THE ROLE OF MHC POLYMORPHISM IN DISEASE/PARASITE RESISTANCE

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SUMMARY

Major histocompatibility complex (MHC) class I and class II loci are highly polymorphic in a majority of species including all major farm animals. There is clear evidence in farm animals as well as in other species that this polymorphism is maintained by some form of balancing selection. Although not yet formally proven, the cause of this selection is most likely pathogen infections. The mechanism promoting MHC diversity is overdominant selection and/or frequency dependent selection. The occurrence of balancing selection at MHC loci implies that it is important to maintain MHC diversity in farm animal populations. It is unlikely that it will be possible to improve general disease resistance by selective breeding for certain MHC haplotypes. It is suggested that a major challenge for future MHC research in farm animals will be to provide a better understanding of the cause for selection at MHC loci.

INTRODUCTION

The scope of this paper is to review the characteristic features of genetic diversity at major histocompatibility complex (MHC) loci, the mechanisms promoting MHC diversity and the significance of the MHC system in relation to livestock production. The paper will complement the comprehensive review on the status of current research as regards associations between MHC polymorphism and disease resistance/immunocompetence presented at this congress by Lunney and Grimm (1994).

MHC molecules have a pivotal role in the vertebrate immune system as regards the recognition of self and non-self (Klein, 1986). MHC class I and class II molecules are found on the cell surface and their only well documented function are to present intracellularly processed peptides to T cells of the immune system. They differ by the fact that class I molecules have a more broad tissue distribution and present peptides which have been synthesized within the antigen presenting cell. Class II molecules are expressed mainly by antigen presenting cells of the immune system (e.g. macrophages and B cells) and bind peptides derived from proteins synthesized outside the antigen presenting cell. Thus, presentation of foreign peptides together with class I reveals that something is wrong within the cell and this activates T killer cells which can eliminate the cell. In contrast, presentation of foreign peptides by class II molecules stimulates T helper cells to mount an immune response which is not directed towards the antigen presenting cell itself.

The MHC was discovered in the mouse as a major locus controlling the rejection of tissues grafts. Since then the MHC system has been described in different orders of vertebrates including mammals, birds, reptiles, amphibians and fishes (see Klein et al., 1993). It appears that the evolution of MHC molecules has accompanied the evolution of a memory based immune system with B and T cells. It has been speculated that the MHC molecules may have other function besides its role in the immune system e.g. during differentiation and embryo development. However, recent transgenic studies clearly show that MHC molecules do not have any crucial house keeping function outside the immune system as transgenic mice in which the expression of MHC class I or class II genes has been eliminated develop normally in a pathogen-free environment (Koller et al., 1990; Grusby et al., 1991).

MHC GENES ARE OFTEN HIGHLY POLYMORPHIC

Extensive MHC diversity has been documented in a number of mammals, birds and lower vertebrates. It appears that MHC polymorphism has been a common phenomenon throughout the evolution

of vertebrate species. There are several characteristic features of the allelic polymorphism observed at MHC loci:

(1.) The number of alleles at each locus is often very high (>50).

(2.) Alleles often differ by a large number of amino acid substitutions (>20 for class II loci and >30 for class I loci). This is in contrast to most other genetic systems where alleles differ by one or a few amino acid substitutions.

(3.) The polymorphism occurs predominantly at functionally important sites, that is at the residues which are involved in peptide binding (Bjorkman et al. 1987; Brown et al., 1988). This is also different from most other systems where genetic polymorphism is more frequent in regions which is less functionally important.

(4.) The MHC polymorphism is functionally relevant. It is very well documented that MHC polymorphism affects the immunological recognition of foreign proteins (see Klein, 1986).

Although extensive MHC diversity has been found in most species studied so far there are populations or species which are more or less monomorphic at MHC loci. Low levels of MHC polymorphism has been documented in the cheetah (O'Brien et al., 1985), Syrian hamster (Streilein, 1987) and some marine mammals (Slade, 1992). We have recently reported on MHC monomorphism at class I and class II loci in a viable and rapidly expanding population of Scandinavian beavers (Ellegren et al., 1993). The moose is an additional example of an apparently highly viable population with restricted MHC diversity (Mikko and Andersson, submitted).

MHC ALLELES HAVE ANCIENT PHYLOGENETIC ROOTS

Klein (1980) was the first to propose that the observed large genetic distances between MHC alleles (i.e. the large number of substitutions between alleles) may be explained by a trans-species mode of evolution. This model implies that MHC alleles are maintained over long evolutionary periods (tens of millions of year). The hypothesis has been corroborated by studies on primates and rodents which has revealed the sharing of MHC alleles between species (Klein et al., 1993). A comparison of DRB alleles in cattle and humans revealed striking sequence similarities also between distantly related mammals (Andersson et al., 1991). However, in this case it was clear that the similarities could not be explained by the existence of shared ancestral alleles as the frequency of synonymous substitutions between species was much higher than the corresponding frequency between alleles within species. Two possible explanations for the observed similarities of MHC allelic sequences between distantly related species are (i.) convergent evolution and (ii.) the sharing of short ancestral sequence motifs (fractions of an exon), but it is difficult to asses the relative importance of these two phenomenon (see Andersson et al., 1991; Gustafsson and Andersson, 1994).

An important conclusion from these studies is that the presence of extensive MHC polymorphism reflects a long evolutionary process and is not due to an exceptionally high mutation rate at these loci (Klein et al. 1993). The basis for the generation of MHC diversity is of course the occurrence of favourable point mutations which apparently is a rare event. There is evidence that intragenic recombination contributes to the generation of MHC diversity by shuffling sequence motifs into a variety allelic forms (Parham et al., 1989; Wakeland et al., 1990). This could be a more rapid process as suggested by the occurrence of novel MHC class I alleles in South American indian tribes (Belich et al., 1992; Watkins et al., 1992). However, this mechanism can only utilize existing sequence polymorphism generated by point mutations. Thus, if a species has lost considerable amount of its MHC diversity it will take a very long time (millions of years) to restore it. This point is well illustrated by our recent analyses of MHC class II polymorphism in the moose. We have observed that both Scandinavian and Canadian populations of moose exhibit very restricted MHC class II polymorphism (Mikko and Andersson, submitted). We have found only seven alleles at a DRB locus among moose from the two continents in contrast to more than 30 alleles at the corresponding locus in cattle. Moreover, the alleles involve only a small number of nucleotide substitutions and the same sequence variants occur in both Europe and North America. Our conclusion is that the moose may have lost much of

its MHC diversity in a population bottleneck occurring before the split between the European and North American subspecies. This event can be dated to at least 10,000 years b.p. when the Bering land bridge disappeared. Our results show that very few, if any, new point mutations have been established in the moose DRB allele pool during the last 10,000 years.

EVIDENCE FOR BALANCING SELECTION AT MHC LOCI

There is now several lines of evidence showing that MHC polymorphism is maintained by some form of balancing selection. Firstly, MHC allele frequencies are distributed more evenly than expected for selectively neutral alleles (Hedrick and Thomson, 1983). Secondly, the relative frequency of non-synonymous substitutions (substitutions changing the amino acid sequence) is significantly higher than the the frequency of synonymous substitutions (substitutions not changing the protein sequence) at the peptide binding site of both MHC class I and class II loci (Hughes and Nei, 1988, 1989). Thirdly, Takahata and Nei (1990) have shown that the long-term persistence time of MHC alleles (millions of years) by far exceeds the one expected for selectively neutral alleles.

What is the cause of the balancing selection at MHC loci? Due to the crucial role of MHC molecules in the immune system the most favoured explanation is that selection at MHC loci is driven by the interaction with pathogens. The basic assumption in this model is that the MHC polymorphism influence the immunological recognition of pathogens and that the MHC type influences disease resistance. Two major selection mechanisms have been invoked for MHC selection. One is overdominant selection implying that heterozygotes have a higher fitness than homozygotes. An immunological argument for this mechanism was put forward by Doherty and Zinkernagel (1975) namely that heterozygotes have a broader immune response due to the presence of two rather than one allelic form of antigen presenting molecules. An alternative mechanism is frequency-dependent selection which implies that the fitness value of an allele is inversely related to its frequency. Bodmer (1972) has proposed that pathogens may adopt to the most common MHC alleles in the population causing a selective advantage for individuals carrying rare MHC alleles. There is not yet any compelling evidence favouring one of these selection mechanism and it is possible that both contribute.

An alternative hypothesis is that MHC mating preference is a major cause for maintaining extensive polymorphism at MHC loci (see Potts and Wakeland, 1993). The argument is that a behaviour has evolved which utilizes the MHC polymorphism as a genetic marker to avoid matings between close relatives.

BALANCING SELECTION AT MHC LOCI IN FARM ANIMALS

There is clear evidence that balancing selection is operating at MHC loci in our farm animal species. Extensive MHC polymorphism at both class I and class II loci has been documented in all major farm animals and the polymorphism observed shows all the signs of balancing selection. I will illustrate this by data on cattle in which both class I and class II loci possess large number of alleles (Andersson and Davies, 1994). Bovine class II DQB and DRB3 alleles have been characterized by DNA sequencing of the highly polymorphic exon 2 (Sigurdardottir et al., 1991, 1992). This analysis showed that the alleles differed by a large number of nucleotide substitutions and that the frequency of non synonymous substitutions were significantly higher than the frequency of synonymous substitutions. The relative excess of non-synonymous substitutions, at both DQB and DRB loci, was in fact more prominent in cattle than that observed at the corresponding loci in humans. An even allele frequency distribution at class II loci deviating significantly from the one expected for selectively neutral alleles was found (Sigurdardottir et al., 1988). Finally, we have observed a significant deficit of MHC class II homozygotes in a sample of young breeding bulls of the Swedish Red and White breed of cattle (Sigurdardottir et al., 1988).

The fact that high MHC diversity has been maintained in farm animals through the domestication process and despite artificial selection for improving production characters has important implications. It is clear that the breeding practices we are using (restricted number of breeding animals and often very biased

sex ratios) cause a reduced effective population size. For instance, the effective population size in American Holstein cattle has been estimated to be at least one magnitude lower than the one in humans (Steele and Georges, 1991). Thus, if balancing selection was not operating at MHC loci in farm animals we would expect reduced MHC diversity due to genetic drift. Moreover, if certain MHC alleles or haplotypes were associated with superior effects on major production traits we would expect to find alleles occurring at a high frequency in selected populations. However, as stated above, we generally find large number of alleles occurring at even allele frequencies. This suggests that MHC alleles are not associated with any large effects on major production traits.

The MHC data on farm animals contribute to the issue whether pathogen-driven selection or mating-type preferences (see above) is the major cause for promoting MHC diversity. Farm animals often experience a high exposure to pathogens whereas mating-type preferences have not played any role since man started to control the breeding of domestic animals. The fact that farm animals show extensive MHC diversity provides a very strong argument against mating-type preferences as the primary cause of selection. (However, the mechanism may well have a secondary role by increasing the proportion of MHC heterozygous offspring which in turn may be more resistant to pathogens.)

MHC DISEASE ASSOCIATIONS

There is a general belief that the selection for MHC polymorphism is related to the resistance to pathogen infections. However, despite the well-documented associations between MHC polymorphism and immune response traits as well as the many convincing MHC disease associations we still lack experimental or empirical data showing the cause of balancing selection. Many MHC disease associations concern non-infectious diseases often with an autoimmune etiology (Tiwari and Terasaki, 1985). Autoimmune diseases often have a late onset and do rarely have significant effects on reproductive success in natural or domesticated populations. There are two prominent examples of associations between MHC polymorphism and infectious disease, that is Marek's disease in chicken (Pazderka et al., 1975; Briles et al., 1977) and malaria in man (Hill et al., 1991). However, none of these examples reveals balancing selection as both involve dominant alleles with increased disease resistance.

We need to ask the question why it has been so difficult to reveal balancing selection at MHC loci through disease association studies. One possible reason is that the selection coefficients may be so low that they can only be revealed in very large studies (Klein et al., 1993). Another possible explanation is that genetic heterogeneity in outbred hosts as well as in the infecting pathogen may obscure significant associations. This means that the relative resistance of an MHC genotype varies between families as well as between strains of the pathogen. Perhaps the most important reason is the lack of appropriate studies which have tried to reveal balancing selection. It is obvious that the fitness of an MHC genotype is not determined by its effect on one specific diseases but on the general resistance against all type of pathogens the species is exposed to. This means that we rather should study the relationship between MHC genotypes and fitness traits like general disease resistance, survival rate and reproductive success in order to have a chance to reveal the cause of selection acting at MHC loci. Such studies, which should involve appropriate sample sizes to have a chance to detect selection, may reveal overdominant selection by comparing the fitness of homozygotes versus heterozygotes and frequency-dependent selection by comparing the fitness of rare versus common haplotypes.

PROSPECTS FOR THE UTILIZATION OF THE MHC SYSTEM IN LIVESTOCK PRODUCTION

As previously discussed (Outteridge, 1993) knowledge of MHC diversity in farm animals will be important for the future development of peptide based vaccines as the immune response to single peptides often show MHC associations. However, it is unlikely that we will be able to improve disease resistance by selective breeding of animals with certain favourable MHC haplotypes. The available evidence strongly suggests that balancing selection is acting on MHC loci in farm animals. This provides an argument for

maintaining MHC diversity in populations of farm animals. Any specific action to maintain MHC diversity will hardly be needed in populations of reasonable size but this may be an option to take into considerations for small populations of farm animals.

Attempts to improve disease resistance in farm animals by increasing the frequency of certain MHC alleles appear doubtful before we have a better understanding of the selection mechanisms affecting the MHC system. The overall consequences on animal health of such applications are impossible to predict. For instance, Xu et al. (1993) have suggested that the dairy industry in the US may save more than $42 million per year by selective breeding bulls carrying MHC alleles associated with resistance to persistent lymphocytosis caused by infection with bovine leukemia virus (BLV). The statement is questionable as the effect on the resistance to other types of infectious diseases is unknown and the selective breeding may have negative effects on the population by reducing the level of MHC diversity.

A major future challenge for MHC research in farm animals will be to design and perform studies with the aim to unravel the selection mechanisms maintaining MHC diversity. This is an important scientific question to solve. Farm animals may be highly suitable for such studies due to the presence of excellent pedigree material, a variety of breeding populations and a strong research interest to study the genetic basis of disease resistance. The development of automated PCR-based typing methods will facilitate large scale screenings. It may in fact be possible to monitor the MHC geneotype of all breeding animals in populations of farm animals. An improved knowledge of the mechanisms promoting MHC polymorphism may open new possibilities and strategies for the utilization of this important system in livestock production.

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