The epidemiology of multiple *Plasmodium falciparum* infections

1. General introduction

M. Tanner*, H.-P. Beck, I. Felger and T. Smith *Swiss Tropical Institute, Socinstrasse 57, 4002 Basel, Switzerland*

Keywords: malaria, *Plasmodium falciparum*, multiple infection, Tanzania

The dynamics and determinants of the development of host defences and immunity to malaria have intrigued researchers from the moment when the parasite's life cycle was understood. Soon after Ross's and Grassi's discoveries, clinicians described exposure-related immunity. Two distinct terms were brought into the discussion of resistance to malaria: (i) *semi-immunity*, referring to a situation of acquired immunity as a consequence of continual exposure controlling infection and leading to low-level chronic parasitaemia, but clearing the infections (ROSS, 1910; reviewed by MCGREGOR, 1986), and (ii) *premunition*, describing protection against superinfection resulting from existing infection (SERGENT & PARROT, 1935). However, the understanding of these 2 phenomena and their interrelationship is still incomplete. This is reflected in the way these terms are often used incoherently, despite their importance for the comprehension of malaria endemicity and protection.

Classical malariological methods have been applied in comprehensive epidemiological projects to study transmission, most effectively in the Garki project (MOLINEAUX & GRAMICCIA, 1980), but have not been able to elucidate the determinants of premunity and semi-immunity. Molecular techniques now offer many new possibilities to generate a deeper understanding of host–parasite interactions and have also been used effectively to enhance our understanding of parasite population biology (reviewed by PAUL & DAY, 1998). These techniques also guide current approaches in vaccine development and/or the characterization of individual parasites in experimental systems or in humans. While our understanding of both host and parasite at the individual level has consequently grown, understanding of premunition and semi-immunity, or acquired immunity, remains fragmentary, particularly at the population level.

Up to now, typing of *Plasmodium falciparum* in human hosts has been concentrated mainly on the diversity of the parasite population (e.g., CONWAY & MCBRIDE, 1991; ARNOT *et al.*, 1994; BABIKER *et al.*, 1995) and the search for markers of parasite virulence, showing that members of some genotype families appear to be associated with morbidity (ENGELBRECHT *et al.*, 1995; ROBERT *et al.*, 1996; KUN *et al.*, 1998). Studies using multi-locus genotyping described complex patterns of multiple infections undergoing rapid change (FÄRNERT *et al.*, 1997). However, few of these studies have quantified the complexity or its dynamics, or compared different population groups. More recently, a series of epidemiological studies in Senegal, Tanzania and Papua New Guinea has indicated the importance of multiplicity of infection, i.e., the number of co-infecting parasite genotypes (CONTAMIN *et al.*, 1995; NTOUMI *et al.*, 1995; AL-YAMAN *et al.*, 1997; BECK *et al.*, 1997). These studies established that (i) the multiplicity of infection within a host appears to depend not only on exposure but also on age, (ii) multiplicity can reach high levels, i.e., up to at least 9 different parasite clones at a given time in one single host, and (iii) multiplicity of infection can be positively associated with protection against mild episodes of malaria.

The Kilombero valley in the Morogoro Region of south-eastern Tanzania is well known as an area of high perennial malaria transmission. Given the great importance of malaria as a public health problem in such situations of very high endemicity, typical of sub-saharan Africa, numerous basic and applied research projects have been undertaken there, ranging from descriptive and analytical to intervention studies, including malaria vaccine trials and trials of different prophylactic regimens and drugs (TANNER *et al.*, 1991; SMITH *et al.*, 1993; ALONSO *et al.*, 1994; HURT *et al.*, 1995; MENENDEZ *et al.*, 1997; HATZ *et al.*, 1998). Most of these studies were community-based and aimed at understanding and quantifying the risks for mild and severe malaria morbidity and at designing strategies that could reduce these risks. Consequently, the studies covered all age groups of the population, but focused on infants and children (KITUA *et al.*, 1996, 1997; MENENDEZ *et al.*, 1997), i.e., those at highest risk and those who are in the process of developing acquired immunity in the complex interplay between (i) loss of fetal and maternal protection, (ii) development of the immune system and (iii) regular challenge with *P. falciparum* infections of different genotypic make-up. The community-based and longitudinal nature of the studies undertaken so far in this area of intense perennial transmission provided the basis for an epidemiological approach to an understanding of the dynamics of multiple *P. falciparum* infections. Large cohorts of infants were followed through their first year of life and later through their childhood and adolescence.

The multiplicity of *P. falciparum* infections was measured and analysed using the highly polymorphic merozoite surface protein 2 locus (*msp2*) of *P. falciparum* as marker gene. Thus, it was possible not only to undertake an in-depth and longitudinal analysis of the dynamics of multiple infections at population level, but also to revisit the concept of premunition. As a result of these studies, we put forward the hypothesis that in young infants host defence against blood-stage infections with *P. falciparum* relies mainly on fever and related cytokine activities, and infections are of relatively short duration. In older children, high multiplicity of *P. falciparum* infection is the feature of chronic, low-level parasitaemia. This in turn appears to confer cross-protection against newly inoculated parasites based on partially genotype-specific responses, that might last only a little longer than the infections themselves. The following series of papers develops this hypothesis, based on the comprehensive analysis of all studies undertaken in the Kilombero valley over the past 10 years compared with findings from other sites of similar or distinctly different endemicity.

Besides suggesting this hypothesis, the findings also highlight the extent to which epidemiological analyses of multiple infections—beyond descriptive presentations of genetic polymorphism—can contribute to insight into the evolution and ecology of *P. falciparum*. There is certainly a need for theoretical studies in this area, and fieldwork on competition between genotypes. This may also trigger the formulation of hypotheses for further research on immunological effector mechanisms, on integrated genetic epidemiology (TIBAYRENC, 1998), and on the dynamics of mixed species

*Author for correspondence; phone: +41 61 284 8283, fax +41 271 7951, e-mail <tanner@ubaclu.unibas.ch>

malarial infections in humans, particularly on the interrelationship between *P. falciparum* and *P. malariae* at the population level.

The following papers do not consider molecular markers of drug resistance. Research on transmissibility, recombination and the relation between different genotypes and their drug sensitivity will become of highest priority once adequate molecular markers for resistance to commonly used antimalarial drugs become available. However, the papers do point to some practical implications which should be considered in malaria control, be it in relation to the short- and long-term effect of insecticide-treated bed nets or the design and application of future malaria vaccines.

We are very grateful to our colleagues working in other endemic areas of Africa for having contributed their own findings on multiple *P. falciparum* infections. It is indeed unusual for experiences from areas of different endemicity to be compiled in one volume. We are equally grateful to the Royal Society of Tropical Medicine and Hygiene for having agreed to collaborate with us in compiling this supplement and for having efficiently supported its production in many ways.

We hope that the present series of papers will contribute to better understanding of the role and dynamics of multiple *P. falciparum* infections among all age groups in different endemic settings, and of the central role that premunition plays in the development of acquired immunity to malaria. We look forward to comments and findings generated in areas of similar or different endemicity that will challenge or support our hypotheses. We are confident that the findings will prove highly relevant to the design of new concepts and strategies of malaria control within the context of the global efforts to 'Roll Back Malaria'.

References

Alonso, P. L., Smith, T., Armstrong Schellenberg, J. R. M., Masanja, H., Mwankusye, S., Urassa, H., Bastos de Azevedo, I., Chongela, J., Kobero, S., Menendez, C., Hurt, N., Thomas, M. C., Lyimo, E., Weiss, N. A., Hayes, R., Kitua, A. Y., Lopez, M. C., Kilama, W. L., Teuscher, T. & Tanner, M. (1994). Randomised trial of efficacy of SPf66 vaccine against *Plasmodium falciparum* malaria in children in southern Tanzania. *Lancet*, **344**, 1175–1181.

Al-Yaman, F., Genton, B., Reeder, J. C., Mokela, D., Anders, R. F. & Alpers, M. P. (1997). Humoral response to defined *Plasmodium falciparum* antigens in cerebral and uncomplicated malaria and their relationship to parasite genotype. *American Journal of Tropical Medicine and Hygiene*, **54**, 430–435.

Arnot, D. E., Roper, C. & Sultan, A. A. (1994). MVR-PCR-analysis of hypervariable DNA sequence variation. *Parasitology Today*, **10**, 324–325.

Babiker, H. A., Satti, G. & Walliker, D. (1995). Genetic changes in the population of *Plasmodium falciparum* in a Sudanese village over a three-year period. *American Journal of Tropical Medicine and Hygiene*, **53**, 7–15.

Beck, H. P., Felger, I., Huber, W., Steiger, S., Smith, T., Weiss, N., Alonso, P. L. & Tanner, M. (1997). Analysis of multiple *Plasmodium falciparum* infections in Tanzanian children during the phase III trial of the malaria vaccine SPf66. *Journal of Infectious Diseases*, **175**, 921–926.

Contamin, H., Fandeur, T., Bonnefoy, S., Skouri, F., Ntoumi, F. & Mercereau-Puijalon, O. (1995). PCR typing of field isolates of *Plasmodium falciparum*. *Journal of Clinical Microbiology*, **33**, 944–951.

Conway, D. J. & McBride, J. S. (1991). Population genetics of *Plasmodium falciparum* within a malaria hyperendemic area. *Parasitology*, **103**, 7–16.

Engelbrecht, F., Felger, I., Genton, B., Alpers, M. & Beck, H.-P. (1995). *Plasmodium falciparum* malaria morbidity is associated with specific merozoite surface antigen 2 genotypes. *Experimental Parasitology*, **81**, 90–96.

Färnert, A., Snounou, G., Rooth, I. & Björkman, A. (1997). Daily dynamics of *Plasmodium falciparum* subpopulations in asymptomatic children in a holoendemic area. *American Journal of Tropical Medicine and Hygiene*, **56**, 538–547.

Hatz, Ch., Abdulla, S., Mull, R., Schellenberg, D., Gathmann, I., Kibatala, P., Beck, H. P., Tanner, M. & Royce, C. (1998). Efficacy and safety of CGP 56697 (artemether and benflumetol) compared with chloroquine to treat acute falciparum malaria in Tanzanian children aged 1–5 years. *Tropical Medicine and International Health*, **3**, 498–504.

Hurt, N., Thein, M., Smith, T., Bordmann, G., Gallati, H., Drees, N., Tanner, M. & Weiss, N. (1995). Immunological markers of childhood fevers in an area of intense and perennial malaria transmission. *Clinical and Experimental Immunology*, **100**, 59–66.

Kitua, A., Smith, T., Alonso, P. L., Masanja, H., Urassa, H., Menendez, C., Kimario, J. & Tanner, M. (1996). *Plasmodium falciparum* malaria in the first year of life in an area of intense and perennial transmission. *Tropical Medicine and International Health*, **1**, 475–484.

Kitua, A., Smith, T., Alonso, P. L., Urassa, H., Masanja, H., Kimario, J. & Tanner, M. (1997). The role of low level *Plasmodium falciparum* parasitaemia in anaemia among infants living in an area of intense and perennial transmission. *Tropical Medicine and International Health*, **2**, 325–333.

Kun, J. F. J., Schmidt-Ott, R. J., Lehman, L. G., Lell, B., Luckner, D., Greve, B., Matousek, P. & Kremsner, P. G. (1998). Merozoite surface antigen 1 and 2 genotypes and rosetting of *Plasmodium falciparum* in severe and mild malaria in Lambaréné, Gabon. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **92**, 110–114.

Menendez, C., Kahigwa, E., Hirt, R., Vounatsou, P., Aponte, J. H., Font, F., Acosta, C. J., Schellenberg, D., Galindo, C. M., Kimario, J., Urassa, H., Brabin, B., Smith, T., Kitua, A. Y., Tanner, M. & Alonso, P. L. (1997). Randomised placebo-controlled trial of iron supplementation and malaria chemoprophylaxis for the prevention of severe anaemia and malaria in Tanzanian infants. *Lancet*, **350**, 844–850.

McGregor, I. (1986). The development and maintenance of immunity to malaria in highly endemic areas. *Clinics in Tropical Medicine and Communicable Diseases*, **1**, 29–53.

Molineaux, L. & Gramiccia, G. (1980). *The Garki Project*. Geneva: World Health Organization.

Ntoumi, F., Contamin, H., Rogier, C., Bonnefoy, S., Trape, J. F. & Mercereau-Puijalon, O. (1995). Age-dependent carriage of multiple *Plasmodium falciparum* merozoite surface antigen-2 alleles in asymptomatic malaria infections. *American Journal of Tropical Medicine and Hygiene*, **52**, 81–88.

Paul, R. E. L. & Day, K. P. (1998). Mating patterns of *Plasmodium falciparum*. *Parasitology Today*, **14**, 197–202.

Robert, F., Ntoumi, F., Angel, G., Candito, D., Rogier, C., Fandeur, T., Sarthou, J.-L. & Mercereau-Puijalon, O. (1996). Extensive genetic diversity of *Plasmodium falciparum* isolates collected from patients with severe malaria in Dakar, Senegal. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **90**, 704–711.

Ross, R. (1910). *The Prevention of Malaria*. London: John Murray.

Sergent, E. & Parrot, L. (1935). L'immunité, la prémunition et la résistance innée. *Archives de l'Institut Pasteur d'Algérie*, **13**, 279–319.

Smith, T. A., Charlwood, J. D., Kihonda, J., Mwankusye, S., Billingsley, P., Meuwissen, J., Lyimo, E., Takken, W., Teuscher, T. & Tanner, M. (1993). Absence of seasonal variation in malaria parasitaemia in an area of intense seasonal transmission. *Acta Tropica*, **54**, 55–72.

Tanner, M., de Savigny, D., Mayombana, C., Hatz, C., Bernier, E., Tayari, S. & Degrémont, A. A. (1991). Morbidity and mortality at Kilombero, Tanzania. In: *Disease and Mortality in Sub-Saharan Africa*, Feachem, R. G. & Jamison, D. T. (editors). Oxford: Oxford University Press, pp. 286–305.

Tibayrenc, M. (1998). Beyond strain typing and molecular epidemiology: integrated genetic epidemiology of infectious diseases. *Parasitology Today*, **14**, 323–329.