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THE VALUE OF SKIN TEST IN Q FEVER CONVALESCENTS AND VACCINEES AS INDICATOR OF ANTIGEN EXPOSURE AND INDUCER OF ANTIBODY RECALL

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Summary. — The skin test (ST) with Q fever chemovaccine revealed more positive reactors than the serological examination by microagglutination (MA) test among humans, who had suffered from Q fever one to eleven years ago or who had been vaccinated with Q fever chemovaccine from three months to four years ago. When examining the sera harvested either at skin-testing or two weeks thereafter by MA test, seroconversion or rise in antibody titres were found to both main *Coxiella burnetii* (C. b.) antigens. The rise or appearance of antibody response was similar in Q fever convalescents and vaccinees, in the latter occuring more often in ST positive than in ST negative individuals. Results of ST at different postvaccination (p.v.) intervals corresponded well to those of lymphocyte transformation (LT) test, but not to those of inhibition of leukocyte migration (ILM) test.

Key words: *Q fever infection, vaccination, skin test, antibody response, cell-mediated immunity*

Introduction

In our studies on field vaccination of persons professionally exposed to Q fever with a Q fever chemovaccine (Brezina *et al.*, 1981; Kazár *et al.*, 1982*a*) we pointed out the value of ST prior to vaccination for predicting the likelihood of p.v. reactions. The present study was aimed to compare the results of ST with those of serological examination by MA test at different intervals after infection and vaccination. In some vaccinees the LT and ILM tests were carried out in parallel.

Materials and Methods

Vaccine and skin-testing. The chemovaccine used was the soluble surface antigenic component extracted by trichloracetic acid (TCA) from highly purified *C.b.* organisms (Brezina and Úrvölgyi, 1961). One vaccine dose represented an amount of soluble material extract from 1 mg of dried *C.b.* cells dissolved in 1 ml of phosphate buffered saline (PBS). The ST was performed by intradermal inoculation on the forearm of 0.1 ml vol of the vaccine diluted 1 : 100 in PBS. The ST was read after 24 hr and was considered positive if there was erythema with a diameter of 0.5 cm or more and/or induration at site of inoculation.

In vitro tests of cell-mediated immunity. The LT test was carried out in tubes as described by Coonrod and Shepard (1971) with lymphocytes separated from the venous heparinized blood by centrifugation on Ficoll-Verografin density gradient (Nyulassi *et al.*, 1974). The cells were resuspended in RPMI medium containing glutamine (30 mg per 100 ml of medium), 2-mercaptoethanol (5×10^{-5} M), penicillin (100 units per ml medium), streptomycine (100 μg per ml), HEPES (2 mmol/l) 7% $NaHCO_3$ (1.5 ml per 100 ml) supplemented by 15% pooled human serum from the AB blood group donors. The cell suspension was diluted to contain $1—2 \times 10^6$ lymphocytes per ml, and 2.0 ml amounts were dispersed into glass tubes for culture. To each tube 50 μl of PBS containing 100 μg of TCA-extracted material from phase I *C.b.* cells was added. In parallel, a positive control, i.e. lymphocytes to which 1 : 100 dilution in RPMI of phytohaemagglutinin (PHA; Wellcome) was added and a negative control with no additives were run. All cultures were incubated at 37 °C for 5 days under 5% CO_2. On the 5th day of incubation 50 μl of PBS containing ^{3}H-thymidine (37kBq, specific activity 990GBq/mmol; ÚVVVR Prague, Czechoslovakia) was added. After incubation overnight at 37 °C and 5% CO_2 atmosphere, the lymphocytes were put onto filters which were washed twice with cold PBS, 5% TCA and methanol. Then the filters were placed into 5 ml of scintillation liquid and activity was counted in Packard-Tricarb (Model 3390). Mean values were calculated from triplicates per each lymphocytes donor. Stimulation index (SI) was determined as the ratio of mean value with PHA or *C.b.* antigen to mean value without antigen. As positive were considered samples with the SI ≥ 3.0.

The ILM test was performed with peripheral blood leukocytes according to the slightly modified method of Mayer *et al.* (1976). Briefly, leukocytes were separated from the plasma after sedimentation of the heparinized blood in the presence of 6% dextrane sulphate. Erythrocytes were disrupted by 5 min incubation with 0.83% NH_4Cl. Remaining leukocytes were repeatedly washed and adjusted to concentration of $3—5 \times 10^7$ cells in enriched RPMI medium. The leukocytes were incubated for 90 min at 37 °C in the presence or absence of 100 μg of specific TCA-extracted *C.b.* antigen. Then the leukocytes were washed out with PBS and the cell sediment was resuspended in 0.2 ml of enriched RPMI medium. Capillaries (d = 1 mm) filled with the cells were sealed at one end in a fine gas flame, centrifuged for 10 min at $60 \times g$, cut at the medium-cell interface and immediately silicone-fixed in plastic chambers containing 1 ml of enriched RPMI medium. The medium was overlaid with paraffin oil and the chambers were incubated overnight at 37 °C. Then the area of leukocyte migration was read and measured planimetrically. Each experiment was performed in triplicates from which mean values were calculated. As positive was considered higher than 20% migration inhibition of the leukocytes preincubated with *C.b.* antigen as compared to that of control leukocytes of each individual.

Serological assay. The non-inactivated sera were examined by the MA test according to Fiset *et al.* (1969) with purified phase I *C.b.* suspensions or with artificial phase II *C.b.* prepared by potassium periodate treatment of purified phase I *C.b.* suspensions (Schramek *et al.*, 1972). As positive were considered sera with antibody titres ≥ 4. The sera were harvested at the time of ST and 2 weeks later. For antibody recall with either *C.b.* antigen we considered seroconversion from negativity to positivity or an at least 4-fold rise of antibody titres in the second serum sample.

Humans chosen for examination were Q fever vaccinees with known date of vaccination or those who suffered from Q fever one to three months ago (Kazár *et al.*, 1982*b*), one year ago (Kazár *et al.*, unpublished data) and 11 years ago (Sádecký *et al.*, 1973) as confirmed serologically by MA and/or complement-fixation (CF) tests at the time of Q fever outbreaks. One group of humans was revealed to overcome Q fever in the past (unknown period of the time = x years) during field vaccination study in Central Slovakia (Brezina *et al.*, 1981). Part of the latter were vaccinated and examined after 4 years p.v. The last, control group consisted of 16 to 17-year-old students who had been expected not to come in contact with *C.b.* during their history.

Results

Comparison of the positivity of ST and MA test in Q fever convalescents and vaccinees at different intervals postinfection (p.i.) and p.v. is shown in Table 1. The ST test was found superior to the MA test at all intervals in-

Table 1. Reactivity of Q fever convalescents and vaccinees in ST and MA test, and recall of MA antibody response in skin-tested individuals

Interval	Number of persons examined	Number and % of positive in		Number and % antibody recals* as detected by MA test with *C.b.*	
		ST	MA test	antigen 1	antigen 2
1—3 months p.i.	48	49 (89.6%)	44 (91.7%)	not tested	not tested
1 years p.i.	22	20 (90.9%)	15 (68.2%)	16 (72.7%)	17 (77.3%)
11 years p.i.	18	16 (88.9%)	3 (16.7%)	10 (55.6%)	10 (55.6%)
X years p.i.	16	15 (93.8%)	11 (68.8%)	6 (37.5%)	10 (62.5%)
1 month p.v.	24	15 (62.5%)	12 (50.0%)	not tested	not trsted
3—9 months p.v.	64	45 (70.3%)	21 (32.8%)	30 (46.9%)	50 (78.1%)
1—2 years p.v.	67	42 (62.7%)	19 (28.4%)	35 (52.2%)	52 (77.6%)
3—4 years p.v.	82	57 (69.5%)	30 (36.6%)	21 (25.6%)	57 (69.5%)
X years p.i. 4 years p.v.	19	18 (94.7%)	13 (68.4%)	14 (73.7%)	16 (84.2%)
control group	32	2 (6.3%)	1 (3.1%)	1 (3.1%)	1 (3.1%)

* Seroconversion from negativity to positivity or 4-fold and higher rise of antibody titres in sera taken at the time of ST and two weeks later, respectively.

vestigated. Though the positivity in the MA test and the negativity in the ST also occurred, this was far less frequent. In Q fever infected subjects, the longer was an interval elapsed from an exposure to *C.b.*, the higher was the sensitivity of ST. In those examined immediately after the outbreak of Q fever (1—3 months p.i.), except one case the positivity in the ST paralleled that in the MA test. Similarly, in Q fever vaccinees at later intervals p.v., the ST was more often positive than the MA test. The highest proportion of ST positivity was observed in those persons who had been infected before vaccination. However, at all intervals individuals occurred negative in both ST and MA test. In the control group, 2 of 32 individuals were ST positive, though one of them could had been exposed to *C.b.* in the past, because he was positive also in the MA test and following the ST he reacted with a rise of antibody titre.

Table 2. Comparison of the results of ST and MA test, number and proportion of antibody recalls in relation to the results of ST in Q fever convalescents

Number of	MA test positive	MA test negative	Total	Antibody recall*
ST positive	28	23	51	39 (76.5%)
ST negative	1	4	5	4 (80.0%)
Total	29	27	56	43 (76.8%)

* MA antibodies directed to both antigen 1 and antigen 2.

Table 3. Comparison of the results of ST and MA test, number and proportion of antibody recalls in relation to the results of ST in Q fever vaccinees

Number of	MA test positive	MA test negative	Total	Antibody recall*
ST positive	62	81	143	122 (84.7%)
ST negative	8	62	70	38 (54.3%)
Total	70	145	213	160 (74.8%)

* MA antibodies directed to both antigen 1 and 2.

Table 1 also shows that the ST caused an antibody recall in both Q fever convalescens and vaccinees. This refers to antibodies directed to both antigen 1 and antigen 2 *C.b.*, though titres of the latter raised more frequently at all intervals p.i. (from 55.6 to 77.3%) and p.v. (from 69.5 to 78.1%). Oddly enough, the higher sensitivity of ST at later intervals was not accompanied by higher frequency of antibody recalls.

Analysis of the number and proportion of antibody recalls in relation to the results of ST in Q fever convalescens and vaccinees, respectively, is

Table 4. Relation of the results of ST to those of MA test and tests reflecting cell-mediated immunity in vitro at different intervals post-vaccination

Postvaccination interval		Positivity (+) and negativity (—) in tests used ST	MA	LT	ILM
weeks	3	+	+	+	+
	5	+	—	+	—
	6	—	—	—	+
	6	+	+	+	—
	8	—	—	—	+
	8	+	+	+	+
	8	+	—	—	+
	15	+	+	+	—
months	6	+	+	—	n.t.
	6	+	+	+	n.t.
	6	+	—	+	n.t.
	6	+	—	+	n.t.
	6	—	—	—	—
	6	—	—	—	—
	9	+	+	+	+
	9	+	—	+	—
	9	+	—	+	+
	12	+	+	+	n.t.
	12	—	—	+	n.t.
Number of positive/ /number of examined		14/19	9/19	13/19	7/13
Accord of the results of ST with those of			MA 14 (73.6%)	LT 16 (84.2%)	ILM 7 (58.3%)

n.t. = not tested.

given in Tables 2 and 3. Whereas no significant difference was found between ST-positive and ST-negative Q fever convalescents, in the group of Q fever vaccinees antibody recalls were more frequent in ST-positive (84.7%) than in ST-negative (54.3%) individuals.

In Table 4, the results of ST and MA test with LT and ILM tests reflecting cell-mediated immunity in vitro are compared at different intervals p.v. with Q fever chemovaccine. As expected, the ST was positive in more vaccinees than the MA test, their results corresponding in 73.6%. High degree of conformity (84.2%) was found also between the results of ST and LT test. In contrast, accordance of results of ILM test was negligible, being in 58.3% only. The low number of examined individuals did not allow a time-dependent analysis of occurrence of particular test; however, the positivity in both ST and LT could be noticed from 3 weeks to 12 months p.v. The ST values for *C.b.* antigen- and PHA-stimulated lymphocytes varied from 3.2 to 15.4 and from 13.3 to 187.3, respectively.

Discussion

Diagnosis of human Q fever depends on laboratory evidence of infection, mainly on the results of serological examination by CF, MA and immunofluorescence tests. Earlier attempts at Q fever diagnosis by ST using corpuscular *C.b.* antigens suffered either from adverse effects of phase I *C.b.* corpuscles (Luoto *et al.*, 1965) or from inconsistent results due to obvious inadequacy of the phase II *C.b.* corpuscles employed (Vivona *et al.*, 1964). Far more promising was the use of soluble *C.b.* antigen extractable by TCA from purified phase I *C.b.* cells (Brezina and Úrvölgyi, 1961), which was devoid of local pathogenic effects upon subcutaneous or intradermal application and at the same time it was proved superior to the methods of serological diagnosis by CF or MA tests (Kudelina and Kambaratov, 1969; Kambaratov *et al.*, 1971; Terentiev and Zeitlenok, 1973; Cracea *et-al.*, 1976, 1978).

In our study using TCA-extracted surface *C.b.* antigen, the positivity of ST has been found to surpass serological positivity by MA test not only in Q fever convalescents, but also in persons vaccinated against Q fever. In Q fever infected subjects our results were comparable with those obtained by above mentioned authors, though in the acute Q fever cases Kambaratov *et al.* (1971) found a greater proportion of positive results with the ST than the CF test, and in a study by Cracea *et al.* (1978) the ST showed less sensitive than the CF test. In Q fever vaccinees, the higher sensitivity of ST in comparison with the MA test was found in all intervals p.v., differences being greater at later intervals. All these results suggest that the ST can serve as reliable tool of Q fever diagnosis supplementary to serological examinations, namely for detection of human exposure to *C.b.* in the more remote past. It should be stressed, however, that ST need not be positive in all persons who were definitely exposed to *C.b.* and positive in serological reactions, respectively.

Of importance is the finding of antibody recall by the ST indicating that intradermal administration of Q fever chemovaccine may act as a booster dose. In our case this occurred in about two thirds of Q fever convalescents and was comparable with a booster effect as regards the CF antibodies in about one third of previously infected Q fever patients (Cracea *et al.*, 1978). Such a booster effect with a predominant phase I IgM antibody response was found also in Q fever convalescents inoculated intradermally with 0.2 μg of phase I killed *C.b.* cells (Peacock *et al.*, 1978). Our observation of antibody recall also in about 3/4 of Q fever vaccinees indicates that the ST may serve not only for detection of previous contacts with *C.b.* antigens, but even for Q fever revaccination.

The specificity of ST follows not only from the fact that it is positive mostly in serologically positive Q fever convalescents and vaccinees, but also from the conformity of its results with those of the LT test. The latter was found positive in serologically negative individuals who acquired Q fever infection in the past (Jerrells *et al.*, 1975) as well as in the volunteers administered subcutaneously a phase I Q fever vaccine (Horwith and Ascher, personal communication). Results of our study suggest that for an assessment of cellular immune response to *C.b.* in humans besides the ST also the LT but not the LMI can be successfully used.

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