Guinea Pig Antibody Response Following A Secondary Injection of *Coxiella burnetti.* (32524)

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In a previous report, the Q fever antibody response of the guinea pig and a number of other animals following a primary injection of *Coxiella burnetti* was described(1). It was found that the guinea pig had a typical response with antibody titers rising relatively quickly to maximums at varying times, depending upon the type of antigens and antigen concentrations employed for testing. After attaining a peak, the antibody titers then decreased slowly over a period of time, easily being detectable as long as 1 year after the initial injection. Little has been published concerning investigations of secondary antibody responses to the Q fever rickettsiae, although Burnet and Freeman(2) reportedly could not induce a secondary rise in antibodies in rabbits primarily and secondarily stimulated by intravenous injection of *C. burnetti.*

It was of interest, therefore, to determine if guinea pigs primarily infected with the rickettsiae and then secondarily stimulated with the same agent would produce detectable secondary rises in titers. This report describes such an experiment.

Materials and methods. Animals. The guinea pigs employed in this study were young adults, weighing 400-500 g at the time of initial inoculation and 750-1050 g at the time of secondary stimulation. All were of the Hartley strain. Each group contained at least 50 animals.

Organism. The California AD strain of *C. burnetti* was used(3). Prior to its use in this study, the organism had been subjected to over 25 consecutive passages through eggs and was in the Phase II (egg adapted) form.

Serologic tests. The complement fixation (CF) test, employing Phase I and II antigens, was used as described previously(1).

Experimental design. All animals were given an intraperitoneal (ip) primary injection of *C. burnetti.* The dosage was 0.2 ml of the 10^5 dilution of infected egg yolk sac slurry which was equivalent to $10^{5.2}$ guinea pig ID_{50} organisms.

At various periods after infection these animals were bled from the orbital sinus and their sera tested for antibodies. After the 125th week the animals were divided into 3 groups. One group was given the same quantity of *C. burnetti* as at first, ($10^{5.2}$), the second group was given $10^{8.2}$ guinea pig ID_{50}'s while the third group was injected with normal yolk sac slurry to serve as controls. Each animal was then bled at regular intervals after the secondary stimulation from the orbital sinus. All sera were tested at the time of initial bleeding and retested at the conclusion of the experiment in order to give comparative results. The mean antibody titers were determined for all groups each time the animals were bled. These were calculated by employing the $\log_2$ of each serum titer to compensate for the manner of dilution, which was 2-fold.

Results. The group given the same number of organisms in the 1° and 2° injections showed a slight decrease in antibody titer of about 1 dilution immediately (2 days) following the 2° injection. This was followed by a 1-3 dilution increase above the original titer for Phase II antibodies 5-8 days later. Phase I antibodies remained unchanged for approximately 3 weeks when increases were evident, with peak titers (averaging 2 dilutions) being found on the 4th week. The group given a much larger number of organisms responded more dramatically producing Phase II antibody titers 5-7 dilutions greater than the yolk sac injected animals which titers remained unchanged. Unfortunately the sera of group 2 were not tested with Phase I antigen. These data are shown in Fig. 1. The Phase I antibody titers were lower, reached their maximum titers, and declined less rapidly than Phase II antibodies with both 1° and 2° injections.

Discussion. The slight decreases in titers (negative antibody phase) observed in the secondarily stimulated guinea pigs were probably due to partial neutralization of the anti-

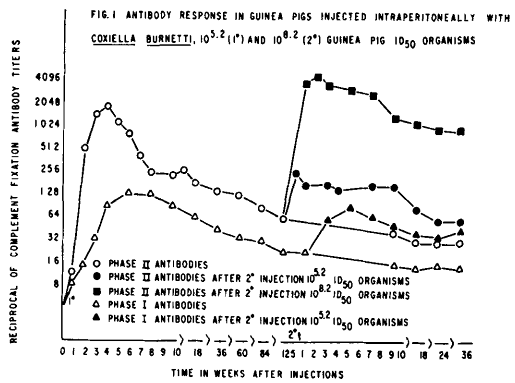

FIG. 1.

bodies by the additional antigen present in the blood, as discussed by Wilson and Miles (4). Following this negative phase, increases in the titers were demonstrated which exceeded the titer values originally observed just before the secondary stimulation and the yolk sac controls by significant amounts, especially in those given the larger number of organisms. This general increase typifies the "classical" secondary response as described for animals injected with substances such as diptheria antitoxin(5,6,7) and bacteria as *Salmonella paratyphi*(4). Employing these latter antigenic substances, however, one usually obtains higher titers than those observed in the present investigation. As pointed out earlier, Burnet and Freeman(2) could induce no detectable secondary rise of agglutinating antibody in iv injected rabbits. At the time of their investigation the agglutination test employed may have been less sensitive than the CF test used in the present study; thus small fluctuations may have occurred in the rabbits which were not readily detected. It is apparent, however, that a typical secondary Q fever antibody response can occur in guinea pigs.

As expected, individual animals yielded variations in antibody titers; 84% of the animals were within ± 1 dilution of the titers reported and 96% were within ± 1.8 dilutions.

In other studies not reported here, a 2° injection of 10^4 guinea pig ID_{50} organisms 90 days after a similar 1° injection failed to produce changes of this magnitude, with titers averaging only 1 dilution higher than noninfected controls, indicating both the time after infection and the number of organisms injected have a bearing on the responses observed.

Summary. Secondary Q fever antibody responses using Phase I and II complement fixing antigens were determined in guinea pigs after intraperitoneal, secondary injection 125 weeks later. As compared with nonsecondarily injected controls, a slight negative antibody phase was observed, followed by significant antibody titers, these titers corresponding in magnitude to the number of organisms injected.

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