THE USE OF THE MHC IN SELECTING FOR DISEASE RESISTANCE

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SUMMARY

Largely as a result of research funded from non-agricultural sources, we now have a remarkably detailed understanding of the vital and fundamental role of the major histocompatibility complex (MHC) in immunity. Unfortunately, however, we do not yet have any MHC knowledge that can be exploited in practical breeding programs. On the positive side, evidence gleaned from much of the non-agricultural MHC research indicates certain changes of emphasis that may bear dividends in domestic animals. In view of the fundamental biological importance of the MHC, it is essential that it continue to be the subject of research in domestic animals. Given the vast resources committed to MHC research in mice and humans, and the case with which discoveries in these species can be translated to domestic species, a relatively small investment by agricultural research funds is likely to pay handsome dividends in the future.

INTRODUCTION

The major histocompatibility complex (MHC) is a cluster of genes which could well be described as the engine room of the immune response; its genes play a number of major roles in enabling mammals and birds to distinguish between "self" and "non-self", and hence to mount an immune response against all things foreign. As such, MHC genes are on the front-line of the body's defences against pathogens and parasites. Because of the similarities of the MHC across species, the first part of this paper will review aspects of the MHC that are common to all species. We will then move on to consider the MHC as a source of markers for disease resistance in animals.

GENERAL FEATURES OF THE MHC

Space does not permit a detailed account of the vital role of MHC gene products in immunity. Readers wanting to sample the excitement of recent discoveries are referred to reviews by Grey et al. (1989), Rothbard (1989), Bjorkman and Parham (1990) and Lawlor et al. (1990).

Briefly, there are two main regions of the MHC, both of which contain genes which encode protein molecules called histoglobulins (Hg for short; Bodmer 1989). The role of Hg molecules is very specific; they pick up fragments of protein from within cells, and "present" these fragments on the surface of the cell, thereby enabling T cells to check whether each fragment is self or non-self. If the latter, an immune response results. Genes in the class I region encode Hgl molecules that present endogenous peptide fragments to cytotoxic T cells.
Without this presentation, a cellular immune response (one of the two arms of the immune response) cannot take place. In contrast, genes within the class II region encode HgII molecules which present exogenous peptide fragments to helper T cells which directly trigger an antibody-mediated (humoral) immune response, but which also indirectly stimulate or suppress cellular immunity.

In most species studied to date, there are several class I loci and several class II loci, with the actual numbers varying considerably between species. In humans, for example, there are three well-documented class I loci (A, B, and C) plus several newly-discovered ones (E, F, and G). At last count, the class II region (called the D region) contained 16 class II loci, but not all of these are functional. A standard nomenclature has now been agreed internationally, with each locus in the D region having a second letter to signify its sub-class (N, O, P, Q, or R), followed by A or B, depending on whether the gene encodes an alpha or a beta protein chain. Thus, along with genes such as DPB and DRB, we now have what surely must be the ultimate: the DNA gene!

One of the most outstanding features of the MHC is the large number of different alleles that have been identified at many of the loci. In humans, for example, a recent count listed 25 alleles at the A locus, 32 at B, 11 at C, and 87 spread across 5 of the class II loci (Anon 1990). Not all of these alleles are present in all populations, but each population has a large proportion of all alleles. This means that heterozygosity is the rule rather than the exception at most MHC loci.

Many MHC alleles have been sequenced in the last few years, giving rise to a fascinating evolutionary picture. Even more fascinating is the observation that the differences between alleles do not occur at random within a gene; in fact, there is a remarkable relationship between sequence diversity and function. Those sections of the gene that encode the portions of the Hg molecule which have the same function in all alleles, e.g. the section that passes through the cell membrane, are extremely similar across all alleles. In contrast, the section of the gene that encodes the groove which actually presents the peptide fragment to T cells is extremely variable between alleles, almost all of the differences between alleles are confined to this one region.

In two landmark studies, Hughes and Nei (1988, 1989) compared the frequency of two different types of nucleotide substitution (synonymous and non-synonymous) in the groove region and in other regions of MHC genes. Synonymous substitutions are those that do not cause a change in the amino acid sequence of the molecule. Conversely, non-synonymous substitutions cause the substitution of one amino acid for another at that site in the Hg molecule, resulting in a different version of the Hg molecule. For both class I and class II genes in humans and mice, Hughes and Nei showed that in the region of the gene corresponding to the groove, non-synonymous substitutions are far more frequent than synonymous substitutions, whereas in other regions of the gene, the exact opposite was true; synonymous substitutions are more common. Yuhki and O'Brien (1990) recently reached similar conclusions from a study of Hg variation in the domestic cat.
The most feasible explanation for these observations is that there is positive selection for diversity in the groove region of Hg molecules, whereas there is selection against diversity in the other regions of the molecule. In other words, there is a selective advantage in having many different grooves, but changes in other parts of Hg are usually disadvantagous, in the sense that it cannot function as well. All this makes sense when we recall that the purpose of the groove is to present peptide fragments to T cells; the greater the variety of grooves, the greater the variety of peptide fragments that can be presented, and hence the greater the chance that a foreign fragment will be detected, and an immune response mounted.

Further evidence for selection comes from various population-genetic studies of class I and class II alleles. The latest of these studies, by Potts and Wakeland (1990), confirms the results of several earlier studies, namely that some form of balancing selection is definitely occurring within the MHC; the polymorphism within the MHC is far greater than would be expected if the alleles were selectively neutral.

What can be the nature of the selection that gives rise to the above picture? Many people look to the following evidence for an explanation.

Disease associations

The MHC was first discovered as the major factor determining graft rejection, and this immediately pointed to an important role in the immune response. Early studies in laboratory animals soon suggested that different MHC genes may be associated with resistance to certain carcinogenic viruses, and this quickly led to an extensive search for associations between MHC molecules and diseases in humans. The result is a long list of diseases (mostly of an auto-immune nature) for which certain MHC molecules confer increased susceptibility (Tiwari and Terasaki 1985). These results have confirmed the vital role of the MHC in immunity. Indeed, recent studies have indicated just how specific this role is. In the case of insulin-dependent diabetes mellitus in humans, for example, Dorman et al (1990) recently showed that the presence or absence of a certain amino acid (aspartic acid) at position 57 in the beta chain of a class II DQ molecule can explain almost all of the worldwide variation in incidence of this type of diabetes, with the presence of aspartic acid conferring strong resistance to the disease. This result shows the remarkably precise role of the MHC in determining disease resistance and susceptibility.

However, most of the well-established MHC/disease associations in humans involve diseases that do not dramatically decrease reproductive ability, and which cannot therefore be responsible for the selection that is acting within the MHC.

In the early days of MHC research, it was suggested that the polymorphism seen today is really the result of MHC associations with infectious diseases that were widespread just a few human generations ago. With many parts of the world now free of such diseases, it has proved difficult for researchers to conduct large-scale studies of associations between MHC molecules and infectious disease in humans. However, a number of studies have now been
completed, and the results are not particularly encouraging; the predicted strong associations have not emerged (Potts and Wakeland 1990).

Domestic animals, of course, are still subjected to many infectious diseases, which means that searches for MHC/disease associations in animals have a major role to play in providing evidence of direct relevance to this question. As we shall see below, the results from domestic animals are similar to those from humans; with one or two notable exceptions, MHC molecules do not appear to be strongly associated with infectious diseases.

What, then, can be the explanation for the selection that occurs at MHC loci? This remains one of the most important unanswered questions facing today's MHC researchers.

**MHC and reproduction**

One possible explanation that has attracted much attention but which is supported by little hard data, is that MHC molecules play an important role in enabling successful implantation of the foetus into the lining of the uterus. The suggestion is that successful implantation involves a controlled immune response, and that the greater the difference in Hg molecules between mother and foetus, the greater the chance of an effective immune response, and hence the greater the chance of a successful implantation. Intriguing indirect evidence for this somewhat unusual suggestion comes from several studies which show that the frequency of natural abortion is greater amongst human couples which share the same Hg molecules (Thomas et al. 1985). Furthermore, several studies in mice have suggested that females prefer the odour of histoincompatible males, and also prefer to mate with such males (Egid and Brown 1989). From a population genetics point of view, the idea of histoincompatibility being favoured is very appealing, since it immediately provides an explanation for the balancing selection that undoubtedly acts on the MHC. However, there is still much debate about the evidence.

**THE MHC OF DOMESTIC ANIMALS**

In the last ten years, there has been an explosion of research into the MHC of domestic animals. From the point of view of funding agencies, this research has been extremely cost-effective, since it has been able to make rapid progress by drawing directly on the results of much more extensively funded research in humans and mice. Indeed, most of the molecular work reported to date in the MHC of domestic animals is the result of using clones from the human MHC directly on DNA extracted from domestic animals. While the details differ from species to species, most of the results are consistent with the general account of the MHC presented above. For detailed reviews of the MHC of domestic animals, readers are referred to Vaiman et al. (1986), Warner (1986), Warner et al. (1987), Spooner et al. (1988), Levy and Charron (1989), Ostergard et al. (1989), Rothschild (1989), van der Zijpp and Egberts (1989), and Warner et al. (1989).

In the following section, the results of MHC/disease association studies in each of the main
Disease associations

MHC/disease association studies in horses were recently summarised by Ostergard et al. (1989). Several associations with sarcoïds (a skin tumour) and one with segregation distortion have been reported. In contrast, no association has been found with laminitis. In a study not included in the above review, McClure et al. (1988) reported an MHC association with arytenoid chondritis but not with laryngeal hemiplegia, umbilical hernia or cryptorchidism. In addition, even with the positive reports, close examination reveals that the picture is not particularly clear cut. For example, the sarcoïd association was with different MHC molecules in different families.

In sheep, disease association studies have concentrated mainly on scrapie in Europe and internal parasites in Australia and New Zealand. The scrapie studies have produced evidence of associations (Millot et al. 1988), but this evidence is hotly disputed (Cullen 1989; Millot 1989a). There have been a number of studies investigating internal parasites, with some claiming associations and others not. In at least some of the former, the apparent association is more a reflection of failure to take account of variation between sires, than of a true MHC association. A similar problem appears to exist in relation to a recent claim of association with *Corynebacterium pseudotuberculosis* infection in French sheep (Millot 1989b); when offspring were pooled across sire families, there appeared to be an association, but when the analysis was conducted within sire families, there was no MHC association. Recent studies by Hulme et al. (1991; internal parasites) and by Litchfield (Raadsma et al. 1991; footrot and flystrike) have made a specific point of allowing for sire effects. The results suggest that the MHC does have an influence on the disease traits studied, but that the effect is not particularly strong.

There have been many association studies in pigs, as summarised by Ostergard et al. (1989), with apparent positive results for piglet mortality, immune response, litter size, ovulation rate and various production traits. Like many studies before them, the recent studies by Mallard...
et al. (1989; immune response) and Gautschi and Gaillard (1990; reproduction and production) will probably be interpreted by some readers as providing further evidence of associations, but in fact provide good illustrations of the limited effect of the MHC on these traits. For example, including MHC genotype in Gautschi and Gaillard's (1990) model for analysis of growth and carcass traits decreased the coefficient of determination ($R^2$) by less than 1%. And in 8 separate comparisons concerning reproductive traits, there was one significant effect with a $P$ value of 0.047. In the immune response study of Mallard et al. (1989), none of the MHC effects appears to be significant after due allowance is made for the number of comparisons conducted.

As detailed by Bacon (1987), the chicken MHC has been the subject of intensive study in relation to disease resistance, and is the species with the strongest and best confirmed MHC associations, most notably with Marek's disease, lymphoid leukosis, cecal coccidiosis, fowl cholera, sarcoma induction by Roux sarcoma virus, and spontaneous autoimmune thyroiditis; and with numerous production traits including hatchability, adult mortality and egg production. However, it is important to note that even with chickens, not all diseases show MHC associations. For example, immunity to *Eimeria tenella* shows no association (Lillehoj et al. 1989).

**DISCUSSION**

As outlined above, there is a long list of diseases with which the MHC appears to be associated. Some of these associations are undoubtedly real, and reflect a very real role for the MHC in contributing to resistance or susceptibility. This is particularly so in chickens. On the other hand, many of the associations are more a reflection of the effects of chance than indicative of a real role for the MHC. This problem is not due solely to researchers failing to take account of statistical problems such as accounting for variation between sire families, and for the number of tests conducted; it is as much due to the fact that even if the authors of a paper choose their words carefully, and make all of the appropriate qualifications, the mere fact that yet another paper has been published on the subject of associations between the MHC and disease, is often interpreted as yet further evidence supporting the existence of real associations. And this problem still tends to exist even if the conclusions of the paper are totally negative, i.e. that there is no association.

It is difficult to summarise all of the published studies briefly, but it seems that even in those cases where there is real statistical significance, the role of the MHC is often insignificantly small. For example, in a study of MHC association with mastitis in cattle by Lunden et al. (1990), there was one set of MHC genes (haplotype) that had a consistent and statistically significant effect on mastitis resistance. As the authors point out, however, eliminating this haplotype from a herd in which it had previously been present in every cow, would decrease the incidence of mastitis by less than one percentage point (from 8.6% to 8.0%).

Even for those associations that have a substantial effect, such as the association with Marek's disease in chickens, there are still many unanswered questions about the exact mechanisms
behind the associations. Furthermore, since many different MHC alleles appear to be favourable in different circumstances and for different diseases, it is often not at all clear as to which of the many MHC alleles is the best one. For these reasons, MHC researchers such as Bacon (1987) are understandably cautious in recommending that breeders should go out and simply create populations homozygous for what might appear to be a favourable MHC allele.

This caution is particularly well founded when we recall the general conclusions about the apparently substantial balancing selection that occurs within the MHC. If natural selection favours heterozygosity at the MHC, then it might pay to think twice before making populations homozygous for particular alleles.

There is, however, a possible solution to this apparent impasse. If heterozygosity is really favourable, then it should be possible for animal breeders to exploit this by designing crossing programs so as to create populations that are heterozygous for the most favourable set of MHC alleles. In order to determine whether this is a sensible approach, it will first be necessary to move away from simply searching for associations with particular MHC molecules, and to start asking whether heterozygosity per se confers advantages in relation to disease resistance. At the same time, it would seem to be a good idea to devote more resources to a thorough investigation of the effects of the MHC on reproduction, particularly in relation to the effect of histoincompatibility between mother and foetus. These two avenues of MHC research might prove to be the most fruitful of all, and may enable informed exploitation of the MHC in breeding for disease resistance and increased productivity.

Finally, it must be clearly stated that the MHC is not the only set of genes concerned with immunity. Others with equally vital roles include genes that encode, for example, antibodies and the T-cell receptor. It seems quite likely that variation at these loci also contributes to variation in resistance to disease. Thus a complete search for potential sources of genetic variation in disease resistance must involve a much wider net than simply MHC genes.

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