IMMUNOLOGY AND MICROBIOLOGY

Effects of Bacterial Endotoxin on α-Synuclein Expression in the Lymph Node Leukocytes of Rats

T. N. Sergeyeva, V. G. Sergeyev, and T. O. Tolstolytskaya*

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The effects of bacterial LPS on the expression of early apoptosis marker (annexin V) and immunoreactive α-synuclein in mononuclear leukocytes of the mesenteric lymph nodes of rats were studied *in vivo* and *in vitro*. Injection of LPS increased the number of lymphocytes with high expression of immunoreactive α-synuclein in cell nucleus and cytoplasm (by 68.6±8.1%) and stimulated apoptosis in this population (by 96.0±3.6% of the control). The expression of α-synuclein in macrophages increased only in the cytoplasm and this increase was not paralleled by stimulation of apoptosis in these cells. The data indicate that LPS-induced elevation of α-synuclein in lymphocytes is a component of the mechanism of programmed death in these cells. The increase of α-synuclein expression in macrophages can be related to processes associated with their stimulation.

Key Words: *α-synuclein; lymphocytes; macrophages; apoptosis; lipopolysaccharide*

α-Synuclein is a protein initially found in neurons [2,3,11] and later in blood monocytes and lymphocytes [5,6], but its physiological function in these cells is unclear. In lymphocytes of patients with Parkinson's disease, enhanced expression of α-synuclein stimulating the proapoptotic effect of glucocorticoids was demonstrated [6]. Since apoptosis of some stimulated lymphocytes is a component of the immune response mechanism [1], it can be hypothesized that α-synuclein serves as a component of the mechanism of programmed death of stimulated lymphocytes during normal immune response.

In order to verify this hypothesis, we studied possible relationship between apoptosis intensity in mesenteric lymph nodes of rats after intraperitoneal injection of bacterial LPS and the level of α-synuclein in cells of these nodes. In addition, we studied (in a special series of *in vitro* experiments) the possibility of direct endotoxin stimulation of α-synuclein synthesis in lymph node cells and the distribution of this protein in various parts of activated cells.

MATERIALS AND METHODS

The study was carried out in 15 male Wistar rats (250-300 g) kept under standard conditions in accordance with regulations on handling of animals, approved by the local ethic committee of the Udmurt University. Animals of the experimental group (n=6) were intraperitoneally injected with 125 μg/kg *Salmonella abortus equi* LPS (Sigma-Aldrich) dissolved in 1 ml saline. Controls (n=6) were intraperitoneally injected with 1 ml sterile saline. After 24 h, the experimental and control rats were narcotized with thiopental, the

Department of Immunology and Cellular Biology, Udmurt State University; *Hematological Laboratory, Hematological Laboratory of Republican Clinical Hospital No. 1, Ministry of Health of the Udmurt Republic, Izhevsk, Russia. ***Address for correspondence:*** cellbio@ya.ru. V. G. Sergeyev

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abdominal cavities were opened, and the mesenteric lymph nodes were resected and crushed in a Petri dish in order to prepare cell suspension. The intensity of fluorescence of immunoreactive α-synuclein and annexin V in isolated cells was analyzed in a BD FACSCanto II flow cytofluorometer.

In a special experimental series, isolated lymph node cells from 3 control animals were cultured (24 h) in RPMI-1460 in histological Lab-Tek boxes in a concentration of 10^6 cell/well. In half of cases the cells were cultured in medium with LPS (0.12 μg/ml). After 24 h the cells were fixed and cytochemically stained with antibodies to α-synuclein (Sigma-Aldrich). The preparations were examined under a Nikon Eclipse 200F fluorescent microscope. Digital images were processed by ImageProPlus 6.0 software. The significance of differences between the samples was evaluated using nonparametric Mann–Whitney *U* test, the differences were considered significant at $p<0.05$ (Statistica 6.0).

RESULTS

The number of apoptotic cells in the nodes 24 h after LPS injection was by 96.0±3.6% higher than in the control ($p<0.001$). The increase in the number of apoptotic cells was seen from enlargement of an additional peak in the histogram of distribution of cells labeled with antibodies to annexin V (Fig. 1, *a*, *b*). Logical gating by nucleus-containing events in two-dimensional histogram of forward and side light scatter showed that the number of dying cells increased mainly in the lymphocyte population of the lymph nodes.

Mononuclear leukocytes with low and high intensity of α-synuclein expression were found in lymph nodes of the control and experimental rats (Fig. 1, *c*, *d*). Injection of LPS led to an increase in the number of cells with high expression of α-synuclein. The number of these cells increased 24 h after LPS injection by 125.9±7.3% ($p<0.001$) in comparison with the control. The increase in the count of cells with

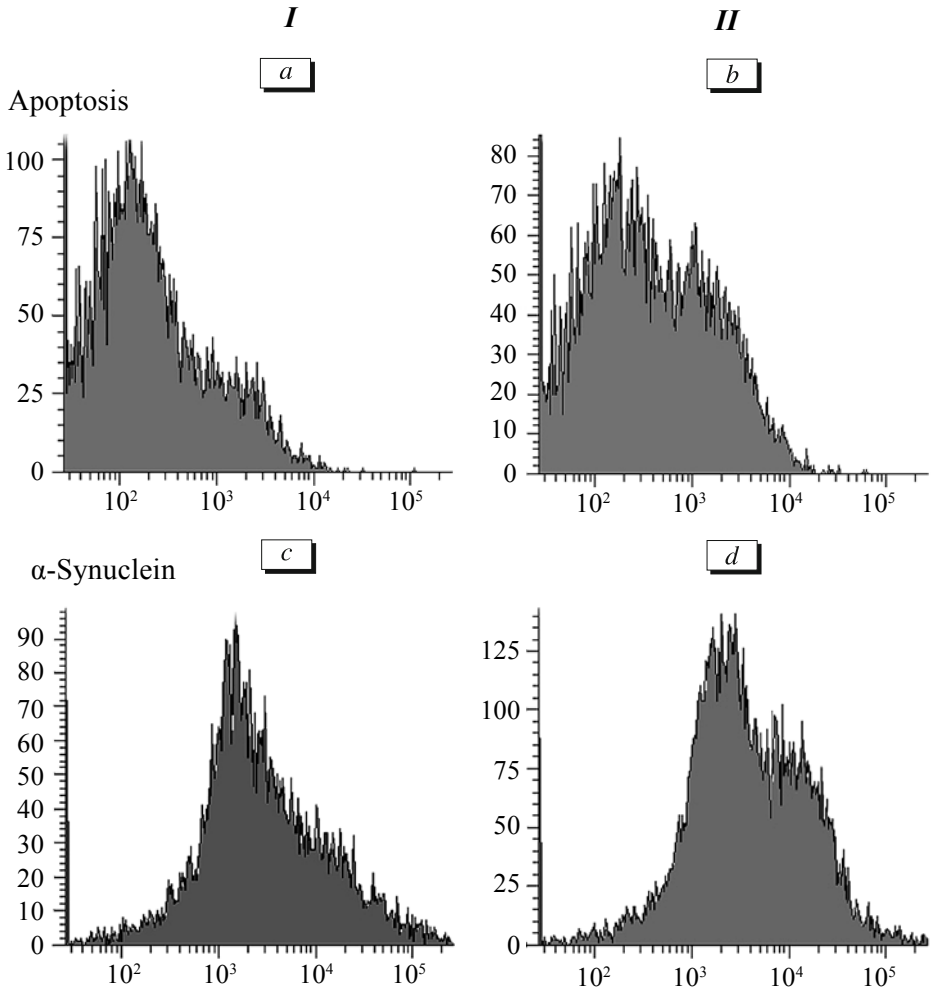

Fig. 1. Quantitative distribution of the rat mesenteric lymph node mononuclear leukocytes expressing with different intensity immunoreactive annexin V (*a*, *b*) and α-synuclein (*c*, *d*) in the control (*I*) and 24 h after LPS injection (*II*). Abscissa: fluorescence intensity; ordinate: number of stained cells.

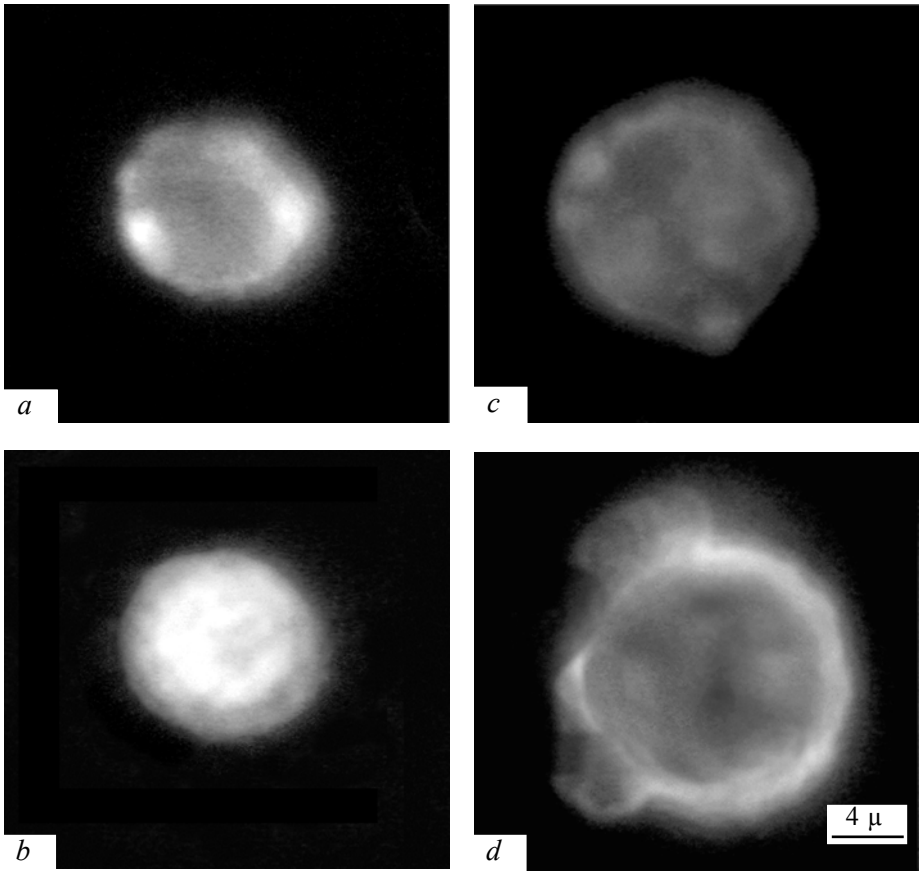

Fig. 2. Expression of immunoreactive α-synuclein in lymphocytes incubated without (*a*) and with (*b*) LPS addition into culture medium and in macrophages incubated without (*c*) and with LPS (*d*).

high expression of immunoreactive α-synuclein was found in the lymphocyte and macrophage populations.

The levels of lymphocytes and macrophages with high content of α-synuclein increased after 24-h culturing of lymph node cells in a medium with LPS (by 68.6±8.1%, $p<0.05$, and by 47.2±7.8%, $p<0.01$ compared to the control, respectively). The immunoreactive protein was found in the cytoplasm and nuclei of lymphocytes and macrophages (Fig. 2). No appreciable differences in the distribution of nuclear and cytoplasmic α-synuclein were found in cells with low expression of this protein. More intense label in macrophages with high summary levels of immunoreactive α-synuclein was mainly due to accumulation of immunoreactive label in the cytoplasm, while in lymphocytes the concentration of immunoreactive protein increased in both the cytoplasm and nuclei.

Previous experiments on neuron culture showed that α-synuclein penetrates into the nuclei, binds to histones [3], and inhibits their acetylation [7], thus reducing the expression of antiapoptotic genes and causing neuron degeneration [8,9]. It is logical to suggest that accumulation of α-synuclein in the lymphocyte nuclei in our experiment can be similarly involved in the mechanisms of their programmed death.

Hence, our results indicate that LPS stimulates the expression of α-synuclein in rat lymph node macrophages and lymphocytes. This protein can be involved into mechanisms of programmed lymphocyte death, on the one hand, and in LPS-induced stimulation of macrophages, on the other.

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