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Clones of different sensitivities in drug-resistant isolates of *Plasmodium falciparum*

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Clones of two Thai isolates of Plasmodium falciparum *were prepared and examined for variations in drug susceptibility and electrophoretic properties of enzymes. Both isolates were found to include mixtures of genetically distinct parasites. Of particular significance was the finding that one isolate (T_9), which on initial testing was resistant to chloroquine, pyrimethamine and sulfadoxine-pyrimethamine, yielded a clone of parasites markedly more sensitive to these three drugs, while five other clones resembled the original isolate in drug susceptibility.*

The spread to *Plasmodium falciparum* of resistance to currently used antimalarial drugs is a major constraint to control programmes in many parts of the world today. Following the confirmation of reports of resistance of this parasite to sulfadoxine-pyrimethamine combinations in south-east Asia and South America (*1*, *2*), resistance to quinine has been reported in south-east Asia (*3*). In addition, chloroquine resistance has spread further in this region and in South America and its appearance in local populations in at least six countries of East Africa was confirmed in 1982 (*4*). However, little is known about the genetics of drug resistance and how it spreads in a natural population. Naturally occurring *P. falciparum* always appears to be a mixture of populations, i.e., including parasites that differ in a considerable number of parameters (*2*). Thus it can be suggested from drug-response curves that a natural isolate may be heterogenous in its sensitivity to a particular drug. The present paper confirms this hypothesis and shows that two isolates of *P. falciparum* from Thailand, which were shown to be resistant to chloroquine, contained parasites that were both resistant and sensitive to the drug.

MATERIALS AND METHODS

Ten clones, as defined by the presence of a single isoenzyme type of each enzyme studied, were established from the two isolates, PB_1 and T_9 by a method based on limiting dilutions (*5*). PB_1 was obtained from a patient in Phra Phutthabat and T_9 from a patient in Ban Mae Sot, near Tak. The isolates were first characterized by enzyme electrophoresis (*6*) and then maintained in continuous culture for several months using the method of Trager & Jensen (*7*). This was followed by cryopreservation in liquid nitrogen.

At the time of isolation, PB_1 contained two forms of adenosine deaminase (ADA-I and ADA-II) and T_9 contained the identical isoenzymes of ADA and two forms of glucose-6-phosphate isomerase (GPI-I and GPI-II), all of which appeared to be present in equal amounts. This suggests that both isolates were mixtures of clones, as indicated by previous studies conducted by Rosario (*5*). These isoenzymes were observed in the isolates during the whole period of study although, during cultivation and cryopreservation, the staining intensity of the respective bands varied. This may indicate that during laboratory maintenance certain clones may be selectively favoured or lost. This observation has been confirmed by the reaction of the two isolates to strain-specific monoclonal antibodies (McBride, personal communication). The two isolates were thawed from liquid nitrogen and maintained in continuous culture for one month before cloning of the isolates was initiated. Prior to cloning, enzyme electrophoresis indicated that PB_1 had similar isoenzyme activities to those present at the time of isolation, whereas T_9 showed similar activities of ADA-I and GPI-I but weak activity for GPI-II and ADA-II. Drug sensitivity testing was also carried out on the isolate prior to cloning.

Cloning was initiated at a parasitaemia of 0.5%, in which 12–18% of the infected cells had two or more parasites. The technique used was that described by Rosario (*5*).

RESULTS AND DISCUSSION

Six clones were established from T_9 and four from PB_1. Each clone was tested for susceptibility to chloroquine and pyrimethamine by the method of

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Table 1. Drug susceptibility and isoenzymes of clones of T_9 isolate of *P. falciparum*

Clones	Enzyme type			Minimum inhibitory concentrations of six drugs (mol/l)					
	GPI	ADA	PEP	Chloroquine	Amodiaquine	Quinine	Mefloquine	Pyrimethamine	Sulfadoxine: pyrimethamine (Fansidar)
$T_{9/97}$	I	II	II	10^{-6}	2.5×10^{-8}	5×10^{-7}	10^{-7}	5×10^{-5}	1.6×10^{-5}: 10^{-6}
$T_{9/98}$	II	I	II	10^{-7}	2.5×10^{-8}	5×10^{-7}	10^{-7}	5×10^{-5}	1.6×10^{-5}: 10^{-6}
$T_{9/101}$	I	II	I	10^{-6}	2.5×10^{-8}	5×10^{-7}	5×10^{-8}	5×10^{-5}	1.6×10^{-5}: 10^{-6}
$T_{9/102}$	I	I	I	10^{-6}	10^{-8}	10^{-7}	5×10^{-8}	5×10^{-5}	1.6×10^{-5}: 10^{-6}
$T_{9/106}$	I	I	I	10^{-6}	2.5×10^{-8}	5×10^{-7}	10^{-7}	5×10^{-5}	1.6×10^{-5}: 10^{-6}
$T_{9/107}$	II	I	II	10^{-8}	10^{-8}	10^{-7}	10^{-7}	10^{-9}	1.6×10^{-9}: 10^{-10}
T_9[a]	II+I	I+II	?	10^{-6}	2.5×10^{-8}	10^{-7}	10^{-7}	5×10^{-5}	1.6×10^{-5}: 10^{-6}

[a] Original isolate before cloning.

Table 2. Drug susceptibility and isoenzymes of clones of PB_1 isolate of *P. falciparum*

Clones	Enzyme type ADA	Minimum inhibitory concentrations of six drugs (mol/l)					
		Chloroquine	Amodiaquine	Quinine	Mefloquine	Pyrimethamine	Sulfadoxine: pyrimethamine (Fansidar)
$PB_{1/1}$	I	10^{-6}	5×10^{-8}	5×10^{-7}	10^{-7}	5×10^{-5}	1.6×10^{-6}: 10^{-7}
$PB_{1/3}$	II	10^{-6}	5×10^{-8}	5×10^{-7}	10^{-7}	5×10^{-5}	1.6×10^{-6}: 10^{-7}
$PB_{1/4}$	II	5×10^{-7}	5×10^{-8}	5×10^{-7}	10^{-7}	5×10^{-5}	1.6×10^{-6}: 10^{-7}
$PB_{1/5}$	II	10^{-6}	5×10^{-8}	5×10^{-7}	10^{-7}	5×10^{-5}	1.6×10^{-6}: 10^{-7}
PB_1[a]	I+II	10^{-6}	5×10^{-8}	5×10^{-7}	10^{-7}	5×10^{-5}	1.6×10^{-6}: 10^{-7}

[a] Original isolate before cloning.

Thaithong & Beale (*8*), to amodiaquine, quinine and mefloquine by the methods described by Thaithong et al. (*9*), and to sulfadoxine-pyrimethamine (Fansidar) using RPMI 1640 medium minus 4-aminobenzoic acid (Thaithong et al., unpublished). In addition, each clone was characterized by gel electrophoresis for ADA and GPI and for peptidase (PEP) in the case of clones from the isolate T_9.

The results (Tables 1 and 2) indicate that, prior to cloning, PB_1 and T_9 were resistant to chloroquine, amodiaquine and pyrimethamine, but sensitive to quinine and mefloquine. They differed, however, in their response to sulfadoxine-pyrimethamine, PB_1 being ten times more sensitive to the drug combination. Following cloning, T_9 was shown to contain one clone ($T_{9/107}$) which was sensitive to chloroquine, pyrimethamine and sulfadoxine-pyrimethamine, four which were resistant to all three of these drugs, and one ($T_{9/98}$) which had an intermediate response to chloroquine but was resistant to pyrimethamine and sulfadoxine-pyrimethamine. Minor differences in the response of the individual clones to amodiaquine, quinine and mefloquine were also observed, but their significance is unknown.

All four clones prepared from PB_1 showed similar patterns of drug sensitivity to the parent isolate, with the exception of $PB_{1/4}$ which was two times more sensitive to chloroquine. All were sensitive to quinine and to mefloquine. Further investigations will be made to determine the relationship between drug sensitivity and isoenzyme patterns in particular clones.

Although the number of clones studied so far has been small, it is clear from the results presented that an isolate of *P. falciparum*, which has been shown to be resistant to a particular drug by *in vivo* or *in vitro* studies, may contain parasites of varying susceptibility including complete sensitivity to this drug. It is of interest to note that clone $T_{9/107}$, which was sensitive to chloroquine, pyrimethamine and sulfadoxine-pyrimethamine, was shown to grow *in vitro* at a slower rate than that observed in chloroquine-resistant clones. This observation has been made on other chloroquine-sensitive clones not reported here, and appears to correlate with the observations made by Rosario et al. (*10*) that chloroquine-resistant parasites of *P. chabaudi* had a selective advantage over those which were chloroquine-sensitive. Differences in the clones have also been observed in their reaction to strain-specific monoclonal antibodies, the results of which will be reported elsewhere.

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RÉSUMÉ

CLONES DE SENSIBILITÉ DIFFÉRENTE DANS DES ISOLEMENTS PHARMACORÉSISTANTS DE *PLASMODIUM FALCIPARUM*

Deux isolements de *Plasmodium falciparum* provenant de Thaïlande (PB_1 venant de Phra Phutthabat, et T_9 venant de Ban Mae Sot, près de Tak) ont été étudiés pour vérifier l'hétérogénéité des parasites sur le plan de la pharmacosensibilité et des propriétés électrophorétiques des enzymes. L'isolement PB_1 était à l'origine dix fois plus sensible à la sulfadoxine-pyriméthamine que T_9, et l'on constatait également de petites différences dans la sensibilité à l'amodiaquine et à la quinine. En revanche, la réponse à la chloroquine, à la méfloquine et à la pyriméthamine était la même dans les deux isolements. On a trouvé dans l'isolement PB_1 deux variants de l'enzyme adénosine-désaminase (ADA-I et ADA-II) et dans l'isolement T_9, outre les deux variants susmentionnés, deux variants de l'enzyme glucose-phosphate-isomérase (GPI-I et GPI-II).

Des clones ont été préparés par la méthode des dilutions limitantes: quatre à partir de PB_1 et six à partir de T_9. Chaque clone a été étudié sur le plan de la sensibilité aux médicaments et caractérisé par électrophorèse des enzymes.

Les clones provenant de PB_1 n'ont guère montré de différences dans leur sensibilité à tous les médicaments essayés. L'un des clones possédait l'enzyme type ADA-I, les trois autres ayant ADA-II.

En ce qui concerne les clones provenant de T_9, l'un ($T_{9/107}$) était nettement plus sensible à la chloroquine, à la pyriméthamine et à la sulfadoxine-pyriméthamine que les cinq autres clones ou que les isolements originels non clonés. Parmi les clones de T_9, on a trouvé différentes combinaisons des variants des enzymes ADA et GPI, ainsi que des variants d'une troisième enzyme, la peptidase (PEP), qui ont été notés au cours de la dernière partie du travail.

Il en est conclu qu'un isolement de *P. falciparum* qui, aux épreuves *in vivo* et *in vitro*, s'est révélé résistant à un ou plusieurs médicaments peut néanmoins contenir des clones de parasites présentant à l'égard des mêmes médicaments des degrés de sensibilité variables, y compris une sensibilité complète.

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