IMMUNOGENICITY AND PROTECTIVE ABILITY OF CORPUSCULAR AND SOLUBLE VACCINES PREPARED FROM DIFFERENT *COXIELLA BURNETII* PHASE I STRAINS

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Summary. – BALB/c mice immunized intraperitoneally (ip) with killed purified *Coxiella burnetii* phase I corpuscular vaccines or trichloroacetic acid (TCA) extracts from phase I corpuscles (soluble vaccines) were protected against ip challenge with both homologous and heterologous *C. burnetii* phase I strains. Though the degree of protection, namely the inhibition of *C. burnetii* multiplication in the mouse spleen slightly varied, in general, corpuscular vaccines provided better protection than soluble ones. Cross-protection was accompanied by comparable levels of cell-mediated immune response as evaluated by lymphocyte transformation test (LTT). However, higher stimulation indices of LTT were obtained with homologous than with heterologous strains. The values of antibody response as determined by enzyme-linked immunosorbent assay (ELISA) were higher with homologous strains too. On average, both antibody-inducing and antibody-binding capabilities of the strains Priscilla and S were lower than those of the Nine Mile and Luga strains, except for values obtained with the antigens from homologous strains.

Key words: *Coxiella burnetii;* corpuscular vaccine; soluble vaccine; immunogenicity; cross-protection; cell-mediated immunity; antibody response

Introduction

Demonstration of heterogeneity in the lipopolysaccharide (LPS) structure (Hackstadt *et al.*, 1985; Hackstadt, 1986) and differences in genome (Vodkin *et al.*, 1986) and plasmid (Samuel *et al.*, 1985) composition of *C. burnetii* phase I strains of different origin raised the problem of cross-immunity between such strains and possibly the necessity of developing the polyvalent Q fever vaccine (Kazár and Řeháček, 1987).

Cross-protection in ip infected guinea pigs between the strains Nine Mile and Priscilla associated with acute and chronic form of Q fever, respectively, was first demonstrated by Moos and Hackstadt (1987). It was confirmed by our cross-protection study on guinea pigs immunized subcutaneously (sc) by corpuscular and soluble vaccines of four *C. burnetii* strains originating from different sources (tick, rodent, domestic animal, man) and associated with acute (strains Nine Mile and Luga) or chronic (strains Priscilla and S) forms of Q fever, and subsequently exposed to infectious aerosol of homologous and heterologous *C. burnetii* strains (Lesný *et al.*, 1991).

The purpose of the present study was to determine the degree of cross-protection between these four different *C. burnetii* isolates and to compare the respective values of cell-mediated and humoral immune response in ip immunized mice. Besides that, the four strains under study were compared for their capability to detect these immune responses, namely in LTT, ELISA and microimmunofluorescence (MIF) tests.

Abbreviations: ELISA = enzyme-linked immunosorbent assay; ip = intraperitoneal(ly); LPS = lipopolysaccharide; LTT = lymphocyte transformation test; MIF = microimmunofluorescence; sc = subcutaneous(ly); SI = stimulation index; TCA = trichloracetic acid

Materials and Methods

Animals. Female BALB/c mice (10 – 12 g) were used throughout the study. The groups of mice (ten per group) were immunized ip with 50 μg of each corpuscular vaccine or with 100 μg equivalent of soluble vaccine.

C. burnetii phase I strains Nine Mile, S and Priscilla were provided by the Rocky Mountain Laboratories, Hamilton, Montana, USA, and the strain Luga was kindly supplied by Prof. A.B. Daiter,

Pasteur Institute, St. Peterburg, Russia. All strains used were in phase I (the 3rd chick embryo yolk sac passage). Phase I corpuscular vaccines consisted of *C. burnetii* corpuscles highly purified by ether extraction and differential centrifugation. Soluble vaccines were prepared by TCA extraction from purified phase I corpuscles as described by Brezina *et al.* (1974).

Protection against *C. burnetii* infection was tested 4 weeks post vaccination in groups of mice (including non-vaccinated controls), by challenging them with 10^6 EID_{50} of each of the strains used. Twenty % spleen suspensions prepared from 5 mice on day 6 post infection (p.i.) were pooled and titrated. The protection was taken for positive when the yield of *C. burnetii* was at least by 2 log EID_{50} units lower than that of controls (Kazár and Schramek, 1985).

LTT. Cell-mediated immunity was evaluated by LTT using mouse splenocytes (from 5 pooled spleens of each group) as indicators of blastogenesis. The radioactivity of incorporated [methyl-^{3}H]thymidine by mouse spleen cells was measured in a liquid scintillation counter (Rackbeta, LKB 1217) and expressed in cpm ± SD. The lymphoproliferative response was expressed as a stimulation index (SI), which was calculated as a ratio of mean cpm in antigen-stimulated vs. control cells (Gajdošová and Brezina, 1989). The experiments were made in triplicates. SI values above or equal to 2 were taken for positive.

ELISA. Humoral immunity was evaluated in 5 pooled sera from each group 4 weeks post vaccination (Peacock *et al.*, 1983) by a modified ELISA (Kováčová *et al.*, 1987). Polystyrene plates (P, Koh-i-noor, České Budějovice, Czech Republic) coated with 100 µl of coating buffer per well containing 5 µg of corpuscular *C. burnetii* phase I or phase II antigen were used. All sera tested were diluted 1:500, 1:1,000, 1:5,000, and 1:10,000. Secondary mouse globulin conjugated with horseradish peroxidase was diluted 1:4,000. After addition of substrate (3,3,5,5-tetramethylbenzidine, Serva) the reaction was stopped with 50 µl of 3 N H_2SO_4. The A_{450} was measured in a spectrophotometer (Labsystem Multiskan MCC/340). The negative-positive cut-off level was calculated from mean values of five negative mouse sera (x ± 3SD).

MIF test for antibodies was carried out only with phase I homologous antigen (Philip *et al.*, 1978). Antibody titers above or equal to 32 were taken for positive.

Results

Cross-protective effects of C. burnetii vaccines

The effectivness of corpuscular and soluble vaccines prepared from four different *C. burnetii* strains in inducing resistance to phase I virulent challenge (10^6 EID_{50}) of each strain used is compared in Table 1. It is expressed as a difference in the yields (in log EID_{50}/ml units) of *C. burnetii* from the pooled spleens of control and immunized mice (underlined values were obtained with homologous strains).

All corpuscular and soluble vaccines exerted a significant (log EID_{50} difference ≥2) protection against all four *C. burnetii* strains used as a challenge. The highest degree of

Table 1. Cross-protective effects of *C. burnetii* corpuscular and soluble vaccines

Vaccine/strain[a]	Difference in yields (log EID_{50}/ml) of *C. burnetii*[b] from the spleens of mice challenged with 10^6 EID_{50} of strain			
	Nine Mile	Luga	Priscilla	S
Corpuscular				
Nine Mile	5.8	5.0	4.0	5.2
Luga	4.0	4.0	2.5	3.7
Priscilla	4.5	3.7	3.8	4.7
S	5.5	5.2	3.5	5.0
Soluble				
Nine Mile	4.8	2.7	3.3	4.2
Luga	4.0	3.0	2.3	2.2
Priscilla	2.8	2.5	3.5	4.0
S	4.3	3.5	3.0	4.2

[a]50 µg of corpuscular and 100 µg equivalent of soluble vaccine, respectively, were applied ip. The values obtained with homologous *C. burnetii* strains are underlined.

[b]Yields (log EID_{50}/ml) of *C. burnetii* strains from control mice were 7.6 (Nine Mile), 7.3 (Luga), 7.8 (S), and 5.6 (Priscilla).

Table 2. Cell-mediated cross-immunity in mice immunized with corpuscular and soluble *C. burnetii* vaccines as evaluated by LTT

Vaccine/strain[a]	SI values with 100 µg of phase I corpuscles of the strain			
	Nine Mile	Luga	Priscilla	S
Corpuscular				
Nine Mile	8.5	5.8	5.6	5.1
Luga	5.7	7.8	5.1	5.3
Priscilla	5.6	4.9	7.2	5.5
S	5.5	4.8	5.3	8.0
Soluble				
Nine Mile	7.6	5.0	4.4	4.0
Luga	4.5	6.9	4.0	4.1
Priscilla	4.7	3.8	6.5	4.4
S	4.2	3.9	4.3	7.3
Control	1.4	1.7	1.3	1.2

[a]50 µg of corpuscular and 100 µg equivalent of soluble vaccine, respectively, were applied ip. The values obtained with homologous *C. burnetii* strains are underlined.

Table 3. Cross-titration by ELISA of sera of mice immunized with corpuscular and soluble *C. burnetii* vaccines

Phase I corpuscular antigen	Approximate antibody titres (x 10^{-3}) in sera of mice immunized with							
	Nine Mile		Luga		Priscilla		S	
	C	S	C	S	C	S	C	S
Nine Mile	≥10	5	7	3	4	1	6	4
Luga	10	5	≥10	4	5	2	4	3
Priscilla	7	2	5	1	9	3	2	3
S	5	2	5	0.5	5	2	≥10	5

C (corpuscular) and S (soluble) refer to 50 µg of corpuscular and 100 µg equivalent of soluble vaccine, respectively, applied ip. The values obtained with homologous *C. burnetii* strains are underlined.

protection (5.8) was found in mice immunized with corpuscular vaccine from the strain Nine Mile and challenged with homologous strain. The lowest protective effects (2.3 and 2.2) were demonstrated with soluble vaccine from the strain Luga and with challenge strains Priscilla and S, respectively. In general, corpuscular vaccines provided better protection than soluble ones, and the protection mediated by a given vaccine was not always higher against the challenge with homologous than with heterologous strains (differences in the degree of protection among the strains varied from 1.2 to 2.1). Of corpuscular vaccines, the most effective was that from the strain Nine Mile, and the least protective was that from the strain Luga. Soluble vaccine from the strain Nine Mile, however, provided the best protection against the strains Nine Mile and S only. In comparing the challenge strains, the best protection was achieved against the strain Nine Mile, except for the mice immunized with corpuscular vaccine from the strain Priscilla and challenged with strain S or for the mice immunized with soluble vaccine from the strain Priscilla and challenged with strains Priscilla or S.

Cross-lymphoproliferative response to C. burnetii vaccines

The cross-lymphoproliferative response of mice immunized with corpuscular and soluble vaccines from the four *C. burnetii* strains under study to corpuscular phase I anti-

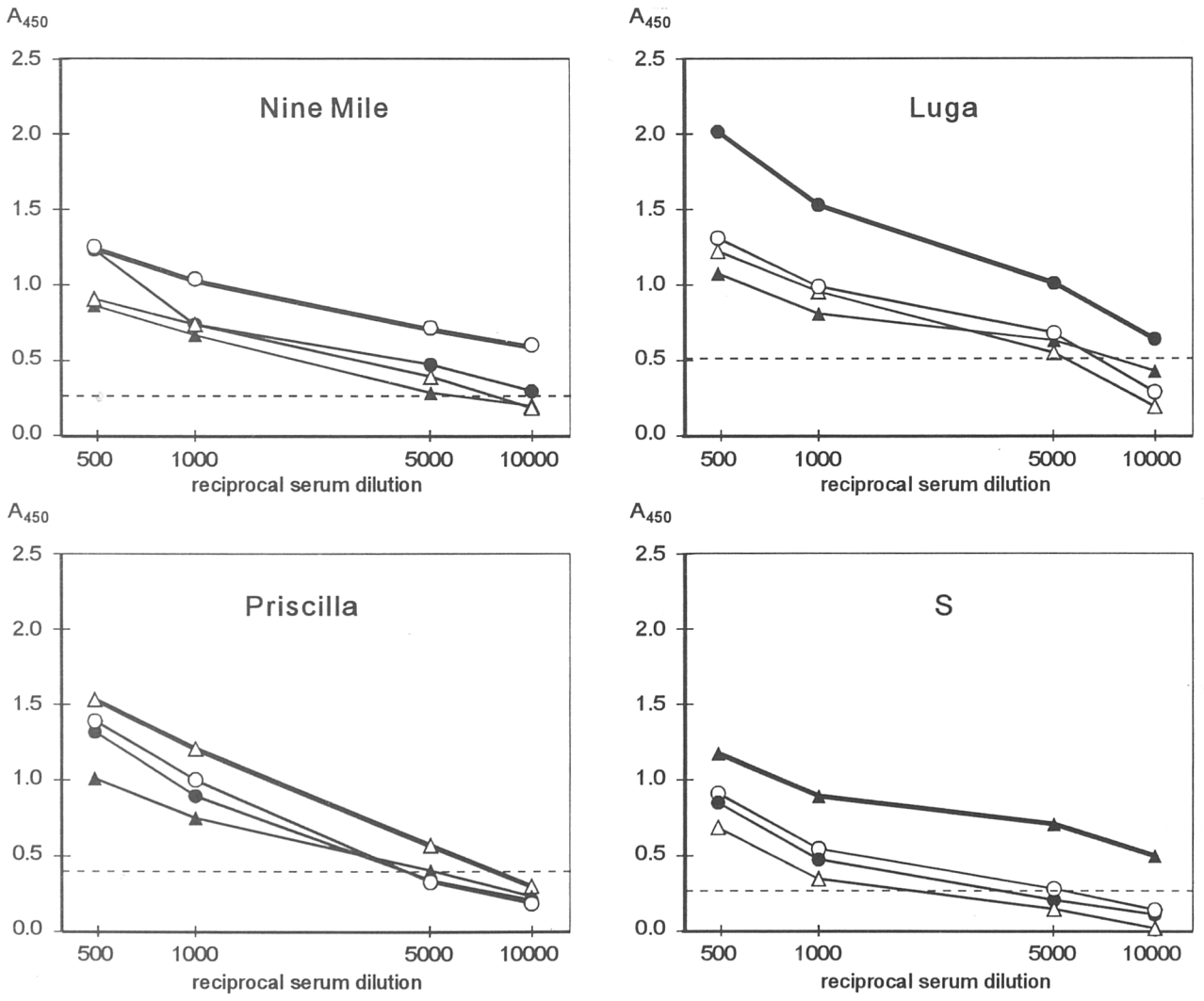

Fig. 1

Detection of phase I antibodies by ELISA in sera of mice immunized with corpuscular vaccines from *C. burnetii* phase I strains by use of corpuscular antigens of corresponding strains

Dashed lines represent (x + 3 SD) values for each antigen used. Strains Nine Mile (○), Luga (●), Priscilla (Δ), S (▲).

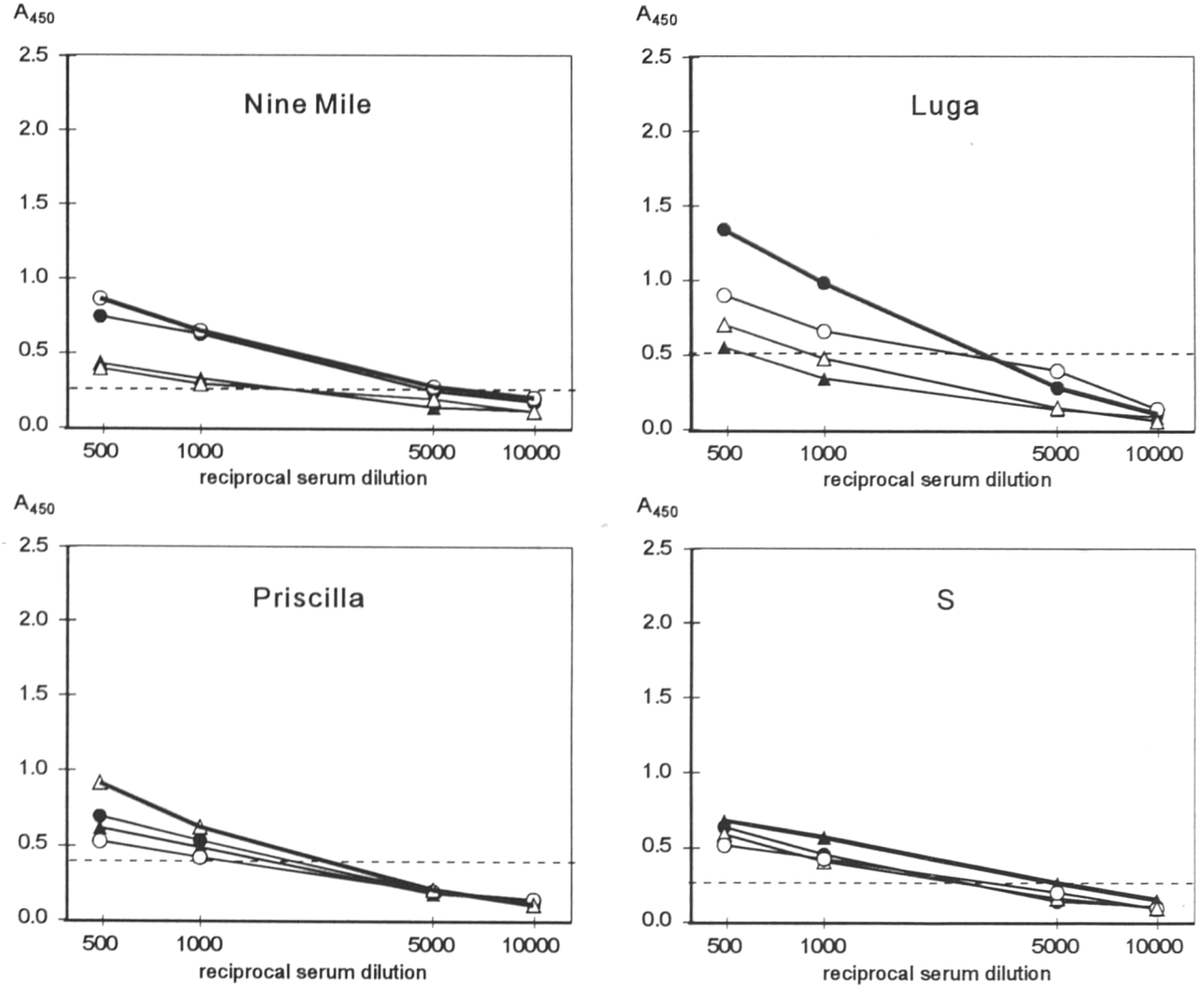

Fig. 2
Detection of phase I antibodies by ELISA in sera of mice immunized with soluble vaccines from *C. burnetii* phase I strains
For the legend see Fig. 1.

gen of these strains is shown in Table 2. In the groups of mice immunized with corpuscular vaccines, the highest proliferation was observed with the vaccine from the Nine Mile strain tested with homologous antigen (SI = 8.5). With other antigens (Luga, Priscilla, S) in this group, the SI values were lower (5.8 – 5.1). Similar situation was in the groups of mice immunized with corpuscular vaccines from the strains Luga, Priscilla and S, where SI values were higher with homologous (8.0 – 7.2) than with heterologous (5.8 – 4.8) antigens.

In all groups of mice immunized with soluble vaccines, the lymphoproliferative response was lower. The highest proliferation was achieved again in the group of mice immunized with the vaccine from the strain Nine Mile tested with homologous antigen (SI = 7.6). With other antigens, the SI values varied from 5.0 to 4.0. In the group of mice immunized with soluble vaccine from the strain S, the SI value with homologous antigen was 7.3, while with other antigens it ranged from 4.3 to 3.9. The mice immunized with soluble vaccine from the strain Luga responded in LTT to homologous antigen with SI value 6.9, whereas to heterologous antigens with SI values from 4.5 to 4.0 only. In the case of Priscilla strain, the SI values were 6.5 with homologous and 4.7 – 3.8 with heterologous antigens, respectively.

Cross-antibody response to C. burnetii vaccines

The results of antibody detection by ELISA in sera of mice immunized with corpuscular and soluble vaccines from four *C. burnetii* strains under study are presented in

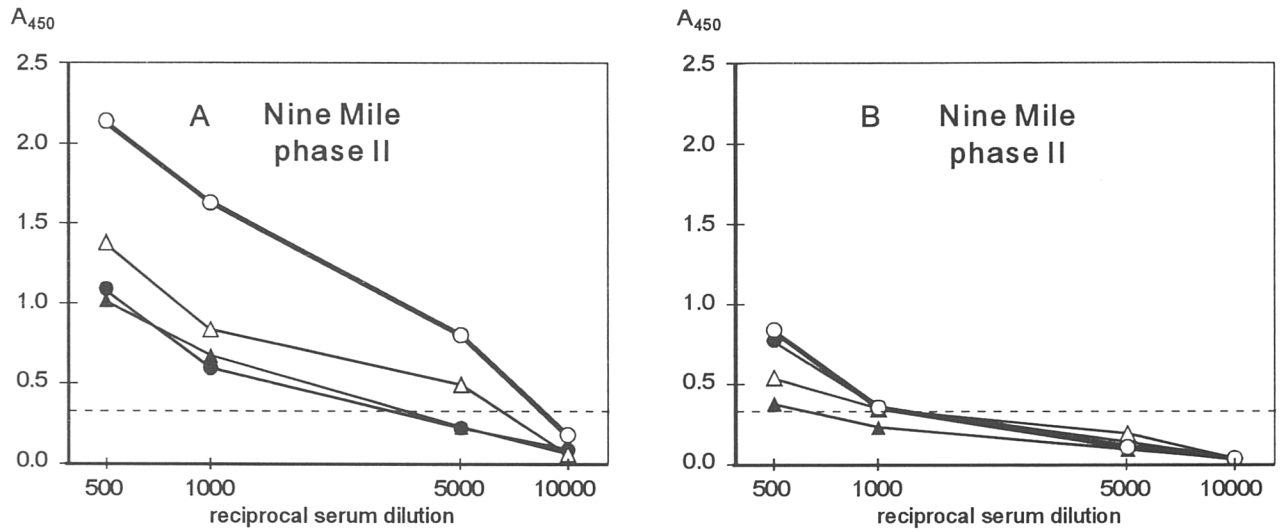

Fig. 3
Detection of phase II antibodies by ELISA in sera of mice immunized with corpuscular and soluble Q fever vaccines
For the legend see Fig. 1.

Figs. 1–3. For detection of phase I antibodies (Figs. 1 and 2), corpuscular antigens of all four strains were used; for detection of phase II antibodies (Fig. 3), only corpuscular antigen of the strain Nine Mile was available.

In all cases, the highest A_{450} values of phase I antibodies were found when the sera were tested with homologous antigen, no matter what kind of vaccine, corpuscular (Fig. 1) or soluble (Fig. 2), was employed for immunization of mice. Soluble vaccines were less immunogenic as evidenced by detection of lower levels of phase I antibodies by corpuscular antigens from both homologous and heterologous strains (Fig. 2). The same can be applied to the levels of phase II antibodies which were markedly lower in the sera of mice immunized with soluble vaccines (Fig. 3).

The results of cross-titration of mouse sera using the calculated approximate antibody titers are summarized in Table 3, from which also differences in both antibody-inducing and antibody-binding capability of four *C. burnetii* strains can be seen. In general, (a) corpuscular and soluble vaccines from the strains Nine Mile and Luga induced higher phase I antibody levels than the vaccines from the strains Priscilla and S except for cases when antibodies were tested with homologous antigens, and (b) soluble vaccine from the strain Luga induced lower phase I antibody levels tested with strain S antigen. At the same time, antigens from the strains Nine Mile and Luga were able to detect higher levels of phase I antibodies in both homologous and heterologous sera. Similar result revealed the MIF test in which the phase I antibody titers in sera of mice immunized with corpuscular vaccines and tested with antigens from homologous strains only were 128 for the strains Nine Mile and Luga, but 64 and 32 for the strains Priscilla and S, respectively.

Discussion

Till the mid 80-ties discussion on the proper Q fever vaccine was aimed at more the way of its preparation rather than at its source, i.e. the *C. burnetii* strain of choice. Apart from a live vaccine consisting of the attenuated *C. burnetii* strain M (Genig *et al.*, 1960), corpuscular phase I vaccine (Marmion *et al.*, 1984), soluble vaccine consisting of the TCA extract from *C. burnetii* phase I corpuscles (Cracea *et al.*, 1973; Brezina *et al.*, 1974), and chloroform-methanol-treated phase I corpuscles residue (Williams and Cantrell, 1982) were considered the candidates for Q fever human specific prophylaxis. As a matter of fact, all these vaccines were successfully used in field vaccination trials (Genig *et al.*, 1965; Kazár *et al.*, 1982; Marmion *et al.*, 1990; Fries *et al.*, 1993); however, the use of attenuated vaccine was not further recommended (Kazár and Řeháček, 1987) because of its relative instability and the possibility of its adverse effects (Johnson *et al.*, 1976; 1977).

Though chronic Q fever human cases were known earlier (Marmion, 1967), only in the mid 80-ties the forms of disease caused by *C. burnetii* was correlated with the plasmid type (Samuel *et al.*, 1985) and antigenic variation in phase I LPS (Hackstadt, 1986). In view of these findings, the question of monovalent vs. polyvalent Q fever vaccine emerged and awaited solution. As follows from the results obtained in this study with four *C. burnetii* strains differing

not only in their origin, but also LPS structure and plasmid composition, a sufficient degree of protection can be achieved to both homologous and heterologous challenge. It applies also to soluble vaccines, though corpuscular vaccines provided better protection, in accord with our previous findings (Kazár and Schramek, 1985). Thus, these results are fully coincident with demonstration of cross-protection between different *C. burnetii* strains in guinea pigs exposed to ip (Moos and Hackstadt, 1987) or by aerosol administered (Lesný *et al.*, 1991) challenge. In view of these findings one may conclude that the monovalent vaccine prepared e.g. from the so far used phase I Nine Mile strain can be effective also against heterologous *C. burnetii* strains.

The concept of the use of monovalent Q fever vaccine, which is of great practical importance, is strongly supported also by recent data demonstrating the lack of pathotype-specific gene in human *C. burnetii* isolates (Stein and Raoult, 1993), the serotyping of *C. burnetii* isolates from acute and chronic Q fever patients by MoAbs (Yu and Raoult, 1994), a doubt about the possibility of plasmid differentiation of *C. burnetii* in "acute" and "chronic" isolates (Thiele and Willems, 1994), and the description of a new plasmid (QpDV) common to *C. burnetii* isolates associated with acute and chronic form of Q fever (Valková and Kazár, 1995). They all indicate that original grouping of *C. burnetii strains* according to their plasmid (type and size) and the disease they cause in humans (Mallavia, 1991) should be reconsidered.

The cross-protection between *C. burnetii* strains under study corresponded to the results of evaluation of cell-mediated and humoral immune responses, though higher SI values and phase I antibody levels were obtained with antigens of homologous than heterologous strains, irrespective of whether mice were immunized with corpuscular or soluble vaccines. However, higher values of cell-mediated and humoral immune responses were observed with corpuscular than soluble vaccines given to mice, indicating again the higher immunogenicity of *C. burnetii* corpuscles than of their extracts.

From the results obtained follows that indices of both cell-mediated (SI assayed by LTT) and humoral immunity (antibodies determined by ELISA) reflected with certain biological variation the protection against phase I virulent *C. burnetii* challenge. In our field studies on detection of post-vaccination immune response in human volunteers vaccinated with a Q fever soluble chemovaccine (Brezina *et al.*, 1974), ELISA was found the most suitable of several serological tests used (Kováčová *et al.*, 1991). The LTT positivity, however, could be detected even 10 years post vaccination (Gajdošová *et al.*, 1991).

Differences were found also in both antibody-inducing and antibody-binding capabilities of *C. burnetii* strains in question. The strains Nine Mile and Luga not only induced higher levels of antibodies than the strains Priscilla and S, but were also able to detect higher phase I antibody levels. In this regard, the strain Priscilla, but not the strain S, differed also from the strains Nine Mile and Luga when hyperimmune rabbit anti-*C. burnetii* sera were examined by MIF test (Kováčová *et al.*, 1994). Therefore, possible differences in the antibody-binding capability of *C. burnetii* strains should be taken into account in considering their use for routine serological examination. On the other hand, for the assessment of the post-vaccination cell-mediated immune response by LTT, corpuscular antigens of different *C. burnetii* strains seem to be of equal value. This is supported by the finding of similar lymphocyte stimulation profiles obtained with the strain Priscilla in human volunteers vaccinated with a corpuscular phase I Henzerling strain vaccine (Izzo *et al.*, 1991).

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