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Role of Macrophages in Innate and Acquired Host Resistance to Experimental Scrub Typhus Infection of Inbred Mice

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Mechanisms of innate resistance to infection with the Gilliam strain of *Rickettsia tsutsugamushi* were examined using congenic strains of mice resistant (C3H/RV) or susceptible (C3H/He) to intraperitoneal infection. Both strains of mice were resistant to infection with 1,000 50% mouse lethal doses of rickettsiae if given intravenously. In both systems rickettsial replication occurred after intravenous infection, as evidenced by an increase in rickettsial numbers in the spleens of infected animals, followed by a decrease in rickettsiae to low levels by day 14 postinfection. Administration of the antimacrophage agents silica and carrageenan to C3H/He mice intravenously rendered these animals susceptible to lethal infection. Neither irradiation nor silica given individually rendered C3H/RV mice susceptible to intravenous infection. However, if silica and irradiation were given together, a lethal infection occurred after intravenous infection. C3H/RV mice became susceptible to lethal infection after sublethal doses of irradiation only if they were infected intraperitoneally. Administration of silica or carrageenan had no effect on the outcome of intraperitoneal infection of these mice with Gilliam rickettsiae. These data suggest that both strains of mice share innate resistance mechanisms to intravenous infection that consist of fixed macrophages. Resistance of C3H/RV mice to intraperitoneal infection, in contrast, apparently was dependent only on an irradiation-sensitive process.

The response of inbred mice to infection with *Rickettsia tsutsugamushi* has been shown to be influenced markedly by the strain of *R. tsutsugamushi*, the genetic background of the mice, and the route of inoculation of the rickettsiae. Studies performed in this laboratory have shown that certain strains of inbred mice are genetically resistant to lethal intraperitoneal (i.p.) infection with the Gilliam strain of *R. tsutsugamushi* (8). Further, this natural resistance to Gilliam rickettsiae is controlled by a single, autosomal dominant gene not linked to the *H-2* complex (9) and is expressed in resistant mice by an apparent limitation of rickettsial replication in the peritoneal cavity (10, 14). The other two prototype strains of *R. tsutsugamushi*, Karp and Kato, are uniformly lethal for all inbred strains of mice tested, regardless of their genetic background, if the rickettsiae are given i.p. (10; unpublished data). Subcutaneous (s.c.) inoculation, however, usually produces a chronic infection with development of immunity to subsequent, otherwise lethal, i.p. challenge with homologous or heterologous strains of scrub typhus rickettsiae (8).

Other models of genetic resistance to infection have been developed with inbred strains of mice and facultative intracellular bacteria such as the BCG strain of *Mycobacterium bovis* (1), *Listeria monocytogenes* (4, 22), and *Salmonella typhimurium* (18). The innate genetic resistance of mice to *L. monocytogenes* apparently is due to the ability of splenic and hepatic macrophages (MΦ) from resistant mice to limit the replication of bacteria (19, 21). This mechanism has been shown to be independent of T-cell function (3, 5) and, interestingly, to be operative in both resistant and susceptible mice. It is critically sensitive to the bacterial challenge dose because the bactericidal activity in susceptible mice is evident only at low doses of bacteria. In further studies, it was concluded that strains of mice resistant to *L. monocytogenes* mount a quantitatively superior inflammatory MΦ response that is sensitive to low doses of irradiation, in addition to the innate ability of fixed MΦ in the spleen and liver to limit bacterial replication (19).

The innate resistance of certain strains of mice to *S. typhimurium* was shown by O'Brien et al. (17) to be abrogated selectively by the administration of silica before bacterial infection, again suggesting a role for MΦ in genetically determined resistance to infectious agents.

The role of MΦ in resistance to rickettsial infection has been evaluated mainly with in vitro methodology. These studies suggest that the

mechanism of acquired immunity to *R. tsutsugamushi* may involve activation of MΦ by soluble T-cell products (15). In a recent study, Nacy and Groves (14) examined the in vivo MΦ response of BALB/c (resistant) and C3H/He (susceptible) mice after i.p. infection with Gilliam rickettsiae. They concluded that C3H/He mice are susceptible to infection due to an inappropriate inflammatory MΦ response rather than to a defect in MΦ function, at least as compared with the response of BALB/c mice.

We have recently described a congenic pair of C3H mice (C3H/RV and C3H/HeDub) that showed dramatic differences in susceptibility to the Gilliam strain of *R. tsutsugamushi* (10). This study demonstrated that the susceptible strain of mice mounted a vigorous inflammatory response in the peritoneal cavity but that the inflammatory cells were incapable of limiting rickettsial replication, again suggesting the importance of appropriate effector cells in controlling rickettsial replication. In this study, we examined the relative importance of resident peritoneal MΦ and inflammatory exudate MΦ in resistance to lethal infection with the Gilliam strain of *R. tsutsugamushi* and assessed the capacity of the fixed splenic and hepatic MΦ population to limit replication of *R. tsutsugamushi* in vivo.

MATERIALS AND METHODS

Mice. Female C3H/He mice were obtained from Flow Laboratories, Inc., Dublin, Va., and used at the age of 8 to 12 weeks. C3H/RV mice were a generous gift from R. Jacoby of Yale University School of Medicine, New Haven, Conn. They were obtained as a breeding pair and were subsequently propagated by Flow Laboratories. Mice were age and sex matched in individual experiments.

Rickettsiae. The Karp strain (egg passage 52) and the Gilliam strain (egg passage 165) of *R. tsutsugamushi* were plaque purified (16) and propagated in embryonated eggs, and infected yolk sac suspensions were prepared and stored at −70°C as previously described (10). Rickettsial titers were expressed at 50% mouse lethal doses (MLD_{50}), based on lethality in C3H/He mice and calculation of the MLD_{50} by the method of Spearman and Karber (6).

Infection of mice. Rickettsial stocks were diluted to the desired concentration in cold brain heart infusion broth. Mice were infected by the administration of 0.1 ml of the diluted rickettsiae i.p. or intravenously (i.v.) into the lateral tail vein.

Quantitation of rickettsiae in infected spleens. At various time intervals after infection, mice were sacrificed by CO_2 asphyxiation, their spleens were removed and weighed, and a 10% (wt/vol) suspension was made in brain heart infusion broth. The tissue was disrupted with a Tenbrock homogenizer, and the resulting homogenates were pooled, quick-frozen, and stored at −70°C. The number of viable rickettsiae was determined by injecting C3H/He mice i.p. with 0.2 ml of spleen homogenate dilutions made in brain heart infusion broth. Mice were observed, and deaths that occurred between 6 and 21 days postinfection were recorded. Data were expressed as the number of MLD_{50} per 20 mg of spleen weight. For each time point postinfection, the spleens of three mice were removed and the homogenates were pooled.

Assessment of immune status after i.v. or s.c. rickettsial infection. Mice were infected with graded doses of rickettsiae either s.c. or i.v., and at various times postinfection representative mice were challenged i.p. with 1,000 MLD_{50} of Gilliam rickettsiae to assess their immunity to rechallenge.

Irradiation of mice. Mice were irradiated in a Plexiglas box with a ^{137}Cs source (Gamma cell 40; Atomic Energy of Canada Ltd., Ottawa, Ontario, Canada).

Pretreatment of mice with anti-MΦ agents. Mice were treated with silica by the procedure of O'Brien et al. (17). Briefly, silica (no. 216; Whittacker, Clark, and Daniels, Plainsfield, N.J.) was washed and dried, and samples were autoclaved. After sterilization, the silica was suspended in Hanks balanced salt solution containing 10% fetal calf serum and sonicated immediately before injection. Each mouse was given the desired dose of silica in 0.2 ml, 24 h before rickettsial infection. In parallel studies, mice were treated immediately after silica administration with 4 mg of poly-2-vinyl pyridine *N*-oxide s.c. to counter the effect of silica.

Quantitation of peripheral blood monocytes. At various times after i.v. infection with 5,000 MLD_{50} of Gilliam rickettsiae, untreated C3H/He and C3H/RV mice and irradiated (500 rads) C3H/RV mice were bled from the retro-orbital sinus, and total leukocyte counts were obtained with an automatic cell counter (Coulter ZBI; Coulter Electronics, Inc., Hialeah, Fla.). Differential counts were performed on Giemsa-stained thin blood smears, and the number of circulating monocytes was calculated by multiplying the percentages obtained from differential counts by the total leukocyte count. In several experiments, monocytes were identified by staining for peroxidase activity, which yielded results similar to those from Giemsa-stained smears.

RESULTS

Effect of route of inoculation on *R. tsutsugamushi* lethality. Initial experiments were performed to establish the pattern of lethality for congenic strains of mice infected i.v. with various doses of the Gilliam strain of *R. tsutsugamushi*. We believed that i.v. administration of rickettsiae should result in preferential infection of the spleens and livers of mice and allow careful quantitation of the replication of the organisms. C3H/He mice, which routinely undergo a lethal infection when inoculated i.p. with 1,000 MLD_{50} of Gilliam rickettsiae, were relatively resistant to the same dose of rickettsiae given i.v. (Table 1). If, however, the i.v. challenge dose was increased 10-fold, all C3H/He mice evidenced a lethal infection. In contrast, C3H/RV mice were resistant to large challenge doses (100,000 MLD_{50}) regardless of the route of inoculation. In all experiments, the viability of the challenge inocula was established by injecting C3H/He

TABLE 1. Susceptibility of C3H/He and C3H/RV mice to i.p. or i.v. infection with the Gilliam strain of *R. tsutsugamushi*

Mouse strain	Route of inoculation	Mortality[a]		
		1,000 MLD_{50}[b]	10,000 MLD_{50}	100,000 MLD_{50}
C3H/He	i.p.	5/5 (100)	5/5 (100)	ND[c]
	i.v.	7/30 (23)	10/10 (100)	ND
C3H/RV	i.p.	0/5 (0)	0/5 (0)	1/10 (10)
	i.v.	0/5 (0)	0/5 (0)	0/5 (0)

[a] Number of deaths/number of animals infected (percent mortality).
[b] Approximate challenge dose based on previous titration of rickettsiae in C3H/He mice.
[c] ND, Not done.

mice i.p. These animals died as expected 10 to 12 days postinfection.

Quantitation of rickettsiae in spleens of infected mice. Spleens of C3H/He and C3H/RV mice infected i.v. contained increasing numbers of rickettsiae postinfection (Fig. 1). The number of rickettsiae peaked at 10 days postinfection and then declined, but there were still substantial numbers of organisms remaining 28 days postinfection, at termination. The rickettsial burden in the spleens of C3H/RV mice developed more slowly than it did in the spleens of C3H/He mice, with detectable numbers of organisms first seen at 5 days after infection. The number of rickettsiae did not reach the peak levels noted in C3H/He mice.

When the numbers of rickettsiae in the spleens of C3H/RV mice were examined after

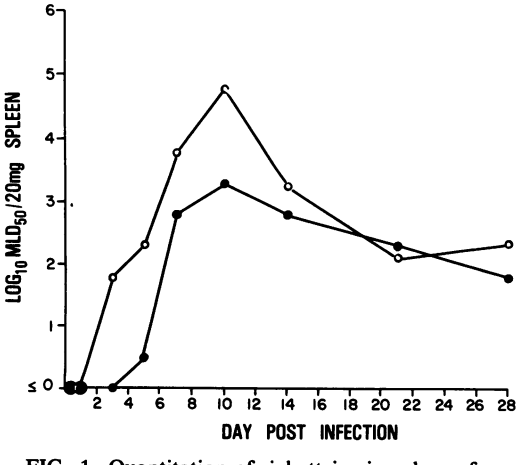

FIG. 1. Quantitation of rickettsiae in spleens from C3H/RV (●) and C3H/He (○) mice infected i.v. with 1,000 MLD_{50} of the Gilliam strain of *R. tsutsugamushi*. Each point represents the mean number of MLD_{50} per 20 mg of spleen obtained from six mice.

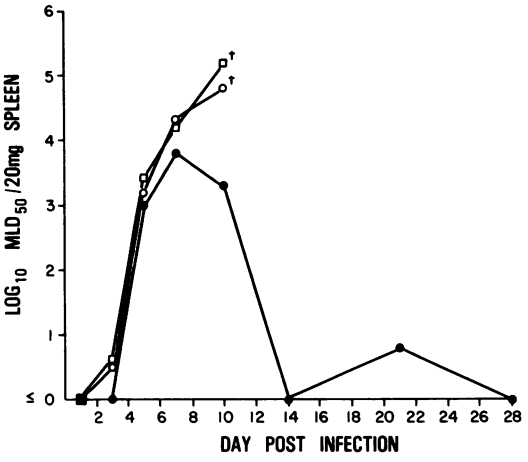

FIG. 2. Quantitation of rickettsiae in spleens from C3H/RV (●) and C3H/He (○) mice infected i.p. with 1,000 MLD_{50} of the Gilliam strain of *R. tsutsugamushi* or C3H/RV (□) mice infected i.p. with 1,000 MLD_{50} of the Karp strain of *R. tsutsugamushi*. Gilliam strain infected C3H/He and Karp strain infected C3H/RV mice died between 11 and 13 days (†) postinfection. Each point represents the mean number of MLD_{50} per 20 mg of spleen obtained from six mice.

i.p. infection with 1,000 MLD_{50} of Gilliam rickettsiae (Fig. 2), the initial pattern of rickettsial proliferation was similar to that in mice infected i.v. However, the number of rickettsiae in the spleens of these animals declined to undetectable levels 14 days postinfection and remained at a low level for the remainder of the experiments. In contrast, C3H/RV mice infected i.p. with Karp rickettsiae, and C3H/He mice infected i.p. with Gilliam rickettsiae, underwent a lethal infection that was characterized by a steady increase in the numbers of rickettsiae in the spleens until the time of death.

Development of acquired immunity after i.v. infection. The data obtained from mice infected i.v. demonstrated a reduction in rickettsial numbers beginning about 10 days postinfection. In an attempt to determine whether this clearance was associated with the development of a specific acquired immunity, mice infected i.v. were rechallenged i.p. with 1,000 MLD_{50} of Gilliam rickettsiae at various times after the primary i.v. infection. It is clear from these data (Fig. 3) that by 10 days after i.v. infection the majority of mice were protected against a lethal i.p. challenge and that by day 14 after i.v. infection all mice were capable of resisting the second challenge. This resistance continued throughout the 28 days that the mice were tested.

Effect of anti-MΦ agents on resistance to Gilliam rickettsial infection. To determine whether spleen MΦ were playing a role in the observed

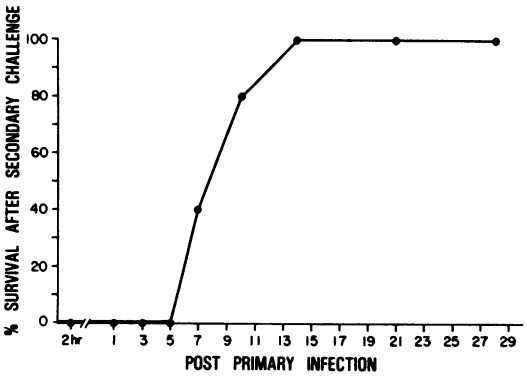

FIG. 3. Development of resistance to i.p. challenge with 1,000 MLD_{50} of the Gilliam strain of *R. tsutsugamushi* at various times after i.v. infection of C3H/He mice with 1,000 MLD_{50} of the Gilliam strain of *R. tsutsugamushi*. Abscissa shows the number of days postinfection.

resistance of C3H/He mice to i.v. infection, mice were depleted of MΦ function by use of established techniques. When C3H/He mice were pretreated with 3 mg of silica i.v. before i.v. administration of 1,000 MLD_{50} of Gilliam rickettsiae (Table 2), they were rendered susceptible to lethal infection. The administration of poly-2-vinyl pyridine *N*-oxide at the same time as silica partially reversed the effect of silica, and the majority of treated mice survived infection. A second agent known to inhibit MΦ function, carrageenan, also rendered mice susceptible to i.v. infection when given before rickettsial inoculation. In no instance did the administration of silica, silica and poly-2-vinyl pyridine *N*-oxide, or carrageenan at the described doses result in the death of uninfected animals.

In contrast to the data obtained with C3H/He mice infected i.v., the administration of 3 mg of silica i.v. to C3H/RV mice had no apparent effect in terms of susceptibility to 1,000 MLD_{50} of Gilliam rickettsiae administered i.v. In further experiments, C3H/RV mice were given increasing doses of silica before infection. Mice treated with 10 mg of silica, which is close to the toxic dose in these mice, still resisted infection with 10,000 MLD_{50} of Gilliam rickettsiae. Carrageenan administered as before also had no effect on the outcome of i.v. infection of C3H/RV mice (data not shown).

TABLE 2. Effect of anti-MΦ agents on resistance of C3H/He mice to i.v. infection with the Gilliam strain of *R. tsutsugamushi*[a]

Pretreatment[b]	Mortality[c]
PBS[d] i.v.	1/6 (8)
3 mg of silica i.v.	5/5 (100)
3 mg of silica i.v. + PVNO[e]	2/5 (40)
1 mg of carrageenan i.v.	5/5 (100)

[a] Mice were given 1,000 MLD_{50} i.v.
[b] Animals were pretreated 24 h before infection as indicated in the table.
[c] Number of deaths/number of animals infected (percent mortality).
[d] PBS, Phosphate-buffered saline.
[e] PVNO, Poly-2-vinyl pyridine *N*-oxide (4 mg) given s.c. at the time of the silica administration.

Effect of irradiation on outcome of i.v. infection. The resistance of C3H/RV mice to Gilliam rickettsiae seemed to be unaffected by classical means of fixed MΦ depletion. Therefore, we decided to employ gamma radiation to impair the immune system. This technique is nonspecific, but it does inhibit the replication of bone marrow precursor cells and can be expected indirectly to influence inflammatory cells arriving at the site of rickettsial infection.

C3H/He and C3H/RV mice were subjected to doses of radiation ranging from 0 to 700 rads. Experiments involved both uninfected animals and those receiving 1,000 MLD_{50} of Gilliam rickettsiae i.v. immediately after irradiation. No lethal radiation effect was observed in normal C3H/He mice until the dosage exceeded 300 rads (Fig. 4). However, irradiation with as little as 100 rads markedly increased mortality after i.v. infection, as compared with infected animals that were not irradiated. At a dose of 300 rads, all animals died after i.v. infection. In parallel experiments, C3H/RV mice were infected i.v. with 1,000 MLD_{50} of Gilliam rickettsiae after various doses of gamma irradiation. In these studies, uninfected C3H/RV mice were less susceptible to irradiation than were C3H/He mice,

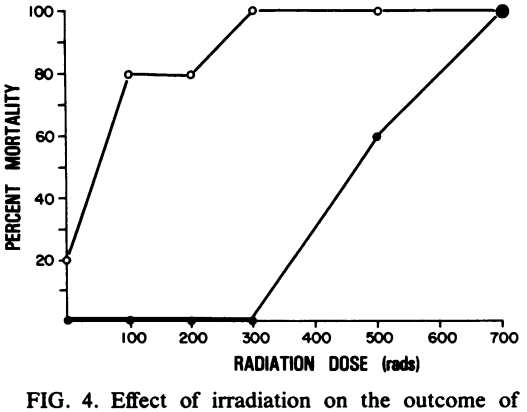

FIG. 4. Effect of irradiation on the outcome of infection with 1,000 MLD_{50} of the Gilliam strain of *R. tsutsugamushi*. Mortality of uninfected C3H/He mice (●) or C3H/He mice infected i.v. with 1,000 MLD_{50} of Gilliam rickettsiae (○) after whole-body irradiation is shown. Five mice were in each group.

TABLE 3. Effect of irradiation and silica given together on resistance of C3H/RV mice to i.v. infection with the Gilliam strain of *R. tsutsugamushi*

Pretreatment[a]	Dose (MLD_{50})[b]	Mortality[c]
500 rads	1,000	0/5 (0)
	5,000	0/5 (0)
5 mg of silica i.v.	1,000	0/5 (0)
	5,000	0/5 (0)
5 mg of silica i.v. + 500 rads	1,000	3/5 (60)
	5,000	5/5 (100)

[a] Animals were pretreated 24 h before infection as indicated in the table.

[b] All doses were administered i.v.

[c] Number of deaths/number of animals infected (percent mortality).

and no radiation dose up to 700 rads eliminated the resistance of C3H/RV mice to 1,000 MLD_{50} of Gilliam rickettsiae given i.v. (data not shown).

To investigate the possibility that a more complex situation exists in C3H/RV mice than in C3H/He mice in terms of resistance to i.v. infection, we used a pretreatment protocol with both silica and irradiation. As high doses of silica and irradiation given together resulted in nonspecific mortality, we employed 500 rads of irradiation and 5 mg of silica. As before, neither silica nor irradiation, given alone, had any effect on the ability of C3H/RV mice to resist i.v. infection with 1,000 or 5,000 MLD_{50} of Gilliam rickettsiae (Table 3). However, when silica and irradiation were given together, mortality was evident after infection with 1,000 MLD_{50} of Gilliam rickettsiae, and all animals died after infection with 5,000 MLD_{50} of Gilliam rickettsiae.

Studies on mechanisms of resistance of C3H/RV mice to i.p. infection. To investigate whether a common mechanism is responsible for the resistance of C3H/RV mice to i.p. infection as well as to i.v. infection, mice were pretreated with anti-MΦ agents or irradiation before infection. As before, administration of silica or carrageenan i.p. before i.p. administration of Gilliam rickettsiae had no effect on the ability of C3H/RV mice to resist the infection, even when relatively large doses of silica were used and the challenge dose was increased to 10,000 MLD_{50} of rickettsiae (data not shown). In further studies, C3H/RV mice were irradiated with various doses of gamma irradiation before i.p. infection with 1,000 MLD_{50} of Gilliam rickettsiae. Radiation doses as low as 300 rads rendered C3H/RV mice susceptible to i.p. infection (Fig. 5), and all animals died after infection when irradiated with 500 rads, although this was itself a nonlethal radiation dose.

Changes in peripheral blood monocyte counts after infection. As data derived from mice treated with silica and irradiation suggested that inflammatory MΦ played a role in the resistance of C3H/RV mice to rickettsiae, C3H/RV mice irradiated with 500 rads, unirradiated C3H/RV mice, and C3H/He mice were evaluated in terms of changes in peripheral blood monocytes after i.v. infection with 5,000 MLD_{50} of Gilliam rickettsiae. As expected, monocytes were absent from the peripheral blood of irradiated C3H/RV mice (Fig. 6) after infection. Unirradiated C3H/RV mice demonstrated a peripheral monocytosis after i.v. infection, with peak numbers of monocytes observed 5 to 7 days after infection. In contrast, total monocyte numbers in peripheral blood of infected, unirradiated C3H/He mice also increased by day 5 but reached a peak level that was only 75% of the day 5 level noted in C3H/RV mice. A greater difference was evident between the two strains 7 days after infection. A similar increase in circulating monocyte counts was observed in C3H/RV mice infected i.p. with Gilliam rickettsiae (data not shown).

DISCUSSION

The mechanism(s) of the genetic resistance of certain inbred strains of mice to the Gilliam strain of *R. tsutsugamushi* is unknown. In a recent study Nacy and Groves (14) provide evidence that susceptibility to infection is correlated with differences in the MΦ response to i.p. infection with scrub typhus rickettsiae, in terms of the presence of peroxidase-positive MΦ in the

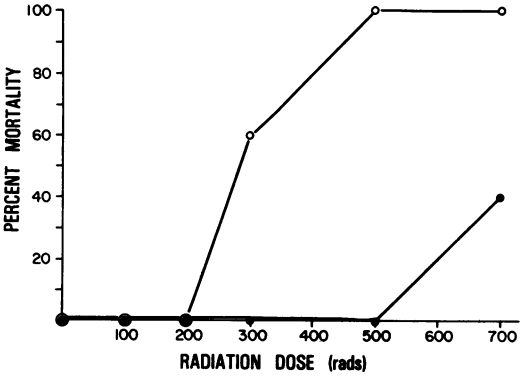

FIG. 5. Effect of irradiation on the outcome of infection with 1,000 MLD_{50} of the Gilliam strain of *R. tsutsugamushi*. Mortality of uninfected C3H/RV mice (●) or of C3H/RV mice infected i.p. with 1,000 MLD_{50} of Gilliam rickettsiae (○) after whole-body irradiation is shown. Five mice were in each group.

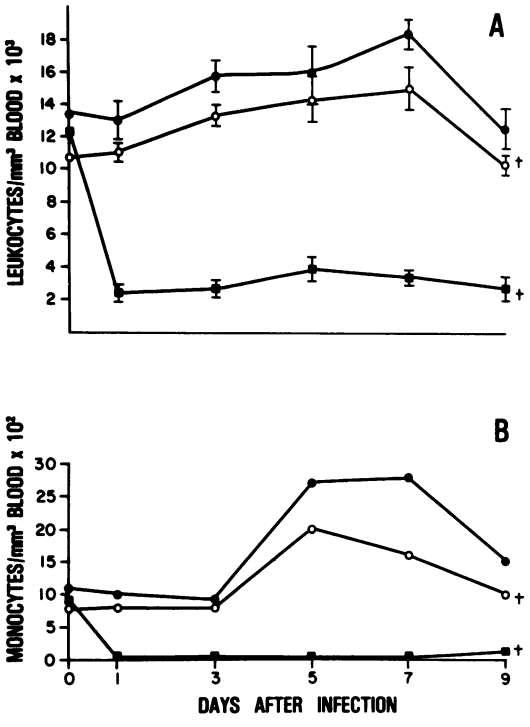

FIG. 6. Quantitation of circulating leukocytes in C3H/RV (●), C3H/He (○), and irradiated (500 rads) C3H/RV (■) mice after i.v. infection with the Gilliam strain of *R. tsutsugamushi*. (A) Total leukocyte count; (B) total calculated monocyte count. Each point represents the mean ± one standard deviation obtained from five mice.

inflammatory exudate. This system is in contrast to that with *Rickettsia akari*, where a defect in MΦ function has been suggested as the mechanism for susceptibility of certain mouse strains (2, 13). In this study we have extended previous work with *R. tsutsugamushi*, using various methods to deplete MΦ in vivo to determine whether differences in susceptibility noted in the congenic C3H/RV-C3H/He model were due to MΦ influences.

The results of this study clearly demonstrate that a mechanism exists in both strains of mice that is capable of limiting the replication of the Gilliam strain of *R. tsutsugamushi*, but in susceptible mice, this mechanism is operative only if rickettsiae are given i.v. at relatively low concentrations. As other studies have shown that organisms given i.v. are primarily cleared by the spleen and liver (19, 21), we followed the fate of rickettsiae injected i.v. by quantitating the numbers of viable rickettsiae in spleen homogenates. These data (Fig. 1) demonstrated that Gilliam rickettsiae are capable of proliferating in the spleens of both strains of infected mice. However, by day 14 the rickettsial infection was adequately controlled by both strains of mice, and there were no fatalities during the 28-day observation period. Replication of scrub typhus organisms also was observed after i.p. infection of these mice. The rickettsial burden in the spleens of mice undergoing a lethal (C3H/He, Gilliam strain; C3H/RV, Karp strain) or nonlethal (C3H/RV, Gilliam strain) infection was essentially indistinguishable during week 1 of infection. However, by day 10, the C3H/RV mice infected with Gilliam rickettsiae were able to reduce the number of rickettsiae in the spleens, whereas the other rickettsia-host interactions resulted in a fulminating, lethal infection.

These data suggested the possibility of a common mechanism in the spleens of both susceptible and resistant mice that inhibited rickettsial replication after low-dose i.v. infection but that was overwhelmed in susceptible mice after high-dose i.v. infection or after continuous assault by rickettsiae emerging from the peritoneal cavity after infection by this route. A similar situation has been described with mice differing in susceptibility to *L. monocytogenes*. A bactericidal mechanism is present in both strains of mice, but this resistance can be overcome in susceptible mice by increasing the dose of infecting organisms (21).

In addition, our data served to answer a lingering question from our initial study of the interaction of rickettsiae with C3H/He and C3H/RV mice (10) and from a study with BALB/c and C3H/HeDub mice (14). In these studies it was observed that Gilliam rickettsiae proliferated abundantly in mononuclear peritoneal cells of susceptible C3H/HeDub mice but that only a small percentage of similar cells from resistant C3H/RV or BALB/c mice showed the presence of rickettsiae after i.p. challenge. It was possible, based on those observations, that the resistance of BALB/c and C3H/RV mice was a purely localized phenomenon restricted to the peritoneal cavity. Our current observations (Fig. 2) documented that both Gilliam and Karp organisms do exit from the peritoneal cavity and initially establish a similar rickettsial burden in the spleen. Thus, rickettsia-host interactions in the peritoneal cavity of resistant mice may be very important after i.p. inoculation, but the final outcome of the infection is apparently not determined solely within this anatomical compartment.

We found that the decline of rickettsiae in the spleen of C3H/He mice after i.v. infection was correlated closely in time with the development of acquired resistance to a secondary i.p. challenge. The kinetics of development of this resistance was essentially the same as that of the

development of cell-mediated immunity and resistance to infection produced by an s.c. immunizing infection, which have been described previously in C3H/He mice (11) and in C3H/RV mice infected i.p. or s.c. (unpublished data). It is likely that the decline in the numbers of rickettsiae in the spleens of C3H/He mice infected i.v. and C3H/RV mice infected i.p. was due to the development of a cell-mediated immunity with the corresponding activation of MΦ which has been suggested as important mediators of rickettsial immunity (15).

Treatment of C3H/He mice with silica or carrageenan before i.v. infection eliminated the observed resistance. However, when C3H/RV mice were pretreated with silica, even at high doses, there was no demonstrable effect on the final outcome of infection. Others have shown that sensitivity to agents such as silica, carrageenan, and dextran sulfate is a characteristic of mature MΦ that comprise the fixed cells of the mononuclear phagocyte system (12, 17). It also has been shown that these cells are relatively radioresistant (7). In contrast, the cells that are attracted to sites of inflammation have been shown to be derived from peripheral blood monocytes that are, in turn, derived from a bone marrow precursor (23, 24), and this process is, accordingly, susceptible to radiation (24). In a recent study, early nonspecific resistance to *L. monocytogenes* was shown to be active in both susceptible and resistant mice, and this resistance was mediated by cells in the susceptible mice that were resistant to relatively high levels of radiation (900 rads) but that were sensitive to dextran sulfate. Mice that were genetically resistant to infection with this bacterium were shown to possess a bactericidal mechanism that was highly radiosensitive but relatively unaffected by dextran sulfate (19). These workers concluded that a superior MΦ turnover, in terms of maturation of promonocytes to circulating monocytes and ultimately to MΦ, is responsible for the genetic advantage of resistant mice.

The resistance of C3H/RV mice to i.p. infection is apparently only susceptible to irradiation (Fig. 5) and suggests that the mechanism is due perhaps to a greater production and more rapid turnover of monocytes, as was seen with *L. monocytogenes* (19). This hypothesis also is supported by the finding that BALB/c mice respond to Gilliam rickettsial infection with a greater peroxidase-positive inflammatory MΦ response in the peritoneal cavity than do susceptible C3H/He mice (14). Previous studies with C3H/RV and C3H/He mice demonstrated that, although C3H/He mice respond to i.p. infection with a greater inflammatory response, the peritoneal exudate cells produced in C3H/He mice are unable to restrict the replication of the infecting rickettsiae (10) based on morphological criteria and direct counting of organisms, using the technique of Silverman et al. (20), thus suggesting that C3H/RV mice are able to resist infection by a more appropriate local or systemic response. The susceptibility of this response to irradiation (Fig. 5) and the more pronounced monocyte response after infection (Fig. 6) suggest that MΦ turnover from precursors is an important facet of the resistance to the Gilliam strain of *R. tsutsugamushi*, although more experiments are required to demonstrate this conclusively.

Although resistance of C3H/RV mice to i.p. infection was found to be susceptible to irradiation, we consistently failed to ablate resistance to i.v. infection with irradiation alone. However, when C3H/RV mice were pretreated with irradiation and silica, they became susceptible to lethal i.v. infection. These data are consistent with the hypothesis that C3H/He and C3H/RV mice share nonspecific effector cells in the spleen and liver, which would be unaffected by radiation. The fact that irradiation alone renders C3H/He mice susceptible to lethal infection after i.v. administration of rickettsiae suggests that the spleen and liver MΦ in these mice provide only temporary restriction of rickettsial growth and are dependent on the ensuing immunologically dependent inflammatory response to clear the organisms. The fact that irradiation alone did not render C3H/RV mice susceptible to i.v. infection suggests that the fixed MΦ population in the spleens and livers of these animals is qualitatively or quantitatively superior to the fixed MΦ population in C3H/He mice and is capable of indefinitely controlling the rickettsiae and maintaining sublethal levels of organisms. Apparently, a similar situation does not exist in the peritoneal cavity, in that irradiation alone rendered mice susceptible to i.p. infection. This suggests that mature peritoneal MΦ are not important in the effector mechanisms involved in protection against i.p. infection. Clearly, further studies of MΦ subpopulations are required to confirm these findings and further delineate the role of different MΦ populations in resistance to *R. tsutsugamushi*.

It is clear that both fixed and inflammatory MΦ are important not only in genetic resistance to *R. tsutsugamushi* infections of inbred mice but also in the development and expression of immune clearance, presumably by a cell-mediated immunity. The relative importance of each MΦ subpopulation is still unclear and warrants further study.

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