

Epidemic Typhus Infection in Cynomolgus Monkeys (*Macaca fascicularis*)†

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A nonhuman primate model of clinical *Rickettsia prowazekii* infection was developed in cynomolgus monkeys (*Macaca fascicularis*). Monkeys infected intravenously with 10^7 plaque-forming units developed clinical signs of illness and pathological changes characteristic of epidemic typhus infection in humans. Increases in total leukocyte counts, serum alkaline phosphatase, blood urea nitrogen, and serum glutamic pyruvate transaminase values were observed. Microscopic examination revealed typical typhus nodules in the brains of two monkeys that died. These data indicated that the cynomolgus monkey is a suitable model for study of the pathogenesis of epidemic typhus infection and may prove valuable in the evaluation of candidate *R. prowazekii* vaccines.

Epidemic typhus is a louse-borne infection of humans characterized by a sudden onset of high fever, headache, rash, weakness, and malaise. Photophobia, delirium, stupor, and coma may occur. The disease often takes a prolonged course lasting 2 to 3 weeks. The incubation time is generally 7 to 14 days. The rickettsiae are carried to the small capillary beds of the brain, skin, heart, and other organs, where they multiply in the endothelial cells. These small capillaries are occluded by swelling and proliferation of endothelial cells, resulting in degeneration with hemorrhage and perivascular accumulation of mononuclear cells or typhus nodules (11).

Rickettsia prowazekii infection in small laboratory animals has been reported (3, 6, 9). Cotton rats and gerbils were found to be most susceptible; some fatal infections occurred; rickettsiae persisted in the brains and kidneys of cotton rats for 35 and 60 days, respectively.

Perez Gallardo and Fox (9) reported that guinea pigs were not susceptible to low doses but were extremely susceptible to high doses of typhus rickettsiae. Very low infecting doses were reported by Ormsbee et al. (8); in our laboratory, low doses of the Breinl strain caused fever and scrotal swelling in Hartley strain guinea pigs (unpublished data).

Studies of epidemic typhus infection in goats, donkeys, and calves showed that these domestic animals are relatively insusceptible (10), although the donkey maintained complement-fix-

ing and toxin-neutralizing titers for more than a year after infection.

Early studies by Nicolle et al. (7) and Anderson and Goldberger (1) established that bonnet, rhesus, and capuchin monkeys were susceptible to epidemic typhus infection. Blood from infected monkeys or humans was used as the inoculum, but the dose of rickettsiae was uncertain.

In 103 rhesus monkeys studied by Anderson and Goldberger, the mean incubation period after intraperitoneal or intravenous inoculation was 8.7 days with a mean duration of illness of 9.1 days (1). Clinical signs of illness in these animals included fever (often greater than 41°C), anorexia, depression, and ruffled fur. Subcutaneous inoculation in a few animals resulted in inconsistent and unpredictable results. Barker et al. (2) described the infection in rhesus and vervet monkeys. Clinical illness and temperature responses were dose-related, and signs varied with the route of infection. The intravenous route resulted in more severe illness than subcutaneous inoculation, and although rhesus monkeys became severely ill after subcutaneous challenge, they did not become febrile. There were no consistent or persistent changes in the clinical laboratory data.

The purpose of this study was to define the clinical signs and pathological changes and determine a dose response to *R. prowazekii* infection in cynomolgus monkeys.

MATERIALS AND METHODS

Laboratory monkeys. Sixteen healthy cynomolgus monkeys (*Macaca fascicularis*) of both sexes, weighing 2.5 to 4.0 kg, were used. They were housed

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in individual cages and fed a commercial ration (Ralston Purina Co., St. Louis, Mo.) and water *ad libitum*. All sampling of blood was done between 8:00 and 9:00 a.m. (before feeding). Monkeys were observed several times daily for clinical signs of illness. Baseline values were established for each monkey during a 2-week period before infection. Mean rectal temperatures were based on five sampling days, and baseline hematology and serum chemistry values were determined from four samples on each monkey. Rectal temperatures were recorded daily for 3 weeks after challenge, and postchallenge hematology and serum chemistry values were measured serially at selected intervals.

Rickettsiae. The Breinl strain of *R. prowazekii* was propagated in the yolk sac of embryonated chicken eggs. Rickettsiae were harvested from surviving eggs when 50% of the infected eggs had died. Rickettsiae were titrated by the plaque assay method of Weinberg et al. (14).

Hematology and serum chemistry. Blood samples collected in ethylenediaminetetraacetic acid were analyzed for total and differential leukocyte counts, hematocrit, total protein, and fibrinogen. Blood urea nitrogen (BUN), serum glutamic pyruvate transaminase (SGPT), and serum alkaline phosphatase were determined by using commercially available kits (Mallinckrodt, Inc., Hazelwood, Mo.).

Electrocardiogram. A six-lead electrocardiogram (ECG) was recorded on each monkey before inoculation and every 2 days during the febrile period. Monkeys were lightly sedated with 10 mg of ketamine hydrochloride per kg for these recordings.

Pathology. Monkeys that died were examined for gross and microscopic lesions. The tissues were fixed in 10% neutral phosphate-buffered Formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin for routine examination.

Experimental design. Sixteen cynomolgus monkeys were randomly placed into one of four groups. All monkeys were injected intravenously, as follows: four with 10^4 plaque-forming units (PFU) of *R. prowazekii* (low dose), four with 10^5 PFU (medium dose) and six with 10^7 PFU (high dose); two monkeys were given 0.5 ml of normal saline as controls.

Statistical analyses. Analysis of variance for repeated measurements was used to detect differences significantly greater than any variability of individual baseline values (5). Significance of repeated measurements was based on the least-significant-difference procedure, with the level of significance set at $P < 0.05$.

RESULTS

Control monkeys remained normal throughout the study. No changes were noted in ECG, hematology, or serum chemistry values. The clinical responses of all monkeys are summarized in Table 1.

All inoculated monkeys became clinically ill with signs ranging from slight anorexia and depression in the low-dose group to severe depression, dissociated appearance, nystagmus, and head twitching in the high-dose group. Clinical signs appeared 2 to 3 days after inoculation and lasted 6 to 12 days. Surviving monkeys showed a marked febrile response beginning 1 to 3 days after inoculation; rectal temperatures as high as 41°C were recorded. Incubation time was longer in the low-dose group than in the two higher-dose groups, and fever was prolonged as the infecting dose was increased. Monkeys that died were initially febrile and then became increasingly hypothermic for 3 to 6 days before death. Three of the six monkeys infected with 10^7 PFU died. The mean time to death was 7.3 days with a range of 5 to 11 days.

The total leukocyte count of surviving monkeys increased from days 7 through 14 to as high as $20,000/\text{mm}^3$, whereas there was no significant leukocyte response in those that eventually died (Fig. 1A). The leukocytosis was due to an absolute neutrophilia with a pronounced left shift in surviving monkeys.

Total protein values of all monkeys remained within normal limits throughout the study. The hematocrit of all monkeys decreased slightly due to repeated bleeding. Fibrinogen concentrations increased slightly in all infected monkeys (from 100 to 200 mg/100 ml to 300 to 400 mg/100 ml).

Serum alkaline phosphatase values in all infected monkeys were elevated on day 7 (as high as 170 IU). Values returned to a normal range of 50 to 70 IU by day 14 in surviving monkeys, but continued to increase until death occurred in the others (Fig. 1B).

BUN concentrations remained normal (15 to 20 mg/100 ml) in the two low-dose groups. In

TABLE 1. Clinical responses of cynomolgus monkeys infected with *R. prowazekii*

Dose (PFU)	No. of monkeys	No. of febrile monkeys	Fever		No. of dead monkeys	Mean days to death (range)
			Mean days to onset (range)	Mean days duration (range)		
Saline controls	2	0	0	0	0	0
10^4	4	4	5.0 (3-9)	3.5 (1-6)	0	0
10^5	4	4	1.5 (1-3)	4.7 (2-6)	0	0
10^7	6	6	1.2 (1-2)	6.3 (5-10)	3	7.3 (5-11)

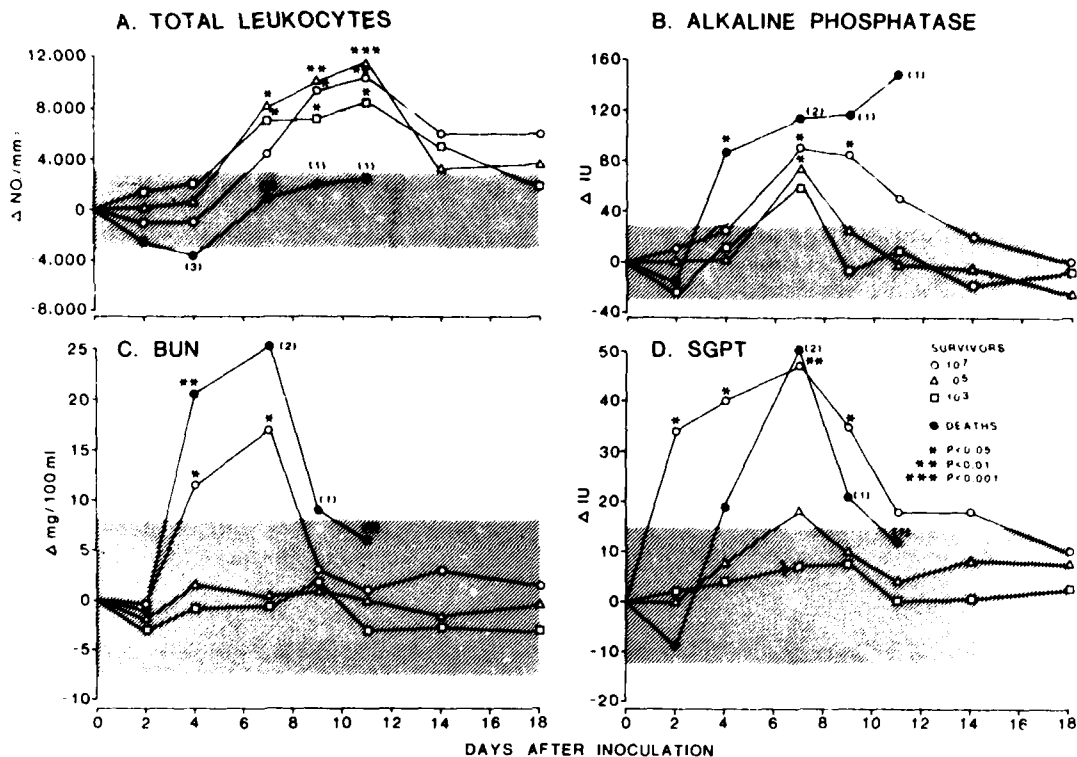


FIG. 1. Changes in total leukocyte counts (A) and serum alkaline phosphatase (B), BUN (C), and SGPT (D) values in cynomolgus monkeys after intravenous inoculation with various doses of *R. prowazekii*. Monkeys that died after challenge with 10^7 PFU are shown separately. Numbers in parentheses represent the number of surviving animals at that point. Shaded area indicates mean + standard deviation values of the prechallenge baseline measurements.

the monkeys infected with 10^7 PFU of *R. prowazekii* the BUN was elevated 4 to 9 days after infection. Values (90 mg/100 ml) were significantly higher ($P < 0.05$) on day 7 in the monkeys that died than in those that survived (Fig. 1C).

SGPT activities increased (from 20 to 80 IU) from days 2 to 9 in the high-dose group. Values in the two lower-dose groups remained within normal baseline limits (Fig. 1D).

The ECG of one monkey showed low-voltage recordings 24 h before death (day 10), when compared to baseline measurements. The R-wave amplitude in lead II was 0.4 mV, one-third lower than in baseline tracings. There were no changes in the ECG of the other monkeys.

Splenomegaly was noted on gross pathological examination of all monkeys that died. Microscopic examination revealed various degrees of vasculitis with perivascular infiltration of lymphocytes and plasma cells in the lungs, spleen, pancreas, liver, kidneys, urinary bladder, and myocardium. There were occasional areas of mononuclear cell infiltration between myocardial fibers in two of the animals. The one mon-

key with low-voltage ECG recordings also had occasional thrombosis of myocardial vessels. Two of the three monkeys had typical typhus nodules in the brain (Fig. 2). These multifocal lesions involved small capillaries with perivascular cuffing and accumulations of neuroglial cells, macrophages, and plasma cells. Mild to moderate meningitis was also observed in one monkey.

DISCUSSION

We have described a cynomolgus monkey model for epidemic typhus infection that closely resembles epidemic typhus infection in humans (12, 13). The intravenous route of inoculation was chosen because previous reports (1, 2) indicated a wide variation in response after subcutaneous infection. A more well-defined dose response, both clinically and pathologically, was desired. Moderate to severe clinical illness with fever was noted in all infected monkeys and was dose-dependent. Skin rash was not observed, which is consistent with the low incidence reported by others (13). Clinical signs of weakness,



FIG. 2. Typical typhus nodules in the brain of a cynomolgus monkey infected with *R. prowazekii*. Stained with hematoxylin and eosin, $\times 480$.

depression, and anorexia were similar to those previously reported (2). Paralysis of the extremities did not occur, although some central nervous system signs were apparent.

A prominent leukocytosis with a left shift was present in all surviving monkeys. It is interesting to note that the monkeys that died did not have this pronounced leukocyte response (Fig. 1A). Mobilization of leukocytes, primarily neutrophils, may play a role in host defense against *R. prowazekii* infection in cynomolgus monkeys.

The increases in BUN in the severely ill monkeys were probably due to dehydration during the febrile period. Increases in BUN have been reported in humans (13). Vasculitis noted in the kidneys on pathological examination could also explain the increased BUN.

Increases in serum alkaline phosphatase and SGPT activities may have been related to the hepatic changes seen on microscopic examination. Increased fibrinogen values may also reflect those hepatic changes.

Electrocardiographic abnormalities are not uncommon in humans with epidemic typhus infection. Changes in the T-wave configuration and prolongation of the P-R interval and low-voltage recordings have been reported (4, 17). In

this study the only change noted was decreased voltage recordings in one monkey. This change may not be significant, although this monkey had moderate thrombosis and vasculitis present in the myocardium.

Pathological changes in the monkeys that died were similar to those observed in humans (11, 12, 16). Focal areas of hemorrhage, necrosis, and thrombosis were seen in small vessels. Pneumonitis, splenitis, and myocarditis were noted; they are not uncommon in humans (11, 12). The focal perivascular lesions observed in the brains of two monkeys were characteristic of the typhus nodules reported in humans (11, 12) and may have contributed to the dissociation, nystagmus, and head twitching seen.

The clinical signs and pathology of *R. prowazekii* infection in cynomolgus monkeys are similar to those seen in humans. The cynomolgus monkey appears to be a suitable model for the study of epidemic typhus infection.

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