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Human leukocyte antigens and natural selection by malaria

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SUMMARY

The extraordinary polymorphism of human leukocyte antigens (HLA) poses a question as to how this remarkable diversity arose and is maintained. The explanation that infectious pathogens are largely responsible is theoretically attractive but clear and consistent associations between HLA alleles and major infectious diseases have rarely been identified. Large case-control studies of HLA types in African children with severe malaria indicate that HLA associations with this parasitic infection do exist and it is becoming possible to investigate the underlying mechanisms by identification of peptide epitopes in parasite antigens. Such analysis reveals how the magnitude and detectability of HLA associations may be influenced by numerous genetic and environmental factors. These complex interactions will give rise to variation over time and space in the selective pressures exerted by infectious diseases and this fluctuation may, in itself, contribute to the maintenance of HLA polymorphism.

1. INTRODUCTION

The major histocompatibility complex (MHC) encodes many of the most polymorphic genes in humans. Those in the class I region encode the highly variable HLA-A, -B and -C proteins which play a central role in the activation of cytotoxic T lymphocytes by presenting short peptides of cytoplasmic origin to these cells. In turn, once activated, these CTL are capable of lysing and killing cells presenting the same antigenic peptide. Hence, this system appears particularly well adapted to defence against intracellular pathogens.

In contrast, the class II region of the MHC encodes HLA-DR, -DQ and -DP gene products which assemble to produce HLA class II molecules that present somewhat longer peptides (10–25 amino acids) to helper T lymphocytes bearing the surface molecule CD4. These helper T cells assist B cells in antibody production and can release a range of cytokines which activate macrophages and other cells. HLA class II molecules are therefore of central importance in regulating immune defence against extracellular as well as some intracellular pathogens.

The pivotal role that MHC molecules play in immune responses against infectious pathogens led to the proposal that the remarkable diversity of MHC molecules had evolved as a result of such host–parasite interactions (Snell 1968; Doherty & Zinkernagel 1975). However, for many years evidence on this point has been controversial. In particular, studies of HLA–disease associations in humans revealed more convincing associations with autoimmune disease than with any infection. In this short review we describe our studies of HLA and malaria in African populations and set these in the context of new molecular insights into the mechanisms of HLA associations with disease in humans. These recent studies help to clarify some of the uncertainties in the older literature and support a major role for pathogen-driven selection in maintaining MHC diversity in human populations.

2. HLA ASSOCIATIONS WITH INFECTIOUS DISEASES

Many studies of HLA associations with infectious diseases have been reported over the last twenty years. The early studies were often very small and could only consider the limited number of HLA alleles known at the time. Clear associations were seldom seen and studies of the same infectious disease in other populations often produced different and apparently conflicting results. Initially, it was believed that diverse HLA associations in different geographical areas might reflect linkage of a common susceptibility gene elsewhere in the MHC to different HLA marker alleles. However, now that it is clear that MHC class I and II proteins function as antigen-presenting molecules and that the HLA loci are the actual immune-response genes, this explanation appears inadequate. Quite reasonably, the claimed

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associations were treated with some scepticism and in 1987 Klein opined that there was no convincing evidence of MHC associations with infectious diseases of humans.

However, with the introduction of more precise molecular typing and the recognition that large studies were required to identify convincing associations, a clearer picture is beginning to emerge. The infectious disease that has been studied most frequently is leprosy. This reflects in part the intriguing variation in the human immune response to the bacillus, leading to polar clinical types, but also the ease of studying a chronic rather than an acute condition. Todd and colleagues (1990) reviewed 24 studies and performed a meta-analysis of the data sets. Although the validity of such pooling might be questioned, the overall conclusion was clear cut. In numerous studies there is a strong association between the HLA class II allele HLA-DR2 and leprosy (of any type). However, in many studies no such association was detected. This heterogeneity was statistically significant. Intriguingly, for another mycobacterial disease, tuberculosis, there are three recent studies identifying an association with the same allele, HLA-DR2 (Bothamley *et al.* 1989; Khomenko *et al.* 1990; Brahmajothi *et al.* 1991). Yet again, a larger number of earlier studies failed to identify this association.

In the last ten years there have also been numerous studies of the influence HLA may have on the risk of HIV infection and on the rate of progression of infected individuals to AIDS. Although these studies have included disappointingly few subjects, a picture of probable heterogeneity in HLA associations is again emerging. An association of the common caucasian haplotype HLA-A1-B8-DR3 with rapid disease progression has been seen in some studies, but in several others HLA-B35 appeared to be a significant risk factor (Scorza Smerali *et al.* 1986; Steel *et al.* 1988; Kaslow *et al.* 1990).

Studies of MHC associations with infectious disease in other species have been limited by a far less detailed characterization of the various alleles and a lack of reagents for their definition. However, in chickens, Briles *et al.*(1977) identified an association between an MHC haplotype and resistance to the herpes virus-induced atypical lymphoma, Marek's disease.

3. HLA STUDIES OF MALARIA IN WEST AFRICA

Our first study of HLA and malaria was of West African children in collaboration with the U.K. Medical Research Council Laboratories in The Gambia. During 1988–1990, over 600 children with severe malaria from the peri-urban Banjul population were studied and compared with 1400 other Gambians (Hill *et al.* 1991). Typing of HLA class I antigens showed that the allele HLA-B53 was under-represented in the children with severe disease (16% of cases) compared to the non-malaria control groups (25% antigen frequency). Although this antigen was also less frequent amongst cases of mild or uncomplicated malaria, this difference was not statistically significant. Analysis of HLA class II region genes identified an association between a haplotype bearing the HLA-DR allele, HLA-DRB1*1302, and resistance to severe malarial anaemia. Cerebral malaria and severe malarial anaemia are the two major clinical manifestations of severe malaria in African children.

Further analysis of HLA class II region genes suggested that the HLA class II association is primarily due to the HLA-DRB1*1302 gene and not to allelic variation in neighbouring loci. Recently, Kwiatkowski and colleagues have analysed one of the HLA class III region genes, TNF, in these samples. It had been speculated that the HLA associations with malaria might relate to variation in this closely linked TNF gene because TNF itself has been implicated in the pathogenesis of severe malaria. However, polymorphisms identified in the TNF gene do not account for the HLA associations with malaria (McGuire *et al.* 1994).

From the odds ratios measured in this case-control study, it is possible to estimate the magnitude of protection associated with the alleles HLA-B53 and HLA-DRB1*1302 (Hill *et al.* 1991). These are in the region of a 40–50% reduction in risk of developing severe malaria. This is clearly smaller than the 90% protection associated with the carrier genotype for sickle haemoglobin. However, because these HLA alleles are much more prevalent in this population than haemoglobin S, a preventive fraction calculation (Bengtsson & Thomson 1981) indicates that at least as many cases of severe malaria may be being prevented by these HLA types being in the population as are prevented by the presence of sickle haemoglobin.

As well as identifying these associations with individual HLA alleles analysis of the overall effect of HLA variation on risk of severe malaria identified a probable influence of HLA class II variation on risk of developing severe malarial anaemia. Subsequently, a further small study of HLA antigens and malaria has been undertaken in a rural area of The Gambia (Bennet *et al.* 1993). Only mild malaria cases were studied and, as in the larger study, no clear association of individual alleles with this syndrome was identified. However, analysis of the combined effects of all class II haplotypes indicated a significant effect of this variation on risk of developing clinical malaria.

4. TEMPORAL VARIATION IN SELECTION?

HLA-B53 is found at highest frequencies in sub-Saharan African countries where malaria transmission is most intense. This suggests that this high frequency might result, at least in part, from natural selection by malaria. If we assume for the moment a constant selection pressure, it is possible to estimate how long it would take for this allele to rise in frequency from the degree of protection associated with it and the population mortality from malaria. To maintain the balanced haemoglobin S polymorphism at a carrier rate of 13% in The Gambia, the fitness of carriers must be 7% greater than that of individuals with normal haemoglobin (Cavalli–Sforza & Bodmer 1971). Taking this difference in fitness as an indicator

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

of the historical death rate from malaria, an estimate compatible with contemporary surveys (Greenwood *et al.* 1987), it can be calculated that to reach the current antigen frequency of HLA-B53 (25%) from an initial allele frequency of, say, 0.001% would take about 7000 years (Nei 1987) with directional selection and a selection coefficient of 0.028 ($0.07 \times [1-$ Odds Ratio]). Because the allele is rare for most of this time, the estimates with dominance and semi-dominance are very similar. Starting at the 0.5% allele frequency which is found in parts of Europe today would take approximately 2500 years. Interestingly, these estimates are similar to the length of time that it was, until recently, believed that malaria had been a major cause of childhood mortality in Africa. It had been assumed that prior to the start of farming in Africa, about 4000–7000 years ago, the population density would have been insufficient to maintain significant malaria transmission (Bruce–Chwatt 1988).

However, this scenario now seems unlikely in the light of new estimates of the transmissibility of malaria (Gupta *et al.* 1994*b*). Because immunity to malaria is incomplete and exposure does not prevent reinfection, it is unlikely that the supply of susceptible individuals would run out. Thus, low population densities in hunter gatherer populations would probably not have ruled out a significant mortality from this pathogen. Hence the time depth of malarial selection in Africa could be much greater than suspected hitherto.

Recent data from our laboratory (Yates, Marsh, Newbold *et al.*, unpublished) on the selection of α thalassaemia by malaria in Africa are consistent with this greater time depth and suggest that malarial selection may have been important for tens of thousands of years. If this has been the case the tidy congruence of the estimate for the time depth of HLA-B53 selection and that of malarial selection is lost. The former estimate is the shorter and it would appear that the HLA-B53 frequency should now be much higher than is observed in Africa had there been a constant degree of selection.

It is worth emphasizing the limitations of these calculations. Our estimates of the original HLA-B53 frequency is very approximate and the measurement of 40% protection in the case-control study has 95% confidence intervals of 19%–57%. It may be the case that HLA-B53 frequencies are influenced by other infectious pathogens and this is ignored in the calculation. However, if malaria has indeed been a major selective force for many tens of thousands of years it appears unlikely that the protective effect of HLA-B53 that we measured in 1988–1990 can have been constant over this period.

5. GEOGRAPHICAL VARIATION IN SELECTION

The geographical distribution of HLA-B53 has been fairly well defined (summarized in Hill *et al.* 1991). It reaches its highest frequencies in west Africa with reports of antigen frequencies up to 40%. In east and central Africa it is also at high frequency of typically 10–20%. Further south in Zimbabwe and South Africa its frequency falls to less than 5%. In other regions it is generally uncommon or absent with frequencies of 1% or less. Thus, rather like the haemoglobin S allele, HLA-B53 is found at highest frequencies in the parts of Africa where malaria transmission is most intense. It is tempting to infer from this distribution that the protective effect of HLA-B53 against malaria may be fairly widespread and consistent within Africa. Following this line of argument one might propose that its rarity in south-east Asia, for instance, where malaria has selected many thalassaemia alleles, simply reflects the same stochastic processes that failed to lead to the selection of haemoglobin S in south-east Asia and Melanesia.

However, we have now had the opportunity to measure the current protective effect of HLA-B53 in another part of Africa. In collaboration with K. Marsh and R. Snow and others at the KEMRI-Wellcome Coastal Research Unit at Kilifi on the Kenyan coast we have been HLA–typing children from another large case-control study designed to measure risk factors for severe malaria in east African children. In Kilifi, HLA-B53 is found in 16% of the general population. Amongst more than 300 children with severe malaria an almost identical frequency of this allele was found (unpublished data). Hence, the protective association found in The Gambia is either absent in this coastal Kenyan region or at least appears to be considerably diminished.

Furthermore, detailed analysis of HLA-DR allelic variation in the Kenyan case-control study has provided further evidence for geographical variation in HLA associations with severe malaria. In The Gambia, HLA-DRB1*1302 was associated with about a 50% reduction in risk of severe malarial anaemia. In Kilifi, a significant protective association with a different HLA-DR type has recently been identified (unpublished data).

Hence, the results on two large African studies of severe malaria are reminiscent of the outcome of many smaller studies of leprosy, HIV infection and tuberculosis. Clear associations identified in some regions may not be found in other geographical areas.

6. MOLECULAR ANALYSIS OF HLA ASSOCIATIONS

This variation in HLA association with infection has important implications for the evolution and maintenance of HLA polymorphism in the human population. It also poses interesting questions as to the molecular basis of this variation which appears more marked than the fairly limited variation in HLA associations with autoimmune disorders.

Indeed, a molecular understanding of the nature of the interaction between parasite epitopes and HLA antigens associated with altered disease susceptibility would be valuable for addressing a further very controversial issue in evolutionary studies of MHC diversity (Hughes & Nei 1992; Hill *et al.* 1992*a*). With the increasing acceptance of the importance of parasite-driven selection in the maintenance of

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

polymorphism in the MHC attention has focused on the type of selection involved. In particular, theoretical studies have addressed the question of whether overdominant selection or frequency-dependent selection may be better at maintaining diversity over long time periods. It is clear that in theory both types of selection could provide a powerful means of preserving polymorphism (Denniston & Crow 1990; Takahata & Nei 1990), although some authors strongly favour one type of selection. Unfortunately, there is almost no evidence from field studies to indicate which is actually operating. However, identification of the molecular mechanisms underlying some MHC associations with infectious pathogens should eventually facilitate an analysis of particular cases.

We have begun to analyse the mechanisms underlying the HLA associations observed with severe malaria in Africa by adopting an approach termed reverse immunogenetics. This utilizes recent information on the specificity of interactions between particular HLA molecules and their bound peptides (Falk *et al.* 1991; Hill *et al.* 1992*b*). For example, we found that peptides bound to HLA-B53 have a strong preference for proline at position 2 of their sequence and that at the carboxy-terminus of the peptide, usually position 9, a large hydrophobic amino acid is preferred. This allowed us to synthesize peptides corresponding to this 'motif' from the sequences of *P. falciparum* antigens known to be expressed during the liver-stage of infection. This stage of infection was chosen as the likely site of interaction between class I molecules and the parasite because infected red blood cells do not express HLA class I molecules. We tested these peptides for binding to HLA-B53 and then used the peptides which bound to identify peptide-specific cytotoxic T lymphocytes (CTL) in the blood of Gambians exposed to *P. falciparum* malaria. We have found that a single malaria peptide is the target of CTL restricted by HLA-B53. This peptide, termed ls6, is a fragment of the malaria antigen LSA-1 (liver-stage antigen-1) which is highly conserved between parasite strains.

Hence, the molecular basis of the protective association between HLA-B53 and severe malaria observed in The Gambia may be as follows. Cytotoxic T lymphocytes are induced by natural infection with sporozoites. In individuals with HLA-B53 these cells target the immunodominant peptide ls6 which is expressed on the surface of hepatocytes infected with a liver-stage parasite. In individuals with other HLA types CTL of other specificities are induced but, possibly because the ls6-B53 complex is more immunogenic than these other peptide-HLA complexes, their CTL are less efficient at lysing infected liver cells. Although this mechanism has not been demonstrated directly with human hepatocytes, in mouse models of malaria CTL have been shown to be protective and to kill infected hepatocytes.

Further work in The Gambia has now led to the identification of malaria epitopes for several further HLA class I molecules (Hill *et al.* 1992*b* and unpublished data). Some of these epitopes are conserved, like ls6, but others show considerable polymorphism. In particular, a HLA-B35 epitope in the circumsporozoite protein (CSP) gene is located in a highly variable region of the molecule.

In principle, it is possible to extend this approach to the analysis of HLA class II associations with malaria. We have recently characterized the sequence features of peptides bound to the HLA-DRB1*1302 molecule associated with resistance to severe malarial anaemia in The Gambia. However, the motifs of peptides bound to HLA-DR molecules are less clear cut than those identified for HLA class I alleles. Hence, a very large number of peptides need to be synthesized to encompass the possible epitopes. This is problematic for a micro-organism as large as a malaria parasite but may be more feasible for viral infections such as hepatitis B, for which a protective HLA-DR association has recently been identified (M. Thursz, H. C. Thomas, A. V. S. Hill *et al.*, unpublished data). An alternative, more challenging option, is to sequence directly individual parasite peptides eluted from the HLA-DR molecules of infected cells. The amounts of these peptides are too small to analyse by conventional sequencers, but the use of new tandem mass spectrometers may make this feasible in the future.

7. WHY MAY HLA ASSOCIATIONS VARY?

The analysis of epitopes for HLA class I molecules in *P. falciparum* suggests one explanation for variation in HLA associations with malaria. There is polymorphism in epitopes in the circumsporozoite protein for some HLA class I molecules including HLA-B35 and HLA-B7. The HLA-B35 epitope has been analysed in some detail. There are at least four variants of this epitope found amongst *P. falciparum* parasites in The Gambia (table 1). Two of these, cp26 and cp29 have been shown to be epitopes for HLA-B35 restricted CTL (Hill *et al.* 1992*b*). Further analysis has shown that cp27 and cp28 fail to bind to HLA-B35, explaining their lack of recognition by the CTL. It is tempting to speculate that the polymorphism observed in this region of CSP has been driven by immune pressure from HLA-B35-restricted CTL with cp27 and cp28 as escape mutants. HLA-B35 is the commonest HLA class I molecule in The Gambia, so that this observation could be extended further to invoke a form of frequency-dependent selection for particular parasite alleles of CSP.

Although parasite epitopes for the HLA-DRB1*302 allele have not yet been identified, it is likely that this allele's association with resistance to severe malarial anaemia is related to an enhanced immune response to a blood-stage *P. falciparum* antigen. The major antigens of the blood stage parasite are characterized by extensive polymorphism, and much of this may relate to immune selection pressures. Furthermore, there is now geographical information on the distribution of some of these parasite variants (Creasey *et al.* 1990). A particular allele of the major merozoite surface antigen, MSA-1, is predominant in The Gambia (Conway & McBride 1991). In contrast, in Kilifi, this allele has recently been found to be

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

present at a much lower frequency (S. Kyes, C. I. N. Newbold, K. Marsh *et al.*, personal communication). Hence, geographical variation in the frequency of parasite alleles could be an important determinant of variable HLA associations.

We have recently analysed possible sequence variation in the HLA-B53 epitope, ls6, amongst parasites in The Gambia and in Kilifi, Kenya. In both areas, no sequence variation was found in the region of the LSA-1 gene that encodes this epitope. Hence, variability of this peptide does not underlie the differential association of this HLA-B53 with resistance to severe malaria in these two areas. However, polymorphism in CTL epitopes may still be relevant to this association. In case-control studies only the *relative* protective efficacies of HLA alleles can be assessed so that a less-effective series of other HLA alleles in The Gambia would give rise to greater apparent protection associated with HLA-B53 in The Gambia than in Kenya. The diminished efficacy of the other HLA types in The Gambia might relate to polymorphism in the parasite gene encoding their particular class I epitopes.

An alternative explanation for geographical variation in HLA associations with malaria relates to differences in transmission intensities in different areas. The intensity of malaria transmission in Kilifi is probably several-fold higher than in the periurban area of The Gambia. Two further features of malaria have been incorporated into a model of the effects of CTL and other interventions on the prevalence of severe and mild malaria in a population (Gupta *et al.*, unpublished). The first is the assumption that there are important differences in virulence between various strains of *P. falciparum* so that common mild strains result in episodes of mild malaria and rare severe strains lead to the rarer severe (cerebral) malaria. The second feature of this model is that blood-stage immunity to disease in malaria is strain-specific. There is evidence to support both of these assumptions.

Figure 1 shows the relationship between the cumulative probability of disease and the number of times an individual has encountered a particular strain of *P. falciparum*. The non-linearity reflects the reduced probability of disease upon encountering a strain that has been met many times before, a consequence of strain-specific blood-stage immunity. The key feature of this model is that interventions at an early stage of infection, e.g. against the liver stage parasite via CTL or against the mosquito by the use of impregnated bed nets (Gupta *et al.* 1994*a*), have a disproportionately greater effect on the incidence of severe malaria than mild malaria. Hence, this type of model may explain the observed greater reduction in severe than mild malaria with bed nets (Alonso *et al.* 1993), and the stronger association of HLA-B53 with resistance to severe than mild malaria (Hill *et al.* 1991). Secondly, in areas of higher transmission, where children have experienced more infections, the slope of the line (figure 1) will be flatter than in a lower-transmission area such as The Gambia. Also, in higher transmission areas, many of the otherwise severe cases of malaria will happen during infancy while there is still protection from maternal antibodies or other protective mechanisms during the early months of life. Both of these factors will have the effect of attenuating differences in the protective efficacy of differential levels of CTL response, and diminish HLA class I associations. A further prediction of the model, that the protective HLA-B53 association in The Gambia should have been more evident in

Table 1. *Amino acid sequences of the four allelic peptides: cp26–cp29*

(These form part of a variable region of the circumsporozite protein of *Plasmodium falciparum* (amino acids 368–375). The sequences are shown using the standard one letter code for amino acids. These four peptides are present in different parasite strains found in The Gambia. Cp26 and cp29 are epitopes for cytotoxic T lymphocytes restricted by the HLA class I molecule HLA-B35. Cp27 and cp28 fail to bind to HLA-B35 and are not epitopes. Cytotoxic T lymphocytes specific for cp26 have been shown to fail to recognize the other three variants. Similarly, cytotoxic T lymphocytes specific for cp29 fail to recognize cp26 – 28.)

	1	2	3	4	5	6	7	8
cp26	K	P	K	D	E	L	D	Y
cp27	K	P	K	D	Q	L	D	Y
cp28	K	P	K	D	Q	L	N	Y
cp29	K	S	K	D	E	L	D	Y

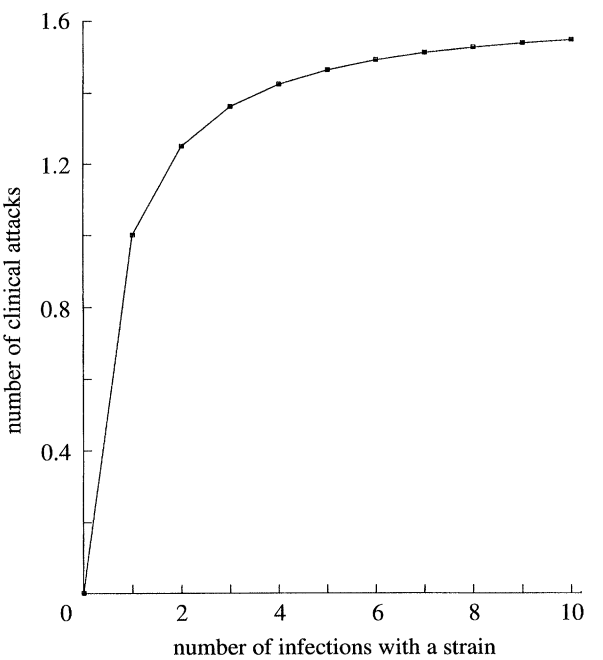

Figure 1. The relationship between the average number of clinical attacks of malaria caused by a single strain of *Plasmodium falciparum* during a year in a given individual and the number of infections caused by that strain. This non-linear relationship reflects an increasing degree of strain-specific anti-disease immunity to a parasite strain with increasing exposure to it.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

younger than older children was indeed observed (unpublished data).

Hence, HLA associations with malaria may be sensitive to the transmission intensity of this infection. It can be seen that in this case the outcome is a function of particular features of the infection such as the presence of immune responses to more than one stage of the life-cycle of the malaria parasite. However, more generally, a multiplicity of different protective immune responses is a feature of host defence against many natural pathogens. It seems likely that the most important protective response may often vary according the host genotype and also the parasite strain. Hence, just as class I and class II restricted responses to the liver stage and blood stage of malaria respectively, may be of varying importance in different geographical areas, if the host uses difference immune clearance mechanisms during the same stage of infection, according to transmission patterns in the locality or according to local parasite genotype, again HLA associations may reflect this heterogeneity.

It is likely that as particular immune defence mechanisms are understood in more detail, we shall develop a clearer picture of how sensitive HLA associations may be to variation in environmental factors and parasite genotype.

8. FLUCTUATING SELECTION

As mentioned above, overdominant selection and frequency-dependent selection are both powerful means by which genetic polymorphism may be maintained in a population. Although it seems likely that both will prove to be important in maintaining such diversity, we shall consider here a third potential mechanism. Forty years ago Levene (1953) and Dempster (1955) found that spatially varying selection could maintain polymorphism without (arithmetic-mean) overdominance, and in 1963 Haldane & Jayakar demonstrated that temporally varying selection coefficients could lead to a permanent polymorphism. This happens provided that the geometric mean fitness of the heterozygote is highest over time. Hedrick (1974), Gillespie (1978, 1985) and Takahata (1981) have developed more detailed models of fluctuating selection incorporating finite population size, mutation and genetic drift.

The general conclusion is that fluctuating selection can lead either to allele loss or preservation of diversity depending on the precise circumstances. Unfortunately, no theoretical work appears to have addressed fluctuating selection and the special circumstances of MHC diversity. It seems likely that conditions may frequently exist in which the 'holding power' (Karlin & Lieberman 1974; Takahata 1981; Gillespie 1985) of fluctuating selection outweighs the increased tendency to lose rare alleles (Karlin & Lieberman 1974; Takahata 1981; Gillespie 1985). There are a large number of fairly frequent HLA alleles in most populations so that the tendency to lose very rare ones may be less relevant, and cyclic variation, which may be more effective than random changes in selection coefficients in maintaining variation (Hedrick 1974), is probably a common feature of host–parasite interactions.

Analysis of fluctuating selection must be particularly relevant to parasite-driven selection and HLA variation. Infectious pathogens frequently appear in epidemics, often of novel antigenic type, so temporally varying selection coefficients are likely to be common. Extensive antigenic diversity exists in the majority of natural human pathogens that have been studied in detail, and there is increasing evidence of geographical variation in parasite allele frequencies. Certain pathogens, such as HIV, also undergo extensive mutation within a single host. Finally, as discussed above, several environmental and genetic factors can lead to different HLA associations with a single infectious pathogen in particular regions.

9. CONCLUSIONS

It is becoming clear that HLA associations with infectious pathogens such as *P. falciparum* exist, but that large studies using molecular HLA typing are required to demonstrate these convincingly. Some of the apparently contradictory results in earlier studies of HLA and infectious diseases may be accounted for by real heterogeneity in HLA associations between different populations. Recently, the use of new molecular immunological techniques has made it possible to begin to dissect the molecular mechanisms underlying these associations and, in turn, such work has revealed possible reasons for heterogeneity in associations. Irrespective of the underlying mechanisms, the observations of variable HLA associations with infection in field studies highlight the relevance of fluctuating selection pressures to the evolutionary maintenance of diversity in the MHC. Further theoretical and experimental studies of fluctuating selection pressures should assist in assessing their importance in maintaining MHC polymorphism.

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