Host Defenses in Experimental Scrub Typhus: Inflammatory Response of Congenic C3H Mice Differing at the Ric Gene

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Two strains of C3H mice differed in their susceptibility to lethal infection with *Rickettsia tsutsugamushi* strain Gilliam. Adult C3H/RV mice were markedly more resistant to lethal infection than C3H/HeDub mice, and both were histocompatible as assessed by mixed-lymphocyte cultures and graft-versus-host responses. The inflammatory response of susceptible C3H/HeDub mice to intraperitoneal infection was evident approximately 5 days postinfection, and the magnitude of the cellular influx increased until death of the animal. The inflammation consisted of an early polymorphonuclear leukocyte response, followed by a mononuclear cell influx which persisted until death of the animal. The C3H/RV mice evidenced similar kinetics of cell influx, but the inflammatory response was significantly reduced in magnitude, and the response of C3H/RV animals to Gilliam was predominantly mononuclear in nature, with little influx of polymorphonuclear leukocytes into the peritoneal cavity. C3H/RV mice were rendered susceptible to Gilliam infection by induction of a nonspecific inflammation with thioglycolate if given 3 days after infection. Conversely, treatment of C3H/HeDub mice with indomethacin, an anti-inflammatory agent, prolonged survival after infection with Gilliam. The results of this study indicate that genetic resistance to Gilliam is not due simply to a greater host response to infection or, conversely, that susceptibility is due to a host response quantitatively lacking in a cellular component necessary for antirickettsial immunity.

Genetic factors have been shown to play an important role in determining the outcome of bacterial (5, 22), viral (3, 6, 14), and rickettsial (1, 11) infections of laboratory animals. Previous work from this laboratory demonstrated that susceptibility of mice to lethal infection with *Rickettsia tsutsugamushi* or *Rickettsia akari* was influenced by both the strain of infecting rickettsiae and the genetic background of the host (1, 11). Resistance to lethal infection with *R. tsutsugamushi* strain Gilliam is controlled by a single autosomal, dominant gene designated *Ric*, which is not linked to the *H-2* locus but has been shown to be on chromosome 5, closely linked to the retinal degeneration gene (11, 11a).

The mechanism of action of the *Ric* gene product is not known at present. However, it has been shown that fibroblastic cells and resident peritoneal macrophages from both susceptible and resistant animals are capable of supporting rickettsial replication (11, 20). Thus, resistance to lethal infection is not due to a physiological inability of host cells to meet the nutritional requirements of these obligate intracellular bacteria. Recovery of resistant mice from infection with scrub typhus rickettsiae is facilitated by a cell-mediated immune response dependent on thymus-derived lymphocytes and activated macrophages (20, 25). However, inbred mice which are susceptible to lethal infection with *R. tsutsugamushi* strain Gilliam do not evidence a gross malfunction in immunocompetency since they can be immunized by subcutaneous inoculation of viable rickettsiae and subsequently resist an otherwise lethal intraperitoneal (i.p.) infection (11).

To assess the effect of the *Ric* gene product on pathogenesis of infection, we used two strains of C3H mice which were found to differ at the *Ric* gene and examined the influence of this gene on the inflammatory response to *R. tsutsugamushi*. This pair of strains (C3H/He and C3H/RV), which have previously been shown to be congenic and differ in susceptibility to certain viral infections (6), provided a model system which minimized the influence of other gene products on the immune response to scrub typhus infection. In this study we have established that the C3H/HeDub and C3H/RV mice differ at the *Ric* gene but not at the histocompatibility loci. The mouse strains used in this study were found to differ only in susceptibility to Gilliam infection, and both were susceptible to *R. tsutsugamushi* strain Karp infection. We have compared the inflammatory response in terms of the cellular influx into the peritoneal cavity after i.p. inoculation of rickettsiae. Experiments were also performed to determine the effect of inflamma-

tory agents on the response of mice resistant to Gilliam infection and, conversely, the effect of the anti-inflammatory agent indomethacin on animals normally susceptible to lethal Gilliam infection.

MATERIALS AND METHODS

Animals. Female C3H/HeDub mice were purchased from Flow Laboratories (Dublin, Va.); C3H/RV mice were obtained initially as a breeding pair from Robert Jacoby, Yale University School of Medicine, New Haven, Conn., and subsequently reared by Flow Laboratories under contract to Walter Reed Army Institute of Research. All mice were used at 6 to 8 weeks of age and weighed 18 to 22 g.

Rickettsiae. The Gilliam (egg passage 165) and Karp (egg passage 52) strains of *R. tsutsugamushi* were plaque purified in L-929 cells as previously described (21) and propagated in embryonated chicken eggs. Infected yolk sac suspensions were prepared and stored as previously described (7). The TA678 strain of *R. tsutsugamushi* was not plaque purified, but otherwise was handled in the same manner as the other strains. Quantification of rickettsiae was accomplished by plaque assay (21) or by inoculation of C3H/He mice and calculation of the 50% mouse lethal dose (MLD_{50}) by the method of Spearman and Karber (8).

Infection. Mice were infected by i.p. injection of 0.2 ml of infected yolk sac suspension diluted to contain 1,000 MLD_{50} or 1,000 plaque-forming units of rickettsiae.

Mixed-lymphocyte cultures. One-way mixed-lymphocyte cultures (MLC) were performed by using a modification of a micro-lymphocyte proliferation assay (15). Briefly, spleen cells were obtained from mice, and single cell suspensions were prepared in RPMI 1640 medium supplemented with 25 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) buffer, 1% glutamine, 50 μg of gentamicin per ml, and 10% heat-inactivated fetal bovine serum (Microbiological Associates, Walkersville, Md.). Responding spleen cells were adjusted to 5 × 10⁶ viable cells per ml, using trypan blue exclusion as the criterion for viability, and 0.1-ml aliquots were added to 96-well microtiter plates (Microtest II, Falcon Plastics, Oxnard, Calif.). The test was performed in triplicate, with wells containing responder cells receiving either media (control) or irradiated (2,500 rads) stimulator cells. Irradiation was performed with a ⁶⁰Co gamma radiation source (Gamma Cell 220, Atomic Energy of Canada Ltd., Ottawa, Ont.). The resulting ratio of responder to stimulator cells was 1:1. Cultures were incubated at 37°C for 96 h in a humidified atmosphere of 5% CO_2 in air. Six hours before termination of incubation, 1.0 μCi of tritiated thymidine (New England Nuclear Corp., Boston, Mass.; specific activity, 6.7 Ci/mmol) was added to each well. Cells were collected on filter strips with a multiple sample harvester, and the amount of [³H]thymidine incorporated into cells was determined by liquid scintillation counting (Packard Prias Tri-Carb liquid scintillation spectrometer). The data were expressed as net counts per minute, which is defined as the counts per minute of experimental cultures minus the counts per minute of control cultures. Stimulation indices were calculated as follows: stimulation index = (counts per minute of responding cells + irradiated stimulating cells)/(counts per minute of responding cells + irradiated autologous cells).

Graft-versus-host response. The ability of spleen cells to produce a graft-versus-host response was determined by using a popliteal node technique (9). Briefly, spleen cell suspensions from donor mice were adjusted to 10⁸ cells per ml in RPMI 1640 medium supplemented with HEPES, glutamine, and gentamicin as before. Five recipient mice were injected in the left footpad with 0.05 ml of heterologous spleen cell suspension. Experiments were controlled by injecting mice with homologous spleen cells, which was found to cause no enlargement of the node compared with the left, uninoculated node. Seven days after footpad injection mice were sacrificed, and the popliteal nodes were removed, trimmed free of fat, and weighed.

Estimation of inflammatory response. At various intervals after infection, animals were sacrificed by CO_2 asphyxiation, and cells were obtained by washing the peritoneal cavity twice with 5 ml of Hanks balanced salt solution supplemented with 50 μg of gentamicin and 10 U of heparin per ml. Peritoneal cells obtained from the two washings were pooled, centrifuged at 500 × g for 10 min at 4°C, and washed once with Hanks balanced salt solution. Cytocentrifuge preparations (Cytospin; Shandon Elliot, Sewickley, Pa.) were Giemsa stained, and differential cell counts were performed. A total of 200 cells were counted in each sample. In some experiments macrophages were quantified by ingestion of latex particles (13) or by the presence of cytoplasmic nonspecific esterase activity (16), and these results were essentially in agreement with the data obtained with morphological criteria. The percentage of mononuclear inflammatory cells containing intracellular rickettsiae was also determined with Giemsa-stained preparations. A total of 200 mononuclear cells were counted in each preparation.

Treatment of mice with inflammatory agents. Nonspecific inflammation was produced in C3H/RV mice by the i.p. injection of 1.0 ml of thioglycolate broth. Mice were challenged at various times after thioglycolate inoculation with 1,000 MLD_{50} of Gilliam strain rickettsiae.

Indomethacin treatment. Indomethacin was a gift of C. Stone (Merck Sharp and Dohme, West Point, Pa.). Stock solutions of indomethacin were prepared in absolute ethanol at a concentration of 10 mg/ml and stored at −70°C until used. Mice were injected i.p. with 100 μg of indomethacin in 0.5 ml of phosphate-buffered saline on alternate days for a period of 7 days before infection. After rickettsial challenge, mice were maintained on indomethacin by incorporating the drug into their drinking water at a concentration of 20 μg/ml.

RESULTS

Resistance of C3H/RV mice to infection with *R. tsutsugamushi* strain gilliam. Initial experiments indicated that C3H/RV mice were

resistant to lethal infection with scrub typhus rickettsiae of the Gilliam strain at doses which routinely killed C3H/HeDub mice. In comparative studies (Table 1), C3H/RV mice were found to be markedly more resistant to lethal infection with Gilliam strain rickettsiae than were C3H/HeDub mice even with very high challenge doses. However, when the Karp strain of *R. tsutsugamushi* was used to infect both strains of mice, no difference in susceptibility was observed.

Mixed-lymphocyte culture and graft-versus-host responses. To ensure that the C3H strains of mice used in this study were histocompatible, mixed-lymphocyte culture and graft-versus-host responses were evaluated. Table 2 shows that no significant stimulation was observed in either assay by reciprocal testing of the C3H pairs. The significant response of lym-

phocytes from BALB/c (H-2^d) mice to C3H (H-2^k) stimulating cells, and the response of both C3H strains to BALB/c stimulating cells, indicated the validity of the negative results of the reciprocal testing of the C3H pairs.

Inflammatory response of C3H mice to Gilliam. Inflammation in response to i.p. infection with Gilliam strain rickettsiae was evidenced by an influx of cells into the peritoneal cavity beginning approximately 5 days after infection (Fig. 1). In susceptible mice (C3H/HeDub), the cellular influx continued to increase through day 10. In all experiments, infection of C3H/HeDub mice with 1,000 MLD$_{50}$ of Gilliam resulted in 100% mortality in 11 to 12 days. Response of the resistant C3H/RV animals was characterized by a total cell response which was significantly less than in C3H/HeDub mice at days 5, 7, and 10 postinfection, using Student's

TABLE 1. *Comparative lethality of R. tsutsugamushi strains Gilliam and Karp for C3H/HeDub and C3H/RV mice*

Mouse strain	Challenge rickettsial strain	No. of deaths/no. of animals challenged (% mortality)			Calculated MLD$_{50}$[a]
		1,000 MLD$_{50}$[b]	10,000 MLD$_{50}$	100,000 MLD$_{50}$	
C3H/HeDub	Gilliam	5/5 (100)	5/5 (100)	5/5 (100)	−9.1 (±0.5)
	Karp	5/5 (100)	5/5 (100)	5/5 (100)	−8.8 (±0.1)
C3H/RV	Gilliam	0/5 (0)	0/5 (0)	1/5 (20)	≤−2.0
	Karp	5/5 (100)	5/5 (100)	5/5 (100)	−8.4 (±0.1)

[a] Log$_{10}$ MLD$_{50}$ (± standard deviation) in test mice calculated from dilutions used to achieve approximate challenge doses.

[b] Approximate challenge (MLD$_{50}$) dose based on previous titration of rickettsial stocks in C3H/HeDub mice. (Log$_{10}$ MLD$_{50}$ for Gilliam = −9.0; log$_{10}$ MLD$_{50}$ for Karp = −8.5.)

TABLE 2. *Mixed lymphocyte culture (MLC) and graft-versus-host (GVH) responses of C3H and BALB/c mice*

Responding strain	Stimulating strain[a]	MLC		GVH node wt (mg ± SD)
		cpm ± SD[b]	SI[c]	
C3H/RV	None	248 ± 83		
	C3H/RV	472 ± 56		2.43 ± 09
	C3H/HeDub	682 ± 73	1.4	2.70 ± 0.9
	BALB/c	2,147 ± 239[d]	4.5	6.98 ± 2.1[d]
C3H/HeDub	None	879 ± 131		
	C3H/HeDub	724 ± 79		2.28 ± 0.3
	C3H/RV	802 ± 100	1.1	2.52 ± 0.2
	BALB/c	2,705 ± 484[d]	3.7	4.33 ± 0.4[d]
BALB/c	None	682 ± 16		
	BALB/c	655 ± 62		2.30 ± 0.4
	C3H/RV	2,448 ± 158[d]	3.7	6.90 ± 1.3[d]
	C3H/HeDub	1,530 ± 411[d]	2.3	4.03 ± 0.9[d]

[a] Spleen cells used as stimulators in MLC were irradiated and added to cultures of responding spleen cells at a ratio of 1:1.

[b] Mean counts per minute of triplicate cultures ± 1 standard deviation (SD).

[c] Stimulation index (SI) = (counts per minute of responding cells + irradiated stimulating cells)/(counts per minute of responding cells + irradiated autologous cells). An SI of ≥2.0 was considered a positive response.

[d] Significantly different from control using Student's t test ($P \leq 0.01$).

t test ($P \leq 0.05$). No difference was observed in the initial (day 0) number of resident peritoneal cells between mouse strains.

To characterize the cellular composition of the inflammatory exudate, differential counts were performed on Giemsa-stained smears to determine the proportion of macrophages, lymphocytes, and polymorphonuclear leukocytes (PMN). Using these data and the total cell counts, the number of each cell type was calculated. These data are presented in Fig. 2. There was no significant difference in the initial proportions of peritoneal cell populations in the two strains of C3H mice. After infection with Gilliam,

FIG. 1. *Kinetics of cellular influx in C3H/HeDub (●) and C3H/RV (○) mice infected i.p. with 1,000 MLD₅₀ R. tsutsugamushi strain Gilliam. Each point represents the mean total cell counts ± 1 standard deviation of nine mice.*

the kinetics of macrophage and lymphocyte influx were similar in both strains of mice, but the number of cells in each subpopulation was significantly ($P \leq 0.01$) greater in the susceptible C3H/HeDub animals at 5, 7, and 10 days after infection. The most striking difference in the inflammatory exudate of the mice was apparent when the PMN response was examined. In susceptible animals, a biphasic PMN response was observed with an early (day 5) peak followed by a late (day 10) influx. In contrast, infection of C3H/RV animals with Gilliam did not produce a notable PMN response at the times studied. In all experiments the percentage of PMN in the peritoneal exudates produced by Gilliam infection of C3H/RV mice never exceeded 8%, compared to a range of 38 to 44% PMN in C3H/HeDub mice (data not shown). As no detectable difference was noted in the total cell number or the cellular composition of C3H/HeDub and C3H/RV resident peritoneal cells, observations in further experiments were begun 3 days postinfection.

While performing the differential counts, it was noted that many of the inflammatory cells from C3H/HeDub mice contained rickettsiae. The number of infected cells in Giemsa-stained preparations was determined, and these data are shown in Fig. 3. It is evident that rickettsiae could be demonstrated within inflammatory cells of C3H/HeDub mice by day 5 and that the percentage of infected cells increased until death of the animals. In contrast, few peritoneal cells from C3H/RV mice contained rickettsiae. The cells from susceptible mice which contained rickettsiae were predominantly macrophages early in the course of infection. By days 7 and 10, both macrophages and lymphocytes contained scrub
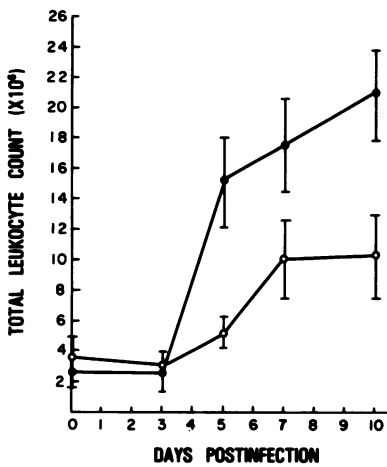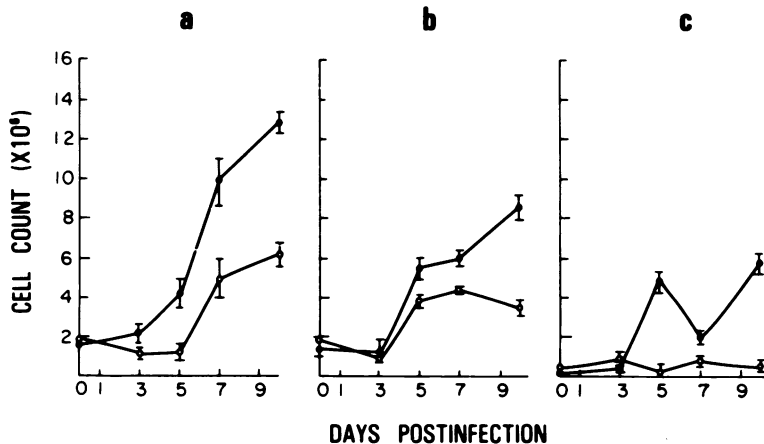

DAYS POSTINFECTION

FIG. 2. *Macrophage (a), lymphocyte (b), and PMN (c) counts in C3H/HeDub (●) and C3H/RV (○) mice infected i.p. with 1,000 MLD₅₀ R. tsutsugamushi strain Gilliam. Each point is the mean cell count ± 1 standard deviation of nine mice. Cell counts were calculated from total cell count and differential data.*
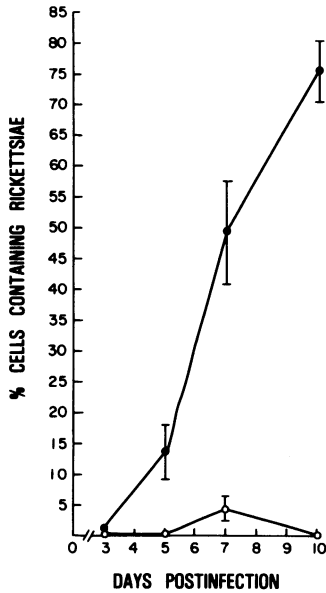
FIG. 3. *Percentage of peritoneal mononuclear cells containing detectable rickettsiae in C3H/HeDub (●) and C3H/RV (○) mice infected i.p. with 1,000 MLD₅₀ R. tsutsugamushi strain Gilliam. Each point represents the mean ± 1 standard deviation of five animals.*

typhus organisms. In C3H/RV animals rickettsiae were found only in the macrophage subpopulation of exudate cells. These results are similar to the data obtained in a separate study comparing the responses of C3H/HeDub mice to BALB/c mice (C. Nacy, personal communication).

Inflammatory response of C3H mice after infection with other strains of *R. tsutsugamushi.* To determine whether inflammatory responses observed after Gilliam infection of C3H/RV and C3H/HeDub mice were representative of the host responses during both lethal and nonlethal infections, the inflammatory response of C3H/RV mice after infection with the lethal Karp strain of rickettsiae was compared with the response in the same animals after infection with the nonlethal Gilliam strain. A similar comparison was made in C3H/HeDub mice after infection with Gilliam, which is lethal for these mice, and infection with strain TA678, which has been shown previously (11) to be nonlethal for C3H/HeDub animals.

The inflammatory responses of C3H/RV mice to Karp and Gilliam are indicated in Fig. 4. As expected, the influx of cells after Karp challenge occurred earlier and was of significantly greater magnitude than the response to Gilliam. Examination of the subpopulation of cells in the inflammatory exudate indicated that the lethal

Karp challenge elicited a cellular influx which was similar to that seen previously in the lethal Gilliam C3H/HeDub model. The influx was composed of macrophages, lymphocytes, and PMN (Fig. 5), and the number of each cell type was greater than that seen in the nonlethal Gilliam infection of C3H/RV animals. The same pattern of results for both total leukocyte influx and subpopulations of cells was observed in C3H/HeDub mice infected with the Gilliam or
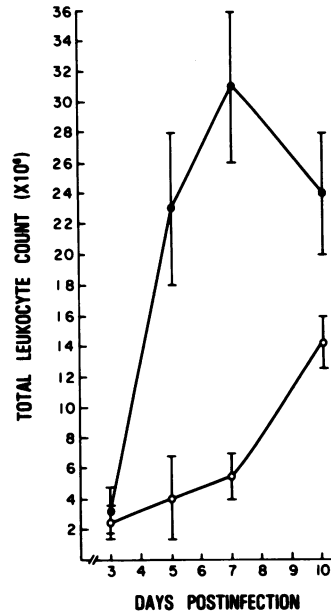

FIG. 4. *Kinetics of cellular influx in C3H/RV mice infected i.p. with 1,000 MLD₅₀ R. tsutsugamushi strain Karp (●) or R. tsutsugamushi strain Gilliam (○). Each point represents the mean total cell count ± 1 standard deviation of five animals.*
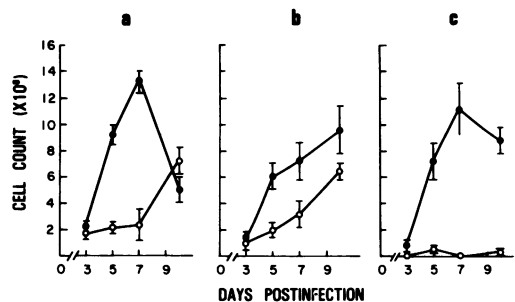

FIG. 5. *Macrophage (a), lymphocyte (b), and PMN (c) counts in C3H/RV mice infected i.p. with 1,000 MLD₅₀ of R. tsutsugamushi strain Karp (●) or R. tsutsugamushi strain Gilliam (○). Each point represents the mean cell count ± 1 standard deviation of five mice. Cell counts were calculated from total cell count and differential count data.*

TA678 strain of scrub typhus rickettsiae (Fig. 6 and 7). The lethal infection initiated by Gilliam in these mice evoked a larger and more rapid cellular influx than did the nonlethal TA678.

Effects of inflammatory and anti-inflammatory agents. It was possible that the magnitude or kinetics of the inflammatory response, or both, modulated the ultimate susceptibility of mice to lethal infection. Therefore, we stimu-

FIG. 6. *Kinetics of cellular influx in C3H/HeDub mice infected i.p. with 1,000 MLD₅₀ R. tsutsugamushi strain Gilliam (●) or 1,000 plaque-forming units of R. tsutsugamushi strain TA678 (○). Each point represents the mean total cell count ± 1 standard deviation of five mice.*

lated a nonspecific inflammation in the peritoneal cavity of C3H/RV mice at various times relative to infection with Gilliam to determine whether this cellular influx would render the animals susceptible to infection. The i.p. administration of thioglycolate before rickettsial challenge did not cause C3H/RV mice to undergo a lethal infection (Table 3). Since the stimulatory activity of this irritant was relatively short-lived, and the response to rickettsial infection was relatively late, we also attempted to induce the inflammatory influx at a more appropriate time relative to rickettsial infection by subsequent administration of thioglycolate on days 3, 5, and 7 postinfection. When thioglycolate was given 3 days postinfection, the net result was a marked reduction of resistance to lethal infection. Thioglycolate given on days 5 and 7 postinfection or 1 day before infection had no effect in terms of lethality. The administration of thioglycolate to uninfected C3H/RV mice (Table 4) produced a short-lived inflammation characterized by an early PMN response (6 h) and a later mononuclear response (48 and 72 h), which agrees with previously published data (18) using nonspecific inflammatory agents.

In the next series of experiments, susceptible mice were treated with indomethacin, an anti-inflammatory agent, to determine whether suppression of the cellular influx would enhance their resistance to rickettsial infection. Animals treated with indomethacin before Gilliam infection and maintained on the drug by incorporating it into their drinking water survived longer than the control animals (Table 5). This differ-
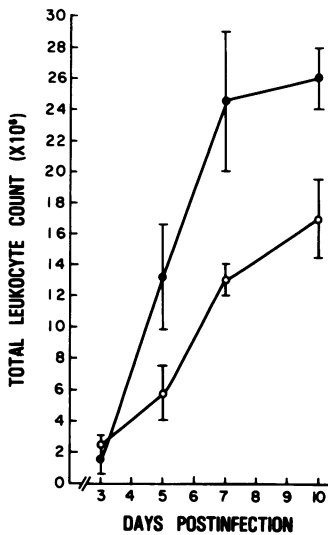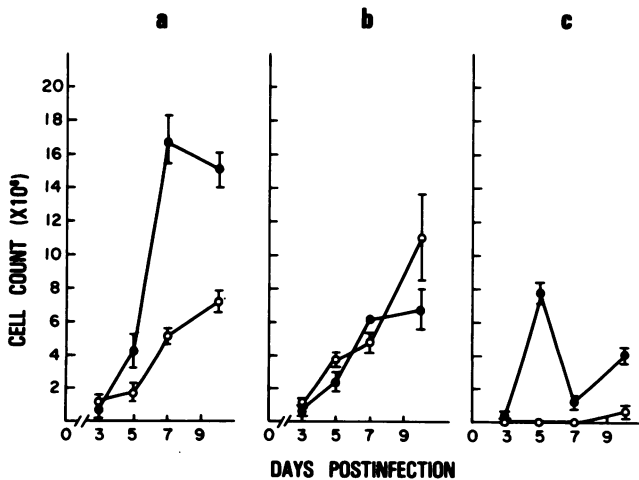

FIG. 7. *Macrophage (a), lymphocyte (b), and PMN (c) counts in C3H/HeDub mice infected i.p. with 1,000 MLD₅₀ R. tsutsugamushi strain Gilliam (●) or 1,000 plaque-forming units of R. tsutsugamushi strain TA678. Each point represents the mean cell count ± 1 standard deviation of five mice. Cell counts were calculated from total cell count and differential data.*

TABLE 3. *Effect of inflammatory agents on the outcome of Gilliam infection in C3H/RV mice*

Stimulant	Treatment day[a]	No. dead/total	Mortality (%)
Phosphate-buffered saline	−1	0/5	0
	0	0/5	0
	+3	0/5	0
	+5	0/5	0
	+7	0/5	0
Thioglycolate	−1	0/5	0
	0	0/5	0
	+3	6/10	60
	+5	0/10	0
	+7	0/10	0

[a] Relative to i.p. infection with 1,000 MLD_{50} of Gilliam.

TABLE 4. *Inflammatory response of C3H/RV mice to thioglycolate[a]*

Time post-inoculation (h)	Total cell count $(\times 10^6)$[b]	Cell type		
		Mφ $(\times 10^6)$[c]	Lymph $(\times 10^6)$	PMN $(\times 10^6)$
0	2.3 ± 0.8	1.1 ± 0.6	1.2 ± 0.2	0
6	5.3 ± 1.0	0.3 ± 0.1	0.9 ± 0.1	4.2 ± 1.2
24	11.3 ± 2.3	0.9 ± 0.3	1.9 ± 0.4	8.5 ± 1.2
48	18.3 ± 1.8	6.8 ± 0.2	6.0 ± 1.2	6.0 ± 0.8
72	11.5 ± 0.8	7.5 ± 0.3	1.2 ± 0.9	2.8 ± 0.6

[a] Each animal was inoculated with 1.0 ml of thioglycolate by the i.p. route.

[b] Mean ± 1 standard deviation of six mice.

[c] Mean number of each cell type ± 1 standard deviation calculated from Giemsa-stained smears and total cell count data.

ence was most apparent at 13 through 16 days after infection. Although all animals treated with indomethacin died, their extended survival time suggested that drug treatment and the accompanying inhibition of the inflammatory response altered the course of Gilliam infection. Indomethacin treatment reduced the cellular influx into the peritoneal cavity of C3H/HeDub mice at days 7 and 10 compared with C3H/HeDub mice treated with the diluent only. The total cell count at 7 days after Gilliam infection of indomethacin-treated mice averaged $7.7 \times 10^6 \pm 0.3$ compared to $10.4 \times 10^6 \pm 0.1$ in vehicle-treated animals. At day 10 the difference was less marked ($12 \times 10^6 \pm 1.1$ versus $14.8 \times 10^6 \pm 0.3$). The most obvious difference in the two groups of animals was seen in the number of PMN at 7 and 10 days postinfection. The indomethacin-treated animals averaged $0.93 \times 10^6 \pm 0.12$ and $0.57 \times 10^6 \pm 0.20$ PMN at days 7 and 10, respectively, compared to $1.44 \times 10^6 \pm 0.20$ and $1.59 \times 10^6 \pm 0.45$ PMN in the vehicle-treated controls.

TABLE 5. *Effect of indomethacin on survival of C3H/HeDub mice infected with Gilliam*

Group	% Survival at days postinfection:							
	10	11	12	13	14	15	16	17
Indomethacin treated (n = 6)	100	100	100	100	67	67	50	0
Control (n = 10)[a]	100	60	25	0	0	0	0	0

[a] Mice treated with ethanol diluted in phosphate-buffered saline.

DISCUSSION

We have examined the inflammatory response in two closely related, histocompatible strains of C3H mice after infection with *R. tsutsugamushi*. C3H/HeDub animals were susceptible to lethal infection with strain Gilliam, whereas C3H/RV mice, possessing the *Ric* gene, were resistant to infection with this strain of scrub typhus rickettsiae.

The inflammatory response of susceptible animals was vigorous and followed the classical pattern of an early PMN response followed by a sustained mononuclear influx. In contrast, the resistant C3H/RV mice were shown to have similar kinetics of total cell influx after infection, but the magnitude of the response was significantly less, and the cellular infiltrate was essentially mononuclear in nature. These patterns of responses seemed to be characteristic of lethal or nonlethal interactions of rickettsiae with the host. Although C3H/RV mice produced a predominantely mononuclear influx after exposure to the nonlethal Gilliam strain, infection of this same strain of mice with the lethal Karp strain elicited a vigorous, early influx of PMN cells into the peritoneal cavity. Similarly, infection of C3H/HeDub mice with the nonlethal TA678 strain produced a mononuclear response typical of a resistant animal, whereas infection with Gilliam strain, which is lethal for these mice, evoked the classical inflammatory response which seems to accompany the initiation of an overwhelming rickettsial infection.

It is possible that the influx of PMN adversely affects the pathogenesis of disease by providing a large population of susceptible cells within·the peritoneal cavity and also serving to transport viable rickettsiae to other sites in the host. The interaction of scrub typhus rickettsiae with mouse PMN has not been carefully studied, but the Gilliam strain is capable of infecting PMN from guinea pigs, and rickettsiae proliferate in the cell cytoplasm after apparently escaping from the phagosome (23). However, other cells resident in the peritoneal cavity are also capable

of supporting rickettsial replication. Mesothelial cells are prominent in the pathology of lethal experimental infection of mice, and strain Karp grows abundantly in these cells after challenge of BALB/c animals (7). In addition, resident peritoneal macrophages from BALB/c mice, cultured in vitro, support the unrestrained growth of *R. tsutsugamushi* strain Gilliam (19). The rickettsiacidal capacity of resident macrophages is realized after interaction with lymphokines, which are thought to activate the macrophage (20). Thus, the presence of PMN in an inflammatory exudate may modulate the pathogenesis of disease by contributing to the population of susceptible cells within the peritoneal cavity. However, it seems unlikely that the lack of PMN in the response of resistant animals to infection is a significant factor in protection, because of the apparent availability of other cell types capable of supporting rickettsial replication.

A nonspecific inflammation was induced in C3H/RV mice with thioglycolate to determine whether the presence of a mixed PMN-mononuclear inflammatory response would render the mice susceptible to Gilliam infection. Our results indicated that the administration of thioglycolate had no influence on the course of infection unless given 3 days after rickettsial challenge. Mice given thioglycolate at other times remained fully competent to prevent lethal infection. Other investigators have reported that administration of thioglycolate influences the course of *Listeria monocytogenes* infection in mice, apparently potentiating lethal infections by alteration of macrophage function and allowing unrestrained bacterial growth within these cells (2). Thus, the effect we observed may be more complex than simply the induction of a cellular influx and perhaps involves a functional alteration of the effector cells, allowing rickettsial replication.

The administration of the anti-inflammatory drug indomethacin in doses previously shown to affect the outcome of microbial infections and tumor growth in mice (18, 24) did prolong survival of susceptible C3H/HeDub mice after infection with Gilliam. Indomethacin has been shown to exert at least part of its anti-inflammatory activity through the inhibition of prostaglandin synthesis by murine peritoneal macrophages (10, 17). Further, it has been demonstrated that these macrophage prostaglandins are potent inhibitors of immune responses (10). In the model system presented in this paper, animals which were shown to be susceptible to lethal Gilliam infection were capable of responding to the infection with the appropriate cell types, i.e., lymphocytes and macrophages, which have been shown to be important in scrub ty-

phus immunity (4, 20, 25). Taken together, the data presented suggest that the defect seen in susceptible animals is not a lack of effector cells at the site of infection, but rather a qualitative change in these cells rendering them noneffective in antirickettsial immunity. Temporary reversal of this defect by indomethacin may be related to a decrease in the influx of cells capable of replicating rickettsiae, but may also be due to its reversal of immunosuppression mediated by prostaglandins. Alternatively, Hanson et al. (12) have shown that cells from C3H/RV mice, which are also resistant to arbo virus group B infection, are more sensitive to interferon treatment than cells from susceptible C3H mice and suggest this as a possible mechanism for resistance to viral infection. It is possible that similar nonspecific mechanisms are operative in our system and responsible for an early restriction of rickettsial replication in C3H/RV mice. This mechanism may be deficient or lacking in C3H/HeDub mice, thus allowing unrestrained replication of rickettsiae which is, in turn, responsible for the observed inflammatory response. Studies in progress addressing these possibilities should help to elucidate the mechanism(s) of action of the *Ric* gene.

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