HLA Class I-associated Resistance to Severe Malaria:

A Parasitological Re-assessment

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The first convincing evidence for a pathogen-driven selection of major histocompatibility complex (MHC) genes was recently reported by Hill and colleagues[1], who showed that the human leukocyte antigen HLA-B53 gene frequency was higher in malaria-endemic areas than in non-endemic areas, this being related to the observation that severe manifestations of malaria were significantly less frequent in people expressing the HLA-B53 antigen.

This observation has met with considerable interest, and debates have ensued about the actual mechanisms that could increase resistance to severe malaria and be controlled by a given HLA-class I molecule[2]. The hypothesis proposed by the authors, and supported to some extent by experimental evidence in murine malaria, relies on an effector mechanism active upon the liver stages, based on the evidence that the intra-hepatic stage is the only one in the parasite life cycle that lies within a cell expressing MHC class I molecules[3]. They proposed, very logically in molecular terms, that MHC class I-restricted cytotoxic T lymphocytes (CTL) specific for a malaria peptide able to associate preferentially with B53 could be responsible for an increased defense against malaria in such individuals, and indeed identified CTLs specific for a highly conserved motif of the liver stage antigen-1 (LSA1)[4].

In parasitological terms, it is difficult, at first glance, to accept this interpretation. Cerebral malaria is universally considered to be the consequence of intraerythrocytic parasitism, either by direct mechanisms (by adherence to capillary wall cells, by agglutination or by rosette formation of mature forms) or indirectly, by triggering the release of various cytokines or other mediators among which tumor necrosis factor (TNF) and nitric oxide ($NO^{\cdot}$) are considered to be the most crucial (for a review, see Ref. 5). In contrast, the intra-hepatic parasitism has never been implicated in the pathology of the disease and, moreover, the parasite load in liver is exceedingly low as compared with that in blood: it is now considered that an infected mosquito injects, on average, 10–15 sporozoites into the bloodstream, and that these result in the formation of approximately 3–7 liver schizonts. This contrasts with the 10^9–10^{11} parasites per liter found in infected blood. Therefore, while the idea that MHC class I-restricted immune responses may act upon the liver stages is logical in molecular terms, their potential influence upon whether or not cerebral complications occur calls for an additional hypothesis: that not all parasite isolates are capable of inducing neurological disorders, but only a proportion of them could do so. This hypothesis appears to be highly likely, but remains to be established unequivocally. In this case, improved defenses against liver stages associated with B53 would lead to the destruction in the liver of a greater proportion of the parasite isolates inoculated by mosquitoes into B53-positive compared with B53-negative individuals. This, in turn, would decrease the proportion of parasite isolates in the bloodstream that might induce cerebral malaria.

We have attempted to investigate this hypothesis at a parasitological level, indirectly, by evaluating resistance to sporozoite inoculation, ie. the frequency of passage of *Plasmodium falciparum* through the liver, in individuals living in an endemic area[6] where the HLA-B53 phenotype is frequent.

Resistance to re-infection by sporozoites in subjects exposed to infected mosquitoes was used as one means to evaluate indirectly the immune defense mechanisms operating at the pre-erythrocytic level, namely against sporozoite and liver stages. In the village of Dielmo (Senegal) where the sporozoite indexes and number of mosquito bites per individual per night has been determined over 40 months by indoor and outdoor human bait captures as described in Ref. 6 (and were further monitored during the four-month study period), we administered a radical cure of bloodstage parasites by quinine (25 mg kg^{-1} per day over seven days) in 165 individuals. We then measured the delay of emergence of asexual parasites in the blood. Quinine has the advantage over other antimalarial drugs of being fully effective in the isolates from the area studied (our unpublished studies), and of having a very short half-life in the body, so the outcome of new infections a few days after the end of treatment would not be influenced.

Entomological data, which present seasonal variations, showed that individuals received an average of 2.91 infective bites per person per week during the four-month study period. Nevertheless, the prevalence of new blood parasite infection within the first two weeks post-treatment was extremely low. Results available over 14 weeks in 147 individuals (Fig. 1) show that most of the individuals studied successfully resisted a large number of sporozoite inocula before becoming positive on thick smears. The mean delay of re-positivation of blood smears was six weeks, and 10% of the cohort studied resisted natural challenges for more than 14 weeks; this corresponds theoretically to 41 consecutive challenges by infective mosquito bites, on average, per individual. There was no relationship between the duration of this delay and either the type of housing or the location of the house in the village, ie. no marked segregation among the various families within the village (data not shown).

The human leukocyte antigens A, B, C, DR and DQ were determined by immunological methods[7]. B53-positive individuals represented 27% of the cohort studied (40 of 147 individuals), a figure very close to the 28% prevalence observed in the nearby Gambian villages studied by Hill *et al.*[1], with no particular imbalance between the various age groups ($\chi^2 = 0.28$; $p = 0.83$). From a parasitological stance, ie. in terms of resistance to sporozoite challenges (Fig. 1), we did not find any statistically significant advantage of harboring the B53 phenotype as compared with other alleles (Logrank test, $p = 0.89$).

Our results, therefore, do not bring parasitological support to the molecular hypothesis that HLA-B53-restricted immune responses against LS antigens would reduce the proportion of new infections emerging from the liver. It should be noted that these results do

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0169-4758/97/$17.00 PII: S0169-4758(96)20062-6

not allow us fully to rule out the initial hypothesis of Hill *et al.* A selective advantage against cerebral malaria mediated by CTL acting upon liver schizonts in B53-positive individuals still remains possible, although it would require an additional hypothesis: that the LS antigens expressed by neurotropic isolates, or neurotropic clones, are presented to the immune system optimally, or solely, by the HLA-B53 molecule, as compared with the antigens from isolates inducing milder forms of malaria. There is no indication yet in favor of particular antigenic differences between isolates found in cerebral and non-cerebral cases. Moreover, for the LS antigens studied to date, only limited or no polymorphism has been found, as compared with many bloodstage antigens particularly in the regions targeted by CTLs (Ref. 4; and P. Druilhe *et al.*, unpublished).

Our experimental measurements may require further consideration, for example the delay of re-positivation following cure may reflect not only an immunity against the pre-erythrocytic stages, but also against asexual bloodstages. We agree that this issue remains to be clarified. However, if the delay of emergence is, at least, the sum of anti-liver stage and anti-bloodstage immunity, then the hypothesis that the defense against the liver stage is increased in B53-positive individuals would be valid only provided it is associated with reduced defences against bloodstages in order to fit with the data we obtained. This is unlikely both in statistical terms and mostly because B53-positive individuals were also at lower risk of severe anemia[1].

Our study was aimed at exploring one of the hypotheses for HLA-restricted disease association that had been proposed and recently supported by epidemiological evidence[1]. However, we have examined only the occurrence of any bloodstage parasite and could not study the occurrence of parasites inducing neurological disorders since these cannot presently be distinguished by any marker (and because the study design in Dielmo included curing malaria in the first hours after detection or presentation of any given case). Others have suggested quite different interpretations of the same data[2] (eg. a reduced risk of developing pathological, rather than protective, immune responses). However, in view of the major role attributed to antibodies, most of these alternative hypotheses would deal with MHC class II-dependent responses. It also remains possible that our knowledge of the implication of MHC class I molecules in immunological defense mechanisms is, as yet, insufficient (eg. they may participate in mechanisms other than CTL) to provide an understanding of their influence under those circumstances. The co-segregation of a particular MHC class-I allele with other genetically dependent factors remains an attractive alternative explanation. This hypothesis was recently explored for the TNF-2 gene promoter which is responsible for a high TNF-α production phenotype[8]. Only a weak, statistically non-significant, negative association was found between TNF-2 and HLA-B53 alleles in this particular study. This remains an interesting area of research, however, as TNF in general and particularly the TNF-2 promoter appears strongly associated with the severity of the disease[8] and also because TNF was recently found to be a crucial mediator in the monocyte-mediated antibody-dependent mechanism of defense against asexual bloodstages[9].

In addition, the influence of B53 may have been masked by other factors known or suspected to influence the course of liver schizont development. These include interferon gamma (IFN-γ) (often found in high concentrations in serum from malaria-endemic areas), MHC class II-restricted antibody responses to sporozoite and/or LS antigens, MHC class II-restricted production of cell granuloma around live schizonts, etc. However, there is no indication that these mechanisms would be less prevalent in B53 individuals than in the remainder of the population.

By leading to the identification of LSA1-specific CTL epitopes able to associate with the B53 molecule, the epidemiological study conducted by Hill *et al.*[1] has been invaluable in substantiating and attracting attention to the role that CTL cells could play in defense against *P. falciparum*-infected hepatocytes. However, we believe that the inference that this can, in turn reduce the incidence of severe malaria remains to be more firmly established.

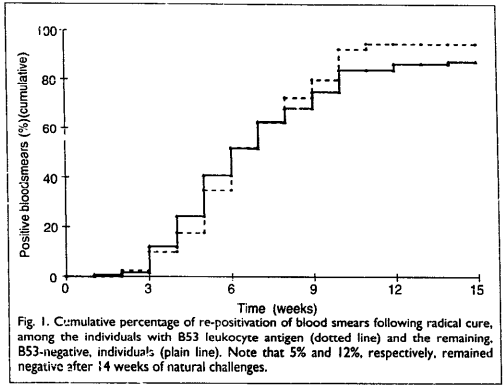

Fig. 1. Cumulative percentage of re-positivation of blood smears following radical cure, among the individuals with B53 leukocyte antigen (dotted line) and the remaining, B53-negative, individuals (plain line). Note that 5% and 12%, respectively, remained negative after 14 weeks of natural challenges.

Acknowledgements
This work received support from the Ministere de la Coopération (grant No. 93-A48) and the EC-std3 program (grant No. CT92-0053).

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