AD-A158 176 STUDIES ON THE ANTIGENIC COMPOSITION OF COXIELLA BURNETII(U) WASHINGTON STATE UNIV PULLMAN D J HINRICHS DEC 79 DADA17-73-C-3090 1/1

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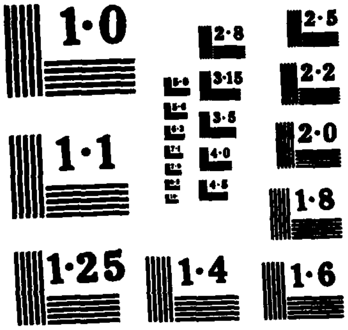

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STUDIES ON THE ANTIGENIC COMPOSITION OF COXIELLA BURNETII

ANNUAL REPORT

DAVID J. HINRICHS

Dec.
~~September~~ 1979

Supported by

US ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701

Contract No. DADA17-73-C-3090

Washington State University
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REPORT DOCUMENTATION PAGE

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1. REPORT NUMBER

2. GOVT ACCESSION NO. AD-A158 176

3. RECIPIENT'S CATALOG NUMBER

4. TITLE (and Subtitle)
Studies on the antigenic composition of *Coxiella burnetii*

5. TYPE OF REPORT & PERIOD COVERED
Annual Progress Report
1 July 1979 - 31 December 1979

6. PERFORMING ORG. REPORT NUMBER

7. AUTHOR(s)
David J. Hinrichs, Ph.D.

8. CONTRACT OR GRANT NUMBER(s)
DADA17-73-C-3090

9. PERFORMING ORGANIZATION NAME AND ADDRESS
Washington State Univeristy
Pullman, Washington 99164

10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
62776A.3M162776A841.00.083

11. CONTROLLING OFFICE NAME AND ADDRESS
U.S. Army Medical Research & Development Command
Fort Detrick, Frederick, MD 21701

12. REPORT DATE
~~September 6~~ Dec, 1979

13. NUMBER OF PAGES
11

14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)

15. SECURITY CLASS. (of this report)
unclassified

15a. DECLASSIFICATION/DOWNGRADING SCHEDULE
N/A

16. DISTRIBUTION STATEMENT (of this Report)

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Accession For
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17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Coxiella burnetii, Q-fever, antigenic analysis, chemical composition, cell-mediated immunity, animal model, in vitro analysis.

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The work described in this annual report covers an extended period of contract support which covers the period July 1, 1979 to December 31, 1979. This period is supported by contract extension of our previous efforts at elucidating the role of cellular and humoral immunity in *Coxiella burnetii* infections. This report details our findings of the role of passive antibody on *C. burnetii* immunity and introduces our initial attempts to adapt the luminol assay to detect activated macrophages that we know are needed to combat *C. burnetii* infections.

During the period covered by this report we have concentrated our efforts in experiments concerned with detecting a role for antibody in C. burnetii infections. From our previous work we have established that the activated macrophage is the critical component for the control of C. burnetii. Our work has consistently pointed to a lymphocyte-macrophage cooperation in the generation of protective immunity in the C. burnetii system that similarly occurs with most other intracellular parasites. That is, following primary vaccination or upon recovery from active disease, a lymphocyte population exists that upon subsequent exposure to specific antigen will produce (release) molecules that have an effect on macrophages. These lymphocyte derived molecules as a class are called lymphokines and some of these lymphokines have a macrophage activating activity. Thus resistance to C. burnetii can be envisioned to occur due to initial contact by a specific antigen sensitive lymphocyte subpopulation with the "protective" antigen(s) of C. burnetii. This leads to the production of a lymphokine that activates a population of macrophages. This activation event is measured by many physiologic changes in the macrophage. One of these activation events gives the macrophage the ability to successfully eliminate parasitic intracellular replication by C. burnetii.

In our experiments with the guinea pig and mouse model systems we have always observed antibodies associated with a cellular immune response. Our previously reported work has failed to show a role for these antibodies independent of the requirement for activated macrophages. We have recently extended our observations in the antibody system by passive transfer studies in normal and athymic mice. Since the early work on neutralizing antibody in Q fever by Abinanti and Marmion (Am. J. Hyg. 66:173, 1957) it has been assumed that antibody has some role in C. burnetii infections. Abinanti's observations were made from results obtained by injecting C. burnetii anti-C. burnetii immune complexes into experimental animals. In repeating the work in the mouse we find that immune complexes of C. burnetii are rapidly cleared and that no disease is evident by spleen smear evaluation. We have recently extended these observations in a series of experiments which tested the role of antibody in C. burnetii infections by varying the time that passively administered antibody was present in the system before or after C. burnetii challenge. As is shown in Table 1 mice that have recovered from C. burnetii challenge will rapidly clear a rechallenge dose of C. burnetii. Animal groups that have the highest antibody titer usually are very efficient in clearing the viable challenge dose. At the time of challenge immunized animals had relatively high titers (256 by microagglutination). This antibody associated clearance of challenge organism coupled with the C. burnetii immune complex observation of Abinanti prompted the following passive transfer experiments. As shown in Tables 2, 3, and 4 we administered anti-C. burnetii homologous antiserum to groups of mice. The recipients of the antiserum were either challenged with C. burnetii 24 hrs after antibody was injected, at the same time that antibody was administered or, 24 hrs before antibody was administered. The titer of antibody that we observed in unchallenged transfer recipients was 64.

As can be seen in Table 2, antibody administered before challenge with C. burnetii had a clearance potentiating effect. This antibody effect was most easily seen at day 14 post challenge, but was evident at day 7. If one administers antibody simultaneously with the C. burnetii challenge dose it is again evident that antibody effects at day 14 are readily detectable but that day 7 effects are not as apparent. If one delays the administration of antibody for 24 hrs following challenge the results are as presented in Table 4. In these experiments it is evident that detectable antibody effects are absent. The C. burnetii infection follows its normal course in mice given immune serum or normal serum. Thus, once the rickettsia are intracellular passive antibody has apparently no effect on the clearance of C. burnetii. This latter situation is similar to what occurs in a natural infection in that specific antibody would not usually be encountered by the invading parasite until some time has passed following infection.

Since the enhanced clearance effect of passively administered antibody is most apparent 14 days post challenge it is possible that the antibody is acting in some way to accelerate the development of the needed cell mediated mechanism. Anti-C. burnetii - C. burnetii immune complexes are probably phagocytized more readily due to the F_c receptor on macrophages. More rapid phagocytosis in the presence of antibody may also lead to more efficient processing of antigenic material with the subsequent development of cell mediated immunity in a shorter time period. Since antibody does not efficiently penetrate living cells, the effect of passively administered antibody 24 hrs after challenge would not have an immediate effect and could perhaps only have some effect on C. burnetii phagocytosis after intracellular release.

In order to test the premise that antibody leads to an increased rate of cell mediated immunity expression we also tested the effect of passive antibody on C. burnetii infection in athymic mice. As can be seen in Table 5, antibody administered 24 hrs prior to C. burnetii challenge has no effect on the course of infection in nude mice but has an enhanced clearing effect on normal mice as also seen in Table 2. The experiments with the athymic mice serve to support our general observations on the role of lymphoid cells and antibodies in the protective mechanisms demonstrable in the C. burnetii system. That is, activated macrophages are essential for immunologic control of C. burnetii replication, macrophages are activated by lymphoid products, antibody may accelerate the destruction of C. burnetii by activated macrophages, antibody-C. burnetii complexes may accelerate the development of cell mediated immunity to C. burnetii, and finally antibody in the absence of activated macrophages does not lead to efficient control of the intracellular replication potential of C. burnetii.

The completion of our studies on the immunologic mechanism of C. burnetii control have demonstrated the need to be able to easily determine when specific cell mediated immunity exists. We feel that detection methods should either evaluate the activation state of the macrophage itself or be able to measure those lymphokines that are critical in the macrophage activation process. The general theme of our contract proposal (pending) relates to the latter approach. We have also completed some initial studies on an assay that may easily identify levels of macrophage activation. If this assay proves out it could allow rapid detection of existing cellular immunity in any system.

We have used the luminol-enhanced chemiluminescence assay employing opsinized zymosån as the phaogyctic particles. Resident peritoneal cells or adherent spleen cells from mice immunized two weeks previously were compared to the same cell populations from non-immune mice (non-activated). Table 6 shows that cells from immune animals exhibited from 6-fold (adherent spleen cells) to 11-fold (peritoneal cells) increased chemiluminescence when compared to non-immune control cell populations. Also, immune peritoneal cells exhibited a 3-fold increase in chemiluminescence compared to non-immune peritoneal cells when the assay was run without any phagocytic particles being present in the assay system. This suggests that either the immune cells have a higher basal rate of production of oxidizing agents and/or that they secrete more of these agents into the medium. We are currently trying to correlate increased chemiluminescence with the ability of macrophages to kill C. burnetii in vitro. These preliminary results suggest that the luminol assay may be an acceptable assay to measure the activation of macrophages by lymphokines in vitro.

TABLE 1

Relative Rate of Clearance of *C. burnetii* in Actively Immunized Balb/C Mice

Immunizing dose of viable[a] *C. burnetii* 10^{-3}	Challenge dose of *C. burnetii*	Relative Spleen Content of Rickettsiae[b]		
		Day 7[c]	Day 14	Day 21
+	10^{-2}	1+-2+ (256)[d]	1+ (512)	± (1024)
-	10^{-2}	4+ (32)	3+ (256)	2+ (1024)
+	10^{-3}	1+ (128)	1+-(±) (256)	± (512)
-	10^{-3}	3+ (16)	2+ (256)	1+ (512)

[a] Balb/C mice were injected with a 10^{-3} dilution of 50% yolk sac suspension and allowed to recover for two weeks prior to challenge.

[b] Scored according to the following protocol: (-) no rickettsiae detected; ± - occasional infected cell seen; 1+ - less than 10 rickettsiae; 2+ - tens of rickettsiae; 3+ - hundreds of rickettsiae; and 4+ - uncountable number of rickettsiae per each field of view. Spleen smear evaluations were reported as the average of three animals.

[c] Days post challenge with *C. burnetii*, phase I.

[d] Microagglutination Ab titer.

TABLE 2

Passive Transfer of Resistance to *C. burnetii* Challenge Mediated by Humoral Antibody Administered 24 h Prior to Challenge

Challenge dose[a] of *C. burnetii*	Immune Serum	Normal Serum	Relative Spleen Content of Rickettsiae[b]	
			Day 7[c]	Day 14
10^{-2}	+	0	3+ (32)[d]	1+ (16)
10^{-2}	0	+	4+ (8)	3+ (128)
10^{-3}	+	0	2+ (16)	(±) (64)
10^{-3}	0	+	3+ (8)	2+ (128)
Serum Control[e]	+	0	(64)	(64)

[a] Animals were treated intravenously with 0.5 ml of immune serum (titer 512) or normal serum 24 h prior to challenge with viable *C. burnetii*.

[b] Scored according to the following protocol: (-) no rickettsiae detected; ± - occasional infected cell seen; 1+ - less than 10 rickettsiae; 2+ - tens of rickettsiae; 3+ - hundreds of rickettsiae; and 4+ - uncountable number of rickettsiae per each field of view. Spleen smear evaluations were reported as the average of three animals.

[c] Days post challenge with *C. burnetii*, phase I.

[d] Microagglutination Ab titer.

[e] Serum obtained from control animals receiving 0.5 ml of immune serum (titer 512) without challenge of *C. burnetii*.

TABLE 3

Passive Transfer of Resistance to *C. burnetii* Challenge Mediated by Humoral Antibody Administered Simultaneously with the Challenge Dose of *C. burnetii*

Challenge dose[a] of *C. burnetii*	Immune Serum	Normal Serum	Relative Spleen Content of Rickettsiae[b]	
			Day 7	Day 14
10^{-2}	+	0	3+ (16)[d]	2+ (32)
10^{-2}	0	+	4+ (8)	3+ (128)
10^{-3}	+	0	3+ (16)	(±) (32)
10^{-3}	0	+	3+ (8)	2+ (128)
Serum Control[e]	+	0	(64)	(64)

[a] Animals were treated intravenously with 0.5 ml of immune serum (titer 512) or normal serum and viable *C. burnetii* simultaneously.

[b] Scored according to the following protocol: (-) no rickettsiae detected; ± - occasional infected cell seen; 1+ - less than 10 rickettsiae; 2+ - tens of rickettsiae; 3+ - hundreds of rickettsiae; and 4+ - uncountable number of rickettsiae per each field of view. Spleen smear evaluations were reported as the average of three animals.

[c] Days post challenge with *C. burnetii*, phase I.

[d] Microagglutination Ab titer.

[e] Serum obtained from control animals receiving 0.5 ml of immune serum (titer 512) without challenge of *C. burnetii*.

TABLE 4

Passive Transfer of Resistance to *C. burnetii* Challenge Mediated by Humoral Antibody Administered 24 h Post Challenge

Challenge dose[a] of *C. burnetii*	Immune Serum	Normal Serum	Relative Spleen Content of Rickettsiae[b]	
			Day 7[c]	Day 14
10^{-2}	+	0	4+ (32)[d]	3+ (64)
10^{-2}	0	+	4+ (16)	3+ (128)
10^{-3}	+	0	3+ (32)	2+ (64)
10^{-3}	0	+	3+ (16)	2+ (128)
Serum Control	+	0	(64)	(64)

[a] Animals were treated intravenously with 0.5 ml of immune serum (titer 512) or normal serum 24 h post challenge with viable *C. burnetii*.

[b] Scored according to the following protocol: (-) no rickettsiae detected; ± - occasional infected cell seen; 1+ - less than 10 rickettsiae; 2+ - tens of rickettsiae; 3+ - hundreds of rickettsiae; and 4+ - uncountable number of rickettsiae per each field of view. Spleen smear evaluations were reported as the average of three animals.

[c] Days post challenge with *C. burnetii*, phase I.

[d] Microagglutination Ab titer.

[e] Serum obtained from control animals receiving 0.5 ml of immune serum (titer 512) without challenge of *C. burnetii*.

TABLE 5

Effect of Passive Transfer of Immune Serum on *C. burnetii* Challenge[a] in Nude (nu/nu) Mice

Mouse Strain	Immune[b] Serum	Normal Serum	Relative Spleen Content of Rickettsiae[b]	
			Day 7	Day 14
nu/nu	+	0	2+-3+ (32)[d]	4+ (16)
nu/nu	0	+	3+ (8)	4+ (16)
Balb/C	+	0	2+ (32)	± (64)
Balb/C	0	+	3+ (16)	1+ (32)

[a] Animals received a challenge dose = 10^{-3} dilution of a 50% yolk sac suspension of *C. burnetii* phase I.

[b] Animals were treated intravenously with 0.5 ml of immune serum (titer 512) or normal serum 24 h prior to *C. burnetii* challenge.

[c] Scored according to the following protocol: (-) no rickettsiae detected; ± - occasional infected cell seen; 1+ - less than 10 rickettsiae; 2+ - tens of rickettsiae; 3+ - hundreds of rickettsiae; and 4+ - uncountable number of rickettsiae per each field of view. Spleen smear evaluations were reported as the average of three animals.

[d] Microagglutination Ab titer.

TABLE 6

Luminol-Enhanced Chemiluminescence Immune[a] and Non-Immune Balb/C Macrophages

Cell Population	Maximal Chemiluminescence Response (counts per minute)[b]
1. Non-immune resident peritoneal cells	14,932
2. Immune resident peritoneal cells	169,759
3. Non-immune adherent spleen cells	33,459
4. Immune adherent spleen cells	192,942
5. Non-immune resident peritoneal cells (no phagocytic particles in assay system)	22,964
6. Immune resident peritoneal cells (no phagocytic particles in assay system)	65,764

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[a] Immune cells were from Balb/C mice immunized 14 days previously.

[b] Scintillation counter set at Out of Coincidence phase, gain 600, window 50-1000.

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