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STUDIES OF THE ANTIGENIC STRUCTURE OF COXIELLA BURNETII

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Summary. — The antigenic structure of *Coxiella burnetii* (*C.b.*) was studied by absorption of human and animal immune sera with *C.b.* organisms in the natural phase II (NPh II) or with artificial phase II (ArPh II) organisms prepared by their treatment with KIO_4. It was found that immune sera absorbed with one type of phase II organisms still reacted with the antigen of another type of phase II organisms as demonstrated in both microagglutination (MA) and complement-fixation (CF) tests.

Key words: *Coxiella burnetii*; *antigenic structure*; *immune sera*; *absorption*

Introduction

Studies on phase variation phenomenon in *C.b.* revealed two main antigenic components (Stocker, 1953; Stoker and Fiset, 1956; Fiset, 1957; Brezina, 1958) which were proposed to be designated as antigen 1 and 2, respectively (Schramek *et al.*, 1978). Later on, a mosaic of antigenic determinants of antigen 2 was supposed (Fiset *et al.*, 1971) and the possibility of existence of further antigenic components in *C.b.* was suggested (Úrvölgyi and Brezina, 1979; 1981).

We have extended our preliminary study on absorption of immune sera (IS) with phase II *C.b.* organisms and brought evidence that the antigenic structure of *C.b.* could be more complex than previously accepted.

Materials and Methods

C. burnetii antigens. *C.b.* strain Nine Mile in phase I (kindly supplied by Dr. R. A. Ormsbee, Rocky Mountain Laboratory, Hamilton, Montana, U.S.A.) after 6 chick embryo (CE) yolk sac passages (EP 6) as well as in phase II (EP 162 in our laboratory) were used for preparation of phase I/Ph IA/ and natural phase II /NPh IIA/ suspensions, respectively. The artificial phase II suspension (ArPh IIA) was prepared by potassium periodate treatment of phase I *C.b.* cells (Schramek *et al.*, 1972). Density of all *C.b.* suspensions which served for absorption experiments and as particular antigens for serologic examination was standardized photometrically to 1 mg/ml according to Fiset *et al.* (1969).

Immune sera. IS were obtained from guinea pigs and rabbits, respectively, inoculated intraperitoneally with 300–1,000 μg of killed phase I *C.b.* or 10^3 EID_{50} of live phase I *C.b.* Guinea pigs were bled 7 days after administration of killed or 14 days after infection with live *C.b.* Rabbit sera were harvested at intervals from the 6th to the 115 th day after immunization. Human sera were collected from patients suffering of Q fever.

Table 1. The antibody titres in IS absorbed with phase II C. burnetii organisms

Serum origin	Antibody titres with antigen ArPh II A	B	C	NPh II A	B	C
Guinea pig						
No. 1*	2048	< 2	512	4096	512	< 2
No. 2*	1024	< 2	512	4096	512	< 2
No. 3**	4096	2	512	4096	64	< 2
Rabbit*	4096	4	512	4096	256	< 2
Human						
No. 1	1024	< 2	128	4096	256	< 2
No. 2	2048	< 2	128	4096	128	< 2

A — non-absorbed serum, B — serum absorbed with ArPh IIA, C — serum absorbed with NPhIIA.
*Immunization with killed phase I C.b. cells;** Immunization with live C.b.

Absorption of IS. For absorption, only those immune sera were employed, which did not contain any detectable (by MA and CF tests) antibodies directed to antigen 1, such as guinea pig and human sera. Rabbit sera had to be pre-absorbed with Ph IA before further absorption studies. Absorption was carried out for 2 hr at 37 °C under stirring and further 18 hr at 4 °C. As many as 40—80 mg of *C.b.* organisms were used for absorbtion with 1 ml of IS. Designation of homologous organisms and antigen, respectively, refers to the same type of phase II *C.b.* antigen used for absorption as well as for serologic examination: heterologous means different phase II preparations used for absorption and serologic examination.

Serologic examination. All absorbed and non-absorbed sera were examined by the MA test according to Fiset *et al.* (1969) and some also in CF test (Brezina, 1958) with PhI antigen and with phase II antigens NPhII and ArPhII.

Results

When non-absorbed immune sera were examied with NPh IIA or ArPh IIA, no substantial differences in antibody titres were observed. Absorbtion of IS with ArPh IIA and NPh IIA, respectively, led to a complete or substantial loss of MA antibody titres with homologous but not with the heterologous antigens (Table 1). Similar resuls were obtained when examining the absorbed IS in CF test. To exclude the eventuality that positive reactivity of absorbed IS with heterologous antigens could be caused by the low quantity of antigens used for absorbtion, one ml volumes of IS were repeatedly absorbed with

Table 2. The MA antibody titres of guinea pig IS after single or repeated absorbtion with phase II C.b. organisms

Immune sera absorbed with	Antibody titres with antigen ArPh II A	B	C	NPh II A	B	C
NPh II	64	< 2	32	< 2	< 2	< 2
ArPh II	< 2	< 2	< 2	32	16	< 2
ArPh II	< 5	< 2	< 2	32	16	< 2

A — serum absorbed with NPh IIA or ArPh IIA; B — serum repeatedly absorbed with ArPh IIA; C — serum repeatedly absorbed with NPh IIA.

Table 3. Kinetics of antibody titres in rabbit IS samples non-absorbed or absorbed with phase II C.b. organisms

Days after immunization	Non-adsorbed serum tested with antigen			Adsorbed serum tested with antigen					
	Ph I	ArPh II	NPh II	ArPh II A	ArPh II B	ArPh II C	NpH II A	NpH II B	NpH II C
6	16	4096	4096	1024	< 2	32	1024	8	< 2
10	256	4096	4096	4096	4	128	4096	128	< 2
17	512	4096	4096	4096	4	512	4096	256	< 2
24	1024	4096	4096	4096	< 2	128	4096	32	< 2
31	1024	4096	4096	1024	< 2	64	2048	2	< 2
72	256	4096	2048	64	< 2	2	< 32	2	< 2
115	64	2048	1024	8	< 2	4	< 8	2	< 2

Titres in MA test (serum dilutions reciprocals). Serum samples absorbed with: A — Ph IA, B — — ArPh IIA, C — NPh IIA.

50—60 mg of either type of phase II organisms. Following single and repeated absorbtion, the same sera were examined in MA tests with homologous and heterologous antigens. As shown in Table 2, IS gave positive though slightly lower reactions with the heterologous antigen, when the same type of phase II organisms were used for single and/or repeated absorbtions respectively; this reactivity was abolished, however, when sera were repeatedly absorbed with the other type of phase II organisms.

Rabbit serum samples harvested at different intervals after immunization, were examined with Ph IA, ArPh IIA and NPh IIA antigens before and after absorbtion with Ph IA, ArPh IIA and NPh IIA *C.b.* suspensions (Table 3). Absorbtion of earlier serum samples (up to 24 days after immunization) with Ph IA led to negligible decrease of antibody titres to both phase II artificial and natural antigens: the decrease was marked, however, in sera from later intervals (days 72 and 115 after immunization). After absorbtion with ArPh IIA or NPh IIA, the sera did not react with homologous antigen at all or in threshold titres only; in contrast, they reacted with the heterologous antigen. This reactivity decreased to the threshold levels or disappeared completely in serum samples from later intervals (days 72 and 115 after immunization).

Discussion

Discovery of two main antigenic components (antigen 1 and antigen 2) in *C.b.* enabled a more efficient serologic diagnosis of Q-fever. Further studies resulted in obtaining natural or artificial phase II *C.b.* organisms of high purity (Fiset *et al.*, 1969; Schramek *et al.*, 1972), which may serve for detection of early antibodies directed to antigen 2. Based on studies of anigenic structure of four *C.b.* strains isolated in different remote geographic regions, it was supposed that antigenic relatedness may be the expression of a mosaic of antigen 2 determinants of phase II *C.b.*, different determinants being changed quantitatively and by space distribution of different *C.b.* strains (Fiset *et al.*, 1971). Recently, immunoelectrophoresis of soluble *C.b.* antigens released from immune complexes with IS revealed at least 6 precipitation lines (Williams *et al.*, 1981).

Our results indicate that sera of immunized laboratory animals as well as of naturally infected humans contain antibodies which could not be completely absorbed with NPh II or ArPh II organisms, as detected by both MA and CF tests. This serological reactivity cannot be exhausted even by repeated absorbtion with enormous amounts of particular phase II *C.b.* organisms. Therefore, the existence of further (in our case two) additional antigenic components capable to induce corresponding antibodies may be expected. The possibility of false reactivity due to denaturation of antigens during *C.b.* purification seems to be improbable, because immune sera were obtained also from animals immunized with live *C.b.* organisms and from humans naturally infected, which therefore could not contain antibodies directed to possibly denaturated antigens.

We suggest that both phase II organisms (natural and artificial) differed in their surface structure. Apparently, individual antigenic components are located in different depth and can be differently unmasked or overlapped depending on chemical treatment or passage history of *C.b.* in chick embryo yolk sac, which may greatly influence virulence, antigenic and physico-chemical properties of *C.b.* strains (Kazár *et al.*, 1974). Since in above-mentioned study by Fiset *et al.* (1971) four *C.b*, strains used differed in the number of yolk sac passages (EP21, 22, 35, and 90), their possibly different surface structure might affect the accessibility of different antigenic components in serological reaction and thereby the results of serological test.

Antigenic components found in our study belong obviously to the sphere of antigen 2, component revealed by antigen from artificial phase II organisms being closer in its location to antigen 1 than the other component. These new antigenic components will probably not play an important role in serological diagnosis of Q fever, but their knowledge may serve for better understanding of antigenic structure of *C.b.* and may explain some deviations in serologic tests using antigen prepared from *C.b.* in natural or artificial phase II (Úrvölgyi and Brezina, 1978).

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