intended to provide a flavour of the work presented at the meeting, and there were many other excellent presentations and posters, a Saturday night ceilidh, and some bright sunshine. After two extremely successful years, this meeting seems to be destined to become an annual event. The generous sponsorship of the following organizations made the meeting possible: British Society for Immunology; Elsevier Trends Journals; Greiner Labortechnik; Bio-Rad; Life technologies; Canberra-Packard; Jouan; Kodak-IBI; and Sigma.

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Comment

Antigenic Diversity and the Transmission Dynamics of *Plasmodium falciparum*: The Clonality/Sexuality Debate Revisited

M. Tibayrenc Michel

A few years ago, the publication of the clonal theory of parasitic protozoa[1] generated a lively debate[2–4], mainly focused on the particular case of *Plasmodium falciparum*. Actually, in that preliminary work[1], this parasite (considered classically as a sexual species) had been selected as a counterexample. Surprisingly, in the few data suitable for population genetics analysis, a notable linkage disequilibrium (nonrandom association of genotypes occurring at different loci) was apparent. This is a classical indication that the populations under study do not undergo random mating. Consequently, the hypothesis of discrete genetic lines in the causative agent of malaria has been cautiously considered[1], with the proposal that linkage could be explained (apart from insufficient sampling) either by the co-existence of uniparental and biparental lineages, or by the presence of sexual cryptic species within the taxon *P. falciparum*. On the other hand, the sense used in population genetics for the term 'clonality' made the hypothesis of uniparental propagation 'acceptable' even in the case of *P. falciparum*. Indeed, in a population-genetics view, a species is clonal when the progeny is genetically identical to the reproducing individual[2], whatever the mating system involved. Several reproduction mechanisms involve meiosis without actual genetic recombination; these include some cases of parthenogenesis, gynogenesis and self-fertilization in haploid organisms[2]. The last mode of reproduction cited (selfing), presented as an alternative hypothesis to the clonal model in the case of *P. falciparum*[4], is actually a particular case of clonality.

Presently, for *P. falciparum*, the currently accepted working hypothesis remains the potentially panmictic model[3], which states that the only possible obstacles to gene flow among parasitic populations are either geographical or temporal, as in the human species. The epidemiological implications of this model are considerable: according to the model, the parasite genotypes are ephemeral individual variants that last only one generation or a few generations. Within the species *P. falciparum*, it is therefore vain to try to distinguish discrete parasite lines that would be genetically isolated from each other. Especially in sympatry (occurrence in the same geographical place), such discrete lines simply cannot exist.

Now in the work by Gupta *et al.* (Ref. 5, and S. Gupta and K.P. Day, this issue), PIESA (parasite induced erythrocyte surface antigen) seroconversion patterns are explained by the existence, in sympatry and in a highly endemic area of Papua New Guinea, of 'independently transmitted strains'. The fact that the antigenic response is isolate-specific 'indicates that there is limited overlap between the variant repertoires of the isolates'. The antigenic diversity is 'reflected by the multitude of strains constituting malaria'.

It has to be clearly stated that these assumptions do question the panmictic model[3]. Indeed if *P. falciparum* was panmictic in that area, the co-existence of such 'independently transmitted strains' would be puzzling. Both sympatry and high endemicity in the parasite population under survey should increase the probability of cross-fertilization between different parasite genotypes, while the 'limited overlap between their variant repertoires' suggests, on the contrary, that these independent strains do not regularly shuffle their genes. Hence, the work by Gupta *et al.* (Ref. 5, and S. Gupta and K.P. Day, this issue) fits well the 'non-panmictic' hypothesis[1,2], that is to say: existence, in *P. falciparum* populations, of discrete, stable multilocus genotypes, whatever their origin (either uniparental propagation, or cryptic speciation, or other as yet unknown reason). Gupta and Day do not favor this hypothesis as a possible explanation of their results, and rather give to the term 'strain' a functional sense, with respect to the host immune response to polymorphic loci. Nevertheless, to make the PIESA model compatible with panmictic assumptions, one would have to accept that this antigen variability is driven by either 'only one gene, or a few, tightly linked genes'. If PIESA genetic background involved 'a large number of unlinked genes', this set of genes should be shuffled apart every generation, which is incompatible with the permanence of independently transmitted strains. The comparison with *Trypanosoma brucei* (S. Gupta and K.P. Day, this issue) is especially telling. Indeed, strong evidence[1,2,6] suggests that this species exhibits a clonal population structure, which can conveniently explain *T. brucei* serodeme circulation pattern mentioned by Gupta and Day to illustrate their model.

Although its goal was different, the work by Gupta *et al.* (Ref. 5, and S. Gupta and K.P. Day, this issue) leads to relaunch the sexuality/clonality debate in

P. falciparum. To my mind, the question of *P. falciparum* population structure is simply an unsettled one: results obtained in our laboratory (B. Abderrazak, PhD Thesis, University of Montpellier, France, 1993) do confirm, in several populations of this parasite, clear linkage disequilibriums that cannot compare with the trivial disequilibriums caused in sexual species (humans for example) by geographical distance and genetic drift. Nevertheless, it is obvious that *P. falciparum* population structure is different from the population structures observed in other species, such as *Giardia*, *Leishmania*, *Toxoplasma* and *Trypanosoma*[1,2]: in the agent of malaria, the linkage mainly involves a limited number of genes, and the levels of significance of the statistical tests exploring linkage are lower than in these other species.

Clearly, the data presently available are far from sufficient to settle the matter. The considerable epidemiological and medical implications of the problem under debate make it worthwhile to undertake all necessary studies to fill this surprising gap in our knowledge of the most fearsome human parasite. Analyzing meiosis products of *P. falciparum* zygotes in the mosquito vector is a valuable approach[7] that will give useful information on this parasite's actual rate of self-fertilization. It appears that in some cases, this rate is high (R. Paul, M. Packer and K.P. Day, unpublished), which favors clonal propagation. Nevertheless, the actual impact of a high selfing rate, downstream from the mosquito, is unforeseeable, and definitely needs to be explored by a 'conventional' population genetic approach[1,2] (B. Abderrazak, op. cit.). Indeed what matters for the epidemiologist is *P. falciparum* population structure in the human host, rather than meiosis in the mosquito. Such a classical population genetics framework will provide a convenient base to test finely the innovative hypotheses proposed by Gupta *et al.*, and will make it possible to decide whether PIESA seroconversion patterns are imputable, totally or partly, to any kind of genetic isolation (either uniparental propagation or cryptic speciation)[1,2]. If this non-panmictic model is verified, its implications will involve not only the PIESA genes, but also the whole genome variability of *P. falciparum*.

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Outlook

Immunity to Malaria Elicited by Hybrid Hepatitis B Virus Core Particles Carrying Circumsporozoite Protein Epitopes

F. Schodel, R. Wirtz, D. Peterson, J. Hughes, R. Warren, J. Sadoff and D. Milich *J. Exp. Med.* 180, 1037–1046

Animals and humans can be protected against malaria by immunization with irradiated sporozoites. The dominant B-cell epitopes of the circumsporozoite (CS) antigens responsible have been identified in repeat regions, and synthetic and recombinant peptides have been prepared as vaccine candidates. When these have been coupled to tetanus toxin, the level of protection against *Plasmodium falciparum* challenge has been low.

Multiply branched CS-derived peptides and multiple antigen peptide systems have been synthesized which elicit high-titre CS repeat-specific antibodies. Schodel and colleagues here report what happens when they insert *P. falciparum* and *P. berghei* CS repeat epitopes into recombinant hybrid hepatitis B virus nucleocapsid antigen (HBcAg) particles.

HBcAg is a particle composed of 180 subunits of a single 21.5 kDa polypeptide, which is highly immunogenic in humans and in experimental animal models. It has been used as a carrier for chemically coupled and recombinant translationally fused peptide epitopes. An internal position has been identified which allows the insertion of heterologous B-cell epitopes without interfering with particle assembly. These inserted epitopes become surface accessible and highly immunogenic, at the same time as reducing HBcAg-specific antigenicity and immunogenicity.

The B- and T-cell immunogenicity were analysed, with a view to answering the following questions: (1) does immunization induce protective immunity against challenge with *P. berghei* in mice? (2) is HBcAg–CS immunogenic in adjuvant? (3) how does pre-existing high-titre, anti-carrier-specific antibodies affect the immunogenicity of HBcAg–CS particles? and (4) how do you explain helper T-cell function? The answers suggest that recombinant HBcAg–CS particles may become a component of future malaria vaccines. ■

Outlook was compiled by T. Saklatvala.