Acta virol. **24** : 218–221, 1980

DETERMINATION OF IMMUNOGLOBULIN CLASSES OF ANTIBODIES AGAINST *COXIELLA BURNETII* BY PROTEIN A FROM *STAPHYLOCOCCUS AUREUS*

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Received December 21, 1979

Summary. — Protein A from *Staphylococcus aureus* was used for differentiating antibodies against *Coxiella burnetii* according to immunoglobulin classes. Interaction of immune serum with protein A resulted in complete (within the sensitivity limits of the methods used) removal of IgG antibody and had practically no effect on the level of IgM antibody.

Key words: Coxiella burnetii; antibody; staphylococcal protein A

A simple and efficient method of differentiation of IgM and IgG antibodies based on their different affinity to protein A from *Staphylococcus aureus* has been introduced recently. Protein A has a marked ability to interact specifically with the Fc fragment of IgG from sera of man and mammals (Kronvall *et al.*, 1970; Kronvall, 1973). Interaction of immune serum with a suspension of *S. aureus* cells containing protein A on their surface or with purified protein A, immobilized on an insoluble carrier, results in the removal of the IgG fraction and thus of this class of antibody. More exactly, protein A reacts with subclasses IgG_1, IgG_2 and IgG_4 which account for 95% of the total concentration of human IgG. A difference in the serological activities between absorbed and original serum indicates the presence of IgG in the latter. The method is especially prospective for the detection of relatively low concentrations of IgM antibody, since the latter is unmasked following the removal of IgG antibody. But it should be taken into account that protein A adsorbs from human and animal sera not only IgG, but also a relatively small proportion of IgM (Grov, 1975, 1976; Goding, 1978). This adsorption of IgM, however, cannot practically affect the results of the method under consideration. Suspensions of *S. aureus* Cowan-I were successfully used in determinations of IgM antibodies in human sera in a number of infections (Ankerst *et al.*, 1974; Mallison *et al.*, 1976; Roggendorf *et al.*, 1976; Chantler *et al.*, 1976; etc.). But we found no report on the use of this method in rickettsial diseases, including Q-fever.

The aim of the present work was the use of protein A for a differentiation of antibodies against *Coxiella burnetii* according to immunoglobulin classes,

which would considerably broaden the diagnostic possibilities of serological reactions in Q-fever (Kazár *et al.*, 1977; Tokarevich, 1978).

Unfortunately there are no data on the identification by protein A of antibody pig serum. It has only been reported that protein A binds guinea pig IgG and insignificant amounts of IgM (Forsgren, 1968). We attempted, therefore, to identify antibodies in sera from infected guinea pigs by a suspension of *S. aureus*. To check the reliability of the method, we not only examined the *S. aureus*-absorbed sera serologically, but also isolated and tested the immunoglobulin fraction adsorbed by protein A and compared the results with those of an electrophoretic analysis.

Sera from guinea pigs infected with *Coxiella burnetii* (strain *Apodemus flavicollis* — Luga in phase I, 3rd chick embryo passage) were examined in the course of infection. To select the dose of inoculum, the *C. burnetii* culture was titrated in 12 g white mice; the titres expressed in log ID_{50} values were calculated according to Reed and Muench. Infection of the mice was checked by immunofluorescence. Six guinea pigs weighing 250—300 g were inoculated intraperitoneally with 1 ml volumes of *C. burnetii* suspensions, corresponding to $2 \times 10^{5.2}$ ID_{50}. Antibodies against *C. burnetii* were determined in complement-fixation (CF) reactions with corpuscular phase II antigen (produced by the N. F. Gamaleya Institute of Epidemiology and Microbiology, U.S.S.R. Academy of Medical Sciences, Moscow).

For antibody differentiation, each serum was divided into 4 parts. One was adsorbed by *S. aureus* suspension, the 2nd was treated with ethanethiol, the 3rd was adsorbed by *S. aureus* suspension and subsequently treated with ethanethiol, and the 4 th served as control. All samples were tested in CF reactions.

Determination of antibody classes by ethanethiol was carried out by the method of Murray *et al.* (1965). When the antibody level in sera treated and untreated with ethanethiol was the same, the antibody was classified as IgG. A 4-fold or higher decrease in antibody titre after ethanethiol treatment as compared with control serum was taken as indicating the presence of IgM antibody.

IgG adsorption was carried out by a suspension of *S. aureus* Cowan-I, previously fixed with 0.5% formaldehyde, according to Kronvall (1973): 0.1 ml of test serum was mixed with 0.5 ml of a 20% staphylococcal suspension and kept for 30 min at room temperature. The staphylococci were sedimented by centrifugation (30 min at $3000 \times g$). Incomplete removal of the staphylococci resulted in anticomplementarity of the test sera.

The immunoglobulin fraction adsorbed on protein A was obtained by affinity chromatography on a column of Sepharose 4B conjugated with protein A by the method of Hjelm *et al.* (1972). Serum passed through the column and the immunoglobulin fraction eluted by glycine buffer pH 3.0 was subjected to polyacrylamide gel electrophoresis. Antibodies in both samples were identified by ethanethiol and staphylococcal suspension (IgA antibody in the sera was not assayed).

The distribution of the different classes of antibodies against phase II *C. burnetii* in the course of infection is illustrated in Fig. 1. Twelve days after inoculation (p.i.) only IgM antibody was detected in the guinea pigs. Twenty days p.i. both methods (ethanethiol and protein A) revealed both IgM and IgG antibodies in the sera. In the early period of infection both methods used for classification of antibody thus yielded comparable results. Forty days p.i. ethanethiol revealed no IgM antibody in any of the animals. The titres of antibody before and after ethanethiol treatment remained the same in some animals or decreased only 2-fold in others. After adsorption of the sera with *S. aureus*, comparatively high antibody titres were also found in all animals. Subsequent treatment of these serum samples with ethanethiol resulted in a loss of serological activity, suggesting the presence

in the test sera of IgM antibody which was unmasked after adsorption of IgG antibody on staphylococcal protein A.

To test this assumption, guinea pig serum obtained 40 days p.i. was subjected to affinity chromatography. The CF titre of this serum was 640 before and after ethanethiol treatment, 40 after adsorption wit *S. aureus*

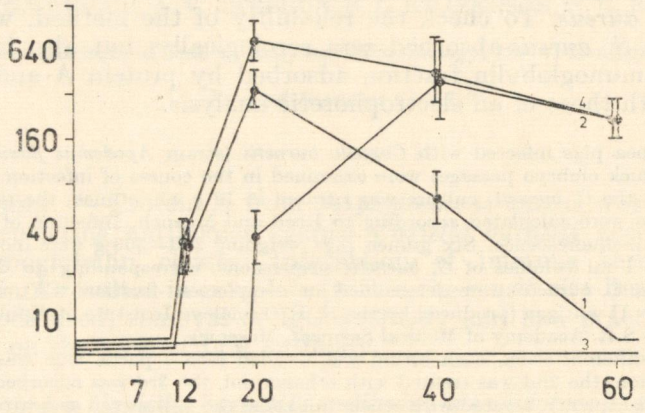

Fig. 1.

Results of examination of guinea pig sera in the course of infection with *C. burnetii*
Abscissa: days p. i.; ordinate: geometrical mean titres (vertical bars: confidence intervals at P = 0.05)
Titres of antibodies against phase II *C. burnetii:*
1 — serum adsorbed with protein A
2 — serum treated with ethanethiol
3 — serum adsorbed with protein A and subsequently treated wit ethanethiol
4 — untreated serum (control)

and < 10 after subsequent treatment with ethanethiol. Electrophoresis of serum passed through the chromatographic column revealed the absence of a fraction corresponding in mobility to IgG, while the IgM fraction was preserved. At the same time the eluate from the column contained a protein electrophoretically identical to IgG, but no detectable amount of IgM (Fig. 2, see Plate XV).

Serological examination of the serum subjected to affinity chromatography revealed only antibody sensitive to ethanethiol treatment. Immunoglobulins, adsorbed on protein A and eluted therefrom, contained only antibody resistant to ethanethiol treatment.

There was a correlation between the electrophoretic and serological examination of serum subjected to affinity chromatography on protein A. Interaction of immune serum with protein A from *S. aureus* resulted in complete (within the sensitivity limits of the methods used) removal of IgG antibody and had practically no effect on the level of IgM antibody.

The coincidence of results of serological and electrophoretic examinations of immune serum that had been subjected to affinity chromatography

indicates that adsorption of sera by *S. aureus* suspensions represents a prospective method of differentiation between IgM and IgG antibodies against *C. burnetii*. The use this method with subsequent treatment of the test samples with reducing substances (in particular ethanethiol) increases the reliability of the results.

Before a definite evaluation, the proposed method should be tested also on other rickettsial diseases.

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