

Mechanisms of Immunity in Typhus Infections

III. Influence of Human Immune Serum and Complement on the Fate of *Rickettsia mooseri* Within Human Macrophages

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Preincubation of *Rickettsia mooseri* with human typhus convalescent serum, which is not rickettsiicidal but which confers passive protection to animals, opsonizes the rickettsiae for enhanced phagocytosis by monocyte-derived human macrophages in cell culture and renders them susceptible to destruction within the macrophages. Nonspecific opsonization by preincubation of the rickettsia with methylated bovine serum albumin enhances phagocytosis, but the rickettsiae are not prepared for intracellular destruction. Instead, they grow within the macrophages and eventually destroy these cells. Thus, immune serum and macrophages, neither of which is capable of killing these rickettsiae alone, act in concert to destroy the virulent organisms. In this system, immune serum appears to exert two distinct, possibly dissociable, actions on the rickettsiae: enhancement of phagocytosis and preparation for intracellular destruction. Complement is not required for this action but, when present with immune serum, markedly enhances phagocytosis of the rickettsiae, often leading to rapid destruction of the macrophage.

Serum from persons convalescent from typhus infection, and resistant to disease upon reexposure to the agent, (i) protects mice on passive transfer against an otherwise lethal typhus infection (C. L. Wisseman, Jr., et al., submitted for publication) and (ii) opsonizes rickettsiae for enhanced phagocytosis (29, 31; C. L. Wisseman, Jr., D. W. Krause, I. B. Fabrikant, and P. A. Machowiak, *J. Infect. Dis.*, submitted for publication), but (iii) fails to render the rickettsia noninfectious for the yolk sac of the embryonated hen egg, even when fortified with complement, i.e., it has no direct rickettsiicidal action (C. L. Wisseman, Jr., et al., submitted for publication). The preceding paper (12) demonstrated that macrophages derived in cell culture from mononuclear cells of human blood, in the presence of normal human serum, did not destroy virulent typhus rickettsiae. Instead, these organisms multiplied freely within the macrophages and eventually destroyed them. Although human immune serum opsonizes typhus rickettsiae for both polymorphonuclear and mononuclear leukocytes (29, 31; Wisseman et al., *J. Infect. Dis.*, submitted for publication), the fate of such opsonized typhus

rickettsiae within the phagocytic cells has not yet been determined. This report is concerned with the interaction in vitro between *Rickettsia mooseri* (*R. typhi*) and macrophages derived from normal and typhus-immune human subjects, in the presence of normal or immune human serum.

MATERIALS AND METHODS

General. The rickettsial suspensions and macrophage cultures from human peripheral blood were prepared as described in the preceding paper (12).

Sera. Normal human serum was collected from healthy male medical students having no history of previous epidemic or murine typhus infection or vaccination. Their sera did not fix complement in the presence of typhus "soluble" antigen.

Immune serum was collected from laboratory personnel who had a history of epidemic or murine typhus infection and whose sera contained complement-fixing antibodies for typhus-soluble antigen. Most of the studies were done with serum from one hyperimmune donor (CLW). Serological and protective properties of serum from this subject have been described in detail elsewhere (C. L. Wisseman, Jr., et al., submitted for publication).

Complement. Complement (C') was prepared from normal Hartley strain guinea pigs bled by cardiac puncture every 10 to 20 days for several months. The serum was separated without delay and immediately frozen in a dry ice-alcohol bath. The sera were pooled,

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checked for sterility and CF antibodies to soluble typhus antigen, refrozen, and stored at -70°C .

Fresh normal human serum (NHS) for use in complement experiments was collected from normal donors, pooled, and frozen in a dry ice-alcohol bath on the day of collection.

Complement activity of these preparations was measured by a microtiter system employing a 50% hemolytic end point ($\text{C}'\text{H}_{50}$) (22). The $\text{C}'\text{H}_{50}$ for the guinea pig complement preparation (GPS) was 1:570 and, for the human complement preparation (fresh NHS), 1:140.

Serum content of media. The medium (Eagle basic medium [BME]), used for both preincubation of rickettsiae with serum and for maintenance of the cell cultures, always contained a final serum concentration of 30%. In experiments with complement, the medium contained 30% of a 1:1 mixture of complement and serum.

Other preparations. Methylated bovine serum albumin (MBSA) and bovine serum albumin (BSA) were prepared in distilled water as 20 mg/ml solutions. When these compounds were used, serum was added to the infecting medium after the rickettsiae had been incubated with the albumin compounds for 30 min.

Statistical analysis. Ninety-five percent confidence limits were determined as previously described (12). Due to the wide variation in cell numbers between Leighton tubes, the nonparametric rank sum test (10) was applied to cell counts of a given area of cover slips in order to determine if the experimental group data (day 1 cover slips) were consistent with the null hypothesis. The P values are given in Tables 2 and 3 along with other data.

RESULTS

Inhibition by immune serum of *R. mooseri* growth in "nonimmune" human macrophages in cell culture. When *R. mooseri* was first incubated for 30 min at room temperature in medium containing serum from a human subject convalescent from murine typhus and was then added to macrophage cell cultures derived from a nonimmune subject, the rickettsiae were consistently taken up by the cells in greater numbers than in similar cultures exposed to *R. mooseri* which had been preincubated in normal human serum. Upon continued incubation of the culture in the presence of medium containing the same serum as was present in the preinfection mixture, i.e., immune or normal, two divergent patterns were clearly and consistently recognized (Fig. 1). Rickettsiae, which had been preincubated with normal serum and which were then introduced into macrophage cultures maintained on normal serum, multiplied within the macrophages and destroyed them, as described in the previous paper (12). In contrast, rickettsiae which had been preincubated with immune serum (IS)

failed to grow and, in fact, disappeared from most of the cells within a day or two, as shown in the first two graphs in Fig. 1. The results were the same regardless of whether fresh or heat-inactivated IS was used. Thus, a combination of typhus-immune human serum and macrophages derived from a nonimmune subject, neither of which is capable of killing virulent *R. mooseri* alone, effectively and rapidly destroyed the rickettsiae. In the rare cell, however, the rickettsiae did multiply, attaining essentially the same growth rate as in the normal serum control after a short lag (graph 3 in Fig. 1). To determine to what extent the intracellular destruction of the rickettsiae depends upon reaction of the organisms with IS prior to entry into the macrophage as opposed to the continued presence of IS in the cell culture medium bathing the infected macrophages during the subsequent incubation period, a second series of experiments was performed (Fig. 2). In these experiments, IS was present in the medium, along with the rickettsiae, (i) during the preinoculation incubation period only, (ii) in the culture medium added only after the cells were infected, or (iii) in both. The results clearly show that the rickettsiae must be reacted with IS prior to entry into the macrophage for the intracellular destruction to occur.

Titration of antirickettsial action of immune serum. A single experiment was performed to determine if the sensitizing action of IS for subsequent intracellular destruction of the rickettsiae could be titrated in a serum dilution "neutralization" type of test. The standard dose of *R. mooseri* was incubated at room temperature for 30 min with fourfold dilutions in BME of the same IS which had been used in all of the experiments thus far described, inoculated into macrophage cell cultures, allowed to adsorb for 2 h, and then incubated and observed as in previous experiments. A very definite relationship between IS concentration and inhibition of growth was observed, but the number of replicates possible with the cells obtained from a single bleeding of one donor was too small to permit the demonstration of a clearcut end point.

Survey of typhus convalescent human sera for antirickettsial action in normal human macrophages. Sera from several human subjects who had experienced either murine or epidemic typhus infections, all with early (1-2 days of disease) chemotherapy, at variable periods of time in the past were examined for their capacity to sensitize *R. mooseri* for destruction or inhibition of growth within macrophage cell cultures derived from a normal

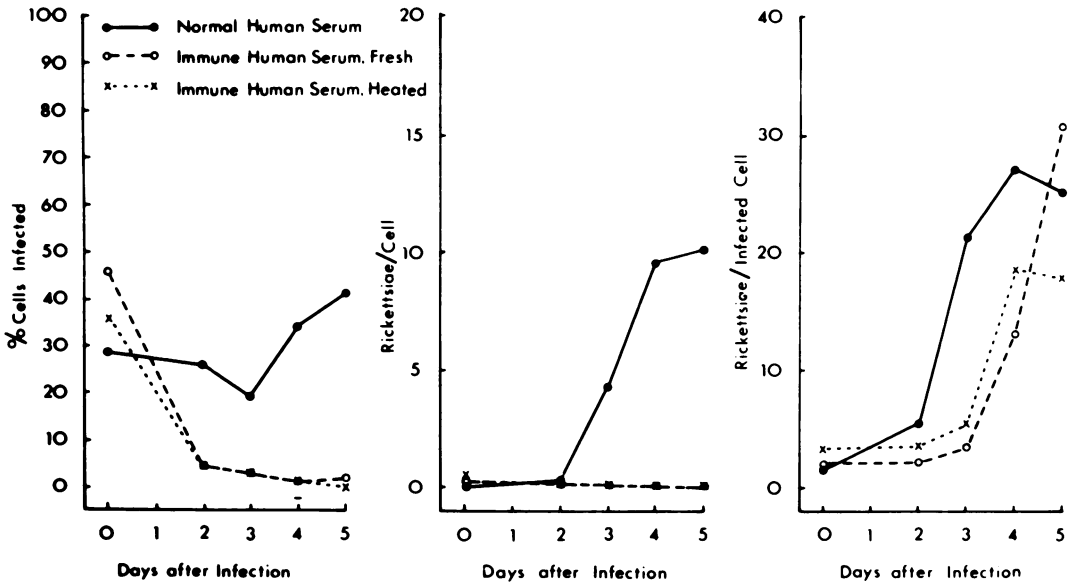


FIG. 1. Destruction by macrophages derived from nonimmune subject of *R. mooseri* sensitized with fresh or heat-inactivated human typhus-immune serum. The rickettsiae were incubated with the sera prior to inoculation into the macrophage cultures, and the corresponding sera were continuously present subsequently in the tissue culture medium.

human subject. The results, in the form of indices comparing each of the three values previously established on day 4 with those obtained on day 1 after infection, are recorded in Table 1. They show that typhus convalescent sera are inhibitory as compared with the NHS control and that there is a suggestion that some degree of specificity is exhibited. The greatest and most important effect was the change in number of rickettsiae per cell despite the fact that the use of the day 1 (24 h) value instead of the day 0 (2 h) value tends to minimize the magnitude of the antiserum effect.

Serum from two of the murine typhus convalescents (numbers 2 and 3) and from the two epidemic typhus convalescents (numbers 9 and 10) had been tested in an earlier study (C. L. Wisseman, Jr., et al., submitted for publication) for their capacity to confer passive protection on normal mice against lethal *R. mooseri* infection and had shown similar trends in specificity, i.e., a high degree of protection in the case of the murine typhus convalescent sera and a substantially lower degree of protection with the epidemic typhus convalescent sera. In neither the in vivo mouse system nor the in vitro macrophage system was there any evidence for a regular correlation with the typhus group-specific, complement-fixing antibody titer.

Effect of nonspecific enhancement of phagocytosis on fate of typhus rickettsiae within nonimmune macrophages. Because *R.*

mooseri may be capable of active penetration into cells as has been described for *R. tsutsugamushi* (7), two alternative mechanisms for the entry of typhus rickettsiae into macrophages in culture are theoretically possible: (i) passively by phagocytosis, and (ii) actively by penetration. To determine if a typhus organism gaining entrance into a macrophage by phagocytosis is automatically destined for intracellular destruction, *R. mooseri* were incubated with MBSA, a basic protein which nonspecifically enhances phagocytosis like certain other basic proteins (21), instead of with IS, prior to inoculation onto macrophage cell cultures. Figure 3 compares the results obtained with MBSA with those obtained with NHS and IS. Rickettsiae pretreated with NHS behaved as expected, i.e., they gained entrance to macrophages and multiplied. Rickettsiae pretreated with IS were taken up in greater numbers by a somewhat greater proportion of the macrophages than in the case of NHS, a manifestation of the opsonizing action of IS, but a large proportion of the cells quickly destroyed the rickettsiae which had gained entrance. At a concentration of 0.1 mg/ml, MBSA also greatly enhanced the uptake of rickettsiae, both in the percentage of cells taking up rickettsiae and in the number of organisms per cell. Indeed, the effect of this MBSA concentration on the uptake of rickettsiae was substantially greater than the particular IS used in this experiment. The higher

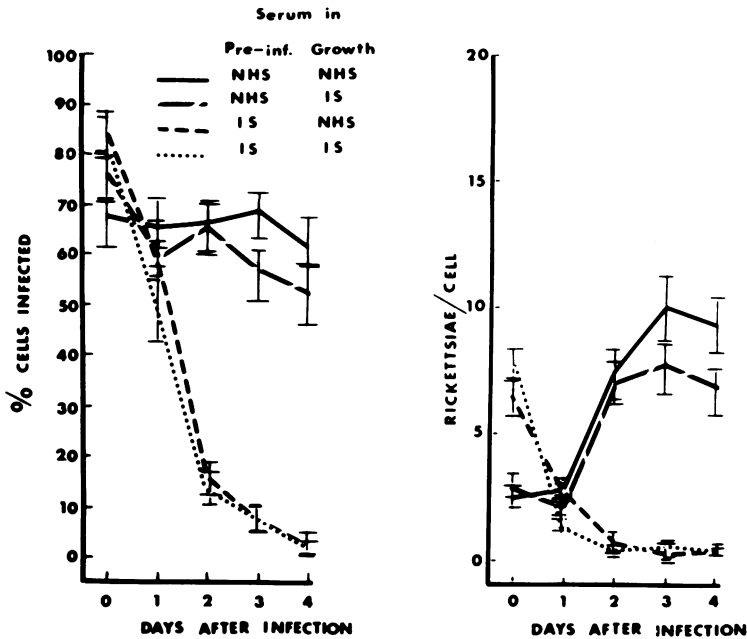


FIG. 2. Dependence of intracellular destruction of *R. mooseri* on reaction of rickettsiae with typhus-immune serum prior to entry into macrophages from a nonimmune human subject. Immune serum introduced into culture medium after rickettsiae have entered the macrophages has no influence on their intracellular growth. Abbreviations: NHS, normal human serum; IS, typhus immune human serum.

TABLE 1. Survey of typhus convalescent human sera for capacity to sensitize *R. mooseri* for antirickettsial action within normal human macrophages^a

Serum		Diagnosis ^b	Complement fixation titer ^c	Indices of fate in macrophages ^d		
No.	Code			Cells infected (%)	Rickettsiae/cell	Rickettsiae/infected cell
1	NHS	Not typhus	<2	1.28	10.4	7.9
2	CLW	MT	64	0.42	2.4	5.9
3	DK	MT	64	0.67	4.0	6.1
4	PR	MT	16	0.77	3.3	4.4
5	MG	MT	8	0.93	4.3	4.6
6	MP	MT	8	0.68	3.9	6.0
7	ZR	MT	8	0.76	4.3	5.7
8	LS	MT?	4	0.77	7.4	9.2
9	RC	ET	8	0.91	8.2	8.5
10	BS	ET	4	0.98	9.7	9.6

^a *R. mooseri* suspension was incubated for 30 min at room temperature in BME containing 30% of the indicated serum and was then allowed to adsorb for 2 h on normal human macrophage cell cultures in Leighton tubes. This mixture was then replaced with BME-30 containing normal serum and incubated at 37 C. Four cover slips from each serum were stained and examined from day 1 and day 4 of incubation.

^b MT, murine typhus; ET, epidemic typhus.

^c Reciprocal dilutions with 8 units of typhus soluble (Group) antigen.

^d Ratio of day 4 value to day 1 value.

MBSA concentration (1.0 mg/ml) was somewhat toxic for the cells, resulting in a lower uptake of rickettsiae more comparable to that with NHS-treated organisms. Despite the enhanced phagocytosis, however, the MBSA-

treated rickettsiae were not destroyed by the macrophages, but instead multiplied in the macrophages at about the same rate as did those treated with NHS. The results of this series of experiments strongly suggest that entry

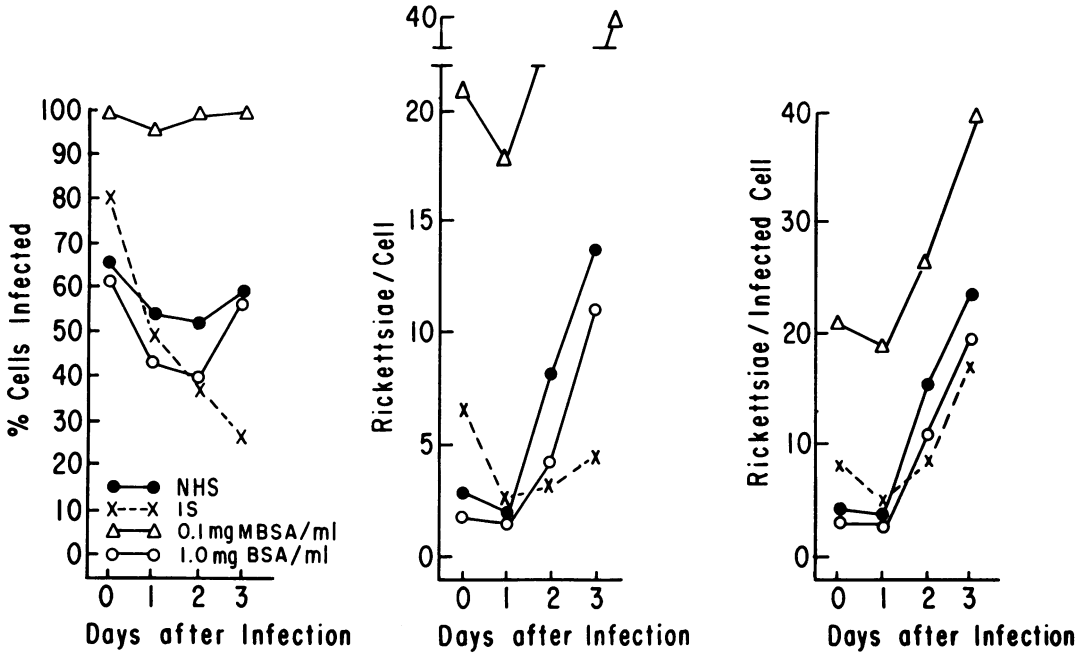


FIG. 3. Dissociation of opsonization from intracellular destruction of *R. mooseri* in macrophages derived from a nonimmune human subject. Both specific and nonspecific opsonization of the rickettsiae by typhus-immune serum and methylated bovine serum albumin, respectively, lead to enhanced phagocytosis, but only the rickettsiae treated with immune serum are destroyed within the macrophages. Abbreviations: NHS, normal human serum; IS, typhus-immune human serum; MBSA, methylated bovine serum albumin; BSA, bovine serum albumin.

of rickettsiae into macrophages by phagocytosis is not automatically followed by intracellular destruction and that IS may have some other specific action on the rickettsiae in addition to its recognized opsonic action to account for the intracellular destruction.

Action of complement. Some unexpected, and as yet unexplained, results were obtained when complement was introduced into the serum-rickettsia-macrophage test system. In the initial series of experiments, fresh guinea pig serum (FGPS) was used as a source of complement. Heat-inactivated NHS or IS was mixed in a 1:1 ratio with FGPS and then added to BME to give a final concentration of 30% serum. Rickettsiae were preincubated in the presence of medium containing serum for 30 min at room temperature and then added to the cells for 2 h at 37 C, 5% CO₂.

Figure 4 shows no marked difference in the growth of *R. mooseri* in the presence of NHS, NHS plus FGPS, or NHS plus heat-inactivated GPS (ΔGPS). Significantly greater phagocytosis occurred with heat-inactivated GPS plus IS on day 0 than with IS alone. The growth pattern for this group was not clearly established since approximately as many rickettsiae per cell were present at day 3 as there were on day 0.

The most surprising result was obtained with IS plus FGPS. This group is not shown in Fig. 4 for the simple reason that by 24 h after infection only a few cells in the entire monolayer were left. After the 2-h infection period, the cells were packed with rickettsiae, but by day 1 most of the cells had disappeared from the cover slip, leaving too few cells to count. This cytotoxic effect was further investigated.

Fresh NHS (prepared as C') was examined along with FGPS to determine if it also had the ability to cause cell disappearance in the presence of IS and rickettsiae or whether this property was unique to FGPS. As seen in Table 2, a definite cell loss was observed in both groups containing FNHS or FGPS plus IS and rickettsiae. No cell loss was observed with heat-inactivated serum or in uninfected cultures.

Several experiments, summarized in Table 3, were then devised to ascertain whether cell disappearance was due to an antigen-antibody-complement reaction or a toxic effect of large numbers of phagocytized rickettsiae.

Rickettsiae were treated with MBSA to increase phagocytosis and thus to determine if increased phagocytosis in the absence of antibody and complement exerted a toxic effect on

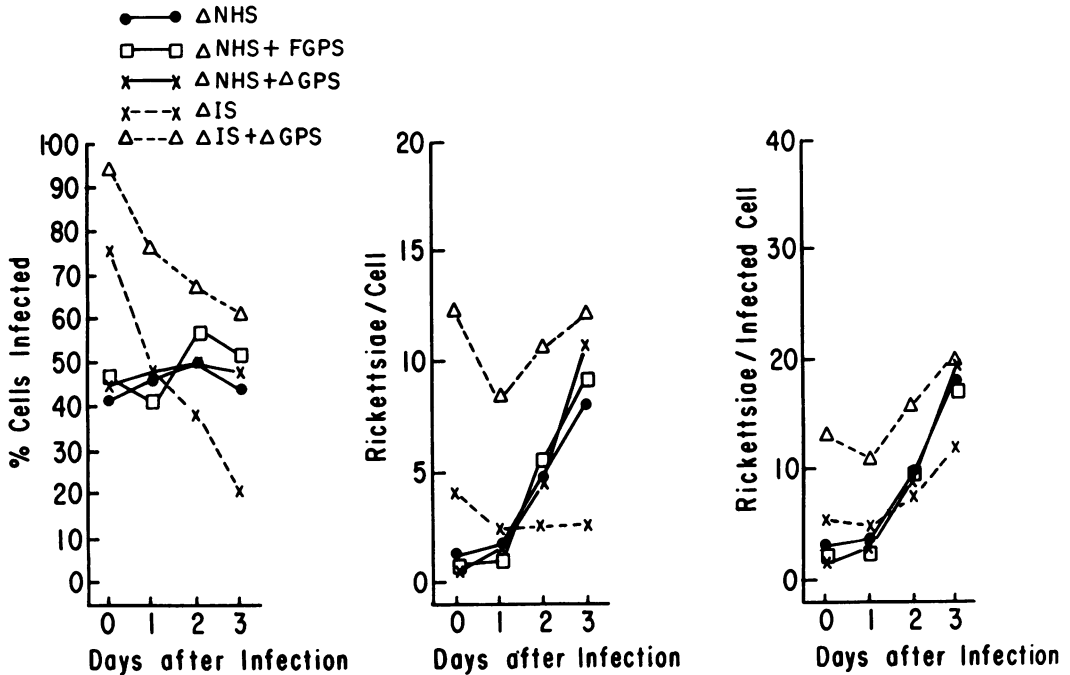


FIG. 4. The effect of the addition of fresh or heat-inactivated normal guinea pig serum to normal or typhus immune human serum on phagocytosis of *R. mooseri* by, and its growth in, macrophages from nonimmune human subject. Abbreviations: Δ NHS, heated normal human serum; Δ IS, heated typhus immune human serum; FGPS, fresh guinea pig serum; Δ GPS, heated guinea pig serum.

TABLE 2. Cell counts^a of infected and noninfected macrophages in the presence of immune serum with added fresh or heat-inactivated normal human serum or guinea pig serum^b

Macrophages	FGPS + IS			Δ GPS + IS			FNHS + IS			Δ NHS + IS		
	D0	D1	P value ^c	D0	D1	P value	D0	D1	P value	D0	D1	P value
Infected (<i>R. mooseri</i>)	276	59	0.036	254	302	0.679	338	198	0.036	203	278	0.571
	195	179		293	262		301	118		242	267	
Noninfected	319	314	0.679	234	315	0.857	312	311	0.857	280	336	0.964
	367	305		235	271		271	371		218	283	

^a 0.6 mm² counted per cover slip.

^b Abbreviations: FGPS, fresh guinea pig serum; IS, immune human serum; Δ GPS, heat-inactivated guinea pig serum; FNHS, fresh normal human serum; Δ NHS, heat-inactivated normal human serum.

^c P value: $n_1 = 2$, experimental group, D1; $n_2 = 6$, control group, D0 and D1.

the cells. However, this experiment did not prove feasible since MBSA would not increase phagocytosis to comparable numbers that this IS and C' did.

Heat-killed and Formalin-killed rickettsiae were prepared to determine if phagocytosis of dead rickettsiae (antigen) would cause a cytolytic effect in the presence of IS and C'. As seen in Table 3, a cell loss was observed after 24 h only in cultures containing live organisms, IS, and C'. None of the other combinations produced an observable cytolytic effect.

Macrophages derived from immune convalescent subjects. Macrophages from immune donors were tested to determine if they differed from cells from nonimmune donors in their interaction with *R. mooseri*. No discernible difference in the growth pattern of *R. mooseri* either in the presence of NHS or IS was observed (Fig. 5).

DISCUSSION

Since typhus convalescent serum is not rickettsiacidal even though it confers strong passive

protection (C. L. Wisseman, Jr., et al., submitted for publication), humoral factors alone cannot be solely responsible for acquired resistance in typhus. However, phagocytosis of *R. mooseri* by both polymorphonuclear neutrophils and mononuclear cells occurs both in vitro and in vivo and is enhanced by IS (29, 31; Wisseman et al., *J. Infect. Dis.*, submitted for publication). Rickettsiae have also been observed within macrophages in the perivascular infiltrates (3, 32). The macrophages in inflammatory lesions of various kinds are now thought to be derived primarily from circulating monocytes which in turn ultimately originate from the bone marrow (6, 11, 27). In the absence of antibodies, monocyte-derived macrophages in cell culture did not destroy typhus rickettsiae; instead, they readily permitted intracellular growth (12).

The present in vitro study demonstrated that *R. mooseri*, which had been sensitized with human typhus convalescent serum, were rapidly destroyed after ingestion by macrophages derived from human blood monocytes. In contrast, similarly sensitized rickettsiae, treated with serum from the same immune donor, retained viability and the capacity to grow in the yolk sac cells of the embryonated hen eggs (C. L. Wisseman, Jr., et al., submitted for publication). Serum must react with the rickettsiae prior to their ingestion by macrophages in order to promote intraphagocytic destruction since IS bathing previously infected macrophages did not inhibit the intracellular growth of rickettsiae. Although complement enhanced the phagocytosis of antibody-sensitized typhus rickettsiae, it did not appear to be required for their intracellular destruction. A similar observation has been made for *Pseudomonas aeruginosa* in mouse macrophages (1). Thus, the destruction of the typhus rickettsiae depends upon the interaction of the rickettsiae with both serum and cellular factors in proper sequence. Neither antiserum nor the macrophage is sufficient of itself to kill these obligate intracellular parasites.

Specific serum opsonins are required for restriction of growth within macrophages of some bacteria under certain conditions (1, 14, 15, 23, 24). However, simple opsonic action of IS does not appear to be the only requirement for intracellular destruction of typhus rickettsiae. The two processes can be dissociated. Thus, nonspecific opsonization with MBSA leads to a comparable enhancement of phagocytosis but fails to render the rickettsiae susceptible to intracellular destruction. Some additional specific action of IS appears to be responsible for preparing the rickettsiae for destruction, even

TABLE 3. Cell counts^a of normal human macrophages allowed to interact with heated, formalinized, or live *R. mooseri* with and without guinea pig serum

Medium	Day 0	Day 1	P value ^b
IS, FGPS, live rickettsiae	191	69	0.022
	173	79	
IS, FGPS, hea-inact. rickettsiae	201	160	0.200
	219	187	
IS, FGPS, Formalin, rickettsiae	156	203	0.956
	199	232	
IS, FGPS, no rickettsiae	177	222	0.556
	201	172	
IS, ΔGPS, live rickettsiae	210	235	0.556
	223	209	
IS, ΔGPS, heat-inact. rickettsiae	232	163	0.089
	271	205	
IS, ΔGPS, Formalin, rickettsiae	231	204	0.089
	214	195	
IS, ΔGPS, no rickettsiae	154	176	0.200
	225	220	
IS, no GPS, live rickettsiae	182	174	
	204	139	

^a 0.3 mm² counted per cover slip.

^b P value: n₁ = 2, experimental group, day 1; n₂ = 8, control group, day 0 only.

though IS does not have a direct rickettsiacidal action. A somewhat similar situation has been described for *Salmonella typhimurium* in mouse peritoneal macrophages, where nonspecific opsonization of the bacteria with bacteriophage failed to lead to the same restriction of intracellular multiplication that followed specific opsonization with antiserum (14).

The mechanism of the apparent destruction of macrophages ingesting very large numbers of antibody-sensitized rickettsiae in the presence of added complement is not yet established (15, 19), but it could be either an unneutralized cytotoxic effect of the very large numbers of ingested organisms or self-destruction of the macrophage by massive release of lysosomal enzymes (28).

Macrophages derived from blood monocytes of typhus convalescent subjects interacted with typhus rickettsiae in a manner indistinguishable from macrophages derived from nonimmune subjects in the in vitro system employed here—i.e., in the absence of specific antibody the rickettsiae grew within the “immune” macrophages and destroyed them, whereas antibody-sensitized rickettsiae were destroyed. However, the possibility exists that any “macrophage cytophilic antibody” (2, 16, 23, 26) which might have existed on the surface of the freshly harvested immune monocytes could

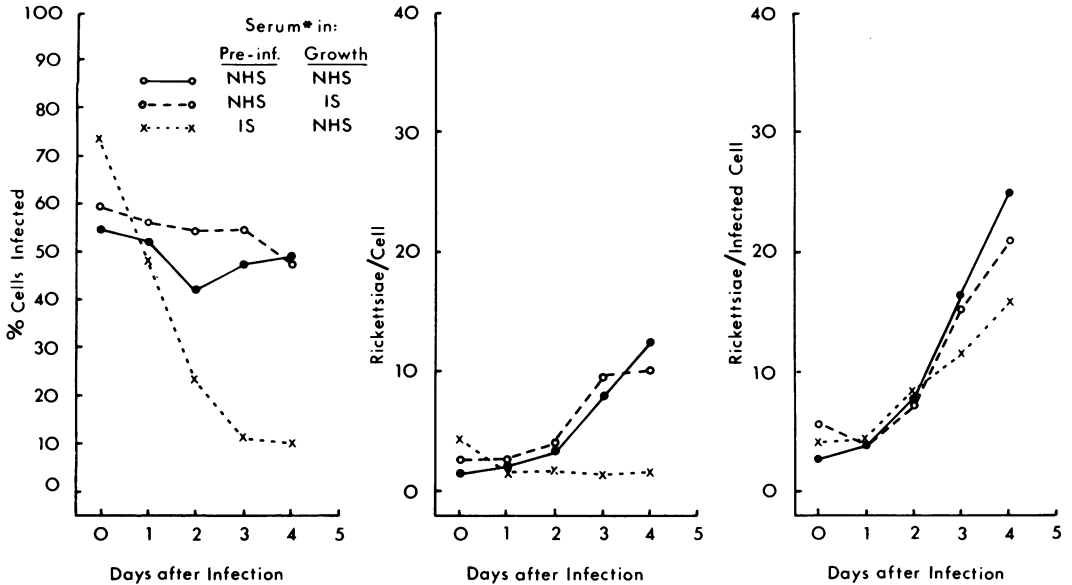


FIG. 5. Failure of macrophages derived from a typhus-immune human subject to restrict intracellular growth of *R. mooseri*. Abbreviations: NHS, normal human serum; IS, typhus immune human serum.

have been lost prior to the introduction of the rickettsiae during the 6 days of in vitro cultivation and transformation in the presence of the antibody-free serum in the medium. Additional studies of a different design are in progress to assess the possible participation of cytophilic antibodies in the rickettsia-macrophage interaction.

Delayed type hypersensitivity, demonstrable by skin test, develops in man as a result of typhus infection (30), and, more recently, blast transformation of lymphocytes from sensitive subjects has been demonstrated in vitro in the presence of typhus antigens (8). Since the macrophage cultures employed in this study were essentially devoid of lymphocytes at the time the rickettsiae were introduced into them, observations on the influence of possible interactions between specifically sensitized lymphocytes and infected macrophages (5, 13, 17, 20) on the fate of the rickettsiae in the absence of antibody were not made at this time. Studies specifically oriented towards an exploration of the role of specific acquired cellular immunity in typhus are being pursued separately.

When antibody-sensitized typhus rickettsiae were introduced into macrophage cell cultures, essentially unrestricted multiplication was observed in the very rare cell. Alternative hypotheses, subject to the experimental approach, may be proposed at this time: e.g., variations in the efficiency and degree of reaction between the rickettsiae and antibodies prior to their entry

into the cells, heterogeneity among the cells or different route of entry into the cells. Rare cells may be present in these cultures which differ from the majority of macrophages, either as variants within the macrophage population itself or as contaminating cells of a different type, which are incapable of destroying antibody-sensitized rickettsiae. These may be the same kind of cells which permitted the attenuated E strain of *R. prowazeki* to grow, whereas the majority of cells in the culture destroyed the attenuated organisms without help from antibody (12). Some precedent exists for heterogeneity among cells of a given type as regards capacity to restrict the growth of intracellular bacteria or to destroy them (9, 17, 24). On the other hand, there is some evidence to suggest differences in the fate of antibody-sensitized rickettsiae in cells of various types, namely, the unrestricted growth of antibody-sensitized typhus rickettsiae in the yolk sac of embryonated hen eggs as opposed to the destruction of such sensitized organisms by macrophages. Finally, the fate of antibody-sensitized *R. mooseri* within the macrophages may depend upon the route or mechanism of entry—i.e., direct penetration versus phagocytosis.

If human macrophages interact with typhus rickettsiae in vivo in the way described in this and the preceding study (12), they can then be visualized, possibly, as playing two, diametrically opposed roles at different stages of the infection, depending upon the presence or ab-

sence of "protective" antibodies. (i) In the early stages of infection, prior to any significant antibody response, macrophages ingesting typhus rickettsiae may actually provide a favorable environment for the invading organisms. Not only do these cells permit the rickettsiae to multiply within their cytoplasm, but they also protect them from other host defense mechanisms, a role somewhat analogous to that ascribed to macrophages in the case of flea-derived plague bacilli and possibly certain other facultative intracellular parasites (4, 25). (ii) After antibodies appear, the role of the macrophage changes to that of an efficient destroyer of the rickettsiae which have reacted with appropriate antibodies.

The observations made in this study, together with those of our preceding two studies, suggest that immunity in typhus, an infection by an obligate intracellular parasite, differs in important ways from immunity in infections by many facultative intracellular bacteria, such as typhoid fever, brucellosis, tularemia, tuberculosis, and listeriosis. In typhus, immunity is strong and long lasting, substantial protection can be passively transferred with IS, and antibody-sensitized organisms are clearly destroyed by macrophages. In the other diseases just named, passive protection with serum is difficult to demonstrate, the fate in macrophages is variable and sometimes controversial, and acquired immunity, such as it is, is currently presumed by many to be primarily "cellular" (18). Without denying the possible participation of cellular immunity in typhus infection, which remains to be clarified, it seems that the potent protective action of humoral antibodies sets this intracellular infection apart from the others just mentioned and probably establishes it as an important exception to the currently popular, probably excessively simplistic, dogma that "immunity to intracellular infections is cellular."

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