Enhancement of the Immunogenicity of Phase I Antigen of Coxiella burnetii

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The immunogenicity of the soluble phase I antigen of Coxiella burnetii for guinea pigs was enhanced by a nuclease-resistant complex of polyriboinosinic-polyribocytidylic acid, poly-L-lysine, and carboxymethyl cellulose.

The soluble phase I antigen of Coxiella burnetii has been shown to be far less reactogenic than the cell wall extracts or intact rickettsiae used for vaccines (1, 5). Investigators in Czechoslovakia (2) and Romania (3) have demonstrated the feasibility of using the soluble phase I antigen as a vaccine for humans. Whereas this antigen may have the advantage of low reactogenicity, it is apparently less immunogenic than the intact organism (1, 5). Enhancement of its protective capacity would increase its potential for use as a vaccine. Polyriboinosinic-polyribocytidylic acid stabilized with poly-L-lysine and carboxymethyl-cellulose [poly(ICLC)] was shown by Houston et al. (4) to significantly potentiate the primary antibody response in rhesus monkeys to inactivated Venezuelan equine encephalomyelitis virus vaccine. Stephen et al. reported that rhesus monkeys treated prophylactically with poly(ICLC) alone did not die after challenge with virulent yellow fever virus (9) and, in a separate study, demonstrated that poly(ICLC) enhanced the antibody response in rhesus monkeys immunized with swine influenza virus subunit vaccine (8).

We have conducted several protection experiments in guinea pigs to determine the effect of poly(ICLC) on the immunogenicity of the phase I antigen of C. burnetii. In an initial test we found that when poly(ICLC) was mixed with antigen before injection, the protective effect of the antigen was significantly reduced. Additional tests confirmed our observation that mixing poly(ICLC) and antigen either reduced or failed to enhance immunogenicity. However, we also found that the immunogenicity of the soluble antigen was enhanced if poly(ICLC) was administered separately from antigen.

In the present studies two doses of phase I antigen (2.5 µg of protein per dose), given 7 days apart, either alone or in combination with poly(ICLC) (300 µg/kg), were administered subcutaneously to groups of guinea pigs (ranging from six to ten per group). Animals were challenged 14 days later with 5 x 10⁵ 50% infective doses of the fourth yolk sac passage of the Henner-Zerling strain. After challenge, temperatures were recorded daily for 10 days; guinea pigs with temperatures above 40.0°C for 2 or more consecutive days were considered unprotected.

Control animals receiving only saline exhibited a typical fever curve (Fig. 1). In contrast, the mean postchallenge temperature of the group that received poly(ICLC) 5 h before antigen did not vary statistically over time from their mean prechallenge temperature; on days 4 and 5 postchallenge their mean temperature was significantly lower (P < 0.01) than that for animals that received antigen alone. In the group that received poly(ICLC) mixed with antigen, protection was not enhanced and the postchallenge temperature curve did not differ significantly from that of animals receiving antigen alone.

Further investigation of the effect of temporal relationship between injection of antigen and poly(ICLC) showed that significant enhancement of protection by poly(ICLC) occurred when the polycation was administered 24 h, as well as 5 h, before antigen but not when it was injected 24 h after the antigen. The importance of time of injection of polynucleotide in relation to antigen was demonstrated by Schmidtke and Johnson (6) in studying the adjuvant effect of polyadenylic-polyuridylic acid on bovine gamma globulin.

Unlike our findings with the soluble phase I antigen, poly(ICLC) enhanced the immunogenicity of a particulate, whole organism, phase I Q fever vaccine (7), equally well when administered combined with vaccine or 5 h earlier. In single-dose experiments this vaccine (containing 1.0 µg of protein) combined with poly(ICLC) (300 µg/kg) protected 100% of 19 guinea pigs tested, as opposed to only 70% of 17 animals that received vaccine alone.

627
Our results indicate that the vaccine potential of the phase I antigen of *C. burnetii* can be enhanced by poly(ICLC), but further investigation is needed to establish the optimal parameters to be used with an appropriate administration regimen.

**LITERATURE CITED**


**Fig. 1.** Effect of stabilized poly(ICLC) on immunogenicity of phase I antigen of *C. burnetii* in guinea pigs (*n* = 6).