

Effect of Sublethal Gamma Radiation on Host Defenses in Experimental Scrub Typhus

DARYL J. KELLY¹* AND JOHN C. REES²‡

Department of Rickettsial Diseases, Walter Reed Army Institute of Research, Washington, D.C. 20307¹; and Department of Biology, Catholic University of America, Washington, D.C. 20010²

Received 3 February 1986/Accepted 21 February 1986

The effect of sublethal gamma radiation on inbred mice chronically infected with scrub typhus rickettsiae was examined. Inbred mice which were inoculated with the Gilliam or Karp strain of *Rickettsia tsutsugamushi* by the subcutaneous route harbored the infection for at least 1 year. Irradiation of these animals at 12 or 52 weeks postinoculation with normally sublethal levels induced a significantly higher percentage of rickettsemic mice (recrudescence) than was seen in the unirradiated, similarly infected control animals. In addition, sublethal irradiation at 12 weeks induced a quantitative increase in total rickettsiae. Homologous antibody titers to the rickettsiae were examined for 5 weeks after irradiation to determine the role of the humoral response in radiation-induced recrudescence. Unirradiated, infected mice showed consistent titers of about 320 throughout the 5-week observation period, and the titer was not affected by exposure of up to 500 rads of gamma radiation. Drug dose-dependent radioprotection and modification of recrudescence was noted in infected, irradiated mice treated with the antiradiation compound S-2-(3-aminopropylamino)ethyl phosphorothioic acid. The results of this investigation supported the conclusion that the recrudescence of a chronic rickettsial infection in the appropriate host after immunological impairment due to gamma radiation can result in an acute, possibly lethal rickettsemia.

Recrudescence of rickettsial infection was first observed in epidemic typhus patients and occurred many years after their primary infection with *Rickettsia prowazekii*. This form of epidemic typhus occurs in the absence of a vector and is known as recrudescence typhus or Brill-Zinsser disease (4). Alterations in the physiological or immunological status of the aging host may play a role in the recrudescence of disease, but a specific mechanism has not been described. This spontaneous recurrence of infection in humans has not been observed in other rickettsial diseases, but there are data to document that *Rickettsia rickettsii* (18) and *R. tsutsugamushi* (23) are harbored in the tissues of humans or laboratory rodents for over a year after primary infection.

Mice experimentally infected with scrub typhus have been induced to recrudescence up to 1 year after infection by impairing immune function with cyclophosphamide (21). This evidence suggests that generalized immunological impairment, which might occur due to exposure to sublethal radiation, may induce recrudescence in chronically infected humans or those immunized with living agents.

It has long been recognized that variations in virulence can be related to the strain of *R. tsutsugamushi* (9-11), the route of inoculation (10), and the genetic susceptibility of the host (9, 16). The mouse scrub typhus model used in this investigation allowed both a thorough examination of radiation-induced rickettsial recrudescence and an examination of the effect of various virulence determinants which bear upon that recrudescence.

The purpose of this study was to examine the effect of gamma radiation on the recrudescence of experimental scrub typhus in mice convalescing from infection with *R. tsu-*

tsugamushi. The parameters selected to examine recrudescence were the temporal onset of rickettsemia, the quantification of circulating rickettsiae, the influence of the humoral response, and the sparing effect of radioprotective drugs.

MATERIALS AND METHODS

Animals. Female BALB/cDub and C3H/HeDub mice were obtained from Flow Laboratories Inc. (Dublin, Va.) and used at an age of 8 to 12 weeks.

Rickettsiae. The Karp (52nd egg passage), and Gilliam (164th egg passage) strains of *R. tsutsugamushi* were plaque purified (17), propagated, stored, and quantified as previously reported (5).

Animal infection. Mice were infected with either the Karp or Gilliam strain of *R. tsutsugamushi* by a single subcutaneous (s.c.) inoculation of 0.2 ml of rickettsial suspension containing 1,000 intraperitoneal (i.p.) 50% mouse lethal doses (MLD₅₀). Previous studies have demonstrated this route of inoculation to be immunizing and nonlethal in mice (22).

Animal irradiation. Mice were exposed to various doses of radiation at 12 weeks or 52 weeks after rickettsial infection by using ¹³⁷Cs radiation (Gammacell 40; Atomic Energy of Canada Ltd., Ottawa, Canada) at a rate of 123 rads per min. Normal and infected mice were observed for 30 days, and the radiation MLD₅₀ was determined by the method of Spearman and Karber (7).

Qualitative evaluation of rickettsemia. At various times after radiation, infected mice were bled from the retroorbital venous plexus with a heparinized pipette. Samples of blood (0.2 ml) were inoculated i.p. into naive susceptible mice. This route of administration can initiate either an immunizing or lethal infection depending on the number of rickettsiae in the challenge inoculum (11). To show whether an immunizing, nonlethal dose was present in the inoculum, recipient animals which survived 28 days after the blood challenge were rechallenged with 1,000 MLD₅₀ of the Karp strain by

* Corresponding author.

† Present address: U.S. Army Medical Research Unit, Institute for Medical Research, Kuala Lumpur, Malaysia.

‡ Present address: Department of Pathology, Washington Hospital Center, Washington, D.C. 20010.

TABLE 1. Effect of gamma radiation on survival of inbred mice infected with *R. tsutsugamushi*

Mouse strain	<i>R. tsutsugamushi</i> strain	No. of 30-day survivors (<i>n</i> = 10) after the following gamma radiation dose (rads):						Radiation MLD ₅₀ ^a	
		450	500	550	600	650	700		800
BALB/cDub	Uninfected control	10	9	10	8	0	0	0	604 (587–623)
C3H/HeDub	Uninfected control	10	10	10	10	3	4	0	653 (633–673)
BALB/cDub	Karp	8	10	3	2	0	0	0	532 (509–556)
BALB/cDub	Gilliam	10	10	3	0	0	1	0	541 (525–558)
C3H/HeDub	Gilliam	10	3	1	1	0	1	0	505 (483–529)

^a Determined by Spearman-Kärber method (7); 95% confidence intervals are shown in parentheses.

the i.p. route (see above). Those mice whose blood induced either death or protection in recipients as noted above were considered to be rickettsemic at the time they were bled. To establish that rickettsiae were responsible for death in recipient animals, a control study was performed in which previously immunized mice which demonstrated specific antibody titers (see below) also received the challenge inoculum. These specifically immune mice did not succumb to infection.

Quantification of rickettsemia. Infected mice were anesthetized with CO₂, partially exsanguinated with heparinized pipettes after incision of the right axillary artery, and then killed by cervical dislocation. Mouse blood was pooled, and 10-fold serial dilutions were injected i.p. into naive mice, five mice per dilution, which were monitored for 28 days. Surviving mice were rechallenged with 1,000 MLD₅₀ of Karp strain i.p. and monitored again for 28 days (see above). The 50% mouse infective dose was determined by using the Spearman-Kärber method of analysis for quantal responses (7). The control consisted of 50% mouse infective dose determination for unirradiated, infected mice.

Modification of recrudescence with radioprotective drugs. The drug S-2(3-aminopropylamino)ethyl phosphorothioic acid was used to determine whether radiation-induced rickettsemia could be modified. Due to the extensive experimentation conducted at Walter Reed Army Institute of Research with this phosphorothioate, it has been identified commonly in the literature as WR 2721. For reader convenience, that nomenclature is also used in this study.

All inoculations of WR 2721 were administered to BALB/cDub and C3H/HeDub mice in a volume of 0.2 ml by the i.p. route. The drug dose was varied, when appropriate, by diluting WR 2721 in distilled water. The drug was prepared fresh each time, 15 to 30 min before inoculation. Normal and infected mice were exposed to various doses of gamma radiation within 30 min after receiving the WR 2721. The survival and percentage of mice demonstrating rickettsemia were monitored as described above.

Influence of the humoral response. Circulating antibody in infected mice was quantified periodically after irradiation. Titers were compared to those observed in similar infected, but unirradiated, animals. Infected mice were bled from the retroorbital venous plexus at various times after irradiation, and sera were pooled and frozen. Rickettsial antibody titers were determined by a microimmunofluorescence test (19). Known positive and negative mouse sera were tested simultaneously as controls.

RESULTS

Mouse scrub typhus model rationale. The mouse scrub typhus models used in this investigation included BALB/cDub mice inoculated s.c. with the Karp strain of *R.*

tsutsugamushi, BALB/cDub mice inoculated s.c. with the Gilliam strain, and C3H/HeDub mice inoculated s.c. with Gilliam strain. These models permitted the comparison of different virulence determinants in this examination of rickettsial recrudescence. The determinants consisted of (i) genetically determined variation in susceptibility of mouse strains to scrub typhus, i.e., BALB/cDub versus C3H/HeDub, and (ii) strain variation of the rickettsiae, i.e., Karp versus Gilliam. Since the i.p. inoculation of either mouse strain with Karp was uniformly lethal, the i.p. versus s.c. routes of inoculation were not compared in chronically infected mice.

Although these determinants have long been individually recognized (3, 9–11, 16), there has not yet been a comparative examination of interrelationships of the individual parameters. Whereas the i.p. inoculation of either mouse strain with 1,000 MLD₅₀ of the Karp strain of *R. tsutsugamushi* was uniformly lethal for those mice, the s.c. inoculation with the same quantity of rickettsiae rendered both mouse strains immune to subsequent i.p. challenge. However, although the i.p. inoculation of the Gilliam strain of *R. tsutsugamushi* was lethal for C3H/HeDub mice, the similar i.p. inoculation of Gilliam rickettsiae into BALB/cDub mice was not lethal but immunized against the normally lethal Karp i.p. challenge.

Effect of radiation on normal and infected mice. The MLD₅₀ of gamma radiation for normal BALB/cDub mice was 604 rads, this was significantly lower than for normal C3H/HeDub mice, which was 653 rads (Table 1) ($P < 0.05$). Mice which had been infected with *R. tsutsugamushi* Karp or Gilliam 12 weeks before radiation exposure died from lower radiation doses than did normal animals. The radiation MLD₅₀ of BALB/cDub mice infected with strain Karp was 532 rads, and similar animals infected with strain Gilliam had a radiation MLD₅₀ of 541 rads. The difference in the effect of radiation on normal and infected animals was even more pronounced in C3H/HeDub mice inoculated with Gilliam. In this case, the radiation MLD₅₀ was substantially diminished and infected animals succumbed after receiving only 505 rads. In further studies the radiation levels used for qualitative and quantitative determinations of rickettsemia were 450 and 500 rads. Those levels proved low enough to have sufficient survivors for the rickettsemia investigations but proved sufficient to induce a sublethal rickettsemia.

Qualitative evaluation of recrudescence. To determine whether sublethal radiation of mice resulted in measured proliferation of rickettsiae in any or all of the mouse scrub typhus systems investigated, whole blood from these animals was examined for the presence of virulent organisms. There was a dramatic increase in the number of BALB/cDub mice undergoing a Karp rickettsemia after exposure to 450 or 500 rads (Fig. 1). This effect was most pronounced 14 days after radiation, but by day 35 the percentage of animals

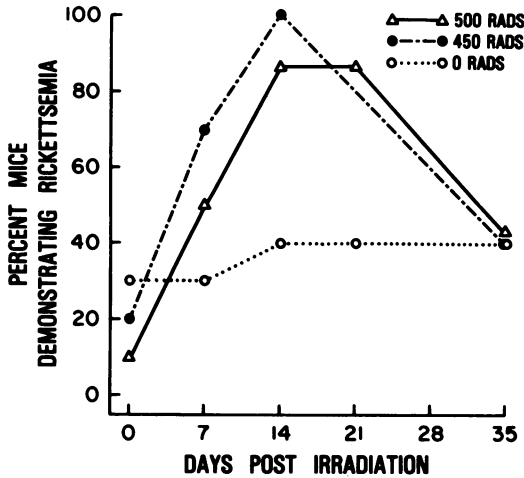


FIG. 1. Qualitative determination of rickettsemia in BALB/cDub mice chronically infected s.c. with 1,000 MLD₅₀ of the Karp strain of *R. tsutsugamushi* 12 weeks before irradiation; n = 10.

exhibiting a rickettsemia had returned to normal values. The effect of irradiation on rickettsemia in Gilliam-infected C3H/HeDub mice was similar to that observed in Karp-infected BALB/cDub animals (Fig. 2). At 14 days after radiation, at least 90% of the mice exhibited virulent Gilliam organisms in the blood. By day 35, the effect of radiation was no longer evident, and only a small percentage of animals showed rickettsemia. In contrast to the BALB/cDub-Karp model system, the C3H/HeDub mice infected with Gilliam demonstrated rickettsemia in a greater percentage of the animals before irradiation. The results obtained with the BALB/cDub-Gilliam model (Fig. 3) indicate that gamma radiation in the range of 450 to 500 rads has little effect on Gilliam rickettsiae. The percentage of unirradiated animals with circulating Gilliam rickettsiae was lower than in the two other models, and exposure to radiation did not cause a substantial increase in the percentage of animals showing rickettsemia through day 35.

We concluded it possible that radiation-induced recu-

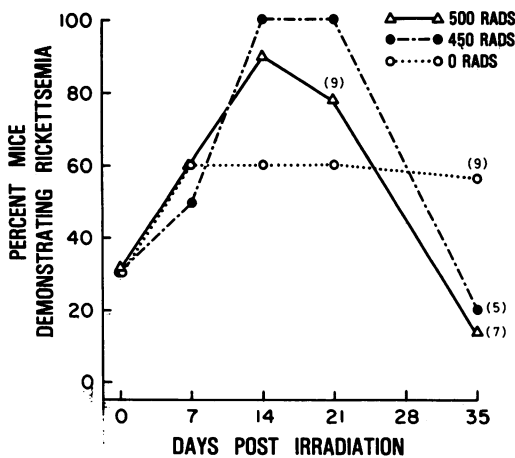


FIG. 2. Qualitative determination of rickettsemia in C3H/HeDub mice chronically infected s.c. with 1,000 MLD₅₀ of the Gilliam strain of *R. tsutsugamushi* 12 weeks before irradiation; n = 10 except where noted in parentheses.

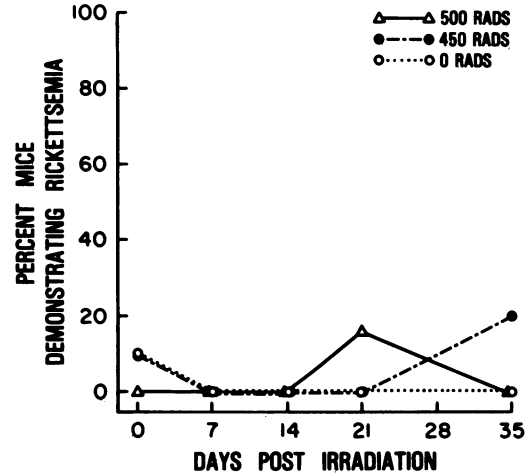


FIG. 3. Qualitative determination of rickettsemia in BALB/cDub mice chronically infected s.c. with 1,000 MLD₅₀ of the Gilliam strain of *R. tsutsugamushi* 12 weeks before irradiation; n = 10.

descent is dependent upon the interval between initial infection and exposure to sublethal gamma radiation. To investigate radiation effects on long-term chronic infection, Karp-infected BALB/cDub mice were exposed to 450 rads 1 year after initial infection. Radiation exposure caused all of these animals to exhibit rickettsemia within 14 days (Fig. 4). Unlike the animals irradiated 12 weeks after infection, surviving mice remained rickettsemic through the 35-day observation period.

Quantification of rickettsemia. The qualitative evaluation of rickettsemia indicated clearly that gamma radiation induced rickettsemia in chronically infected animals. To more fully investigate the degree of rickettsemia, we quantified the virulent organisms circulating in the blood after various doses of radiation. This investigation included the BALB/cDub-Karp and C3H/HeDub-Gilliam model systems, since measurable differences in these two models were noted in the qualitative evaluation of rickettsemia. The

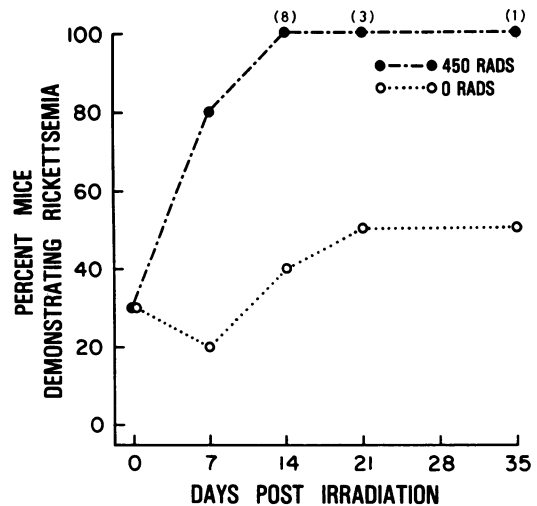


FIG. 4. Qualitative determination of rickettsemia in BALB/cDub mice chronically infected s.c. with 1,000 MLD₅₀ of the Karp strain of *R. tsutsugamushi* 52 weeks before irradiation; n = 10 except where noted in parentheses.

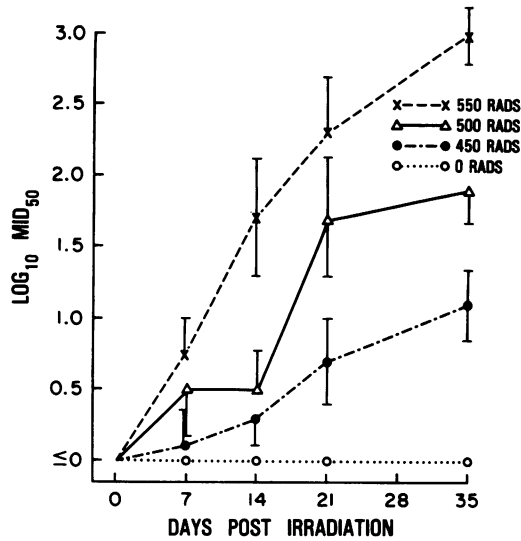


FIG. 5. Quantitative determination of rickettsemia in BALB/cDub mice chronically infected s.c. with 1,000 MLD₅₀ of the Karp strain of *R. tsutsugamushi* 12 weeks before irradiation. Each point represents the mean 50% mouse infective dose (MID₅₀) per 0.2 ml of pooled whole blood obtained from five mice ± standard deviation by using Spearman-Kärber method (7).

BALB/cDub-Gilliam model was excluded, however, since the qualitative data (Fig. 3) indicated minimal rickettsemia. Figure 5 shows the level of Karp rickettsemia in BALB/cDub mice after exposure to 450, 500, or 550 rads of gamma radiation. The number of organisms in the blood was related to the radiation dose, and at approximately the radiation MLD₅₀ for infected BALB/cDub mice (532 rads) rickettsiae were detected at a concentration of 10³ organisms per ml. In these experiments, control infected animals had very low levels of circulating rickettsiae (0 to 2 infected whole blood recipients per time point; MID₅₀ < 0 by the Spearman-Kärber method), and the radiation effect was interpreted easily. However, our experience from qualitative rickettsemia studies suggested that about 25% of nonirradiated, infected BALB/cDub mice normally sustained detect-

able levels of virulent Karp organisms in the blood (day 0 in Fig. 1).

Infection of C3H/HeDub mice with Gilliam produced a pattern of rickettsemia very similar to that observed with Karp-infected BALB/cDub mice (data not shown). However, the response observed with 500 rads was nearly identical to that seen at 550 rads. Both radiation doses produced a Gilliam rickettsemia which exceeded 10^{2.5} virulent organisms per ml of whole blood. The radiation MLD₅₀ of infected C3H/HeDub mice was 505 rads, and doses in excess of this had little further effect on induction of rickettsemia.

Humoral antibody. Quantification of circulating antibody in infected mice was accomplished periodically after irradiation. Titers were compared with those observed in similar infected, but unirradiated, animals. Circulating antibody was produced by infected animals before irradiation, since titers remained stable in both irradiated and unirradiated animals, but subsequent *in vivo* events which permitted high levels of virulent rickettsiae to circulate in the blood in the presence of specific antibody were uncertain. Table 2 summarizes the indirect fluorescent antibody titers observed in Karp-infected BALB/cDub mice and Gilliam-infected C3H/HeDub mice. There was little variation in the titers observed in the BALB/cDub-Karp model system. Unirradiated, infected animals showed a consistent titer of approximately 320 throughout the observation period, and the titer was not affected by exposure of animals to 450 or 500 rads of gamma radiation. A similar phenomenon was observed in C3H/HeDub mice infected with Gilliam. It is clear that in both model systems there was substantial antibody present in the blood on days 14 and 21, which represented the period of maximum rickettsemia in irradiated animals.

Effect of radioprotective drug. The antiradiation compound WR 2721 increased the radiation MLD₅₀ in C3H/HeDub mice. Protection was dose dependent; and at the largest dose employed, 500 mg/kg, all animals tested successfully resisted 800 rads of gamma radiation (Table 3). The upper limit of radiation protection was not determined, but even these results present a dramatic difference from those observed in untreated C3H/HeDub animals, where uniform lethality was observed after exposure of mice to 750 or 800 rads (Table 1). A similar dose-dependent, radioprotective effect was found in BALB/cDub mice. All uninfected BALB/cDub animals receiving 500 mg/kg survived the 800-rad dose of gamma radiation, whereas all untreated mice succumbed to that radiation dose.

TABLE 2. Homologous immunofluorescence assay antibody titers in infected mice after gamma irradiation

Mouse strain	<i>R. tsutsugamushi</i> strain	Days post-irradiation	Titer after the following gamma radiation dose (rads) ^a :		
			0	450	500
BALB/cDub	Karp	0	640	640	320
		7	320	320	320
		14	320	320	320
		21	320	320	320
		35	320	640	160
C3H/HeDub	Gilliam	0	640	640	320
		7	160	640	320
		14	320	640	320
		21	320	320	320
		35	160	640	320

^a Radiation was administered to mice 12 weeks after infection. Titers are shown as the reciprocals of the greatest serum dilutions demonstrating fluorescence with the homologous antigen; n = 5.

TABLE 3. Effect of radioprotective drug WR 2721 on survival of mice after exposure to 800 rads of gamma irradiation

Mouse strain	Drug dose (mg/kg)	No. of survivors/total (% surviving) after 30 days	
		Uninfected mice	Infected mice ^a
BALB/cDub	0	0/10 (0)	0/15 (0)
	125	4/15 (26.6)	0/15 (0)
	250	13/14 (92.9)	14/15 (93.3)
	500	14/14 (100)	12/14 (85.5)
C3H/HeDub	0	0/15 (0)	0/15 (0)
	125	6/15 (40)	1/15 (6.7)
	250	15/15 (100)	13/15 (86.7)
	500	15/15 (100)	15/15 (100)

^a BALB/c mice chronically infected with *R. tsutsugamushi* Karp; C3H/HeDub mice chronically infected with *R. tsutsugamushi* Gilliam.

The sparing effect seen in uninfected animals was paralleled in Gilliam-infected C3H/HeDub mice. As might be expected, the magnitude of the sparing effect at each intermediate drug dose was less for chronically infected animals than for uninfected mice. At the maximum dose of WR 2721, Gilliam-infected C3H/HeDub mice were sufficiently protected from gamma radiation so that no deaths were observed after exposure of chronically infected mice to 800 rads, whereas the Karp-infected BALB/cDub animals receiving the same drug dose were less protected.

Occurrence of rickettsemia after prophylaxis with WR 2721. WR 2721 protected most infected mice from a radiation-induced lethal recrudescence of rickettsiae. It was anticipated that this protection might be mirrored by a qualitative reduction in the number of mice experiencing rickettsemia after radiation exposure. Figure 6 suggests that suppression of rickettsemia is a correlate of drug protection. The entire population of untreated Gilliam-infected C3H/HeDub mice quickly evidenced rickettsemia after exposure to 800 rads of gamma radiation, and by day 14 all had died. Those receiving 500 mg of WR 2721 per kg showed a modest increase in rickettsemia and maintained a level of chronicity seen earlier in infected, but unirradiated and untreated, animals (Fig. 2). A large percentage of animals receiving the intermediate dosage (250 mg/kg) became rickettsemic by day 14, but drug protection was apparently sufficient for animals to control recrudescence, and the number of mice evidencing virulent rickettsiae in their blood returned to the normal, chronic level by day 35.

Similar results were noted in Karp-infected BALB/cDub mice (Fig. 7). All untreated animals died by day 14; therefore, rickettsemia could not be monitored beyond day 7, at which time the percent rickettsemia had risen substantially from the chronic level. The increase in rickettsemia after treatment with 500 mg of WR 2721 per kg was slightly higher than in the C3H/HeDub-Gilliam system. Also similar to that system, BALB/cDub mice receiving the 250-mg/kg intermediate dose peaked by day 14 but gradually diminished by day 35.

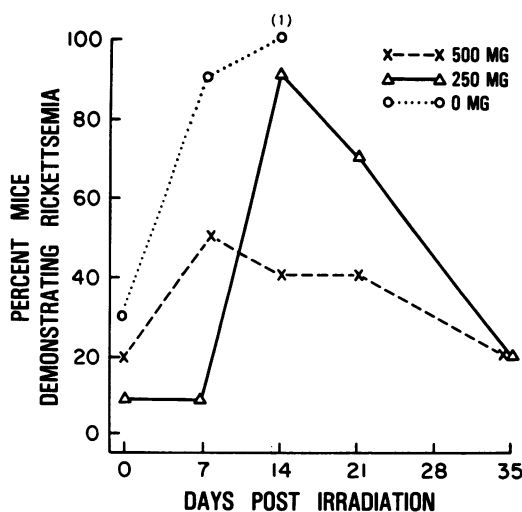


FIG. 6. Qualitative determination of rickettsemia in C3H/HeDub mice chronically infected s.c. with 1,000 MLD₅₀ of the Gilliam strain *R. tsutsugamushi* 12 weeks prior to irradiation. Mice received various doses of the radioprotective drug WR 2721 followed by 800 rads of gamma radiation; $n = 10$ except where noted in parentheses.

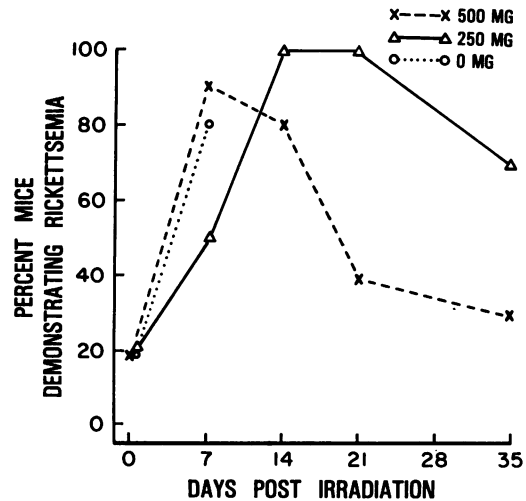


FIG. 7. Qualitative determination of rickettsemia in BALB/cDub mice chronically infected s.c. with 1,000 MLD₅₀ of the Karp strain of *R. tsutsugamushi* 12 weeks before irradiation. Mice received various doses of the radio-protective drug WR 2721 followed by 800 rads of gamma radiation; $n = 10$.

DISCUSSION

The data presented here clearly indicate that mice chronically infected with viable *R. tsutsugamushi* were induced by radiation to demonstrate a recrudescence of infection as evidenced by rickettsemia. This radiation-induced rickettsemia was observed up to 1 year after the initial infection and was lethal to animals irradiated at normally sublethal levels. The rickettsial recrudescence was evidenced by a substantially greater percentage of rickettsemic mice during the period after irradiation as well as by a quantal increase of rickettsiae.

Previous experiments from this laboratory have focused upon the effect of virulence determinants on acute and chronic scrub typhus infection through the examination of magnitude and duration of rickettsemia (14). It was not clear whether those parameters were responsive to some mechanism of immune interdiction such as gamma irradiation. There is little published work which examines the effects of irradiation of animals which are chronically infected with rickettsiae. Zinsser, who distinguished epidemic typhus from the recrudescence form of that disease (26), X irradiated rabbits and rats so as to affect the resistance of the animals while permitting them to live long enough for adequate multiplication of subsequently inoculated rickettsiae (27). Susceptibility of X-irradiated mice and guinea pigs to the attenuated E strain of *R. prowazekii* has been examined (I. B. Fabrikant, Ph.D. thesis, University of Maryland, 1966). In that study it was reported that sublethal doses of whole body X irradiation only slightly enhanced guinea pig susceptibility to infection with the strain.

The increased susceptibility of infected animals to gamma radiation could have resulted from an impairment of host physiological processes due to the rickettsial infection. However, mice sustain chronic experimental scrub typhus infections for periods in excess of 12 months (8), and subjective appraisal indicates no overt decline in the health and vitality of chronically infected animals. It seemed more likely to us that the apparent decrease in radiation MLD₅₀ in infected mice (Table 1) is due to reduced immune function

caused by sublethal irradiation and resulting in recrudescence of the chronic rickettsial infections.

The *in vivo* target in animals subjected to sublethal irradiation appears to be the immune system (2). The lymphocyte is one of the most radiosensitive of mammalian cells (2, 15). Radiation-induced depletion of lymphocytes *in vivo* is dose dependent up to the point of lethal irradiation; B cells are more radiosensitive than T cells (2). Indeed, our studies show a radiation dose-dependent quantal response of circulating rickettsiae (Fig. 5), although a precise correlation has not been proven.

In the course of this study specific circulating antibody to *R. tsutsugamushi* was present in elevated titers (≥ 160); however, the presence of antibody failed to protect the animals, since mice demonstrating antibody were rickettsemic after irradiation and frequently died with normally sublethal levels of radiation. The prolonged presence of elevated antibody levels in mice chronically infected with *R. tsutsugamushi* is consistent with the results of others (M. G. Groves, unpublished data), but the precise role of antibody in limiting rickettsial replication during infection is unknown. Shirai et al. (20) indicated that the passive transfer of sera afforded protection against acute homologous scrub infection and hypothesized that antibody may be necessary to preclude recrudescence of the disease after infection. However, acquired resistance to infection can be transferred with T cell-enriched lymphocyte populations alone before the development of detectable antibody (20). Jerrells and Eisemann have shown that T-cell function is necessary even in the presence of specific antibody (12). Only when T-cell function for resistance was restored did "nude" BALB/c mice that were protected with specific antisera survive an i.p. challenge of 1,000 MLD₅₀ of Karp rickettsiae (13).

WR 2721 was used to determine whether radiation-induced rickettsemia could be modified. The proposed mechanism of action of this phosphorothioate compound appears related to an ability to scavenge radiation-induced free radicals (1). Generally, the cause of damage within the immune system is molecular oxygen induced to the free radical state, which potentiates many of the radiation effects. This compound has been demonstrated by Davidson et al. to protect mice, dogs, and rhesus monkeys against gamma and X irradiation (6). Yuhas (24, 25) has demonstrated that the drug protects a variety of normal tissues, whereas solid animal tumors are not protected. Employing a dose of 600 mg/kg, Davidson et al. (6) demonstrated the drug would protect ICR mice against 825 rads (X ray) for 3 h after inoculation. In our studies this drug successfully protected both BALB/cDub and C3H/HeDub mice from normally lethal levels of radiation, although the chronically infected BALB/cDub mice were less protected. It seems possible that rickettsial recrudescence in these infected animals adds to their physiological stress and causes greater numbers of deaths than in the uninfected animals receiving the same doses of radiation and drug. In addition to the reduced lethality, drug-treated, infected animals were qualitatively less rickettsemic than were untreated, chronically infected mice. WR 2721 clearly modified radiation-induced lethality and rickettsial recrudescence in the model system studied.

The results of this study emphasize the role of differential host susceptibility in radiation-induced rickettsial recrudescence. BALB/cDub and C3H/HeDub mice chronically infected with Karp and Gilliam strains of *R. tsutsugamushi*, which are virulent for the respective inbred mouse strains when inoculated by the i.p. route, recrudescence after irradiation. In contrast, BALB/c mice chronically infected with the

Gilliam strain, which is avirulent for that inbred mouse strain, fail to recrudescence after irradiation. The precise mechanism defining differential susceptibility to recrudescence remains undetermined. Clearly further evaluation of rickettsial recrudescence must be defined within the parameters of genetic background of the host.

ACKNOWLEDGMENTS

We thank Joseph V. Osterman for his invaluable assistance and advice throughout the course of this study and David Davidson and Marie Grenan for their suggestions and for providing the WR 2721. We also thank Thomas Jerrells, George E. Lewis, Jr., and David R. Franz for their critical review of the manuscript.

LITERATURE CITED

- Alexander, P., Z. M. Bacq, S. F. Cousins, M. Fox, A. Herve, and J. Lazar. 1955. Mode of action of some substances which protect against the lethal effect of x-rays. *Radiat. Res.* 2:392-415.
- Anderson, R. E., and N. L. Warner. 1976. Ionizing radiation and the immune response. *Adv. Immunol.* 24:215-323.
- Blake, F. G., K. F. Maxey, J. F. Sadusk, Jr., G. M. Kohls, and E. J. Bell. 1945. Studies on tsutsugamushi disease (scrub typhus, mite-borne typhus) in New Guinea and adjacent islands: epidemiology, clinical observations, and etiology in the Dobadura area. *Am. J. Hyg.* 41:243-373.
- Brill, N. E. 1910. An acute infectious disease of unknown origin. *Am. J. Med. Sci.* 139:484-502.
- Catanzaro, P. J., A. Shirai, P. K. Hildebrandt, and J. V. Osterman. 1976. Host defenses in experimental scrub typhus: histopathological correlates. *Infect. Immun.* 13:861-875.
- Davidson, D. E., M. M. Grenan, and T. R. Sweeney. 1980. Biological characteristics of some improved radioprotectors, p. 309-320. *In* L. W. Brady (ed.), *Radiation sensitizers: their use in the clinical management of cancer*. Masson Publishing U.S.A., Inc., New York.
- Finney, D. J. 1964. Alternate methods of analysis for quantal responses, p. 524. *In* *Statistical method in biological assay*, 2nd ed. Hafner Publishing Co., New York.
- Fox, J. P. 1948. The long persistence of *Rickettsia orientalis* in the blood and tissues of infected animals. *J. Immunol.* 59:109-114.
- Groves, M. G., and J. V. Osterman. 1978. Host defenses in experimental scrub typhus: genetics of natural resistance to infection. *Infect. Immun.* 19:583-588.
- Irons, E. N., and H. E. Armstrong. 1947. Scrub typhus in Dutch New Guinea. *Ann. Intern. Med.* 26:201-220.
- Jackson, E. B., and J. E. Smadel. 1951. Immunization against scrub typhus. II. Preparation of lyophilized living vaccine. *Am. J. Hyg.* 53:326-331.
- Jerrells, T. R., and C. S. Eisemann. 1983. Role of T lymphocytes in production of antibody to antigens of *Rickettsia tsutsugamushi* and other *Rickettsia* species. *Infect. Immun.* 41:666-674.
- Jerrells, T. R., B. A. Palmer, and J. G. MacMillan. 1984. Cellular mechanisms of innate and acquired immunity to *Rickettsia tsutsugamushi*, p. 277-281. *In* L. Leive and D. Schlesinger (ed.), *Microbiology—1984*. American Society for Microbiology, Washington, D.C.
- Jerrells, T. R., B. A. Palmer, and J. V. Osterman. 1983. Gamma-irradiated scrub typhus immunogens: development of cell-mediated immunity after vaccination of inbred mice. *Infect. Immun.* 39:262-269.
- Kataoka, Y., and T. Sado. 1975. The radiosensitivity of T and B lymphocytes in mice. *Immunology* 29:121-130.
- Kekcheeva, N. G., and I. N. Kokorin. 1976. Different allotypic susceptibility of mice to *Rickettsia tsutsugamushi*. *Acta Virol.* 20:142-146.
- Oaks, S. C., Jr., J. V. Osterman, and F. M. Hetrick. 1977. Plaque assay and cloning of scrub typhus rickettsiae in irradiated L-929 cells. *J. Clin. Microbiol.* 6:76-80.

18. **Parker, R. T., P. G. Menon, A. M. Merideth, M. J. Snyder, and T. E. Woodward.** 1954. Persistence of *Rickettsia rickettsii* in a patient recovered from Rocky Mountain spotted fever. *J. Immunol.* **73**:383-386.
19. **Phillip, R. N., E. A. Casper, R. A. Ormsbee, M. G. Peacock, and W. Burgdorfer.** 1976. Microimmunofluorescence test for the serological study of Rocky Mountain spotted fever and typhus. *J. Clin. Microbiol.* **3**:51-61.
20. **Shirai, A., P. J. Catanzaro, S. M. Phillips, and J. V. Osterman.** 1976. Host defenses in experimental scrub typhus: role of cellular immunity in heterologous protection. *Infect. Immun.* **14**:39-46.
21. **Shirai, A., T. C. Chan, E. Gan, and D. L. Huxsoll.** 1979. Persistence and reactivation of *Rickettsia tsutsugamushi* infections in laboratory mice. *Jpn. J. Med. Sci. Biol.* **32**:179-184.
22. **Shishido, A., M. Ohtawara, S. Tateno, S. Mizuno, M. Ogura, and M. Kitaoka.** 1958. The nature of immunity against scrub typhus in mice. I. The resistance of mice, surviving subcutaneous infection of scrub typhus rickettsia, to intraperitoneal reinfection of the same agent. *Jpn. J. Med. Sci. Biol.* **11**:383-399.
23. **Smadel, J. E., H. L. Ley, F. H. Dierks, and J. A. P. Cameron.** 1952. Persistence of *Rickettsia tsutsugamushi* in tissues of patients recovered from scrub typhus. *Am. J. Hyg.* **56**:294-302.
24. **Yuhus, J. M.** 1972. Improvement of lung tumor radiotherapy through differential chemoprotection of normal and malignant tissues. *J. Natl. Cancer Inst.* **48**:1255-1257.
25. **Yuhus, J. M.** 1973. Radiotherapy of experimental lung tumors in the presence and absence of a radioprotective drug, S-2(3-aminopropylamino)ethyl phosphorothioic acid (WR 2721). *J. Natl. Cancer Inst.* **50**:69-78.
26. **Zinsser, H.** 1934. Varieties of typhus virus and epidemiology of the American form of European typhus fever (Brill's disease). *Am. J. Hyg.* **20**:513-532.
27. **Zinsser, H., and M. R. Castaneda.** 1932. A method of obtaining large amounts of *Rickettsia provaceki* by X-ray radiation of rats. *Proc. Soc. Exp. Biol. Med.* **29**:840-844.