Genetic analysis of host–parasite coevolution in human malaria

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SUMMARY

Recent twin studies of clinical malaria and immune responses to malaria antigens have underscored the importance of both major histocompatibility complex (MHC) and non-MHC genes in determining variable susceptibility and immune responsiveness. By using a combination of whole genome genetic linkage studies of families and candidate genes analysis, non-MHC genes are being mapped and identified. Human leucocyte antigen (HLA) genotype was found to affect susceptibility to severe malaria in a large study of West African children. T lymphocytes that may mediate such resistance have been identified and their target antigens and epitopes characterized. Some of these epitopes show substantial polymorphism, which appears to result from immune selection pressure. Natural variant epitopes have been found to escape T-cell recognition in cytolytic and other T-cell assays. More recently a novel immune escape mechanism has been described in viral infections, altered peptide ligand antagonism, whereby variants of a T-cell epitope can downregulate or ablate a T-cell response to the index peptide. The likely implications of such immune escape mechanisms for the population structure of malaria parasites, for HLA associations with malaria infection and disease, and for the design of new malaria vaccines, are discussed. The evolutionary consequences of such molecular interactions can be assessed by using mathematical models that capture the dynamic interplay of variable host and parasite molecules. Combined genetic, immunological and mathematical analysis of host and parasite variants in natural populations can identify some mechanisms driving host–parasite coevolution.

1. INTRODUCTION

Analysis of host–parasite interactions in laboratory models of infectious diseases has provided detailed insights into just how complex molecular mechanisms of resistance may be, even in these supposedly simple systems. Genetic analysis of gene knockout mice has recently been providing important mechanistic insights, as illustrated by other contributors to this volume. However, even in these fairly well-defined models many surprising results have turned up and others no doubt remain in store. Against this background, attempting to unravel the complexities of the interaction of Plasmodium falciparum with its human host at a molecular level may appear rather optimistic and overambitious. However, there are advantages to the study of natural host–parasite interactions. Increasing knowledge of the molecular basis of host–parasite interactions in model systems presents pressing questions concerning which of these are of significance in natural infections. Despite its complexity, malaria in humans provides molecular evidence of a long-running encounter between a resourceful parasite and a great array of host defence mechanisms. Analysis of these has in the past provided fascinating insights into the evolution of human genetic variation.

This short review firstly outlines some recent advances in the study of human genetic resistance to malaria, focusing particularly on the role of variable immune response genes encoded both within and outside the human major histocompatibility complex (MHC). There follows a summary of recent analysis of some complex interactions between variable components of the P. falciparum parasite that interact with particular genetic variants of the human leucocyte antigen (HLA) system. It is proposed that combined genetic and functional analysis of both host and parasite polymorphism in natural field settings is required to understand the coevolution of this variation. Finally, the probable relevance of these findings for the design of interventions against malaria by using new subunit vaccines is suggested.

2. HOST GENETIC FACTORS IN MALARIA

Malaria provides several of the classical examples of human genetic polymorphisms that affect disease susceptibility. Sickle haemoglobin, G6PD deficiency and the Duffy blood group are well represented in textbooks. However, the discovery of these striking examples preceded any formal estimate of the magnitude of the
of HLA associations with resistance to severe malaria (Hill et al. 1991). More recently, polymorphism in the promoter of another MHC gene, tumour necrosis factor, was found to affect the risk of cerebral malaria (McGuire et al. 1994). However it has been surprisingly difficult to detect an influence of HLA and other major histocompatibility complex genes on the magnitude of immune responses to malarial antigens in field studies. Indeed, in general, cellular immune responses to malarial antigens show marked heterogeneity in specificity, type and magnitude; the relative importance of MHC polymorphism and other genetic factors in accounting for this heterogeneity has been unclear.

Substantial insight into the role of host genetics in immune response to malarial antigens has recently been provided by twin studies. A few years ago a small twin study suggested that monozygotic (MZ) twins were more concordant than dizygotic (DZ) twins for certain antibody response to malaria (Sjoberg et al. 1992). Recently, Jepson et al. (1997a) have reported a large study of 267 Gambian twin pairs that provided heritability estimates for several immune responses and an estimate of the relative importance of MHC and non-MHC genes in their determination. Comparison of the intraclass correlation coefficients of lymphoproliferative responses for MZ and DZ twins showed higher values in the former for all 15 malarial antigens or epitopes studied. Calculated heritability estimates ranged from 0.04 to 0.80. Comparison of the same MZ twin values with HLA-identical DZ twins still showed substantially higher values in the former and permitted estimation of the contribution of non-MHC genes. The analysis provided evidence that MHC genes do affect immune responses to several of the malarial antigens studied but, interestingly, the contribution of non-MHC genes was predominant. Cumulatively, the non-MHC genes accounted for on average about 80% of the genetic component.

4. NON-MHC IMMUNE RESPONSE GENES

The identity of these non-MHC genes that affect immune responses to malaria is unclear. Inspection of the list of non-MHC genes that affect susceptibility to the disease provides no likely candidates, suggesting that other genes remain to be identified. Two approaches are currently being pursued to this end. A family study is being undertaken of a large number of Gambian dizygotic twin pairs with measured immune response to malaria antigens. Linkage analysis of these quantitative traits found to have high heritabilities is being performed with a panel of microsatellite markers spanning the entire human genome. This study should allow localization of major genes affecting these phenotypes, to be followed by fine mapping aimed at gene identification. The second approach is to study polymorphisms in individual candidate genes that are considered likely to affect immune responsiveness, such as variation in key cytokine genes and immune receptors.

This candidate gene approach is less systematic than genome scanning but is more powerful in detecting small effects because association rather than linkage is
measured. It is more likely to succeed when there is already detailed biological information available on the phenotype being studied. Such candidate-gene studies are now in progress for several infectious diseases; this approach has recently identified a non-MHC gene that may influence immune responses and resistance to several infectious diseases. The vitamin D receptor was initially studied as an infectious disease candidate gene in a large control study of tuberculosis in The Gambia (R. Bellamy et al., unpublished data). The choice of this candidate gene was based on clinical, epidemiological and in vitro data that suggested a role for vitamin D in tuberculosis (TB) resistance (Rook 1998). The finding of a variant allele associated with TB resistance has led to further studies of several infectious diseases with associations now identified for leprosy, persistent hepatitis B virus infection and HIV infection as well as tuberculosis (R. Bellamy, S. Ali, S. Roy et al., unpublished data). Analysis of the vitamin D receptor association with leprosy type has led to the suggestion that this gene may have a qualitative effect on the immune response to leprosy as well as perhaps to other pathogens (S. Roy et al. unpublished data). The marked immunomodulatory effects of the active form of vitamin D both in vitro and in vivo are consistent with a role for functional variation of this receptor gene in immunity to several foreign pathogens. However, it is likely that this is but one example of a large number of non-MHC genes that affect immune responses. Identification of these and analysis of their role in malaria immunity and resistance will be of interest.

5. POLYMORPHISM IN PLASMODIUM FALCIPARUM

Some of the first evidence that sequence variation in the malaria parasite may be the consequence of immune selection pressure came from analysis of the circumsporozoite (CS) protein of Plasmodium falciparum. This is the major surface protein of sporozoites, the form of the parasite inoculated by mosquitoes. The CS gene encodes a largely conserved central repeat, of amino acid sequence NNP, that is the major antibody or B-cell epitope (figure 1). In the carboxy-terminal region of the CS protein are two major T-cell epitope regions termed TH2R and TH3R. These are recognized by both CD4+ HLA class II restricted T cells and CD8+ HLA class I restricted T cells (Nardin & Nussenzweig 1993). Lockyer et al. (1989) analysed sequence variation in these epitopes from five malaria-infected Gambian children. Marked polymorphism was found in both regions (table 2). Furthermore, there was a striking excess of non-synonymous (coding) changes compared with synonymous or silent changes in these epitope regions. This excess provided evidence that these regions were under selection pressure by the human immune system. Variation was found at numerous positions and it was concluded that too many alleles were likely to exist to hope to include all in a subunit vaccine. Subsequent studies have found a more limited number of alleles of these regions in Brazil and one area of Papua New Guinea, and relatively few further allelic types have been reported (Shi et al. 1992; Doolan et al. 1992). Hence this polymorphism appears finite and has not prevented continuing attempts to develop CS as a malaria vaccine. However, it is clear that variants of both the TH2R and the TH3R region affect human T-cell recognition for both CD4+ and CD8+ T-cell responses (Guttinger et al. 1988; Hill et al. 1992); this result is consistent with the proposal that this variation has evolved through immune selection.

These polymorphic regions were initially identified as epitopes for proliferative CD4+ T-cell responses before techniques for reliable detection of low levels of CD8+ T-lymphocyte responses in humans were developed. However, it was proposed that the main selection pressure was mediated by CD8+ rather than by CD4+ T-lymphocytes because only the former were viewed as effector cells (Good et al. 1988). If CD4+ proliferative T cells simply acted to help antibody formation or other T-cell responses there would usually be little or no advantage to a parasite with a new escape variant in this epitope, because help from other T cells recognizing the non-variant epitope would be sufficient. However, it is now clear that CD4+ T cells can act as effector cells in human malaria either as lytic cells or, more likely, through the release of interferon-γ (Nardin and Nussenzweig 1993). It is likely that the protective efficacy of the recently described CS vaccine, RTS,S (Stoute et al. 1997), is mediated at least partly by such CD4+ effector T cells. Indeed inspection of the span of polymorphism within the TH2R and TH3R regions, up to 14 amino acids, is in keeping with the size of HLA class II restricted T-cell epitopes. Sequence variation is also found in another major pre-erythrocytic antigen of P. falciparum, thrombospondin-related adhesive protein (TRAP), but is less marked (Robson et al. 1990). Sequence variation in other pre-erythrocytic antigens has been less fully characterized but may be more limited than in the CST-cell epitopes.

6. T LYMPHOCYTES RECOGNIZE LIVER-STAGE ANTIGENS OF P. FALCIPARUM

The initial impetus for the characterization of human T-cell responses to pre-erythrocytic malaria antigens came from studies of mouse models of malaria. It has been known for thirty years that irradiated sporozoites can be used to immunize and
protect mice and some other species against sporozoite challenge. Analysis of lymphocyte subsets revealed that in most mouse strains this protection is mediated by CD8+ T lymphocytes. In fewer cases an important role for CD4+ T lymphocytes was identifiable (Nardin & Nussenzweig 1993). However, these rodent models of malaria employ parasites that are only very distantly related to \textit{Plasmodium falciparum}.

Evidence that CD8+ T cells might be of protective relevance in human \textit{P. falciparum} malaria was provided indirectly. In a large Gambian case–control study of human genetic risk factors for severe malaria, a protective HLA class I antigen association was found for the allele HLA-B53 (Hill \textit{et al.} 1991). Analysis of flanking polymorphisms indicated that the HLA-B53 gene itself was most likely to be responsible, implying a protective role for CD8+ T cells restricted by this HLA type. Subsequent immunological studies identified such CD8+ T cells to a conserved parasite epitope in the same population (Hill \textit{et al.} 1992). These cells are often referred to as cytotoxic T lymphocytes, reflecting the lysis assay usually employed for their detection. However, CD8+ T cells are known to have several effector mechanisms and it is uncertain which is of most protective relevance in human malaria.

There has now been extensive characterization of the human CD8+ T-cell response to natural infection by \textit{P. falciparum} as well as to immunization by irradiated sporozoites. Six pre-erythrocytic antigens have been found to contain cytotoxic T lymphocyte (CTL) epitopes (table 3) (Aidoo \textit{et al.} 1995, unpublished data).

The circulating frequencies of these cells in peripheral blood of individuals in endemic areas is low, as revealed by limiting dilution assay analysis (Plebanski \textit{et al.} 1997). This is true even in areas with very high transmission levels of malaria (Lalvani \textit{et al.} 1996). This may reflect the low immunogenicity of the very small number of sporozoites inoculated by a typical infectious mosquito bite. As for CD4+ T-cell responses, there is considerable variation between individuals in the specificity and magnitude of their T-cell responses that is not explained by HLA variation (Taylor \textit{et al.} 1996). None the less, the CD8+ T-cell response is strongly restricted by HLA class I antigens and no promiscuous CTL epitopes have been identified.

The protective relevance of these CD8+ T cells in humans may be assessed directly in either of two ways. Firstly a prospective study of children in an endemic area could be undertaken, searching for a negative correlation between the magnitude of the CD8+ T-cell response and subsequent disease episodes. This has not

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Table 2. \textit{Sequence polymorphism in the TH2R and TH3R regions of the \textit{P. falciparum} circumsporozoite protein}

Source: Lockyer \textit{et al.} (1989); Shi \textit{et al.} (1992). The amino acids are shown in single letter code with their sequence positions.

(a) TH2R region

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(b) TH3R region

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Table 3. \textit{Pre-erythrocytic antigens of \textit{Plasmodium falciparum} that have been found to contain CD8+ T-cell epitopes recognized by malaria-immune Africans}


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been attempted because of the low sensitivity of standard CTL assays and their requirement for relatively large blood samples. However, the recent introduction of a new more sensitive technique for the detection of these cells may overcome this problem. This assay involves the detection of single cells secreting γ-interferon with an ELISPOT assay that appears to be many times more sensitive than standard lysis assays (Miyahara et al. 1995; Lalvani et al. 1997). The second approach would be to induce these CD8+ T cells by vaccination and monitor for increased resistance to malaria infection or disease. This strategy has been hampered by the poor immunogenicity of most available vaccine delivery systems for inducing CD8+ T cells in humans. However, again, this problem may be resolved by the use of some promising new delivery systems. The protective efficacy of CD4+ T cells against pre-erythrocytic malaria antigens could be assessed in similar fashion, particularly as some new vaccine adjuvants are better at inducing CD4+ than CD8+ T-cell responses.

Of particular interest for the coevolution of host and parasite diversity has been the extent of polymorphism in epitopes defined by effector T cells in malaria. Surprisingly little epitope mapping has been performed for CD4+ cell responses apart from studies of the CS epitopes (above). However, numerous epitopes for CD8+ T cells have been identified in six pre-erythrocytic antigens of P. falciparum. Most of these epitopes appear to be in relatively conserved region of these antigens (Aidoo et al. 1995) with the important exception of the CTL epitopes in the CS protein. Four CD8+ epitopes have been found in the latter, one a seldom recognized peptide in a conserved region and the other three encoded by polymorphic segments of the 3′ region of the gene. The first epitope identified, cp26, contains eight amino acids, and is restricted by the HLA class I molecule HLA-B35 (Hill et al. 1992). Three naturally occurring variants of this sequence, cp27, cp28 and cp29, are frequently found among parasite strains in Africa. However, CD8+ T cells specific for cp26 failed to cross-recognize all three of these variants on cytotoxic T-cell assays. Furthermore, CD8+ T cells specific for cp29 failed to recognize any of cp27, cp27 or cp28. Hence there is marked specificity in CD8+ T-cell responses to this and other (A. V. S. Hill et al., unpublished data) regions of the CS protein. This implies that CTL induced by vaccines containing this epitope would almost certainly not protect against heterologous sporozoite challenge.

7. ALTERED PEPTIDE LIGAND ANTAGONISM

The specificity of T-cell responses for allelic variants of the P. falciparum CS protein suggests that one option for vaccine design might be to include several variants of an epitope in a vaccine construct. However, the discovery of altered peptide ligand (APL) antagonism of human T-cell responses (figure 2) may present a
problem for this strategy. APL antagonism was first described for human CD4+ T-cell responses to variants of an influenza haemagglutinin epitope (De Magistris et al. 1992). HLA-DRA1-restricted cloned T cells were rendered non-responsive to the index peptide by co-incubation with low concentrations of analogues representing minor variants of the epitope. APL antagonism was subsequently demonstrated for other class I as well as class II-restricted T-cell responses in mice and humans (Sette et al. 1994). A closely related phenomenon of partial T-cell activation has been characterized by P. M. Allen and colleagues, who showed that variant peptides often induce some but not all of the signals required for T-cell activation (Sloan-Lancaster & Allen 1996). For example, a wild-type peptide could induce both interleukin-4 production and lymphoproliferation whereas a variant peptide induced only the former response.

The molecular mechanisms underlying APL antagonism remain incompletely understood but there is much interest in why such a mechanism might have evolved. Natural variants of both HIV and the hepatitis B virus have been found to antagonize CD8+ T cell responses to their corresponding epitopes, suggesting that APL antagonism is employed as an immune escape mechanism by these viruses (Bertoletti et al. 1994; Kienemann et al. 1994). However, Davenport (1995) has pointed out that often it would be of more benefit to a particular pathogen to evolve variants that simply failed to bind to HLA molecules rather than antagonistic variants. One possible advantage of antagonism is that mutually antagonistic strains might benefit each other by reciprocally downregulating immune responses to the other. Because many malaria infections involve mixtures of clones, this possibility might be of particular relevance to this disease.

The characterization of common variant epitopes for both CD4+ and CD8+ T cells in the CS protein of malaria makes this antigen a prime candidate for studies of the role of altered peptide-ligand antagonism in human malaria; recent detailed T-cell studies support the importance of APL antagonism in human immune responses to this antigen (M. Plebanski et al., unpublished data).

Another malaria antigen that has been studied in detail as a vaccine candidate is that encoding a major surface protein of the malaria parasite's life cycle, such as during hepatocyte infection. The genetic characteristics of early blood-stage malaria parasites are typed by sampling of peripheral blood. These parasites make it possible to determine whether clearer HLA association might be found with infection or disease caused by a particular pathogen strain rather than with all strains. Malaria offers what may be a uniquely powerful opportunity to perform this analysis as a result of the sequence of life-cycle stages of Plasmodium. The genetic characteristics of early blood-stage malaria parasites are typed by sampling of peripheral blood. These parasites make it possible to determine whether clearer HLA association might be found with infection or disease caused by a particular pathogen strain rather than with all strains.

8. HLA ASSOCIATIONS WITH MALARIA AND WITH PARASITE TYPE

HLA associations with susceptibility to several infectious diseases have now been described (reviewed by Hill (1997)). All of these studies have considered together disease caused by all variants of the infectious pathogen. Now that strains or alleles of many microorganisms are readily typeable at the sequence level, it is possible to determine whether clearer HLA association might be found with infection or disease caused by a particular pathogen strain rather than with all strains.

Malaria offers what may be a uniquely powerful opportunity to perform this analysis as a result of the sequence of life-cycle stages of Plasmodium. The genetic characteristics of early blood-stage malaria parasites are typed by sampling of peripheral blood. These parasites make it possible to determine whether clearer HLA association might be found with infection or disease caused by a particular pathogen strain rather than with all strains.
parameter values for such mathematical models can be estimated from genetic and immunological studies.

9. PERSPECTIVE

There are clearly numerous determinants of the nature and extent of immune responses to a pathogen that infects all individuals. Host and parasite genetic factors are both of importance; the virulence of a particular parasite strain will depend in part on the genetic makeup of the host. Twin studies have now allowed precise estimates to be made of the heritabilities of several immune responses to major *P. falciparum* antigens. Numerous genes affect these immune responses; cumulatively, those outside the MHC are of greater importance than those within. HLA type is important but not of overriding importance. Identification of these other non-MHC immune response genes and analysis of their mechanisms is a priority for a fuller understanding of the determinants of variable host–parasite interactions. Techniques are now available for mapping and eventually identifying these genes. This process may well be complicated by the interpopulation heterogeneity that is well recognized in HLA studies of infectious diseases. However, the magnitude of the non-MHC genetic effect on several immune responses suggests that it should be possible to isolate major relevant genes.

Considerable complexity is to be expected in the interaction of these non-MHC genes with malaria, as illustrated by the analysis of MHC-regulated effects. Furthermore, epistatic interactions between genes are to be expected, as seen in many simpler systems (Green 1984; Lark et al. 1995) and in some theoretical models of the evolution of resistance genes for infectious pathogens. The expected consequence of such epistasis is that single-gene effects are likely to vary from population to population according to the prevalence of alleles at other loci. Such genetic heterogeneity is to be expected and may explain some apparently conflicting results observed in studies of different populations. The practical implication of such expected population differences is that one large study is often of more value than numerous small studies in different populations.

We write at a time when exciting studies of the population structure of *P. falciparum* and relevant immunological mechanisms are ongoing. It appears likely that altered peptide-ligand antagonism may play an important role in shaping the extent and specificities of at least some T-lymphocyte responses to *P. falciparum*. In turn, the precise details of these molecular interactions may have major implications for our understanding of the coevolution of some interacting host and parasite variants. It is interesting that the host immune response focused on here, against the liver stage of malaria, is only one of many that probably play a role in protective immunity to malaria. Indeed the major strain-structuring immune response is more likely to be against an antigenically varying molecule expressed at the blood stage of infection (Gupta et al. 1994). This raises the possibility that there may be a variety of interactions structuring the population of malaria parasites determined by the interaction of host immune response genes with particular stage-specific antigens of the parasite. Importantly, the effects of such interaction on the parasite population structure would be identifiable only by typing the parasite epitope of interest, or a closely linked marker. Thus we suggest that there may be many such niches of host–parasite interaction in malaria and other infections that humans have had to adapt to. These will be best searched for by simultaneous typing of interacting host and parasite variants in field populations. Precise mathematical modelling of these dynamic interactions will be required. In the longer term such combined molecular, cellular and mathematical analysis of host–parasite interaction in the field should help uncover the mechanisms that commonly drive host–parasite coevolution.

There is now evidence from studies of several infectious diseases that HLA association may vary substantially from one population to another. Various possible reasons for this genetic heterogeneity have been discussed. We have proposed elsewhere that polymorphism in infectious pathogens may be a major factor (Hill et al. 1994). Evidence that pathogens can subvert host immune response by using altered peptide-ligand antagonism adds yet another possible mechanism by which HLA associations may differ geographically. The practical consequence of this is that replication of suggested HLA associations is ideally performed in the same ecological context. The evolutionary consequence is that selection pressures on HLA frequencies exerted by infectious pathogens are more likely to fluctuate both spatially and temporally.

Finally, such host–parasite interactions have implications for the design of vaccines against malaria. The main barrier to the development of an effective malaria vaccine remains the poor immunogenicity in humans of available delivery systems for inducing T-cell responses, particularly of CD8+ T cells. However, the additional problem of polymorphism in the T-cell epitopes of some malaria vaccine candidates has been well appreciated and debated (Good et al. 1988). Optimists have emphasized the finding of some cross-reactivity in T-cell response to variants of these epitopes. Others, pointing out that cross-reactivity is limited and that the polymorphism is probably the result of immune selection pressure, have advocated including an array of common variant epitopes in a subunit vaccine to produce immunity to the great majority of parasite types (Doolan et al. 1992). However, if there is frequently altered peptide-ligand antagonism between common variant epitopes such a strategy will also fail.

The issue is timely, as the leading current malaria vaccine candidate, RTS,S, is a fusion protein of hepatitis B surface antigen and the highly polymorphic CS protein. Preliminary evidence indicates that the promising efficacy of this construct against homologous sporozoite challenge may relate to T-cell, rather than antibody, responses (Stoute et al. 1997). If these are focused, as usual, on highly polymorphic T-cell epitopes of the CS protein, escape variants are likely to be selected and CD4+ T-cell antagonism might even
pose an additional problem. If this does turn out to be the case, what alternative strategy might be employed? Fortunately, in other pre-erythrocytic antigens several conserved T-cell epitopes have been identified. It is possible that these are more subdominant epitopes that have been under less strong selection pressure to vary. Polypeptide vaccines consisting of long strings of such epitopes in tandem are now being designed and appear immunogenic (S. C. Gilbert et al., unpublished data). Epitope-based vaccines have to include enough peptides to encompass the polymorphism of human HLA types, but such extensive constructs may be feasible.

The complexities of host–parasite interactions, well illustrated by studies of malaria, have provoked both admiration of the seemingly infinite resourcefulness of parasites and occasional despair about the feasibility of the control of parasitic disease by vaccination. Neither response is quite appropriate. Complex immune evasion mechanisms identify both the target antigens and the mechanisms of protective immunity. Once the immune escape mechanisms are understood and the difficult immunogenicity problem is solved, useful vaccines will follow.

REFERENCES


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