Short Communication

PERSISTENCE AND REACTIVATION OF RICKETTSIA TSUTSUGAMUSHI INFECTIONS IN LABORATORY MICE*

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SUMMARY: The persistence of R. tsutsugamushi in tissues of experimentally infected mice for 565 days was demonstrated. Reactivation of apparently dormant infections was accomplished by inoculating the mice with a heterologous strain of the organism or treatment with cyclophosphamide.

Previous studies of Rickettsia tsutsugamushi infections in rodents suggest that persistent or dormant infections may be common (Webster, 1940; Kohls et al., 1945; Fox, 1948; Rights et al., 1948; Kouwenaar and Esseveld, 1949). In endemic areas, a large number of wild rodents have yielded isolates from blood or tissue pools or both. Smadel et al. 1952) described the occurrence of R. tsutsugamushi organisms in lymph nodes of a patient 1–2 years after infection. Little is known about the persistence of scrub typhus organisms in a host and its effect on resistance to subsequent infection with R. tsutsugamushi.

In an earlier study from this Unit (D.M. Robinson, personal communication), silvered leaf monkeys, infected with different strains of scrub typhus organisms, were challenged with homologous and heterologous strains, when the antibody titer fell to ≥1:10 (approximately one year post-infection). No animals were rickettsemic at the time of challenge. Two animals infected initially with TA678 strain and challenged with TC586 strain yielded blood isolates which were antigenically related to both TA678 and TC586. It has been postulated that the challenge inoculation activated a dormant primary infection.

This experiment was designed to determine the persistence of scrub typhus infections and the reactivation of such persistent infections by inoculating the mice with a heterologous strain of R. tsutsugamushi; by infecting the mice with a non-rickettsial organism, Plasmodium berghei; and by introducing an immunosuppressant, cyclophosphamide.

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All mice were 18 to 20-g outbred females of the ICR strain, produced by the Division of Laboratory Animal Resources, Institute for Medical Research, Kuala Lumpur, Malaysia.

The TA678 and TC586 strains of *R. tsutsugamushi* were isolated from Thailand rodents and chiggers, respectively (Robinson et al., 1976). The former is a distinct prototype strain which is avirulent for mice. TC586 strain is similar to Gilliam strain in its antigenic and pathogenic properties. Both strains were grown in embryonated hens' eggs from leukosis-free flocks (Spafas Inc., Norwich, Conn.). The TA678 inoculum contained $10^{3.4}$ 50% mouse infectious doses (MID$_{50}$) per 0.2 ml, and the TC586 inoculum contained $10^2$ MID$_{50}$ per 0.2 ml. The MID$_{50}$ was determined as previously described (Catanzaro et al., 1976). All inoculations were made intraperitoneally. The TA678 and TC586 strains were used to simulate the conditions in the earlier primate study.

*P. berghei* was obtained from the blood of an infected mouse (Dr. V. Thomas, Department of Parasitology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia). Each inoculum contained $2 \times 10^4$ organisms per 0.2 ml.

Cyclophosphamide was purchased as Endoxan-Asta® (Asta-Werke Aq, Chemische Fabrik, Brackwede, Federal Republic of Germany) in 200-mg vials for injection. Each mouse received ip injections of 8 mg/0.2 ml and 4 mg/0.2 ml 3 days apart.

Sera were examined for the presence of antibody to *R. tsutsugamushi* by the indirect immunofluorescent (IFA) test (Robinson et al., 1976). Strains of scrub typhus rickettsiae present in the infected mice were identified by the direct fluorescent antibody (DFA) technique (Iida et al., 1966).

A large number of mice were infected with the avirulent strain of TA678. Tests were performed to insure that the mice were infected solely with TA678 strain. A group of glycogen-treated mice (Robinson et al., 1977) were infected with the inoculum, and thrice weekly for 4 weeks, peritoneal exudates from three mice were collected and examined for the presence of rickettsial organisms by the DFA test. Ten infected mice were bled individually from the retrorbital plexus on a weekly basis for 4 weeks and monthly thereafter to measure antibody response. When the titer fell below 1:5, a group of mice were bled to determine the presence of rickettsemia, and liver, spleen, kidney and lymph nodes were harvested to determine the presence of organisms in tissues. The remaining mice were divided into three large groups. One group was inoculated with the heterologous TC586 strain, the second with *P. berghei*, and the third group was given cyclophosphamide. Thrice weekly for 3 weeks, four mice were bled and each of the four samples was inoculated into three glycogen-treated mice. When sick or on day 14, mice were killed. The peritoneal fluids from the three mice were pooled and processed for examination by the DFA technique. A suspension prepared from blood, liver and spleen from selected mice was inoculated immediately into glycogen-treated mice (2nd passage). Blood and tissues from other mice were stored at $-70 \, ^\circ$ C. The peritoneal fluids from the 2nd passage mice were also pooled when the rodents became ill, or on day 14, and were processed
for examination by the DFA technique.

Results showed that peritoneal cells prepared from TA678-infected mice 4 to 23 days post-inoculation contained rickettsiae which reacted only with anti-TA678 serum. The sera were also tested, and only antibody to TA678 was detected.

Maximum titers of 1:25 to TA678 were attained on day 14 post-inoculation, and by day 90, the titers had fallen to 1:5. After 260 days post-infection when the antibody titers were below 1:5, mice were not rickettsemic, but 4 of 10 tissue pools were positive for rickettsiae. On day 565 post-infection, 10 additional mice were examined. Although none of the mice were rickettsemic, 6 of 10 tissue pools contained rickettsiae.

The findings in three groups of mice, given TC586, *P. berghei*, and cyclophosphamide, respectively, on day 565 post-inoculation, are summarized in Table I. In the group of mice inoculated with the TC586 strain, TA678 organisms were seen in a minimal number of mice only after the 2nd passage. However, cyclophosphamide reactivated TA678 infection in most of the mice tested, and all rickettsemias were apparent in the first mouse passage. The findings also indicate that the mice remained rickettsemic for at least 18 days. There were no organisms detected in peritoneal fluids from mice given *P. berghei*; however, the infectivity of the inoculum was so high that no mice survived beyond the 11th day.

The long persistence of rickettsial organisms in tissues of man is well-

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**Table I**

*Reactivation of TA678 rickettsiae in mice by inoculation with heterologous TC586 strain, inoculation with Plasmodium berghei, and treatment with cyclophosphamide*  

<table>
<thead>
<tr>
<th>Days post-inoculation/Treatment</th>
<th>TC 586*</th>
<th>P. berghei</th>
<th>Cyclophosphamide</th>
</tr>
</thead>
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<tr>
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<td>4/4</td>
</tr>
<tr>
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</tr>
<tr>
<td>13</td>
<td>0/4</td>
<td>—***</td>
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<td>—</td>
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<tr>
<td>20</td>
<td>0/4</td>
<td>—</td>
<td>0/4</td>
</tr>
</tbody>
</table>

* TC586 organisms were detected in the blood of all mice with the exception of mice examined 2 days after inoculation when blood of only 1/4 contained TC586 organism.  
** Number of mice in which TA678 organisms were found in the blood/total number of mice examined.  
*** Not done because no *P. berghei*-infected mice survived beyond the 11th day.
exemplified in Brill-Zinsser disease, in which *R. prowazeki* is reactivated many years after the initial infection. Smadel and coworkers (1952) recovered *R. tsutsugamushi* organisms from the lymph node of a patient who 15 months earlier was ill with scrub typhus. Persistent *R. typhi* and *R. tsutsugamushi* infections in experimental hosts for extended periods of time have been well documented (Lepine and Sautter, 1936; Philip and Parker, 1938; Webster, 1940; Kohls et al., 1945; Fox, 1948; Rights et al., 1948; Kouwenaar and Esseveld, 1949). Our findings demonstrated the presence of *R. tsutsugamushi* in the tissues of mice infected 19 months previously.

Kuwata (1952) observed that mice initially infected with S13 strain of *R. tsutsugamushi* did not develop disease after inoculation with the virulent Agano-X strain, in spite of the invasion of the latter strain into the tissues. These organisms persisted in the tissues for longer than 180 days. In our study, we did not measure the persistence of the challenge strain, but rather demonstrated the reactivation of the original strain. Reactivation of TA678 by inoculation of TC586 could have occurred in more mice but may not have been detected due to the greater numbers of the latter strain. TC586 produced rickettsemias which persisted throughout the 20 day observation period. It is conceivable that in mice, TC586 may grow more rapidly and to higher titer than the TA678 strain.

Cyclophosphamide has the properties of lengthening the survival time of tissue grafts (Fox, 1964), suppressing humoral antibody production (Frisch and Davies, 1966), and producing tolerance (Aisenberg, 1967). This drug also increased the virulence and enhanced the growth of *R. prowazeki*, *R. akari* and *Coxiella burnetii* (Kazar et al., 1971) as well as *R. sennetsu* (Tachibana and Kobayashi, 1975) in mice. In a previous study in this laboratory, increased virulence of an avirulent strain of *R. tsutsugamushi* was demonstrated in mice treated with cyclophosphamide, but there was no correlation with an increased number of organisms (D. M. Robinson, personal communication). Our study showed an immediate reactivation of the TA678 infection after the administration of cyclophosphamide. The mechanism of reactivation is not known. One can speculate that there may exist a delicate balance between the infecting agent and the host's immune system, which can be tipped in the favor of the invader by the immunosuppressive action of the drug.

Hayashi and Watanabe (1948) induced rickettsemia in a patient, who had recovered from infection with the Pescadores strain of *R. tsutsugamushi*, by injecting typhoid vaccine intravenously 34 days after defervescence. They concluded that “the Tsutsugamushi virus of Pescadores strain not only hides in the body of patient for fairly long period after the recovery, but also can appear in the circulating blood under fever due to some other causes.” We attempted to reactivate the TA678 organisms by inoculation of *P. berghei*, a non-rickettsial, intracellular, obligate parasite, but unfortunately, the dosage given was too high to allow the mice to survive for a minimum of 30 days.

The persistence of the *R. tsutsugamushi* organisms in the tissues of mice and the reactivation of such by the inoculation of a heterologous strain of scrub
typhus rickettsiae and by treatment with cyclophosphamide have been noted. In this study, the failure to reactivate the latent rickettsial infection by inoculation with P. berghei may have been due to the overwhelming numbers of malarial organisms in the inoculum and the resultant early death of the mice. Nevertheless, the possibility of reactivation by other infections cannot be overlooked. Studies of febrile illnesses in rural Malaysia have revealed a large number of dual infections involving R. tsutsugamushi and other unrelated organisms (unpublished data). One can only speculate that some of these infections may have resulted from reactivation of an earlier infection.

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References


