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COXIELLA BURNETII ANTIGENS FOR DETECTION OF Q FEVER ANTIBODIES BY ELISA IN HUMAN SERA

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Summary. — Different concentrations of chemically-treated (by potassium periodate oxidation or mild acid hydrolysis) purified phase I *Coxiella burnetii* (*C.b.*) corpuscles and natural (untreated) purified phase I and phase II *C.b.* corpuscles were compared by ELISA for detection of both phase I (directed to antigen 1) and phase II (directed to antigen 2) antibodies in human Q fever convalescent sera. As to the absorbance values the most sensitive was the antigen obtained by mild acid hydrolysis (0.1 mol/l HCl of phase I corpuscles for 30 min at 100 °C) followed by phase I corpuscular antigen (treated with 0.01 mol/l potassium periodate for 4 hr at 45 °C). The natural phase I (the 3rd egg passage) and phase II (the 162th egg passage) *C.b.* corpuscles gave lower absorbance with some sera not even distinguishing the 6th egg passage (EP6 or EP3 in phase I) from EP 162 or from negative controls.

Key words: serological tests; ELISA; antibody detection; Coxiella burnetii antigens; Q fever

Introduction

ELISA is widely used in serological diagnosis of various human and animal infections; recently it has been also applied for detection of Q fever antibodies mostly by means of natural *C.b.* antigens (Cracea *et al.*, 1983; Krauss *et al.*, 1985; Field *et al.*, 1983; Behymer *et al.*, 1985; Roges and Edlinger, 1985; Schmeer *et al.*, 1985). Results from our Institute (Gallo *et al.*, 1985) indicated that chemically treated phase I *C.b.* antigens were more suitable than natural ones for detection of antibodies to *C.b.* in rabbit sera. In the present study, treated antigens, namely those obtained by oxidation or mild acid hydrolysis of phase I *C.b.* corpuscles, were compared with natural phase I and phase II *C.b.* cells for detection of Q fever antibodies by ELISA in human convalescent sera.

Materials and Methods

Coxiella burnetii (*C.b.*) *strain, passages and antigens.* Purified (by differential centrifugation and ether extraction) egg-grown *C.b.* strain Nine Mile in phase I (EP3 and EP6) or in phase II (EP162 in our laboratory) killed by formalin or phenol served as natural phase I or phase II antigen. Phase I corpuscles from EP3 were subjected to mild acid hydrolysis (treatment with

0.1 mol/l HCl as described by Schramek *et al.*, 1978) for 15, 30, 45, 60, 75 and 90 min at 100 °C. Phase I corpuscles from EP6 were treated with 0.01 mol/l KIO_4 for 2, 4, 6, 8, 10, 12 and 14 hr at 45 °C (Schramek *et al.*, 1972). Following either treatment all specimens obtained were adjusted to a 1 mg/ml concentration similar as were the untreated phase I and phase II *C.b.* corpuscles.

Sera. Human Q fever-positive convalescent sera used in our experiments were from patients with atypical pneumonia. Q fever was diagnosed according to a higher than 4-fold increase of antibody titres in the serum samples collected at the onset of the disease and 2 weeks later as detected by complement-fixation (CF), microagglutination (MA) and microimmunofluorescence (MIF) tests (Kazár *et al.*, 1982). The serum samples were harvested 4 months from the beginning of the disease which was proved by MA test. As control, two human sera were employed for ELISA; both were negative in all tests. The sera were diluted in phosphate buffered saline (PBS) containing 0.05 % Tween 20 (LACHEMA) solution supplemented with 2 % polyvinylpyrrolidine (SERVA).

ELISA was performed by the indirect method (Voller *et al.*, 1979). Antigenic preparations diluted in carbonate-bicarbonate buffer, pH 9.6, were coated to flat-bottomed plastic microtitre plates (KOH-I-NOOR, Dalečín, Czechoslovakia). Following adsorption for 4 hr at 37 °C, the plates were washed 3 times with PBS-Tween solution, pH 7.4, and after addition of the tested sera (diluted 1 : 1000) they were incubated for 18 hr at 4 °C. Then the plates were washed 3 times with PBS-Tween and pig anti-human IgG peroxidase-labelled globulin — SwAHu /IgG/ Px (SEVAC, Prague) diluted 1 : 1000 in PBS-Tween 20-polyvinylpyrrolidine solution containing 0.2 % bovine serum albumin (IMUNA, Šarišské Michaľany, Czechoslovakia), was added. After incubation for 4 hr at 37 °C the plates were washed, followed by addition of a 0.05 % concentration of orthophenylen-diamine (OPD, SERVA) prepared in 2.9 % sodium citrate, pH 5.0, containing 0.01 % H_2O_2 as substrate. The reaction which proceeded in dark was evaluated after 30 min without stopping.

The volume of reagents in each step was 200 µl per well. All antigen and serum dilutions were tested 4 times. Absorbance was determined by spectrophotometer Dynatech at the wavelength of 490 nm (OD_{490}). The values of non-specific reactions (background values) were substracted from significant deviation of the measured values at OD_{490} never exceeded 0.1.

Other serological tests. Positivity and negativity of sera tests was determined based on their examination by MA test performed according to Fiset *et al.* (1969).

Results

Acid-treated C.b. antigen

In the first series of our experiments we aimed at the finding of optimal conditions for the use of acid-treated *C.b.* antigen to determine Q fever antibodies by ELISA as to suitable antigen concentration and duration of the mild acid hydrolysis.

When testing three human Q fever convalescent sera (diluted 1 : 1000) with increasing concentrations (from 10 to 100 µg/ml) of phase I *C.b.* corpuscules treated with 0.1 mol/l HCl for 30 min at 100 °C, the highest optimal absorbance for all sera tested was found with antigenic concentration of 50 µg/ml, i.e. 10 µg per well (Fig. 1-I). To determine the optimal duration of acid-treatment, phase I *C.b.* corpuscles were subjected to mild acid hydrolysis for different intervals (from 15 to 90 min); 50 µg of resulting antigen was tested in ELISA to determine the absorbance with three Q fever-positive sera. The optimal interval was found based on the highest OD values with the antigen obtained following 30 min treatment (Fig. 2).

Periodate-treated C.b. antigen

Similar approach as described for acid-treated *C.b.* antigen revealed that optimal concentration of phase I *C.b.* corpuscles treated with periodate in

ELISA ranged from 40 to 100 μg/ml (Fig. 1-II), the optimal interval of periodate treatment being 4 hr at 50 μg/ml antigen concentration for all three Q fever-positive sera tested (Fig. 3).

Comparison of the suitability of chemically-treated and natural C.b. antigens for ELISA

To compare the suitability of chemically-treated and natural *C.b.* antigens for determination of antibodies by ELISA, 14 Q fever positive and 2 Q fever negative sera (diluted 1 : 1000) were tested with 50 μg/ml of acid-treated (EP3, 30 min), periodate-treated (EP6, 4hr) and natural *C.b.* antigens (from EP3, and EP162, corresponding to phase I and phase II, respectively). Natural *C.b.* preparations were used also in 50 μg/ml concentration, which was found suitable for detection of antibodies in hyperimmune rabbit sera. As shown in Fig. 4, the highest absorbance values were found with acid--treated antigen followed by periodate-treated antigen. The OD values obtained with natural phase II antigen were much lower, namely when compa-

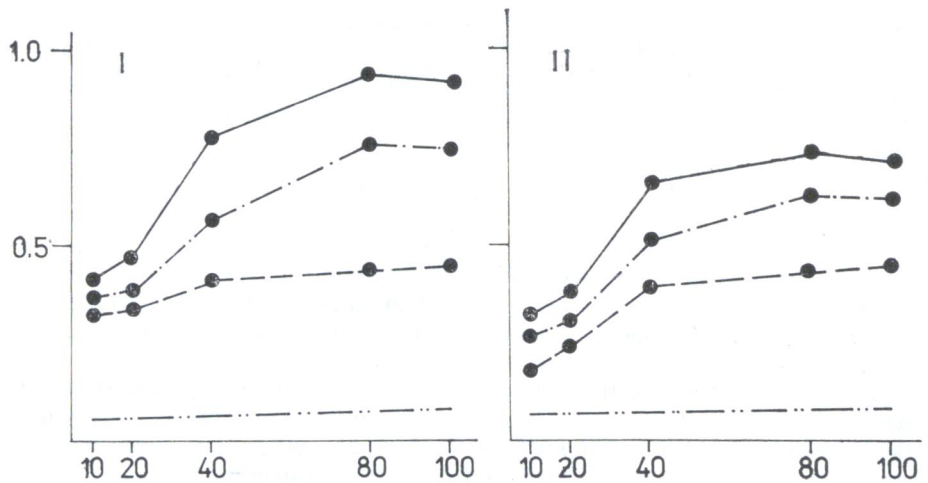

Fig. 1.

I. Dependence of absorbance values in ELISA on the different concentrations of HCl-treated (for 30 min) *C.b.* antigen with three Q fever-positive and one negative human sera (diluted 1 : 1000).

Abscissa: antigen concentration (μg/ml).

Ordinate: OD values at 490 nm.

II. Dependence of absorbance values in ELISA on the different concentrations of KIO_4-treated (for 4 hr) *C.b.* antigen with three Q fever-positive and one negative human sera (diluted 1 : 1000).

Abscissa: antigen concentration (μg/ml).

Ordinate: OD values at 490 nm.

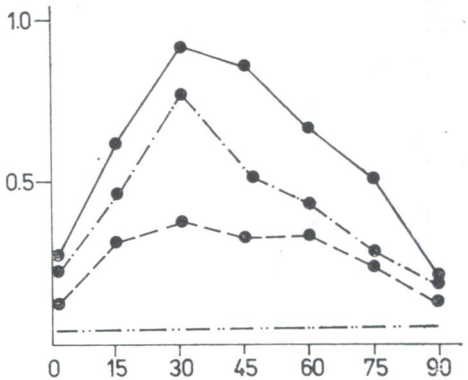

Fig. 2.
Dependence of absorbance values for three Q fever positive human sera reacting in ELISA with HCl-treated *C.b.*. antigen on duration of the treatment
Abscissa: duration (min) of HCl treatment.
Ordinate: OD values at 490 nm.

red with those of acid-treated antigen. The lowest absorbance values were observed with natural phase I antigen, the OD values with most sera even not exceeding negative controls.

Discussion

The diagnostic value of ELISA known for many infectious diseases was confirmed also for Q fever. This test was found to be more sensitive for detection of specific IgM and IgG antibodies than CF test in human sera (Cracea *et al.*, 1983; Field *et al.*, 1983; Krauss *et al.*, 1985) at lest as sensitive as MA test in surveillance of Q fever antibodies in sera of domestic animals (Behymer *et al.*, 1985) and correlated well with results of examination of human sera by MIF test (Roger and Edlinger, 1985). Moreover, its ability to detect IgM, IgG_1 and IgG_2 antibodies allowed to differentiate not only between acute and convalescent sera, but in cattle also between the post--infection and post-vaccination antibody response (Schmeer *et al.*, 1985).

In Q fever the situation is more complicated by the existence of phase I and phase II *C.b.* antigens; corresponding antibodies may differentiate the

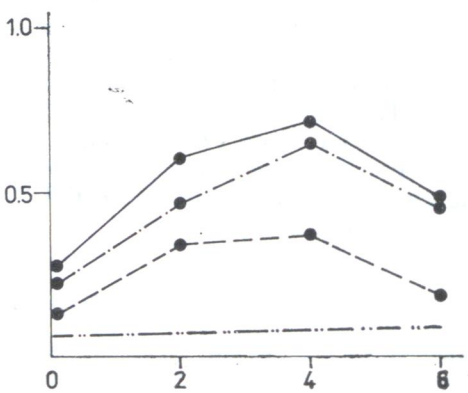

Fig. 3.
Dependence of absorbance values for three Q fever positive human sera reacting in ELISA with KIO_4-treated *C.b.* antigen on duration of the treatment.
Abscissa: duration (hr) of KIO_4 treatment.
Ordinate: OD values at 490 nm.

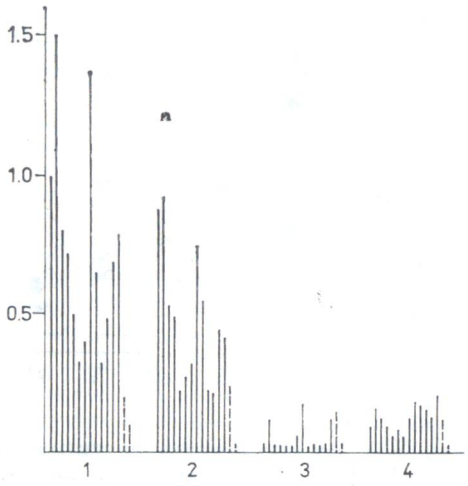

Fig. 4.
Comparison of absorbance values with chemically-treated and natural *C.b.* antigens in ELISA for 14 Q fever-positive and 2 Q fever-negative human sera (diluted 1 : 1000)
Abscissa: antigens used in 50 μg/ml concentration as follows: 1 — HCl--treated (for 30 min) antigen; 2 — KIO_4--treated (for 4 hr) antigen; 3 — phase I natural (EP3) antigen; 4 — phase II natural (EP162) antigen.
Ordinate: OD values at 490 nm for the 16 sera tested.
Full lines: positive sera; interrupted lines: negative sera.

acute and chronic forms of infection (Peacock *et al.*, 1983). For this reason the antigen type used in ELISA is of importance. In the most of above--mentioned studies phase II antigen was employed, except for the study by Schmeer *et al.* (1985) who used for antibody detection in cattle the mixture of phase I and phase II *C.b.* antigens. Suitability of different *C.b.* antigen preparations for ELISA was compared by Ruppaner *et al.* (1985), who for routine Q fever surveillance in domestic animals recommended a trichloro-acetic acid extract (TCAE) from pure phase I cells. This preparation along with acid and periodate-treated antigens was found to be the most sensitive also for detection of antibodies in hyperimmune rabbit sera (Gallo *et al.*, 1985). In our study sensitivity of chemically-treated (by HCl and KIO_4) antigens surpassed that of natural *C.b.* antigens also when used for detection of antibodies in human sera. Preliminary studies with TCAE indicated the suitability of this antigen preparation (Kováčová *et al.*, to be published).

In this connection questions arise, why chemically-treated antigens are more sensitive and which antibodies they detect. One possibility is that chemical treatment of phase I cells depending on its duration destroys a part of phase I antigen epitopes and at the same time it unmasks epitopes of phase II antigen, so that increased sensitivity of such antigen may be due to availability of both phase I and phase II epitopes (Schramek *et al.*, 1978). To answer these questions definitively, more detailed studies are required using monoclonal antibodies directed to different *C.b.* antigen determinants (Williams *et al.*, 1984).

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