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Inverse Associations of Human Leukocyte Antigen and Malaria Parasite Types in Two West African Populations

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Differences in allelic associations between populations continue to cause difficulties in the mapping and identification of susceptibility genes for complex polygenic diseases. Although well recognized, the basis of such interpopulation differences is poorly understood. We present an example of an inverse allelic association of an immune response genotype to an infectious disease in two neighboring West African populations. In this case, both the key environmental contributor, i.e., the malaria parasite, and a major biological mechanism are well defined. We show that this surprising result fits well with the predictions of a mathematical model describing the population genetics and dynamics of this interaction.

Cytotoxic T-lymphocyte (CTL) responses against *Plasmodium falciparum* antigens expressed at the early liver stage of infection may have an effect on the distribution among human hosts of parasites bearing particular alleles. Epitopes from liver-stage proteins are presented by HLA class I molecules on the hepatocyte, rendering the parasitized hepatocyte susceptible to killing by CTLs. Polymorphism in parasite proteins during the liver stage of infection can result in escape from or immunological antagonism (3) to CTL killing (5). We have previously described an association of parasite population structure, HLA, and altered peptide ligand (APL) antagonism in The Gambia (5). The most common HLA class I molecule in this population is HLA-B*35, and CTLs restricted by this allele often recognize an epitope from the highly polymorphic Th3R region of the circumsporozoite protein. Four such circumsporozoite allelic variants are found in The Gambia, of which two (cp26 and cp29) bind HLA-B*35 and elicit CTL responses and the other two (cp27 and cp28) fail to bind to HLA-B*35.

Parasites bearing the HLA-B*35 epitopes were found together in mixed infections more frequently than would be expected from their prevalence within the population, regardless of the HLA type of the host. We also found that these two epitopes, cp26 and cp29, are minimally cross-reactive and mutually antagonistic both at the effector level and during the primary induction of CTL responses (5). Thus, although immunity to one of the epitopes can develop in HLA-B*35-bearing hosts after a single infection with a strain bearing that epitope, no immunity results after a mixed infection with strains bearing both cp26 and cp29 epitopes in hosts of that HLA type. A mathematical model was used to demonstrate the effect of APL antagonism on the parasite population structure in both HLA-B*35- and non-HLA-B*35-bearing hosts. This showed that APL antagonism results in the appearance of a large proportion of cp26-cp29 mixed infections, generated in HLA-B*35-bearing hosts as a result of the advantage conferred to the parasite by mutual antagonism of the host immune response but rapidly spreading to the whole host population (5).

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MATERIALS AND METHODS

Blood sampling for DNA extraction was approved by an internal ethical review board. Parasites were typed for 115 of the children studied by hybridization of specific labeled oligonucleotides, as described previously (5). The remainder were typed by the ligation detection reaction (LDR), details of which have been described previously (6) For this procedure, we used the same PCR primers plus three sets of LDR probes to detect polymorphisms at three regions of the HLA-B*35 epitope. (i) For the P/S polymorphism at position 2, we used Th3R-2P (AAAAGCCTGGCTCTGCTAATAAAC), TH3R-2S (GCCTGGCT CTGCTGGTAAAT), and the common probe TH3R-2com (CTAAAGACSAA TTARATTATGCAAATGA). (ii) For the E/Q polymorphism at position 5, we used TH3R-5E (GGCTCTGCTAATAAACCTAAAGACG), TH3R-5E (AGC TCTGCTGGTAAATCTAAAGACG), TH3R-5Q (AAGGCTCTGCTAATAA ACCTAAAGACC), and the common probe TH3R-5com (AATTARATTATG CAAATGATATTGAAAAA). (iii) For the D/N polymorphism at position 7, we used TH3R-7D (AATCTGCTAATAAACCTAAAGACSAATTAG), TH3R-7D(cp29) (AAACTGCTGGTAAATCTAAAGACGAATTAG), TH3R-7N (AAA ATCTGCTAATAAACCTAAAGACCAATTAA), and the common probe Th3R-7com (ATTATGCAAATGATATTGAAAAAAAAAT). Parasites with each of three polymorphisms were typed separately. Where probes overlapped known polymorphisms, either a probe synthesized with a mixture of the possible bases at that position or two probes with both possible versions were used. Allele-specific probes were labeled at the 5′ end with 6-carboxyfluorescein, and bases were added to the 5′ end to create ligation products of different lengths; for example, TH3R-2P plus TH3Rcom is 52 bp, whereas TH3R-2S plus TH3Rcom is 48 bp. The LDR was carried out at an annealing temperature of 64°C.

TABLE 1. Frequency of infection with parasites bearing cp26 to cp29 in 305 children in Mali

Parasite type(s)[a]	No. of children with parasites		
	Observed	Expected at PR of[b]:	
		50%	10%
cp26 only	52	70	90*
cp27 only	152	117*	135
cp28 only	10	10	15
cp29 only	3	38*	52*
cp26, cp27	7	30*	6
cp26, cp28	0	3	1
cp26, cp29	59	10***	2****
cp27, cp28	10	4	1**
cp27, cp29	7	16	3
cp28, cp29	0	1	0
cp26, cp27, cp28	1	1	0
cp26, cp27, cp29	4	4	0*
cp26, cp28, cp29	0	0	0
cp27, cp28, cp29	0	1	0
cp26, cp27, cp28, cp29	0	0	0

[a] Parasites were typed by PCR and oligonucleotide hybridization, as described previously (5). The expected frequencies of single and mixed infections with each of the strains, assuming random mixing, are shown for different parasite rates (PR) in the population.

[b] Analysis of the distribution showed cp26 and cp29 together significantly more often than expected at a PR of 10% or 50% (*, $\chi^2 = 10$ to 50; **, $\chi^2 = 50$ to 100; ***, $\chi^2 = 100$ to 1,000; ****, $\chi^2 > 1{,}000$.)

TABLE 2. Frequency of cp26 and cp29 in Malians with HLA-B*35 compared to that in the rest of the population[a]

Parasite type	Children with parasites with HLA-B*35			
	Present ($n = 126$)		Absent ($n = 263$)	
	n	%	*n*	%
cp26	31	25	92	35
cp27	69	55	108	41
cp28	10	8	10	4
cp29	16	12	53	20
cp26 or cp29	47	37	145	55

[a] All the children for which the HLA type was known were divided into two groups based on the presence of HLA-B*35, and the numbers of children with infections with each parasite type were then scored. Thus, a child with a mixed infection of cp26 and cp27 would be included in each of those categories but not in the cp28 or cp29 category. The total scores for infections with either cp26 or cp29 were then determined for each group of children. There is a decreased occurrence of cp26 and cp29 both together and separately in individuals with HLA-B*35 ($P < 0.001$).

RESULTS AND DISCUSSION

The population of Mali in West Africa has a frequency of HLA-B*35 similar to that of the Gambian population, but there are significant differences in the frequencies of other HLA class I alleles (1, 8). We detected antigen frequencies of 30 and 27% in The Gambia and Mali, respectively. We typed blood-stage *P. falciparum* parasites from 305 children from Mali, who were also assessed for the presence or absence of HLA-B*35. First, we found (Table 1) that the prevalence of the four parasite types is very similar to that in The Gambia, with cp27 being the most common allele, followed by cp26, cp29, and cp28, in order of decreasing prevalence. Second, there is a marked cohabitation of cp26 and cp29 in the Malian cohort in both HLA-B*35- and non-HLA-B*35-bearing hosts (Table 1), again as found in The Gambia. However, the striking difference between the two studies is that, in Mali, cp26 and cp29 were found to be less, not more, common in hosts with HLA-B*35 (Table 2). In Mali, 37% of infections in HLA-B*35-bearing hosts were with either cp26 or cp29, compared to 55% in non-HLA-B*35-bearing hosts ($P = 0.0009$; odds ratio, 0.48 [95% confidence intervals, 0.31 to 0.77]). The corresponding figures for The Gambia were 51% in HLA-B*35-bearing hosts and 42% in non-HLA-B*35-bearing hosts (5). The Gambian samples and 115 of the Malian samples were typed by PCR and hybridization of labeled oligonucleotide probes specific for the four parasite variants. The remainder of the Malian samples were typed by LDR (4), and some of the original samples were retyped using this method. Excellent agreement was found between the results obtained by the two methods as well as with results from sequencing some of the samples.

The cohabitation of parasites bearing the two HLA-B*35 epitopes and the difference in prevalence of these parasites between HLA-B*35- and non-HLA-B*35-bearing hosts suggest that the same forces are acting to structure the parasite population in both human populations. The main observed difference is that, in Mali, the prevalence of cp26 and cp29, either singly or together, is higher in non-HLA-B*35-bearing hosts than in HLA-B*35-bearing hosts, whereas the inverse is true for hosts in The Gambia. Such a potential inversion of HLA association was explicit in a previously published simulation result (see Fig. 2 of reference 5). The mathematical model behind this simulation essentially demonstrates that the altered parasite population structure will be reflected in both HLA-B*35- and non-HLA-B*35-bearing hosts, but the prevalence in the latter will be determined by the relative efficacy of their non-HLA-B*35 class I-restricted CTL responses. The observed inversion may thus have resulted from a lower average efficacy of class I-restricted CTL responses in the non-HLA-B*35-bearing population in Mali (compared to that of The Gambia), which would be consistent with the observation that the frequencies of non-HLA-B*35 class I alleles are different between these two countries. HLA associations have been identified for several infectious and noninfectious diseases (7, 9). Associations have been sought less frequently for particular strains or epitopes of infectious pathogens (2). CTL epitopes often display immunodominance whereby a single preferred epitope is selected from a large protein or pathogen. Thus, HLA associations might be observed more readily for an immunodominant epitope than for a disease. However, such associations with variant pathogen epitopes may be very sensitive to the frequency of the HLA types in the host population studied and may also be complicated by the presence of APL antagonism to T-cell receptors. The evolutionary outcome of this complex array of interactions may be difficult to intuit and specific to each population.

This example of the potential of relatively simple and here well-defined biological interactions to generate marked population differences in disease associations underscores the importance of identifying genetic contributors to complex disease in a population-specific manner.

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REFERENCES

1. **Allsopp, C. E., R. M. Harding, C. Taylor, M. Bunce, D. Kwiatkowski, N. Anstey, D. Brewster, A. J. McMichael, B. M. Greenwood, and A. V. Hill.** 1992. Interethnic genetic differentiation in Africa: HLA class I antigens in The Gambia. Am. J. Hum. Genet. **50:**411–421.
2. **Apple, R. J., H. A. Erlich, W. Klitz, M. M. Manos, T. M. Becker, and C. M. Wheeler.** 1994. HLA DR-DQ associations with cervical carcinoma show papillomavirus-type specificity. Nat. Genet. **6:**157–162.
3. **Bertoletti, A., A. Sette, F. V. Chisari, A. Penna, M. Levrero, M. De Carli, F. Fiaccadori, and C. Ferrari.** 1994. Natural variants of cytotoxic epitopes are T-cell receptor antagonists for antiviral cytotoxic T cells. Nature **369:** 407–410.
4. **Day, D. J., P. W. Speiser, P. C. White, and F. Barany.** 1995. Detection of steroid 21-hydroxylase alleles using gene-specific PCR and a multiplexed ligation detection reaction. Genomics **29:**152–162.
5. **Gilbert, S. C., M. Plebanski, S. Gupta, J. Morris, M. Cox, M. Aidoo, D. Kwiatkowski, B. M. Greenwood, H. C. Whittle, and A. V. Hill.** 1998. Association of malaria parasite population structure, HLA, and immunological antagonism. Science **279:**1173–1177.
6. **Hennig, B. J., S. Hellier, A. J. Frodsham, L. Zhang, P. Klenerman, S. Knapp, M. Wright, H. C. Thomas, M. Thursz, and A. V. Hill.** 2002. Association of low-density lipoprotein receptor polymorphisms and outcome of hepatitis C infection. Genes Immun. **3:**359–367.
7. **Hill, A. V.** 1998. The immunogenetics of human infectious diseases. Annu. Rev. Immunol. **16:**593–617.
8. **Piazza, A., L. Sgaramella-Zonta, P. Gluckman, and L. L. Cavalli-Sforza.** 1975. The Fifth Histocompatibility Workshop gene frequency data: a phylogenetic analysis. Tissue Antigens **5:**445–463.
9. **Ryder, L. P., A. Svejgaard, and J. Dausset.** 1981. Genetics of HLA disease association. Annu. Rev. Genet. **15:**169–187.

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