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Murine and Epidemic Typhus Rickettsiae: How Close Is Their Relationship?

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Typhus fever has occurred globally as epidemic and endemic disorders. In 1910, Brill reported a typhus-like illness which Zinsser and others determined to be recurrent epidemic typhus fever. Maxcy, in 1926, proposed rodents and fleas as reservoir and vector, respectively, of endemic typhus, which Dyer confirmed in 1930.

Animals experimentally infected with epidemic typhus (*Rickettsia prowazeki*) are immune to murine typhus (*Rickettsia typhi*) and vice versa. Similar solid cross-immunity exists for humans. The two diseases are clinically similar in pathologic and serologic reactions.

Human epidemic typhus presumably involved a man-louse-man cycle without an animal reservoir. This concept is now questioned. Antibodies to *R. prowazeki* have been reported in livestock in Africa, rats in Manila, and from flying squirrels and humans in the United States. *R. prowazeki* was recovered from blood specimens of goats, sheep, from ixodid ticks, louse, and flea ectoparasites of flying squirrels, and tissues of flying squirrels. More than 20 cases of squirrel-related acute epidemic typhus have been reported in the United States. *R. prowazeki* has not been recovered from human cases.

Chemical studies of *R. prowazeki* and *R. typhi* show genetic similarities but differences in genome size and degree of hybridization suggest that interconversions between the two agents do not occur rapidly in nature. It is proposed that, with time, their relatedness will become even closer.

Epidemic typhus fever has been feared as a medical scourge for centuries. However, not until 1909, three years after Ricketts' incrimination of the tick as a vector of Rocky Mountain spotted fever [1], was the louse transmission of typhus reported by Nicolle [2]. There were recognized clinical differences between rickettsial infections. Brill, from 1898 to 1910, studied and reported the clinical and laboratory results of 221 cases of an illness which resembled typhoid fever to most persons [3]. To him, it appeared to be a modified form of classic epidemic typhus fever. From the time of Brill's report, a sporadic typhus illness was designated globally as endemic typhus, to distinguish it from the serious classical form. In the United States, and particularly the southeastern coastal states, this sporadic illness was called either endemic typhus or, erroneously, Brill's disease.

Maxcy, a wise epidemiologist, helped clarify matters by studying cases in the South and proposed that the endemic type had its agent reservoir in rodents and an arthropod as its probable vector. His remarkable conclusion in 1926 was: "Obviously, the rodents upon which suspicion immediately falls are rats and mice, and the

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parasite intermediaries which are first suspected are fleas, mites or possibly ticks" [4].

This unique epidemiologic prediction was confirmed in 1930 by Dyer and his associates, who trapped rats, in Baltimore, taken in the locale of typhus patients [5]. Rat brains, when injected into guinea pigs, yielded causative rickettsiae, as did fleas taken from these rodents.

The murine reservoir and flea transmission of a rickettsia was established; the clinical illness was noted to be similar to epidemic typhus, milder in its course, and generally non-fatal except in older persons. Epidemic typhus was generally associated with a severe or fatal illness.

Similarities between the two diseases were very apparent. Through the work of Maxcy [4], Dyer [5], Paullin [6], Zinsser [7], Castaneda [8], Mooser [9], and Anderson [10], various epidemiologic, ecologic, and clinical features were clarified. The rickettsiae of epidemic typhus (*Rickettsia prowazeki*) and murine typhus (*Rickettsia typhimooseri*), had the same morphologic and staining characteristics. Reactions in guinea pigs were noted to be similar although murine rickettsiae caused a more diffuse swelling of the tunica vaginalis in male animals and masses of intracellular rickettsiae called Neill-Mooser bodies [11]. Soon it was noted that animals recovered from murine typhus were solidly immune to virulent epidemic-type rickettsiae and vice versa. French and other European investigators quickly noted, on epidemiologic grounds, that patients naturally convalescent from murine typhus fever were immune to the more severe epidemic illness.

During the mid-1930s, Zinsser and his associates [8] in Boston and New York evaluated patients with the Brill-type disease. It was apparent that the clinical manifestations were milder than the classic type. Several strains of rickettsiae were isolated in guinea pigs with blood from such patients, and in animals, the disease simulated the findings of epidemic typhus, including the characteristic histologic findings of vasculitis. Moreover, such convalescent animals were immune to epidemic or murine typhus challenge.

An odd finding in patients was the low or negative titers to proteus OX19 antigens. Epidemiologically, it was noted that most patients (95 percent) with Brill's disease gave a history of having contracted classic typhus in epidemic countries in Europe in earlier life. Zinsser reasoned correctly that Brill's disease was simply a recurrence of their illness, milder, but nevertheless recurrent epidemic typhus fever. His concept included the correct hypothesis that *R. prowazeki* had remained viable in the human host for many years and was able to cause relapse. This illness is now correctly called Brill-Zinsser disease.

To follow were further clinching facts which proved Zinsser correct. Murray, Snyder, and associates later applied lice to patients with Brill's disease and regularly isolated the causative rickettsiae characterized as *R. prowazeki* [12]. When better antigens and more definitive serologic procedures became available, it was shown that Brill's patients developed epidemic typhus antibodies when tested by complement fixation (CF) techniques. Also, the specific antibody which developed in Brill-Zinsser patients appeared in high titer after several days of recurrent illness and was of the 7S antibody type and not IgM, the usual antibody response after an initial attack [13].

In all serologic testing between murine, epidemic, or Brill-Zinsser patients, there are cross antigen/antibody reactions, i.e., a patient with epidemic or murine typhus shows an antibody response to the heterologic antigen. Usually the titer of antibody is higher with the homologous antigen.

It is now time to discuss more intimate relationships of murine and epidemic typhus and ask whether they are ever the same.

CROSS IMMUNITY BETWEEN MURINE AND EPIDEMIC TYPHUS FEVER IN HUMANS

In North Africa from 1942 to 1944, epidemic typhus was rampant among the civilian population; the British military forces experienced fatal cases. A trial of the available epidemic typhus vaccines was conducted which included inactivated epidemic typhus vaccine and a living attenuated type of murine typhus rickettsiae made from infected flea feces.

As a sequel to the epidemic typhus vaccine studies in volunteers, there were seven persons convalescent from murine typhus who consented to receive an infectious challenge of viable epidemc typhus rickettsiae to evaluate their immune status. The infectious strain was *R. prowazeki*; one ml of a 20 percent suspension of guinea pig brain tissue prepared on the fourth febrile day was given subcutaneously. The inoculum contained approximately 10^7 infectious guinea pig doses of viable *R. prowazeki* administered to the volunteers six to nine months after their recovery from fully confirmed murine typhus fever. The CF titer to murine and epidemic typhus antigens at the time of infectious challenge ranged from 40 to 320. (Refer to Table 1). Macaca monkeys were used as controls. In this study, conducted simultaneously, each of two control monkeys died of typhus infection.

None of the seven murine typhus convalescents experienced any clinical reaction; they were solidly immune. Each showed a local reaction of redness and induration at the injection site in the upper arm similar to a reaction of delayed hypersensitivity. There was no significant rise in CF antibody titers to either the murine or epidemic typhus antigens after the infectious challenge.

This evaluation in seven murine typhus convalescent patients clearly established the existence of solid immunity between murine and epidemic infections in humans. The pertinent serologic reactions are shown in Table 1. These studies, heretofore unreported, were performed in collaboration with Georges Blanc and Marcel Baltazard (each is now deceased).

TABLE 1
Murine-Epidemic Typhus Fever
Cross Immunity Studies in Murine Convalescents

Volunteer #[a]	Highest Pre-Infection Titer[b]		Titer at Infection		Highest Post-Infection Titer		Clinical Reaction (Post-Infection)
	Murine	Epidemic	Murine	Epidemic	Murine	Epidemic	
1	640	80	320	20	320	20	0
2	80	40	80	0	80	10	0
3	640	40	320	10	640	10	0
4	320	40	160	0	80	10	0
5	1280	160	160	10	80	10	0
6	1280	160	80	40	80	20	0
7	320	20	40	10	40	0	0
Controls Macac							
1			0	0	640		Fever 10 days
2			0	0	320		Died 17th day

[a]1,2,3,4—Six months conv.; 5,6,7—Nine months conv.
[b]Comp. Fix. titers, Wilmington, Breinl antigens

ANTIBODY TO *R. PROWAZEKI* IN RATS IN MANILA, P.I.

An unsolved vignette involves a serologic paradox which was noted in the sera of rats trapped in Manila, Philippine Islands, during the American reoccupation. The finding, although not fully appreciated at that time, presumably emphasized the intimacy of the two rickettsial strains, *R. prowazeki* and *R. typhi* (mooseri).

Several sporadic human cases of murine typhus were identified for the first time in Manila [14,15]. Rats (*Rattus norvegicus*) were trapped, anesthesized, bled, and allowed to recover. Rats whose sera showed CF antibody to typhus antigens were sacrificed. Suspensions of the pooled brains were inoculated into guinea pigs and yielded typhus rickettsiae in several trials. Of special interest was the unanticipated finding that titers of CF antibody in rat sera were higher for epidemic thyphus (Breinl) than murine typhus (Wilmington) antigens. This unusual reaction was reconfirmed in Manila and also by Plotz at the Rickettsial Diseases Laboratory, Walter Reed Army Institute of Research. This paradox, higher antibody titers to epidemic than to murine typhus antibodies in rats, was unique.

Later, Dr. Edward Murray informed me of a similar experimental finding in cotton rats [personal communication].

The artifact was not fully appreciated at the time and was thought possibly to be attributable to the crude antigens used which caused strong cross-over serologic reactions. Unfortunately, the rickettsiae isolated from the wild rats in Manila were not studied further because of wartime difficulties which precluded proper agent preservation and transfer to base laboratories. Nevertheless, the observed natural finding could have significance and possibly relate to the more recent studies of *R. prowazeki* infection in flying squirrels.

In 1938, Brigham and Dyer, using the Wilmington strain of *R. typhi* (mooseri) showed the following animals susceptible to typhus infection [16]: opossum (*Didelphis virginiana*), old-field mouse (*Peromyscus polionotus*), cotton mouse (*Peromyscus gossypinus*), golden mouse (*Peromyscus nutalli aureolus*), wood rat (*Neotoma floridnarubida*), cotton rat (*Sigmodon hispicus*), rice rat (*Oryzomys palustris*), and flying squirrel (*Glaucomys volans saturatus*). Brigham conducted a serologic study of Weil-Felix and complement fixation reactions in wild rats to evaluate the incidence of typhus infestation [17]. Most were Norway (*R. norvegicus*); a few were *R. rattus*. Proteus OX19 agglutinins and CF antibodies, indicative of murine typhus infection, were present. Unfortunately, the CF antigen was not described. It is presumed that the test employed the Wilmington strain of *R. typhi* (mooseri). Results with an epidemic typhus antigen were not reported.

R. PROWAZEKI AS A NATURAL INFECTION IN THE UNITED STATES

For years, it was accepted that *R. prowazeki* had no animal or arthropod reservoir and that the cycle of infection simply involved man-louse-man. This hypothesis was questioned in early 1950, when Giroud and other French rickettsiologists suggested an extra human cycle of *R. prowazeki* based on their finding of antibodies to this rickettsiae in livestock from various parts of Africa and the isolation of epidemic typhus rickettsiae from the blood of goats, sheep, and ixodid ticks [18,19,20,21, 22,23]. Burgdorfer et al. [24] were unable to recover *R. prowazeki* from 2,624 ticks collected in Central and Southern Ethiopia, nor was there serologic evidence suggesting the presence of this agent in any of the ticks examined. Nevertheless, the possibility of an extra human source of the epidemic typhus agent remained.

In 1975, epidemic typhus rickettsiae were isolated from six flying squirrels in

Florida and in four from Virginia [25,26]. Sonenshine and his associates identified a widespread natural infection of *R. prowazeki* in the flying squirrel, *Glaucomys volans*. The ectoparasites of this squirrel, lice (*Neohaemato pinus sciuropteri*) and fleas (*Orchopeas howard*), were infected, suggesting the flying squirrel louse and flea as possible natural vectors. Serologic tests of flying squirrel sera revealed a maximum incidence of seroconversion in the fall and early winter months, coincident with the maximum increase in abundance of the suspected arthropod vectors [27]. McDade and his associates, from 1976 to 1982, have identified, by serologic techniques, the presence of twenty-two human infections to *R. prowazeki* in the United States [28]. Clinical data and serum specimens submitted by various physicians to the Center for Disease Control, Atlanta, revealed clinical illnesses compatible with mild to moderate louse-borne typhus fever occurring in persons living in rural environments during winter months. Most cases occurred in eastern and southeastern states. Serum specimens revealed IgM antibodies specific for *R. prowazeki* indicative of recent and not recrudescent illness of the Brill-Zinsser type. Fully one-half of the patients reported had contact with flying squirrels. None of the clinical illnesses were fatal; headache, fever, and rash occurred in most cases and the therapeutic response to either tetracycline or chloramphenicol was good. This identification of enzootic foci of *R. prowazeki* in proximity to suspected human cases of epidemic typhus suggests a natural source of infection other than man-louse-man.

The findings of epidemic typhus rickettsiae in flying squirrels, their ectoparasites and antibodies to *R. prowazeki* in squirrels, and possibly in rats raises important public health questions. The validity of man as the sole reservoir of epidemic typhus is suspect.

Could an antigenic shift from *R. typhi* (mooseri) to *R. prowazeki* have occurred in flying squirrels and their ectoparasites which resulted in a milder human type of epidemic typhus? Zinsser and others struggled with this possibility [7]. Hans Zinsser felt that murine and epidemic typhus rickettsiae were distinct varieties of agents, distinguishable by biologic methods, each of which could occur as endemic or epidemic infections. His studies in Boston and in Mexico with his collaborator, Castaneda, showed the louse capable of epidemic transmission of murine rickettsiae [7,8]. Such strains passed through the cycle, man-louse-man, were temporarily, not permanently, modified in the direction of European or classical type characteristics. Zinsser's studies of Brill's disease convinced him that secondary cases of epidemic typhus could result from louse infestation and transmission from recrudescent cases; yet, he did not believe that latent human infections were the only source of origin or reservoir of the classical European illness [8]. (The wise concepts of Zinsser are apparent.)

Biochemical studies of rickettsiae by Dasch, Wisseman, and others are very relevant to the problem. When comparing antigenic strains of *R. typhi* and *R. prowazeki,* Dasch et al. [29] noted similarities in heat lability, sensitivity to proteases, insensitivity to periodate and glycosidases, isoelectric points, and mobilities in neutral Davis polyacrylamide gels.

Wisseman's work embraced the genetic relatedness of various rickettsiae through evaluation of the percent guanine + cytosine composition of the respective DNAs of *R. typhi* and *R. prowazeki*, their genome size, and the degree of DNA:DNA hybridization [30,31]. Their G + C content is identical, their genome size is nearly the same (each smaller than other rickettsiae such as *Rickettsia rickettsii* or *Rickettsia canada*). The degree of hybridization between *R. typhi* and *R. prowazeki* nearly match when tested by optical or radiolable techniques. Wisseman computes their

genetic relatedness as about 70 percent with small differences in genome size and degree of hybridization. These findings would militate against a rapid interconversion between *R. typhi* (mooseri) and *R. prowazeki*. Yet, the possibility of a slower conversion might have occurred over centuries by proper shift and linkage of their genetic machinery in squirrels, rats, other mammals, and their ectoparasites.

Currently, *R. prowazeki* and *R. typhi* (mooseri) are different. Perhaps they started out as one, shifted their characteristics through chemical interaction in mammals and/or insects, and will in future centuries become identical twins.

The opportunity to contribute a short note in honor of Dorothy Horstmann is a memorable privilege. Her contributions to the noble cause of epidemiology and preventive medicine place her at the level of those not to be forgotten.

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