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# Effects of *Coxiella burnetii* endotoxin on granulocyte functions

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Lipopolysaccharides (LPS) exhibit the discussed ability to prime polymorphonuclear leukocytes (PMN) for enhanced release of oxidative radicals upon subsequent stimulation [1]. The hypothesis that a modification in LPS structure could alter the resulting priming suggested to study a natural example of chemical and immunological variation represented by *Coxiella burnetii (C.b.)* LPS in its virulent phase I (LPS I) and avirulent phase II (LPS II). We studied the effect of both LPS I and II (Dr. S. Schramek, Bratislava) on the human granulocyte oxidative burst and aggregation. LPS (10ng ÷ 2.5μg/ml) were incubated in a shaking bath with PMN in different experimental conditions (0°, 37 °C; 0 ÷ 60 min); the burst was stimulated by 1 μM FMLP or PMA, 0.5 μg/ml and evaluated as superoxide production. Aggregation was tested by a platelet aggregometer on PMN incubated with LPS (1 ÷ 5 μg/ml) in the above conditions and stimulated with 1 μM FMLP plus cytochalasin B (5 μg/ml). *C.b.* LPS I and LPS II were not per se a stimulus for superoxide release but, when evaluated in their ability to prime PMN upon either FMLP or PMA, LPS I was almost completely devoid of activity, whereeas PMN were capable of being primed by a wide range of LPS II concentrations. The priming was dependent on the time (by 60 min it was in 70% maximal), on the temperature of incubation (no effect at 0 °C) and on the dose up 0.25 μg/ml (30 $O_2^-$ nmoles in 10 min by FMLP from $10^6$ not primed PMN vs. 95 $O_2^-$ nmoles from PMN primed by LPS II, 0.25 μg/ml at 37 °C for 1 h). To further evaluate the mechanism of priming, we used Polymyxin B (PB) which neutralizes LPS by binding to lipid A, the toxic moiety of endotoxin. A PB dose-dependent disappareance of the priming was observed when LPS II was directly challenged with the antibiotic, but a less inhibition was appreciated with PB added to PMN prior or at beginning of LPS incubation. This finding might depend on the ability of PB to disrupt the endotoxin and to weakly compete with it for membrane receptor sites. Moreover, only PMN preincubated with LPS II appeared well aggregated after 1 hr, while both controls and LPS

I pretreated-PMN were going to a complete disaggregation.

PMN priming could be triggered by granule acyloxyacylhydrolases which selectively remove nonhydroxylated fatty acids from lipid A [2] by activating therefore the protein kinase C. Since fatty acid profile of the two *C.b.* LPSs appears to be similar, the different behaviour could be explained in our test systems by a more ready availability of lipid A from LPS II.

## References

1. Guthrie LA, McPhail LC, Henson PM and Johnston RB Jr: Priming of neutrophils for enhanced release of oxygen metabolites by bacterial lipopolysaccharide. J. Exp. Med. 160: 1656, 1984
2. Munford RS and Hall CL: Detoxification of bacterial lipopolysaccharide by a human neutrophil enzyme. Science 234: 203, 1986