Combined Epidemic Typhus and Q-Fever Vaccine: Adjuvant Effect Of *Coxiella burneti.* (32317)

J. A. Morris, C. L. Wisseman, C. G. Aulisio, E. B. Jackson, and J. E. Smadel*

Department of HEW, U. S. Public Health Service, National Institutes of Health, Division of Biologics Standards, Laboratory of Virology and Rickettsiology, Bethesda, Md. and School of Medicine, University of Maryland, Baltimore, Md.

This note reports results obtained in tests in guinea pigs and in man on the antibody responses to Cox-type epidemic typhus vaccine combined with formalin-killed Q-fever rickettsiae and compares these with the antibody responses to conventional Cox-type epidemic typhus vaccine. The findings show that the Q-fever rickettsiae acted as an immunologic adjuvant in the combined product, potentiating the antibody response to the epidemic typhus component.

Materials and methods. Epidemic typhus vaccine. A detailed description of methods employed to prepare the concentrated, partially purified, epidemic typhus vaccine used in this work is given elsewhere(1). Briefly, yolk sac tissues harvested from chick embryos infected with the Breinl strain of *Rickettsia prowazeki* were suspended in sufficient sterile saline to make a 10% suspension and treated with formalin. The sediment obtained by high speed centrifugation was resuspended in 1/10 the original volume in saline containing merthiolate. This was extracted with ether and the resulting aqueous phase constituted the concentrated vaccine.

Typhus vaccine potency assay. The epidemic typhus vaccine was assayed for potency in mouse protection, toxin neutralization and complement fixation tests employing standard procedures described elsewhere(1,2,3). The $\log_{10}$ index of the vaccine potency obtained in the mouse protection test was 2.7, *i.e.,* 1.0 ml of a 1:500 dilution of vaccine when injected into mice would be expected to protect 50% of the test mice against an intravenous challenge of 2 units of epidemic typhus toxin. Pooled serum obtained from vaccinated guinea pigs yielded a toxin neutralization (TN) antibody titer of 1:64 and a complement fixing (CF) antibody titer of 1:128 against 2 units of epidemic typhus soluble antigen.

Q-fever vaccine. The Q-fever vaccine consisted of a formalin-killed 10% suspension of yolk sacs infected with the 22nd egg passage of the Henzerling strain of *Coxiella burneti* and was prepared by the Division of Biologics Products, Walter Reed Army Institute of Research, following the methods described earlier(4). The organisms at this passage level were largely in phase 2, but some residual rickettsiae in phase 1 were probably also present. When used as a CF antigen with 2 units of Q-fever antibody, the vaccine had a titer of 1:8.

Guinea pigs were a mixed breed and were maintained before and during use under specific pathogen-free conditions. At the time of vaccination they weighed 300 to 400 g.

Volunteers were young, adult, male, first and second year medical students at the University of Maryland, Baltimore, who gave negative reactions at 48 hours and at 7 days when examined in Q-fever sensitivity tests(5) by intradermal injection of 0.05 ml of Q-fever vaccine diluted 1:100.

Vaccination and bleeding schedules. After collection of prevaccination blood specimens the guinea pigs and volunteers were given the volumes and antigen amounts by the subcutaneous route according to the vaccination schedules shown in Tables I and II. Blood specimens were collected from the vaccinated guinea pigs and men 7, 14 and 21 days later.

Serologic testing. The guinea pig and human sera were separated from the clot on the day of collection and stored at —20°C until examined 1 to 5 months later. The conduct of the epidemic typhus CF and TN and the Q-fever CF tests was as described earlier(1,3,4). CF antibody titers were expressed as the reciprocal of the highest dilution of serum

* Deceased

giving 3+ or 4+ fixation of complement in tests with 2 units of antigen and 2 full units of complement with overnight incubation at 4°C before addition of the hemolytic system.

Results. Serologic findings in guinea pigs. The serologic response in guinea pigs injected with concentrated epidemic typhus vaccine mixed with an equal volume of killed Q-fever rickettsiae was compared with that obtained in guinea pigs receiving one or the other of the components making up the combined product. Vaccine dosage, injection schedules, antibody levels for individual guinea pigs and the geometric mean titer (GMT) for each of the 3 test groups of guinea pigs are given in Table I. At 14 days after the second injection of vaccine, a striking enhancement of about 3.5- and 5-fold, respectively, of epidemic typhus CF and TN antibodies was observed in the animals which received the combined epidemic typhus-Q-fever product as compared with those which received the typhus component only. In contrast, no significant difference in Q-fever CF antibody response was found to exist between the animals which received the combined vaccine and those which received the Q-fever component only. These results strongly indicate that the *C. burneti* exerted an adjuvant action for the typhus component in the mixed vaccine.

Responses in man. Groups of 18 to 23 young, adult, male, medical students were injected with aliquots of the same preparations that had been examined in guinea pigs. Vaccine dosage schedules and the serologic responses as measured at 21 days after the administration of the first dose of vaccine are given in Table II. None of the 83 volunteers developed local or systemic reaction in the 3 weeks following vaccination. It is seen in Table II that members in Group III who were injected with two 1-ml doses of the combined preparation received the same antigen mass that was administered to members in Group I (Q-fever rickettsiae only) and in Group II (epidemic typhus vaccine only). Further, it is noted that members in Group IV received only ¼ the amount of each of the 2 components that were administered to members in the other 3 groups. The combined product when given in full dosage (Group III) in-

TABLE I. Determination in Guinea Pigs of Effect on Antibody Response of Addition of Q-Fever Rickettsiae to Epidemic Typhus Vaccine.

Vaccines	Vaccine assay values: Mouse protection index (log_{10})	Vaccine assay values: Vaccinated guinea pig serum titers TN	Vaccine assay values: Vaccinated guinea pig serum titers CF	Reciprocal of serum titers of individual guinea pigs (at 21 days)									Geometric mean titer
Q-fever alone*	—	—	—	0/256‡	0/128	0/128	0/128	0/128	0/64	0/64	0/64	0/64	0/110
				0	0	0	0	0	0	0	0	0	0
Epidemic typhus alone*	2.7	1:64	1:128	256/0	256/0	256/0	128/0	128/0	64/0	64/0	64/0		140/0
				64	64	32	64	64	64	64	32		54
Q-fever and epidemic typhus combined†	—	—	—	4096/128	1024/128	1024/64	512/64	512/64	256/512	256/128	256/128	128/128	512/119
				128	256	256	512	256	512	512	512	64	276

* 2 0.5 ml subcutaneous doses on days 0, 7.

† 2 1.0 ml subcutaneous doses on days 0, 7.

‡ Epidemic typhus CF titer / Q-fever CF titer / Epidemic typhus TN titer.

TABLE II. Determination in Volunteers of Effect on Antibody Response of Addition of Q-Fever Rickettsiae to Epidemic Typhus Vaccine.

Group	Vaccine and dosage schedule*	Reciprocal of serum titers in individual volunteers (at 21 days)											No. volunteers: In group	No. volunteers: With antibody rises	Geometric mean titer
I	Q-fever alone	0/16†	0/8	0/8	0/4	0/4	0/4	0/4	0/2	0/2	0/2	0/0			
		0	0	0	0	0	0	0	0	0	0	0	21	0/10	0/0.2
	2 0.5 ml doses on days 0, 14	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0			0	0
		0	0	0	0	0	0	0	0	0	0				
II	Epidemic typhus alone	64/0	64/0	32/0	32/0	32/0	32/0	32/0	32/0	32/0	32/0	32/0			
		64	32	64	64	32	32	32	32	16	16	4	21	20/0	14/0
	2 0.5 ml doses on days 0, 14	16/0	16/0	16/0	8/0	8/0	8/0	8/0	8/0	8/0	0/0			21	22
		32	16	16	64	32	16	16	4	4	16				
III	Q-fever and epidemic typhus combined	256/0	256/2	128/0	128/4	64/0	32/0	32/0	32/0	32/0	32/0	32/0	32/0		
		256	128	128	32	64	128	64	64	32	32	32	32	23	21/3	16/0.02
	2 1.0 ml doses on days 0, 14	32/2	32/0	32/0	16/0	16/0	16/0	8/0	4/0	4/0	0/0	0/0			23	42
		32	32	16	64	32	32	32	32	8	32	16			
IV	Q-fever and epidemic typhus combined	64/4	64/0	64/0	64/0	64/0	32/0	16/0	16/0	16/0					
		128	128	64	32	8	8	32	16	4			18	15/1	5/0.01
	1 0.5 ml dose on day 0	8/0	8/0	4/0	4/0	4/0	4/0	0/0	0/0	0/0				16	9
		16	16	64	32	16	4	4	0	0					

* Administered subcutaneously.

† $\frac{\text{Epidemic CF / Q-fever CF}}{\text{Epidemic TN}}$.

duced epidemic typhus TN antibody in all 23 recipients and induced epidemic typhus CF antibody in all except 2 individuals. The GMT of TN antibody in Group III was 42 or about 2 times as great as the GMT value of 22 for this antibody in Group II. The difference in the GMT of the CF antibody was not as marked as that recorded for the TN antibody; however, if the individual responses in the 2 groups are matched it is seen that there were 2 non-responders in Group III and 1 in Group II; 2 individuals with titers that were lower in Group III than in Group II; 7 with titers that were the same and 12 with titers that were 1- to 4-fold higher in Group III than in Group II. Individuals in Group IV received only a single dose of vaccine consisting of ¼ the epidemic typhus antigen mass that was administered to members in Group II. This dosage elicited an antibody response in 16 of the 18 recipients in Group IV but the GMT of the CF and TN antibodies was approximately 2.5 to 3 times higher in Group II than in Group IV.

Discussion. The addition of *Coxiella burneti* to the list of immunologic adjuvants that already includes aluminum and calcium compounds, water-in-oil emulsions, endotoxins and bacteria is in itself of little importance. However, the observation that the addition of killed *C. burneti* to inactivated epidemic typhus vaccine potentiates the antibody response to the typhus component of the mixture assumes importance in any consideration concerned with the continued development of a bivalent Q-fever-epidemic typhus vaccine. Such an objective is worthy of consideration if for no other reason than to reduce the number of injections needed to establish effective immunity in populations at risk to infection with the etiologic agents of the two diseases. One of the original intents of this study was to explore the feasibility of combining 2 rickettsial vaccines into a single product which would yield an acceptable antibody response to both components. This not only was accomplished for both components when guinea pigs were vaccinated with the combined *R. prowazeki-C. burneti* preparation but also yielded striking evidence that the Q-fever component served as an effective adjuvant in guinea pigs for the epidemic typhus antigens.

When tested in humans who had no prior experience with either epidemic typhus or Q-fever antigens, an excellent CF antibody response was elicited by the epidemic typhus vaccine when used alone and in the combined preparation. However, the adjuvant effect of the *C. burneti* for the typhus antigens in the combined preparation was not evident to the same degree as had been observed in guinea pigs, although there did appear to be a slight trend towards higher epidemic typhus antibody titers in the recipients of the combined vaccine. The low antibody response in man to the *C. burneti* preparation, when used alone or in combination with epidemic typhus vaccine, is consistent with the experience of others(6). In any case, the compatability of the 2 vaccines in a mixture appears to be established.

Years of experience with Q-fever vaccine has resulted in the definition of the major dangers associated with its use in man and these risks must be weighed against benefits which might be gained by use of *C. burneti* as an adjuvant in epidemic typhus vaccine or as one of the components in a bivalent epidemic typhus-Q-fever vaccine. Use of *C. burneti* as a component in a combined vaccine should present no problem not associated with its use as a monovalent vaccine. The same careful attention to detect persons who either by vaccination or infection have had prior experience with Q-fever rickettsiae must be exercised in any circumstance involving use of a vaccine containing Q-fever antigens. Almost all groups who have used this immunogen in such sensitized people have reported severe local and systemic reactions (6). In the present work each of the volunteers prior to their participation in the study were shown by skin tests not to have had experience with Q-fever antigens and not to possess Q-fever CF antibody in their prevaccination sera. In none of the volunteers were untoward reactions observed in the 3 weeks following injection of Q-fever material.

Except for aluminum potassium sulfate and aluminum hydroxide, immunologic adjuvants have not had wide acceptance for use in

man. The objections to their use are numerous and include data recorded in animal studies which link water-in-mineral oil adjuvants to autoimmune disease, allergy and cancer. Moreover, there are unresolved questions concerned with the long term effects of retention of mineral oil in tissues. *C. burneti* presents another kind of immunologic adjuvant and is one of rickettsial origin which together with those of bacterial origin (*Brucella abortus*(7), *Bordetella pertussis*(8)) might constitute a family of adjuvants of biologic origin deserving of intensive study to isolate and to characterize the immunologic potentiating factors. In each of the 3 instances mentioned the enhancing organism is itself an agent of disease against which an effective immunity is desirable.

Summary. 1. Two doses of a partially purified, concentrated, formalin-killed epidemic typhus vaccine yielded excellent antibody responses in guinea pigs and in man. Even a single dose of this vaccine elicited an appreciable antibody response in man. 2. The compatibility of the epidemic typhus vaccine with a formalin-killed Q-fever vaccine administered as a mixture has been established in both guinea pigs and in man. 3. The *C. burneti* component of the combined vaccine definitely serves as an immunologic adjuvant for the epidemic typhus antigen in guinea pigs and may enhance the antibody response to the epidemic typhus component to some degree in man.

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Alterations in Erythrocyte Phospholipids Produced by Environmental Change.* (32318)

WILLIAM H. R. NYE AND GUIDO V. MARINETTI (Introduced by Lawrence E. Young)
(Technical Assistance by Bonnie Fratta)
Departments of Medicine and Biochemistry, University of Rochester School of Medicine and Dentistry, Rochester, N. Y.

Phospholipids undoubtedly play an important structural and functional role in the erythrocyte membrane. The percentage relationships among individual membrane phospholipids, their fatty acid composition and the permeability characteristics of the membranes vary among mammalian species(1). Although changes in the fatty acids of individual membrane phospholipids are readily produced by dietary manipulation in human subjects(2) and in animals(1,3,4), phospholipid composition itself is felt to be characteristic of, and fixed in, each species(1,5). In man, careful study has shown no variation in red cell phospholipid composition with dietary manipulation(2), in malnutrition(6), and in a variety of hematological disorders (4), with the exceptions of a-beta-lipoproteinemia(6) and of four cases of hereditary nonspherocytic hemolytic disease(7). The existence of abnormal plasma phospholipids in a-beta-lipoproteinemia and the ready exchange of P^{32}-labelled phospholipids between

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