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INDIRECT HAEMOLYSIS TEST IN Q-FEVER

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Summary. — Sheep erythrocytes sensibilized with the lipopolysaccharide (LPS) extracted from *Coxiella burnetii* phase 1 cells were used in indirect haemolysis test (IHT). The test was highly sensitive and specific. Its use seemed reasonable when following the chronic course of Q-fever by serologic methods.

Key words: *C. burnetii*; *serologic diagnosis*; *chronic infection*

Chronic persistence of *Coxiella burnetii* in the convalescent patients opens the opportunity for a chronic course of this infection. The detection of specific antibodies allows to recognize such course of the disease. Here we describe our attempts to use the indirect haemolysis test (IHT) for this purpose.

The antigen for complement fixation test (CFT), for indirect haemagglutination test (IHAT) and for IHT was prepared from *C. burnetii* phase I cells, strain "Luga" (from *Apodemus flavicollis*). The coxiella were obtained from the mouse spleen. The coxiella suspension containing $2 \times 10^{6.2}$ ID_{50} was inoculated to 7 rabbits (weighing 2—2.5 kg) in 1 ml aliquots. The animals were reinfected within 120—240 days, their sera were drawn at different intervals during 360 days.

To test the specificity of IHT, control sera of 9 rabbits and 21 guinea pigs and 27 human sera from patients with Brill disease were compared with 60 sera of Q-fever convalescents. Sera of experimentally infected rabbits were examined in IHT, in IHAT, and CF test using the phase I *C. burnetii* antigen; all other animal sera were tested in IHT and CF test with phase I and phase II *C. burnetii* antigens. The sera of Q-fever convalescents were examined in parallel by IHT, IHAT, and CF test against both phase I and phase II antigens.

For performing IHT sheep erythrocytes sensibilized with 2 mg/ml LPS solution prepared from phase I *C. burnetii* cells as described by Schramek and Galanos (1981). Erythrocytes were washed three times in physiological saline (pH 7.2) and resuspended in veronal buffer.

The microtest reaction was performed in a vol of 125 μl. The sera were diluted 1 : 5 in physiological saline, inactivated at 56°C for 30 min and adsorbed to sheep erythrocytes. Then the erythrocytes were suspended in veronal buffer. Guinea pig serum was used as complement in dilution 1 : 10 after adsorption to sheep erythrocytes.

Negative controls were made in the absence of serum to exclude spontaneous agglutination; positive control was made with previously titrated homologous immune serum. Positive sera were checked for reactivity with uncounted sheep erythrocytes. In addition, some rabbit sera, repeatedly infected with *C. burnetii* were separately adsorbed to: 1. spleen erythrocytes from noninfected mice; 2. sheep erythrocytes; 3. commercial *C. burnetii* phase II antigen; 4. phase I *C. burnetii* antigen. The results were read according to usual procedures.

In IHT all sera of noninfected animals as well as the sera of patients with recurrent typhoid exanthema showed negative results. In contrast, the sera from *C. burnetii*-infected animals and convalescent sera from Q-fever patients were positive. The positive sera in IHT showed negative reaction with the uncoated by LPS sheep erythrocytes. The specific reactivity of sera was abolished after their adsorption with the *Coxiella burnetii* phase I antigen.

Comparing the dynamics of antibody formation to *C. burnetii* phase I cells detected by IHT, IHAT, and CF test in infected rabbits it was found that after first antigen administration IHT and IHAT revealed antibodies earlier and for a less prolonged interval than CF test. After repeated antigen administration the antibody levels as determined by IHAT and IHT were higher than detected by CF test; after 3rd antigen injection these differences were significant (Fig. 1). However, the duration of positive antibody detection by IHAT and IHT was shorter after primoinoculation as well as after booster injections as compared to that by CF test.

In patients with Q-fever and in convalescents positive results of IHAT and IHT were detected from day 4 since the onset of disease, because they

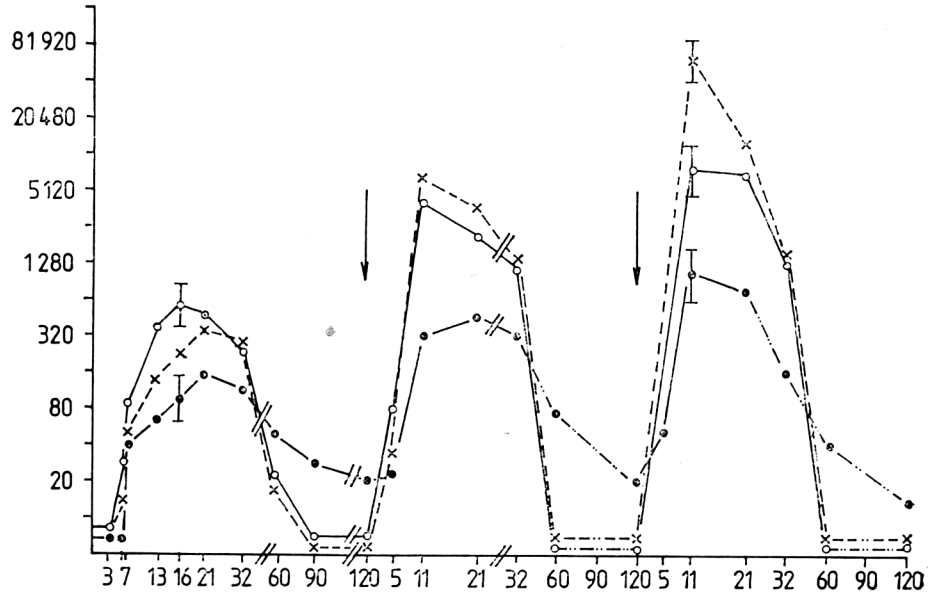

Fig. 1

Comparison of IHT, IHAT, and CF test in the course of rabbit infection with *C. burnetii*
(× — — ×) antibodies detected by IHT (phase I *C. burnetii*)
(○ — — ○) antibodies detected by IHAT
(● — — ●) CF antibodies
Arrows: *C. burnetii* administration
Abscissa: days; ordinate: serum dilution reciprocals
Significant differences (at $p = 0.05$) are marked.

detect only phase I antibodies. On weeks 4—8 of the disease the results of IHT and IHAT were in agreement. By means of these tests *C. burnetii* phase antibodies were found in 58% of serum samples while by CF test was positive in 25% of these samples only. The mean geometric antibody titres as detected by IHT and IHAT were considerably higher than determined by CF test (1 : 388 and 1 : 549, respectively, in comparison with 1 : 32).

The negative results of IHT and IHAT found on days 50—120 since the beginning of the disease confirmed the experimental data in animals revealing relatively short and transient serum antibody levels detected by abovementioned reactions. When examining four highly positive samples (IHT antibody titre 1 : 12 000) two were positive for *C. burnetii* confirming chronic rickettsiaemia.

We believe that IHT is highly specific and sensitive. This reaction, along with IHAT is suitable for detection of phase I antibodies. In addition to its simplicity, the reaction allows to determine the antibodies at early post-infection intervals.

Reference

Schramek, S., and Galanos, C. (1981): Lipid component A of *Coxiella burnetii* derived lipopolysaccharides. *Acta virol.* **25**, 230—234.