HLA ANTIGENS AND MALARIA AT SAN LAZARO HOSPITAL MANILA, PHILIPPINES

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INTRODUCTION

Malaria continues to be an important disease in Southeast Asia and elsewhere in the world and *Plasmodium falciparum* remains a primary source of worldwide mortality (WHO, 1974).

Infected individuals exhibit symptoms and signs ranging from a symptomless parasitemia, to mild pyrexia, to renal and liver failure, and to cerebral involvement. Clearly, the pathogenesis and pathophysiology of malaria cannot be simplified in terms of pyrogen activity, increased reticuloendothelial activity and anemia. Certainly, many factors influence an individual's susceptibility or resistance to the malaria parasite.

Increasing interest in the field of malaria research has greatly added to our understanding of malarial infections. In the area of immunology, for instance, it has been suggested that cerebral malaria and the tropical splenomegaly syndrome may be linked to an immune complex disorder (WHO, 1975). Other studies have also looked into the possibility of defining genetic markers such as RBC antigens and other blood group factors which could confer protective immunity to the host against infections. Conclusive evidence has been presented by several investigators on the Duffy factor Fy which correlates with resistance to *P. vivax* infection (Miller *et al.*, 1976). Sickle cell traits among some African populations have also been known to protect these populations from malaria (Allison, 1954). On the other hand, glucose-6-phosphate dehydrogenase deficiency in some populations may trigger hemolytic anemia when exposed to oxidant antimalarial drugs (Luzzatto, 1979).

The possibility that the human leucocyte antigen (HLA) may play a protective role in populations exposed to malaria was suggested by Piazza *et al.*, (1973) and Osoba *et al.*, (1979). The HLA complex has been given much attention lately in relation to disease susceptibility linkages and the present study attempts to further document possible genetic predisposition to malarial infection.

MATERIALS AND METHODS

The population studied included Filipinos who were screened for HLA-A and B antigens for the period covering April 1982 to September 1983. All were in-patients at the San Lazaro Hospital and volunteers. No attempts were made to sex-age match the populations. Possible ethnic differences were eliminated.

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A total of the 211 individuals were included in the study; 68 were confirmed *P. falciparum* cases, 77 were *P. vivax* and 66 considered normal control subjects, i.e. individuals with no known history of malaria. The latter group were from members of the staff, prospective donors to a kidney transplant program and patients seen in the referral hospital for causes other than malaria. Infants, young children and the aged were excluded. All subjects were unrelated individuals but all came from the same homogeneous population.

The diagnosis of malaria was confirmed by the presence of malarial parasites in peripheral blood smears and all were admitted to the malaria ward of the San Lazaro Hospital in Manila. Treated and well patients, seen in the out-patient department for follow-up studies, were also bled and typed.

Lymphocytes for HLA typing were separated from peripheral venous blood samples using density gradient (Histopaque) centrifugation. Viability was determined by the trypan-blue exclusion test and samples with viability of less than 90% were excluded. Samples with yields of less than 6×10^6 of lymphocytes were also excluded from the test.

Histocompatibility antigen typing was done by the standard U.S. National Institutes of Health (NIH) method of lymphocytotoxicity test. HLA antisera used in the study were provided by NIH and the U.S. Navy Tissue Bank, National Naval Medical Center both located in Bethesda, Maryland, U.S.A. Seventy-five selected antisera were available to determine 33 HLA antigens, and of these, 16 were specific for the HLA-A locus antigens and 17 for the HLA-B locus antigens. The A locus antigens represented were A1, 2, 3, 9, 10, 11, 28, 29, w23, w24, w25, w26, w30, w31, w32, and w33 and the B locus antigens, B5, 7, 8, 12, 13, 14, 15, 17, 27, 37, 40, w16, w21, w22, and w4 and w6. Each specificity was defined by at least two sera.

Statistical analysis was done to determine differences in HLA antigen phenotype frequencies between different groups of malaria patients and controls. The chi-square test was employed for this purpose. The test contains continuity correction for small numbers described by Yates. The significance level was arbitrarily set at 0.05.

RESULTS

The Filipino population expectedly showed similar restricted HLA polymorphism reported earlier by other investigators (Payne *et al.*, 1973; Smith *et al.*, 1975; Chan *et al.*, 1979). This restriction involved four A locus antigens (HLA-A2, 9, 10, 11) and five B locus antigens (HLA-B5, 12, 15, w16, 40) accounting for a majority of the HLA-A and B loci. All of the above investigators selected random individuals who were from a homogeneous population coming from the Luzon and the Visayan regions. No minority groups or aborigines were included in their studies.

Table 1 shows the study populations by malaria type and the HLA specificities observed in these individuals.

An interesting result involves the antigen HLA-B27. This phenotype occurred in 10% of *P. vivax* cases and 11% in the general population. Among the *P. falcifarum* cases, however, B27 was absent. This observation was statistically significant, $p < 0.01$.

An examination was made of antigen combinations, i.e., phenotypes appearing for a particular individual and the common antigen combinations in the general populations were A2/A9-A9(w24), A9(w24)/A10, A2/B5, A2/B40, A2/B12, A9-A9(w24)/B40, A9-A9(w24)/B5 and B5/B40. Table 2 presents a representative listing of the frequencies

observed when antigen combinations were tabulated.

Table 1

HLA antigen frequency in study population.

Antigens	*P. vivax* (77)	*P. falciparum*(68)	Control(66)
A1	1*	2	3
A2	58	53	64
A3	0	0	0
A9	74	69	64
Aw23	15	19	6
Aw24	35	38	26
unspecified	23	12	32
A10	35	46	33
Aw25	1	0	0
Aw26	9	18	6
unspecified	25	28	27
A11	15	21	12
A28	0	0	3
A29	0	0	0
Aw30	0	2	0
Aw31	0	0	0
Aw32	0	0	0
Aw33	0	0	0
B5**	26	38	33
B7	1	7	3
B8	4	3	3
B12**	20	18	11
B13	3	4	5
B14	0	0	0
B15	14	13	11
B17	7	4	2
B18	1	3	0
B27	10	0***	11
Bw37	0	0	0
B40	49	52	64
Bw16**	21	15	14
Bw21**	3	12	5
Bw22	1	0	2

*Phenotype frequency (%).

**Individuals with these supertypic specificities could not be clearly assigned to the associated subspecificities.

***$p \leq 0.05$, $p \leq 0.01$. (Fisher's ezact test).

Table 2

HLA antigen combination frequencies (representative list).

Antigen combinations	*P. vivax*	*P. falciparum*	Control
A2/A9	9	7	15
A2/A9(w23)	8	5	2
A2/A9(w24)	12	12	7
A2/A10	9	4	7
A2/A11	3	4	3
A9/A10	4	1	2
w23/A10	1	3	0
w24/A10	5	8	5
A10/A11	1	2	0
A2/B5	10	17	16
A2/B17	5	3	1
A2/B40	21	16	25
A2/Bw16	6	7	4
A2/B27	4	0	6
A2/B15	5	6	3
A2/B12	11	9	6
A9/Bw16	4	3	4
A9/B5	4	6	6
A9/B40	10	6	15
w23/B5	8	7	3
w24/B40	12	15	13
w24/B15	6	4	2
w24/Bw16	8	4	2
w24/B12	5	4	1
w24/B5	3	11*	5
A10/B5	2	6	7
A10/B15	2	1	5
A10/Bw21	1	4	3
w26/B40	3	5	4
A11/Bw16	4	3	2
A11/B40	7	4	5
B5/B40	11	10	11
B15/B40	4	4	3
B16/B40	7	7	2

*$p \leq 0.05$.

A comparison was made of the frequencies of antigen combinations in the general populations with those in the malaria groups and some combinations were found to occur more or less frequently than in the control group. These were found to be statistically significant but because the population size was not large enough, no inference can be considered valid.

The combination A9(w24)B5 was significantly higher among the *P. falciparum* group than that found in the *P. vivax* group and the control group. Eleven in the *P. falciparum* group had this antigen combination compared to only 3 in the *P. vivax* group and 5 in the control group.

DISCUSSION

To define in more concrete terms the genetic influence of an individual's susceptibility to a disease, susceptibility or resistance needs to be a result of a single gene defect. Further, the gene products, of which HLA marker is one, must if incriminated, be describable and identifiable. In many disease entities, defining specific genetic influence is difficult if not impossible because of the numerous factors which tend to determine the response of a host to a disease. Infectious diseases, in particular, are characterized by the interplay of various variables between the host and parasite or pathogen.

Certain HLA antigens have been well documented to assume a direct association with susceptibility to a disease. In some cases, the relationships are so strong that HLA typing may play a role in diagnosis. Autoimmune diseases and rheumatologic disorders are examples of diseases known to have such specific associations. Lesser associations involving studies with infectious diseases have been determined and results of these investigations have been equivocal except in a few diseases (Kaslow and Shaw, 1981).

HLA-B27 has been implicated in several disease associations particularly in rheumatologic disorders. This includes ankylosing spondylitis, Reiter's disease, psoriatic arthritis, juvenile rheumatoid arthritis, and *yersinia* arthritis (Braun, 1979). In some cases, e.g. ankylosing spondylitis and Reiter's disease, the association is so well defined that typing for B27 has been resorted to as a diagnostic aid. In general, increased frequency of the antigen was associated with disease susceptibility. In the present study, decreased or absent frequency of B27 was seen with increased susceptibility to acquiring *P. falciparum* infection. There is statistical evidence for this, however, since the sample size is small, no valid conclusion can be made without regard to the probability of chance.

The frequencies for antigen combinations are rather small when compared to frequencies of antigens taken singly. This is because it is not possible to detect antigens which are undefined by current methods. HLA-A and B loci antigens are not by any means completely defined and the procurement of specific antisera has not made this task easier. Also, sera defined in a particular ethnic group may be a non-specific reactor when tested with another ethnic group.

The much more complex systems involving interactions between the host and infectious pathogens can not be explained directly by current methods used in determining subtle genetic influences of the host. Statistical analysis can not be relied upon solely to detect specific associations. Credibility can only be given statistics if large sample sizes of unrelated individuals are used for the study. The probability of chance associations can be great if fewer sample sizes are employed. Further, detailed family studies are also required to define subtle influences of genetic

susceptibility to the acquisition of clinical expression of such diseases. Further studies need to be done before conclusive inferences are made to define associations.

Whereas, these preliminary results can not be construed to give concrete evidence for association between HLA antigen specificities and susceptibility to malaria infection, there are at least enough grounds to warrant further studies. Sample sizes have to be expanded further and family studies are needed. It is also clear from other studies such as in leprosy (de Vries *et al.*, 1980; Fine *et al.*, 1979) that the more recently defined DR locus antigens may be stronger indicators of linkage between the HLA region and disease susceptibility.

SUMMARY

Human leucocyte antigens (HLA) were used as genetic markers in an attempt to determine possible host genetic susceptibility or resistance to malarial infections. HLA-A and B typing on lymphocytes from 68 confirmed *P. falciparum* and 77 *P. vivax* cases was compared with that found in 66 control subjects with no known history of malaria. A significant deviation was observed in the distribution of HLA-B27. This phenotype was absent in the *P. falciparum* group although found present in the *P. vivax* group (10%) and the control group (11%). Also, the combination of A9(w24) and B5 was significantly higher among the *P. falciparum* group than that found in the *P. vivax* and control groups. These findings require confirmation but do suggest the possibility of genetic susceptibility and that extensive genetic studies might be worth investigating.

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