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ONSET AND DURATION OF IMMUNITY IN GUINEA PIGS AND MICE INDUCED WITH DIFFERENT Q FEVER VACCINES

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Summary. — Protective effects of different types of Q fever vaccines, namely untreated *Coxiella burnetii* phase I cells (*Cb* I) or *Cb* I cells treated with chloroform-methanol (CM) mixture (*Cb* I-CM) and of a Q fever chemovaccine obtained by trichloroacetic acid extraction (TCAE) from intact *Cb* I cells, were compared in mice and guinea pigs at different intervals after intraperitoneal (i. p.) or subcutaneous (s.c.) immunizations. The highest degree of protection at all intervals studied was achieved with *Cb* I cells, irrespective of the route of immunization and i. p. or aerosol challenge. This vaccine exerted a protective effect in guinea pigs and mice as early as after one or two weeks post-immunization, the effect lasting for at least 40 weeks in mice (i. p. challenge) and 12 months in guinea pigs (aerosol challenge). Addition of small amount of *Cb* I cells to TCAE increased resistance of guinea pigs to aerosol challenge. Degree, onset and duration of protection to either type of virulent challenge afforded by *Cb* I-CM cells and TCAE was similar, but when compared with that of *Cb* I cells it was lower, started later (from the 2nd week in guinea pigs and the 3rd week in mice), and in mice it lasted for a shorter period (20 weeks only). The resistance to virulent challenge in guinea pigs did not depend on the levels of microagglutination (MA) antibodies and in mice it was reflected by delayed type hypersensitivity (DTH) reaction and adoptively transferred splenocytes, rather than by MA antibody titres and passive transfer of immune sera to recipient mice. In evaluating the suitability of Q fever vaccine and immunity to *C.b.* not only type and dose of the vaccine tested, but also the species of laboratory animals, the routes of immunization and infection can be decisive.

Key words: *Q fever vaccines*; *Coxiella burnetii antigens*; *experimental immunity*; *guinea pigs and mice*

Introduction

In the series of our recent papers performed mostly as collaborative studies, histological and ultrastructural changes in the mouse liver and spleen (Jakubovský *et al.*, 1985; Kokorin *et al.*, 1985), effects on non-specific host

resistance (Macela *et al.*, 1985) along with some adverse effects (Kazár and Schramek, 1984) as well as protective effects against virulent challenge (Kazár and Schramek, 1985; Votruba *et al.*, 1985) of different *C.b.* antigenic preparations, namely *Cb* I cells, *Cb* I-CM cells and TCAE, were investigated. The most pronounced protective effects were observed in the 4th week post-immunization, when the degree of protection afforded by *Cb* I-CM cells and TCAE was comparable to that induced by *Cb* I cells. The purpose of this study was to compare the degree of specific resistance induced in guinea pigs and mice by these three *C.b.* antigenic preparations at different intervals post-immunization and to find out whether it could be affected by different routes of immunization and infection, respectively.

Materials and Methods

C. burnetii Nine Mile strain serologically in phase I (the 3rd egg passage) grown and titrated in chick embryo yolk sac was stored at −60 °C. It also served as the source for purification of killed *Cb* I cells, from which *Cb* I-CM cells (Kazár *et al.*, 1983) and TCAE (Brezina and Urvölgyi, 1962) were prepared. The antigenic *C.b.* preparations were lyophilized so that for immunization purpose their defined masses (from 30 to 300 μg for *Cb* I and *Cb* I-CM cells and from 30 to 100 μg of TCAE) could be used.

Animals, their immunization and infection. Guinea pigs and C3H mice weighing 250−300 g and 18−20 g, respectively, were immunized and infected i. p., except in certain experiments in which guinea pigs were immunized s. c. and exposed to aerosol challenge. *C.b.* infectious dose for i. p. inoculated mice was 10^4 EID_{50}/0.5 ml, for i. p. infected guinea pigs 10^2 or 10^5 EID_{50}/ml. Challenge aerosol doses contained approximately 10^5 and 10^2 EID_{50} of *C.b.* Generation of infectious aerosol and calculation of infectious dose was previously described (Votruba *et al.*, 1985).

Determination of protective effects in guinea pigs and mice. To determine the vaccine protective effects in guinea pigs, febrility indices (FI) were employed. They were calculated according to the formula FI = FRT/FRC × 100, where FRT represented the values of febrile reactions from animals inoculated with the vaccine tested and FRC those from control animals which were exposed to the same dose of *C.b.* challenge under the same conditions. The FR of individual guinea pigs were measured in rectum daily in the morning and recorded up to day 15 post-infection (p. i.). The FR (over 39.5 °C) were graphically depicted, the pattern on the standard paper was cut out by scissors, the mass of papers obtained from one group of guinea pigs was weighed and divided by the number of animals in the given group. As positive were considered the FI with values lower than 50.

In mice, specific resistance to virulent challenge was based on evaluation of the degree of *C.b.* multiplication in the spleen on day 6 p. i. The values were scored as previously described (Kazár *et al.*, 1973), where sign − represented no rickettsiae, (+) single rickettsiae (occasionally seen), + less than ten, ++ tens, +++ hundreds, and ++++ uncountable number of rickettsiae per one field of view in 20 fields evaluated. At the same time, 20% spleen suspension in brain-heart infusion pooled from 4−6 mice were prepared, stored at −20 °C and titrated in chick embryo yolk sacs to determine *C.b.* yields from spleens of immunized and control mice. As resistant were considered groups of mice, from which the yield of *C.b.* was at least by 2 log EID_{50} units lower then that from controls.

Passive immunity transfers with sera and splenocytes of investigated mice were carried out as described (Kazár *et al.*, 1977). Briefly, 10^8 splenocytes or sera diluted 1 : 4 in phosphate buffered saline (pH 7.2) pooled from 4−6 immunized or control mice were inoculated i. p. into recipient mice, which were given 24 hr later 10^4 EID_{50} of phase I virulent *C.b.* strain. The degree of C.b. multiplication in the mouse spleen on day 6 p. i. was evaluated as mentioned above.

Assay of DTH reaction was performed as described (Kazár *et al.*, 1982*a*) with 10 μg of TCAE inoculated into hind foot-pad using the method of Kitamura (1980). It was read after 24 hr by weighing legs of individual mice out in the knee joint and calculating difference in mass increase of the test (left) versus the control PBS-inoculated (right) leg in each mouse group. Differences

in the means of values obtained from 4—5 mice in each group and their standard deviations were evaluated by Student's t-test. Probability values of $P < 0.05$ were considered as significant.

Serological examination by MA test was done according to Fiset *et al.* (1969) with sera pooled from 4—6 mice in each group or with individual guinea pig sera which were tested with phase I and artificial (Schramek *et al.*, 1972) phase II *C.b.* antigens. Titres $\geqslant 2$ were considered as positive; for calculation of mean geometrie titres of MA antibodies in guinea pigs, the values of negative sera were considered to be 1.

Results

Resistance to i. p. challenge of guinea pigs immunized i. p. with different types of Q fever vaccine

Guinea pigs were immunized i. p. with 30 μg of *Cb* I or *Cb* I-CM cells and with 60 μg of TCAE. On days 3, 7, 14 and 28 they were bled to obtain samples for serological examination and divided into two groups (each consisting of 4—6 animals) which were infected i. p. with 10^5 or 10^2 EID_{50}/ml of virulent phase I Nine Mile *C.b.* strain. Febrile reactions were recorded daily in the morning up to the 15th day p. i. and FI evaluated as described in Materials and Methods. Sera were examined for the presence of MA antibodies.

As shown in Table 1, *Cb* I cells exerted protective effect as early as on day 7 post-immunization, the degree of protection increased at later intervals. This effect was less pronounced against the higher infectious dose, on day 7 being at the border of significance. The degree of protection afforded by *Cb* I-CM cells and TCAE did not practically differ when using the higher or the lower infectious doses. It started later and on day 14 it was somewhat higher after immunization with *Cb* I-CM cells than with TCAE. On day 28, however, the protective effects of *Cb* I-CM cells and TCAE were similar, but at all intervals investigated lower than those of *Cb* I cells. The latter also induced the earliest appearance (on day 7) and the highest levels (MGT of 1,435.0, day 28) of phase II MA antibodies. Phase I MA antibodies (data

Table 1. Onset of resistance to i.p. challenge in guinea pigs immunized i.p. with different types of Q fever vaccine

Guinea pigs immunized with	*C.b.* challenge (EID_{50}/ml)	FI and MGT of phase II MA antibodies* on days p.i.			
		3	7	14	28
Cb I cells	10^5	69.5	49.2	15.3	3.4
	10^2	76.0	28.0	12.0	0.0
		(1.0)	(8.0)	(128.0)	(1435.0)
Cb I-CM cells	10^5	79.6	71.2	32.2	23.7
	10^2	80.0	68.0	28.0	20.0
		(1.0)	(2.0)	(3.7)	(20.8)
TCAE	10^5	88.1	74.6	47.5	15.2
	10^2	92.0	72.0	44.0	24.0
		(1.0)	(2.2)	(4.8)	(11.3)

*Given in parentheses

Table 2. Duration of resistance to aerosol challenge in guinea pigs immunized s.c. with different types of Q fever vaccine

Guinea pigs immunized with	*C.b.* challenge (EID_{50})	FI, % of surviving animals* and MGT of phase II MA antibodies** in months post-immunization		
		1	6	12
Cb cells	10^5	6.9 **90.0**	5.7 **80.0**	4.8 **30.0**
	10^2	4.8 (119.4)	3.1 (18.4)	0.0 (3.9)
Cb I-CM cells	10^5	36.8 **90.0**	48.9 **60.0**	33.3 **33.3**
	10^2	8.1 (18.4)	9.4 (2.0)	21.4 (1.6)
TCAE	10^5	16.1 **100.0**	17.1 **77.7**	42.9 **50.0**
	10^2	14.5 (20.4)	6.3 (1.6)	39.3 (1.3)
Cb I cells + TCAE	10^5	19.5 **100.0**	15.7 **90.0**	9.5 **62.5**
	10^2	9.7 (13.0)	0.0 (5.6)	3.6 (2.0)

* Data bold type; **data given in parentheses

not shown in Table 1) appeared first on day 14 after immunization with *Cb* I cells and on day 28 after immunization with *Cb* I-CM cells and TCAE, the latter vaccines inducing again much lower antibody titres. Though the increase in phase II MA antibody titres was accompanied by an increased resistance to virulent challenge, fair protection was achieved also at intervals when antibody levels were low (day 7 after immunization with *Cb* I cells and day 14 after immunization with *Cb* I-CM cells and TCAE, respectively).

Resistance to aerosol challenge in guinea pigs immunized subcutaneously with different types of Q fever vaccines

Guinea pigs were immunized subcutaneously with 30 μg of *Cb* I and *Cb* I-CM cells, 60 μg of TCAE and a combination of 10 μg of *Cb* I cells with 30 μg of TCAE. At intervals 1, 6 and 12 months post-immunization, they were bled to determine MA antibody response and again divided into two groups each consisting of 8—10 animals, which were exposed to aerosol challenge containing about 10^5 or 10^2 EID_{50} of phase I *C.b.* Along with evaluation of FI, death of animals was daily recorded up to day 15 p.i.

As follows from Table 2, the highest degree of protection at all intervals was observed in guinea pigs immunized with *Cb* I cells, regardless the challenge dose. The protection provided by *Cb* I-CM cells and TCAE against the higher challenge dose was much lower, in case of *Cb* I-CM cells at 6 months post-immunization interval and in case of TCAE at 12 months post-immunization interval approaching the critical (50) value of FI. Admission of *Cb* I cells to TCAE increased its protective potency namely at later intervals (even though the concentration of components of this "mixed"

vaccine was lower than doses of individual vaccines). The protective ability of *Cb* I-CM cells and TCAE decreased with the time elapsed from immunization. This can also be applied to the proportion of survival of those guinea pigs which were exposed to higher dose of aerosol challenge. As to the phase II MA (and also not presented phase I MA) antibody levels, they decreased markedly at later post-immunization periods, which, however, did not affect the high degree of protection induced by *Cb* I cells and the "mixed" vaccine.

Similar results (data not presented) were obtained with guinea pigs which were exposed to *C.b.* infectious aerosol 3 and 9 months after immunization with 30 and 300 μg of *Cb* I-CM cells and with 100 μg of TCAE.

Protection in mice against i.p. challenge after i.p. immunization with different types of Q fever vaccine

Mice were immunized i. p. with 300 μg of *Cb* I or *Cb* I-CM cells and with 100 μg of TCAE. By 1, 2, 3, 4, 12, 20 and 40 weeks post-immunization, 5—6 mice in each group were infected i. p. with 10^4 EID_{50} in 0.5 ml of virulent phase I *C.b.* strain to determine protective effects of Q fever vaccine tested (based on the degree of *C.b.* multiplication in the mouse spleen and difference in the yields of *C.b.* from the spleen of control and immunized mice). At the same time portions of mice (5—6 in each group) were bled and dissected to obtain serum and splenocytes for passive immunity transfers and to determine the levels of phase I and phase II MA antibodies. In parallel, in another portion of mice (4—5 in each group) also DTH reaction was evaluated.

Table 3 summarizes the kinetics of development of above mentioned immunity correlates in mice immunized with Q fever vaccines under study. As similar as in the experiments with guinea pigs, the most pronounced protective effects were found in mice immunized with *Cb* I cells, in which the first marked protection was detected earlier (the 2nd week post-immunization) and lasted during the whole observation period, i. e. up to the 40th week post-immunization. Resistance to virulent challenge was at all intervals accompanied by high levels of both phase I and phase II MA antibodies, capability to transfer protection by serum and splenocytes from immunized mice, and high values of DTH reaction. Again, the protective effect of *Cb* I-CM cells and TCAE was similar, but the protection that they induced started later (the 3rd week post-immunization), was of shorter duration (the 20th week post-immunization) and was never so marked as that observed with *Cb* I cells. Other immunity correlates were also lowered at all intervals, passive immunity transfer by splenocytes and values of DTH reaction corresponding better to the degree of specific resistance than passive immunity transfer by serum and the levels of MA antibodies.

Discussion

As stated by the Editorial in Lancet (1984), and as follows from a proportion of papers dealing with *C.b.* presented at the IIIrd International

Table 3. Onset and duration of protection in mice against i.p. challenge after i.p. immunization with different types of Q fever vaccine

Week post-immuni-	Immunity correlates in mice immunized with *Cb* I cells						*Cb* I-CM cells		
	1	2	3	4	5	6	1	2	3
1	+1.8	++	+++	2	128	> 0.05	++1.3	+++	+++
2	(+)3.8	(+)	+	32	1024	< 0.05	++1.6	++	++
3	(+)4.8	–	(+)	128	4096	< 0.05	+2.8	+	++
4	−5.3	–	–	256	4096	< 0.05	(+)4.0	+	+
12	−4.5	–	+)	64	4096	< 0.05	+2.2	+	+++
20	(+)3.7	–	(+)	32	2048	< 0.05	+2.0	++	+++
40	−4.0	–	–	32	1024	< 0.05	++1.5	++	++++
control mice	+++	++++	+++	< 2	< 2	> 0.05			

Symposium on Rickettsiae and Rickettsial Diseases in Smolenice (1984) and at the Meeting of the American Society for Rickettsiology (1985) indicate that this agent is still in the focus of interest and that Q fever still posses an important public health problem requiring proper protective measures including vaccination. Apart from live attenuated Q fever vaccines, whose disadvantages we discussed elsewhere (Kazár *et al.*, 1982*b*), three types of *C.b.* antigenic preparations which were or could be used for vaccination of man are available, i. e. purified killed *Cb* I cells which appeared to be safe and effective in preventing Q fever in the abattoir (Marmion *et al.*, 1984), TCAE from intact *Cb* I cells used in Romania and Czechoslovakia (Cracea *et al.*, 1973; Brezina *et al.*, 1974; Kazár *et al.*, 1982*b*) and eventually *Cb* I-CM cells (Williams and Cantrell, 1982; Kazár *et al.*, 1983). These three types of Q fever vaccine were compared in our study, the results of which demonstrated that the degree of protection they induced, its onset and duration depended not only on the type and dose of the vaccine used and on the challenge dose of *C.b.*, but also on the animal species and the routes of immunization and infection, that should also be taken into consideration when evaluating vaccine potency.

Field studies with Q fever corpuscular phase I vaccine — *Cb* I cells (Marmion *et al.*, 1984) and soluble chemovaccine — TCAE (Kazár *et al.*, 1982*b*) gave comparable results as to their immunogenicity (as measured by post-vaccination antibody response) and reactogenicity (as reflected by the occurrence of local and systemic post-vaccination reactions). We developed, used and recommended the Q fever chemovaccine (Brezina *et al.*, 1974), because Q fever corpuscular vaccines used in the past caused severe local reactions, namely in the subjects with previous *C.b.* exposure (Lackman *et al.*, 1962; Bell *et al.*, 1964). These untoward effects can be reduced, however, when excluding seropositive and skin test-positive individuals from vaccination not only in the case of Q fever chemovaccine (Kazár *et al.*, 1982*b*), but also from application of a small dose (30 μg) of inactivated *Cb* I cells

Table 3 continued

Cb I-CM cells			Immunity correlates in mice immunized with TCAE					
4	5	6	1	2	3	4	5	6
2	16	> 0.05	+++0.1	++++	+++	2	8	> 0.05
4	32	> 0.05	++1.8	++	++	2	32	> 0.05
4	64	< 0.05	+3.0	+	++	2	64	< 0.05
8	128	< 0.05	(+)3.5	+	++	4	256	< 0.05
4	8	< 0.05	+2.3	++	++	2	4	< 0.05
2	2	< 0.05	+2.1	++	+++	2	2	< 0.05
2	2	< 0.05	++1.7	++	+++	< 2	< 2	< 0.05

1 — Degree of *C.b.* multiplication in spleen of immunized mice and difference (in log EID_{50} units) in the yield of *C.b.* from the spleen of control and immunized mice
2 — Degree of *C.b.* multiplication in spleen of mice to which 24 hr earlier 10^8 splenocytes from immunized mice were adoptively transferred
3 — Degree of *C.b.* multiplication in spleen of mice inoculated 24 hr earlier with the serum from immunized mice (diluted 1 : 4)
4 — Titres of phase I MA antibodies
5 — Titres of phase II MA antibodies
6 — P value of DTH reaction

(Marmion *et al.*, 1984). Higher degree of protection, its earlier onset and longer duration observed in our presented study favour then the use of *Cb* I cells rather than TCAE or *Cb* I-CM cells for specific Q fever prevention. Situation is further complicated by antigenic differences among *C.b.* strains of different origin (Hackstadt, 1986), which may require the use of pooled *C.b.* corpuscles or TCAE from representatives of different antigenic groups. In this case it would be probably more feasible to prepare a proper mixture of soluble TCAE than of *Cb* I corpuscular vaccine. Since the addition of small amount (10 μg) of *Cb* I cells to TCAE increased its protective effects to natural aerosol challenge in our experiments in guinea pigs, one possibility could be the use of a combined vaccine consisting of TCAE from different *C.b.* strains and *Cb* I cells of the strain typical for area in which Q fever vaccination is considered.

As to the last type of possible Q fever vaccine in question, i. e. *Cb* I-CM cells, no data on its use for vaccination in man are available. In laboratory animals it is far less reactogenic than *Cb* I cells (Kazár and Schramek, 1984), however, its antigenicity in man and various animal species is also lower (Kazár *et al.*, to be published). One may conclude, finally, that further studies are necessary to decide which *C.b.* antigenic preparation is the most suitable to fulfill the requirements of high immunogenicity and low reactogenicity of the Q fever vaccine.

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