resource for cattle for 270 bovine immune-related genes with real-time PCR primer sequences (http://gowhite.ans.msu.edu/public_php/gd-bovine-immunology.php). Thus, much needed and useful information will hopefully become available in the next few years.

Candidate genes might also be identified through a functional genomics approach using microarrays and comparing the expression of genes in animals differing in vaccine response or disease resistance. In addition, microarray technology offers new opportunities to investigate host–pathogen interactions and to identify genes relevant to vaccinology (Aujame *et al*., 2002). Identifying genes involved in responses to vaccines could lead to new approaches for vaccine design; for example, as novel immunomodulators and immunopotentiators. Both host and pathogen arrays suitable for livestock are

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becoming available. In my own laboratory we are assembling a macrophage focused microarray consisting of 5000 cDNAs selected from a normalized library derived from *Bos taurus* and *B. indicus* resting, stimulated and infected monocyte/macrophages (McGuire *et al*., 2002; http://www.ark-genomics.org/projects/00006abs.html). Two other microarrays for cattle have also been reported which contain further cDNAs of immunological relevance (Yao *et al*., 2001; Band *et al*., 2002). Watkins and colleagues (2002) have created an array consisting of 75-mer oligonucleotides of ruminant immunoinflammatory relevance. In addition, Hernandez and colleagues (2003) report on the use of a human microarray to investigate immune responsiveness in cattle.

Identification of T-cell epitopes and MHC binding sites can be aided by the use of various algorithms, at least for humans (Sturniolo *et al*., 1999; De Groot *et al*., 2001). The integration of these algorithms together with microarray data on the expression of pathogen genes and sequence information holds much promise for the identification of vaccine candidates. Recently, Vordermeier *et al*. (2003) have described the identification of so-called promiscuous bovine $CD4^+$ T-cell epitopes in a *Mycobacterium bovis* antigen using a human prediction program for human MHC (*HLA*) *DR* alleles (ProPred) (Singh and Raghava, 2001). ProPred is also available as an on-line web tool for predicting human MHC class I restricted epitopes (Singh and Raghava, 2003) and De Groot *et al*. (2003) have refined two algorithms developed for humans, by screening peptides eluted from a *BoLA* class I allele. These studies suggest that, where the pathogen sequence is known, it may be possible to determine regions that are stimulatory across many bovine haplotypes.

Thus, various approaches can be taken to identify genes underlying variation in vaccine response. However, genetically informative populations and an ability to identify meaningful phenotypes are also required. Field data, if available, might be sufficient; alternatively, studies can be performed on specific lines/breeds and their crosses. The simplest part of such studies should aim to show that significant heritabilities in vaccine response exist, and then aim to identify genes/chromosomal regions controlling the traits of interest through QTL analysis and/or genetic association/linkage studies with candidate genes/markers.

Together with colleagues at Roslin Institute and Glasgow University, I am adopting these approaches to determine the role of both the bovine MHC and non-MHC genes in terms of responses to vaccines and model antigens. In particular, a QTL study is investigating responses to various antigens, including a peptide derived from FMDV as well as vaccines for viral respiratory pathogens in a specific population of crossbred cattle. The results so far indicate that both MHC and non-MHC genes play a role in regulating immune traits in cattle. Ultimately, the aim is to identify the non-MHC genes and quantify their relative contributions to the immune response.

BoLA: relationship to immune responsiveness and vaccination

The current *BoLA* map is not as detailed as that for humans and mice, and it is found on chromosome 23 (cattle have 29 chromosomes plus X and Y) (Lewin *et al*., 1999). Although it is similar in many respects to those in other species, with similar genes residing within it, there are some notable differences. At the gross level, there is an insertion which has sent the processing-associated genes much further away from the classical class II genes (Lewin *et al*., 1999). Also, Bovidae have some novel non-classical genes, including *DYA*, *DYB* and *DIB*, some of which may have functional significance as they are expressed in bovine dendritic cells (Ballingall *et al*., 2001), but they have limited, if any, polymorphism. Alleles of *BoLA* genes can be found at http://www.projects.roslin.ac.uk/bola/.

BoLA class I

Originally, BoLA class I typing with alloantisera suggested that each haplotype expressed only one dominant class I molecule (Spooner *et al*., 1979). However, biochemical and molecular analysis has revealed that the situation with *BoLA* class I genes is very complex, with different haplotypes carrying different numbers of loci, with no obvious lineages associated with particular loci and with variable degrees of gene expression (Ellis and Ballingall, 1999). Currently, there are 28 full-length class I cDNAs sequenced (http://www.projects.roslin.ac.uk/bola/mhc1seq.html). It is clear that BoLA class I restricted cytotoxic T cells (CTL) play a role in protective responses to various intracellular pathogens, including bovine respiratory syncytial virus (Gaddum *et al*., 2003), *Theileria annulata* (Preston *et al*., 1999) and *T. parva* (McKeever *et al*., 1999). Antigenic variation in CTL targets has been reported in human studies, suggesting that CTL play an important role in protection against intracellular pathogens (Borrow and Shaw, 1998). CTL generally recognize a restricted set of immunodominant epitopes, the selection of which is determined by the MHC class I types, and evidence suggests that this may also be the case for *T. parva* (McKeever *et al*., 1999). Thus, vaccines for intracellular pathogens may need to induce CTL. However only a few BoLA class I anchor motifs have been identified; several CTL epitope candidates have been suggested but have not been formally identified (Ellis and Ballingall, 1999; Hegde and Srikumaran, 2000; De Groot *et al*., 2003). Recently, *BoLA* class I transgenic mice have been created, which could be used to screen

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CTL epitope-containing candidate vaccines (Russell *et al*., 2002). However, overcoming variability in BoLA class I interactions with vaccine candidates is probably not going to be straightforward.

BoLA class II

For *BoLA* class II genes there are *DR* and *DQ* loci but no *DP* loci. There is a non-polymorphic *DRA* gene together with three *DRB* genes, although only one, *DRB3*, seems to be both expressed and functional. *DQ* is more complicated: in about half of the haplotypes that have been tested there are duplicated *DQA* and *DQB* genes. It is clear that, in cattle, *BoLA* class II polymorphism plays a role in observed variability in immune responsiveness (Glass *et al*., 1991, 2000; Glass and Millar, 1994, 1995; Casati *et al*., 1995; van Lierop *et al*., 1995a; Newman *et al*., 1996; Garcia-Briones *et al*., 2000; Sitte *et al*., 2002). There are many alleles for *DRB3* (104 alleles with 50 major types) and for *DQA* (47 alleles and 20 major types) and *DQB* (49 alleles and 30 major types) (http://www.projects.roslin.ac.uk/bola/) (Fig. 1). Currently, the most frequently used method for determining polymorphism of *BoLA* class II is by PCR-RFLP of the second exon (van Eijk *et al*., 1992; Ballingall *et al*., 1997). More recently, Miltiadou *et al*. (2003) have developed a sequence-based typing method for *DRB3* exon 2 together with a program called Haplo-Finder. As with epitopes determined by *BoLA* class I alleles, there are only a few reports on *BoLA* class II allele-specific T-cell epitopes (e.g. Collen, 1994; Court *et al*., 1998; Haghparast, 2000; Fogg *et al*., 2001; Collen *et al*., 2002; Vordermeier *et al*., 2003), although a recent study has suggested binding motifs for two *BoLA* class II *DR* alleles (Sharif *et al*., 2003).

Although BoLA class II polymorphism has been associated with disease resistance (e.g. Xu *et al*., 1993; Sharif *et al*., 2000), and indeed used successfully to cull dermatophilosis-susceptible animals (Maillard *et al*., 2003), very few vaccine studies have investigated whether *BoLA* class II alleles can account for variation in responsiveness to vaccines. Newman and colleagues (1996) reported that *BoLA* and other genes accounted for a significant amount of the variation in response to a *Brucella abortus* vaccine. In collaboration with Spanish and Argentinean colleagues, we have shown that *BoLA* class II polymorphism does play a role in determining the degree of protection against FMDV provided by vaccination with FMDV-derived peptides (Garcia-Briones *et al*., 2000). In a third study, variation in response to a commercial cattle sub-unit tick vaccine was associated with BoLA type (Sitte *et al*. 2002).

Immune responsiveness to FMDV-derived peptides

In my laboratory, studies on the role of BoLA class II and immune responsiveness have concentrated on a

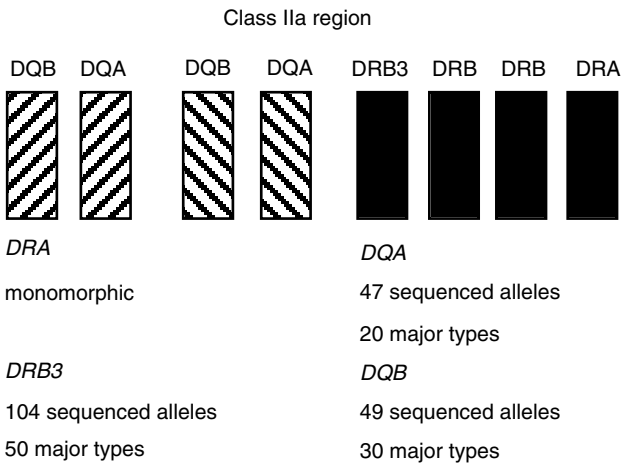

Fig. 1. BoLA class II genes and polymorphism.

model vaccinal 40-mer peptide derived from VP1 of FMDV. This peptide consists of two non-continuous sequences derived from FMDV VP1, one of which forms a loop and is adjacent to the other in the native virus particle. These regions are the focus of neutralizing antibody. This peptide and similar ones have been shown to protect a proportion of cattle (around 30–40%) (DiMarchi *et al*., 1986; Taboga *et al*., 1997). The reasons why not all cattle are protected are manifold and probably include genetic factors, particularly the MHC. Although this FMDV peptide and variants of it induce neutralizing antibody, no clear correlation between the level of neutralizing antibody and protection has been observed, in contrast to the situation with inactivated virus vaccines (Sobrino *et al*., 2001). Different antibody isotypes may be associated with different functions, and it is possible that these peptides have a propensity to induce different antibody isotypes than the viral vaccines (Mulcahy *et al*., 1990; Taboga *et al*., 1997). It seems likely that virus-specific $CD4^+$ T cells and possibly $CD8^+$ T cells are also essential for protection (Collen, 1994; Childerstone *et al*., 1999; Sobrino *et al*., 2001). It is probable that other mechanisms of protection are important as well; for example, the production of cytokines such as interferon-γ (IFN-γ), as this cytokine has been shown to inhibit FMDV replication *in vitro* (Zhang *et al*., 2002). Amadori *et al*. (1992) have shown a correlation between protection against FMDV and the production of interleukin (IL)-2 and IFN-γ by T cells *in vitro*. T cells derived from various BoLA class II types responding to the inactivated viral vaccine do produce IFN-γ and not IL-4 (van Lierop *et al*., 1995a) and our own more recent studies indicate that vaccination with the 40-mer peptide also induces IFN-γ production by peptide specific T cells derived from animals expressing a broad spectrum of different BoLA types (Fig. 2). One further explanation may account for the lack of protection induced by the peptide: infection with the virus does not induce T-cell

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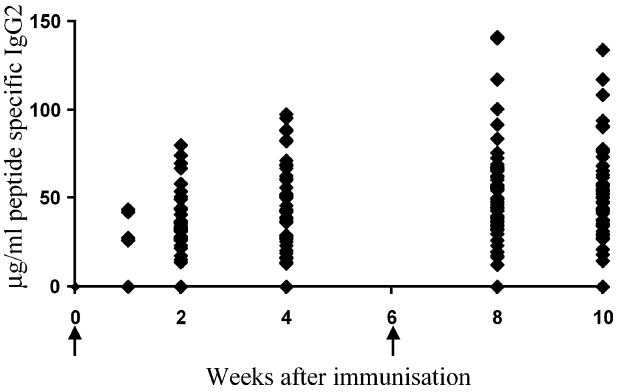

Fig. 2. Peptide-specific IgG2 response by the first cohort of F_2 Holstein/Charolais cross animals. Each point at each time point represents an individual animal. Antibody responses were measured by ELISA with FMDV15 as antigen, and calibrated to µg/ml with serum standards.

responses directed to the loop region of VP1, possibly reflecting differences in processing of the virus particle and the peptide (van Lierop *et al*., 1995b). However, my own studies have shown that at least some peptide specific T-cell clones recognise the native virus (unpublished observations). As animals with different BoLA types were used in the various studies cited, any differences observed between studies might be accounted for by the different binding properties of *BoLA* class II alleles.

FMDV peptide as a probe for BoLA class II function

We have shown that most *BoLA* class II haplotypes examined were associated with FMDV-specific T-cell and antibody responses, although a few haplotypes were associated with low or non-responsiveness, even in the presence of another haplotype which conferred responsiveness (Glass *et al*., 1991; Glass and Millar, 1994). There were consistent qualitative differences between haplotypes, which suggested that different BoLA DR and/or DQ molecules bind different fragments of the 40-mer peptide with different affinities. Most animals tested have a high proportion of T cells specific for the loop region, which in the native virus particle is also the focus of B-cell responses and neutralizing antibody. Nonetheless, possession of one particular allele, *BoLA DRB3*18*, directed the T-cell response to a 'spacer' sequence (added to the peptide for conformational reasons) (Glass and Millar, 1995). Even though high levels of neutralizing antibody are produced in animals expressing this allele and vaccinated with FMDV-derived peptide, they are not protected against challenge with live virus (García-Briones *et al*., 2000), possibly because the BoLA DRB3*18-restricted T cells induced by the peptide do not cross-react with the virus. This lack of correlation between T-cell proliferative responses, BoLA type and antibody responses, including anti-peptide, anti-virus and neutralizing antibody, was also observed in our other studies (García-Briones *et al*., 2000; Glass and Millar, 1994, 1995). However, further results from the vaccine trial indicate that the MHC does play a part in determining the response to the peptide, as haplotypes 1, 3 and 7 are associated with high serum neutralizing titer and confer some degree of protection, whereas animals expressing haplotype 12 produce low serum neutralizing titers in response to the peptide and are not protected against live viral challenge (García-Briones *et al*., 2000).

DQ

Most studies on T-cell responsiveness consider DR-restricted responses. However, at least as far as responses to the model antigen derived from FDMV are concerned, both DR and DQ play a role. It is still unclear if DR and DQ play different roles in immunity. In humans, *DQ* alleles are associated with autoimmunity and also with suppressive responses. DQ molecules are differentially expressed on human antigen-presenting cells and have differently shaped binding clefts (Paliakasis *et al*., 1996; Raddrizzani *et al*., 1997). The role of DQ in cattle is unknown but both anti-DR and anti-DQ antibodies inhibit T-cell proliferation (Glass *et al*., 2000), and many of the peptide-specific $CD4^+$ T cell clones isolated from responder animals were DQ-restricted (Glass *et al*., 2000). As the functions of these clones were not assessed, it is not known if the DQ-restricted clones have different cytokine profiles, for example.

As both *DQA* and *DQB* are polymorphic and both loci, if present, are expressed, there is the possibility of intra- and inter-haplotype pairings between the DQ α and β chains. In a clonal analysis of T-cell clones specific for the FMDV peptide, evidence was presented that indeed such mixed pairings do occur and are functionally important *in vivo* (Glass *et al*., 2000). Further analysis of other cattle immunized with this peptide revealed that, of those animals defined as being high responders, the majority were heterozygous for haplotypes with duplicated *DQ* genes. Thus, it may be that extra restriction elements simply increase the chances of having high-affinity binders for a given peptide. However, this would have to be confirmed with other antigens.

As with BoLA class I, BoLA class II-restricted $CD4^+$ responses tended to be biased towards particular restriction elements (Glass and Millar, 1994, 1995; Glass *et al*., 2000), suggesting that particular T-cell epitopes are immunodominant, perhaps reflecting peptide affinities for the binding cleft of particular BoLA class II molecules. Identification of 'promiscuous' epitopes on the native virus particle, which are recognized by T cells

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from a wide range of BoLA types, is needed as a first step towards a protective synthetic vaccine. This may be relatively straightforward, given that the sequence of FMDV is known, and an approach similar to that described by Vordermeier *et al.* (2003) could be undertaken. Candidate T-cell epitopic regions on the virus particle may be discontinuous; however, new technologies in peptide chemistry have now made it possible to synthesize these (Meloen *et al.*, 2001). Additionally, the ability to synthesize long peptides that are correctly folded and biologically active (Demotz *et al.*, 2001) would suggest that peptides may yet become the basis of effective vaccines.

Unfortunately, there is a dearth of immunological tools, such as monoclonal antibodies specific for polymorphic determinants on BoLA class II molecules, transfectants and tetramers, which could be used to answer these questions. BoLA tetramers are in the pipeline (S. A. Ellis, personal communication) but these are limited to peptides identified as both binding to a specific *BoLA* allele and forming a T-cell epitope.

The role of non-MHC genes in immune responsiveness to vaccines

The MHC has long been an important focus for immune responsiveness studies in both humans and livestock. However, with the advances in genomics it has now become possible to begin to quantify the relative contributions that MHC and non-MHC genes play in determining responses to vaccines. Certainly in humans, a significant proportion of the genetic component of variation in the immune response is caused by non-MHC genes (Jepson *et al.*, 1997; Hohler *et al.*, 2002). There have been many studies on disease resistance, and QTL and genes have been identified that play an important role in man and mouse (Hill, 2001), but much less is known in livestock species, particularly in terms of genes, their polymorphism and the role they might play in vaccine responsiveness. Most studies in cattle have considered disease resistance or health traits (recent papers include Adams and Templeton, 1998; Holmskov *et al.*, 1998; Ambrose *et al.*, 1999; Teale, 1999; Frisch *et al.*, 2000; Kelm *et al.*, 2001; Gasbarre *et al.*, 2001; Detilleux, 2002; Behnke *et al.*, 2003). The recessive BLAD allele is the only non-MHC gene in cattle pinpointed to have disease consequences (Shuster *et al.*, 1992). Natural resistance-associated macrophage protein (*NRAMP* or *SLC11A1* [solute carrier family 11, member 1]) has been mooted as likely to play an important role in the resistance of cattle to intracellular pathogens (Adams and Templeton, 1998) and a preliminary study has correlated microsatellite markers in linkage with *SLC11A1* with resistance and susceptibility to bovine tuberculosis (Zanotti *et al.*, 2002). One study has reported that variation in the antibody responses of cattle to vaccination with a *Brucella abortus* live attenuated vaccine is dependent on both *BoLA* and non-*BoLA* genes, although no non-*BoLA* genes were identified (Newman *et al.*, 1996).

Quantitative trait loci

The identification and analysis of genes controlling quantitative traits has become feasible with the discovery of highly informative DNA markers and the development of new genomics techniques. The attractiveness of the whole-genome approach is that many major genes involved in determining variation could be located, including the possibility of identifying contributory genes that were not previously suspected of involvement. The existence of detailed genetic maps in cattle makes the identification of the loci harboring genes controlling quantitative traits (QTL) possible in this species. Localization of QTL to chromosomal regions by a genome mapping approach is the first step in identifying the genes controlling the traits. While the map position serves as a starting point for identifying the genes themselves, markers flanking the QTL could be used directly to enhance selection programs.

One way to attempt to identify genes controlling quantitative traits such as responsiveness to vaccines is to produce a cross of extreme types and then to genotype and phenotype the offspring. Most of the cattle crosses set up in this way have been created to investigate performance traits such as meat quality, milk production and growth. However, some of these crosses have also been used to identify QTL associated with health traits, particularly mastitis, the most common disease of dairy cattle (Ashwell and Van Tassell, 1999; Heyen *et al.*, 1999; Klungland *et al.*, 2001; Boichard *et al.*, 2003; Brunner *et al.*, 2003; Kuhn *et al.*, 2003). A QTL study has identified regions of the bovine genome associated with bovine spongiform encephalopathy (Hernandez-Sanchez *et al.*, 2002) and a cattle cross between *Bos taurus* (resistant) and *Bos indicus* (susceptible) animals has identified QTL for trypanotolerance (Hanotte *et al.*, 2003). Gasbarre *et al.* (2002) have reported preliminary findings for QTL affecting parasite resistance. None of these QTL studies has yet identified the genes underlying these disease traits.

Roslin Institute QTL study

At Roslin Institute, a cross has been set up between two extremes of cattle, Holstein (dairy type) and Charolais (beef type) and genotyped with 186 microsatellite markers that give an average 20 cM coverage and a minimum informativeness of 0.5. The phenotypic traits include performance traits such as growth, feed intake (Lagonigro *et al.*, 2003) and milk production, as well as

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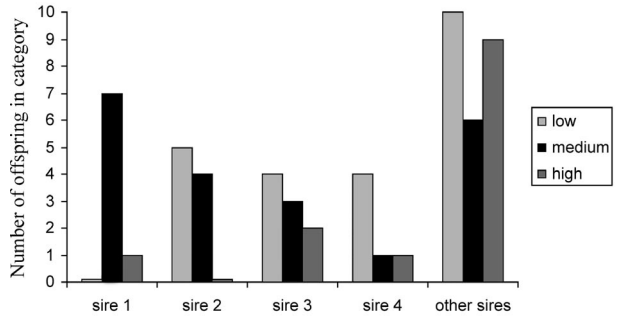

Fig. 3. IFN-γ responses in peripheral blood T cells derived from the first cohort of F_2 Holstein/Charolais cross animals were categorized according to high, medium and low responsiveness. They were then divided into sire groups. Offspring from different sires had significantly different responses ($P < 0.05$; Pearson's χ^2 analysis). Whole-blood assays were used and supernatants collected after 24 hours' incubation with 1 μg/ml FMDV peptide. Commercial kits for bovine IFN-γ were obtained from Biosource and IFN-γ concentrations were calculated using recombinant ovine IFN-γ as a standard.

mastitis incidence as a disease trait in F_2 animals. Various immune traits, encompassing innate, humoral and acquired immunity, are being measured and include T-cell proliferation to *Staphylococcus aureus*, neutrophil phagocytosis (Young *et al*., 2000; Young, 2002), antibody responses following vaccination with commercial vaccines for the respiratory pathogens bovine respiratory syncytial virus, parainfluenza-3 virus and bovine herpesvirus 1, proliferative responses to the T-cell mitogens concanavalin A and phytohaemagglutinin, as well as T-cell and antibody responses to the 40-mer vaccinal FMDV peptide. One advantage of performing all of these measures on the same animals is that it will enable us to correlate the immune responses to different antigens, in addition to linking performance traits with the immune traits. In addition we plan to create an immune index by combining the immune parameters with the production trait data. Such an index would potentially be invaluable in selecting individuals for superior immune competence.

However, because the immune experiments must not influence the performance traits in this study, this has limited the immune parameters measured and no live experimental challenge has been conducted. Instead, we have measured innate traits that do not require any challenge, or used natural challenge, or administered commercial vaccines, or the FMDV peptide emulsified in Freund's incomplete adjuvant. The immune traits also had to be measurable in blood samples, and be amenable to high-throughput assays. Although it might be argued that the use of the Holstein/Charolais cross will not be informative for immune traits (because these breeds are extremes for performance rather than immune indicators), in fact we have found considerable variability in all of the immune parameters measured to date, and have found significant sire and cross effects for all traits assessed so far (O'Neill *et al*., 2003; Young *et al*., 2000;Young, 2002) highly suggestive of genetic regulation, making the possibility of detecting related QTLs likely. We are also *BoLA* typing the F2 animals by the SBT method developed by Miltiadou *et al*. (2003), as although one of the microsatellite markers is within the MHC region, it is unlikely to be sufficiently informative to assess MHC effects on the immune traits. So far 143 of a total of 500 animals have been typed and 23 different *DRB3* alleles distinguished.

The first cohort of females were immunized with the FMDV peptide in Freund's incomplete adjuvant on two separate occasions, and both cellular and humoral responses were determined. The analysis consisted of T-cell proliferation, T-cell-generated IFN-γ, and peptide-specific antibody isotypes (IgM, IgG_1, IgG_2 and IgA). The cell-mediated responses were all measured using whole-blood assays, thus minimizing *in vitro* manipulations. All animals were naive to the peptide prior to immunization. For all parameters measured, wide variation was seen in response, from complete non-responders in terms of all parameters to very high responders (Figs 2 and 3). In the first cohort (56 animals) analysed for DRB3, effects were observed only for antibody response to peptide (IgG_1, $P = 0.05$).

Of all the parameters measured in the first cohort, significant sire effects were seen for the IFN-γ response ($P = 0.05$), IgG_2 ($P = 0.02$) and IgG_1:IgG_2 ratio ($P = 0.002$), suggesting that genetic factors other than MHC may be regulating the responses to peptide. These studies give confidence that QTL controlling these responses will be identified following analysis of the remaining cohorts.

Concluding remarks

In conclusion, it is clear that both *MHC* and non-*MHC* genes play a role in determining the responses of cattle to vaccination. Cattle have a complex set of *BoLA* class I and class II genes, with *DQ*-duplicated haplotypes giving rise to more expressed restriction elements than might be predicted from the number of genes present. In the future, both QTL studies and microarray experiments will be used to identify non-*MHC* genes, as well as to quantify the relative contributions of different genes that play a role in vaccine responsiveness. Ultimately, these studies should provide valuable information that will be essential for the development of more effective and safe vaccines for livestock world-wide.

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