Acta virol. **28**: 48–52, 1984

PHAGOCYTOSIS OF COXIELLA BURNETII BY *HYALOMMA DROMEDARII* TICK HAEMOCYTES

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Received March 17, 1983

Summary. — Haemocytes of laboratory bred half-engorged *Hyalomma dromedarii* ticks phagocytized intracoelomally inoculated *Coxiella burnetii* organisms. Significantly higher phagocytosis of phase II than phase I *C. burnetii* was observed, irrespective of whether live, killed untreated or killed organisms treated with chloroform-methanol (CM) mixture were used. However, HCl- or KIO_4-treated phase I cells were phagocytized to a similar extent as phase II cells. More consistent results were obtained with haemocytes of male than of female ticks. Phagocytosis of phase I killed and live organisms was significantly increased by their preincubation with phase I but not with phase II immune rabbit sera.

Key words: Coxiella burnetii; phagocytosis; opsonization; tick haemocytes

Introduction

Phase variation in *Coxiella burnetii (C. b.)*, originally described as serological phenomenon (Stoker and Fiset, 1956), was later found to be more complex. Phase I to phase II conversion is accompanied by the change of several biological and physicochemical properties of *C. b.* cells (Kazár *et al.*, 1974). The most pronounced feature of the phase state of *C. b.* is a clearcut difference in nonspecific phagocytosis of phase I and phase II organisms as demonstrated with rabbit (Brezina and Kazár, 1965) and human (Wisseman *et al.*, 1967) polymorphonuclear leukocytes as well as with guinea pig and mouse macrophages (Kazár *et al.*, 1975; Kishimoto *et al.*, 1976).

We tried to find out whether such a difference in phagocytosis of *C. b.* cells could be demonstrated also with tick haemocytes and whether opsonization of *C. b.* with immune sera, as known in above mentioned human and animal systems, would also function in them.

Materials and Methods

Coxiella burnetii strain Nine Mile serologically in phase I (3 chick embryo yolk sac passages — —Ep3) or in phase II (Ep162 in our laboratory) was partially purified by differential centrifugation and stored at −60 °C. Either suspension contained approximately 10^7 EID_{50}/ml of

live rickettsiae. Highly purified formalin-killed *C. b.* organisms were prepared from the same egg passage of Nine Mile strain. The part of them was subjected to either CM-treatment (Schramek and Mayer, 1982), or KIO_4-treatment (Schramek *et al.*, 1978). All killed *C. b.* suspensions were adjusted to protein concentration of 1 mg/ml.

Hyalomma dromedarii ticks bred in our laboratory were half-engorged by feeding on rabbits for 6 days prior to intracoelomal inoculation with C. b. organisms (males with 0.02 ml and females with 0.05 ml, respectively). Six and 25 hr after inoculation the coxal part of the front leg pair was amputated; drops of the haemolymph were allowed to dry, fixed with methanol for 5 min and stained according to Gimenez. The total number of haemocytes obtained from one tick varied from 20,000 to 30,000 per mm .

Phagocytosis of C. b. was determined by counting the proportion of haemocytes containing clearly visible *C. b.* cells. In each smear at least 100 haemocytes were evaluated; each counting was performed in 4—8 samples. Opsonizing effects of phase I and phase II immune sera (obtained from rabbits immunized with killed phase I and phase II *C. b.* organisms, respectively) were determined in haemocytes harvested from male ticks 24 hr after inoculation (of 0.02 ml/vol) from a mixture consisting of 0.1 ml of *C. b.* cells (either live or killed) and of 0.1 ml immune serum, which had been preincubated for 30 min at 37 °C.

Results

As shown in Table 1, male as well as female haemocytes were able to phagocytize live, killed untreated and killed but chemically treated *C. b.* organisms of either phase. However, a marked difference in phagocytosis of phase I (Fig. 1) and phase II (Fig. 2) organisms was observed, namely by male haemocytes. Whereas phase II organisms were rapidly phagocytized by 85.4 $\pm$ 12.1 male haemocytes, phase I organisms only by 12.1 $\pm$ 7.1 of these cells. The difference was not so distinct with female haemocytes which phagocytized phase II organisms in 64.6 $\pm$ 11.2 but phase I organisms in 29.6 $\pm$ $\pm$ 11.1. A higher degree of phagocytosis was observed in both males and females by 24 hr after inoculation than by 6 hr with each material investigated. CM-treatment of phase I and phase II *C. b.* organisms did not cause any significant change in phagocytosis. However, HCl- or KIO_4-treatment of phase I cells caused that they were phagocytized alike to phase II cells. The degree of phagocytosis of live phase I and phase II *C. b.* organisms was similar to that of killed *C. b.* organisms.

Opsonizing effects of immune rabbit sera were studied only on male haemocytes harvested 24 hr after inoculation of mixtures of live or killed *C. b.* organisms with immune rabbit sera (Table 2). A marked increase in proportion of haemocytes phagocytizing phase I killed or live organisms was observed only providing they were preincubated with phase I immune serum, i.e. a serum containing antibodies directed to the surface antigen. Because of the high degree of nonspecific phagocytosis, no significant increase in phagocytosis of phase II live and killed *C. b.* was noticed after their preincubation with either phase I or phase II immune sera.

Discussion

Phagocytosis plays an important role in hosts' resistance to bacterial infections and its degree depends markedly on the surface properties of bacterial cells. The bacterial cells with higher surface tension than that of

Table 1. Nonspecific phagocytosis of phase I and phase II C. burnetii organisms by Hyalomma dromedarii haemocytes in vivo

C. burnetii		Per cent haemocytes containing *C. burnetii* (M ± S.D.)*			
		males		females	
		6 hr	24 hr	6 hr	24 hr
phase I	killed	12.1 ± 7.1	15.1 ± 8.8	28.4 ± 15.3	29.6 ± 11.1
phase II	killed	67.6 ± 15.5	85.4 ± 12.1	42.9 ± 7.6	64.6 ± 11.2
phase I CM-treated	killed	10.3 ± 8.5	11.3 ± 10.9	33.9 ± 18.1	28.8 ± 13.0
phase II CM-treated	killed	70.8 ± 17.3	88.8 ± 11.3	42.8 ± 15.7	51.6 ± 22.0
phase I KIO_4-treated	killed	59.3 ± 10.8	80.1 ± 12.9	43.6 ± 11.1	67.5 ± 10.5
phase I HCl-treated	killed	65.1 ± 23.4	84.8 ± 13.7	43.1 ± 15.5	69.6 ± 11.1

* mean ± standard deviation calculated from 8 samples

phagocytic cells are hydrophobic, and therefore they are readily phagocytized also in the absence of specific antibodies (van Oss and Gilman, 1972). *C. b.* organisms in phase II possess obviously similar surface properties resulting in a high degree of nonspecific phagocytosis and the surface tension of phase I organisms can be increased by an addition of corresponding immune serum leading to their increased phagocytosis, as found with human and animal polymorphonuclear leukocytes and macrophages (Brezina and Kazár, 1965; Wisseman *et al.*, 1967; Kazár *et al.*, 1975; Kishimoto *et al.*, 1976). Our results clearly show that differences observed in phagocytosis of phase I and phase II *C. b.* in mammalian systems are valid also for tick haemocytes. We found that opsonization with corresponding immune serum of phase I *C. b.* organisms occuring at phagocytosis by mammalian phagocytes can also be found with tick haemocytes. Chemical treatment of phase I *C. b.* cells with HCl or KIO_3 causing damage to the surface antigen determining the phase I state influenced phagocytosis so that phase I *C. b.* cells behaved as phase II cells. In contrast, CM-treatment of phase I cells which probably affects the surface phospholipids did not change their behaviour during phagocytosis.

Phagocytosis is one of the main functions of haemocytes of the arthropods (Crossley, 1975), playing both the nutritive and defense role. There has not been known so far in which extent tick haemocytes may contribute to the defence against microorganisms, namely against those which for the ticks serve as vectors. The possibility of multiplication of an infectious agent within haemocytes and of its distribution throughout the tick body must also be taken into account. The functional ability of haemocytes may depend on their morphology, but some morphological criteria differentiating haemocytes (Dolp, 1971; Balashov, 1979, Brinton and Burgdorfer, 1971) registered

Table 2. Opsonization by rabbit imune sera of phase I and phase II C. burnetii organisms for phagocytosis by Hyalomma dromedarii male haemocytes after 24 hr

C. burnetii preincubation	Per cent haemocytes containing *C. burnetii* (M ± S.D)* killed	live
phase I organisms with		
phase I serum	78.1 ± 10.0 (8)	54.8 ± 9.5 (8)
phase II serum	21.8 ± 11.7 (8)	27.0 ± 13.4 (8)
no serum	16.2 ± 7.8 (8)	13.0 ± 9.9 (8)
phase II organisms with		
phase I serum	76.3 ± 12.5 (5)	72.5 ± 19.1 (4)
phase II serum	75.0 ± 12.9 (4)	66.0 ± 16.7 (5)
no serum	75.4 ± 12.1 (4)	79.0 ± 12.2 (8)

* calculated from 4—8 samples

also in our experiments, could be hardly sufficient for their proper functional characterization. One can assume that we dealt with the cells which were elicited to clot the haemolymph in the wound after limb amputation, as found in *Orithodorus moubata* ticks (Diehl, personal communication). The population of haemocytes obtained from *Hyalomma dromedarii* males seemed to be more homogenous morphologically than that harvested from females and in our phagocytosis experiments it gave more consistent results. Apparently, such factors as sex, physiological state and species of ticks should be taken into consideration. It is worth mentioning that similar preliminary results as presented in this paper we have obtained also with haemocytes of *D. reticulatus* species.

The model used may serve for studies of intime relationships between *C. b.* and ticks not only in terms of further elucidation of phase variation phenomenon in *C. b.*, but also as regards its multiplication, distribution and persistence in ticks. To obtain more comprehensive data on such relationships and on the possible defense functions of ticks, in future a more detailed characterization (ultrastructural, bochemical, etc.) of haemocytes themselves and of their interaction with pathogenic microorganisms is necessary.

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Explanation of Micrographs (Plate IV):

Fig. 1. Phagocytosis of phase I *C. b.* cells by male *H. dromedarii* haemocytes. The *C. b.* cells are mostly non-phagocytized remaining free in the haemolymph. Gimenéz staining, ×600.

Fig. 2. Phagocytosis of phase II *C. b.* cells by male *H. dromedarii haemocytes.* The haemocytes are packed with dense masses of *C. b.* cells. Gimenéz staining, ×600.