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EFFECT OF COXIELLA BURNETII ON THE STIMULATION OF HEXOSE MONOPHOSPHATE SHUNT AND ON SUPEROXIDE ANION PRODUCTION IN HUMAN POLYMORPHONUCLEAR LEUKOCYTES

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Summary. — Killed *Coxiella burnetii (C.b.)* cells in phase II but not inphase I had a mild stimulatory effect on hexose monophosphate shunt (HMPS) and superoxide anion production by human polymorphonuclear (PMN) leukocytes. Preincubation of *C. b.* cells of either phase with serum of a leukocyte donor lacking detectable antibodies to *C.b.* did not affect the studied activities of PMN leukocytes. In contrast, both HMPS stimulation and superoxide production were enhanced by specific opsonization of *C.b.* cells with rabbit immune sera containing corresponding phase I and/or phase II antibodies. Stimulatory effect was observed also with lipopolysaccharide-protein-phospholipid (LPS-Pr-Pl) complex but not with lipopolysaccharide-protein (LPS-Pr) complex and with purified lipopolysaccharide (LPS) isolated from phase I *C.b.* cells. Possible consequences of these findings for explanation of *C.b.* resistance to intracellular killing by professional phagocytes are discussed.

Key words: Coxiella burnetii; polymorphonuclear leukocytes; enzymatic activation; intracellular killing.

Introduction

C.b., the causative agent of Q fever is a strict obligatory intracellular parasite of eukaryotes. It is unique among Rickettsia possessing the phenomenon of phase variation (Stocker and Fiset, 1956). Virulent "wild" *C. b.* strains existing in nature in phase I, convert to phase II during repeated passages on chick embryonated eggs in laboratory. This conversion is accompanied by a change of various properties of *C.b.* (Kazár *et al.*, 1974). It has been found that killed *C.b.* phase II cells are readily phagocytized to a greater extent than phase I cells by PMN leukocytes, the phagocytosis of the latter being increased markedly by their preincubation with phase I immune serum (Brezina and Kazár, 1965; Wisseman *et al.*, 1967). Unlike the majority of bacterial parasites, *C.b.* multiplies within the phagolysosomes

(Hackstadt and Williams, 1981), but little is known on the mechanism(s) that accounts for the intracellular survival of *C.b.*

The present study was undertaken to determine whether killed *C.b.* after interaction with human PMN leukocytes may stimulate their hexose monophosphate shunt and superoxide generation. Such a stimulation is considered to reflect the bactericidal activity of PMN cells (Klebanoff, 1975, 1982; Roos, 1980; Ferenčík and Bergendi, 1984) and may be important for the intracellular survival of *C.b.* as well.

Materials and Methods

Rickettsiae. C. b. strain Nine Mile in phase I (5 yolk sac passages, EP 5) and in phase II (EP 163 in our laboratory), as well as phase I strains 48 (EP 3), L 35 (EP 4) and Florian S (EP 6) were propagated in the chick embryo yolk sac. *C. b.* cells were killed by formalin or phenol and purified as described (Schramek *et al.*, 1978). As stimuli of HMPS activation and superoxide production were used the following *C. b.* preparations: purified phase I and phase II cells, LPS-Pr-Pl complex extracted from purified phase I *C. b.* cells by trichloracetic acid (Brezina and Úrvölgyi, 1961), LPS-Pr complex extracted by trichloracetic acid from *C. b.* cells pretreated by chloroform-methanol (CM) mixture (Kazár *et al.*, 1983), purified LPS isolated from phase I *C. b.* cells by phenol (Schramek and Brezina, 1976) and *C. b.* phospholipid (Pl) which was obtained by CM extraction from intact phase I *C. b.* cells. For isolation of control Pl preparation, non-infected chick embryo yolk sacs were also treated with CM mixture. Pl extracts were evaporated and dry residuum was emulgated in PBS.

Human PMN leukocytes were separated from heparin (25 U/ml) anticoagulated blood obtained from normal healthy volunteers by the method of Boyum (1967) with the use of Dextran T-500 (Pharmacia, Uppsla) sedimentation. Contaminating erythrocytes were removed by hypotonic lysis, isolated leukocytes were washed twice with phosphate-buffered saline (PBS), pH 7.4, containing 5 U heparin/ml (PBSH) and finaly suspended in PBS, which contained 0.1% glucose (PBSHG) at the concetration of 1×10^7/ml. Differential cell counts with Wright-Giemsa stain showed that at least 94% of the cells were PMN leukocytes.

Opsonized zymosan was prepared by incubating 0.5 ml of fresh homologous serum with 1 mg zymosan (ZKL, Trenčín) in 1.0 ml PBS at 37 °C for 30 min. After three times washing with 1.0 ml PBS and resuspending in 1.0 ml PBSHG, the suspension contained about 1×10^8 zymosan particles. For similarly performed opsonisation of *C. b.* corpuscular preparations, serum of the PMN leukocytes donor and specific rabbit antiserum containing both phase I and phase II antibodies were used.

Stimulation of HMPS was measured on the basis of ability to reduce 3-(4-iodophenyl)-2-(4-nitrophenyl)-5-phenyl-tetrazolium chloride (INT; Lachema, Brno). The total INT reduction was assayed after 30 min. incubation of 2×10^6 PMN leukocytes with 0.1% INT at 37 °C in presence or absence of zymosan or different *C. b.* preparations by measuring the coloured formazan extracted into acetone at 485 nm (Lokaj and Obůrková, 1975). The concetration in reacting mixture of zymosan, *C. b.* cells, LPS preparations and Pl extracted from uninfected chick embryo yolk sacs was 50 pg per one leukocyte. In the case of *C. b.* Pl amount used per one leukocyte was extracted from 50 pg of phase I *C. b.* cells.

Production of superoxide was estimated on the principle of INT reduction to formazan by PMN leukocytes without and with the presence of superoxide dismutase (Ferenčík *et al.*, 1984). Superoxide dismutase (Miles Labs.) was used in the concentration of 10 μg per 10^6 PMN leukocytes.

Results and Discussion

Results of the effects of different *C.b.* preparations or zymosan on the stimulation of HMPS and superoxide production are summarized in Table 1. When compared with a marked stimulatory effect of zymosan, different *C. b.* strains of high virulent phase I stimulated neither HMPS nor superoxide

Table 1. The effect of various Coxiella burnetii preparations on stimulation of hexose monophosphate shunt (HMPS) and superoxide anion production by human PMN leukocytes

Stimulus	HMPS stimulation	Superoxide production	n
Zymosan ops. DS	100	100	10
Zymosan nonops.	57.7 ± 5.1	66.0 ± 6.8	10
C.b. I nonops.	−4.8 ± 3.1	−5.9 ± 4.5	10
C.b. I ops. DS	0.5 ± 4.2	−2.6 ± 5.2	8
C.b. I ops. RbAb	65.3 ± 19.6	85.3 ± 24.5	8
C.b. II nonops.	11.7 10.2	12.2 ± 9.8	10
C.b. II ops. DS	14.9 ± 9.4	11.4 ± 7.9	10
C.b. ops. RbAb	41.8 ± 17.0	49.3 ± 15.5	10
LPS-Pr-Pl	68.0 ± 11.4	57.1 ± 9.4	7
LPS-Pr	4.5 ± 5.1	8.5 ± 6.8	5
LPS	3.9 ± 6.1	13.2 ± 8.1	7
Pl-*C.b.* I	35.0 ± 6.5	44.5 ± 10.6	4
Pl-y.s.	−17.4 ± 5.2	−11.8 ± 4.2	4
LPS-Pr + Pl-*C.b.* I	33.6 ± 7.4	48.2 ± 12.3	3
LPS-Pr + Pl-y.s.	−2.9 ± 5.8	1.4 ± 6.7	3
LPS + Pl-*C.b.* I	24.2 ± 9.3	31.6 ± 13.5	3
LPS + Pl-y.s.	−8.1 − 6.4	−6.3 − 5.8	3

Figures represent percentage of HMPS stimulation and superoxide production after different stimuli compared to that induced by opsonized zymosan which was considered to be 100% (mean ± SD). Mean value of absorbance (at 485 nm in 1 ml cuvette) of reduced INT after 30 min incubation of PMN leukocytes with zymosan was 0.432 ± 0.086.
n — number of experiments. *C.b.* I = Coxiella burmetii, — phase I; II — Coxiella burnetii = phase II; non-ops. = non-opsonized; ops. DS = opsonized with serum of leukocyte donor; ops. RbAb = opsonized with rabbit antiserum against *C.b.*; LPS-Pr-Pl = lipopolysaccharide-protein-phospholipid complex prepared from *C.b.* I; LPS-Pr = lipopolysaccharide-protein complex prepared from *C.b.* I; LPS = lipopolysaccharide; Pl-*C.b.* I = phospholipid prepared from *C.b.* I; Pl-y.s. = phospholipid prepared from pure chick embryo yolk sac.

production (or they had rather a mild inhibitory effect), whereas *C.b.* cells of low virulent phase II displayed a low stimulatory effect (about 10% of that of zymosan) on both activities of PMN leukocytes under study. Opsonization of *C.b.* cells of either phase with serum of PMN leukocytes donor containing no detectable antibodies against *C.b.* (non-specific opsonization) did not affect significantly their original stimulatory activity. In contrast, specific opsonization of *C.b.* cells with rabbit serum containing both phase I and phase II antibodies resulted in a distinct increase of stimulatory activity of phase I cells, while in the case of phase II cells the increase was not so striking. The results obtained are in accord with those on phagocytosis of specifically or non-specifically opsonized *C.b.* cells by professional phagocytes, in which the phagocyting activity and specific opsonization depended on the phase state of *C.b.* cells (Brezina and Kazár, 1965; Wisseman *et al.*, 1967; Kazár *et al.*, 1975; Kishimoto *et al.*, 1976). Though in our experiments killed *C. b.* cells were employed, one may suggest that similar results could be also achieved with the use of live *C.b.* cells, because with regard

to the phase state of *C.b.* cells no substantial difference in the phagocytosis of live or killed *C.b.* cells was observed (Kazár *et al.*, 1975; Škultétyová *et al.*, 1978). Importance of specific opsonization of *C. b.* follows also from the findings of phagolysosomal fusion and subsequent degradation of phase I *C.b.* within the macrophages only providing *C.b.* cells were opsonized with specific immune serum (Little *et al.*, 1983).

The phase I state of *C.b.* cells is determined by the presence of the surface antigen 1 of LPS nature (Schramek *et al.*, 1978). This was extracted in different forms, namely as LPS-Pr-P1 complex, LPS-Pr complex or pure LPS. Investigation of the stimulatory activity of these LPS forms on enzymatic systems under study revealed that only complete LPS-Pr-P1 complex possessed a stimulatory effect on both HMPS and superoxide production. For such a stimulation the specific *C.b.* P1 seemed to be responsible, because a control P1 extracted from uninfected chick embryo sacs, used for the growth of *C.b.* had no stimulatory but inhibitory effect. Inability of expression of the stimulatory effect of P1 in intact phase I *C.b.* cells can be explained by structural localization of LPS-Pr-P1 complex and steric hindrance of P1 molecule.

C.b. as an obligatory intracellular bacterium is known to persist within phagocytic cells (Khavkin and Amosenkova, 1969; Ariel *et al.*, 1973; Baca *et al.*, 1981; Hackstadt and Williams, 1981). The results indicate that one of the possible explanation of survival of virulent natural form of phase I *C.b.* cells within polymorphs would be their inability to activate the phagocyte metabolism, especially superoxide production and HMPS in the absence of specific phase I antibodies. Such activation is necessary for complementization of both main groups of antibacterial substances, i.e. reactive products of oxygen and constituents of lysosomes. Another explanation of *C.b.* survival within phagocytes is its superoxidase dismutase nad catalase activities which may split superoxide and hydrogen peroxide generating in phagosomes during phagocytosis (Akporiaye and Baca, 1983).

The specific antibodies against phase I are produced in the late (convalescent) stage of Q fever infection and may contribute to the killing of *C.b.* cells by macrophages rather than PMN leukocytes, which appear in the early phases of inflammatory process. The investigation of enzymatic activation in macrophages by specifically and non-specifically opsonized *C.b.* cells will be the purpose of our further study.

Just before submitting this manuscript for publication we became aware of the results of Akporiaye and Baca (1983) indicating that superoxide dismutase and catalase activities in *C.b.* may contribute to their ability to survive within phagocytes.

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