THE EFFECT OF MOUSE AGE ON THE DETERMINATION OF RICKETTSIA TSUTSUGAMUSHI VIRULENCE

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SUMMARY: The effect of age on the susceptibility of ICR mice to lethal intra-peritoneal (ip), Rickettsia tsutsugamushi infections was tested with five virulent strains —Karp, Kato, Gilliam, TA763, and TH1817— and three strains of reduced virulence—TA678, TA686, and TA716. Susceptibility differences were noted only in the ICR mice inoculated with two of the strains of reduced virulence, TA716 and TA678. With both strains, mice in the 12-weeks and younger age groups had lower death rates than did mice in the 21-weeks and older age groups. Also, CBA/CaJ mice of varying ages were inoculated intravenously with large doses of the Gilliam strain to determine the effect of age on susceptibility to acute death syndrome (ADS). A progressive increase in ADS resistance was seen in the 4-, 8-, 12-, and 16-week-old age groups. This study indicates that the age of mice used to test the virulence of R. tsutsugamushi strains may be an important consideration, especially when testing the ip lethality of strains of reduced virulence.

The laboratory mouse is commonly used in the study of scrub typhus to isolate and characterize the causative agent, Rickettsia tsutsugamushi (Bell, Bennett and Whitman, 1946; Smadel, Jackson and Cruise, 1949; Elisberg and Bozeman, 1969; Shirai and Wisseman, 1975). Large studies often require that mice be accumulated over a period of several weeks, leading to a considerable disparity in their ages. Despite the extensive use of mice in scrub typhus research and the past interest in R. tsutsugamushi virulence for mice, specific information on the effect of age on mouse susceptibility to infection is lacking. Therefore, we tested the susceptibility of outbred mice of varying ages to intraperitoneal (ip) R. tsutsugamushi infection.

Eight prototype strains were prepared, stored, and assessed for infectivity using methods reported previously (Groves and Osterman, 1978). ICR mice, ages 4, 8, 12, 21, 23, and 26 weeks, were infected intraperitoneally with 3, 10-fold varying dosages (5 mice/dose) of each rickettsial strain, and the total number of lethal infections within each age group recorded. Dosages for the virulent

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strains (Karp, Kato, Gilliam, TA763, and TH1817) varied between $10^{0.5}$ and $10^{4.0}$ MID$_{50}$, and dosages for the strains of reduced virulence (TA678, TA686, and TA716) varied between $10^{4.5}$ and $10^{8.0}$ MID$_{50}$. All inoculations were done on the same day, and each prototype strain was administered to all age groups within a 30 minute period.

No susceptibility differences were observed with the virulent strains of *R. tsutsugamushi*. Even very low dosages of these strains, $\leq 10$ MID$_{50}$, were lethal for all ages. Also, no age related susceptibility was noted with the less virulent TA686 strain. However, significantly fewer deaths were observed in mice 12-weeks and younger than in ones 21-weeks and older infected with strains TA678 and TA716 (Table I, Exp. 1). The younger mice receiving TA678 had a death rate of $2/45$ versus $10/45$ for the old mice ($p<0.02$). Death rates for the younger and older age-mice receiving TA716, were $12/45$ and $34/45$ respectively ($p<0.01$).

**TABLE I**

Results of ip infection of ICR mice with strains *TA678 and TA716*

<table>
<thead>
<tr>
<th>Strain</th>
<th>Experiment</th>
<th>Age in weeks</th>
<th>4</th>
<th>8</th>
<th>12</th>
<th>21</th>
<th>23</th>
<th>26</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0/15* (0%)</td>
<td>2/15 (13%)</td>
<td>0/15 (0%)</td>
<td>3/15 (20%)</td>
<td>5/15 (33%)</td>
<td>2/15 (13%)</td>
</tr>
<tr>
<td>TA678</td>
<td>1</td>
<td></td>
<td>3/45 (7%)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td></td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2/15 (13%)</td>
<td>6/15 (40%)</td>
<td>4/15 (27%)</td>
<td>11/15 (73%)</td>
<td>12/15 (80%)</td>
<td>11/15 (73%)</td>
</tr>
<tr>
<td>TA716</td>
<td>1</td>
<td></td>
<td>26/45 (58%)</td>
<td>—</td>
<td>—</td>
<td>37/45 (82%)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td></td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

* Dead/Total (% lethal infection).

Because age related resistance to TA678 and TA716 was unexpected, we repeated the infections with these strains using 8- and 23-week-old mice as representatives of the younger and older age groups (Table I, Exp. 2). As in the first experiment, death rates were lower in the younger mice—$3/45$ versus $23/45$ with TA678 infections ($p<0.01$) and $26/45$ versus $37/45$ with TA716 infections ($p<0.02$).

In addition to ip infections, we studied the effect of mouse age on susceptibility to Gilliam-induced, acute death syndrome (ADS). This phenomenon, sometimes referred to as the “toxic effect”, is the rapid death of mice following the intravenous (iv) inoculation of high concentrations of certain *Rickettsia spp* (Elisberg and Bozeman, 1969). Among the scrub typhus rickettsiae, it has only been reported for the Gilliam strain (Smadel et al., 1946).

Because susceptibility to Gilliam-induced ADS is genetically determined in mice, inbred CBA/CaJ mice were used in this study (Groves, Rosenstreich and Osterman, 1980). CBA/CaJ mice, ages 4, 8, 12, 16, 20 and 26 weeks, were
TABLE II

Mean death times of inbred CRA/CaJ mice inoculated intravenously with strain Gilliam

<table>
<thead>
<tr>
<th>Mean death time (Standard error of the mean)</th>
<th>Age in weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minutes</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>(±e)</td>
</tr>
</tbody>
</table>

* p<0.01 compared to all groups 12 weeks and older.
** p<0.05 compared to all groups 16 weeks and older.

inoculated intravenously with 0.2 ml of a Gilliam suspension containing 10^{8.8} MID_{50}, and the time of death recorded. Each age group consisted of five mice. All mice died in less than 150 min following iv inoculation. The 4-week-old CBA/CaJ mice had a mean death time that was significantly lower than the 12-, 16-, 20-, and 26-week-old groups (p<0.01) (Table II). The 8- and 12-week-old CBA/CaJ mice also had a significantly lower mean time of death than the 16-, 20- and 26-week-old groups (p<0.05).

The virulence of a *R. tsutsugamushi* isolate for mice is commonly determined by ip infection and a cursory review of the literature reveals that such studies most frequently use mice between the ages of 6 and 14 weeks. Our findings indicate that the comparison of mouse virulence within this age range should be consistent, and an even wider age range could be used for virulent strains. However, older mice, 21 weeks and above, appear for unknown reasons to be more sensitive to some *R. tsutsugamushi* strains of reduced virulence. This increased sensitivity to infection did not appear to be progressive with age, since there was a definite delineation between the 12-week-old and younger groups and the 21-week-old and older groups.

The progressive increase in mean death times seen in the 4- through 16-week-old mouse groups in the Gilliam-induced ADS study may have been related to the rapid growth of the mice during this period. CBA/CaJ mice in our laboratory typically progress from 14 g at 4 weeks to 24 g at 16 weeks. Logically, the inoculation of an extremely large dose of rickettsiae in an amount known to cause acute death should kill small animals more quickly.

The exact relationship between the mouse virulence of a *R. tsutsugamushi* isolate and its virulence for humans is unknown. A correlation in the variation of clinical scrub typhus severity and a variation in the mouse virulence of corresponding isolates has been reported (Irons, 1946; Carley et al., 1955; Doherty, 1956). However, *R. tsutsugamushi* strains of reduced mouse virulence isolated from sources other than human cases of scrub typhus have never been inoculated into man. *R. tsutsugamushi* strain Gilliam, originally isolated from a patient contracting the disease in Burma and believed to have lost virulence for mice by serial passage in embryonated eggs, was tested in human volunteers as a possible attenuated vaccine (Smadel et al., 1951). Unfortunately, this strain
was found to cause clinical disease indistinguishable from naturally acquired scrub typhus. However, the recent discovery that genetic resistance to Gilliam infection is widespread among inbred and outbred mice (Groves and Osterman, 1978; Groves et al., 1980) leads us to wonder if the apparent loss of virulence observed by Smadel and co-workers was due more to the genetic makeup of their mice rather than to an alteration of the rickettsiae.

The ability to predict the human virulence of \( R. \) tsutsugamushi isolates based on a trait that is easily assayable in the laboratory is important in the selection of possible vaccine strains. Mouse virulence may well prove to be that trait, since there is currently no evidence to the contrary. If so, our study indicates that the age of the mice used to test rickettsial strains, especially those of reduced virulence, may be an important consideration.

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**References**


