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STUDIES ON THE ANTIGENIC COMPOSITION OF 'COXIELLA BURNETII'.(U)
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DAVID J. HINRICHS

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STUDIES ON THE ANTIGENIC COMPOSITION OF COXIELLA BURNETII

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It has been possible to employ the mouse as a model for vaccine development in the Coxiella burnetii system. Resistance to re-infection can be demonstrated in infected-recovered mice as well as mice that have been immunized with formalin-killed cells. This immunity can be ascertained by accelerated clearance of viable C. burnetii from spleen and liver in challenged animals. The accelerated clearance is apparently not due to antibody levels alone. (continued)

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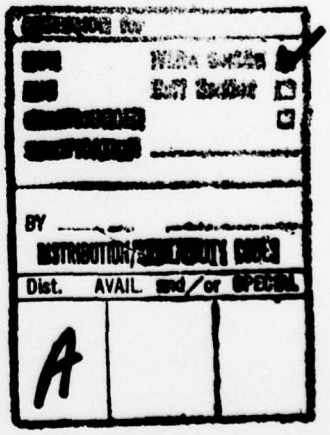
20. Abstract

Studies with passively administered antibody in normal mice or infection studies in the nude mouse system indicate a cooperative need with a functioning lymphocytic-macrophage system. We have isolated by Triton X-100 extraction, material from C. burnetii that is immunogenic and has some protective activity. This is a preliminary observation that is currently being confirmed.



Studies on the Antigenic Composition of *Coxiella burnetii*

Summary

During the period covered by this annual report we have focused our efforts on generating the baseline data necessary to evaluate our sub-unit vaccine preparations in the mouse model. In this regard we have determined the levels of the humoral and cellular immune responses that occur following immunization with the formalin-killed whole-cell preparation of *Coxiella burnetii* (WCP). We have also determined that it is possible to evaluate protective immunity in the mouse system by observing by light microscopy the presence of rickettsia in stained spleen smears. Thus at 7 days post infection virtually all animals tested have abundant intracellular (and some extracellular) rickettsia in their spleens. Number of rickettsia decrease and by day 21 are not apparent; however, these spleens contain rickettsia and are still infective, as confirmed by egg passage and cell culture infectivity in vitro.

In this regard we have determined that mice that have recovered from a *C. burnetii* infection or animals that have been immunized with WCP contained virtually no spleen associated rickettsia. Thus accelerated clearance of challenge doses of rickettsia would appear to be a viable assay for *C. burnetii* derived vaccine products.

Although we have had to abort some of our recent experiments (see below) we have generated some preliminary data indicating that a simple Triton X-100 extract of *C. burnetii* provokes high levels of circulating antibody in immunized mice. We are currently making preparations for a full scale examination of the immunogenic and protective potential of these products.

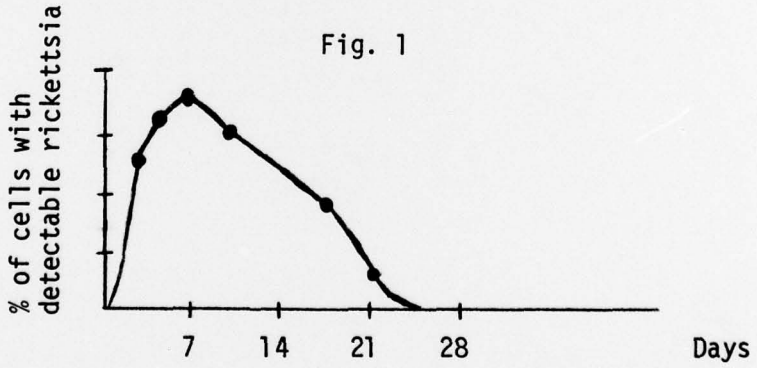
We have evaluated in some recent experiments the role of passively administered anti *C. burnetii* antibody in affecting the clearance rate of intravenously administered viable *C. burnetii*. In our hands, as assayed by accelerated clearance in the mouse system, no observable effect on the clearance rate of *C. burnetii* could be seen in mice passively immunized with antibody. The lack of antibody effectiveness is also apparent in that nude mice do not clear *C. burnetii* even in the presence of high antibody titers. Finally, and unexpectedly, we have developed what is apparently a continuous macrophage cell line derived from the guinea pig. This apparent macrophage line contains Fc receptors, has active phagocytic activity, is esterase positive and is susceptible

to infectious destruction by C. burnetii in vitro. Our current "hands on" efforts are devoted to the analysis of polyacrylamide gel electrophoresis runs of the SDS solubilized proteins of Phase I and Phase II C. burnetii. These results are compared with the products that we are in the process of obtaining from Triton X-100 extraction procedures as specified in our current contract request.

Progress to Date

Our goal during the time period covered by this annual report was to establish baseline conditions in the laboratory mouse such that this common experimental animal could be employed as a test animal in the study of C. burnetii sub-unit vaccines. We have established that various mouse strains (outbred Swiss Webster, inbred Balb/c and C_3H) are in reality not innately resistant to infection with C. burnetii. Mice, in fact, go through a period of illness following intravenous injection with viable Phase I C. burnetii. The illness, although short term and subjective in nature, can be readily confirmed by following the rise and fall of C. burnetii in stained impression smears of infected mice spleens. As Fig. 1 indicates, following infection the number of spleen cells that contain C. burnetii increases through 7 days and then show a steady decline until 21 days at which time virtually no rickettsia are present.

Fig. 1



We have taken spleens at days 5, 7, 9, 11, and 20 post infection, minced these spleens with a tissue homogenizer, lyzed the spleen cell suspension and then infected eggs and guinea pig macrophages maintained in vitro with dilutions of the spleen lysate. These assays both demonstrate that the rickettsia that are observed microscopically are indeed viable and infectious. Thus the mouse is not innately resistant to C. burnetii infection and can be employed as a suitable animal model.

We have further established that once mice have recovered from the C. burnetii infection they become immune (for at least 30 days) to subsequent re-infection. These recovered mice following re-infection rapidly clear the injected rickettsia. At day 7 post infection immune mice have few spleen cells with intracellular C. burnetii and when we have assayed these spleens for viable infectious C. burnetii we have been unable to detect them by in vitro assays.

The development of immunity in these animals (above) can also be correlated with some in vitro assays of a functioning immune system. By day 10, micro-agglutination titers are consistently in the 100 - 400 range. These antibody titers are detected with C. burnetii formalin-killed cell-Phase I (WCP-I). The antibody levels remain elevated through the testing period of 30 days. Significantly, the infected-recovered mice also display readily detectable levels of antigen-sensitive lymphocytes. Thus lymphocyte transformation stimulation indices can reach 15 by day 10-14 and remain detectable throughout the testing period. We have cultured splenic lymphocytes in microtiter employing WCP-I and the trichloroacetic acid soluble extract of C. burnetii (TCA-Ag). As might also be expected the background activity of cells without antigen is initially high but drops to normal by day 21. We feel that this indicates antigen carryover into the culture system as well as an indication of the cellular activity in the spleen during the initial phases of the infection. As previously reported we still consistently observe in the mouse lymphocyte transformation system mitogenic activity of WCP-I and the TCA-Ag when tested in normal mouse spleen cultures. This may be associated with the lipopolysaccharide content of C. burnetii. However we have tested WCP-II preparations and have observed minimal to no mitogenic activity. We feel this observation correlates well with our previous observations of the mitogenicity of the TCA-Ag preparation. (In immune animals the stimulation indices are 4-5 fold higher than mitogenic levels.) We have conducted the same assays in our baseline data gathering process following immunization of mice with WCP-I. This is an effective immunogen in the guinea pig and Dr. R. Kieshimoto (USAMRIID) has shown it to produce protective immunity in this laboratory animal. As might have been expected, 7 days after mice have received a 20 μg dose of WCP-I they show circulating levels of anti WCP-I antibody. The response is variable and ranges from less than 20 to an occasional microagglutination titer of 400. These immunized mice also respond in lymphocyte transformation. Table 1 summarizes some of our observations that we have generated as necessary preliminaries to the testing of our sub-unit vaccine preparations.

Table 1

*C. burnetii immunization	Antibody produced M.A. titer	Lymphocyte transformation indices	Accelerated clearance
Viable C. burnetii as immunogen	64 - 256	8-fold	yes
WCP-I 1 µg	8	no	not apparent
WCP-I 10 µg	variable 128	6 - 10	yes
WCP-I 100 µg	32	4 - 6	yes
+WCP-I 10µg,10µg	64	15 - 20	yes

*All immunized groups were assayed 14 days following the last immunization. Animals were tested for accelerated clearance 7 days following challenge.

+Immunization Day 1 10 µg, Day 15 10 µg, Test Day 30

It is apparent from these observations that immunity in the mouse to C. burnetii does develop and that this immunity can be produced by a non-viable preparation of C. burnetii. This immunity is demonstrable when cellular and humoral immunity is also detectable. Our studies also demonstrate the threshold dose required for induction of immunity and also show that a booster injection raises the levels of immunity.

Attempts made in sub-unit vaccine development have been initially promising. Preliminary data indicate that Triton X-100 is capable of extracting C. burnetii protein that is at least immunogenic in nature. That is, when we immunized our first group of mice with microgram quantities of a detergent extracted preparation antibody reactive against WCP-I was produced to an average titer of 124. This initial preparation was then employed as a vaccine to induce accelerated clearance in the mouse. This initial study was not conclusive in that the experimental group was small and when challenged and assayed at day 7 4/10 animals contained few spleen associated rickettsia. However 6/10 showed no vaccine effect.

We have recently had to abort some of our clearance studies. As the reader may realize, the Department of Bacteriology and Public Health moved into new accommodations this past year. One of the features of the move was access to

laboratories designed for work with P-3 classified microorganisms. After the move it was determined that no consideration had been made for testing or changing the high-efficiency air-filter system. After the necessary buck-passing it was determined the system had to be modified such that guidelines could be followed. This re-engineering has started and is scheduled to be completed by the latter part of May.

In the interim we have curtailed our protective studies and are currently concentrating on gathering large amounts of WCP-I in order to have sufficient starting material for our planned Triton X-100 extraction procedures that are outlined in our current contract request. Since our single observation with the Triton extract showed antibody formation but variable protective immunity (as measured by clearance rates) we have initiated a study of passive antibody effects on C. burnetii clearance. We injected mice with homologous anti C. burnetii in sufficient quantity that when tested the recipients had a passive titer of not less than 64. Forty-eight hours after the administration of the antiserum the animals were challenged with viable C. burnetii. Spleens were assayed at day 4 and day 7. These experiments indicate that passively administered antibody is not sufficient to prevent the initiation of the rickettsial infection and does not promote the accelerated clearance of C. burnetii. In this regard we have also demonstrated that the nude mouse is capable of producing substantial levels (titer 246 and higher) of anti WCP-I antibody. These athymic mice however do not clear the organism. We find evidence of substantial accumulation of spleen and liver rickettsia 30-40 days after initial infection. We have also observed 40% mortality by 6 weeks in C. burnetii infected nude mice. (These later observations have not been rigorously attributed to C. burnetii. What the nude mouse lacks in T cells is made up for in the caveats associated with work in this strain.) The observation with passive antibody effects in normal mice and the response of the nude mouse may imply a full cooperation of the humoral and cellular immune system in C. burnetii immunity. This statement will become more or less apparent following the analysis that we will include in the Triton sub-unit study.

An unrelated development during this reporting period is our isolation of an apparent continuous cell line. We have maintained in tissue culture guinea pig macrophages for C. burnetii infectivity studies. Macrophages normally have a very low (if any) replication rate in vitro. The normal cells however will allow C. burnetii infection and replication. Since macrophages do not divide

in vitro it is necessary to obtain cells for study directly from the animal. There currently are available three mouse macrophage cell lines and reports relative to these lines indicate that they have potential for the study of many immunologic events. During our work with guinea pig derived macrophages Robert Humphres noted substantial metabolic activity in one of the control macrophage cultures. Further studies indicated that cell division was apparent in the culture that these cells could be subcultured and that microscopically they had the appearance of macrophages. We have done some studies which indicate that these cells are macrophage-like. We have determined that the cells have Fc and complement receptors, they stain esterase positive with α naphthyl butyrate as substrate, are phagocytic, and will support the intracellular replication of C. burnetii. This last observation is dependent on controlling the "macrophage" metabolic activity since a lower pH in vitro inhibits C. burnetii infectivity and then the macrophage can outgrow the rickettsia. We have not been able, as yet, to hold the cell-line in liquid N_2. If studies can be achieved then greater access to this putative guinea pig macrophage cell line can be had by other interested investigators.

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