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Association between HLA type and antibody response to malaria sporozoite and gametocyte epitopes is not evident in immune Papua New Guineans

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SUMMARY

HLA-A,B,C and DR types were determined for 46 adults living in the Madang area of Papua New Guinea. Sera from these individuals were tested by ELISA for antibodies against: (i) sonicated schizont extract of *Plasmodium falciparum*; (ii) circumsporozoite repeat regions of *P. falciparum* and *P. vivax*; and (iii) epitopes on the 230 and 48/45 kD gametocyte antigens of *P. falciparum*. All sera were from highly immune individuals and reacted strongly to the schizont antigen. The proportions responding to circumsporozoite repeat regions were 60·7% and 23·9% for *P. falciparum* and *P. vivax*, respectively. Between 32·6 and 47·8% of adults responded to each gametocyte epitope as assessed by inhibition of monoclonal antibodies. The limited number of alleles present at each HLA locus which is characteristic of coastal Papua New Guinea was observed. Five HLA-DR alleles were detected, of which only three (HLA-DR2, 4 and w5) were present at frequencies over 0·12. All individuals possessed at least one DR2,4 or w5 allele, and 96% of individuals possessed DR2, or 4 or both. There was no evidence for association between HLA type and antibody response to circumsporozoite repeat regions or the gametocyte epitopes. Homozygotes for DR2 and 4 were able to respond to each antigen. These results imply that either there is no HLA restriction of the response to these antigens or that each DR type is responding to a different variant of the T-epitope. Even in the latter case the results are encouraging for the prospects of inclusion of an HLA-restricted T-epitope in a malaria vaccine for Papua New Guinea since a limited number of versions would be required to cover a population with an HLA profile similar to that in Madang.

Keywords malaria HLA gametes sporozoites Papua New Guinea

INTRODUCTION

Malaria (*Plasmodium* spp) is transmitted from humans to mosquitoes when they take up the gametocyte stages of the parasite in the bloodmeal. Gametocytes emerge from the erythrocytes to form gametes which undergo fertilization and further development in the mosquito over the next 10 days, resulting in sporozoite stages of the parasite in the salivary glands. The sporozoites initiate a new infection when they are inoculated into the bloodsteam when the mosquito next feeds. Since the antigens which appear on the gamete surface are synthesized during the gametocyte stage in the blood, people are exposed to gamete surface antigens, as well as to surface antigens of the sporozoite stages. However, antibody responses to gamete antigens of *Plasmodium falciparum* are highly variable between individuals living in hyperendemic areas (Graves *et al.*, 1988b). Responses to gamete surface antigens, as measured by immunoprecipitation, do not correlate with antibody responses to intracellular gametocyte proteins (Carter *et al.*, 1989). Similarly, antibodies to the circumsporozoite repeat region (CSR) in individuals living in endemic areas increase in prevalence with age, but often do not reach 100% prevalence despite frequent exposure to malaria inoculations (Nardin *et al.*, 1979; Hoffman *et al.*, 1986; del Guidice *et al.*, 1987).

In mice, the immune response to the *P. falciparum* circumsporozoite (CS) protein and the gamete surface proteins is under the control or Ir genes (Good *et al.*, 1986, 1988a; del Guidice *et al.*, 1986). Two Ia-restricted T helper cell sites on the CS protein have been identified, and correlate with polymorphic regions of the molecule (Good *et al.*, 1988b). Immune Gambians also responded to one of the T helper sites (Good *et al.*, 1988c). A cytotoxic lymphocyte (CTL) T-epitope which is H-2 class I restricted has also been identified (Good *et al.*, 1988a; Kumar *et al.*, 1988).

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It has been suggested that the lack of antibody response of certain humans to sporozoite and gamete antigens is associated with HLA type (Good *et al.*, 1988a; Carter *et al.*, 1989). Individuals lacking a DR or DQ type capable of binding to a T helper epitope on the antigen in question would be unable to mount an effective antibody response. Similarly, absence of required class I molecules would limit the ability of cytotoxic lymphocytes to kill parasites (Schofield *et al.*, 1987, Kumar *et al.*, 1988). The discovery of major histocompatibility complex (MHC) restriction of antibody responses in mice to CS proteins has made vaccinating against malaria seem less feasible.

Studies with human malaria in inbred mice may have limited relevance to human populations living in endemic areas, for three reasons. Firstly, most people are heterozygous for alleles at the HLA loci. Secondly, human malaria can be expected to have exerted selective pressure on the HLA system, leading to the elimination of non-responder alleles (although the parasites may also have been expected to change in response to selective pressure). Thirdly, in certain populations at least (for instance Papua New Guinea) there are few alleles present at each HLA locus. This limited variation, combined with heterozygosity, makes it unlikely that variation between individuals in HLA type could account for the large differences in response to sporozoite or gamete antigens.

There has been a report of association between a DR allele and hypermalarious splenomegaly (HMS) (regarded as an atypical response to malaria infection) in Papua New Guinea (Bhatia & Crane, 1985). In the Watut people of Morobe Province (highlanders who have only recently migrated to a malarious region), DR2 is more frequent in patients with gross splenomegaly than in those with moderate splenic enlargement. Since DR2 is very prevalent in coastal Papua New Guineans (who do not have HMS), DR2 itself cannot be the factor responsible for the over-reaction of the immune system which leads to HMS, unless the DR2 alleles are actually of different subtypes. It has been suggested that such a factor was associated with DR2 in the founding population of Papua New Guinea but has been eliminated from present-day coastal populations by selection due to malaria.

We investigated the association between HLA type and antibody responses to malaria sporozoite and gametocyte epitopes in individuals from the Madang area, where malaria is hyperendemic with year-round transmission (Cattani *et al.*, 1986). The sporozoite inoculation rate varies between villages, but ranged between 68 and 526 infectious bites per person per year (Burkot *et al.*, 1988). Malaria prevalence in adults in the area averaged 22·2% for *P. falciparum* and 4·7% for *P. vivax* in 1983–1985 (Graves *et al.*, 1988a).

MATERIALS AND METHODS

Forty-six individuals were recruited for the study from three villages near Madang, on the north coast of Papua New Guinea (17 from Sah, 17 from Agan and 12 from Umuin). Agan and Umuin are located 2 km apart on the coast, and Sah is 9 km inland from these two villages. All individuals were aged over 15 years and those selected in each village were members of two to four related nuclear families. Venous blood samples (45 ml) were drawn; a small aliquot was clotted for serum and the rest was heparinized and air freighted the same day to Goroka, where tissue typing procedures for HLA-A, B, C and DR were performed by micro-lymphocytotoxicity assays as described by Bhatia, Gorogo & Koki (1984).

Table 1. Antigen frequencies in three villages near Madang, Papua New Guinea

	No. Persons with antigen	Antigen frequency
	($n=46$)	
HLA-A		
11	33	0·717
24	27	0·587
w34	21	0·457
HLA-B		
13	11	0·239
27	2	0·043
w56	24	0·522
w60	10	0·217
w61	2	0·043
w62	21	0·457
HLA-C		
w1	7	0·152
w3	4	0·087
w4	17	0·370
w7	24	0·522
Null	8	0·174
HLA-DR	($n=44$)	
1	5	0·114
2	29	0·659
4	18	0·409
w5	19	0·432
w14	4	0·091

Serum from each individual was tested by ELISA for the presence of antibodies recognizing the following antigens: (i) Sonicated total extract of schizonts of the FCQ-27 (Papua New Guinea) strain of *P. falciparum* as described by Graves *et al.* (1989). Sera were tested at 1:1000 dilution. (ii) The circumsporozoite repeat regions of *P. falciparum* and *P. vivax* as described by Ballou *et al.* (1987) and Burkot *et al.* (1989). The sera were tested at 1:100 dilution and the ELISA value for each serum obtained by comparison with a standard curve of serial dilutions of a control serum. Cut-off values for positivity (mean $\pm$ 2 s.d.) were determined from a panel of 32 sera from Brisbane Blood Bank and were as follows: *P. falciparum* IgG 8·0; IgM 5·3; *P. vivax* IgG 4·1; and IgM 2·8. (iii) Two epitopes on the 48/45-kD gamete antigen recognized by the monoclonal antibodies (MoAbs) IIC5-B10 and IA3-B8 (Rener *et al.*, 1983) and one on the 230-kD gamete antigen recognized by MoAb 28F1 (Vermeulen *et al.*, 1985) using MoAb-based competitive ELISA, as described by Graves *et al.* (1988c). Sera were tested at 1:10 dilution against the MoAbs IIC5-B10, IA3-B8 and 28F1. IIC5-B10 and IA3-B8 were conjugated with horseradish peroxidase, and 28F1 was conjugated with biotin and detected with streptavidin peroxidase. The antigen was NP40-extracted, PBS-dialysed gametocyte antigen of the X-10 strain of *P. falciparum* (Walliker *et al.*, 1987) which was used because the FCQ-27 strain does not produce gametocytes in culture. Mean binding in the presence of each serum was expressed as the percentage of control binding, which was the mean of 12 replicates of a pool of

Table 2. Frequencies of HLA class I and class II antigens in groups with and without antibody to *Plasmodium falciparum* circumsporozoite repeat (CSR) region

	Anti-*P. falciparum* sporozoite IgG			Anti-*P. falciparum* sporozoite IgM		
	−	+	*P**	−	+	*P**
	(*n*=18)	(*n*=28)		(*n*=39)	(*n*=7)	
HLA-A						
11	0·722	0·714	0·262	0·718	0·714	0·346
24	0·500	0·643	0·154	0·538	0·857	0·105
w34	0·611	0·357	0·060	0·487	0·286	0·209
HLA-B						
13	0·278	0·214	0·242	0·231	0·286	0·334
27	0·000	0·071	0·365	0·051	0·000	0·716
w56	0·556	0·500	0·902	0·538	0·429	0·277
w60	0·278	0·179	0·207	0·205	0·286	0·317
w61	0·056	0·036	0·487	0·051	0·000	0·716
w62	0·500	0·429	0·098	0·436	0·444	0·399
HLA-C						
w1	0·222	0·107	0·187	0·179	0·000	0·287
w3	0·111	0·071	0·369	0·103	0·000	0·504
w4	0·388	0·357	0·239	0·359	0·429	0·302
w7	0·555	0·500	0·223	0·538	0·429	0·277
Null	0·111	0·214	0·221	0·154	0·286	0·159
HLA-DR	(*n*=18)	(*n*=26)		(*n*=39)	(*n*=5)	
1	0·056	0·154	0·248	0·128	0·000	0·530
2	0·667	0·654	0·193	0·641	0·800	0·328
4	0·444	0·385	0·226	0·410	0·400	0·366
w5	0·389	0·462	0·218	0·385	0·800	0·089
w14	0·167	0·038	0·156	0·103	0·000	0·606

* Fisher's exact test.

Table 3. Frequencies of HLA class I and class II antigens in groups with and without antibody to *Plasmodium vivax* circumsporozoite repeat (CSR) region

	Anti-*P. vivax* sporozoite IgG			Anti-*P. vivax* sporozoite IgM		
	−	+	*P**	−	+	*P**
	(*n*=38)	(*n*=8)		(*n*=41)	(*n*=5)	
HLA-A						
11	0·684	0·875	0·213	0·732	0·600	0·311
24	0·605	0·500	0·261	0·585	0·600	0·365
w34	0·500	0·250	0·143	0·439	0·600	0·291
HLA-B						
13	0·211	0·375	0·205	0·244	0·200	0·420
27	0·026	0·125	0·294	0·049	0·000	0·792
w56	0·579	0·250	0·079	0·439	0·800	0·128
w60	0·211	0·250	0·336	0·220	0·200	0·430
w61	0·026	0·125	0·294	0·049	0·000	0·792
w62	0·421	0·625	0·179	0·488	0·200	0·129
HLA-C						
w1	0·026	0·125	0·413	0·171	0·000	0·420
w3	0·079	0·125	0·414	0·073	0·200	0·327
w4	0·342	0·500	0·217	0·390	0·200	0·295
w7	0·578	0·125	0·079	0·439	0·800	0·128
Null	0·158	0·250	0·296	0·195	0·000	0·366
HLA-DR	(*n*=36)	(*n*=8)		(*n*=39)	(*n*=5)	
1	0·139	0·000	0·347	0·125	0·000	0·530
2	0·667	0·625	0·305	0·692	0·400	0·170
4	0·389	0·500	0·258	0·341	0·600	0·220
w5	0·444	0·375	0·291	0·400	0·600	0·268
w14	0·111	0·000	0·444	0·100	0·000	0·606

* Fisher's exact test.

10 non-immune sera run on the same plate. Each assay was repeated two or three times on separate occasions and the results were averaged. An inhibitory serum was defined as one giving less than the mean binding minus 2 s.d. observed with a panel of 26 sera from Brisbane Blood Bank, i.e. 65·9% of control, and an enhancing serum was defined as one giving more than 134·1% of control binding.

RESULTS

All sera were strongly positive to *P. falciparum* when tested by ELISA to sonicated schizont antigen of the FCQ-27 strain. At 1:1000 dilution, the OD at 410 nm ranged from 0·344 to 1·663. The mean OD of eight negative controls (London Blood Bank sera) was 0·093 ± 0·013 s.d. Mean ODs in Sah, Agan and Umuin villages of 0·796, 0·888 and 0·817, respectively, were not significantly different (one-way ANOVA, $F_{2,43}=0·36$, not significant).

Proportions of individuals recognizing CS proteins by antibody class were *P. falciparum* IgG, 60·7%; *P. falciparum* IgM, 15·2%; *P. vivax* IgG, 17·4%; and *P. vivax* IgM, 10·9%. The mean units and range of antibody activity were *P. falciparum*—IgG, 43·6 (range 8·9–117·8), IgM, 16·2 (10·7–24·3); *P. vivax*—IgG, 17·7 (4·4–29·0), IgM, 11·9 (4·4–20·8). There was no correlation between the anti-*P. falciparum* schizont ELISA value and the units of antisporozoite antibody activity for this species ($r=0·1932$, $P=0·198$).

The proportion of individuals responding to the target epitope of each anti-gamete MoAb (or an overlapping epitope) was as follows: 32·6%, 47·8% and 37·0% of individuals inhibited binding of IIC5-B10, IA3-B8 and 28F1, respectively, and 17·4% and 4·3% enhanced binding of IIC5-B10 and IA3-B8. No enhancement was seen with 28F1. There was no significant correlation between binding of any MoAb in the presence of a serum and the anti-asexual ELISA titre of the serum.

The HLA antigens detected in these subjects are shown in Table 1. Three HLA-A antigens were present in moderate frequencies (A11, A24 and Aw34). HLA-Bw56 and HLA-Bw62 were in the majority, as was HLA-Cw7. There was a high proportion (0·174) of HLA-C null alleles. Only five DR types were encountered, DR1, 2, 4, w5 and w14. Of these, DR2 was the most prevalent but both DR4 and w5 were present at frequencies over 0·4. There were three technical failures of DR typing, but in one of these cases the DR type was assigned from knowledge of the parental haplotypes.

Frequencies of anti-*P. falciparum* CSR antibody positive and negative individuals by each of the HLA alleles at each locus are given in Table 2. Differences between the frequency of responders possessing or not possessing a particular antigen were tested by Fisher's exact test. No significant increase or decrease in responders to *P. falciparum* CSR was detected in any

Table 4. Frequencies of HLA class I and class II antigens in groups with and without antibody to *Plasmodium falciparum* epitopes on 48/45-kD gamete protein

	IIC5-B10				IA3-B8			
	Enhance	Normal	Inhibit	*P**	Enhance	Normal	Inhibit	*P**
	(*n*=8)	(*n*=23)	(*n*=15)		(*n*=2)	(*n*=22)	(*n*=22)	
HLA-A								
11	0·375	0·826	0·733	0·247	0·500	0·682	0·773	0·213
24	0·875	0·478	0·600	0·202	1·000	0·545	0·591	0·228
w34	0·625	0·435	0·400	0·258	0·000	0·500	0·455	1·000
HLA-B								
13	0·250	0·174	0·333	0·163	1·000	0·136	0·273	0·162
27	0·000	0·087	0·000	0·400	0·000	0·091	0·000	0·244
w56	0·875	0·522	0·333	0·141	0·500	0·591	0·455	0·546
w60	0·250	0·304	0·067	0·075	0·000	0·273	0·182	0·220
w61	0·000	0·043	0·067	0·491	0·000	0·000	0·091	0·244
w62	0·000	0·565	0·533	0·256	0·000	0·455	0·500	1·000
HLA-C								
w1	0·000	0·174	0·200	0·319	0·000	0·136	0·182	0·294
w3	0·250	0·087	0·000	0·360	0·000	0·136	0·045	0·250
w4	0·250	0·391	0·400	0·264	1·000	0·318	0·364	0·237
w7	0·875	0·522	0·333	0·141	0·500	0·545	0·500	1·000
Null	0·000	0·174	0·267	0·247	0·000	0·091	0·273	0·097
HLA-DR	(*n*=8)	(*n*=22)	(*n*=14)		(*n*=2)	(*n*=21)	(*n*=21)	
1	0·000	0·136	0·143	0·372	0·000	0·095	0·143	0·328
2	0·750	0·682	0·571	0·222	0·500	0·714	0·619	0·209
4	0·250	0·500	0·357	0·193	0·500	0·429	0·381	0·235
w5	0·875	0·273	0·429	0·179	1·000	0·429	0·381	0·235
w14	0·000	0·136	0·071	0·366	0·000	0·048	0·143	0·250

* Fisher's exact test.

HLA allele group. Results for *P. vivax* were similar (Table 3). Similar analysis for responses to gamete epitopes showed no significant differences in frequencies (Tables 4 and 5). In the case of the antibodies IIC5-B10 and IA3-B8, the comparison was made between the frequencies in those persons inhibiting or not inhibiting the MoAbs; those sera enhancing MoAb binding were excluded.

If lack of response is recessive, the inability of a particular allele to respond will be masked by other alleles present in heterozygotes, and homozygotes will be the only informative subjects. Numbers of responders amongst homozygotes for HLA-A, B and DR are shown in Table 6. HLA-C is not given because the presence of null alleles makes the identification of homozygotes questionable. Both DR2 and 4 homozygotes are capable of responding to each species of sporozoite and to each gamete epitope tested. In the sample there were no DRw5 homozygotes; however, there was no evidence of deficiency in responders to sporozoites or gametes among individuals carrying DRw5 (Tables 2–5).

DISCUSSION

Antibody responses to malaria sporozoite and gamete epitopes have been studied in 46 adults living in a hyperendemic area of Papua New Guinea. Proportions of individuals responding (IgG or IgM) to sporozoite antigens were 60·7% and 23·9% for *P. falciparum* and *P. vivax*, respectively. Between 32·6 and 47·8% of individuals responded to each gamete epitope as assessed by inhibition of MoAbs in ELISA. These proportions are much higher than has been previously reported for *P. falciparum* using immunoprecipitation studies (Graves *et al.*, 1988b). A number of sera enhanced the binding of MoAbs IIC5-B10 and IA3-B8 in the ELISA. This phenomenon has been previously noted (Graves *et al.*, 1988b) and possibly reflects binding of sera to different epitopes on the molecule resulting in conformational changes in the antigen. The phenomenon of enhancement may also be due to reactions between the labelled monoclonal antibody, non-specific factors in the serum and the coated crude antigens.

There is no evidence for association between HLA type and antibody response to the CSR regions of *P. falciparum* and *P. vivax* or epitopes on the 48/45-kD or 230-kD gamete antigens of *P. falciparum*. There were only three HLA-DR antigens present at high frequency (over 0·4) in this population: DR2, 4 and w5. The other two alleles present, DR1 and w14, were at frequencies of below 0·12. All individuals tested possessed at least one DR2, 4 or w5 allele, and 96% of individuals possessed either DR2, 4 or both.

Although the participants in this study were members of three (unrelated) extended families, this does not appear to be the reason for the limited number of alleles detected. Frequencies of HLA antigens detected compare well with previous studies of coastal Papua New Guineans (Crane *et al.*, 1985). The restricted number of alleles present, the presence of HLA-C null

Table 5. Frequencies of HLA class I and class II antigens in groups with and without antibody to *Plasmodium falciparum* epitope on 230 kD gamete protein

	28F1		
	Normal	Inhibit	P*
	(n=29)	(n=17)	
HLA-A			
11	0·690	0·765	0·234
24	0·586	0·588	0·243
w34	0·414	0·529	0·182
HLA-B			
13	0·276	0·176	0·219
27	0·069	0·000	0·392
w56	0·586	0·412	0·127
w60	0·241	0·176	0·260
w61	0·000	0·118	0·131
w62	0·414	0·529	0·182
HLA-C			
w1	0·103	0·235	0·163
w3	0·103	0·059	0·381
w4	0·464	0·294	0·184
w7	0·552	0·471	0·211
Null	0·103	0·294	0·087
HLA-DR	(n=28)	(n=16)	
1	0·107	0·125	0·362
2	0·679	0·625	0·381
4	0·500	0·313	0·184
w5	0·429	0·438	0·211
w14	0·036	0·188	0·087

* Fisher's exact test.

alleles (indicating an allele undetectable with present reagents) and the preponderance of the DR2 allele have all been noted previously.

The responses of homozygotes in our sample were examined since they would be informative if ability to respond is recessive. However, there have been reports of dominant HLA-linked immune suppressive loci in humans (Sasazuki *et al.*, 1980), in which case homozygotes are no more informative than heterozygotes. At least some homozygotes for DR2 and 4 responded to CSR antigens of both malaria species and to the three gamete epitopes tested. There were no DRw5 homozygotes, and thus its ability to respond is unclear, although there is no suggestion of a deficiency of responders amongst DRw5 heterozygotes. However, even if DRw5 was unable to respond, the 96% of the population who possess DR2 or 4 would be expected to be capable of mounting a T helper response to these antigens. These results are very encouraging for the prospects of including an HLA restricted T epitope in a synthetic malaria vaccine, since only three versions would be required to cover the Madang population.

In view of the polymorphism observed in the major human T helper epitope of the *P. falciparum* CS protein (Good *et al.*, 1988b c), it is possible that there is HLA restriction of the response, but that each DR type is responding to a different variant of the T epitope and making antibodies to the same B epitope (the CSR). It is also possible that parasite T epitope variants are circulating which are not recognized by any of the DR types in the human population, although selection pressure by malaria would make this unlikely. The absence of anti-CSR and anti-gamete surface antigen antibody in some highly immune adults may be due to short-lived antibody response and/or low immunogenicity rather than genetically determined inability to respond.

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Table 6. Response of homozygotes for HLA-A, B and DR to the circumsporozoite protein repeats and to gametocyte epitopes

				Sporozoite CSR gametocyte epitopes				
		No. positive to		IIC5-B10		IA3-B8		
	No. homozygotes	*P.f.*	*P.v.*	Enhance	Inhibit	Enhance	Inhibit	28F1 Inhibit
HLA-A								
11	8	6	2	0	4	0	3	2
24	3	2	1	1	0	1	1	0
HLA-B								
13	3	2	1	1	2	1	1	1
w56	13	9	1	4	5	0	6	5
w60	1	0	0	0	0	0	1	1
w62	5	3	0	0	2	0	3	3
HLA-DR								
2	8	6	2	1	4	0	4	1
4	5	2	2	0	4	0	3	3

CSR, circumsporozoite repeat; *P.f.*, *Plasmodium falciparium*; *P.v.*, *Plasmodium vivax*.

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