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Experimental Scrub Typhus Immunogens: Gamma-Irradiated and Formalinized Rickettsiae

GEORGE H. G. EISENBERG, JR., AND JOSEPH V. OSTERMAN*

Department of Hazardous Microorganisms, Walter Reed Army Institute of Research, Washington, D.C. 20012

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Scrub typhus immunogens were prepared by exposing infected yolk sac suspensions of *Rickettsia tsutsugamushi* to various doses of gamma radiation. Mouse lethality was abolished at doses greater than 200 krads, whereas immunogenicity of the suspensions, as shown by mouse protection tests, was diminished relatively little by radiation doses in the 200- to 400-krad range. Using a 300-krad gamma dose to provide a safety factor, immunogens were prepared and their protective capacity was contrasted with formalinized scrub typhus immunogens prepared by conventional techniques. Formalinized suspensions afforded mice only partial protection against intraperitoneal challenge with 1,000 50% mouse lethal doses of the virulent homologous strain and no significant protection against similar challenge with an equally virulent heterologous strain. Using the same strains, radiation-inactivated preparations provided 100% protection against 10,000 50% mouse lethal doses of the homologous strain and 70% protection against challenge with the same dose of a heterologous strain. Neither immunogen was a potent stimulator of antibody production as measured by the complement-fixation test. Cell-transfer studies using inbred mice indicated a role for cell-mediated immunity after vaccination with gamma-irradiated immunogens, but no cell-mediated protection could be demonstrated after vaccination with formalin-inactivated rickettsiae.

Primary scrub typhus infection renders humans solidly immune to reinfection by the homologous strain of *Rickettsia tsutsugamushi* for at least 1 year, but protection against the several known heterologous strains wanes rapidly, with susceptibility to disease reappearing within months (24, 25). A similar period of protection against homologous challenge is seen in rodent animal models (9), but the duration of heterologous immunity is less clearly defined, since most studies (4, 12, 22, 23) have tested heterologous resistance within 1 to 2 months after initial infection. Attempts to develop a safe, effective vaccine for scrub typhus have not been successful, although at least three conceptual approaches have been employed: infection with viable rickettsiae considered to be "attenuated" by laboratory or clinical criteria; infection with pathogenic rickettsiae combined with chemoprophylaxis to prevent overt clinical disease; and immunization with chemically inactivated organisms.

Vaccination with strains of *R. tsutsugamushi* considered to be attenuated by laboratory (12, 16) or clinical (13) criteria resulted in scrub typhus infections that were indistinguishable from those occurring after natural exposure (13, 14, 25). Inoculation of pathogenic rickettsiae combined with chemoprophylaxis had limited practical applicability because the technique required a delicate balance between the individual and the rickettsial infection to allow sufficient replication of the organism for adequate immunogenicity while suppressing overt disease by chemotherapy (16). Immunization with formalin- or merthiolate-treated rickettsiae was ineffective in providing protection for humans against natural infection (3, 6) but did protect laboratory animals against moderate homologous challenge (2, 10, 18, 26).

Since chemically inactivated immunogens elicited little heterologous protection (20) and only moderate homologous protection in laboratory animals, we explored inactivation by a physical process. Ultraviolet light has been employed to inactivate rickettsiae (1, 8), but the necessity of preparing thin films of rickettsial suspensions for exposure to a light source detracted from the usefulness of this technique. Gamma radiation has been used previously for preparation of immunogens from protozoan parasites (15, 21) and is known to inactivate bacteria (11). Since its penetrating capacity facilitates preparation of large volumes of immunogen, it was chosen for use in this study.

Immunogens were prepared from infected

yolk sac suspensions of *R. tsutsugamushi* either by exposure to lethal doses of gamma radiation or, for comparison, by addition of formalin and merthiolate. We chose to duplicate the formalinization techniques and the vaccination and challenge schedules of Smadel et al. (26) to insure that comparisons were valid with respect to the cápabilities of the formalinized immunogens and to provide a sense of historical continuity to the research. The relative immunizing capacities of the two types of immunogens were contrasted by vaccination of mice followed by homologous and heterologous challenge with highly virulent strains of scrub typhus rickettsiae. These protection tests showed that radiation-inactivated preparations were far superior to those containing formalin-killed rickettsiae.

MATERIALS AND METHODS

Mice. Male and female weanling ICR mice (Walter Reed Army Institute of Research, Washington, D.C.) were used for the majority of these studies. Female BALB/c mice (Flow Laboratories, Dublin, Va.), 18 to 22 g, were used in spleen cell transfer experiments.

Rickettsiae. The Karp strain (47th to 55th egg passages) and Kato strain (161st egg passage) of *R. tsutsugamushi* were propagated, stored, and quantified using methods previously reported (7). The 50% mouse lethal dose (MLD_{50}) was determined in ICR mice. Only those suspensions having a titer $\geq 10^8$ MLD_{50}/g of yolk sac were used in this study.

Immunogens. Infected yolk sac suspensions of the Karp strain were used to prepare all immunogens. Formalinization was accomplished by the technique of Smadel et al. (26). A double-strength solution of formalin and merthiolate in diluent was added to freshly prepared yolk sac suspensions, achieving final concentrations of 10% infected yolk sac, 0.1% formalin, and 0.01% merthiolate. The resulting formalinized immunogens were stored at 4°C and used 3 to 8 weeks after preparation. Radiation-inactivated immunogens were prepared by exposing frozen 20% yolk sac suspensions of rickettsiae in a ^{60}Co gamma irradiator (Gamma Cell 220, Atomic Energy of Canada Limited, Ottawa, Canada). Immunogens prepared by this method were used immediately after irradiation.

Determination of the effect of gamma radiation on lethality and protective capacity of Karp suspensions. The gamma radiation dose required for complete inactivation of scrub typhus rickettsiae was determined by exposing frozen rickettsial suspensions to various radiation dose in the range of 1 to 400 krads and then titrating the irradiated suspensions in mice to quantify survivors, expressing the results as MLD_{50}. Mice injected with rickettsiae irradiated with 200 to 400 krads were challenged 24 days later with 1,000 MLD_{50} of the Karp strain and then observed for deaths occurring 6 to 21 days postchallenge. Using these mortality data, the dilution of immunogen protecting 50% of the mice from 1,000-MLD_{50} challenge was calculated by the method of Reed and Muench (19). At that dilution, the quantity of irradiated rickettsiae present, defined as the 50% protective dose (PD_{50}), was determined by calculation of the antilog of the difference between the $\log_{10}$ of the protective dilution and the $\log_{10}$ MLD_{50} of the unirradiated suspension.

In vitro tests for the presence of replicating organisms in immunogen preparations. Rickettsial suspensions used as immunogens also were examined by two in vitro techniques to detect surviving organisms. The infection assay was similar to that previously described by Bozeman et al. (5) but utilized L-929 cells whose growth was arrested by treatment with 3,000 rads of gamma radiation (27). The cells were mixed in suspension with thoroughly washed untreated or inactivated scrub typhus organisms at a multiplicity of infection of 1 MLD_{50} of the Karp strain/cell. Rickettsiae were adsorbed for 1.75 h at 26°C, and then the mixture were diluted with Eagle minimal essential medium and centrifuged for 10 min at 240 × *g*. The pellet of infected cells was resuspended to 8 × 10^4 cells/ml in Eagle minimal essential medium containing 25 nM HEPES (*N*-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid) supplemented with 10% fetal calf serum and 1% glutamine (all growth media were purchased from Microbiological Associates, Bethesda, Md.). An initial portion was removed and centrifuged in a Cytospin centrifuge (Shandon-Elliot, Sewickley, Pa.) to prepare a slide for microscope examination. The remaining suspension was added in 2.5-ml portions to 60-mm plastic culture dishes (Falcon Plastics, Oxnard, Calif.) containing sterile glass cover slips and incubated at 34°C in a humidified atmosphere of 5% CO_2 in air. At 1, 3, 5, and 7 days postinfection, cover slips were removed, washed in physiological saline, fixed in absolute methanol, and stained with Giemsa stain. Two hundred cells on each slide were observed, and the number of rickettsiae/cell was determined. The plaque assay was a modification of the technique previously described for *R. conori* (17). Medium 199 supplemented with 10% fetal calf serum and 1% L-glutamine (Microbiological Associates, Bethesda, Md.) was used for maintenance of irradiated L-929 cells. The monolayers required incubation for 18 days at 34°C for plaque formation.

Vaccination and challenge. All injections were given intraperitoneally (i.p.). For mouse protection tests, ICR mice were vaccinated with formalinized immunogens by administration of three injections of 0.5 ml each, given at 5-day intervals. Radiation-inactivated immunogens were administered in 0.2-ml injections employing three regimens: one injection, two injections given 10 days apart, and three injections given at 5-day intervals. The animals were challenged 24 days after initial administration of immunogen by injection of 0.2 ml of Karp or Kato suspensions serially diluted in 10-fold increments to cover the desired range in MLD_{50} of rickettsiae. Five to ten animals were challenged with each dilution, and mice dying within the period of 6 to 21 days postchallenge were used for calculation of the MLD_{50} of the challenge inoculum. Immunity indices (26) were obtained by subtracting the MLD_{50} observed in

control mice from that observed in the vaccinated group.

Spleen cell transfer. Separate groups of BALB/c mice were vaccinated with both types of immunogen using the three-injection regimen. Employing techniques previously described (22), transfer of one mouse equivalent of spleen cells from vaccinated donors to normal recipients by i.p. injection was performed on day 24 after initiation of the vaccination regimen. Spleen cell recipients and vaccinated mice from the donor pool were challenged 8 h later using serial 10-fold dilutions of Karp or Kato suspensions. Normal controls were challenged with 1,000 MLD_{50} of each strain. Each challenge dilution was tested in a group of five mice.

Serology. At the time of challenge, pooled serum specimens were obtained from vaccinated ICR mice that had been bled from the ophthalmic venous plexus. Pooled serum specimens were obtained from vaccinated BALB/c mice by exsanguination of spleen cell donors. In all experiments, mice surviving challenge of 100 to 10,000 MLD_{50} were exsanguinated at 4 to 6 weeks postchallenge. The serum obtained was pooled by type of vaccination and challenge strain employed and stored at −40°C. Complement-fixation tests were performed in microtiter plates as previously described (22).

RESULTS

Protection with formalinized Karp immunogens. Mouse protection tests showed that formalinized immunogens prepared from two different Karp suspensions provided vaccinated mice with similar levels of protection against homologous challenge, although the preparation containing greater numbers of rickettsiae evidenced a higher immunity index (Table 1). Absolute protection was never achieved, and most surviving mice showed signs of distress during the challenge period. The suspension providing the greatest protection against homologous challenge afforded insignificant protection against challenge with the heterologous Kato strain.

Effect of gamma radiation on lethality and immunogenicity of Karp suspensions. The effect of increasing doses of gamma radiation on the lethality of two different Karp strain preparations is shown in Table 2. The calculated 100% lethal dose was 180 to 190 krads (Table 3) but, on one occasion, four mice inoculated with a suspension receiving 200 krads died. The application of a radiation dose in excess of 200 krads was obviously desirable to provide a safety factor, but it was also important to know the effect of higher doses on the immunogenicity of the rickettsiae. The effects of increased radiation doses on immunogenicity (Table 4) were comparable for the two suspensions and indicated a reciprocal relation between gamma radiation dose and immunogenicity (i.e., an increase in quantity of rickettsiae required for protection indicates a decrease in immunogenicity). However, the decrease in immunogenicity seen with increasing radiation dose was small in comparison to the corresponding decrease in MLD_{50} of the suspension. Referring to Tables 2 and 4, it can be seen that a 200-krad dose caused a decrease of approximately 10^8 MLD_{50}, whereas application of an additional 200-krad dose caused only a modest increase in the PD_{50}. Since an increase of 100 krads over the calculated 100% lethal dose had relatively little effect on immunogenicity, it was used to provide the necessary safety factor. Therefore,

TABLE 2. *Effect of gamma radiation on lethality of Karp suspensions*

Gamma radiation dose (krads)	Log_{10} MLD_{50}/g of yolk sac	
	1[a]	2
0	−8.3	−8.3
1	−8.0	
5	−8.0	
10	−7.2	
25		−7.3
50		−7.2
100	−3.7	−3.8
150		≥ −0.7
200	≥ −0.7	≥ −0.7

[a] Suspension.

TABLE 1. *Protection of ICR mice vaccinated with formalinized Karp immunogens*

No. of MLD_{50}[a] of formalinized rickettsiae/injection	Challenge strain	No. of survivors/no. of vaccinated mice challenged					Log_{10} MLD_{50} in vaccinated mice[c]	Log_{10} MLD_{50} in control mice[c]	Immunity index
		10,000[b]	1,000	100	10	1			
3.0×10^7	Karp	1/10	7/10	6/9	9/9	8/10	−5.4	−8.3	2.9
1.8×10^8	Karp	5/10	10/10	10/10	10/10	9/10	−4.5	−8.0	3.5
	Kato	0/5	1/10	1/10	1/10	1/10	≤ −7.6	−8.1	≤0.5

[a] Number of MLD_{50} was determined by titration of suspensions before formalinization.
[b] Approximate challenge dose (MLD_{50}).
[c] Values are based on exact challenge doses, which were determined from titration of inoculum in control mice and dilution factors used to achieve the approximate challenge doses noted.

TABLE 3. *Regression analysis of radiation dose-Karp lethality curve*

Suspension	Log_{10} MLD_{50} of unirradiated suspension/g of yolk sac	100% Lethal gamma radiation dose (krads)[a]	Slope (log_{10} MLD_{50}/g of yolk sac/krads)	Correlation coefficient
1	−8.1	180	4.4×10^{-2}	0.99
2	−8.6	190	4.4×10^{2}	0.96

[a] Point at which there will be ≤1 surviving rickettsia/g of irradiated yolk sac.

TABLE 4. *Effect of lethal doses of gamma radiation on immunogenicity of Karp suspensions*

Gamma radiation dose (krads)	PD_{50}[a]	
	1[b]	2
200	1.1×10^{6}	
250		2.5×10^{6}
300	4.0×10^{6}	3.8×10^{6}
400	1.5×10^{7}	7.9×10^{6}

[a] Number of MLD_{50} of irradiated rickettsiae required to protect 50% of vaccinated mice from a 1,000-MLD_{50} homologous challenge.

[b] Suspension.

a 300-krad dose was employed for the remainder of these studies.

Examination of inactivated rickettsial suspensions for the presence of replicating organisms. It was recognized that the inactivation procedures employed, particularly gamma irradiation, could have resulted in the survival of a small number of resistant organisms that did not express their lethal potential when inoculated into mice because the vastly greater number of inactivated organisms initiated an immune response capable of suppressing proliferation. Therefore, two in vitro techniques were employed to search for replicating organisms in the immunogen preparations. The growth of untreated, gamma-irradiated, and formalinized rickettsiae in L-929 cells is shown in Table 5. Both untreated and irradiated organisms rapidly entered the cytoplasm of cells, but uptake of formalinized rickettsiae was not observed. Untreated rickettsiae continued to multiply throughout the incubation period, but there was no indication of replication by the irradiated Karp. There was, apparently, gradual elimination of irradiated intracellular organisms, since the percentage of cells evidencing a light infection decreased during the observation period. This technique allowed detailed observation of the fate of a representative inoculum of gamma-irradiated rickettsiae, but the possibility remained that the sample was not large enough to contain one of a small number of viable organisms. The plaque assay was more suitable for screening large numbers of rickettsiae for radiation-resistant survivors. Portions of a Karp suspension having a titer of 4.8×10^{8} MLD_{50}/g of yolk sac were assayed before and after irradiation with 300 krads. The preexposure titer was 2.7×10^{8} plaque-forming units/g of yolk sac. After irradiation, the Karp suspension was diluted 1:2, and each of 32 plates was seeded with 0.1 ml of inoculum. This represented a total of 0.32 g of infected yolk sac containing 1.5×10^{8} potential MLD_{50}, a number that exceeds the amount of rickettsiae contained in one injection of irradiated immunogen. No plaques were observed on any plate.

Protection with irradiated Karp immunogens. Previous experiments (Table 4) established that one injection of 4×10^{6} MLD_{50} of rickettsiae irradiated with the selected dosage afforded mice protection against homologous challenge of 1,000 MLD_{50}. Approximately the same level of protection, evidenced by an immunity index of 2.9 (Table 1), required the injection of a total of 9×10^{7} MLD_{50} of formalinized rickettsiae. This indicated that the irradiated rickettsiae were considerably more immunogenic than the formalinized organisms. Studies were initiated to determine if increasing the number of irradiated rickettsiae and modifying the injection regimen would result in further enhancement of protection. Homologous protec-

TABLE 5. *Growth of untreated and inactivated Karp in irradiated L-929 cells*

Treatment	Severity of cell infection[a]	% Cells infected				
		0[b]	1	3	5	7
Untreated	H	0	0	3	12	30
	M	34	31	44	50	40
	L	62	60	30	11	13
	O	4	9	23	27	17
Gamma radiation (300 krads)	H	0	0	0	0	0
	M	0	0	0	0	0
	L	27	19	6	3	2
	O	73	81	94	97	98
Formalin	H	0	–[c]	–	–	–
	M	0	–	–	–	–
	L	0	–	–	–	–
	O	100	–	–	–	–

[a] Evaluation of infected cells was made on the following basis: Heavy infection (H), >20 rickettsiae/cell; moderate infection (M), 6 to 20 rickettsiae/cell; light infection (L), 1 to 5 rickettsiae/cell; and uninfected (0), no visible rickettsiae.

[b] Day postinfection.

[c] –, Not done.

tion was studied after vaccination with one and three injections of either 10^6 or 10^7 radiation-inactivated rickettsiae (Table 6). Increase in either concentration of rickettsiae or number of injections resulted in increased protection. The immunity indices indicate that protection was heightened 100-fold by increasing the regimen from one injection of 10^6 rickettsiae to either three injections of 10^6 or one injection of 10^7 organisms. Since challenges calculated to contain greater than 10,000 MLD_{50} were not employed, immunity indices do not clearly differentiate the further increase in protection that may have been achieved by use of three injections of 10^7 irradiated Karp. However, from Table 6 it can be seen that this latter regimen provided absolute protection against homologous challenge with at least 10,000 MLD_{50} of Karp. In addition, mice showed no signs of illness throughout the observation period following challenge.

As a result of the excellent homologous protection demonstrated in this experiment, additional tests were performed to evaluate the protective effect of a two-injection regimen against homologous challenge and the effectiveness of all three regimens against the heterologous challenge. As can be seen from the immunity indices in Table 6, all regimens provided substantial protection against heterologous challenge, but a large increase in protection was seen when more injections of immunogen were administered. Within the limits of the experiment, both multiple-injection regimens appear to provide approximately the same level of protection. Both schedules protected vaccinated mice against homologous and heterologous challenges of 10,000 MLD_{50}. Homologous protection was absolute, with no morbidity observed at any time after challenge. Mice challenged with the heterologous Kato strain showed signs of distress and generally some died at each challenge level, although the immunity indices were of similar magnitude to those seen after homologous challenge.

Cell-mediated immunity. Studies previously reported by this laboratory (22) indicated that spleen lymphocytes obtained from mice surviving infection with small doses of Gilliam strain are able to protect normal recipient mice against heterologous challenge with the Karp strain. Considering these observations, experiments were performed to determine if differences in immunogenicity observed between formalinized and irradiated rickettsiae were due to their capacities to activate a cell-mediated immune response. Neither homologous nor heterologous protection was observed in mice that received spleen cells from donors vaccinated with formalinized organisms (Table 7). On the other hand, 80% of the mice receiving cells from donors vaccinated with irradiated immunogens survived subsequent homologous challenge of 1,000 MLD_{50}, and 100% of the recipients resisted homologous challenge of 100 MLD_{50}. Spleen cell recipients challenged with the heterologous Kato strain showed little resistance, with only 20% surviving 100 MLD_{50} challenge.

Serology. The results from studies of mice vaccinated using the three-injection regimen are shown in Table 8. Neither type of immunogen proved to be a potent stimulator of antibody production as assayed by the complement-fixation test. On the other hand, mice were capable

TABLE 6. *Protection of ICR mice vaccinated with irradiated Karp immunogens*

No. of MLD_{50}[a] of irradiated rickettsiae/injection	No. of injections	Challenge strain	No. of survivors/no. of vaccinated mice challenged			Log_{10} MLD_{50} in vaccinated mice[c]	Log_{10} MLD_{50} in control mice[c]	Immunity index
			10,000[b]	1,000	100			
1.6×10^6	1	Karp	2/10	3/10	2/8	−6.2	−8.8	2.6
	3		7/10	9/10	8/8	≥ −4.2	−8.8	≥4.6
1.6×10^7	1	Karp	7/10	9/10	10/10	≥ −4.2	−8.8	≥4.6
	3		10/10	10/10	11/11	≥ −3.9	−8.8	≥4.9
1.6×10^7	2	Karp	10/10	9/9	10/10	≥ −3.9	−8.2	≥4.3
	1	Kato	2/8	1/10	5/10	−5.0	−7.7	2.7
	2		8/8	9/10	7/10	≥ −3.2	−7.9	≥4.7
	3		7/10	8/10	5/10	−3.6	−7.7	4.1

[a] Number of MLD_{50} was determined by titration of suspensions before irradiation.
[b] Approximate challenge dose (MLD_{50}).
[c] Values based on exact challenge doses, which were determined from titration of inoculum in control mice and dilution factors used to achieve the approximate challenge doses noted.

TABLE 7. *Survival of BALB/c mice receiving spleen cells from donors vaccinated with Karp immunogens*

Type of immunogen	No. of MLD_{50}[a] of inactivated rickettsiae/injection	Challenge strain	Mouse status[b]	No. of survivors/no. of vaccinated mice challenged		
				10,000[c]	1,000	100
Formalinized	1.8×10^8	Karp	Donor	0/5	2/5	2/5
			Recipient	0/5	0/5	0/5
		Kato	Donor	0/5	0/5	0/5
			Recipient	0/5	0/5	0/5
	None	Karp	Control		0/5	
		Kato	Control		0/5	
Gamma-irradiated	7.9×10^7	Karp	Donor	5/5	5/5	5/5
			Recipient	0/5	4/5	5/5
		Kato	Donor	4/5	5/5	3/5
			Recipient	0/5	0/5	1/5
	None	Karp	Control		0/4	
		Kato	Control		0/5	

[a] Values are expressed as MLD_{50} based on titration of suspensions before inactivation. All mice received three i.p. injections of immunogen.

[b] Donor, vaccinated mouse; recipient, normal mouse receiving i.p. injection of one mouse-equivalent of spleen cells from donor mouse; control, normal mouse.

[c] Approximate challenge dose (MLD_{50}).

TABLE 8. *Complement-fixation titers of mice vaccinated with Karp immunogens and of protected mice surviving subsequent challenge*

Type of immunogen	Mouse strain	Titer on day of challenge		Challenge strain	Titer of survivors 4 to 6 weeks postchallenge	
		Karp[a]	Kato		Karp[a]	Kato
Formalinized[b]	ICR	10[c]	<10	Karp	640	20
				Kato	80	160
	BALB/c	10[d]	<10[d]			
Irradiated[b]	ICR	10	<10	Karp	320	20
				Kato	20	80
	BALB/c	20[d]	<10[d]	Karp	320[e]	<10[e]

[a] Strain of *R. tsutsugamushi* used to make CF antigen.

[b] All mice received three i.p. injections.

[c] Titers expressed as reciprocal of highest dilution showing hemolysis ≤50%. Lowest dilution tested was 1:10.

[d] The antibody titer reported for BALB/c mice on day of challenge represents the humoral response of donor mice at the time their spleens were removed and transferred to recipient mice, who were challenged 8 h later with the Karp strain.

[e] The antibody titer reported for BALB/c mice 4 to 6 weeks postchallenge represents the humoral response of surviving mice that received one spleen equivalent of cells from an immunized BALB/c donor followed 8 h later by challenge with the Karp strain.

of responding against the challenge strain and showed excellent antibody titers when tested 4 to 6 weeks postchallenge.

DISCUSSION

We have shown that the lethality of scrub typhus rickettsiae could be abolished by application of gamma radiation doses in excess of 200 krads, producing immunogens that were markedly superior to formalinized rickettsiae in protection of mice against both homologous and heterologous challenge. Not only were the mice resistant to larger challenge doses, but the protection was achieved with fewer rickettsiae. Resistance to homologous challenge of 1,000 MLD_{50} required 9×10^7 MLD_{50} of formalinized rickettsie, whereas a similar level of protection was achieved with 4×10^6 MLD_{50} of irradiated organisms. When multiple-injection regimens were used with the irradiated immunogens, they routinely elicited homologous protection levels higher than those achieved with formalinized suspensions, but it has not yet been determined if this enhanced protection is due to the temporal regimen employed or is simply the result of accumulating a larger amount of immunogen in the host. The homologous protection with irradiated immunogens was absolute to at least 10,000 MLD_{50}, whereas formalin-killed rickettsiae were unable to routinely induce absolute immunity at any challenge level. The differences in protection levels induced by the two types of immunogens were even more

striking when heterologous challenge was employed. Vaccination with formalinized rickettsiae provided negligible protection against heterologous challenge, the results being quite similar to those reported by Jackson and Smadel (12). On the other hand, although absolute immunity was not observed, use of multiple-injection regimens with radition-inactivated organisms resulted in protection of the majority of mice against the heterologous Kato strain.

The mechanisms responsible for the heightened protection observed after vaccination with radiation-inactivated scrub typhus immunogens remain undefined. It is possible that radiation inactivation simply causes less structural damage to peripheral macromolecules than does chemical inactivation, thereby allowing a more effective response by the immune system. Alternatively, it is possible that scrub typhus rickettsiae inactivated by exposure to ionizing radiation retain some physiological capabilities important in stimulating host defenses. We have shown that gamma-irradiated rickettsiae enter cells more readily than formalinized organisms, and it is possible that this capacity influences their immunogenicity. Numerous studies have shown that mice surviving active infection are solidly immune to homologous challenge for extended periods of time (9) and to heterologous challenge for shorter periods of time (4, 12, 23). Recent studies performed in this laboratory have shown that cell-mediated immunity plays an important role in the heterologous protection (22). Our spleen-cell transfer data revealed that significant levels of homologous protection could be provided to recipients by injection of cells from mice vaccinated with irradiated suspensions, although the failure to achieve substantial heterologous protection required consideration of the possibility that some transferred cells produced antibody that aided in protection against homologous challenge. No protection was observed in mice receiving cells from donors vaccinated with formalinized suspensions. These observations, taken in conjunction with the low antibody titers observed after vaccination with either gamma-irradiated or formalinized rickettsiae, suggest that differences in immunogenicity are related to stimulation of cell-mediated immunity and that properties shared by both infectious and irradiated rickettsiae enhance this type of host response.

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