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Incipient resistance of *Plasmodium falciparum to* chloroquine among a semi-immune population of the United Republic of Tanzania

2. The impact of chloroquine used as a chemosuppressant on the immune status of the population

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Decreased sensitivity and incipient resistance of Plasmodium falciparum *strains to chloroquine have been reported from Mto-wa-Mbu, in the north-east of the United Republic of Tanzania. In this locality the population had been exposed to chloroquine pressure for about two decades, in the form of medicated salt and through easy availability of the drug itself. In an attempt to find out whether such chemosuppression had influenced the immune response of the population, two seroepidemiological surveys were carried out in March 1981 and March 1982; the second survey was performed to confirm the results obtained in the first one. The humoral immunological response was measured by the immunofluorescent antibody technique. In the absence of information on the immunological profile that existed in the area prior to the introduction of chloroquine in 1960, the results of the present surveys were compared with those obtained in another locality in the north-east of the United Republic of Tanzania in 1967, and in the West Kiang district of Gambia in 1965. The two areas used for comparison exhibited a malaria endemicity similar to that prevailing in Mto-wa-Mbu prior to the introduction of the medicated salt. The results from Mto-wa-Mbu showed a significantly lower proportion of subjects with positive titres and a lower geometric mean titre in all age groups.*

A reduction in the humoral immunological response might be explained by the drug pressure that has been exerted in the area for many years. The depressed immune response found at Mto-wa-Mbu, however, was so marked that other factors may have contributed to its establishment.

In view of the importance of these findings, it is recommended that further, longitudinal serological studies be conducted in the field to assess the effects of chemosuppression on the immune response of the protected populations.

The decreased sensitivity of strains of *Plasmodium falciparum* to chloroquine in Mto-wa-Mbu, a village in the north-east of the United Republic of Tanzania which had been exposed to chloroquine pressure for about two decades, and the presence of strains resistant to chloroquine at RI level have been confirmed by *in vivo* and *in vitro* studies and reported in a previous paper (*1*).

At the time these studies were carried out, blood specimens were taken from a sample of the population in order to determine the serological profile of the local residents. This was in order to find out whether the chemosuppression exerted by the chloroquinized salt for many years and the easy access to chloroquine tablets at the local dispensary and village shops had affected the immune status of the population. A reduced immunological defence might have been partly responsible for the change in the sensitivity of the local *P. falciparum* strains to chloroquine.

A second seroepidemiological survey was carried out in Mto-wa-Mbu in March 1982. The results of the two surveys are presented and discussed in the present paper.

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METHODS

At both surveys, blood samples were taken from the same 300 persons who had been examined to assess the malaria prevalence in the area. The blood was taken using microhaematocrit tubes and transferred to filter paper strips. These were dried and conveyed to a specialized laboratory 3 weeks later, where one of the present authors (P.A.T.) carried out the indirect immunofluorescent antibody (IFA) test, as described previously (*2*). The antigen was prepared using red cells parasitized with schizonts of *P. falciparum*. The parasite had been isolated from a patient who had contracted the disease in Gambia, and had been maintained in culture for some years following the technique of Trager & Jensen (*3*). Synchronization by sorbitol was carried out prior to the preparation of the antigenic slides.

The seroepidemiological results obtained at Mto-wa-Mbu have been compared with those from Muheza-Ubembe, another locality in the north-eastern part of the United Republic of Tanzania (*4*), and with those from the West Kiang district of Gambia (*5*). In the studies in Muheza-Ubembe, *P. fieldi* was used as antigen for the IFA technique (*6*). In Gambia, thin blood films from patients with *P. falciparum* parasitaemia of not less than 30 000 trophozoites per mm^3 constituted the antigen (*5*).

RESULTS

Table 1 shows the parasite rates and the IFA titres found in residents in Mto-wa-Mbu, in March 1981. It appears that at the time of the examination, the prevalence of the disease in the area was high, indicating a hyperendemic situation. However, the percentage of patients with a positive IFA titre was not as high as might be expected in an area with high malaria endemicity, and the geometric mean titre was consistently low in all the age groups.

The results of the IFA test were more striking when they were analysed according to the results of parasitological examination, separately for children and adults. From Table 2, it can be seen that the geometric mean antibody titre was very low for the children, with practically no difference between those with positive or negative slides. Among the adults (15 years of age and over), the geometric mean titre was again low, but with a slightly higher value among subjects with a negative blood film.

Fig. 1 shows the percentage of each age group who were parasitologically or serologically positive, while Fig. 2 shows the titre distribution in the two age groups, separately for those with and without parasitaemia. The percentage of children with a negative titre ($< 1:20$) was surprisingly high, both for subjects with a positive, and those with a negative blood film, and the percentage with a low positive titre (1:20–1:80) was much greater than the percentage with a higher value ($\geq 1:160$). In the adult group, the percentage with a negative immunological response was lower than that in the children's group and almost identical for subjects with a positive or negative blood film. The large number of immuno-negative subjects was unexpected in a hyperendemic area, where the humoral immunological response is usually positive, confirming that practically all subjects have been in contact with the parasite. Low antibody titres were more prevalent than high values, even among the adults.

Because of these unexpected findings, the survey was repeated under the same conditions exactly one year later in March 1982. The results of this second survey are presented in Tables 3 and 4. The parasito-

Table 1. Parasite rate and results of IFA tests on blood samples taken from residents of Mto-wa-Mbu, United Republic of Tanzania, March–April 1981

Age group (years)	No. of people examined	Parasite rate	IFA titre								Percentage with positive titre	Geometric mean titre
			< 20	20	40	80	160	320	640	1280		
< 1	20	50.0	12	5	1	1	1				40.0	16.2
1	11	45.5	6	2	2			1			45.5	20.0
2–4	39	53.8	17	7	8	4	1	1	1		56.4	24.3
5–9	48	66.7	29	7		8	3	1			39.6	20.0
10–14	34	52.9	12	2	7	3	3	4	2	1	64.7	47.1
≥ 15	148	37.8	24	13	24	31	21	19	15	1	83.8	74.9
Total	300	47.3	100	36	42	47	29	26	18	2	66.7	42.8

Table 2. IFA test results in residents of Mto-wa-Mbu, according to the results of the parasitological examination, March–April 1981

Age group (years)	Parasitological result	No. of people examined	IFA titre								Percentage with positive titre	Geometric mean titre
			< 20	20	40	80	160	320	640	1280		
0–14	Positive	85	39	15	10	11	4	5	1		54.1	25.5
	Negative	67	37	8	8	5	4	2	2	1	44.8	23.8
	Total	152	76	23	18	16	8	7	3	1	50.0	24.8
⩾ 15	Positive	56	9	6	9	11	10	8	3		83.9	68.1
	Negative	92	15	7	15	20	11	11	12	1	83.7	79.4
	Total	148	24	13	24	31	21	19	15	1	83.8	74.9

logical and serological age profiles observed at the second survey are also shown in Fig. 1. The similarity of the findings of the two surveys is evident, and in particular, the surprisingly low number of serologically positive individuals observed in the first survey is not invalidated by the results of the second survey.

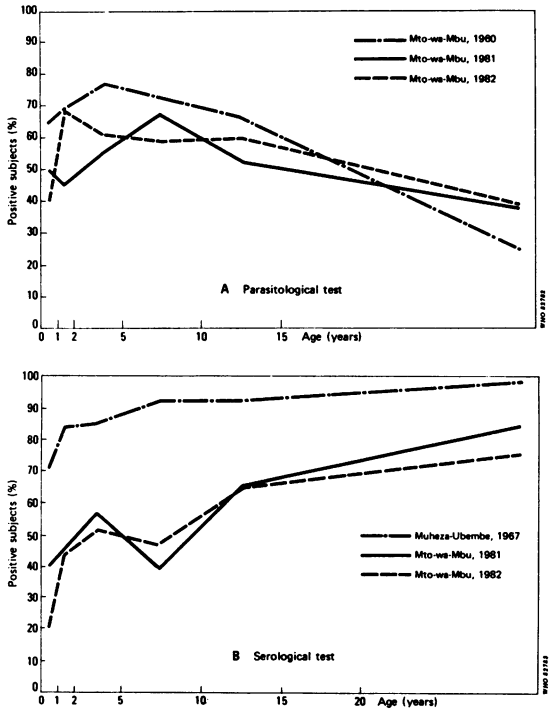

Fig. 1. Percentage of subjects with a positive parasitological or serological result, according to age, in Mto-wa-Mbu, 1960, 1981, and 1982, and in Muheza-Ubembe, 1967.

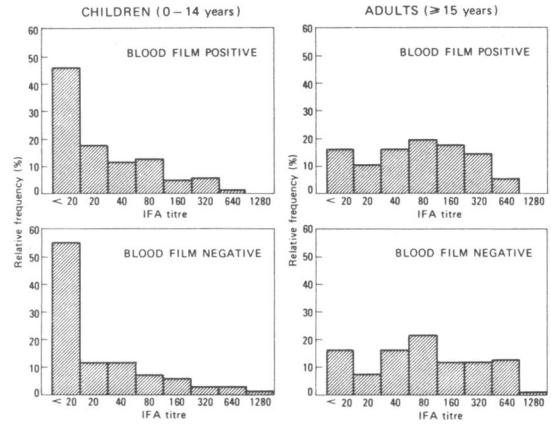

Fig. 2. IFA titre distribution in adults and children in Mto-wa-Mbu, March–April 1981.

DISCUSSION

Chemosuppression of malaria, especially among vulnerable groups of the population, is considered an important activity which may help reduce malaria morbidity, especially in areas where vector control measures are precluded by the lack of human and financial resources. Although this activity has been carried out in the past, either in the form of medicated salt projects (*7–10*) or through regular administration of drugs to selected groups of the population (*11–13*), key questions such as whether and how chemotherapy influences or depends on the immune response and its role in selecting drug-resistant parasites, remain to be answered.

In hyperendemic areas, fluorescent antibody levels are usually high in newborn infants, then fall in the weeks following birth and remain low during the first

Table 3. Parasite rate and results of IFA tests on blood samples taken from residents of Mto-wa-Mbu, March 1982

Age group (years)	No. of people examined	Parasite rate	IFA titre								Percentage with positive titre	Geometric mean titre
			< 20	20	40	80	160	320	640	1280		
< 1	15	40.0	12	1	2						20.0	12.6
1	16	68.8	9	4	3						43.8	15.4
2-4	29	62.1	14	9	5		1				51.7	17.3
5-9	49	59.2	26	9	9	2	2			1	46.9	19.7
10-14	45	60.0	16	13	10	3	2		1		64.4	23.7
⩾ 15	146	39.0	37	33	30	20	20	3	3		74.7	35.4
Total	300	49.3	114	69	59	25	25	3	4	1	62.0	25.7

Table 4. IFA test results in residents of Mto-wa-Mbu, according to the results of the parasitological examination, March 1982

Age group (years)	Parasito-logical result	No. of people examined	IFA titre								Percentage with positive titre	Geometric mean titre
			< 20	20	40	80	160	320	640	1280		
0-14	Positive	91	32	25	24	5	3		1	1	64.8	23.6
	Negative	63	45	11	5		2				28.6	13.8
	Total	154	77	36	29	5	5		1	1	50.0	18.9
⩾ 15	Positive	57	9	11	15	10	11		1		84.2	43.6
	Negative	89	28	22	15	10	9	3	2		68.5	30.9
	Total	146	37	33	30	20	20	3	3		74.7	35.4

year of life (*5*). With advancing age and increased exposure to a high degree of transmission, there is a progressive rise in the IFA titre (*5, 14, 15*). However, the results presented in this paper indicate that there was a reduced immunological response among the population of Mto-wa-Mbu.

Such a statement could be fully substantiated only by comparing the parasite rates and the immunological status of the population before and after the introduction of the medicated salt. Unfortunately, although parasite rates are available, the immunological status of the population prior to the introduction of the drug in 1961 is unknown.

An attempt has been made to circumvent this difficulty by using data obtained in other hyperendemic areas. In 1967, parasitological and seroepidemiological surveys were carried out in Muheza-Ubembe, a highly endemic malarious area in the north-east of the United Republic of Tanzania (*4*). The results of these surveys have been compared with those of the parasitological survey carried out at Mto-wa-Mbu in 1960 (Table 5), and it appears that the endemicity of malaria in Mto-wa-Mbu was certainly equal to and probably higher than that found in Muheza-Ubembe. Both areas exhibited perennial transmission with seasonal fluctuations, with the main malaria vectors being *Anopheles gambiae* s.l. and *A. funestus*, and with *P. falciparum* being the most prevalent species. Thus, a comparison of the percentages of subjects with positive titres in Muheza in 1967 and in Mto-wa-Mbu in 1981 would appear to be justifiable.

Fig. 1(A) displays the age-specific parasite rates observed in Mto-wa-Mbu in 1960, before the introduction of chloroquine, and in 1981 and 1982. The observed decrease in the level of parasitaemia in children and increase of the parasite rate in adults (*1*) are consistent with the serological findings, as shown in Fig. 1(B). The proportion of each age group with a positive titre was substantially and constantly lower in Mto-wa-Mbu in 1981 and 1982 than in Muheza in 1967; the humoral immunological response in Mto-wa-Mbu remained unusually low even in the adults.

In Mto-wa-Mbu, the highest parasite rate and the lowest proportion of serologically positive subjects

Table 5. Results of the parasitological survey in Mto-wa-Mbu in 1960, and the parasitological and serological surveys in Muheza-Ubembe in 1967

Mto-wa-Mbu, 1960			Muheza-Ubembe, 1967			
Age group (years)	No. of people examined	Parasite rate	Age group (years)	No. of people examined	Parasite rate	Percentage with positive titre
< 1	34	64.7	< 1	25	36.0	71
1	74	68.9	1	21	42.9	84
2–5	91	76.9	2–4	102	61.8	85
6–10	180	71.7	5–9	124	48.4	92
11–15	84	65.5	10–19	120	45.0	92
⩾ 16	243	24.7	⩾ 20	403	22.4	98
Total	706	54.8		795	35.9	94

were found in the 5–9 year age group; the reasons for this are not known.

A comparison was also made of the titre distribution among children less than 5 years old in Mto-wa-Mbu with that found in a similar group from the West Kiang district of Gambia in 1965. This latter area was hyperendemic and no chemosuppressive measures had been implemented. The results are shown in Fig. 3, separately for children with and without parasitaemia. In subjects with positive blood films, 40% of the Mto-wa-Mbu group had a negative IFA titre compared with only 1.7% of those from the West Kiang district. High titres were significantly much more prevalent in West Kiang (⩾ 1:100) than in Mto-wa-Mbu (⩾ 1:80). As regards the children with negative blood films, 57.1% of the Mto-wa-Mbu group were seronegative, compared with 30.4% of the subjects from the West Kiang district. It therefore appears that the child population of Mto-wa-Mbu exhibited a much reduced humoral immunological response.

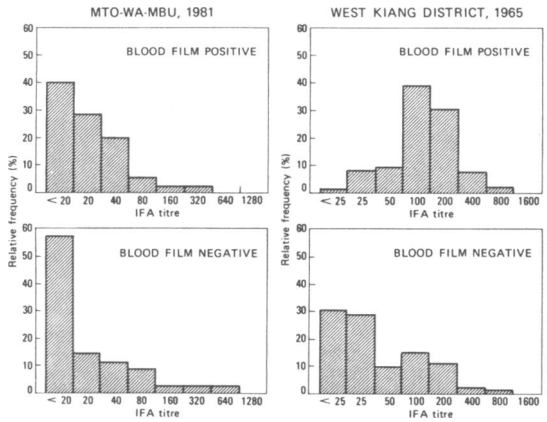

Fig. 3. Comparison of titre distributions among children under 5 years old, in Mto-wa-Mbu, 1981, and in the West Kiang district of Gambia, 1965.

Several factors may have been responsible for the different immunological profiles obtained in the three areas, namely (*a*) differences in the level of endemicity prevailing in the areas at the time the serological tests were performed; (*b*) discrepancies in the IFA results, which were obtained using different antigens; or (*c*) reduced sensitivity of the IFA test system used at Mto-wa-Mbu.

It has already been noted that the level of malaria endemicity found at Mto-wa-Mbu in 1960 was similar to that measured at Muheza-Ubembe in 1967. It may further be assumed that the epidemiological situations in Mto-wa-Mbu (1960) and the West Kiang district of Gambia (1965) were also comparable, since they had similar prevalence, both areas had perennial transmission with a seasonal increase during the wet months, and *P. falciparum* was the most prevalent malaria species in both areas.

The endemic situation in Mto-wa-Mbu does not appear to have changed in recent years. Parasitological surveys carried out in 1978, 1979, 1980, and 1982 did not show significant differences from the 1981 findings.

With regard to the antigens used in the different areas, the highest FA response would have been expected with the *P. falciparum* (local strains) antigen used at West Kiang, followed by the cultured *P. falciparum* antigen used in Mto-wa-Mbu, and the *P. fieldi* in Muheza. It has already been demonstrated, however, that the average FA response with *P. fieldi* is very similar to that obtained with *P. falciparum* (*16–18*). Although the two different antigens may have had some bearing on the results, the FA response found in Mto-wa-Mbu was signi-

ficantly lower than that observed in Muheza-Ubembe despite the fact that the homologous and, therefore, more specific antigen was used. The difference in the humoral immunological response observed in Mto-wa-Mbu and the West Kiang district was, on the other hand, very marked; it is unlikely that this could be related only to different antigen specificity.

There is no reason to suspect that the sensitivity of the IFA assay system used in Mto-wa-Mbu was at fault. The IFA test is carried out routinely in the reference laboratory, not only for seroepidemiological studies but also for diagnostic purposes and for the screening of healthy blood donors. The sensitivity of the antigen and of the reagents are checked frequently in order to verify the validity and reliability of the test. During the second serological survey in March 1982, a number of precautionary measures were taken to avoid all possible causes of error. The results obtained confirmed to a large extent the original findings.

There is not, as yet, an immunological technique that can unquestionably relate antibody levels to protective immunity. The IFA technique is recognized as the best and most valid tool for the determination of the presence of antibodies to human malaria and has proved useful in estimating the development, persistence, and specificity of these antibodies. It is however not yet possible to say whether the humoral immunological response measured with the IFA technique is directly related to protective immunity. It can only be assumed that any significant change in the level of IFA antibodies may correspond to a similar change in protective immunity.

Previous longitudinal studies have proved that chemosuppression reduces the immunological response. It has been shown in the Gambia that after administration of antimalarial drugs to mothers and their infants, from the time of their birth for a period of 1 year, the FA levels of the mothers were markedly reduced and the children were serologically negative (*19*). In another study in Senegal, conducted among the child population of one village, this observation was confirmed.[a] In a research project in northern Nigeria, studies were carried out on the humoral immune response to malaria before, during, and after the application of control measures (spraying of residual insecticide and mass drug administration). At the end of the intervention phase, which lasted two years, the *P. falciparum* IFA titres in the protected population had decreased. A comparison between antibody titres in the protected and unprotected population showed a very significant difference in all age groups, and especially in the younger subjects (*20*). During the post-intervention phase there was convincing epidemiological evidence that the intervention was followed by a loss of parasitological immunity (*21*).

The results of the investigations carried out at Mto-wa-Mbu seem to provide further evidence that drugs, when given for long periods, have a negative influence on the immunological response of the population. However, the humoral immunological response of Mto-wa-Mbu's population was exceptionally low.

Seronegative reactions have often been observed in parasite carriers in hyperendemic malarious areas, and several possible reasons have been suggested. One explanation is that occasionally there may be a complete or partial immunological unresponsiveness (*22*). Any attempt to relate the lack of immunological response in Mto-wa-Mbu to the drug pressure exerted for many consecutive years can only be speculative, though *in vivo* studies have proved that the sensitivity of the *P. falciparum* strains in the locality has greatly diminished after many years of chemosuppression (*1*). It seems logical to suppose that the increased amount of chloroquine now required to eliminate the parasite from the peripheral blood circulation is the result of a certain loss of protective immunity. This loss may have been due to reduced antigenic stimulation as a consequence of the effect of the drug, such stimulation being necessary to maintain high levels of humoral antibodies (*23*).

The significance and interpretation of the results obtained in Mto-wa-Mbu are open to discussion; however, it is clear that more field studies are urgently required, particularly in view of the practical implications of the findings presented here.

[a] MATTERN, P. ET AL. *Chimioprophylaxie antipalustre et anticorps fluorescents.* WHO unpublished document, WHO/MAL/67.609, 1967.

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RÉSUMÉ

APPARITION D'UNE RÉSISTANCE DE *PLASMODIUM FALCIPARUM* À LA CHLOROQUINE AU SEIN D'UNE POPULATION SEMI-IMMUNE DE LA RÉPUBLIQUE-UNIE DE TANZANIE. 2. IMPACT DE LA CHLOROQUINE UTILISÉE COMME MÉDICAMENT CHIMIOPROPHYLACTIQUE SUR L'ÉTAT IMMUNITAIRE DE LA POPULATION

Une dimunition de la sensibilité et un début de résistance à la chloroquine des souches de *P. falciparum* ont été signalés à Mto-wa-Mbu, une localité du nord-est de la République-Unie de Tanzanie. Dans cette localité l'emploi de sels médicamenteux à base de chloroquine et l'accès facile à ce remède pour le traitement du paludisme remontaient à une vingtaine d'années.

Deux enquêtes séro-épidémiologiques ont été exécutées en mars 1981 et mars 1982 afin de mesurer par la technique d'immunofluorescence (IF) l'influence éventuelle de la chimioprophylaxie sur la réponse immunitaire de la population. En l'absence d'information sur le profil immunologique de la population de cette région avant l'introduction de la chloroquine (1960), les résultats des deux enquêtes ont été comparés à ceux obtenus en 1967 dans une autre localité du nord-est de la République-Unie de Tanzanie et en 1965 dans le district de West Kiang en Gambie. Dans ces deux territoires de référence le niveau d'endémicité paludienne était en effet le même qu'à Mto-wa-Mbu avant l'introduction du sel médicamenteux. Par comparaison avec ce profil immunologique de ces deux territoires, les résultats obtenus en 1981 et 1982 à Mto-wa-Mbu ont révélé une diminution hautement significative de la proportion des sujets à titre positif ainsi que des titres moyens systématiquement inférieurs dans tous les groupes d'âge.

Une réduction de la réponse immunitaire pourrait s'expliquer par la pression que le médicament a exercée sans interruption dans le territoire pendant de nombreuses années. Cependant une chute aussi importante dépassait toute attente et il n'est pas exclu que d'autres facteurs aient pu y contribuer.

Etant donné l'importance épidémiologique de ces observations, il est hautement recommandé d'entreprendre des études sérologiques longitudinales sur le terrain afin d'évaluer les effets possibles de la chimioprophylaxie sur la réponse immunitaire des populations protégées.

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