Effect of Various *E. coli* LPS Chemotypes on Apoptosis and Activation of Human Neutrophils

M. G. Vinokurov*,, M. M. Yurinskaya*,**, I. R. Prokhorenko*,***, and S. V. Grachev*,***

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 142, No. 8, pp. 136-138, August, 2006
Original article submitted March 24, 2006

We studied the effects of various *Escherichia coli* LPS chemotypes on the production of reactive oxygen species and regulation of apoptosis in human neutrophils. A correlation was found between the increase in chemiluminescence (Re-LPS<Ra-LPS<S-LPS) and lengthening of the polysaccharide chain in endotoxins. Shortening of the polysaccharide chain was associated with more pronounced inhibition of neutrophil apoptosis under the influence of endotoxins, which correlated with the increase in hydrophobicity of these chemotypes.

Key Words: *endotoxin chemotypes; neutrophils; apoptosis; reactive oxygen species*

Endotoxins (LPS) play an important role in the development of sepsis and endotoxin shock caused by gram-negative bacteria. Endotoxins are released from the wall of gram-negative bacteria into the circulation. They interact with cells responsible for innate immunity [1]. These target cells express surface membrane CD14 receptors. The interaction of endotoxins with LPS-binding protein and CD14 receptor initiates assembly of the receptor complex including Tool-like receptor 4, MD2, CD11b, CD18, MyD88, and other proteins and receptors in the membrane of target cells [3]. Stimulation of neutrophils with LPS induces generation of reactive oxygen species (ROS) [1] and increases the lifetime of these cells. These changes are associated with the inhibition of apoptosis under the influence of endotoxins [13].

LPS have similar structure. *E. coli* LPS consists of a hydrophobic component, lipid A, that forms the external layer in the outer membrane, phosphorylated non-repeating oligosaccharide (core oligosaccharide, OS), and polysaccharide located above the surface of the bacterial cell (O-antigen) and identified by serotyping. Most clinical isolates of *E. coli* carry S-LPS, which includes a 3-component structure of LPS. The R-type endotoxin lacks O-antigen and sometimes contains shortened core oligosaccharide. Core OS is divided into the outer and inner core region. The inner core consists of only 2-keto-3-deoxy-D-manno-octulonic acid (KDO) or includes also heptose. Enterobacteria are lettered to illustrate the impairment of biosynthesis in the outer core. This classification provides information about structural characteristics of the outer core. Core-defective structures are designated as Rb- and Re-chemotypes. The Ra-chemotype includes structures with a normal outer core.

The influence of core OS and LPS polysaccharide (O-antigen) on endotoxin-induced pathological processes is less studied than the effect of lipid A [12]. A direct correlation was found between the length of the polysaccharide chain (hydrophobicity of endotoxin) and induction of cytokines [5,10]. The interaction of chemotypes of various LPS with myeloid cells is poorly understood [9]. There are no comparative data on the effect of LPS on the regulation of apoptosis in human neutrophils.

*I. M. Sechenov Moscow Medical Academy; **Institute of Cell Biophysics, Russian Academy of Sciences, Pushchino; ***Institute of Fundamental Problems of Biology, Russian Academy of Sciences, Pushchino. ***Address for correspondence:*** mgvinokurov@rambler.ru. M. G. Vinokurov

0007-4888/06/1422-0173

Here we compared the effects of various chemotypes of *E. coli* LPS (S-LPS, Ra-LPS, Rc-LPS, and Re-LPS) on ROS production by neutrophils and regulation of apoptosis in these cells.

MATERIALS AND METHODS

Experiments were performed with Re-LPS from *E. coli* JM103, Rc-LPS from *E. coli* J5, and Ra-LPS from *E. coli* EH100 (bacterial R-chemotype), and LPS from *E. coli* O55:B5 (S-chemotype, Sigma). Re-LPS was isolated from *E. coli* JM103 by extraction with a phenol-chloroform-petroleum ether mixture (method of Galanos) [6]. Preparations of Re-LPS were tested for the content of proteins and nucleins by absorption at 260 and 280 nm [14].

The blood was taken from the cubital vein of healthy donors and placed in vacutainers with heparin. Peripheral blood neutrophils were isolated by differential centrifugation on a Ficoll-Verografin gradient (1.119 and 1.077). The purity of isolated neutrophils was ~98%.

Viability of isolated neutrophils was studied by flow cytofluorometry with a fluorescent probe propidium iodide (30 μM). The percentage of cells impermeable for propidium iodide in the presence of 40 μg/ml digitonin was determined. Cell viability was 98-99%.

To study apoptosis, isolated neutrophils (10^6 cells/ml) were cultured in RPMI-1640 medium containing 1% L-glutamine, 1% penicillin, 1% streptomycin, and 10% heat-inactivated fetal bovine serum. Culturing was performed at 37°C and 5% CO_2 for 9 h.

The cells with fragmented DNA were detected by flow cytofluorometry. By the end of culturing, these cells were fixed with 70% ethanol and stored at -20°C. Fixed cells were pelleted by centrifugation. The supernatant was removed and the pellet was resuspended in a solution of 100 mM NaCl and 100 mM Tris-HCl (pH 7.4) containing 1 μg/ml fluorescent probe Hoechst-33258. Staining was performed at 37°C for 30-40 min. The cells with degraded DNA were identified in histograms obtained on a flow cytofluorometer (to the left of peak G1). Viability of cultured neutrophils was 95-98%.

ROS generation in neutrophils was studied by the chemiluminescence method. Neutrophils (0.2×10^6 cells/ml) were placed in Eppendorf tubes with Hanks medium containing 0.5 μM NaN_3, 2% autologous serum, and 3.5×10^{-4} M luminol. They were maintained in a temperature-controlled cuvette of a 1250 LKB luminometer. fMLP (10^{-6} M) served as the activator of ROS generation. Neutrophils were incubated for 25 min before addition of fMLP.

The results were analyzed by Student's *t* test. The differences between mean values were significant at $p<0.001$.

RESULTS

In series I, we studied the effect of various chemotypes of *E. coli* LPS on ROS generation by neutrophils. The test endotoxins significantly increased luminol-dependent chemiluminescence (CL) compared to the control (Fig. 1). *E. coli* S-LPS was most potent in increasing ROS generation by neutrophils. The ability of endotoxins to induce the rise in CL increased in the following order: control<Rc-LPS<Re-LPS<Ra-LPS<S-LPS. The differences between numerical values of CL for Re-LPS (219%) and Rc-LPS (209%) were much lower compared to those for Ra-LPS (256%) and S-LPS (285%, Fig. 1).

In series II, we compared the effects of various chemotypes on apoptosis in cultured neutrophils. The ability of endotoxins to inhibit apoptosis decreased in the following order: Re-LPS>Rc-LPS>Ra-LPS>S-LPS (Fig. 2). These data indicate that endotoxin consisting of lipid A and 2 KDO moieties produced the most pronounced inhibitory effect on apoptosis in neutrophils.

A correlation was found between variations in CL (S-LPS>Ra-LPS>Re-LPS, Fig. 1) and increase in the length of the polysaccharide chain in LPS chemotypes. Published data show that binding of LPS to specific receptors on myeloid cells is followed by rapid internalization of endotoxin molecules. Internalization of 5% cell-bound LPS occurs over 1 min [8]. The kinetics of internalization of LPS chemotypes correlated with lengthening of the polysaccharide chain in LPS and decrease in hydrophobicity of endotoxins [2]. However, the rate of

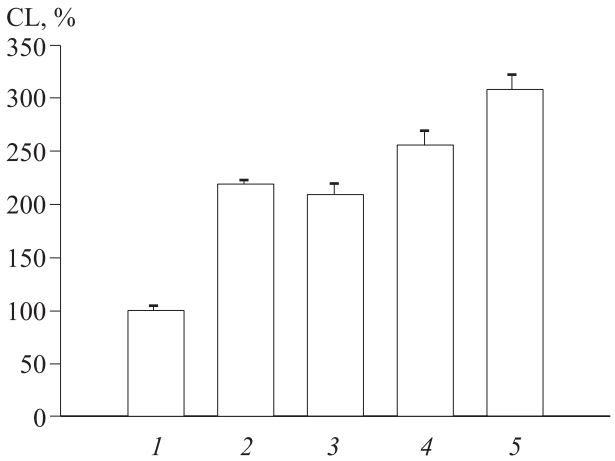

Fig. 1. Effect of various *E. coli* LPS chemotypes (20 ng/ml) on ROS generation by neutrophils. Here and in Fig. 2: control (*1*), Re-LPS (*2*), Rc-LPS (*3*), Ra-LPS (*4*), and S-LPS (*5*).

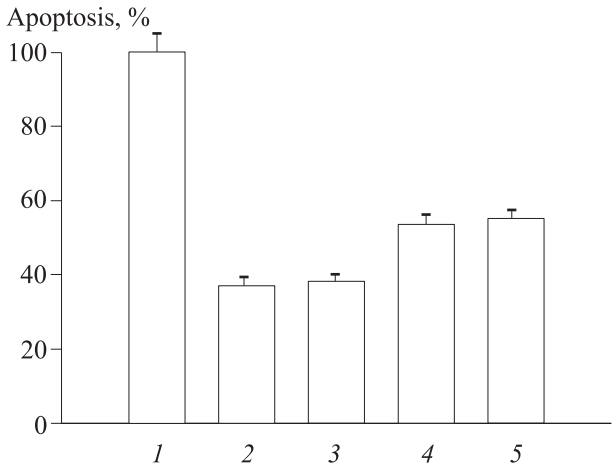

Fig. 2. Effect of various *E. coli* LPS chemotypes (200 ng/ml) on apoptosis in neutrophils.

internalization for lipid A is lower compared to that for Re-LPS and Rd-LPS. The observed differences are probably associated with structural characteristics of these compounds [9]. CL of neutrophils in the presence of Rc-LPS was higher compared to that observed in experiments with Re-LPS (Fig. 1). It was probably related to structural characteristics of endotoxins and their interaction with endotoxin receptors on myeloid cells. CL of neutrophils increased in the presence of Re-LPS and Ra-LPS, which is consistent with published data [9]. The kinetics of internalization of S-LPS and activation of ROS generation by myeloid cells under the influence of this endotoxin is similar. By contrast, Re-LPS is characterized by slow internalization [8,9]. High hydrophobicity of Re-LPS probably contributes to the formation of large aggregates in the medium.

The relationship exists between intracellular signal pathways of ROS generation by neutrophils and regulation of apoptosis. Endotoxins accelerate proteosomal degradation of activated caspase-3 in neutrophils [11] and increase the inhibition of extracellular regulatory caspase [7]. Maximum inhibition of apoptosis in neutrophils was typical of Re-LPS with the shortest polysaccharide chain (compared to other chemotypes, Fig. 2). Moreover, Re-LPS is characterized by a slower internalization compared Ra-LPS [9] and S-LPS [8]. The increase in ROS generation under the influence of the test chemotypes (S-LPS>Ra-LPS>Rc-LPS>Re-LPS>control) is probably related to the interaction of these LPS with endotoxin receptors. These changes correlate with the length of the polysaccharide chain in LPS. Shortening of the polysaccharide chain is accompanied by a greater inhibition of apoptosis in neutrophils under the influence of the test endotoxins, which correlates with the increase in hydrophobicity of these chemotypes.

REFERENCES

1. S. V. Grachev, S. G. Pak, V. A. Malov, *et al*., *Ter. Arkhiv*, No. 11, 84-89 (2003).
2. K. Brandenburg, A. Schromm, and M. H. J. Koch, *Bacterial Endotoxins: Lipopolysaccharides, from Genes to Therapy*, Eds. J. Levin *et al*., New York (1995), pp. 167-182.
3. A. Christian and Z. Ulrich, *Trends Glycosci. Glycothechnol*., **14**, No. 76, 69-86 (2002).
4. A. M. Condliffe, E. Kitchen, and E. R. Chilvers, *Clin. Sci. (Lond.)*, **94**, No. 5, 461-471 (1998).
5. W. Feist, A. J. Ulmer, and J. Musehold, *Immunobiology*, **179**, Nos. 4-5, 293-307 (1989).
6. C. Galanos, O. Luderitz, and O. Westphal, *Eur. J. Biochem*., **9**, No. 2, 245-249 (1969).
7. S. J. Gardai, B. B. Whitlock, Y. Q. Xiao, *et al*., *J. Biol. Chem*., **279**, No. 43, 44,695-44,703 (2004).
8. R. L. Kitchens and R. S. Munford, *J. Immunol*., **160**, No. 4, 1920-1928 (1998).
9. A. Lentschat, V. T. El-Samalouti, J. Schletter, *et al*., *Infect. Immun*., **67**, No. 5, 2515-2521 (1999).
10. H. Loppnow, H. Brade, and I. Durbaum, *J. Immunol*., **142**, No. 9, 3229-3238 (1989).
11. L. Mica, L. Harter, O. Trentz, *et al*., *J. Am. Coll. Surg*., **199**, No. 4, 595-602 (2004).
12. S. Muller-Loennies, U. Zahringer, U. Seydel, *et al*., *Prog. Clin. Biol. Res*., **397**, 51-72 (1998).
13. K. Sheth, J. Friel, B. Nolan, *et al*., *Surgery*, **130**, No. 2, 242-248 (2001).
14. C. J. R. Thorne, *Techniques in Protein and Enzyme Biochemistry*, Eds. H. L. Kornberg, *et al*., Pt. 1, Amsterdam (1978), pp. 1-18.