Studies on humoral and cellular immune responses in humans from areas where *Plasmodium falciparum* malaria is endemic

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Epidemiological studies, based on longitudinal surveys of human communities from endemic areas of Senegal and Brazil, have provided more precise criteria for defining premunition. Humoral and cellular immune responses of individuals with different levels of premunition have been investigated using recombinant antigens and peptides corresponding to vaccine-candidate antigens.

FIELD STUDIES ON ANTI-DISEASE AND ANTI-PARASITE PREMUNITION

The present analysis of the protective role of parasite antigens is essentially based on comparative studies of the immune responses in protected and susceptible individuals exposed to malarial infection. These studies are based on longitudinal, clinical-parasitological surveys, with continuous follow-up, of human populations from two endemic areas: (1) two villages (Dielmo and N'Diop) in a holo- and hyper-endemic area of Senegal (Fontenille *et al.*, 1997); and (2) three sites (Candeias, Urupa and Porto Chuelo) in Rondonia, Brazil, which experience hypo-endemic malaria with epidemic episodes (Camargo *et al.*, 1994). Although many previous studies have used age as the main criterion in the definition of the development of premunition, the initial results of the surveys in Senegal and Brazil have shown the limitations of this approach and indicated more precise criteria.

For example, although the two study villages in Senegal, in the Side Saloum area, are only 5 km apart, their populations differ considerably in terms of malarial epidemiology. The presence of a permanent stream running through Dielmo provides permanent breeding sites for *Anopheles* mosquitoes (*Fontenille et al.*, 1997) and these, in turn, permit intense and perennial malarial transmission (around 200 infective bites/person-year). In N'Diop, however, there is no such stream and intense transmission only occurs in the rainy season (around 20 infective bites/person-year, con-
centrated in the 4-month rainy period). Not surprisingly, the annual incidences of malaria attacks and parasite indexes both differ considerably between villages. Although adults from N'Diop have relatively more malaria attacks, the young children from Dielmo (aged <5 years) have twice as many attacks as their counterparts in N'Diop. In N'Diop, children aged >5 years continue to have a high frequency of malaria attacks until they are 12–14 years old (Rogier and Trape, 1996; Trape and Rogier, 1996).

Another interesting observation concerns the evolution of clinical immunity. It is accepted that premunition is characterized by a decrease in the number of malaria attacks. Attempts were made to see if, as this age-dependent immunity developed, the intensity of malaria-related symptoms (sudoresis, vomiting etc) and signs (temperature) also decreased in the immune adults. However, detailed studies only indicated a reduction in the duration of the symptoms (unpubl. obs.).

Other observations concern the non-specificity of clinical immunity conferred by malarial parasites. In Dielmo, for example, clinical attacks of Plasmodium malariae malaria are quite rare even though the prevalence of P. malariae in the blood of the local children is high (Trape et al., 1994). Similarly in Rondonia, although clear-cut premunition was not apparent in various cross-sectional surveys (Camargo et al., 1996), the results of clinical investigations indicate interference between P. falciparum and P. vivax infections (unpubl. obs.).

In the light of these and other observations, the immune status (at the level of clinical and anti-parasite immunity) is now defined individually, in relation to the evolution of the infection in the child or adult (asymptomatic or symptomatic; stable or unstable parasitaemia) in the periods before and after the collection of sera and/or cells.

**HLA TYPING**

After typing the HLA-class-I antigens in the villagers from Dielmo, HLA-A, -B, -C, -DR and -DQ were investigated in 116 from the Serere ethnic group in the village (Dieye et al., 1996). The frequencies of distribution of the 25 different alleles identified in the Dielmo Serere were not found to be significantly different from those previously recorded for Mandinka groups from Senegal and for Serere and Mandinka groups from The Gambia.

**IMMUNOLOGICAL STUDIES IN THE ENDEMIC AREAS**

Recently, the studies in Dielmo have focused on the characterization of the isotype-specific antibody responses against total and specific P. falciparum antigens, because it has been shown that the protective antibodies are not neutralizing antibodies but cytophilic (i.e. they bind to the Fc receptors of macrophages) and that the observed anti-parasite activity depends on the monocyte-mediated, antibody-dependent mechanisms of the ADC1 and/or opsonization/phagocytosis (Dubois and Pereira da Silva, 1995). The antigens studied were Pf72 (HSP70), Pf332, R45 and AARP I (all isolated and characterized at the Institut Pasteur; Dubois and Pereira da Silva, 1995), and MSP-1 (isolated and characterized at the National Institute for Medical Research; Holder, 1988). Serum samples from three, cross-sectional surveys in Dielmo (representing 145 villagers of all age groups) were used for analysis of antibody isotypes. Initially, total antigen of P. falciparum was used for measuring total antimalarial antibodies of IgM and IgG classes and of IgG subclasses, by ELISA.

Adults were found to have higher levels of specific antibodies than children, IgM, IgG2 and IgG3 accounting for the difference. Levels of specific IgG1, IgG2, IgG3 and IgG4 differed significantly between the period of lowest transmission and the period of highest transmission (reflecting a 20-fold difference in the number of infective bites/person-night). No particular isotype distribution pattern was apparently associated with any given level of parasitaemia. The relationship between optical density (OD) in the ELISA for each isotype
and the risk of clinical malarial attack (observed in the period following collection of the relevant serum sample) was tested using a Poisson regression model. Only increases in the OD for IgG₃ were found to be associated with a significant reduced risk of an attack (Aribot et al., 1996). Although the specific activity of total IgG is therefore not indicative of any given level of protection against malaria, that of IgG₃ alone is significantly associated with relative susceptibility. Antigen-specific IgG₃ levels are now being analysed, and the preliminary results indicate an increase in anti-R45 and anti-MSP-3 antibodies of the IgG₃ isotype in individuals with reduced risk of malaria attacks. The measurement of antibodies against the different fragments of the C-terminal part of the MSP-1 antigens, particularly the 42- and 19-kDa processing products (Blackman et al., 1991), is also in progress. Recent studies have also been concentrated on the analysis of the in-vitro effects of human antibodies purified from the blood of residents from the endemic areas. Competitive experiments are being performed in which the purified IgG and monoclonal antibodies directed against different regions of the MSP-1 molecule can be compared and their effects on the invasion of red blood cells by merozoites can be investigated.

The Rondonia samples analysed so far show an increase in the level of antibodies against E200 (Pf332) and Pf72 antigens with increasing age and exposure to malarial infections. However, no associations are yet apparent between levels of antibody to any antigen and premunition, although isotype analysis has only just begun.

REFERENCES


