RESEARCH NOTE

IMPROVEMENT OF GROWTH OF *PLASMODIUM FALCIPARUM* FRESH CLINICAL ISOLATES BY USING AN ESTABLISHED SERUM-FREE MEDIUM, GIT

T Furuta[1], M Kimura[2], T Tsunoda[3], T Kikuchi[1] and S Kojima[1]

[1]Departments of Parasitology; [2]Infectious Diseases and Applied Immunology, The Institute of Medical Science, The University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8639; [3]Department of Infectious Diseases, Tokyo Metropolitan Ebara General Hospital, 4-5-10 Higashiyukigaya, Ohta-ku, Tokyo 145-0065, Japan

Abstract. In the present study, we have tried to establish continuous cultures of fresh clinical isolates of *P. falciparum* by using a serum-free meduim, GIT. To examine the ability of GIT to support the parasite growth, the growth of various *P. falciparum* isolates including two laboratory strains of *P. falciparum*, FCR3 and Kl was compared in both of GIT and RPMI 1640 medium supplemented by 10% human serum (RPMI-HS). Growth rates of various *P. falciparum* expressed as fold increases were compared in GIT and RPMI-HS, and the maximum growth rates of *P. falciparum* were 72 in GIT and 35 in RPMI-HS during the culture for 8 days. Growth rate of the clinical isolates varied individually in both culture media, with average growth rates of parasites being 15.9 in GIT and 8.8 in RPMI-HS, respectively (not significant). Growth rates of FCR3 and Kl strains were 28.0 and 6.6 in GIT, and 10 and 7.5 in RPMI-HS. After 30 days culture of *P. falciparum* in GIT, 9 of 12 clinical isolates still continuously propagated but other three isolates disappeared. Despite variation of the *P. falciparum* isolates in their abilities to multiply in GIT, our experiments suggested that GIT is useful for culture of fresh clinical isolates of *P. falciparum* that are derived from geographically distinct areas as well as laboratory strains used commonly in laboratory research.

INTRODUCTION

Continuous *in vitro* cultivation of erythrocytic stages of *Plasmodium falciparum* by using RPMI 1640 medium supplemented with human serum has been effectively applied for malaria research (Trager and Jensen, 1976). Recent studies have attempted to replace human serum with different components or commercially available serum supplements in the cultivation of *P. falciparum* (Asahi *et al*, 1996; Flores *et al*, 1997; Lingnau *et al*, 1994; Kilunga Kubata *et al*, 1998). Although several suggestions have arisen as to which factors of human serum are necessary for growth, the results have been conflicting, and the complete identification has not yet been carried out. The ability of serum-free media to support parasite growth also varied among parasite strains used in the experiments. Previous studies have tested only laboratory strains of *P. falciparum* that were maintained *in vitro* for long term and commonly used in laboratory research, however, it has not been examined yet whether those serum free-media are useful for culture of fresh clinical isolates of *P. falciparum* as well as for that of laboratory-established *P. falciparum* strains. A recent study indicated that GIT, an established serum-free medium, could support the growth of laboratory *P. falciparum* strains *in vitro* without any supplement (Asahi and Kanazawa, 1994). However, GIT has never been tested for cultivation of *P. falciparum* that are newly isolated from malaria patients. It is important to examine this point because the ability of serum-free media to support parasite growth varies among malaria parasite strains.

In the present study, we have tried to establish continuous cultures of fresh clinical isolates of *P. falciparum* by using GIT. To examine the ability of GIT to support the parasite growth, the growth of various *P. falciparum* isolates including two laboratory strains of *P. falciparum*, FCR3 (drug resistance, Gambia) and K1(drug resistance,Thailand) was compared in both of GIT and RPMI 1640 medium supplemented by 10% human serum. *P. falciparum*-infected blood samples were collected from 12 malaria patients admitted to the Hospital of Institute of Medical Science, the University of Tokyo before they received anti-malarial drug treatments. Suspected countries where they contracted malaria were those of Southeast Asia and

Table 1
Growth rate of *P. falciparum* (fold increase).

No. isolates	Suspected countries[a]	GIT	RPMI 1640 with 10% human serum
1	Kenya[b]	72	1
2	Madagascar	30	35
3	Kenya	19.3	23
4	Ghana	17.2	21.4
5	Tanzania[c]	13.8	1.9
6	Mali	8.8	3.5
7	Tanzania	8	0.5
8	Lao PDR	6	8
9	Ghana	5.4	5.9
10	Tanzania	5.4	2.3
11	Ghana	3.4	0.1
12	Papua New Guinea	1.8	3.3
FCR3		2.8	10
K1		6.6	7.5

[a]Suspected countries where infection took place.
[b]Infected blood had been stored at 4ºC for 2 days before the culture was started.
[c]The parasite donor had been treated with sulfadoxine-pyrimethamine 1 day before the blood was taken.

Africa. *P. falciparum* -infected erythrocytes, and uninfected erythrocytes (type B+), obtained from one single donor, were washed three times with GIT (Wako Pure Chemical Industries Ltd, Osaka, Japan) or RPMI 1640 containing glutamine (Gibco BRL, Life Technologies, Inc, Grand Island, NY, USA), 25 mM 4-(2-hydroxyethyl)-piperazine ethanesulfonic acid, sodium salt (HEPES Na, Sigma Chemical Co, St Louis, MO, USA), 24 mM $NaHCO_3$, 100 mg/ml streptomycin sulfate (Sigma). The RPMI 1640 medium was supplemented with 10% (v/v) human serum (RPMI-HS). Mixture of infected and uninfected erythrocytes (see below) were distributed in a 24 well culture plate (1 ml suspension/well) at a hematocrit value of 3%, and the culture was performed for 8 days under a humidified atmosphere of 5% CO_2, 5% O_2 and 90% N_2, at 37ºC, with daily medium changes. Synchronization of the FCR3 and K1 erythrocytic stages in culture was achieved by successive exposures to 5% (w/v) D-sorbitol (Sigma) according to Lambros and Vanderberg (1979). The parasitemia was initially adjusted to 0.1 to 0.5% by adding uninfected cells, subcultures were performed on the fourth day after starting the culture. For assessment of the parasite growth, samples were taken at daily medium change, thin blood smears were made and stained with Giemsa. More than 1,000 erythrocytes were examined to determine the percentage of parasitized cells (parasitemia) for the calculation of a growth rate. The growth rate was determined by dividing the parasitemia of the test sample at a time indicated by the initial parasitemia at 0 day.

As shown in Table 1, growth rates of various *P. falciparum* expressed as fold increases were compared in GIT and RPMI-HS. The maximum growth rates of *P. falciparum* was 72 in GIT and 35 in RPMI-HS during the culture for 8 days. All *P. falciparum* in GIT and 8 of 12 *P. falciparum* isolates in RPMI-HS were continuously propagated in this period. Growth rate of the clinical isolates varied individually in both culture media, with average growth rates of parasites being 15.9 in GIT and 8.8 in RPMI-HS, respectively (not significant). Six out of 12 isolates showed a higher growth rate in GIT than that in RPMI-HS, whereas 6 of 12 isolates did so in RPMI-HS than in GIT. Growth rates of FCR3 and K1 strains were 28.0 and 6.6 in GIT, and 10 and 7.5 in RPMI-HS respectively. After 30 days culture of *P. falciparum* in GIT, 9 of 12 clinical isolates still continuously propagated but other three isolates disappeared. In Giemsa-stained smears, asexual forms of *P. falciparum* that were grown in GIT were morphologically normal, and pigment comparable with that seen in RPMI-HS was formed. Numbers of merozoites in schizonts were same in both culture

media (data not shown).

Despite variation of the *P. falciparum* isolates in their abilities to multiply in GIT, our experiments showed that GIT is useful for culture of fresh clinical isolates of *P. falciparum* that are derived from geographically distinct areas as well as laboratory strains used commonly in laboratory research. Most of the isolates propagated for more than 30 days in GIT, and furthermore, those GIT-adapted parasites were stable upon freezing at -80ºC and subsequent thawing in either GIT or RPMI-HS. It has been reported that GIT is an all-purpose medium and can support growth of many kinds of mammalian and hybridoma cells (Sasaki *et al*, 1985), and that the mechanism of support of parasite growth by GIT is not yet clear. However, it has been suggested that components of a 55-70% ammonium sulphate fraction of adult bovine serum contained in GIT give good effects for intraerythrocytic growth of *P. falciparum* (Asahi *et al*, 1996). Since GIT also supports growth of *Leishmania* parasites *in vitro* without any supplements (Yamazaki *et al*, 1994), this medium could be a useful tool for culture of other protozoan organisms.

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