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A Nonhuman Primate Model for Severe Human Malaria: *Plasmodium coatneyi* - Infected Japanese Macaque (*Macaca fuscata*)

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A major factor in the pathogenesis of severe human malaria with cerebral involvement due to *Plasmodium falciparum* is blockade of microvessels by sequestration of parasitized red blood cells (PRBCs). However, pathogenesis of rodent malaria does not appear to be the same as that of human falciparum malaria because erythrocytes infected with rodent malaria parasites do not form knobs or show PRBC sequestration in microvessels. Aikawa and others have demonstrated that rhesus monkey (*Macaca mulatta*) infected with *P. coatneyi* or *P. fragile*, and squirrel monkey (*Saimiri sciureus*) infected with *P. falciparum* produced PRBC sequestration in the cerebral microvessels which were very similar to that of human cases. We recently showed that the *P. coatneyi* - infected Japanese macaque could be used as a primate model for severe human malaria, including cerebral pathology. In the investigation reported here, we conducted clinical and pathological observations of *P. coatneyi* - infected Japanese macaque, and *in vivo* and *in vitro* studies on our primate model. This study also includes a discussion on the applicability of our primate model to the study of severe human malaria.

Clinical observations: The monkeys infected with *P. coatneyi* developed a fulminating acute infection and became moribund. When the animals became moribund, typical clinical signs of acute severe malaria such as restlessness, severe anemia, dark-colored urine, shivering, dyspnea, and cyanosis were observed. Several hours after developing these symptoms, the monkey became lethargic and comatose.

***In vivo* studies**: Plasma levels of TNF-α, IFN-γ, soluble intracellular adhesion molecule-1 (ICAM-1) and soluble vascular cell adhesion molecule-1 (VCAM-1) were high at the time when the animals became moribund. In addition to the responses shown above, extremely lowered plasma glucose levels in the infected monkeys were observed according to the development of high parasitemia. Moreover, we analyzed proportion of lymphocyte subpopulations in peripheral blood during the course of infection. $CD4^+$ T cell showed little change at the level of population, while $CD8^+$ T cell decreased in population when the animals became moribund.

***In vitro* studies**: We studied the spontaneous rosette formation of PRBCs and the binding of PRBCs to human umbilical vein endothelial cells (HUVECs) that express ICAM-1. Rosette formation involved 88% of PRBCs after 30 hrs of incubation. Spontaneous rosettes were formed when the ring-stage parasites developed into late trophozoites or schizonts. A rosette usually consisted of a PRBC surrounded by three or more uninfected erythrocytes. In electron micrographs, interaction with adjacent uninfected erythrocytes in rosettes appeared to be mediated by the knob of PRBCs. The binding of PRBCs to HUVECs was observed in 222 cells per 500 cells, and the number of bound PRBC per HUVEC was 1～7 PRBCs.

Pathological observations: Light microscopic observation of the autopsy tissue from infected monkey showed PRBC sequestration leading to microvascular clogging in the brain and other organs. Particularly, prominent sequestration of the infected erythrocytes was uniformly distributed in capillaries of the heart and lungs. Electron microscopy revealed multiple electron dense knobs protruding from the membrane of PRBC, and

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PRBCs adhered to the endothelial cells in microvessels by means of the knob. These microvessels with sequestered PRBCs were shown by immunohistochemical analysis to possess the ICAM-1.

These findings were remarkably similar to those seen in severe human cases. Our nonhuman primate model may serve as a promising candidate for the assessment of severe haman malaria.