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Research Note

Plasmodium falciparum: Selection of Infected Erythrocytes Adhesive to Amelanotic Melanoma Cells C32

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The erythrocytes infected with mature stages of *Plasmodium falciparum* (P.f.) adhere to the endothelium of venules and capillaries via knobs, which causes intravascular sequestration (Miller, 1969). *In vitro* correlate of sequestration was developed by using cultured human endothelial cells (Udeinya *et al.*, 1981) and amelanotic melanoma cells C32 (Schmidt *et al.*, 1982). Fresh isolates of P.f. from humans or Aotus monkeys were used for these experiments. The adhering ability in vitro of different P.f. isolates was variable (Udeinya *et al.*, 1983).

Strains of cultured parasites with stable adhering ability are necessary for studying the mechanism of cytoadherence of infected erythrocytes. We attempted to select infected erythrocytes capable of adhering to amelanotic melanoma cells, which was a useful substitute for endothelial cells in adhering studies requiring large numbers of target cells. Two isolates of P.f., FCW-1 and MAG, were provided by Prof. M. Suzuki, School of Medicine, Gunma University, Japan. FCW-1 was carried back from Liverpool School of Tropical Medicine in 1986. MAG was isolated from Malagasy patients at Gunma University in 1987. Amalanotic melanoma cell line, No. CRL 1585, designated C32, was purchased from the American Type Culture Collection. The selection procedures were based on the idea that first knob-positive infected erythrocytes were selected from continuous culture of the parasites by using the gelatin floatation method (Jensen, 1978) and then infected erythrocytes adhesive to the melanoma cells were selected from the knob-positive infected erythrocytes. Briefly, two isolates were maintained in continuous culture according to standard procedures (Trager and Jensen, 1976) except for the use of a CO_2-N_2 gas incubator instead of candle jars. The gas mixture was adjusted to 5% CO_2, 5% O_2 and balanced N_2. Infected erythrocytes in the culture with more than 3% parasitemia were suspended in 0.5% gelatin/RPMI 1640 medium to a 10% Ht in 10 ml tubes and placed in a 37°C incubator. After 30 min knob-positive erythrocytes containing trophozoites and schizonts were concentrated in the supernatant. A part of the concentrated erythrocytes were resuspended in complete medium (RPMI 1640 medium supplemented with 25 mM HEPES, 20 mM sodium bicarbonate, 25 μg/ml gentamicin sulfate and 10% human type O serum, pH 7.4) and inoculated into the melanoma cell culture in 100 mm dishes. The remainder was used in the adherence assay. After 90 min incubation at 37°C in a 5% CO_2 incubator, the erythrocytes adhered to the melanoma cells were left by removing non-adherent erythrocytes. Fresh erythrocytes were added to the culture and the dishes were incubated in the CO_2-N_2 gas incubator for 24 h. Then, overlaid erythrocytes were transferred into new culture dishes without melanoma cells and cultured until parasitemia reached more than 3%. The same procedures were repeated.

The adherence assay was performed on cover

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slips in duplicate. The concentrated erythrocytes were suspended in the complete medium to a 1% Ht and inoculated into two wells of 24-well tissue culture plate (Nunc). Each well contained target cells grown on Thermanox cover slips (Lux). The plate was incubated at 37°C in a CO_2 incubator for 90 min with gently rocking by hand every 30 min. At the end of incubation, the cover slips were taken out, gently washed in RPMI 1640 medium, dried, fixed in methanol, and stained with Giemsa. The number of adhering erythrocytes per 100 target cells was determined by a light microscopy and was expressed as the adherence rate on the basis of 1% parasitemia.

More than 98% erythrocytes adhering to the melanoma cells were infected with trophozoite and schizont stage of the parasite. The adherence rate of erythrocytes infected with FCW-1 increased from 1.1 in the initial assay to 20.5 after nine repeats of the selection procedures. The adherence rate of MAG infected erythrocytes also increased from 0.3 to 20.7 after five repeats (Fig. 1). Both isolates showed one fluctuation of the adherence rate before reaching the final level although the reason was not clear. To compare the adherent ability of infected erythrocytes to amelanotic melanoma cells and to endothelial cells, three samples of the MAG infected erythrocytes were simultaneously applied to the endothelial cells and the melanoma cells. Endothelial cells were prepared from umbilical vein of a newborn baby according to the method of Kan *et al.*, 1985. The adherence rate to the endothelial cells also increased by the selection procedures, though the degree was far lower than that to the melanoma cells. When the adherence rate exceeded 20, both isolates were subjected to the continuous culture to examine the stability of their adherent ability. The adherence rate of both isolates remained at a high level for at least 69 days though MAG showed a little fluctuation of the adherence rate (Fig. 2). Thus, infected erythrocytes having an ability to adhere to the target cells can be selected by the procedures described in the present work. Knob phenotype of erythrocytes infected with MAG or FCW-1 was examined by the scanning electron microscopy during the continuous culture after

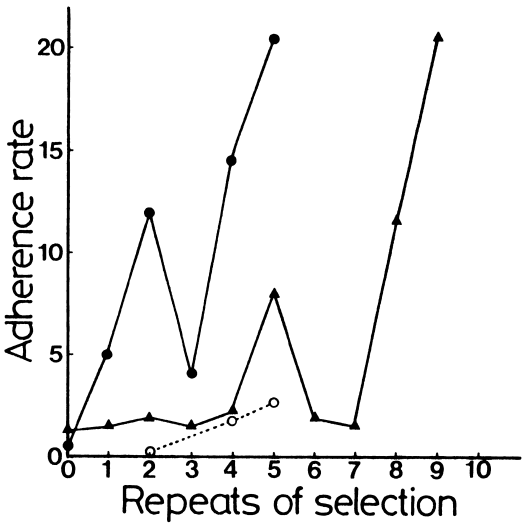

Fig. 1 Adherence rate of MAG or FCW-1 infected erythrocytes during repeated selection. A repeat of selection is a series of the gelatin floatation method and the adherence to amelanotic melanoma cells C32. Adherence rate is number of infected erythrocytes adhering to 100 target cells on the basis of 1% parasitemia.
—●—; MAG infected erythrocytes adhered to C32.
—▲—; FCW-1 infected erythrocytes adhered to C32.
—○—; MAG infected erythrocytes adhered to human umbilical vein endothelial cells.

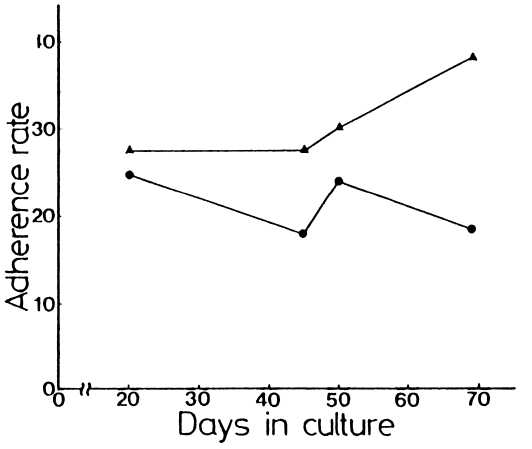

Fig. 2 Stability of the adherence rate of MAG or FCW-1 infected erythrocytes to amelanotic melanoma cells C32. After the last selection the isolates were maintained in continuous culture and were examined their adherence rate four times.
—●—; MAG infected erythrocytes.
—▲—; FCW-1 infected erythrocytes.

the last selection. Both the isolates infected erythrocytes were knob-positive. Recently cytoadherence of knobless infected erythrocytes was reported (Udomsangpetch *et al.* 1989). Although whether the parasites which induces no knobs on the surface on infected erythrocytes play an important role in natural infection is still unknown at present, knobless infected erythrocytes may be also useful for studying the mechanism of the cytoadherence of infected erythrocytes.

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