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Mechanisms of Immunity in Typhus Infection: Some Characteristics of Intradermal *Rickettsia mooseri* Infection in Normal and Immune Guinea Pigs

JAMES R. MURPHY,† CHARLES L. WISSEMAN, JR.,* AND PAUL FISET
Department of Microbiology, University of Maryland School of Medicine, Baltimore, Maryland 21201

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Rickettsia mooseri infection in skin at sites of intradermal inoculation was studied in nonimmune and immune guinea pigs with respect to dynamics of infection, localization of rickettsiae within tissues, and gross and microscopic pathology. Intradermal inoculation of *R. mooseri* into nonimmune guinea pigs resulted in gross lesions which, in magnitude, were directly related to the number of rickettsiae inoculated. The lesions progressively enlarged through 3 or 4 days and remained enlarged through at least 7 days. Histological examination revealed an early acute inflammation which progressed to a predominantly monocyte-macrophage inflammation and subsequently condensed into lymphocyte-containing granulomatous foci. Rickettsiae in the skin at sites of inoculation increased in numbers from 6 h through 3 days, in parallel with the increasing diffuse monocyte-macrophage inflammatory response, and then declined markedly on days 4 or 5 as granulomatous foci appeared. Some rickettsiae, however, persisted through at least day 7. Fluorescent-antibody studies suggested that *R. mooseri* infected only a subset of cells available, i.e., cells associated with the microvascular system. Dissemination of infection was demonstrated by the presence of rickettsiae in the skin at sites distant from the point of inoculation. Immune guinea pigs, made immune by intradermal infection with *R. mooseri* 12 days before intradermal challenge, displayed an accelerated response. The lesions were maximal by 24 to 48 h and subsequently regressed. The inflammatory response of immune guinea pigs was of greater magnitude than the response of similarly challenged nonimmune guinea pigs, and the response from acute inflammation through the formation of granulomatous lesions was accelerated. The number of rickettsiae in the skin of immune guinea pigs declined steadily from the time of inoculation, until no rickettsiae were recovered on or after day 3. Furthermore, dissemination of rickettsiae to sites in skin distant from the site of inoculation was not demonstrable. These results are discussed in terms of pathogenesis and of immunity to typhus.

In a previous study (13) the intradermal (i.d.) inoculation of *Rickettsia mooseri* (*R. typhi*) into guinea pigs was shown to result in an infection beginning at the site of inoculation and spreading to regional lymph nodes and finally through the blood to various internal organs. This sequence was accompanied by gross pathological changes locally, regionally, and distantly in some organs and by the development of a humoral antibody response. Infection, as measured by rickettsial content, appeared to wax, wane, or persist in various tissues at different levels independent of a measurable antibody response. In particular, the organisms in the skin at the site of inoculation attained peak titers early and then declined rapidly at about the time antibodies became detectable in the serum. Moreover, the organisms disappeared from this site at a time when systemic spread of infection was not yet demonstrable. Thus, the cutaneous site of primary rickettsia-host interactions appeared to us, as it did to Castaneda (5, 6) many years ago, to be uniquely adapted to a study of host defenses to rickettsial infection. The skin of the back (13), low in bacterial contaminants, was chosen as the site for detailed studies of the local rickettsia-host interaction, after i.d. inoculation, the subject of this paper.

MATERIALS AND METHODS

Rickettsiae. Two seeds of *R. mooseri*, Wilmington strain, were used. Seed 1 was the same yolk sac-grown *R. mooseri* (12EP/15GP/5EP) used in previous stud-

† Present address: Trudeau Institute, Saranac Lake, NY 12983.

ies (13, 14). A partial characterization of this seed and the methods employed for characterization have been published previously (14).

Seed 2 was derived from seed 1. Three days after intraperitoneal inoculation of 5.1×10^6 plaque-forming units (PFU) of seed 1 into a Hartley guinea pig, heparinized blood was collected and used to infect BSC-1 cells in monolayer culture. Infected cell cultures were maintained at 32°C in medium 199 (Grand Island Biological Co., Grand Island, N.Y.), supplemented with 10% fetal calf serum. Monolayers from the fourth BSC-1 cell passage were scraped from the flask surface after 6 days of incubation. After centrifugation, the pelleted cells were resuspended in 3.7% brain heart infusion (Baltimore Biological Laboratories, Cockeysville, Md.), homogenized at 4°C in an Omni mixer (Ivan Sorval, Newton, Conn.), and dispensed into ampoules which were flame sealed, rapidly shell frozen in a dry ice-alcohol mixture, and stored at −70°C. This tissue culture-grown (12EP/15GP/5EP/1GP/4BSC-1) seed had a titer of 4.6×10^6 PFU/ml. The methods employed for characterization have been published previously (13).

Sucrose-phosphate-glutamate solution (4) was employed as a diluent for seed 1 and 3.7% brain heart infusion was employed as diluent for seed 2. Normal yolk sac diluted with sucrose-phosphate-glutamate was employed as a noninfectious control inoculum.

Some characteristics of the inocula of the two seeds employed in this study are recorded in Table 1. Guinea pigs infected with the monkey kidney cell seed preparation did not develop hypersensitivity reactions to normal yolk sacs.

Animals. Male Hartley strain guinea pigs weighing 300 to 500 g were purchased from a commercial supplier (R. C. Rosecrans, Hamilton, Mont.). The animals were housed individually, fed Purina guinea pig chow (Ralston Purina Co., St. Louis, Mo.), and provided with water ad libitum.

Infection. A model based on multiple inoculations of two doses of rickettsiae was selected for the study of the events in the skin after i.d. inoculation of nonimmune and immune guinea pigs. The pattern of inoculation is presented in Fig. 1. Three sites along the cephalad region of the back received a high (8.2×10^6 PFU) dose of rickettsiae, three sites along the caudad portion of the back received a low (8.2×10^4 PFU) dose of rickettsiae, and two sites intermediate to the high- and low-dose inoculations were employed as uninfected controls. At 1 to 2 h before inoculation, the back was shaved with electric clippers and further denuded with a depilatory agent (Nair, Carter Products, New York, N.Y.). The denuded sites were rinsed with tap water and dried. All inocula were contained in a volume of 0.1 ml and were administered through a 26-gauge needle.

When animals were to be immunized by infection before challenge in the skin of the back, they were inoculated i.d. on the outer aspect of the right thigh as

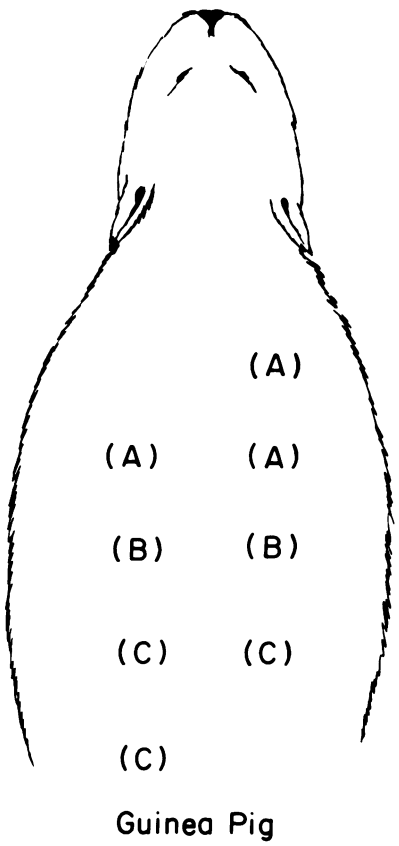

FIG. 1. *Diagram of pattern of i.d. R. mooseri injection sites: (A) sites receiving 8.20×10^6 PFU of R. mooseri, (B) sites receiving no challenge, and (C) sites receiving 8.20×10^4 PFU of R. mooseri.*

TABLE 1. *Some characteristics of immunizing and/or challenge doses of R. mooseri*

Passage history	Seed designation	Dilution (diluent)[a]	Rickettsial content (in 0.1 ml)		
			PFU	Guinea pig i.d. ID_{50}[b]	Rickettsial particles
12 EP/15GP/5EP	1				
High dose	(challenge)	1:3.1 (SPG)	8.20×10^6	8.54×10^6	3.96×10^8
Low dose		1:310 (SPG)	8.20×10^4	8.54×10^4	3.96×10^6
12EP/15GP/5EP/1GP/4BSC-1	2 (immunizing)	1:13.4 (BHI)	3.46×10^4	1.65×10^5	1.82×10^7

[a] Diluents: SPG, sucrose-phosphate-glutamate; BHI, brain heart infusion.
[b] ID_{50}, 50% infective dose.

previously described (13).

Skin biopsy procedures. Three immune and three nonimmune guinea pigs were sacrificed at each time point. Eight-millimeter circular punch biopsies of the inoculation sites, three from the high rickettsial dose sites (see below), three from the low rickettsial dose sites, and two from uninoculated control sites, were taken at different times after inoculation in the manner previously described (14). Two biopsy specimens of each dose level and one of the control biopsies were processed for quantitation of rickettsiae (14), and one specimen from each dose level and one of the control biopsies were processed for histological examination. A second group of animals was employed to provide biopsies which were processed for fluorescent-antibody staining.

Quantitation of rickettsiae. The chicken embryo cell plaque assay was employed for enumeration of viable rickettsiae. This assay and the preparation of samples for plaque assay have been described in detail elsewhere (14). Particle counts were made by a modification of the method of Silberman and Fiset (18), and guinea pig 50% infective dose titrations were performed as previously described (13).

Histological techniques. Biopsy specimens were bisected, fixed in Zenker-Formalin, and embedded in paraffin by standard procedures (12). Sections (7 μm) were stained with hematoxylin eosin.

Fluorescent-antibody studies. A fluorescein-conjugated guinea pig anti-*R. mooseri* serum was prepared from serum pooled from animals convalescent from *R. mooseri* infection. The globulin fraction of this pool, obtained by two sequential 50% saturated ammonium sulfate precipitations, was resuspended in phosphate-buffered saline (PBS, pH 7.5), dialyzed against PBS, and conjugated with fluorescein isothiocyanate by standard techniques (8). The conjugate gave brilliant fluorescence with *R. mooseri* in infected tissue culture cells.

Frozen sections (7 μm) were cut from skin biopsies embedded in OTCR compound (Ames, Elkart, Ind.), fixed in cold acetone, and rehydrated in PBS (pH 7.5). The sections were flooded with the fluorescein-conjugated guinea pig anti-*R. mooseri* serum diluted to working concentration in PBS containing 10% (wt/vol) homogenized normal yolk sac and 0.2% (wt/vol) Evans blue, incubated for 30 min at 37°C, rinsed thoroughly in PBS, and mounted under a no. 1 cover slip in a mixture of 90% glycerine and 10% PBS. The sections were examined under an American Optical Co. fluorescence microscope equipped with a vertical UV illumination system.

Frozen sections of normal guinea pig skin exposed to the anti-*R. mooseri* fluorescein-conjugated serum did not stain, and preincubation of infected sections with unconjugated anti-*R. mooseri* serum markedly reduced fluorescence.

Measurements. The area of erythema or induration at sites of *R. mooseri* inoculation was estimated from measurements of the diameter of these reactions on an axis perpendicular to the spine. The areas of erythema or induration about sites receiving identical inoculations on one animal were used to determine the mean reaction of the animal. Then, the values from the animals within a group were used to calculate the mean reaction and the standard error for the group.

RESULTS

Observations on nonimmune animals. (i) Gross pathology. A reproducible sequence of events detectable by gross examination occurred at the sites of rickettsial inoculation but not at control sites distant from the point of inoculation after the initial bleb and erythema observed at all inoculation sites disappeared. The responses consisted of erythema, induration and necrosis (Fig. 2 and Table 2).

Thus, with both rickettsial doses, erythema was detectable about 1 day after infection, attained maximum areas at about days 3 and 5 for high- and low-dose sites, respectively, and persisted in both through day 7. Induration, first detectable at about day 2 with the high dose and at about day 3 with the low dose, persisted through day 7 in both instances. The erythema and induration were greater at the high-dose than at the low-dose inoculation sites. Necrosis was observed only with the high dose. It was first detectable about day 2 after inoculation and was maximum between about days 3 and 4. It eventually yielded a lesion with raised, indurated edges and central necrosis covered by a black eschar.

(ii) Histopathology. Examination of tissue sections prepared from inoculation sites between 6 h and 7 days after infection showed a dose-dependent inflammatory reaction which underwent a distinct series of changes with respect to time—namely, an early acute cellular infiltrate in which polymorphonuclear leukocytes (PMN) and larger mononuclear (MN) cells were observed with about equal frequency, followed by a diffuse, dominantly large MN infiltrate, and finally the appearance of focal granulomatous lesions.

Although present at 6 h, PMN were not seen in significant numbers after day 1 in either low- or high-dose inoculation sites. A substantial increase in the number of large MN cells continued through day 3, at which time increasing numbers of small MN cells, presumably lymphocytes, were seen. Between 5 and 6 days focal, compact lesions, most often in association with blood vessels, appeared within the diffuse MN inflammation. Large macrophage-type MN cells were the major cell type seen in focal lesions, although some small MN cells, lymphocytes, and a few plasma cells, eosinophilic PMN, and multinucleated cells were also identified.

(iii) Rickettsiae in skin. Titration of rickettsiae in biopsies taken from inoculated and uninoculated skin sites revealed a reproducible

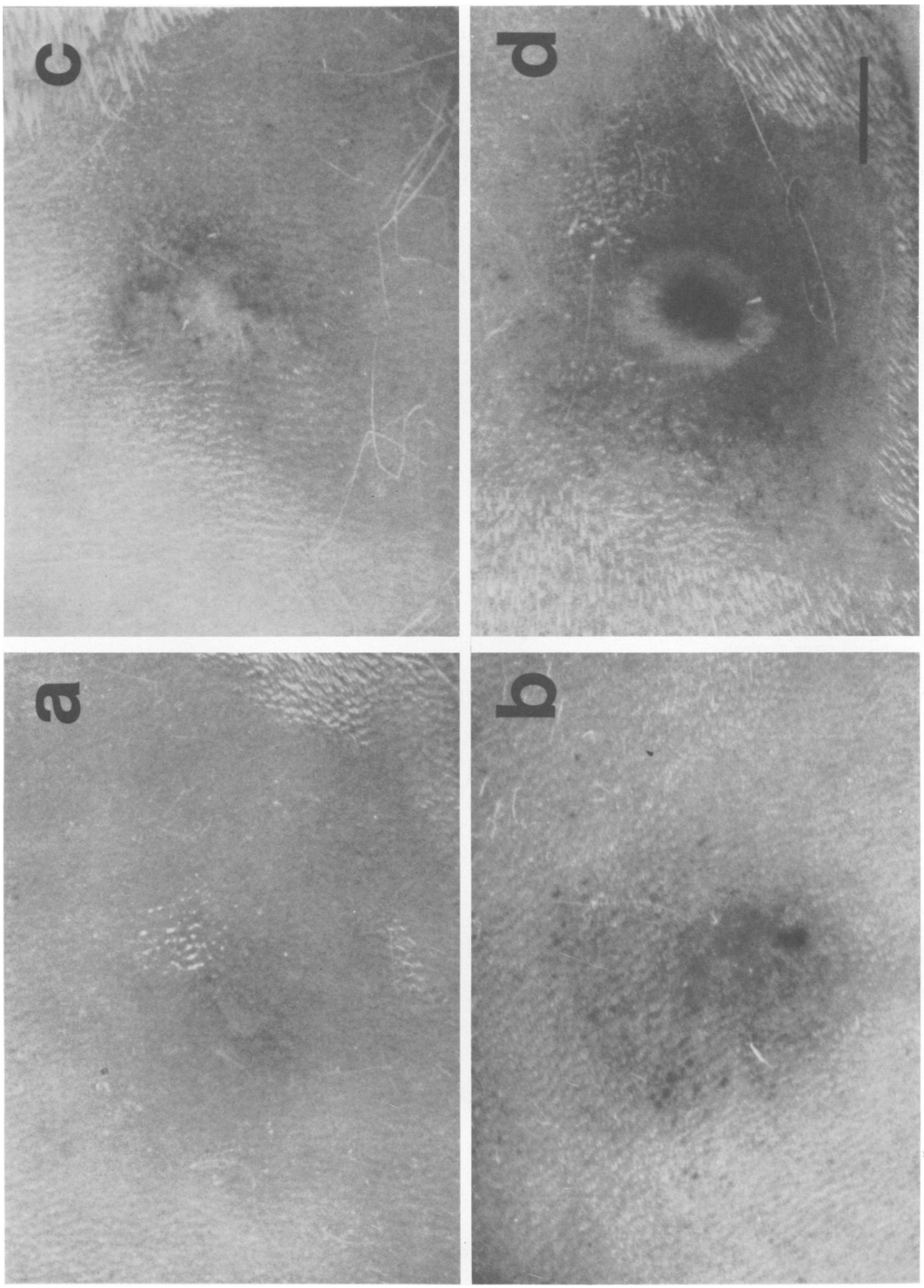

FIG. 2. *Appearance of skin lesions on nonimmune guinea pigs: (a) 1 day after low-dose inoculation, (b) 3 days after low-dose inoculation, (c) 1 day after high-dose inoculation, and (d) 3 days after high-dose inoculation. Bar = 0.5 cm.*

pattern (Fig. 3). Notable were (i) recovery of only about 1.0% of the inoculum at 6 h after inoculation, (ii) subsequent steady increase in number of PFU reaching a maximum at about 3 days, (iii) rapid decline in number of PFU between days 4 and 5, and (iv) persistence of low numbers of rickettsial PFU through at least day 7. The pattern was essentially the same in both high- and low-dose inoculation sites. However, the rate of increase in number of PFU was greater in the low-dose sites, so that maximum titer at day 3 approached that in the high-dose sites.

Rickettsiae were also recoverable from uninoculated distant skin sites, i.e., between the sites of infection (Fig. 1). Unfortunately, the 6-h samples were lost because of bacterial contamination in the plates. However, rickettsiae were isolated

TABLE 2. *Effect of number of rickettsiae inoculated on the development of erythema and induration at site of i.d. inoculation*

Challenge	Effect	Mean area (mm^2) on postinfection day:						
		1	2	3	4	5	6	7
8.20×10^6 PFU of *R. mooseri*	Erythema	10.3[a]	30.0	70.6	60.9	65.6	56.0	62.8
		(2.1)[b]	(5.4)	(5.2)	(7.6)	(8.4)	(5.0)	(6.3)
	Induration	0.0	6.9	25.9	31.0	29.5	27.3	29.0
		(1.8)	(4.6)	(4.6)	(6.0)	(4.1)	(1.5)	(4.1)
8.20×10^4 PFU of *R. mooseri*	Erythema	6.2	9.6	41.8	47.4	49.9	40.1	33.7
		(0.9)	(1.1)	(14.0)	(13.2)	(13.4)	(9.1)	(1.6)
	Induration	0.0	1.9	9.2	19.2	14.3	17.8	17.1
			(0.3)	(3.5)	(7.7)	(2.9)	(4.3)	(3.9)
Normal yolk sac[c]	Erythema	0.4	0.0	0.0	0.5	1.4	0.0	0.0
		(0.4)			(0.4)	(1.0)		(0.4)
	Induration	0.0	0.0	0.0	0.0	0.0	0.0	0.0

[a] Mean of five animals.
[b] Standard error of mean.
[c] Uninfected yolk sac suspension diluted in sucrose-phosphate-glutamate to equivalent of yolk sac concentration as that in the 8.20×10^6 PFU *R. mooseri* challenge.

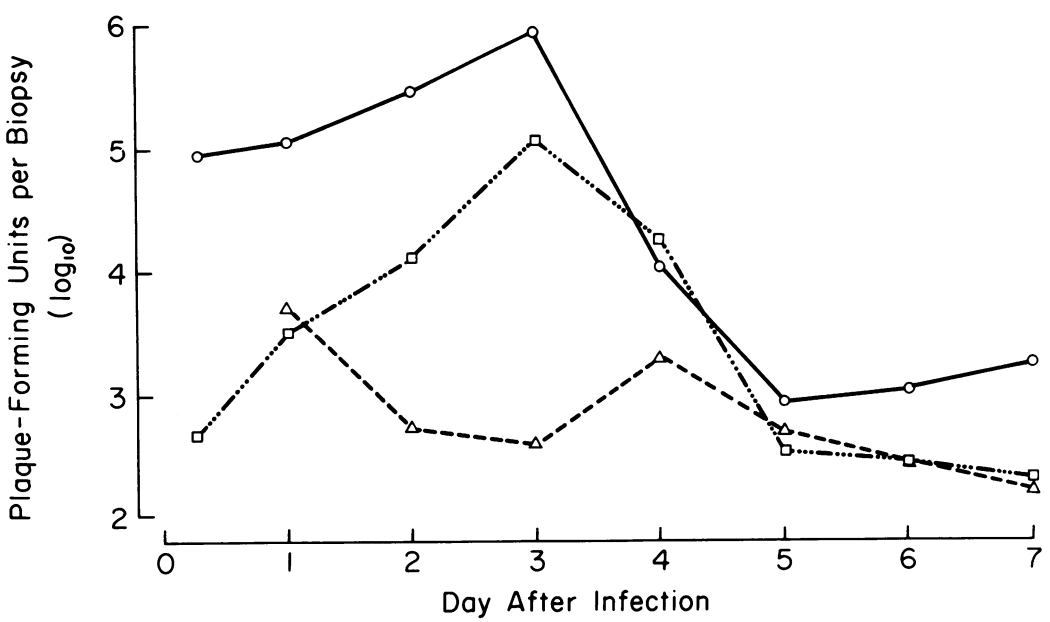

FIG. 3. *Dynamics of R. mooseri skin infection of nonimmune guinea pigs. Symbols:* (○) *PFU per biopsy of high-dose inoculation sites,* (□) *PFU per biopsy of low-dose inoculation sites, and* (△) *PFU per biopsy of uninjected sites distant from inoculation sites.*

from uninoculated sites at 1 day after infection in numbers equivalent to those in the low-dose sites. In contrast to the events at sites of inoculation, the number did not increase in the uninoculated sites and, if anything, declined slightly over the 7-day observation period. The numbers of PFU recoverable from both inoculated and uninoculated sites were similar between 4 and 7 days after inoculation.

Examination of frozen sections of biopsies for rickettsial bodies (RLB) by staining with fluorescein-conjugated anti-*R. mooseri* serum revealed the following patterns which differed somewhat from the information obtained from assays of viable rickettsiae. Thus, in the high-dose sites at 6 h after inoculation, RLB were found to be diffusely distributed in the dermis and subjacent areolar tissue. By day 1 the numbers had decreased, but the distribution was unchanged. At 2 days, however, the distribution pattern had changed. RLB were confined to the dermis, and foci consisting of multiple RLB were detected. By 3 days and continuing through 4 and 5 days, the numbers of RLB had increased and had assumed a reticular distribution consistent with the distribution of the microvascular system. However, by 7 days, the number of foci of fluorescent RLB had decreased markedly. The pattern seen in the low-dose sites was similar to that just described, except that the number of RLB detectable early, i.e., at 6 h and 1 day, was very low.

Observations on immune animals. In a preliminary study, guinea pigs were infected i.d. in the hind limb with either 8.2×10^6, 8.2×10^4, or 8.2×10^2 PFU of yolk sac-grown *R. mooseri* (12EP/15GP/5EP seed). By day 7 after primary infection, the animals had already developed the capacity to resist the development of the characteristic lesions in the skin of the back. This resistance continued undiminished through day 28, the last observation point. Figure 4 shows the marked difference between nonimmune and immune animals in the appearance of sites of *R. mooseri* inoculation at 1 and 3 days after challenge. However, hypersensitivity to normal yolk sac component was demonstrable by day 14 and detracted from the observations.

On the basis of the preliminary experiment just described and a previous study (14), the following model was selected for the subsequent studies on immunity as manifested in guinea pig skin. Normal animals were infected i.d. on outer aspect of the thigh with 3.5×10^4 PFU of a tissue culture-grown seed of *R. mooseri* (12EP/15GP/5EP/4BSC-1) (Table 1) to avoid sensitizing the animals to yolk sac components. At 12 days after primary infection, these animals were challenged in the skin of the back with high and low doses (Table 1) of the yolk sac-grown *R. mooseri* seed (12EP/15GP/5EP), with the same pattern of inoculations presented in Fig. 1. Observations of the kind described for nonimmune animals were then made in these immune animals. The pattern of these observations as recorded in the immune animals differed markedly from that observed in nonimmune animals.

(i) Gross pathology. At the high-dose inoculation sites, erythema was visible by 6 h, attained a maximum at day 1, and had disappeared by day 3. The pattern of appearance and disappearance of erythema at the low-dose sites was similar but of lesser magnitude.

Induration detectable by palpation was minimal and was found only at the sites of high-dose inoculation, where it was maximal at day 1 and greatly diminished at day 2 but persisted through day 7. Central necrosis was not observed in the immune animals.

(ii) Histopathology. Massive perivascular accumulation of inflammatory cells, consisting of substantial numbers of PMN leukocytes and large MN cells and a few eosinophilic PMN cells, was present at 6 h in the high-dose sites. By day 1, these sites showed an increasing area of diffuse MN cell infiltration, cellular debris, some hemorrhage, and a substantial decrease in relative number of PMN. A large MN cell infiltration of similar magnitude was present by day 2, but by this time there were occasional multinucleate giant cells and an increasing number of small MN cells, presumably lymphocytes. Between days 2 and 7 there was some resolution of the inflammatory response, with the diffuse MN infiltrate giving way to condensed, dominantly perivascular, granulomatous foci. The histopathological changes at the sites of low-dose inoculation were similar but of lesser magnitude. No histopathological changes were seen in sections of skin prepared from distant uninoculated sites.

(iii) Fate of the rickettsiae. Examination of inoculation sites for viable rickettsiae (Fig. 5) revealed rapid clearance of rickettsiae which fell below detectable levels by day 1 in the low-dose inoculation sites and by day 2 in high-dose sites and remained below detectable levels through day 6. Rickettsiae were never recovered from uninoculated distant skin sites.

Diffusely distributed RLB were detected by fluorescent antibody in both low- and high-dose sites at 6 h. None was detected after this in the low-dose sites. At day 1, the number was markedly reduced in the high-dose sites, and by day 3 rickettsiae were no longer visible.

DISCUSSION

These studies demonstrated that the course

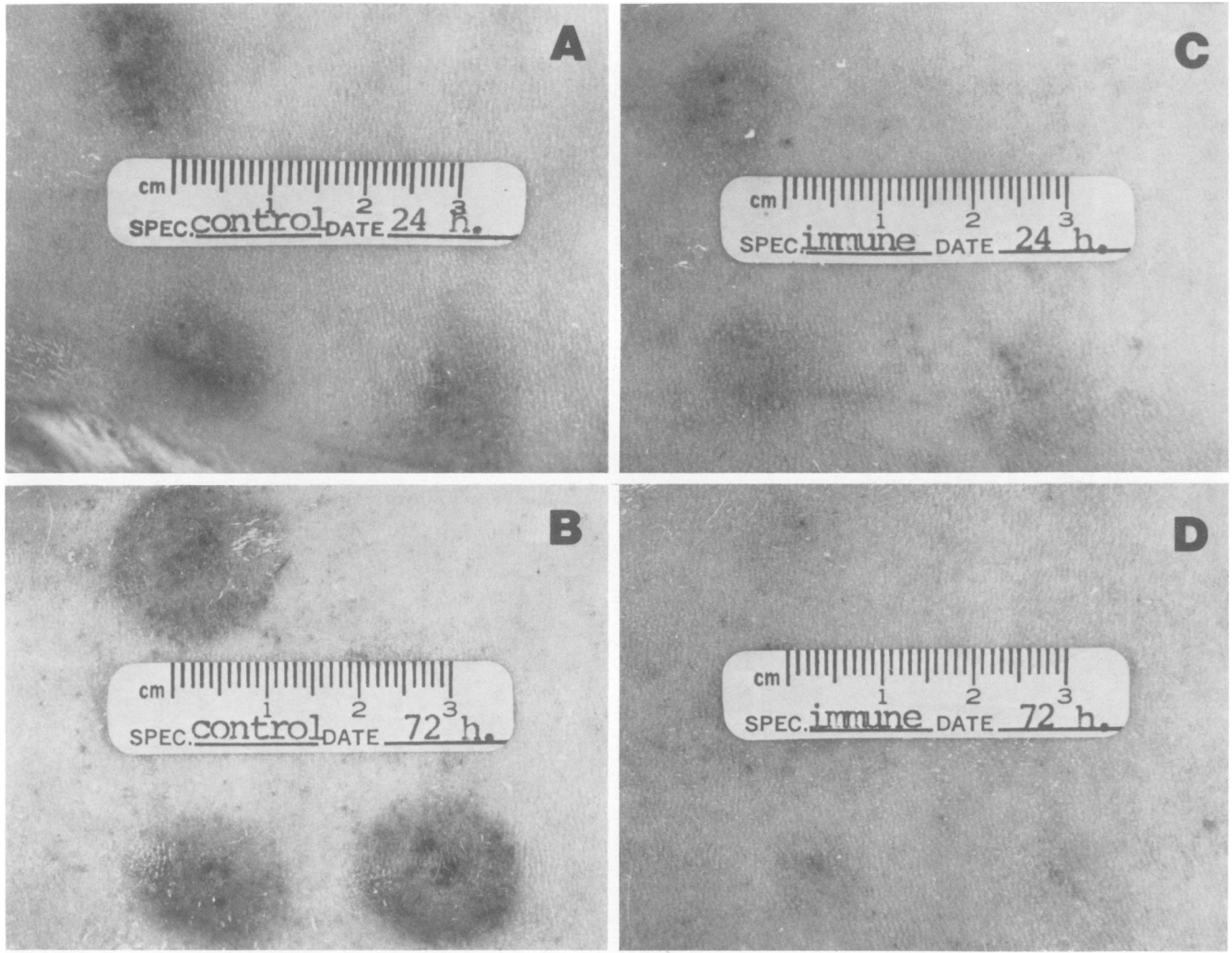

FIG. 4. *Appearance of skin lesions at low-dose inoculation sites on nonimmune and immune guinea pigs: (A) 1 day after inoculation of a nonimmune guinea pig, (B) 3 days after inoculation of a nonimmune guinea pig, (C) 1 day after inoculation of an immune guinea pig, and (D) 3 days after inoculation of an immune guinea pig.*

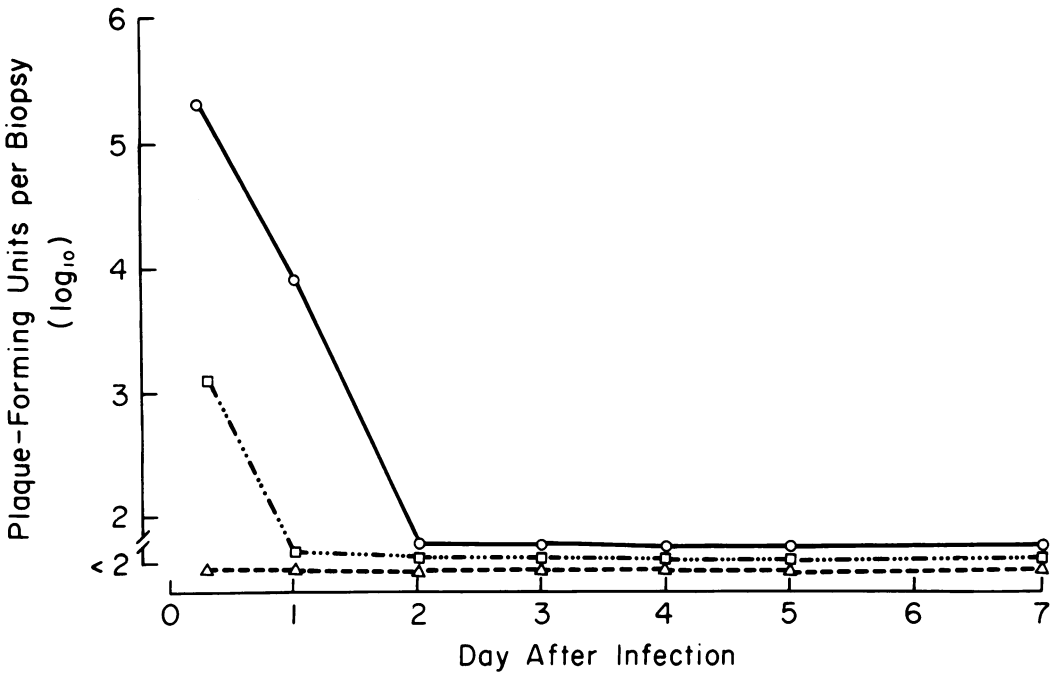

FIG. 5. *Dynamics of R. mooseri skin infection of immune guinea pigs. Symbols:* (○) *PFU per biopsy of high-dose inoculation sites,* (□) *PFU per biopsy of low-dose inoculation sites, and* (△) *PFU per biopsy of uninjected sites distant from inoculation sites.*

of *R. mooseri* infection at sites of inoculation in skin differed in a marked and reproducible fashion between nonimmune and immune guinea pigs. In normal guinea pig skin, rickettsiae established a progressive infection from 6 h after inoculation through day 3. In contrast, rickettsiae inoculated into the skin of immune guinea pigs declined in number and were cleared over the same time period. Gross and microscopic observations revealed a different pattern of responses to i.d. *R. mooseri* inoculation between normal and immune guinea pigs, although the cellular composition of the lesions during the period of rickettsial clearance was qualitatively similar in both instances.

Nonimmune animals. On the basis of these studies, i.d. *R. mooseri* infection of nonimmune guinea pigs may be divided into four sequential phases which encompass the conversion of a nonimmune animal into an animal with an acquired capacity to control *R. mooseri.*

Phase 1. Phase 1 comprised the interval between inoculation of rickettsiae and about 6 h after infection. The acute inflammatory response to the rickettsial inoculation, proportional to the number of viable organisms inoculated and consisting mainly of PMN and monocyte cells, and the failure of similar concentrations of normal yolk sac to induce an equivalent response (J. R. Murphy, unpublished data) indicate that a component of the rickettsial preparation induced a rapid and marked host response. Similar rapidly developing acute inflammatory responses have been demonstrated by others in guinea pig skin after i.d. infection (6), in abraded human skin exposed to purified rickettsial suspension (22), in the lungs after intranasal infection (11, 16), and in the peritoneal cavity and tunica vaginalis after intraperitoneal infection (17; Y. El Batawi, Ph.D. Thesis, University of Maryland, Baltimore, Md., 1963).

Phase 2. In the second phase, i.e., from 6 h through day 3, the surviving, presumably intracellular, rickettsiae multiplied in an apparently unrestricted manner in the face of an increasing inflammatory response which was composed almost exclusively of monocyte-macrophage-type cells and at a rate similar to that observed in vitro in cell culture (C. L. Wisseman, Jr., et al., manuscript in preparation).

The pattern of rickettsial growth demonstrated by the fluorescent-antibody technique suggested that *R. mooseri* were associated with the microvascular tissues in skin and that they accumulated in substantial numbers at these sites between 6 h and 3 days.

A similar pattern, i.e., increasing numbers of rickettsiae in areas of increasing monocyte-macrophage cell inflammation, had been demonstrated by Castaneda in guinea pig skin with *R.*

prowazeki (5), by El Batawi with peritoneal *R. mooseri* infection of the guinea pig (Ph.D. thesis), by Catanzaro et al. (7) with *R. tsutsugamushi* in mouse liver, and by Pinkerton (17) with *R. prowazeki* in peritoneal infection. Moreover, human monocyte-derived macrophages in cell culture are incapable of destroying virulent *R. mooseri* or *R. prowazeki* in the absence of antibody (2, 10). Thus, evidence from the present and other studies suggested that the accumulation of large MN cells, professional phagocytes, about foci of rickettsial infection did not herald in itself the development of a capacity to control infection during this period.

Phase 3. During the interval from days 3 through 5 after infection, the rickettsiae not only ceased to grow in the inoculation site but also declined markedly in titer. This local reduction in infection was (i) associated with a condensation of the hitherto diffuse MN cell infiltrate into discrete foci of more complex composition which included some lymphocytes (see phase 4 below), (ii) paralleled the development of a capacity to resist i.d. challenge at sites distant (back) from the site of primary infection (hind limb) (13), and (iii) occurred shortly before serum antibody was demonstrable (13). Thus, the development of a capacity to control *R. mooseri* infection in skin at sites of i.d. inoculation may have been a manifestation of the development of systemic immunity.

Phase 4. The fourth phase of i.d. *R. mooseri* infection in nonimmune guinea pig skin, days 5 through 7 (the last day of observation of the present study), was marked by continued condensation of the inflammatory response into discrete granulomatous foci and the persistence of a relatively stable but reduced number of rickettsiae. The cellular composition of these relatively condensed and at times perivascular lesions differed from the earlier predominantly monocyte-macrophage-type inflammation in that, although monocyte-macrophage cells remained the dominant cell type, lymphocytes, plasma cells, occasional eosinophils, and multinucleated cells were present.

Some caution is indicated in interpretation of the results of both the fluorescent-antibody studies and the titrations of the viable rickettsiae in the biopsies from the early stages of infection. It is likely (i) that less than 10% of the RLB contained in the inoculum were viable (14) and (ii) that less than 10 viable *R. mooseri* constitute one guinea pig i.d. 50% infective dose (14; C. L. Wisseman, Jr., et al., unpublished data). Thus, the bulk of the rickettsiae contained in the inoculum was nonviable but probably retained certain endotoxic and immunogenic properties.

By using the information available about the *R. mooseri* seed employed here (14), it is possible to calculate that each high-dose (8.2×10^6 PFU) inoculation site received approximately 3.9×10^8 RLB (viable and nonviable) and that each guinea pig received a total of approximately 12×10^8 RLB. These facts have an important bearing on certain aspects of lesion development, immune response, and interpretation of results, as follows.

(i) Although "toxic" actions of viable rickettsiae and possibly materials released as a result of rickettsia-host cell membrane interaction (3, 9, 21) may have contributed to the induction of the rapidly developing early inflammatory response, a major contributor to the initial inflammatory response could have been the endotoxic component present in the nonviable rickettsiae (20; C. L. Wisseman, Jr., et al., unpublished data). It is clear, however, that the phagocytic cells and other components of the acute inflammatory response are not capable of eliminating viable rickettsiae completely.

(ii) The cumulative mass of preformed *R. mooseri* antigens contained in the inoculum (12×10^8 RLB) exceeds the minimum dose of killed rickettsiae required to raise an antibody response in the guinea pig (C. L. Wisseman, Jr., et al., unpublished data; J. R. Murphy, Ph.D. Thesis, University of Maryland, Baltimore, Md., 1977). However, these numbers of rickettsiae appear not to sensitize the guinea pig for delayed-type hypersensitivity (L. Gluck, R. W. I. Kessel, and C. L. Wisseman, Jr., manuscript in preparation). It is clear, therefore, that the immunity raised during the course of those studies which employed high doses of *R. mooseri* may have resulted from either infection, immunization with nonliving rickettsiae, or both. The relative contributions of these potentially differing types of immune responses to the observed results is not known.

(iii) The bulk of the diffusely distributed RLB visible by the fluorescent-antibody technique in the early stages (probably at least through day 1) of lesion development most likely consisted of nonviable rickettsiae, and the observed early decline (phase 1 to 2) in numbers of visible RLB probably reflects clearance of these nonviable organisms by phagocytic host cells (see i above). These observations, therefore, do not indicate an efficient, nonspecific host defense mechanism against viable *R. mooseri* (see iv below). Instead, they probably reflect the anticipated capacity of the inflammatory response to deal with nonviable foreign materials. On the other hand, the distribution and apparent increase in numbers of RLB at and after day 2 probably reflect the

true infection pattern.

(iv) Because less than about 10 viable rickettsiae regularly infect 50% of the animals, these data strongly suggest that (a) *R. mooseri* is efficient in circumventing nonspecific defense mechanisms of the guinea pig and, conversely, (b) that the nonspecific mechanisms of the acute inflammatory response inherently are incapable of significant restriction of *R. mooseri* infection. Moreover, in vitro studies have shown that human macrophages do not restrict *R. mooseri* replication in the absence of antibodies (2, 10). Therefore, our failure to recover from the inoculation sites at 6 h more than a small fraction of the inoculated viable rickettsiae is not likely to be a reflection of killing of rickettsiae by nonspecific defense mechanisms. Rather, technical and other phenomena probably account for the apparent loss of viable rickettsiae—e.g., (a) rapid dissemination of rickettsiae on day 1 to skin sites distant from the point of inoculation and (b) interference with plaque formation by host cell fragments, presumably membranes in the homogenates (14, 23). Thus, although certain aspects of the early interactions between rickettsiae and host are at present uninterpretable, on a relative basis the subsequent increase and decline of rickettsiae (PFU) in the skin of the nonimmune animals and the progressive decline of rickettsiae in the immune animals probably reflect accurately the progression of infection and its control by host responses.

Immune animals. In contrast to the events described above, guinea pigs with immunity to *R. mooseri* acquired from a previous infection responded to i.d. inoculation of *R. mooseri* in a markedly different manner with respect to (i) the infectious process, (ii) the cytological dynamics of lesion formation, and (iii) the evolution and regression of the grossly observable lesion. The responses observed in immune guinea pigs were rapid, and a discrete sequential pattern was difficult to discern.

Thus, by comparison with the course of events in nonimmune guinea pigs, immune guinea pigs demonstrated, after i.d. inoculation with viable *R. mooseri,* an accelerated inflammatory response, enhanced in cell numbers, which progressed within 48 h from the early PMN-rich exudate at 6 h to discrete granulomatous perivascular foci, consisting mostly of cells of the monocyte-macrophage series but also containing a few PMN, lymphocytes, and eosinophils.

Despite the early accelerated PMN-dominant response and the presence of humoral antibodies, the number of viable rickettsiae at 6 h was comparable to that observed in the nonimmune animals. However, between 6 and 24 h, coincident with the progressive appearance of cells of the monocyte-macrophage series as well as a few lymphocytes in the inoculation site, the rickettsial titer dropped markedly and was below detectable levels by 48 h. Moreover, spread of rickettsiae appeared to be restricted because no rickettsiae were detected at skin sites distant from the point of inoculation.

Although this study has not directly identified the mechanism(s) of host control of rickettsiae in skin at sites of i.d. inoculation, the correlation between clearance of rickettsiae and the appearance of perivascular granulomatous lesions, a tissue response which, at times, has been associated with an expression of cell-mediated immunity (1, 15, 19), suggests that the thymus-dependent arm of the immune response may have contributed to control of this rickettsial infection. Furthermore a tissue response typical of Arthus-type antibody-mediated reactions was not observed (during primary infection) at the time that developing control of infection was demonstrated. Thus, the present study suggested a possible role for a cell-mediated immune mechanism, despite the fact that previous studies (13) failed to detect by skin test the development of delayed-type hypersensitivity. However, other studies in these laboratories (L. Gluck, R. W. I. Kessel, and C. L. Wisseman, Jr., manuscript in preparation) have demonstrated the development, during the course of *R. mooseri* infection in the guinea pig initiated by intraperitoneal inoculation, a serum factor which blocks the expression of delayed-type hypersensitivity.

The results of these and previous (13) studies suggest that the period of rapid elimination of rickettsiae from i.d. sites of inoculation has the following common features for both primary infection and second homologous challenge of an animal with established immunity: (i) presence of cells of the monocyte-macrophage series; (ii) the probable presence of humoral antibody; and (iii) lymphocytes. The relative contributions of these components of the host's immunological defenses to the clearance of rickettsiae from tissues is the subject of another report (J. R. Murphy, C. L. Wisseman, Jr., and P. Fiset, manuscript in preparation).

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