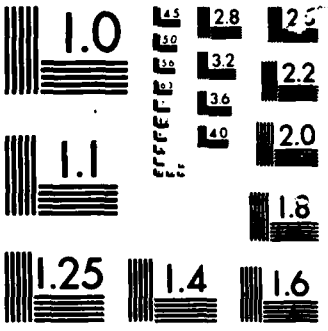
AD-A168 354 MECHANISMS OF PROTECTIVE IMMUNOGENICILTY OF MICROBIAL VACCINES OF MILITARY MEDICAL SIGNIFICANCE(U) CALIFORNIA UNIV IRVINE DEPT OF MEDICINE M S ASCHER ET AL. 1/1

UNCLASSIFIED 15 MAY 84 DAMD17-80-C-0154 F/G 6/15 NL



SECURITY CLASSIFICATION

1. REPORT NUMBER	AD-A168 354 — SION NO.
	READ INSTRUCTIONS BEFORE COMPLETING FORM
	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Sub) Mechanisms of Protective Immunogenicity of Microbial Vaccines of Military Medical Significance	5. TYPE OF REPORT & PERIOD COVERED Final (1 Sept. 1980-31 Dec. 82 Annual(31 Dec.1981-31 Dec. 82
	6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) Michael S. Ascher, M.D. Monique A. Berman, Ph.D.	8. CONTRACT OR GRANT NUMBER(s) DAMD17-80-C-0154
9. PERFORMING ORGANIZATION NAME AND ADDRESS Department of Medicine University of California, Irvine	10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 62770A.3M162770A871.BB.079
11. CONTROLLING OFFICE NAME AND ADDRESS U.S. Army Medical Research and Development Command Fort Detrick, Frederick, MD 21701-5012	12. REPORT DATE 15 May 1984
	13. NUMBER OF PAGES
14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office)	15. SECURITY CLASS. (of this report) Unclassified
	15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release, distribution unlimited

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)

Q Fever, cellular immunology, vaccines, human studies, skin testing, delayed hypersensitivity, lymphocyte transformation, immunofluorescence.

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

Initial clinical studies of Phase I Q fever vaccine (IND #610) and skin test were performed under this Contract. A total of 242 volunteers received skin tests and 22 received vaccine at two dose levels. Skin test reactions were classified according to the duration of persistence of erythema and skin test results were correlated with serologic and in vitro lymphocyte stimulation results. A role for lymphocyte testing in predicting skin test reactions was

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

defined. In response to vaccine, local reactions were minimal and without abscess or granuloma formation. Immunologic results show consistent antibody responses and variable lymphocyte transformation responses to Q fever antigens.

Dermal granulomatous skin reactions seen to Q fever vaccine antigens in immune guinea pigs have been analysed for their immunologic basis. New information has been obtained on the interrelations between antigens of C. burnetii and their relative contributions to granuloma formation. A purified fraction of C. burnetii obtained by extraction with chloroform and methanol (CMR) has been characterized as a skin and lymphocyte antigen in man and guinea pigs. CMR is devoid of granuloma reactivity but carries the determinant for delayed hypersensitivity and lymphocyte stimulation.

A solid phase (FIAX) immunofluorescent assay procedures was developed for serodiagnosis of Q fever and used in the evaluation of volunteers responses to Q fever vaccine and skin test as well as in an intensive national surveillance program.

Mechanisms of Protective Immunogenicity of Microbial Vaccines of Military Medical Significance

Annual and Final Summary Report

Michael S. Ascher, M.D.
Monique A. Berman, Ph.D.

15 May 1984

Supported by

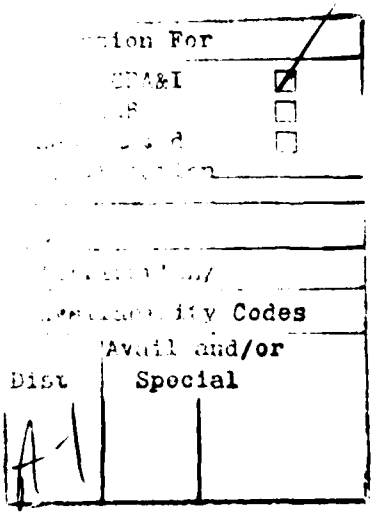
U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701-5012

Contract No. DAMD17-80-C-0154

University of California, Irvine
Irvine, California, 92717

FOREWORD

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).



SUMMARY

Initial clinical studies of Phase I Q fever vaccine (IND #610) and skin test were performed under this contract. A total of 242 volunteers received skin tests and 22 received vaccine at two dose levels. Skin test reactions were classified according to the duration of persistence of erythema and skin test results were correlated with serologic and *in vitro* lymphocyte stimulation results. A role for lymphocyte testing in predicting skin test reactions was defined. In response to vaccine, local reactions were minimal and without abscess or granuloma formation. Immunologic results show consistent antibody responses and variable lymphocyte transformation responses to Q fever antigens.

Dermal granulomatous skin reactions seen to Q fever vaccine antigens in immune guinea pigs have been analyzed for their immunologic basis. New information has been obtained on the interrelations between antigens of *C. burnetii* and their relative contributions to granuloma formation. A purified fraction of *C. burnetii* obtained by extraction with chloroform and methanol (CMR) has been characterized as a skin and lymphocyte antigen in man and guinea pigs. CMR is devoid of granuloma reactivity but carries the determinant for delayed hypersensitivity and lymphocyte stimulation.

A solid phase (FIAX) immunofluorescent assay procedures was developed for serodiagnosis of Q fever and used in the evaluation of volunteers responses to Q fever vaccine and skin test as well as in an intensive national surveillance program.

PROGRESS

1. Clinical Studies of Q Fever Vaccine

In this contract we conducted the initial studies of the IND 610 Q fever vaccine and skin test in man. 242 individuals received skin tests and 22 received vaccine. No serious adverse reactions have been noted in these subjects. Local reactions have been limited to transient erythema and pain without evidence of abscess or granuloma formation. Immunologic results show the appearance of antibody measured by FIAX immunofluorescence in all vaccinated subjects. These results are published. (Ascher, M.S., Berman, M.A., Ruppanner, R. Initial Clinical and Immunological Evaluation in Man of a New Phase I Q Fever Vaccine and Skin Test. J Inf Dis 148:214-222, 1983.)

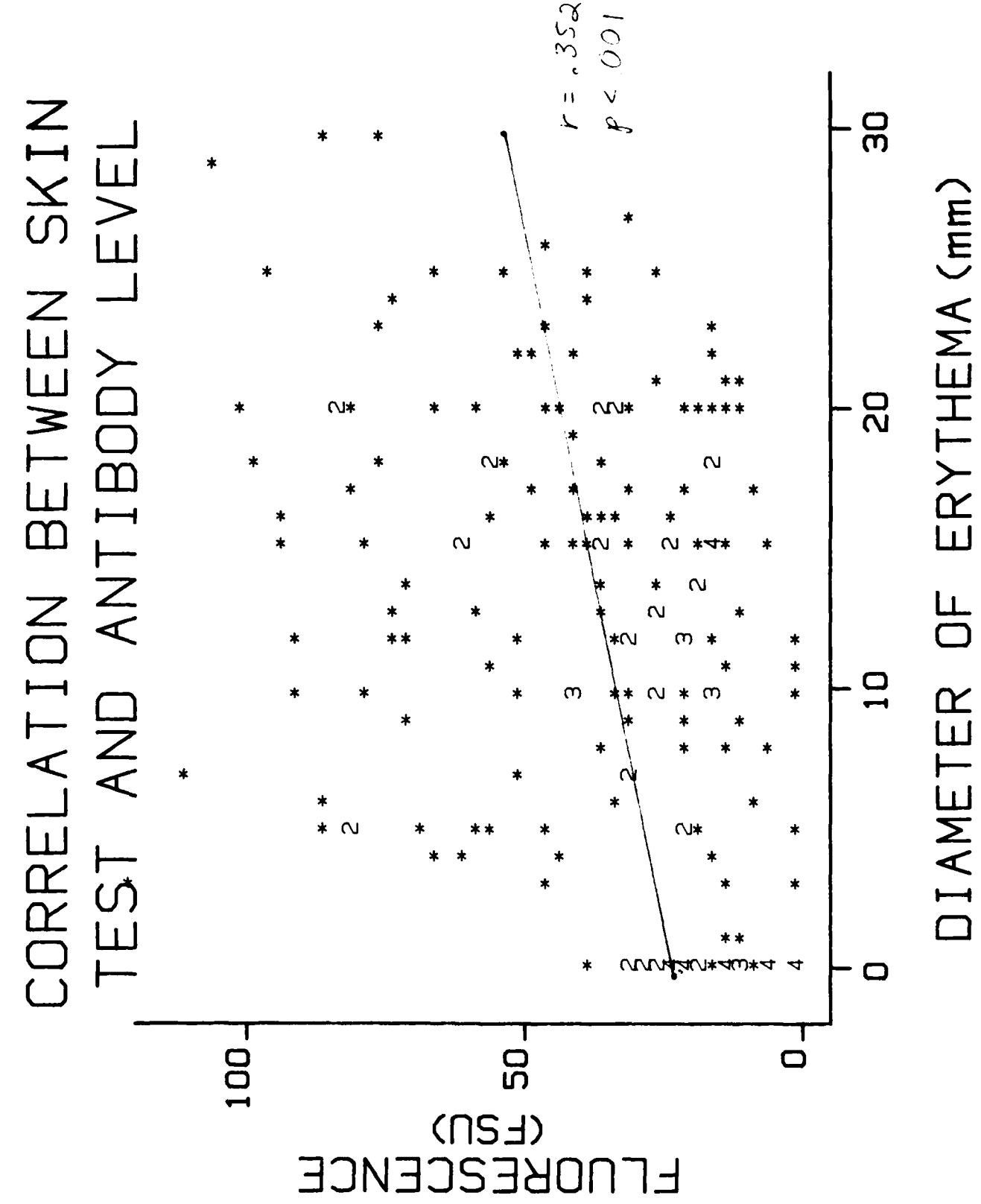
Since our publication, the relationships between skin test results and serologic results have been analyzed in a larger group of individuals now comprising a total of 242 subjects. In the series of skin tested individuals, we have correlated skin test erythema diameter at 24 hours and the level of antibody measured by FIAX immunofluorescence. As presented in Table 1 and Figure 1, the relationship previously described still holds with the addition that some reactions read as only 4 mm at 24 hours are seen in individuals carrying antibody suggesting that these reactions must be considered significant. The overall correlation remains the same in this larger sample (r = .351, p= 0.001).

TABLE 1

CROSSTABULATION OF ANTIBODY LEVELS AND SKIN TEST DIAMETER

	FIAX Immunofluorescence		
MM at 24 hrs.	Negative 0 to 37	Positive 38 +	Total
0 to 3	37 92.5	3 7.5	40
4 to 9	16 53.3	14 46.7	30
10 to 19	48 59.3	33 40.7	81
20+	17 41.5	24 58.5	41
Total	118 61.5	74 38.5	192

Figure 1


CORRELATION BETWEEN SKIN
TEST AND ANTIBODY LEVEL
r = .352
p < 001
FLUORESCENCE
(FSU)
100
50
0
0
10
20
30
DIAMETER OF ERYTHEMA (mm)

The next relationship we have been able to address is based on our observation that skin test types in individuals vary. We have classified skin reactions into 6 types based on the kinetics and intensity of development of erythema and induration. The types of reactions are classified as follows:

A) negative throughout 7 days of observation.
B) less than 4 mm at 25 hours becoming larger at 48.
C) greater than 10 mm at 24 hours becoming negative at 48 hours.
D) greater than 10 mm at 24 hours becoming smaller but not negative at 48 hours.
E) greater than 10 mm at 24 hours remaining the same at 48 hours.
F) positive at 24 hours becoming larger at 48 hours.

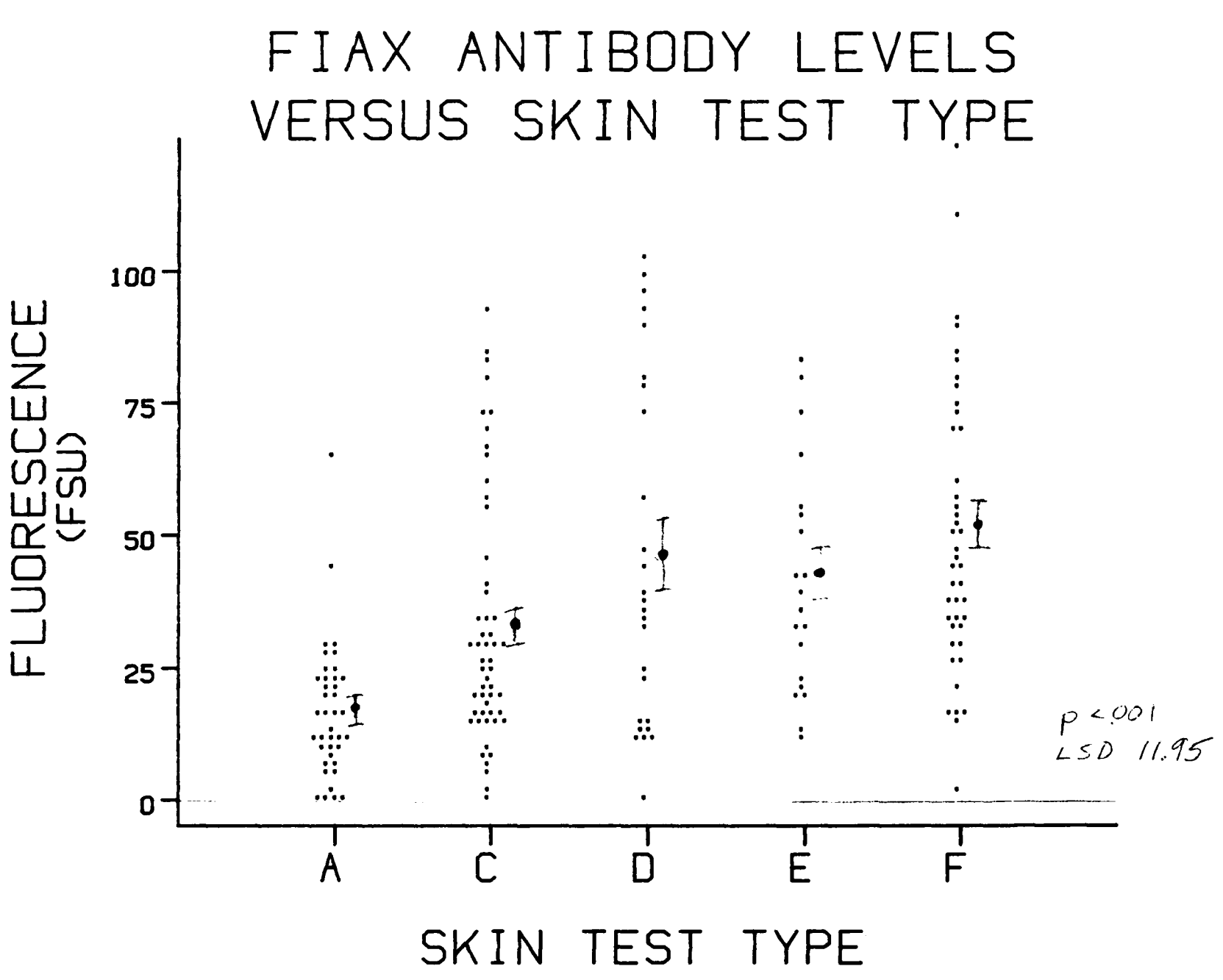
Based on this classification scheme we have tested the hypothesis that the type of skin reaction may correlate with the presence of antibody or its change after skin testing. One theoretical consideration is that circulation antibody might have a greater frequency on the Jones-Mote or type C reactions consistent with a suppressive effect of the humoral immune system on cellular immune functions. In contrast to this prediction, we find a direct correlation between the intensity of skin reactions and the frequency of antibody. The evanescent reactions (type C) had an antibody frequency of only 28% whereas the "solid" type F reactions had a frequency of 65%. The full breakdown of these data is presented in Table 2 and the quantitative relationship between these data is presented in Figure 2.

TABLE 2

DISTRIBUTION OF ANTIBODY RESULTS BY SKIN TEST TYPE

	FIAX Immunofluorescence		
Skin test type	Negative 0-37u	Positive 38+u	Total
A - Neg	37 94.9	2 5.1	39
C - Pos then 0	38 71.7	15 28.3	53
D - Pos then less	13 50.0	13 50.0	26
E - Pos then same	10 50.0	10 50.0	20
F - Pos then more	15 34.9	28 65.1	43
Column	113	68	181

Figure 2


FIAX ANTIBODY LEVELS
VERSUS SKIN TEST TYPE
FLUORESCENCE
(FSU)
100
75
50
25
0
A
C
D
E
F
SKIN TEST TYPE
p < .001
LSD 11.95

Both the frequency of positive antibody (Table 2) and its level (Figure 2, one-way analysis of variance, $p<0.05$) are increased in higher grade reactions. The overall frequency of positive antibody in skin test positives is still only 47%.

Although antibody was not a very good predictor of skin reactivity, the possibility existed that the booster effects on antibody due to skin testing might be related to skin test type or intensity. In 60 individuals who were seronegative prior to skin test, we were able to obtain sera after skin test alone and have analyzed the change in antibody in FIAX units with respect to the intensity and type of skin reaction. In contrast to the results obtained with antibody prior to skin tests, there was no significant relationship between change in antibody and skin test type.

Finally, we have had the opportunity to analyze the antibody levels measured against the major virulence component of the organism, the phase I antigen. Phase I antibodies rarely persist after normal convalescence. There was a good correlation between the phase I antibody and the phase II antibody (data not shown) but there was no additional predictive relationship between the phase I antibody and skin test size type which was not already reflected in correlations with the phase II antibody.

Skin testing

One observation in the early trial was the low degree of skin test conversion obtained after vaccine. Although positive skin test reactions were seen in the individuals who experienced adverse local vaccine reactions, the skin test results were low grade and frequently not of a quality typical of delayed hypersensitivity. Further studies should be aimed at the effects of booster immunizations or skin test reactions and lymphocyte stimulation.

In addition, we have demonstrated a rise in antibody after skin testing alone in all individuals, regardless of skin test type as discussed above. These findings speak highly for the immunogenic potential of this vaccine since a dose as small as 20 ng boosts antibody in immunes. Previous studies have demonstrated antibody rises after a higher dose of vaccine given to skin test positive humans who developed rather severe local skin reactions. These recent findings validate the activity of the skin test reagent and strengthen the case for its continued use in clinical trials versus screening potential vaccines by serologic means.

Lymphocyte testing

More important prospects for a solution to the difficulty with serologic screening, however, come from our recent observations with the lymphocyte transformation test in these individuals. We have addressed in detail the relationships between various humoral and cellular immune tests and skin

reactivity in the group of 74 skin tested individuals. There was a good overall correlation between FIAX antibody status and skin reactivity, but 22 out of 59 individuals who were antibody negative and positive skin tests in our test group. We have analyzed the correlations between serologic, skin test and lymphocyte data in the 27 individuals on whom complete studies were performed. Strong positive correlations were found between FIAX antibody status, skin reactivity and LT to both specific Phase I and Phase II antigens. LT results with the nonspecific mitogen PHA failed to correlate with any of these variables. Because all variables were highly intercorrelated, we then performed partial correlation analysis to assess the independence of these effects. Significant independent correlations were found between FIAX antibody and QII LT, between FIAX antibody and skin reactivity, and between skin reactivity and QI LT. Finally discriminant analysis was applied to determine the ability of the immunologic tests to predict skin reactivity. The best predictor was found to be FIAX antibody status, but a significant additional contribution was made by Q I LT. No other factors added to the accuracy of classification. Overall, all 17 skin test negative individuals and 7 of 10 skin test positive individuals were correctly classified by the discriminant function using FIAX antibody and phase I LT results even though 5 of the latter group were antibody negative. Both FIAX antibody and QII-LT testing have a high proportion of false-negatives and false positives respectively and Phase I-LT is the best overall predictor of skin reactivity when analyzed in this way.

2. Studies of Dermal Granulomatous Hypersensitivity in Q Fever Immune Guinea Pigs

Studies on the granulomatous skin reactions seen to Q fever antigens in immune guinea pigs have concentrated on testing a variety of antigenic preparations of C. burnetii for their relative ability to induce or elicit such reactions. The findings of the studies are published. (Ascher, M.S., Berman, M.A., Parker, D., Turk, J.L. Experimental Model for Dermal Granulomatous Hypersensitivity in Q Fever. Inf Imm 39:388-393, 1983.) (Ascher, M.S., Williams, J.C., Berman, M.A. Dermal Granulomatous Hypersensitivity in Q Fever: Comparative Studies of the Granulomatous Potential of Whole Cells of Coxiella burnetii, Phase I and Subfractions. Inf Imm 42:887-889, 1983.) (Ascher, M.S., Williams, J.C., Berman, M.A.. Cellular Immunologic Analysis of Coxiella burnetii Antigens. Microbiology 84.) In summary, the skin reactions were all qualitatively similar when either Phase I vaccines, Phase II vaccines, or TCA soluble and insoluble extracts were used to skin test Phase I immune animals. When animals were immunized with the various preparations and skin tested with whole cell Phase I vaccine, it was the group of Phase II immune animals which showed accelerated granuloma formation at three weeks. Parallel lymphocyte transformations studies revealed a high degree of cross reactivity between Phase I vaccine and TCA residue with a very selective response of Phase II immune animals to Phase II vaccine. These findings together suggest that the metabolic alteration which results in phase variation to Phase II produces

preferential expression of a specific "Phase II antigen" which is a significant contributor to the granulomatous reaction. One fraction, the residue of extraction by chloroform and methanol (CMR) elicits delayed hypersensitivity in sensitized guinea pigs with little or no granulomatous activity compared to whole cell products. These studies have also shown that the antigenic determinant responsible for induction and stimulation of lymphocyte reactivity in vitro is heavily represented in the CMR fraction. These findings lead to the proposal for initial testing of CMR vaccine in man.

3. FIAX Immunofluorescence Testing for Q Fever

In the two years of the contract our laboratory performed biosurveillance testing on over 1000 samples from 23 institutions inside and outside of California using our published FIAX technique (Ascher, M.S., Horwith, G.S., Thornton, M.F., Greenwood, J.R., Berman, M.A. A Rapid Immunofluorescent Procedure for Serodiagnosis of Q Fever in Mice, Guinea Pigs, Sheep and Humans. Diagnostic Immunology 1:33-38, 1983). positive samples clearly illustrate the sensitivity of FIAX testing and its reliability for screening serum samples. Although there is little increase in fluorescence over the ı6-128 CF range, there is little increase in fluorescence between CF titers of 8 and 16, the critical point of discrimination between "positive" and "negative" samples. Parallel testing of 40 of our samples by the unit at USAMRIID revealed agreement between their micro IF test and our FIAX results in 38 of 40 specimens.

As a result of recent laboratory outbreaks, two features of Q fever are bound to receive more attention in the future, the chronic disease state and endocarditis. The hallmark of these states is the presence of high titer specific antibody to phase I C. burnetii antigen. Given the fact that we have over 100 random positive sera on hand, we elected to survey a group of them for the presence of phase I antibody by FIAX. The thought was that a high phase I FIAX value may provide an early predictor of those at risk for chronic manifestations of Q fever. The results of this testing revealed a straightforward correlation between the two tests with no evidence of high phase I titers in this pilot group. The highest Phase I FIAX value was in a vaccinee. Two other samples with high positive phase II FIAX from suspect chronic cases were negative for phase I antibody. Since the publication of the manuscript, we have used the FIAX assay to characterize the class of immunoglobulin produced in response to infection or immunization. When two sequential sets of sera were compared, one from a clinical case and one from a vaccinee, equivalent FIAX values were obtained using a conjugated antiserum reactive against total immunoglobulin. When the IgG and IgM antibody classes were assayed separately the response of the clinical case was almost entirely IgG and the vaccinee's response IgM confirming previous results.

One additional observation with the FIAX assay in evaluating vaccine recipients has been that the fluctuations seen in antibody

levels after vaccine were greater than might be expected from this stable assay. Testing of immunoglobulin class of antibody retrospectively revealed sharp peaks of the IgM antibody associated with repeated skin tests. This finding may be useful in the follow-up of vaccinated individuals for evidence of subsequent infection.

We have also applied the FIAX assay to sera obtained from four different clinical situations 1) recent mild disease 2) vaccination 3) granulomatous hepatitis and 4) endocarditis. The titration of the quantitative FA result shows an extremely high titer of antibody in the chronic disease. We would therefore include a higher dilution in the evaluation of maximally reactive sera to screen for chronic disease. This result has direct utility in the clinical laboratory setting where FIAX technology is available and lessens the requirement for tedious dilution required in the standard microscopically read FA test.

4. Q Fever and Public Health

In addition to the recognized risk to military personnel of Q fever in natural exposure settings or biologic warfare, a number of institutional outbreaks have occurred associated with contact of humans with infected experimental sheep. The risk of this situation was first highlighted by an outbreak in 1979 at the University of California, San Francisco involving 150 cases and one death.

In 1980, year the UCSF outbreak, similar epidemics were noted at the University of Colorado Medical Center and at the Letterman Army Institute of Research in San Francisco (Ascher, unpublished). The next year, 1981, saw an outbreak reported from Bristol, England and in 1982 an identical situation occurred at the Hospital for Sick Children in Toronto, Canada. As was pointed out by Meikeljohn, these outbreaks were not always obvious, but in each case some individuals became severely ill, alerting physicians to look for the cause of the problem. The number of institutions with grants for conduct of sheep research was estimated by NIH to be 274 in 1981 with a total budget of $29 million. Every one of these units has the potential for Q fever transmission. The topic of laboratory acquired Q fever has been on the program of the American Association of Laboratory Animal Science national meeting and was a major symposium topic at the International Congress of Laboratory Animal Science in Vancouver in August of 1983. This symposium resulted in the formulation of an updated information statement (Grant, C.G., Ascher, M.S., Bernard, K.W., Ruppanner, R., Vellend, H. Q Fever and Experimental Sheep. Infection Control 6:122-123, 1985.)

END

DTIC

7-86