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Original article

Blood typing in *Saimiri sciureus* monkeys: influence of anti-red blood cell alloantibodies on *Plasmodium falciparum* parasitaemia in vivo

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Abstract

The *Saimiri sciureus* monkey is a well-established host for experimental studies with human malaria parasites. During the course of iterative inoculations with *Plasmodium falciparum* parasitised red blood cells (RBC), anti-RBC alloantibodies were detected in the sera of two of eight *Saimiri* monkeys. These anti-RBC antibodies were further used to investigate RBC phenotypes in 35 colony-reared *Saimiri* monkeys by flow cytometry. Three RBC phenotypes (named I–III) were observed. Their distribution was I (86%), II (11%) and III (3%). Using the Palo Alto FUP-2 strain, a variant *P. falciparum* line insensitive to hyperimmune serum and the passive transfer of anti-RBC alloantibodies, a dramatic drop in parasite growth was documented in an incompatible monkey.

Keywords: Red blood cells; Anti-red blood cell alloimmune sera; Flow cytofluotometry; Blood groups; *Plasmodium falciparum*; Passive transfer; *Saimiri* monkey

1. Introduction

The *Saimiri sciureus* monkey has been used for several decades as an experimental host for studies on human malaria, vaccine development and pathogenicity studies [1–9]. In this model, as in humans, antibodies play an important role in the protective immune response elicited after repeated *Plasmodium falciparum* blood stage inoculation. Protection has been correlated with the capacity of antibodies to promote phagocytosis of *P. falciparum* parasitised red blood cells (RBC) [10–13]. During iterative inoculations of *P. falciparum* parasitised RBC in recipient animals to generate hyperimmune sera, we detected anti-RBC alloantibodies (anti-RBC-alloAbs) in two out of eight monkeys. These allosera were further used as reagents for the RBC typing. They identified three major RBC phenotypes in *Saimiri* monkeys from our breeding colony. Passive transfer of sera containing anti-RBC-alloAbs in an incompatible animal harbouring *P. falciparum* resulted in a dramatic drop in parasite multiplication.

2. Materials and methods

2.1. Animals

Randomly selected, 4-year-old male *S. sciureus* monkeys (of karyotype 14-7 and Guyanese phenotype) bred in the animal facility at the Institut Pasteur, French Guiana, were used [5]. All monkeys were splenectomised 1–3 months before their first inoculation with *P. falciparum* blood stages. Splenectomy was performed after lateral laparotomy under anaesthesia with a combination of medetomidine (Pfizer Corp., France) and ketamine chlorhydrate (Merieux, France). Fresh

Abbreviations: anti-RBC-alloAbs, anti-red blood cell alloantibodies; HIS, hyperimmune serum; RBCs, red blood cells.

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or cryopreserved *P. falciparum*—parasitised blood inoculation and blood sample collection were performed intravenously under light ketamine anaesthesia. At the time of blood inoculation no patent haemomycoplasmosis infection was detected. All animal handling procedures were performed under the regulations of the Institut Pasteur Ethics and Animal Use Committee.

2.2. Parasites

The *P. falciparum* parasite strain FUP-2 [14], derived from the Uganda Palo Alto FUP-1 strain of *P. falciparum* adapted to splenectomised *Saimiri* monkeys was used [3,15]. Giemsa-stained thin blood films were performed daily to measure the parasitemia and record the proportion of various development stages (ring-, trophozoite-, and schizont-parasitised RBCs) of *P. falciparum*.

2.3. Antisera

Sera were collected from splenectomised *Saimiri* monkeys S 91480 and S 90114, undergoing inoculations with parasitised blood originated from monkeys subjected to a primary infection with *P. falciparum* FUP-2 parasites (Table 1). Successive infections were performed by intravenous injection of 1×10^8 *P. falciparum* FUP-2-parasitised-RBCs. Individual sera collected before and approximately 60 days after each inoculation, were incubated for 30 min at 56 °C, aliquoted and stored at –20 °C. Sera collected from monkey S 91480 (IS 91480) and monkey S 90114 (IS 90114) following their seventh challenge with parasitised blood were used as primary reagents for RBC phenotyping. The HIS 2 pool of hyperimmune sera was made by pooling equal volumes of sera collected from six splenectomised *Saimiri* monkeys rendered resistant to a challenge of 1×10^8 *P. falciparum* FUP-2-parasitised-RBCs. Resistance was achieved following several rounds of drug-cured parasitism with FUP-2-parasitised-RBCs from primary infected monkeys. The emergence of anti-RBC-alloAbs was not detected in these *Saimiri* monkeys.

2.4. RBC phenotyping by flow cytometry

RBC surface indirect immunolabelling and analysis by flow cytometry were performed as described [12]. Blood samples were washed twice with 0.9% saline. Five microlitres RBC pellets were resuspended in 100 µl of a 1/50 dilution of IS 91480, IS 90114 alloimmune serum or autologous serum, in FACSflow solution (Becton-Dickinson, San Jose, CA, USA) containing 2% foetal calf serum (FACSflow-S), and incubated at room temperature for 30 min. After washing with FACSflow-S, the RBCs were incubated for 30 min with 50 µg/ml of a mouse monoclonal antibody reacting with *Saimiri* monkey IgG (mAb3F11/G10) [12]. After washing with FACSflow-S, the RBCs were incubated for another 30 min at room temperature with phycoerythrin (PE) conjugated sheep anti-mouse IgG (Sigma-Aldrich, St Quentin, France) at 1/50 dilution. Finally, the RBCs were washed twice with FACSflow-S. Cytometric analysis was performed using a FACScan flow cytometer (Becton-Dickinson). Fluorescence parameters were recorded with logarithmic amplifications. List mode data from 10,000 cells were stored and processed with LYSIS software (BDIS).

3. Results

3.1. Emergence of anti-RBC-alloAbs in Saimiri monkeys following iterative P. falciparum parasitised blood inoculations

Fig. 1 shows the progressive acquisition of anti-RBC-alloAbs by *Saimiri* monkey S 91480, following successive inoculations with *P. falciparum* FUP-2 parasitised blood from various donor monkeys (Table 1). RBCs from an unparasitised donor (S 91007) were analysed by flow cytometry for surface labelling after incubation with serum from the S 91480 monkey collected after three, five and seven inoculations. The weak signal detected after the third infection (Fig. 1B), was markedly increased after the fifth and the seventh infection (Fig. 1C, D, respectively), indicating both

Table 1
Collection of sera from two *Saimiri* monkeys undergoing repeated inoculations with *P. falciparum* FUP-2 parasitised RBC

	P. falciparum FUP-2 infected blood from *Saimiri* monkeys								
	S 90070	S 91480 cryoctable	S 90114	S 90020	S 91059	S 91025	S 91049	S 92062	S 91007
Saimiri monkey S 91480	Infection 1	Infection 2	Infection 3	Infection 4	Infection 5	Infection 6	Infection 7	Infection 8	
Code of immune serum after infection	IS 1	IS 2	IS 3	IS 4	IS 5	IS 6	IS 7 **IS 91480**	IS 8	
Saimiri monkey S 90114	Infection 1			Infection 2	Infection 3	Infection 4	Infection 5	Infection 6	Infection 7
Code of immune serum after infection	IS 1			IS 2	IS 3	IS 4	IS 5	IS 6	IS 7 **IS 90114**

Splenectomised monkeys S 91480 and S 90114 were inoculated intravenously with 1×10^8 *P. falciparum* FUP-2-parasitised-RBC from monkeys subjected to a primary infection. Individual sera were collected approximately 60 days after each infection. Sera from monkey S 91480 (IS 91480) and monkey S 90114 (IS 90114) following their seventh challenge with infected blood were used as primary reagents for RBC phenotyping.