

Rickettsial Infectious Antibody Complexes: Detection by Antiglobulin Plaque Reduction Technique

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Rickettsia rickettsii and *R. australis* formed infectious antibody complexes when reacted with homologous convalescent serum. The addition of antiglobulin to these complexes showed neutralization of plaques proportional to the concentration of rickettsial immune serum.

Laboratory diagnosis of rickettsial disease is usually made by isolation of the organism or demonstration of specific immune response during convalescence (3). Commonly used are complement fixation (CF), fluorescent-antibody techniques, and microagglutination. In efforts to develop additional diagnostic tools, we observed that serum containing demonstrable CF antibody would not neutralize in vitro the same spotted fever species that induced the immunity. Reviewing the literature yielded little information on in vitro methods to measure rickettsial neutralizing antibody and none for the spotted fever group (1, 6). We have investigated this enigma in a rhesus monkey infection model of Rocky Mountain spotted fever and Queensland (Australia) tick fever.

Stocks of rickettsiae were prepared from infected chicken egg yolk sacs by homogenization with equal volumes of sucrose-phosphate-glutamate (SPG) buffer (2). Antibodies against *Rickettsia australis* were prepared in rhesus monkeys infected with 10^6 organisms and back-challenged at 4 weeks. Sera were collected 4 weeks after challenge. Antibodies against *R. rickettsii* were prepared in rhesus monkeys immunized with a single dose of inactivated duck embryo cell-grown vaccine (7) and challenged at 4 weeks with a lethal dose of *R. rickettsii*; sera were collected 8 weeks after challenge. Antiserum to rhesus monkey immunoglobulins was prepared in goats. All sera were heat-inactivated for 30 min at 56 C prior to use.

For *R. australis* plaque reduction experiments, convalescent sera were pooled and diluted serially by twofold increments in Hanks balanced salt solution with 10% calf serum. Challenge rickettsiae were diluted with SPG buffer to a concentration of approximately 200 plaque-forming units per 0.1 ml. An equal volume of rickettsiae was added to each dilution of convalescent serum and incubated overnight

at 4 C. An equal volume of goat-antimonkey immunoglobulins diluted 1:20 then was added, and the test was held at room temperature for 25 min. Controls included both types of diluent, normal monkey serum, normal goat serum, normal monkey serum plus goat antiglobulin, goat antiglobulin plus diluent, and guinea pig complement added to immune serum. After room temperature incubation, 0.1 ml of each mixture was inoculated into duplicate 25-cm² flasks of chick embryo cell culture. They were allowed to adsorb at room temperature for 30 min. The agar overlay technique was essentially that of Weinberg et al. (9). Each flask was overlaid with 5 ml of minimal essential medium with Earle balanced salts containing a final concentration of 0.5% agarose (Marine Colloids, Inc., Rockland, Me.). Inoculated flasks were incubated at 35 C for 5 days. A second agar overlay containing 1:10,000 neutral red stain was then added, and the plaques were read the following day.

R. rickettsii plaque reduction experiments were performed essentially by the same methods. However, the first incubation was for 30 min at 35 C in a 5% CO₂ atmosphere.

The dose response of the summary data from repeated experiments with *R. australis* and *R. rickettsii* is presented in Fig. 1 and 2, respectively. Regression lines were generated assuming Poisson weights of maximal likelihood (4). Control reactions verified that plaque reduction resulted only when rickettsiae were incubated with convalescent serum and when goat-antimonkey immunoglobulin was added to the mixture.

Ricketts (8) reported that spotted fever immunity could be passively transferred by serum from convalescent to normal animals. We have demonstrated in vitro that convalescent serum will directly bind but not neutralize spotted fever rickettsiae. Evidently, antibody can com-

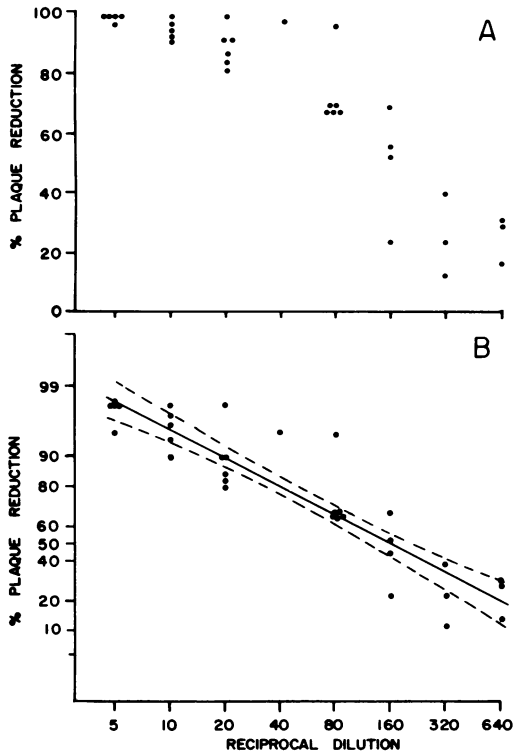


FIG. 1. Dose response (A, linear; B, regression on a probit scale with 95% confidence limits) of *R. australis* plaque reduction as a function of dilution of convalescent serum. After initial incubation, a 1:20 dilution of goat-antimonkey immunoglobulin was added to each reaction tube.

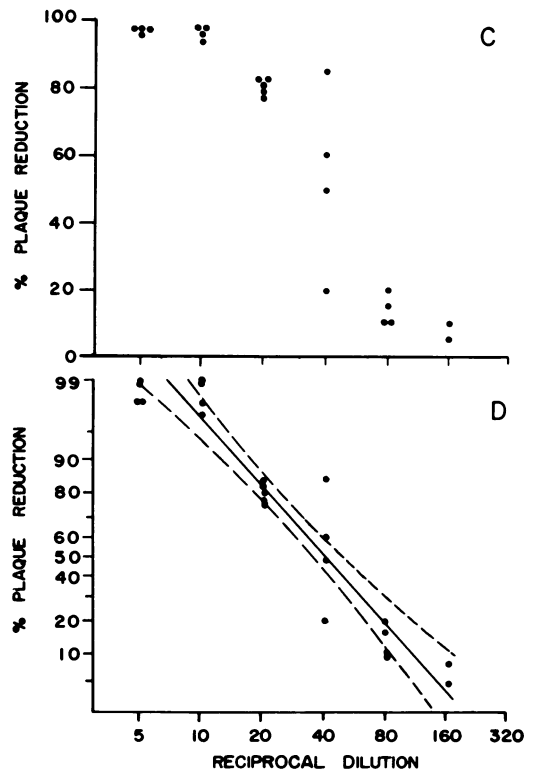


FIG. 2. Dose response (C, linear scale; D, regression on a probit scale with 95% confidence limits) of *R. rickettsii* plaque reduction as a function of dilution of convalescent serum. After initial incubation, a 1:20 dilution of goat-antimonkey immunoglobulin was added to each reaction tube.

bine with rickettsiae, forming infectious complexes without affecting the number of plaque-forming units. A similarity for this phenomenon was recently reported by Gambrill and Wiseman (5), who demonstrated that typhus rickettsiae exposed to immune serum were infectious for eggs but were killed by cultured human phagocytes. Rickettsiae not exposed to immune serum replicated within and killed the phagocytes.

This is the first report of rickettsial plaque reduction. No cross-neutralization has been observed between *R. australis* and *R. rickettsii*. The neutralization technique is not advised for routine clinical laboratory detection of rickettsial antibody. Rather, its importance lies in the fact that the existence of infectious rickettsial antibody complexes can be demonstrated. We are presently investigating whether the existence of such infectious complexes can be detected in vivo during the course of spotted fever disease in guinea pigs and monkeys.

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