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Characterization of Factors Determining *Rickettsia tsutsugamushi* Pathogenicity for Mice

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Pathogenicity of *Rickettsia tsutsugamushi* for laboratory mice is known to be influenced by at least three factors: (i) route of inoculation, (ii) antigenic strain, and (iii) natural resistance of the host. By using Karp, Gilliam, and Kato strains of *R. tsutsugamushi*, we examined the effect of these three pathogenicity factors on the kinetics of infection and the development of immunity in BALB/cDub and C3H/HeDub mice. The appearance of rickettsemia in the pathogenic infections generally preceded infections of reduced pathogenicity by 1 to 2 days in both magnitude and time of onset. Mice infected by the subcutaneous route with normally pathogenic rickettsiae, i.e., Gilliam-infected C3H/HeDub mice and Karp-infected BALB/cDub mice, consistently maintained a detectable rickettsemia over a 1-year period. Rickettsiae were recovered from the spleens of 95% (19 of 20) of these mice 52 weeks postinfection. In contrast, mice with infections of reduced pathogenicity, i.e., BALB/cDub mice infected by intraperitoneal and subcutaneous inoculation with Gilliam, did not have detectable rickettsemia from week 20 through week 52 postinfection except for a single mouse on week 44 postinfection. Rickettsiae were detected in the spleens of only 40% (8 of 20) of these mice after 1 year. In both Gilliam-infected mouse strains, protection against heterologous challenge with Karp or Kato rickettsial strains was incomplete up to 7 days postimmunization. Infections of reduced pathogenicity did not result from an enhanced systemic immune response by the host. The onset of the humoral response was not different for the pathogenic and reduced-pathogenicity infections. Pathogenicity differences seemed to result from the more rapid growth of the rickettsiae in the pathogenic infections.

The laboratory mouse is used extensively in the study of scrub typhus to isolate and maintain the etiologic agent, *Rickettsia tsutsugamushi* (7); to test the efficacy of antibiotics against the organism (32, 33); to determine the virulence of an isolate (12, 29); and to characterize the antigenic variations of the agent by various in vivo procedures (1, 25, 30). More recently, studies on the immunity produced by either infection (18, 27) or vaccination (6, 17, 19), on the effect of antibiotic therapy on immunity (26), on the pathogenesis of infection (5, 8), and on the effect of immune impairment on recrudescence (20, 28) have emphasized the value of the mouse as an animal model for the disease rather than as just a useful aid for studying the biology of the rickettsiae.

Investigators have recognized that the route of inoculation (3, 10, 20) and strain of organism (13, 14, 20) can alter the observed pathogenicity of *R. tsutsugamushi*. A third pathogenicity factor, the genetic susceptibility of the host, has also been recognized (10, 16, 17). Natural resistance in mice to the Gilliam strain of *R. tsutsugamushi* is controlled by the single, autosomal, dominant *Ric* gene located on chromosome 5 and closely linked to the retinal degeneration gene (10, 11).

The widespread use of mice in the study of scrub typhus and the importance of observed pathogenicity in characterizing rickettsial strains, defining immunity, and determining the efficacy of chemotherapeutic agents prompted us to perform additional studies concerning the influence of the pathogenicity factors on the kinetics of infections and immunity. The results of these studies are reported here.

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MATERIALS AND METHODS

Animals. Female BALB/cDub and C3H/HeDub mice (Flow Laboratories, Inc., McLean, Va.), 18 to 24 g, were used throughout the study.

Rickettsiae. The Gilliam (131st egg passage), Karp (56th egg passage), and Kato (197th egg passage) strains of *R. tsutsugamushi* were propagated, stored, and quantified as previously reported (5).

Animal infection. Mice were infected with the Karp, Gilliam, or Kato prototype strains of *R. tsutsugamushi* by single intraperitoneal (i.p.) or subcutaneous (s.c.) inoculation of 0.2 ml of rickettsial suspension containing either 1,000 or 10,000 50% i.p. mouse lethal doses (MLD_{50}). Previous studies have shown the s.c. route of inoculation to be immunizing and nonlethal in mice (31). Immunized mice were challenged with 1,000 MLD_{50} of either Karp or Kato strains by i.p. inoculation.

Pathogenicity model. The BALB/cDub and C3H/HeDub strains of inbred mice and the Karp and Gilliam prototype strains of *R. tsutsugamushi* were used to devise our model system (Fig. 1). BALB/cDub mice are genetically resistant to Gilliam i.p. infection, whereas C3H/HeDub mice are genetically susceptible (10). Both mouse strains are uniformly susceptible to i.p. infection with the Karp strain (10). The s.c. administration of either rickettsial strain at dosages of 10,000 MLD_{50} or greater than the minimum lethal i.p. dose produces a nonlethal, immunizing infection in both BALB/cDub and C3H/HeDub mice (10). Pathogenicity variation due to differing rickettsial strains was examined by contrasting the lethal, BALB/cDub-Karp i.p. combination with the nonlethal, BALB/cDub-Gilliam i.p. combination. Likewise, the effect of the mouse strain was studied by contrasting the lethal C3H/HeDub-Gilliam i.p. combination with the nonlethal BALB/cDub-Gilliam i.p. combination. Route-of-inoculation studies were done by comparing i.p.

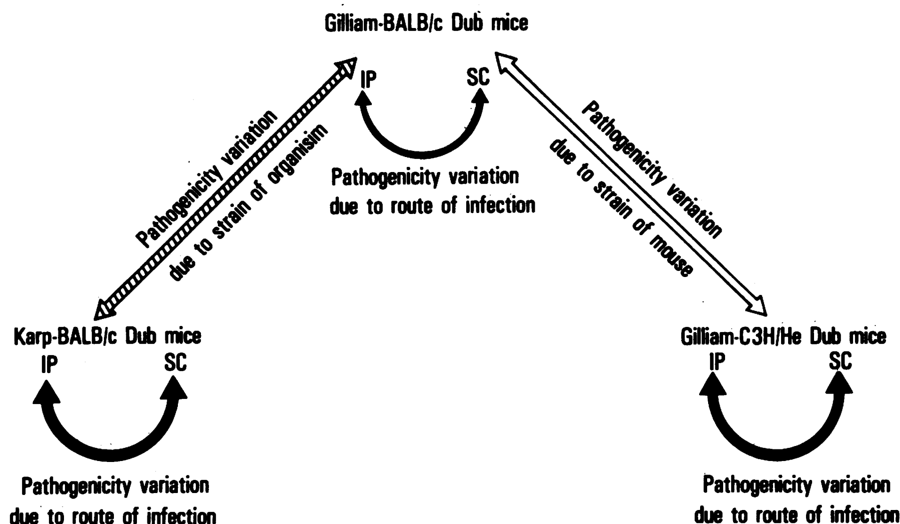

FIG. 1. Model system for studying pathogenicity factors of *R. tsutsugamushi*. The i.p. inoculation of Karp into BALB/cDub mice and the i.p. inoculation of Gilliam into C3H/HeDub mice represent lethal infections. All other infections are nonlethal. The pathogenicity pairings used to study the pathogenicity factors were as follows: (⇔) effect of rickettsial strain, (⇔) effect of mouse strain, (⟷) effect of route of infection.

and s.c. infections in either of two combinations, BALB/cDub-Karp or C3H/HeDub-Gilliam. In chronicity experiments resistant BALB/cDub mice inoculated i.p. and s.c. with the Gilliam strain were used.

Rickettsemia determinations. Quantitative determinations were used to follow acute infections as previously reported (20). Rickettsemia was quantified on days 1 through 5, 7, 9, 21, and 35 postinfection (p.i.). Qualitative determinations were used to follow chronically infected mice over a 1-year period as previously described (20). Thirty mice per group were inoculated either s.c. or i.p. with the Karp or Gilliam strain of *R. tsutsugamushi*. Five mice from each group were tested for rickettsemia on days 1 to 10 p.i.; 10 mice from each group were tested biweekly through week 16 and then every 4 weeks through week 52. This method required testing each animal more than once; thus, the mice that were selected for rebleeding were those which had been least recently bled.

Transfer of disrupted spleen cells. Mice were tested for chronic rickettsial infections. Homogenized spleen cell suspensions were prepared as previously described (26) and transferred i.p. to normal C3H/HeDub mice. Animals that died from the original inoculation or that survived challenge 28 days later with 1,000 MLD_{50} of Karp were judged to harbor *R. tsutsugamushi* infections.

Serology. Infections were confirmed by the microtiter complement fixation (CF) (27) and indirect fluorescent antibody (IFA) (4) tests. Pooled sera of five mice from each of the chronically infected groups in the qualitative rickettsemia study were examined for IFA and CF antibodies.

Serum neutralization test. A modification of the neutralization test of Bennett et al. (2) was used. Briefly, undiluted normal control sera or experimental sera were added in equal volumes to standardized 10-fold dilutions of rickettsial yolk-sac suspensions. The serum-rickettsia suspensions were mixed, incubated in a 37°C water bath for 30 min, and then placed in an ice bath until needed. Five C3H/HeDub mice per dilution were then inoculated i.p. with 0.2 ml of the appropriate mixture. Mice were observed for 21 days. An MLD_{50} of each titration was calculated by the method of Reed and Muench (24). A neutralization index was then calculated for each test serum by determining the difference between the $\log_{10}$ of the test serum MLD_{50} and the $\log_{10}$ of the normal control serum MLD_{50}. Sera were collected for testing on days 14, 21, and 28 p.i.

RESULTS

Effect of pathogenicity determinants on rickettsemia in acute infections (quantitative study). Pathogenicity variations due to strain of rickettsia, strain of mouse, and route of infection (Fig. 1) were compared in replicate studies in which the rickettsemias of the lethal BALB/cDub-Karp i.p. and C3H/HeDub-Gilliam i.p. and nonlethal BALB/cDub-Gilliam i.p., BALB/cDub-Gilliam s.c., BALB/cDub-Karp s.c., and C3H/HeDub-Gilliam s.c. combinations were monitored. Rickettsemias of the BALB/cDub-Karp i.p. lethal group preceded by 1 to 2 days and were of greater magnitude than those of the nonlethal infections. However, the rickettsiae were detected initially on day 5 p.i. in the blood of mice from both the lethal C3H/HeDub-Gilliam i.p. and nonlethal C3H/HeDub-Gilliam s.c. groups. Figure 2 shows the quantitation of rickettsiae in whole blood collected from BALB/cDub mice infected with the Karp or Gilliam strain of *R. tsutsugamushi*. Whereas the rickettsemic BALB/cDub mice infected with Karp uniformly died by day 9 p.i., those infected with Gilliam survived. The differences in lethality of these two groups contrasted with pathogenicity differences due to strain of organism (Fig. 1). The results shown in Fig. 2 are also typical of the responses observed for mouse strain differences and for route-of-inoculation differences in the Karp-infected BALB/cDub mice. The onset of quantifiable rickettsemias for the lethal infections was day 4 or 5 p.i., and the onset for the nonlethal infections was day 7 p.i. Maximum rickettsemia was reached on day 14 p.i. in all nonlethal infections. The highest rickettsemia, >10,000 MLD_{50} per 0.2 ml of whole blood, was observed in the BALB/cDub-Karp s.c. group (data not shown), and the lowest rickettsemia at day 14 p.i. was $10^{3.5}$ MLD_{50} in the BALB/cDub-Gilliam i.p. group (Fig. 2). Only the rickettsemias of the BALB/cDub-Gilliam i.p. and BALB/cDub-Gilliam s.c. mice fell below quantifiable levels by day 35 p.i.

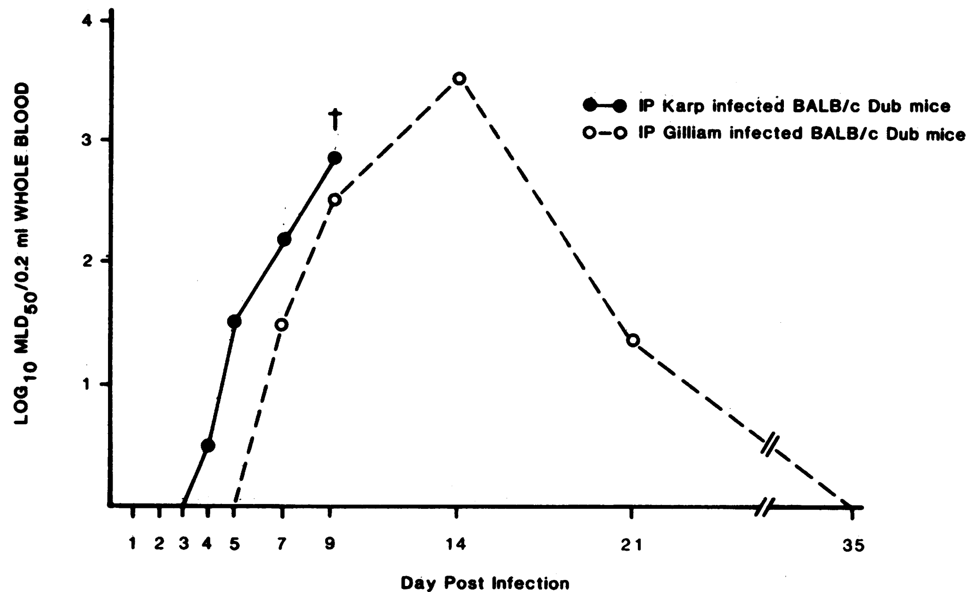

FIG. 2. Quantitation of rickettsiae in whole blood from BALB/cDub mice infected i.p. with 10,000 MLD_{50} of the Gilliam or Karp strain of *R. tsutsugamushi*. Each point represents the mean number of MLD_{50} per 0.2 ml of whole blood obtained from five mice. Lethally (Karp) infected mice all succumbed by day 9 p.i. (†).

Effect of pathogenicity factors on rickettsemia in acute and chronic infections (qualitative study). Rickettsemia was detectable in all i.p. inoculated groups on day 3 p.i. and in all s.c. inoculated groups on day 4 p.i. (Table 1). Otherwise, there appeared to be no dramatic differences between other pathogenicity factor pairings early in the infection.

Beginning on day 14 p.i., rickettsemia was determined for the four surviving combinations every 2 weeks through week 16 p.i. and then every 4 weeks until 52 weeks p.i. (Fig. 3). For purposes of clarity the rickettsemia determinations for the i.p. Gilliam-infected BALB/cDub mice are not shown in Fig. 3, because they paralleled the data from the s.c. Gilliam infection. The most striking observation was that rickettsemia was constantly detectable in the two combinations in which the i.p. route of infection is lethal (BALB/cDub-Karp and C3H/HeDub-Gilliam). This is in marked contrast to the Gilliam-infected BALB/cDub mice (a nonlethal i.p. infection), which had detectable rickettsemias only on weeks 16 and 44 p.i.; in both instances, it was only one mouse.

One year after the initial infection, 10 animals from each surviving group were sacrificed, and their spleens were examined for rickettsiae by the transfer of disrupted spleen cells (Table 2). Not surprisingly, the two groups that maintained detectable rickettsemias throughout the year-long study, BALB/cDub-Karp and C3H/HeDub-Gilliam, had a high percentage of infected spleens, 100 and 90%, respectively. Interestingly, a small percentage of the i.p. and s.c. Gilliam-inoculated BALB/cDub mice also possessed infected spleens despite the almost total absence of rickettsiae in the blood of these two groups after week 14 p.i.

Serological studies. Two chronically infected groups, BALB/cDub-Karp and C3H/HeDub-Gilliam, that maintained constant rickettsemia throughout the qualitative study and had a high percentage of infected spleens after a year yielded high antibody titers that remained elevated (Table 3). In contrast, the titers of the i.p. and s.c. inoculated BALB/cDub-Gilliam groups declined after week 16 p.i.

Serum neutralization of rickettsiae. Serum neutralizing antibody against homologous challenge was measured in the four nonlethal pairings. In replicate studies, steadily rising neutralizing indices were observed with sera from the s.c. inoculated C3H/HeDub-Gilliam, i.p. inoculated BALB/cDub-Gilliam, and s.c. inoculated BALB/cDub-Karp groups (Table 4). Sera from the s.c. inoculated BALB/cDub-Gilliam mice showed only a slight rise in the neutralizing index.

In vivo protection against heterologous rickettsial strains. The effect of mouse strain on acquisition of heterologous in

TABLE 1. Qualitative determination of rickettsemia in inbred mice acutely infected with 10,000 MLD_{50} of *R. tsutsugamushi*

Mouse strain	*R. tsutsugamushi* strain	Route of inoculation	% of mice with rickettsemia on the following day p.i.[a]: 3	4	5	6	7
C3H/HeDub	Gilliam	i.p.	80	80	100	100	100
		s.c.		20	100	100	100
BALB/cDub	Gilliam	i.p.	60	40	80	100	100
		s.c.		40	100	100	100
	Karp	i.p.	40	80	100	100	100
		s.c.		20	80	60	100

[a] Rickettsemia was not detected before day 3 p.i. All groups remained rickettsemic on days 8 to 10 p.i. Five mice were tested per group.

TABLE 2. Detection of *R. tsutsugamushi* in the spleens and blood of chronically infected mice[a]

Mouse strain	*R. tsutsugamushi* strain	Route of inoculation	% of spleens infected[b]	% of rickettsemic mice
C3H/HeDub	Gilliam	s.c.	90	50
BALB/cDub	Gilliam	s.c.	20	0
		i.p.	30	0
	Karp	s.c.	100	30

[a] Tests were done 52 weeks p.i.
[b] $n = 10$.

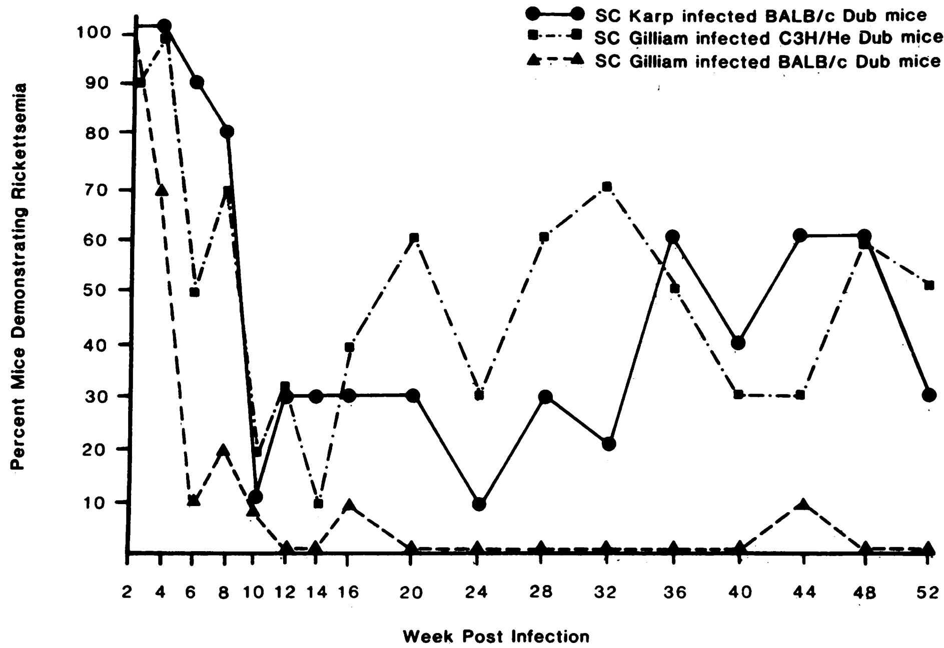

FIG. 3. Qualitative determination of rickettsemia in C3H/HeDub and BALB/cDub mice infected s.c. with 10,000 MLD_{50} of the Gilliam or Karp strain of *R. tsutsugamushi* (n = 5 on days 1 to 10, n = 10 from 2 to 52 weeks).

vivo protection was examined by immunizing Gilliam-resistant BALB/cDub and Gilliam-susceptible C3H/HeDub mice with the Gilliam strain and challenging with the Karp strain. As a control, the same mouse strains were immunized and challenged with Karp to establish that the BALB/cDub mice did not possess an innate ability to respond to all rickettsial antigens better than did the C3H/HeDub mice. Gilliam immunization did not stimulate heterologous protection more quickly in the BALB/cDub mice than in the C3H/HeDub mice (Table 5). On the contrary, the C3H/HeDub mice were able to withstand both homologous and heterologous challenges earlier than were the BALB/cDub mice.

The effect of *R. tsutsugamushi* strain on the development of heterologous protection was also examined by immunizing BALB/cDub mice with either the Karp or Gilliam strain and challenging with the Kato strain. C3H/HeDub mice were similarly immunized and challenged as a control to establish that neither the Karp nor the Gilliam strain preferentially protected mice against Kato infections. The onset of heterologous protection produced by immunization with either the Gilliam or the Karp strain in the Gilliam-resistant BALB/cDub mice was not demonstrably different (Table 6).

DISCUSSION

The lethally i.p. Karp-inoculated BALB/cDub and Gilliam-inoculated C3H/HeDub mice developed rickettsemias sooner and sustained higher rickettsemia levels before death than did their nonlethally infected counterparts when compared for pathogenicity variations due to differences in mouse strain, rickettsial strain, and, in the case of the Karp-inoculated BALB/cDub mice, route of inoculation. Only in the s.c. and i.p. Gilliam-inoculated C3H/HeDub mice did rickettsemia levels for the two groups parallel each other. Even in these mice, the lethally i.p. inoculated mice developed rickettsemias sooner in the qualitative study than did the s.c. inoculated ones (Table 1).

Catanzaro et al. (5) suggested that survival of scrub typhus infections in mice depends on a delicate balance between the proliferation of the organism and the intensity of

TABLE 3. Homologous immunofluorescence assay and complement fixation antibody titers in mice infected with *R. tsutsugamushi*

Mouse strain	*R. tsutsugamushi* strain	Route of inoculation	Antibody test	Titer at the following week p.i.[a]:								
				1	2	4	8	12	16	32	40	48
C3H/HeDub	Gilliam	s.c.	IFA	20	320	640	2,560	2,560	2,560	1,280	1,280	1,280
			CF	<10	20	640	640	320	80	160	80	80
BALB/cDub	Gilliam	i.p.	IFA	10	160	640	640	640	1,280	320	320	160
			CF	<10	40	160	80	80	40	10	10	10
		s.c.	IFA	10	80	640	1,280	1,280	1,280	320	320	320
			CF	<10	10	640	80	80	40	40	10	10
	Karp	s.c.	IFA	10	20	640	640	1,280	2,560	1,280	2,560	2,560
			CF	<10	80	>1,280	>1,280	>1,280	>1,280	1,280	1,280	1,280

[a] Titers are shown as the reciprocals of the greatest serum dilutions demonstrating reactivity with the homologous antigens; n = 5.

TABLE 4. Comparison of homologous neutralization indices of sera collected at weekly intervals

Mouse strain	R. tsutsugamushi strain	Route of inoculation	Neutralization index[a] on the following day p.i.:		
			14	21	28
C3H/HeDub	Gilliam	s.c.	1.8	3.0	3.4
BALB/cDub	Gilliam	s.c.	0.1	1.2	1.0
		i.p.	0.2	2.2	3.2
	Karp	s.c.	0.2	0.8	3.2

[a] Neutralization index to homologous antigen ($\log_{10}$); mean of duplicate experiments.

TABLE 5. Effect of mouse strain on aquisition of heterologous in vivo protection

Mouse strain	Immunizing R. tsutsugamushi strain[a]	No. of survivors on the following challenge day p.i.[b]:					
		2	3	4	5	6	7
C3H/HeDub	Karp (homologous)	2	5	5	5	5	5
BALB/cDub	Karp (homologous)	0	1	1	4	4	5
C3H/HeDub	Gilliam (heterologous)	0	0	0	3	5	5
BALB/cDub	Gilliam (heterologous)	0	0	0	1	3	4

[a] Mice were immunized s.c. with 1,000 MLD_{50} of *R. tsutsugamushi*.
[b] All mice were challenged i.p. with 1,000 MLD_{50} of the Karp strain of *R. tsutsugamushi*; all challenged nonimmune control mice died by day 10 p.i. Five mice were tested per group.

the immune response of the host. Similarly, one can speculate that the rickettsemias observed in the lethal scrub typhus infections resulted from more rapid growth of the organisms, a decreased host immune response, immunosuppression or inhibition of T-lymphocyte functions as recently suggested by Jerrells et al. (15, 16), or a combination of these factors.

Our studies do not support a contention that there is a decrease in the systemic immune response in lethally infected mice when the pathogenicity factors of mouse strain are compared. Lethally infected mice succumbed to infection before their immune responses could be assessed; therefore, they could not be directly compared with their nonlethally infected counterparts. However, the response of BALB/cDub mice to Gilliam and Karp antigens could be compared by s.c. infecting the mice. Using this approach, the onset of humoral response as measured by IFA, CF, and homologous serum neutralization tests and of T-lymphocyte response as measured by early in vivo heterologous protection was not quicker in the nonlethal BALB/cDub-Gilliam pairing than in the potentially lethal C3H/HeDub-Gilliam and BALB/cDub-Karp pairings.

To contrast immunity by route of inoculation, the temporal protection conferred by i.p. and s.c. Gilliam infections was compared in BALB/cDub mice. Rickettsiae inoculated s.c., which consistently show reduced pathogenicity for mice regardless of mouse or rickettsial strain, failed to stimulate either a more rapid or a greater humoral response than did rickettsiae inoculated i.p. Comparison of T-lymphocyte response by using the in vivo heterologous protection test was not done. Shirai et al. have shown that early heterologous protection in the mouse is a function of the T lymphocyte (27). Using similar methods to ours, they reported that BALB/cDub mice inoculated i.p. with 1,000 MLD_{50} of Gilliam were protected against i.p. challenges of 1,000 MLD_{50} of Karp beginning on day 3 postvaccination. In contrast, we found that BALB/cDub mice inoculated s.c. with 1,000 MLD_{50} of Gilliam were incompletely protected against a similar Karp challenge on day 7 postvaccination. Although this supports the B-lymphocyte results, this comparison appears to be invalid due to the potential for compartmentalizing a local immune response after i.p. vaccination with subsequent i.p. challenge. Additional evidence that s.c. inoculation does not stimulate an accelerated immune response was reported by Plotz and co-workers (23). They produced a killed scrub typhus vaccine that provided protection when inoculated i.p. into mice but proved ineffective when administered s.c. Consistent with this finding, Jerrells reported a transient immunosuppression in s.c. inoculated BALB/c mice regardless of the infecting strain (15). He also reported a differential influx of inflammatory macrophages into the spleen of s.c. infected BALB/cDub mice; the Gilliam strain produced a lesser influx than did either the Karp or the Kato strain (15).

Although we found no difference in the systemic immune responses between lethal and nonlethal infections, this does not preclude a difference in the local immune response. Indeed, reports of other investigators would indicate that the localized immune reaction at the inoculation site may be one of the controlling factors differentiating lethal and nonlethal infections. Kokorkin et al. (21) reported that genetically resistant mice, such as BALB/cDub mice, can suppress Gilliam proliferation in the peritoneal cavity through the evolution of rickettsiacidal macrophages, whereas susceptible mice, such as C3H/HeDub mice, cannot. Likewise, Cantanzaro and associates (5), in contrasting lethal i.p. Karp and nonlethal i.p. Gilliam infections in BALB/cDub mice, reported that a similar immunologic mechanism was responsible for the observed virulence differences of the two rickettsial strains.

The well-documented capability of the mouse peritoneal cavity to mount an intense immunological reaction to *R. tsutsugamushi* infection (5, 17, 18, 21) and the absence of reports of a similar reaction in the subcutaneous tissues argue against an immunologic mechanism accounting for the decrease in pathogenicity of s.c. inoculated rickettsiae. A more plausible explanation may be that subcutaneous tissue does not present as favorable an environment for the proliferation of *R. tsutsugamushi* as the peritoneal cavity. The latter organ is rich in mesothelial cells, a cell type that readily supports abundant ricksettsial growth (21, 22), and is constantly bathed in fluid that readily serves to transmit infectious particles from cell to cell. Kundin et al. (22), using a direct fluorescent antibody test on serial frozen sections of

TABLE 6. Effect of *R. tsutsugamushi* strain on the aquisition of heterologous in vivo protection

Mouse strain	Immunizing R. tsutsugamushi strain[a]	No. of survivors on the following challenge day p.i.[b]:					
		2	3	4	5	6	7
C3H/HeDub	Gilliam	0	0	0	1	3	3
	Karp	0	0	0	0	3	4
BALB/cDub	Gilliam	0	0	0	0	2	4
	Karp	0	0	0	0	1	4

[a] Mice were immunized s.c. with 1,000 MLD_{50} of *R. tsutsugamushi*.
[b] All mice were challenged i.p. with 1,000 MLD_{50} of the Kato strain of *R. tsutsugamushi*; all challenged nonimmune control mice died by day 12 p.i. Five mice were tested per group.

weanling mice infected with *R. tsutsugamushi*, could not detect rickettsial antigen in s.c. inoculated mice until day 26 p.i. but could readily detect it as early as day 12 p.i. in i.p. inoculated animals.

The persistence of rickettsiae in infected mice was not surprising and has been well documented by others (9, 20, 28). The Karp strain of *R. tsutsugamushi* has been recovered from the blood and tissues of mice 610 days p.i. We observed persistent blood infections of 1 year only in mice infected s.c. with rickettsiae that would have induced lethal infections if inoculated i.p. The rickettsemias persisted in the presence of high IFA and CF antibody titers. With isolated exceptions, Gilliam-inoculated BALB/cDub mice did not show detectable rickettsemias after week 20 p.i. regardless of the route of inoculation. The decrease in the number of detectable rickettsemias was accompanied by a concomitant drop in serum antibody. Despite the inability to detect rickettsemias and the decreasing antibody levels, 5 of 20 C3H/HeDub mice inoculated with Gilliam harbored detectable rickettsiae in their spleens. Although it appears likely that at least some of the mice with spleens that tested negative for *R. tsutsugamushi* may have been cleared of the infection, there exists the possibility that they harbored rickettsiae below detectable levels. Shirai et al. (28) also observed falling antibody levels and were unable to detect rickettsiae in the tissues of 40% of rickettsemia-negative mice 565 days p.i. with the nonpathogenic strain *R. tsutsugamushi* TA678 (29). They were, however, able to reactivate a rickettsemia in a high percentage of these mice by treatment with cyclophosphamide. These results would indicate that even nonpathogenic rickettsial strains cannot be or are rarely cleared in the mouse.

Clearly, the utility of the mouse models described here is that they permit the manipulation of the course of a *R. tsutsugamushi* infection, thus enabling greater, in-depth study of the mechanism of host resistance as well as pathogenesis of the organism.

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