

## Characteristics of *Rickettsia mooseri* Infection of Normal and Immune Mice

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*Rickettsia mooseri* infection initiated by subcutaneous injection has been studied in BALB/c mice with the objective of developing a model for the study of immune mechanisms. Characterization of infection included the following: measurement of the replication, dissemination, and clearance of rickettsiae; measurement of correlates of the immune response, including humoral antibody, hypersensitivity to subcutaneously inoculated rickettsial antigen, and activation of nonspecific macrophage microbicidal capacity; and measurement of resistance to a second homologous challenge. Local infection at the site of subcutaneous injection progressed through day 5 and was controlled by day 7. Systemic infection as determined by the presence of rickettsiae in spleen was first detected on day 7 and progressed through day 14; however, rickettsiae persisted in this organ at reduced numbers through at least day 28. Control of the local infection at the site of subcutaneous injection occurred at about the time humoral antibodies and hypersensitivity reactions to subcutaneously injected rickettsial antigens became demonstrable and was paralleled by a capacity to resist homologous subcutaneous challenge at a site distant from that of the primary infection. Systemic infection progressed in spite of this acquired immune capacity and was controlled in the spleen in parallel with the development of enhanced macrophage microbicidal capacity in the liver. The results show that an acquired immunity is capable of restricting rickettsial growth at subcutaneous sites at a time when rickettsiae are increasing in titer in deep organs.

Typhus fever (*Rickettsia prowazekii* infection) of humans usually develops 10 or more days after exposure to this obligate intracellular bacterial parasite and, in the absence of specific antibiotic therapy, may persist for about 2 to 3 weeks thereafter (19). Cell-mediated immunity, measured as delayed hypersensitivity to *R. prowazekii* antigens, is regularly demonstrable in convalescence and may be present at the onset of disease, but is not demonstrable during the febrile disease (22). On the other hand, antibodies are not detectable until near the end of week 1 of disease (16), and the disseminated focal cutaneous vascular lesions begin to show the typical perivascular accumulation of dominantly mononuclear cells during this time (24). Nevertheless, rickettsiae are demonstrable microscopically in swollen endothelial cells from about 3 to 5 days until 13 to 15 days after onset (24) and by louse feeding between about days 4 and 13 after onset (3, 24). The reasons that the immune response fails to eliminate rickettsiae and limit disease more rapidly have not been explored.

In previous reports (6-9), we have used *Rickettsia mooseri* (*Rickettsia typhi*) infection of guinea pigs as a model of human typhus to address some of these questions. Guinea pigs rapidly (within 5 to 7 days) develop both anti-rickettsial antibodies and an antibody-independent cell-mediated capacity to eliminate rickettsiae from intradermal sites of infection. These capacities, however, do not prevent the later development of systemic infection and perivascular round cell infiltrates ("typhus nodules"). Our attempts to identify the mediators and effectors of the immune response and the mechanisms employed by the rickettsiae to avoid elimination were limited by the relative paucity of techniques for analyzing cellular defenses in guinea pigs. Because such techniques are more fully developed for mice, we

have characterized the course of infection and immune response of BALB/c mice to *R. mooseri* challenge to determine whether this is a suitable model for investigating immunity to typhus rickettsiae.

### MATERIALS AND METHODS

**Animals.** Female BALB/c mice weighing 18 to 22 g (Flow Laboratories, Dublin, Va.) were used for all experiments on immunity. ICR mice of both sexes, used for some infectivity titrations, were obtained from a colony at the University of Maryland at Baltimore (breeding stock obtained from the Trudeau Institute, Saranac Lake, N.Y.). Mice were provided with water and food (Purina Rodent Chow, Ralston Purina Co., St. Louis, Mo.) ad libitum.

**Rickettsiae.** The two seeds employed in this study were derived from a plaque-purified preparation of *R. mooseri* Wilmington which had been passaged: 42 times in yolk sacs, 3 times in tissue cultures, and 2 times again in yolk sacs (42E/3TC/2E). One seed was prepared by homogenizing infected yolk sacs of specific pathogen-free chicken eggs (SPAFAS, Norwich, Conn.) in 3.7% brain heart infusion broth (BHI) (BBL Microbiology Systems, Cockeysville, Md.) to produce a 20% (wt/vol) suspension (42E/3TC/3E). The other was prepared from homogenates of roller bottle cultures of infected L cells (ATCC929, CCL1.1) suspended in BHI (42E/3TC/3E/1TC). Convenient volumes of these were stored at -70°C in flame-sealed glass ampoules.

The conventional PFU titer, centrifuged PFU (cPFU) titer, and rickettsial body (RLB)/cPFU ratio (see below) of the yolk sac seed were  $1.48 \times 10^9$ /ml,  $7.29 \times 10^9$ /ml, and 1.84:1, respectively; those of the L cell seed were  $2.36 \times 10^7$ /ml,  $2.63 \times 10^8$ /ml, and 3.41:1, respectively. To minimize the possibility that culture medium and host cell contaminants might interfere with experiments, the two rickettsial seeds were used as follows. The L cell seed, diluted to working concentrations in BHI, was used for all immunizing

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infections. The yolk sac seed, diluted in sucrose-phosphate-glutamate solution (1) to working concentrations, was used for all challenge infections. This same yolk sac material was the source of antigens for hypersensitivity tests (see below).

**Collection and preparation of specimens.** Serum from blood obtained by cardiac puncture was stored at  $-20^{\circ}\text{C}$  until tested. Blood collected from the tail onto strips of Whatman no. 4 filter paper (0.5 by 3 in. [ca. 12.7 by 76.2 mm]) in a series of discrete drops was allowed to air dry and was then stored at  $-20^{\circ}\text{C}$  until tested. Spleens collected at a given interval from a group of similarly treated mice were pooled; 50% (wt/vol) homogenates in BHI, produced with a motor-driven Teflon pestle-glass tube homogenizer (VirTis, Gardiner, N.Y.), were stored at  $-70^{\circ}\text{C}$  until tested. Feet were dipped in 70% alcohol for 30 s, rinsed extensively in sterile saline, and then amputated at the ankle. Feet of mice within a group of similarly treated animals were pooled, minced with scissors, suspended in  $4^{\circ}\text{C}$  BHI, and homogenized with a VirTis tissue grinder to produce at 50% (wt/vol) suspension which was subjected to a low-speed centrifugation to remove debris. The resulting suspension was quick-frozen in a dry ice-alcohol mixture and stored at  $-70^{\circ}\text{C}$  until tested.

**Quantitation of rickettsiae.** The absolute RLB content in working seeds was determined by the method of Silverman et al. (18). A modification (10) of the chicken embryo cell plaque assay method of Wike et al. (20, 21) was used routinely to determine PFU. Since this method has a plaque efficiency of less than 1.0, a centrifuge method modified from that of Wike et al. (21) and Ormsbee et al. (14) was used to estimate the absolute number of infectious units (cPFU). When specimens contained bacterial contaminants or contained very low numbers of rickettsiae, rickettsial content was determined by mouse titration. One milliliter of undiluted or of serial 10-fold dilutions of homogenates of mouse feet and some mouse spleens was inoculated intraperitoneally (i.p.) into ICR mice (five per dilution). Seroconversion from  $<1:40$  to  $\geq 1:40$  in the indirect fluorescent antibody test (see below) at 28 days after inoculation was used to calculate the mouse i.p. 50% infectious dose by the method of Reed and Muench (17). The i.p.  $\text{ID}_{50}$  of the Wilmington strain of *R. mooseri* for outbred Swiss mice was found by Ormsbee et al. (14) to be 2 cPFU.

**Mouse s.c.  $\text{ID}_{50}$  titrations.** Groups of female BALB/c mice (10 per dilution) were inoculated subcutaneously (s.c.) in the left hind footpad with decimal dilutions of the L cell seed in a volume of 0.05 ml. The mouse s.c.  $\text{ID}_{50}$  was calculated on the basis of seroconversions as above.

**Serological procedures.** Anti-*R. mooseri* antibodies were measured by an indirect fluorescent antibody test which drew upon the experience of Elisberg and Bozeman (4) and introduced the use of antigen slides bearing acetone-fixed infected chicken embryo cells containing 5 to 15 intracellular rickettsiae. Sera were serially diluted in phosphate-buffered saline (PBS) (pH 7.4) containing 10% normal yolk sac homogenate (wt/vol) (PBS-NYS). Blood collected on Whatman no. 4 filter paper was eluted from 3-mm disks in a ratio of 0.025 ml of PBS-NYS per disk to give an initial dilution of about 1:40. The test was performed by conventional indirect procedures, using fluorescein-conjugated anti-mouse immunoglobulin (Cappel Laboratories, Downingtown, Pa.) diluted to working concentration in PBS-NYS containing 0.1% Evans blue. Fluorescence of discrete morphologically identifiable intracytoplasmic rickettsiae was scored as positive.

***R. mooseri* antigen.** Purified *R. mooseri* particulate antigen was prepared from a 20% (wt/vol) yolk sac suspension in

BHI by a method recently described by Hanson et al. (5). The purified rickettsiae suspended in PBS containing 0.2% Formalin were adjusted to a concentration of 1 mg (dry weight)/ml and stored at  $4$  to  $8^{\circ}\text{C}$ . Before use, the antigen was washed three times in PBS to remove soluble Formalin and adjusted with PBS to the desired concentration.

**Hypersensitivity tests.** *R. mooseri* particulate antigen (20  $\mu\text{g}$  in 0.05 ml of PBS) was injected s.c. into the plantar surface of the right hind footpad, the thickness of which was measured before injection and at 6 and 24 h after injection, using dial gauge calipers. Formalin-inactivated *R. mooseri* antigen caused a variable degree of foot swelling when inoculated into normal BALB/c mice; hence, normal mice were inoculated with antigen in parallel with each of the treatment groups. The difference between the increased foot thickness in *R. mooseri*-infected mice, assumed to result from nonspecific swelling plus the specific response to the antigen, and that of normal mice, assumed to result from nonspecific swelling only, is designated as the specific increase in foot thickness and is considered to be a measure of specific hypersensitivity to the antigen.

**Foot swelling reaction to viable *R. mooseri*.** Changes in foot thickness of normal mice or immune mice after challenge in the plantar surface with viable *R. mooseri* were determined by using dial gauge calipers. Foot thickness was measured before and at times after inoculation, and the differences in these measurements were recorded (see Fig. 1).

**Assay of macrophage bactericidal activity.** Changes in macrophage-effected bactericidal capacity were measured as changes in nonspecific resistance to *Listeria monocytogenes* (13). *L. monocytogenes* EGD, obtained from Maurice J. Lefford, Trudeau Institute, Saranac Lake, N.Y., was grown in Trypticase soy broth (BBL Microbiology Systems) and stored at  $-70^{\circ}\text{C}$ . Before use, a culture was thawed and diluted in sterile saline to  $5 \times 10^5$  CFU/ml, and 0.2 ml ( $10^5$  CFU) was injected intravenously (i.v.). After 24 h, the livers were removed and separately homogenized with a motor-driven Teflon pestle-glass tube homogenizer. Viable counts of *L. monocytogenes* were obtained by inoculating dilutions of organ homogenate on Trypticase soy agar and counting colonies after overnight incubation at  $37^{\circ}\text{C}$ . The geometric mean of the viable counts was obtained for each group, and the difference between the means of *R. mooseri*-infected and normal mice was used as a measure (resistance index) of macrophage-effected resistance to *L. monocytogenes* (13).

## RESULTS

**Infectivity of *R. mooseri* for BALB/c mice.** The susceptibility of BALB/c mice to *R. mooseri* was tested by s.c. inoculation of decimal dilutions of the L cell seed into the left hind footpad and determination of serological conversion 28 days later. As measured by the centrifugation plaque assay, 2.30 viable rickettsiae comprised 1 s.c.  $\text{ID}_{50}$ .

**Selection of a model infection.** Groups of mice were inoculated as above with graded doses of *R. mooseri* and were bled at intervals through 28 days. Table 1 shows that 100% of the mice inoculated with  $10^4$  PFU developed antibody by day 9 of infection. Seroconversion in groups receiving smaller doses was slower and less synchronous.

The uniform seroconversion of mice challenged with  $10^4$  PFU suggested that infections initiated with this dose would be suitable as a model for immunological studies. However, the possibility existed that the antibody response to this dose may have been due to the antigenic mass of the total number of rickettsiae inoculated (live plus dead) and not just to the infection ( $10^4$  PFU =  $3.80 \times 10^5$  RLB or about 0.026  $\mu\text{g}$  [dry

weight] of organisms [14]). Therefore, groups of mice which were inoculated s.c. in the left hind footpad with doses of Formalin-killed *R. mooseri* antigen were bled at weekly intervals. The minimum dose of killed antigen which elicited an antibody response by day 28 was 0.1  $\mu\text{g}$  ( $1.47 \times 10^6$  RLB), and this resulted in only a 30% seroconversion (Table 2). Thus, the antibody response (Table 1) after inoculation of  $10^4$  PFU of *R. mooseri* (0.026  $\mu\text{g}$ ) was in response to infection.

**Relationship between infection and aspects of the host response.** Figure 1 shows that *R. mooseri* infection progressed and resolved rapidly (e.g., within 7 days) at the s.c. site of challenge, but splenic infection developed later and progressed more slowly, with maximum titers of rickettsiae at day 14, followed by a 10-fold reduction by day 21 and a persistence of this number of rickettsiae through at least day 28 (Fig. 1A). By day 7, when the s.c. infection at the site of challenge had been controlled, mice possessed a capacity to resist s.c. challenges delivered at distant sites (Fig. 1B); mice also had serum antibody and evidence of immediate and delayed hypersensitivity to *R. mooseri* antigens (Fig. 1C). All of these measures of immunity persisted through at least day 28, but their early development did not prevent the progress of splenic infection through day 14. Partial control of *R. mooseri* in the spleen was paralleled by a transient enhanced nonspecific macrophage microbicidal activity in the liver (Fig. 1D).

## DISCUSSION

This study shows that within 1 week of the s.c. introduction of a relatively small number of rickettsiae, BALB/c mice have an enhanced capacity to resist a homologous challenge delivered to a site distant from that employed for the primary infection. This observation, coupled with the evidence that infection at sites of primary infection is controlled by day 5 and the demonstration in vivo on day 7 of significant levels of hypersensitivity reactions to injections of killed *R. mooseri* and in vitro of anti-*R. mooseri* antibody, albeit in low titer, give credence to the proposition that these obligate intracellular bacterial parasites are strongly immunogenic and rapidly cause the generation of a protective systemically disseminated anti-*R. mooseri* immunological defense.

The kinetics of the development and deployment of this antibacterial resistance mechanism are not unusually rapid if viewed from the perspective of the dynamics of the immune response to some facultative intracellular bacteria. For ex-

TABLE 2. Dynamics of the antibody response to s.c. inoculation of increasing doses of Formalin-killed *R. mooseri* (yolk sac seed, ICR mice)

| Dose<br>$\mu\text{g}^a$ | RLB <sup>b</sup>   | % Seroconversion <sup>c</sup> on day after inoculation: |     |     |     |     |
|-------------------------|--------------------|---------------------------------------------------------|-----|-----|-----|-----|
|                         |                    | 0                                                       | 7   | 14  | 21  | 28  |
| 30                      | $4.41 \times 10^8$ | 0                                                       | 100 | 100 | 100 | 100 |
| 10                      | $1.47 \times 10^8$ | 0                                                       | 100 | 100 | 100 | 100 |
| 3                       | $4.41 \times 10^7$ | 0                                                       | 100 | 100 | 100 | 100 |
| 1                       | $1.47 \times 10^7$ | 0                                                       | 100 | 100 | 100 | 100 |
| 0.1                     | $1.47 \times 10^6$ | 0                                                       | 0   | 0   | 30  | 30  |
| 0.01                    | $1.47 \times 10^5$ | 0                                                       | 0   | 0   | 0   | 0   |
| None                    |                    | 0                                                       | 0   | 0   | 0   | 0   |

<sup>a</sup> Dry weight.

<sup>b</sup> Calculated by using a value of  $1.47 \times 10^7$  RLB/ $\mu\text{g}$  as determined by Ormsbee et al. (14).

<sup>c</sup> Animals with an indirect fluorescent antibody titer of  $\geq 1:40$  (five mice per group).

ample, the T cells which are the mediators of immunity to *L. monocytogenes* are generated in large numbers by days 4 to 6 of infection (12), and available evidence supports the view that at least one of the rapidly produced mediators of immunity to *R. mooseri* is a T cell (Murphy, Ph.D. thesis, University of Maryland, Baltimore, 1976), possibly one which is phenotypically similar to that which mediates the anti-*L. monocytogenes* response. However, unlike the anti-*L. monocytogenes* response, which rapidly provides systemic resistance to this bacterium, the rapidly developing antirickettsial defense mechanism appears to be of limited efficacy in that, whereas clearance of rickettsiae from sites of primary inoculation or of distant s.c. second challenge correlates with this rapidly developing immunity, systemic infection, measured in this study as rickettsiae in the spleen, develops and progresses in the face of the early immune response. We emphasize this discordance of the development and expression of immunity between sites of infection because it is possible that such a segregation of acquisition and expression of immunity may play an important role in the pathogenesis of rickettsial infections of humans. Direct or inferential evidence supports the view that rickettsiae grow at a skin site of initiation of infection for a relatively short time, after which they are controlled or eliminated (6). During the latter part of this initial sequence or relatively soon after its completion, systemic infection and overt disease occur. For example, it is clear that overt clinical typhus fevers develop and persist in the face of building or mature immune responses (6, 19, 22–24). Although the precise dynamics of the infectious processes and the immune responses may differ for each organism, it may be that this phenomenon holds in principle for infections caused by other members of the genus *Rickettsia*. These observations raise questions of the capacities and limitations of immunological defenses to rickettsial infections, and, because the primary pathological lesion of these infections is a round cell perivascular infiltrate (typhus nodule), some have questioned whether there is an immunological component to this pathology (23). Resolution of these points is of central importance to the design of appropriate immunoprophylactic procedures and of therapy regimens. Studies of the relationship between course of *R. mooseri* infection and immune response in guinea pigs (6–9) and rats (S. Arango-Jaramillo, Ph.D. thesis, University of Maryland, Baltimore, 1979) provide further evidence of the similarities among mammals of the relationship between course of infection and immune response as cited above.

TABLE 1. Dynamics of the antibody response after s.c. inoculation of increasing doses of live *R. mooseri* (L cell seed, BALB/c mice)

| Dose of rickettsiae <sup>a</sup><br>(PFU) | Seroconversion <sup>b</sup> on day after infection: |     |     |     |     |     |
|-------------------------------------------|-----------------------------------------------------|-----|-----|-----|-----|-----|
|                                           | 0–3–6                                               | 9   | 12  | 15  | 21  | 28  |
| $10^4$                                    | 0                                                   | 100 | 100 | 100 | 100 | 100 |
| $10^3$                                    | 0                                                   | 0   | 80  | 90  | 100 | 100 |
| $10^2$                                    | 0                                                   | 0   | 77  | 88  | 88  | 100 |
| 10                                        | 0                                                   | 0   | 0   | 55  | 77  | 88  |
| 1                                         | 0                                                   | 0   | 0   | 10  | 50  | 80  |
| $10^{-1}$                                 | 0                                                   | 0   | 0   | 0   | 0   | 30  |
| $10^{-2}$                                 | 0                                                   | 0   | 0   | 0   | 0   | 0   |
| None                                      | 0                                                   | 0   | 0   | 0   | 0   | 0   |

<sup>a</sup> Conventional plaque count; for this seed, 1 PFU = 11.1 cPFU = 38.0 RLB.

<sup>b</sup> Animals with an indirect fluorescent antibody titer of  $\geq 1:40$  (10 mice per group).

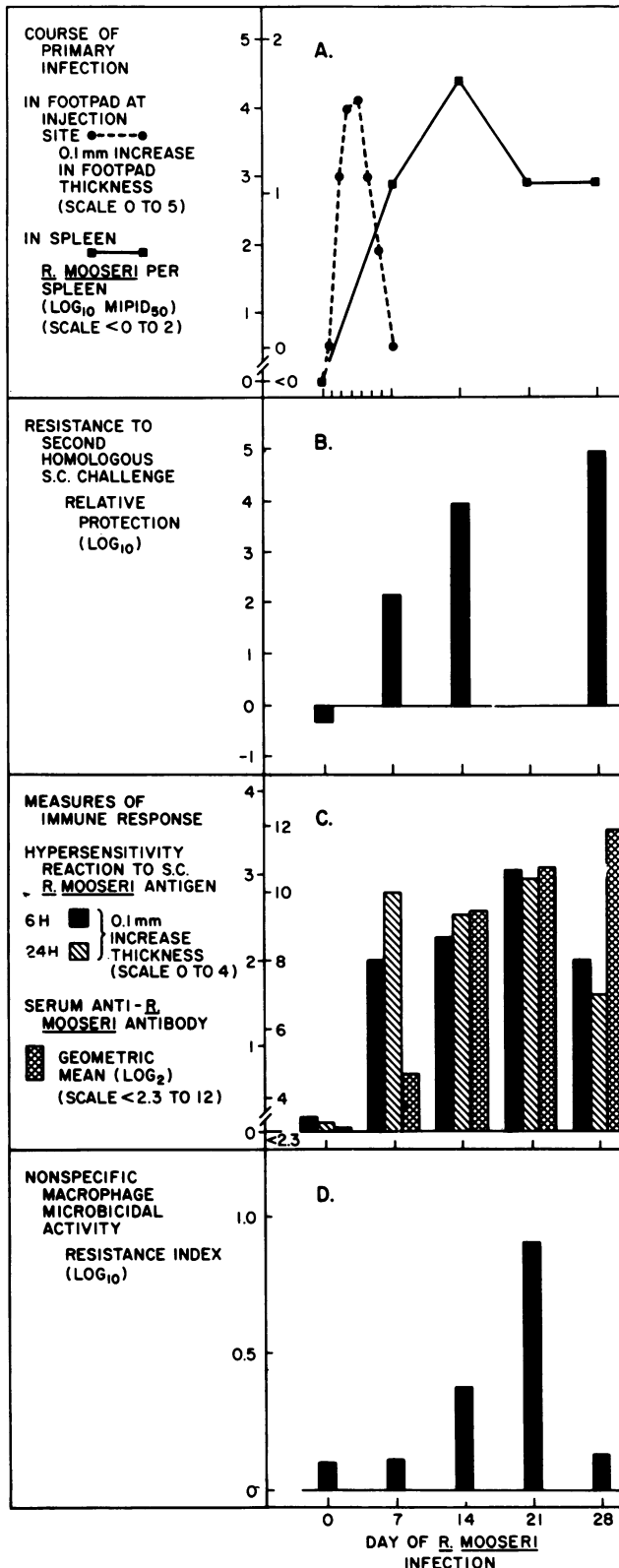


FIG. 1. Course of *R. mooseri* infection of BALB/c mice and its relationship to aspects of the host response. In all experiments, primary infection was initiated by the s.c. injection of rickettsiae into the plantar surface of the left hind footpad; the infectious challenge contained 10<sup>4</sup> PFU of *R. mooseri* except in the experiment in (A), in which a challenge of 10<sup>5</sup> PFU was used to follow the local

In this study, we attempted to correlate the expression of immunity measured as clearance of rickettsiae or control of rickettsial growth rate with selected parameters of immune responsiveness in an effort to explain the asynchronous expression of immunity between sites of infection. The degree of systemic protective immunity measured as inhibition of rickettsial growth at sites of second challenge (Fig. 1B) was found to increase markedly during the interval from day 7 to day 28 after primary infection. This increase in degree of immunity paralleled an increase in serum anti-*R. mooseri* antibodies and was not directly related to the magnitude of 24-h hypersensitivity to killed *R. mooseri* antigen, thus suggesting that antibody titer as measured by the serological test might be correlated with the degree of immunity. This relationship has been observed previously in studies of *R. mooseri* infection of guinea pigs (9), but attempts (Murphy and Wissemann, Abstr. Annu. Meet. Am. Soc. Microbiol. 1976, D52, p.60) to prove that humoral factors were responsible for rapid elimination of rickettsiae from sites of challenge yielded negative results. In contrast, direct evidence was obtained showing that control of infection at challenge sites in guinea pig skin was lymphocyte mediated (8). If a similar, presumably T cell-mediated, mechanism is functioning in mice, it seems reasonable to suggest that there should have been a direct rather than the observed relationship between 24-h hypersensitivity and degree of protection from footpad challenge as is seen in parallel with the expression of T cell-mediated defenses to some other bacteria (11). Although an explanation for the dissociation between degree of 24-h hypersensitivity and protection is not available from these studies, it should be noted that guinea pigs, unlike mice, express their lymphocyte-mediated defense to local *R. mooseri* infection in the complete absence of a 24-h hypersensitivity response (6). Moreover, although the cellular components of the delayed hypersensitivity reaction have been shown to include those responsible for the activation of macrophages for enhanced microbicidal action (15), there is evidence that such reactions may not always reflect cell-mediated antibacterial immunity (2).

infection at the injection site. (A) The course of infection at s.c. sites of injection and in the spleen. The splenic infection is presented directly as PFU per spleen (five mice per data point); the local infection in the footpad was monitored by measuring the swelling of the foot. Previous studies (6-8) demonstrated that local reactions are indications of rickettsial growth and killing. Significant ( $P \leq 0.01$ ) increases in footpad thickness were observed at days 2 to 6 but not at days 1 and 7 (five mice per data point). (B) Demonstration that mice develop by day 7 and retain thereafter at least through day 28 of infection an enhanced capacity to restrict the growth of a homologous challenge of 10<sup>5</sup> PFU delivered to a site distant from that used for the primary infection (the right hind footpad). Relative protection = log<sub>10</sub> i.p. ID<sub>50</sub> (C) - log<sub>10</sub> i.p. ID<sub>50</sub> (T), where C is the number of mouse i.p. ID<sub>50</sub> per foot of *R. mooseri*-inoculated normal mice, and T is the number of mouse i.p. ID<sub>50</sub> per foot after second challenge (measured 72 h after the 10<sup>5</sup>-PFU challenge) of mice previously infected with *R. mooseri*. There were four mice per group per time point. (C) Relationship of measures of the anti-*R. mooseri* immune response to the course of infection. Foot swelling reactions at 6 and 24 h after s.c. injection of Formalin-killed *R. mooseri* and serum indirect fluorescent antibody titers are shown. There were five mice per time point. The hypersensitivity responses on days 7 to 28 were statistically significant ( $P \leq 0.01$ ). (D) Demonstration of enhanced nonspecific macrophage-mediated microbicidal activity in the liver on days 14 ( $P \leq 0.05$ ) and 21 ( $P \leq 0.01$ ) of *R. mooseri* infection. There were five mice per group.

Attempts to correlate the number of *R. mooseri* in spleen with serum antibody as measured by the indirect fluorescent antibody test or 6-h or 24-h hypersensitivity responses failed to establish a direct relationship between any of these correlates of immunity and the number of rickettsiae. Therefore, the studies were extended to include the measurement of nonspecific macrophage microbicidal activity. A direct relationship among appearance in liver of macrophages with enhanced microbicidal capacities, the arrest of the progression of infection in spleen, and a subsequent reduction in rickettsial numbers was demonstrated. However, after infection was controlled, a reduced number of rickettsiae persisted in the spleen in the absence of activated macrophages. Whether the observed relationship between control of splenic infection and the appearance of activated macrophages is causal or coincidental remains to be determined.

Naturally acquired rickettsial infections often result from contamination of skin of a susceptible host with arthropod-borne rickettsiae. These rickettsiae appear to replicate locally before systemic dissemination and appear to show, in every instance where detailed analysis has been conducted, that the complex interrelationships with the immune response are as summarized above. We have attempted to mimic this natural sequence in a manner sensitive to laboratory analysis by introducing in this and previous (6-9) studies relatively small numbers of rickettsiae by peripheral routes. This results in a sequential evolution of infection and immune response (Fig. 1) (6). In contrast, we also have demonstrated that the i.v. or i.p. routes of injection of rickettsiae (6; Y. A. El Batawi, Ph.D. thesis, University of Maryland, Baltimore, 1964), which are less natural routes, result in infections which present a markedly different manner and produce immune response with different dynamics. Our cumulative experience suggests that laboratory models can be selected which might artificially amplify or mask the significance of aspects of the host-parasite interaction, including immunological defense mechanisms. The basis of our selection of the model based on peripheral injection of relatively small numbers of rickettsiae, addressed in detail herein, is our belief that, of the systems we have tested, it most faithfully represents the sequence of events which occur in naturally acquired rickettsial infections.

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