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EFFECT OF LYSOZYME ON THE IMMUNE RESPONSE OF GUINEA PIGS TO THE SOLUBLE PHASE 1 ANTIGEN OF *COXIELLA BURNETII*

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Summary. — Pretreatment of guinea pigs with lysozyme prior to vaccination with the phase 1 antigen of *Coxiella burnetii* enhanced antibody response and protection against challenge. An observed effect on macrophage migration suggests that the role of lysozyme includes stimulation of cell-mediated immunity.

Key words: Coxiella burnetii; lysozyme; enhanced protection; antigen 1

The surface antigen of *Coxiella burnetii* has been shown to be far less reactogenic than cell wall or whole cell preparations of the rickettsia (Anacker *et al.*, 1962). Brezina *et al.* (1974) and Cracea *et al.* (1973) demonstrated the feasibility of using this so-called "soluble" antigen (antigen 1) as a vaccine for humans. Although this antigen has the advantage of low reactogenicity, it is about 10 times less immunogenic than the intact whole organism (Anacker *et al.*, 1962).

We reported earlier (Wachter *et al.*, 1978) that the immunogenicity of antigen 1 was enhanced when the complex of polyriboninosinic-polyribocytidylic acid, poly-L-lysine and carboxymethyl-cellulose (poly(ICLC)) was administered to guinea pigs prior to antigen. In more recent tests we have found that pretreatment of guinea pigs with lysozyme prior to vaccination with the antigen produced a higher antibody response and increased protection against challenge. Also, an observed effect on macrophage migration suggests that lysozyme may stimulate the cellular immune response. The effect of lysozyme on the immune response of guinea pigs to antigen 1 of *C. burnetii* is described in this paper.

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals" as promulgated by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

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In a series of five similar, but not identical, experiments, guinea pigs (8 or 10 per group for a total of 92) were inoculated subcutaneously (s.c.) with two doses, 14 days apart, of antigen only or of lysozyme followed by antigen 4 or 5 hr later. Saline and lysozyme control groups (a total of 63 guinea pigs) were included. In some additional tests we varied the time of administration of lysozyme relative to antigen. A dialyzed trichloroacetic acid extract of concentrated, partially purified phase I *C. burnetii*, Henzerling strain, was employed as the antigen. Lysozyme (3× crystalline egg white, Sigma Chemical Co., St. Louis, Mo.) was administered at both the first and second dose interval. Doses of antigen ranged from 2 to 14 μg of protein, as determined by the method of Lowry *et al.* (1951); doses of lysozyme ranged from 12.5 to 250 μg. Doses used in each test are listed in Table 1. Serum samples collected 14 days after the second inoculation were assayed for antibody by the microagglutination (MA) (Fiset *et al.*, 1969) and complement fixation (CF) (Casey, 1966) tests. Guinea pigs were challenged intraperitoneally 28—45 days after the second dose with 5×10^5 EID_{50} of phase I *C. burnetii* (fourth yolk-sac passage og the Henzerling strain). Temperatures were recorded once daily for 10 days; animals with temperatures >40.0 °C for two or more consecutive days were considered unprotected.

The effect of pretreatment of guinea pigs with lysozyme on protection against Q fever by antigen 1 is indicated in Table 1. No optimal dosage combination was found; lower dose levels were as effective as higher levels. Fifty-nine percent of 46 guinea pigs that received antigen only were protected compared to 83 % of 46 that received lysozyme prior to antigen ($P < 0.02$). The time of administration of lysozyme relative to antigen was important: injection of lysozyme 24 or 48 hr before antigen or at the same time (at a different site or mixed with antigen) either had no effect or reduced protection, as compared to guinea pigs that received antigen only.

Table 1. Effect of pretreatment of guinea pigs with lysozyme on protection against Q fever by antigen 1 of C. burnetii

Treatment	Animals with fever/total	Fever days/animal
Saline	40/41	4.3
Lysozyme*	17/22	3.0
Antigen**	19/46	1.4
Lysozyme* prior to antigen**	8/46	0.78

*Lysozyme (μg) employed for first and second doses, respectively, for tests 1 through 5: 12.5, 12.5; 25, 25; 12, 25; 12.5 25; 50, 250.

**Antigen (μg protein) employed for first and second doses, respectively, for tests 1 through 5: 3.5, 3.5; 3.5, 7.0; 3.5, 14.0; 7.0, 7.0; 2.0, 6.0.
The data shown represent the mean of five tests.

For the same guinea pigs referred to in Table 1 the effect of lysozyme on antibody response was determined on sera collected 14 days after the second dose. Table 2 shows the geometric mean titers and percent animals responding for phase 1 and 2 MA antibodies and phase 2 CF antibody (phase 1 CF antibody is not produced at detectable levels from immunization with the phase 1 antigen). The most pronounced difference was seen with phase 2 CF antibody ($P < 0.001$); differences for phase 1 and phase 2 MA antibody titers were also significant, $P < 0.05$ and $P < 0.01$, respectively.

Table 2. Effect of pretreatment of guinea pigs with lysozyme on antibody response to antigen 1 of C. burnetii

Antibody	Treatment		Geometric mean titer	Percent positive
	Antigen	Lysozyme		
Phase 2 CF	+		4.5	42
	+	+	12.2	78
Phase 1 MA	+		3.9	63
	+	+	6.3	82
Phase 2 MA	+		28.6	74
	+	+	39.8	98

*Mean of same 5 tests and same doses as for Table 1.

To investigate the possibility that lysozyme increased protection by stimulation of cellular immune mechanisms, we applied the macrophage migration-inhibition technique to peritoneal cells from 4 groups of guinea pigs (4 per group). Comparison was made between one group that received two doses, 2.0 and 6.0 μg (protein) of antigen only, 14 days apart, and a group that received lysozyme, 50 and 250 μg, 5 hr before each dose of antigen. Peritoneal exudate cells were harvested, processed, and employed in the agarose droplet method of Harrington and Stastny (1973) as applied by Kishimoto and Burger (1977) to detect direct MMI. Cells were collected 4 days after intraperitoneal injection of 25 ml of sterile sodium caseinate. Half of the animals were started on test, i.e., given caseinate, one week, and half, 2 weeks after the second dose of antigen. In the absence of apparent differences, results from the two time periods were combined for purposes of analysis and presentation. Twenty replicate agarose droplets containing exudate cells were prepared from the cells harvested from each guinea pig. Subsets of five droplets each were overlaid with 0.2 ml of medium 199 (with calf serum) or with 0.2 ml of medium containing (a) 100 μg/ml lysozyme, (b) 20 μg/ml antigen, or (c) 100 μg/ml lysozyme and 20 μg/ml antigen. Cultures were incubated, droplets examined, and migration inhibition calculated as described by Kishimoto and Burger (1977).

The migration-inhibition of macrophages from guinea pigs that received antigen only was much less than the inhibition of macrophages from animals that received lysozyme prior to antigen (Table 3). Also, inhibition observed in subsets of droplets in the test system where lysozyme plus antigen were employed as additives was substantially greater then in subsets with antigen alone; this was especially pronounced with macrophages from guinea pigs that received the lysozyme-antigen regimen. Also in this group, lysozyme itself produced limited inhibition.

Active immunity to Q fever has been reported to depend on both humoral and cellular responses (Kishimoto *et al.*, 1978). Other recent research has indicated that cellular immune mechanisms are exclusively responsible for

Table 3. Migration inhibition of macrophages from guinea pigs vaccinated with antigen 1 of C. burnetii, with and without prior administration of lysozyme

Antigen used in test system	Percent migration inhibition	
	Guinea pigs receiving antigen only	Guinea pigs receiving lysozyme before antigen
Lysozyme, 100 μg/ml	0	13
Antigen, 20 μg/ml	9	22
Lysozyme, 100 μg/ml + antigen, 20 μg/ml	20	57

protection against Q fever (Ascher *et al.*, to be published). The increase in CF antibody and the effect on macrophage-migration, which we have observed, suggest that the role of lysozyme in enhancing protection in the guinea pig host against Q fever could include a stimulation of humoral response and cell-mediated immunity.

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