The epidemiology of multiple *Plasmodium falciparum* infections

8. Effect of iron supplementation and malaria prophylaxis in infants on *Plasmodium falciparum* genotypes and multiplicity of infection

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Abstract

During a randomized placebo-controlled trial of chemoprophylaxis against *Plasmodium falciparum* malaria and iron supplementation, in infants living under conditions of intense transmission, all samples of *P. falciparum* obtained from children aged 5 and 8 months were genotyped by polymerase chain reaction–restriction fragment length polymorphism analysis for the *msp2* locus. One hundred and six blood samples were analysed for the number of concurrent infections (multiplicity), and the allelic family of each *msp2* genotype was determined. Mean multiplicity of infection was, overall, 2·76 infections/child, and it was significantly reduced in infants receiving chemoprophylaxis. This finding might help to explain the rebound effect in morbidity observed after prophylaxis was ended. Iron supplementation did not affect multiplicity of infection. In infants receiving placebo only, or placebo and iron supplementation, a significant positive association was observed between the number of infections and parasite densities (Spearman's ρ=0·25, P-0·047). This association was lost in the group receiving chemoprophylaxis alone, or in combination with iron. This study showed a significant association of FC27-like *msp2* alleles with prospective risk of clinical malaria in children (relative risk=1·487, P=0·013). Such an association was also found for the present risk of clinical malaria in infants receiving prophylaxis (odds ratio=3·84, P=0·026), which might imply that chemoprophylaxis may impair the development of premunition.

Keywords: malaria, *Plasmodium falciparum*, multiple infection, genotypes, *msp2* gene, Deltaprim™, dapsone, pyrimethamine, iron supplementation, premunition, Tanzania

Introduction

Anaemia is the major cause of severe malaria morbidity in Tanzanian children living under conditions of high malaria transmission (KITUA *et al.*, 1996; MENENDEZ *et al.*, 1997). Chemoprophylaxis has been recommended to reduce malaria morbidity in infants, and iron supplementation has been recommended to prevent the adverse effects of iron deficiencies (AMERICAN ASSOCIATION, 1978). However, both recommendations have elicited substantial controversy. Prophylaxis for young children in holoendemic areas has been questioned (GREENWOOD *et al.*, 1995), because of its effect on the development of semi-immunity that gradually builds up either because of frequent infections with antigenically distinct *Plasmodium falciparum* parasites (GUPTA *et al.*, 1994) or because of the chronicity of infections (SMITH *et al.*, 1999b). It has been speculated that long-term chemoprophylaxis, similarly to exposure-reducing interventions, might interfere with the acquisition of infections or their chronicity, and thus impair the development of naturally acquired semi-immunity. Furthermore, removal of such interventions would result in a rebound effect, shifting the malaria-attributable morbidity and mortality pattern to older ages (SNOW & MARSH, 1995).

With respect to iron supplementation, controversial evidence exists (OPPENHEIMER, 1989; HERSHKO, 1996). Several studies showed decreased susceptibility to malaria in iron-deficient or anaemic children (MURRAY *et al.*, 1978; OPPENHEIMER *et al.*, 1986), whereas others did not find such an association (BATES *et al.*, 1987; MENENDEZ *et al.*, 1994). Most microorganisms are heavily dependent on external iron provision (WEINBERG, 1978), and reduction in available iron may also put parasites at a disadvantage. Therefore, one might speculate that a sufficient supply of iron would enable parasite infections to grow to higher densities. This could increase host susceptibility or morbidity, or favour multiple infections, with unknown consequences.

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A recent randomized placebo-controlled trial of chemoprophylaxis and iron supplementation in infants living in Ifakara, Tanzania, an area holoendemic for malaria, clearly showed the beneficial effects of both chemoprophylaxis and iron supplementation in children less than one year of age (MENENDEZ *et al.*, 1997). In this study, children receiving chemoprophylaxis had a reduced risk of severe anaemia and of clinical malaria episodes. Iron supplementation significantly reduced the number of severely anaemic children, but did not increase susceptibility to malaria. Within this study we investigated whether chemoprophylaxis or iron supplementation would affect multiplicity of infections, and whether the development of premunition would be affected. We also tested whether genotypes of different *msp2* allelic families were differentially affected by either of these interventions.

Material and Methods

The study took place in Ifakara town in south-eastern Tanzania, an area holoendemic for malaria. A detailed description of the trial has been given by MENENDEZ *et al.* (1997). Briefly, 832 infants were enrolled at birth after informed consent of their parents and randomly allocated to one of 4 groups: (i) 204 children received oral iron supplementation (2 mg/kg/d) plus weekly placebo syrup (group IP): (ii) 208 children received 2·5 mL Deltaprim™ syrup (pyrimethamine 3·125 mg+dapsone 25 mg/25 mL; Wellcome, South Africa) weekly and daily placebo syrup (group DP); (iii) 213 children received weekly Deltaprim™ and daily iron supplementation (group DI); and (iv) 207 children received daily and weekly placebo syrup (group PP). All children were followed by cross-sectional surveys conducted at 2, 5, 8 and 12 months of age, and clinical episodes of all children were recorded by a passive case detection system at the local hospital. A clinical malaria episode was defined as axillary temperature >37·4°C and *P. falciparum* parasitaemia, with no density cut-off level. During the cross-sectional surveys, and at each visit with presumptive malaria, a finger-prick blood sample was collected for microscopical assessment and subsequent polymerase chain reaction–restriction fragment length polymor-

phism (PCR–RFLP) analysis. Haematological and malariological indices and sickle cell status were determined as described by MENENDEZ *et al.* (1997).

The genotyping study was carried out on all samples obtained during the cross-sectional surveys from children aged 5 and 8 months who had been found to be positive for *P. falciparum* by microscopy. Deoxyribonucleic acid (DNA) was prepared and used for PCR amplification of the *msp2* locus as described by FELGER *et al.* (1999a). The number of concurrent infections and the individual genotypes of *msp2* were determined.

The total number of multiple infections and the number of infections belonging to either of the 2 allelic families (FC27-like, 3D7-like) were analysed with respect to survey (age), intervention group, present and prospective risk of clinical malaria, parasite density, packed cell volume (PCV), and sickle cell status.

Data analysis was carried out with STATA™ v5.0 statistical software (STATA Corp., Texas, USA). The Wilcoxon test was applied to compare the average number of total, FC27-like and 3D7-like infections between the intervention groups and between the surveys. Spearman's correlation coefficients were calculated to assess associations between multiplicity of infection and parasite densities or PCV levels.

Logistic regression models were fitted to assess the significance of the relationship between the number of infections and the current risk of clinical malaria at the cross-sectional survey or during 15 d afterwards. Poisson regression models were also fitted to evaluate the effect of the total multiplicity or of the multiplicity of FC27-like and 3D7-like infections on the subsequent incidence of first clinical malaria episodes during the first year of life and after the fifth month or eighth month, depending on the survey. The time at risk was calculated for each child as the period between the survey and the date of the first or only clinical malaria episode, or the date of withdrawal, or the end of its first year of life. If a child had a clinical episode at the time of the cross-sectional survey or during the following 2 weeks, the time at risk was considered to begin 28 d after the survey.

Results

In the cross-sectional survey at month 5, 52 samples were found to be parasite-positive by microscopy, and there were 60 such samples in the survey at month 8. A PCR product was obtained from 49 month 5 samples and from 57 month 8 samples. The mean number of concurrent total, FC27-like and 3D7-like infections in the sample from these 2 surveys is shown in Table 1. A significant decrease in multiplicity between the surveys at months 5 and 8 was observed, which was due to a decrease in numbers of 3D7-alleles (Table 1). The age effect on mean multiplicity was not significant when tested for the individual intervention groups, most probably due to the small numbers in each group. However, mean multiplicity was lower at month 8 than at month 5 in all intervention groups, and data for both surveys were combined for the further analysis.

Mean multiplicity in the different intervention groups is shown in Table 2. Since no significant difference was observed between the IP and PP groups, or between the DP and DI groups, we combined all data for further analysis into 2 groups, IP/PP and DI/DP. Mean multiplicity of infections was significantly reduced in children receiving chemoprophylaxis (DI/DP) compared with children in the IP/PP group (Table 2). Except for the age-dependent reduction of mean multiplicity of 3D7-like infections, no further specific effect on the distribution of genotypes of either allelic family between the different intervention groups was found.

Parasite densities in the IP/PP group were significantly positively correlated with the number of concurrent infections (Table 3). However, this correlation was entirely due to a positive correlation of parasite density with the number of concurrent infections of the FC27-type, and 3D7-like infections showed no correlation. This trend was also observed in the DI/DP group, but did not reach significance (Table 3).

Children with febrile episodes and parasitaemia in both groups (IP/PP, n=17 and DI/DP, n=5) showed non-significant higher multiplicity than asymptomatic children (n=47 and n=37, respectively). Logistic regression models suggested a trend that the risk of clinical malaria might increase with increasing multiplicity [likelihood ratio test (LRT): χ_1^2=3·412, P=0·065]. This effect was found to be higher in the IP/PP group (LRT: χ_1^2=3·51, P=0·061) but there was no statistically significant interaction between the intervention group and the number of infections (Table 4). This risk was mainly determined by the number of FC27-like infections (LRT: χ_1^2=3·64, P=0·056). This model showed a trend for higher risk of clinical malaria in the DI/DP group (LRT: χ_1^2=3·51, P=0·061), and also suggested a possible interaction between the intervention group and the number of FC27-like infections (LRT: χ_1^2=2·287, P=0·13). Separate logistic regression models within the 2 groups showed that for every acquired FC27-like infection the risk of clinical malaria in the DI/DP group increased 3·84 times [95% confidence interval (CI) 1·07–13·85, P=0·026], whereas it increased only 1·38

Table 1. Average number of concurrent *Plasmodium falciparum* infections in all intervention groups at both surveys

Survey	No. of subjects	Multiplicity of genotypes[a] All	FC27-like	3D7-like
5 months	49	3·12(1·32)	0·86(0·94)	2·25(1·16)
8 months	57	2·44(1·12)	0·72(0·80)	1·72(1·10)
Wilcoxon's Z	–	2·55	0·600	2·790
P	–	0·011	0·548	0·005

[a]Mean (SD in parentheses).

Table 2. Number of all *Plasmodium falciparum* infections according to intervention group

Intervention	No. PCR[a]-positive	Multiplicity[b]	Wilcoxon's Z	P
Deltaprim™/iron	19	2·26(1·10)	0·771	0·441
Deltaprim™/placebo	23	2·57(1·31)		
Iron/placebo	28	3·04(1·43)	0·056	0·955
Placebo/placebo	36	2·92(1·11)		
Both groups with Deltaprim™	42	2·43(1·21)	2·19	0·029
Both groups without Deltaprim™	64	2·97(1·25)		

[a]Polymerase chain reaction.
[b]Mean (SD in parentheses).

Table 3. Spearman's correlation of *Plasmodium falciparum* parasite densities and number of concurrent infections

	Placebo+placebo/iron+placebo group (n=64)			Deltaprim™+placebo/Deltaprim™+iron group (n=42)		
	All genotypes	FC27-like genotypes	3D7-like genotypes	All genotypes	FC27-like genotypes	3D7-like genotypes
Spearman's ρ	0·250	0·295	0·038	0·134	0·262	–0·014
P	0·047	0·019	0·76	0·39	0·09	0·93

Table 4. Logistic models to assess the association of total number of *Plasmodium falciparum* infections with the present risk of clinical malaria

Outcome variable	Explanatory variable	Odds ratio	95% Confidence interval	P[a]
Risk of clinical malaria[b]	No. of infections			
	In IP/PP group[c,e]	1·486	0·934–2·365	0·086
	In DI/DP group[d,e]	1·326	0·623–2·825	0·467
	No. of FC27-like infections			
	In IP/PP group[c,f]	1·355	0·739–2·484	0·328
	In DI/DP group[d,f]	3·84	1·065–13·85	0·026
	No. of 3D7-like infections			
	In IP/PP group[c,e]	1·292	0·806–2·071	0·286
	In DI/DP group[d,e]	0·685	0·264–1·782	0·419

[a]Likelihood ratio test (LRT).
[b]Clinical malaria: *P. falciparum* parasitaemia plus temperature >37·4°C at the cross-sectional survey or 15 d later.
[c]Iron+placebo/placebo+placebo.
[d]Deltaprim™+iron/Deltaprim™+placebo.
[e]No interaction between intervention group and numbers of infections.
[f]Interaction between intervention group and numbers of FC27-like infections of borderline significance (LRT: χ^2=1·29, P=0·13).

Table 5. Poisson models to assess the association of multiple *Plasmodium falciparum* infections with the prospective risk of first clinical malaria episodes occurring in the subsequent observation period between the cross-sectional surveys and the first year of life

Outcome variable	Explanatory variable	Relative risk	95% Confidence interval	P[a]
Incidence rate of prospective first clinical malaria episode[b]	Nos. of concurrent infections			
	All	1·235	0·924–1·651	0·163
	FC27-like	1·847	1·167–2·923	0·013
	3D7-like	0·953	0·659–1·377	0·796

[a]Likelihood ratio test. Interactions between intervention groups and number of infections not significant.
[b]Clinical malaria: *P. falciparum* parasitaemia plus temperature >37·4°C.

times in the IP/PP group (95% CI 0·66–2·92, P=0·403) (Table 4). The models showed no evidence of an association between the number of 3D7-like infections and the risk of clinical malaria in either intervention group.

We also wanted to assess whether the number of concurrent infections (all, FC27-like, or 3D7-like) would determine the subsequent period to the first clinical malaria episode. Poisson regression showed no evidence that the total number of infections had an effect on the incidence rate of first clinical malaria episodes after the cross-sectional survey (Table 5). However, numbers of concurrent FC27-like infections significantly determined the incidence rate of first episodes, but there was no statistically significant difference in the incidence rate between the intervention groups. 3D7-like infections did not determine the incidence rate of first clinical episodes in either group (Table 5).

Because of reduced anaemia in both the iron supplementation group and the Deltaprim™ group (MENENDEZ *et al.*, 1997), we tested whether anaemia was also determined by the number of concurrent infections. Spearman's correlations between PCV values and numbers of concurrent infections for each intervention group showed that neither multiplicity nor numbers of genotypes belonging to the 2 allelic families affected the PCV values (data not shown).

Within the tested samples, 8 sickle cell gene carriers were detected. Globin genotypes showed no significant effect on numbers of concurrent infections (Wilcoxon's Z=1·369, P=0·171), although there was a reduction in FC27-like infections in the 8 carriers of the sickle cell trait (mean number of infections 0·375) compared with 96 children with normal or fetal haemoglobin (mean number 0·833).

Discussion

The present study was intended to elucidate the effect of interventions on the infecting *P. falciparum* population. We tested whether naturally-occurring mechanisms leading to the development of semi-immunity or premunition might be affected by these interventions. Genotyping of parasites and enumeration of concurrent infections have proven to be powerful techniques to add to our understanding of the dynamics of infections and the development from a non-immune infant to a semi-immune child (AL-YAMAN *et al.*, 1997; BECK *et al.*, 1997; FELGER *et al.*, 1999b; SMITH *et al.*, 1999a, 1999b). Thus, the finding of a significant reduction of multiplicity in children receiving Deltaprim™ prophylaxis may partly help to explain the rebound effect in morbidity seen in the observation period after the end of prophylactic treatment (MENENDEZ *et al.*, 1997). Reduction of blood-stage infections may reduce the number of concurrent infections and thus might impair the development of premunition. In another study, in which no intervention was made, we have shown that infants aged 5–8 months already harbour a mean of 2·7 infections (FELGER *et al*, 1999b). This is in agreement with the observed multiplicity in children receiving no chemoprophylaxis, and provides evidence that iron supplementation does not support the establishment of a higher number of infections. In the same study in in-

fants, little age-dependency of multiplicity was observed during the first year of life, though there was a small increase at the end of the first year (SMITH *et al.*, 1999a). Thus the reason for the observed reduction in multiplicity in blood samples from the cross-sectional surveys conducted at months 5 and 8 in all groups remains unclear. One could speculate that the reduction might have been due to the frequent hospital attendance of all the children in this study, which could have reduced the malaria prevalence because they were more frequently under medical care. Whatever effect was responsible for this reduction, it is interesting that it seemed to act only on 3D7-like infections, while FC27-like infections were not affected.

A further important finding of this study is that parasite densities were positively correlated with the number of concurrent infections and in particular with infections due to parasites with the FC27-type *msp2* gene. This trend was also observed in our previous analysis of multiplicity of infections during the SPf66 malaria vaccine trial (BECK *et al.*, 1997) and in a study in Papua New Guinea by ENGELBRECHT *et al.* (1995). In this context it is also noteworthy that infants infected with FC27-alleles had, on average, significantly higher parasite densities in all intervention groups than infants with infections without FC27-like alleles (Wilcoxon's Z=–2·63, P=0·009). There was no statistically significant difference in the average parasite densities between infants with or without 3D7-like alleles (Wilcoxon's Z=0·37, P=0·715). However, such an effect of FC27-like infections on parasite density was not found in infants from Idete (FELGER *et al.*, 1999b). It is interesting to observe that the correlation between density and numbers of all genotypes was lost in children receiving prophylaxis, and that the correlation with numbers of FC27-like genotypes was smaller than in children without prophylaxis. Such a loss of correlation implies that Deltaprim™ prophylaxis controls or reduces high parasite densities, which are mostly reached when a child is infected with parasites of the FC27-like genotype. This effect may also be one of the mechanisms leading to the observed reduction in morbidity (MENENDEZ *et al.*, 1997).

The observation of an association between increased risk of present clinical malaria, or the incidence rate of first episodes, and numbers of FC27-like infections is in concordance with findings from Papua New Guinea (ENGELBRECHT *et al.*, 1995), but no such association could be observed in a recent study by KUN and colleagues (1998), who compared severe malaria cases with mild malaria cases in Gabon. The association between the higher risk for current clinical episodes and FC27-like infections in infants receiving prophylaxis might hint that the development of premunition is impaired in these children. Nevertheless, the overall reduction in multiplicity, both of FC27-like and 3D7-like infections, would have contributed to the immediate beneficial effect of chemoprophylaxis.

The lack of an association between 3D7-like infections and risk of clinical malaria, however, is in contrast to the situation in infants from Idete (FELGER *et al.*, 1999b). Because we used only samples with positive PCR results in the analysis, we cannot determine whether the increased risk of clinical malaria with increasing numbers of concurrent FC27-like infections was indeed an increase, or whether children with 3D7-like infections were actually protected against clinical malaria, as was observed by AL-YAMAN and colleagues (1997) in a study comparing infected and uninfected children in Papua New Guinea. Selecting only samples positive for *P. falciparum* for this analysis may have introduced a bias. Indeed, Kaplan–Meyer survival curves indicated that the selected children differed from the total sample in the median time to their first clinical episode (*P. falciparum*-positive samples, median time 115 d, 95% CI 71–398; *P. falciparum*-negative samples, median time 379 d, 95C 354–437; log rank test, χ_1^2=16·9, P<0·0001).

In conclusion, we were able to show in this study that iron supplementation did not increase or affect the multiplicity of infection. Furthermore, it did not support increased growth of certain genotypes compared with the other intervention groups. Thus the only effect of iron-supplementation was the reduction of anaemia, which is clearly beneficial. We also showed that chemoprophylaxis reduced multiplicity in the infections occurring in infants. There is some evidence that this reduction might interfere with the development of premunition. Other studies have suggested that semi-immunity might be acquired by frequent infections stimulating responses which will control parasite growth but will not eliminate infection (SMITH *et al.*, 1999b). Therefore, by preventing multiple infections from becoming established, chemoprophylaxis might reduce the acquisition of premunition. On the other hand, Deltaprim™ prophylaxis did reduce morbidity in these infants in their first months. There was subsequently a rebound effect, but the actual clinical episodes may not have been as severe for older children as they would have been for infants (MOLINEAUX, 1997).

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References

Al-Yaman, F., Genton, B., Reeder, J., Anders, R., Smith, T. & Alpers, M. P. (1997). Reduced risk of clinical malaria in a highly endemic area in children infected with multiple clones of *Plasmodium falciparum*: a prospective community study. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **91**, 602–605.

American Association [of Paediatrics Committee on Nutrition] (1978). Relationship between iron status and incidence of infection in infancy. *Paediatrics*, **62**, 246–250.

Bates, C. J., Powers, H. J., Lamb, W. H., Gelman, W. & Webb, E. (1987). Effect of supplementary vitamins and iron on malaria indices in rural Gambian children. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **81**, 286–291.

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 trial of the malaria vaccine SPf66. *Journal of Infectious Diseases*, **175**, 921–926.

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.

Felger, I., Irion, A., Steiger, S. & Beck, H.-P. (1999a). The epidemiology of multiple *Plasmodium falciparum* infections 2. Genotypes of merozoite surface protein 2 of *Plasmodium falciparum* in Tanzania. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **93**, supplement 1, S1/3–S1/9.

Felger, I., Smith, T., Edoh, D., Kitua, A., Alonso, P. L., Tanner, M. & Beck, H.-P. (1999b). The epidemiology of multiple *Plasmodium falciparum* infections 6. Multiple *Plasmodium falciparum* infections in Tanzanian infants. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **93**, supplement 1, S1/29–S1/34.

Greenwood, B. M., David, P. H., Otoo-Forbes, L. N., Allen, S. J., Alonso, P. L., Armstrong Schellenberg, J. R., Byass, P., Hurwitz, M., Menon, A. & Snow, R. W. (1995). Mortality and morbidity from malaria after stopping malaria chemoprophylaxis. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **89**, 629–633.

Gupta, S., Trenholme, K., Anderson, R. M. & Day, K. P. (1994). Antigenic diversity and the transmission dynamics of *Plasmodium falciparum*. *Science*, **263**, 961–963.

Hershko, C. (1996). Iron and infection. In: *Iron Nutrition in Health and Disease*, Hallberg, I. & Asp, N. G. (editors). London: John Libbey & Company, pp. 231–238.

Kitua, A. Y., Smith, T., Alonso, P. L., Masanja, H., Menendez,

C., Urassa, H., 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.

Kun, J. F. J., Schmitt-Ott, R. J., Lehman, L. G., Lell, B., Luckner, D., Greve, B., Matousek, P. & Kemsner, 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., Todd, J., Alonso, P. L., Francis, N., Lulat, S., Ceesay, S., M'Boge, B. & Greenwood, B. M. (1994). The effects of iron supplementation during pregnancy, given by traditional birth attendants, or the prevalence of anaemia and malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **88**, 590–593.

Menendez, C., Kahigwa, E., Hirt, R., Vounatsou, P., Font, F., Aponte, J., Acosta, C. J., Schellenberg, D., Galindo, C. M., Kimario, J., Masanja, H., Urassa, H., Fumado, V., 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.

Molineaux, L. (1997). Nature's experiment: what implications for malaria prevention? *Lancet*, **349**, 1636–1637.

Murray, M. J., Murray, A. B., Murray, M. B. & Murray, C. J. (1978). The adverse effect of iron repletion on the course of certain infections. *British Medical Journal*, **ii**, 1113–1114.

Oppenheimer, S. J., Gibson, F. D., Macfarlane, S. B. J., Moody, J. B., Harrison, C., Spencer, A. & Bunari, O. (1986). Iron supplementation increases prevalence and effects of malaria: report on clinical studies in Papua New Guinea. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **80**, 603–612.

Oppenheimer, S. J. (1989). Iron and infection: the clinical evidence. *Acta Paediatrica Scandinavica*, **361**, 53–62.

Smith, T., Beck, H.-P., Kitua, A., Mwankusye, S., Felger, I., Fraser-Hurt, N., Irion, A., Alonso, P., Teuscher, T. & Tanner, M. (1999a). The epidemiology of multiple *Plasmodium falciparum* infections 4. The multiplicity of infection with *Plasmodium falciparum* and other malariological indices in an area of high endemicity. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **93**, supplement 1, S1/15–S1/20.

Smith, T., Felger, I., Tanner, M. & Beck, H.-P. (1999b). The epidemiology of multiple *Plasmodium falciparum* infections 11. Premunition in *Plasmodium falciparum* infections: insights from studies of multiple infections. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **93**, supplement 1, S1/59–S1/64.

Snow, R. W. & Marsh, K. (1995). Will reducing *Plasmodium falciparum* transmission alter malaria mortality among African children? *Parasitology Today*, **11**, 188–190.

Weinberg, E. D. (1978). Iron and infection. *Microbiological Reviews*, **42**, 45–66.