Administration of LPS-Stimulated Autologous Macrophages Induces α-Synuclein Aggregation in Dopaminergic Neurons of Rat Brain

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Administration of autologous macrophages isolated from the abdominal cavity and stimulated *in vitro* with bacterial LPS to rats increased blood level of antibodies against α-synuclein. Antibody titer reached its maximum during week 5 of the experiment and exceeded the levels of anti-α-synuclein antibodies appeared in response to transplantation of non-stimulated macrophages. Brain immunohistochemistry showed that additional administration of LPS (250 μg/kg) to animals during week 4 after injection of LPS-stimulated macrophages led to α-synuclein accumulation in 9.4±3.2% dopaminergic neurons of the substantia nigra. These findings attest to induction of humoral immune response to α-synuclein in rats after administration of autologous LPS-stimulated macrophages, which can affect α-synuclein metabolism in dopaminergic neurons of the brain.

Key Words: *α-synuclein; macrophages; dopaminergic neurons*

Parkinson's disease (PD) is a neurodegenerative disorder caused by hyperproduction or modification of α-synuclein protein in dopaminergic neurons of the substantia nigra [1,3]. In only 5-7% cases these disturbances are associated with genetic mutations [4], while in all other cases the etiology of PD remains unclear. Inflammation at the periphery and immune reaction were previously proposed to serve as possible etiological factors of PD [2,5]. However, specific conditions under which inflammation can initiate autoimmune destruction of dopaminergic neurons in the brain are still not determined and no molecular of cellular mechanisms underlying this autoimmune reaction were proposed for consideration.

Previous data concerning macrophages ability to intensify α-synuclein synthesis in response to bacterial antigens [7] allowed us to hypothesize that macrophages are able to initiate humoral response not only against bacterial antigens, but also to endogenous α-synuclein. It is logical to assume that under conditions of inflammation-induced disturbances in blood-brain barrier integrity, these antibodies may penetrate nerve tissue parenchyma and affect protein metabolism in substantia nigra neurons.

To test this hypothesis, we studied the capacity of rat intraperitoneal macrophages activated with bacterial LPS *in vitro* to induce humoral response against α-synuclein after their transfer to the host animal, which in turn received additional injection of LPS in a high dose. Possible disturbances in α-synuclein metabolism in the nervous tissue were estimated by accumulation of inclusions of this protein in the cytoplasm of dopaminergic neurons in the substantia nigra.

MATERIALS AND METHODS

Experiments were carried out on 16 male Wistar rats weighing 250-300 g kept under standard conditions in compliance with the rules of animal handling established by local ethical committee of Udmurt State University. Leukocyte suspension was obtained from

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16 animals using the method of intraperitoneal lavage. For this purpose, sterile physiological solution (20 ml) was injected into the peritoneal cavity and after 5-min massage collected back with a syringe. Cell suspension (containing generally lymphocytes and macrophages) obtained from each animal was centrifuged, washed, and incubated in individual Petri dishes in RPMI-1460 in concentration 2×10^6 cells per dish for 1 h. Nonadherent cells (primarily lymphocytes) were washed out and remaining macrophages were incubated in RPMI-1460 without (8 control animals) or with (8 experimental animals) *Salmonella abortus equi* 0.12 μg/ml LPS (Sigma-Aldrich). After 24-h culturing, adherent cells were harvested using 0.02% versene solution with 0.25% trypsin and intraperitoneally administered to the animal, from which they were obtained. The blood was taken once a week for 2 month after cell transfer via heart punction. The titer of antibodies against α-synuclein was measured by ELISA using α-synuclein protein (Sigma-Aldrich) precipitated in polystyrene plates. After 4 weeks, 3 animals from each group received LPS in a dose of 250 μg/kg body weight. Upon completion of the experiment, rats were intracardialy perfused with Bouin solution, brain was removed and 14 μ cryostat the brain sections were obtained. Expression of immunoreactive α-synuclein and tyrosine hydroxylase was assessed on the sections using indirect immunohistochemistry (Sigma-Aldrich antibodies). Preparations were examined under fluorescent microscope Nikon Eclipse F200. Fluorescence intensity and number of immunopositive cells were measured using software for computer image processing ImagePro Plus 6.0. Significance of differences between mean group values was assessed using Student's *t* test and Statistica 6.0 software.

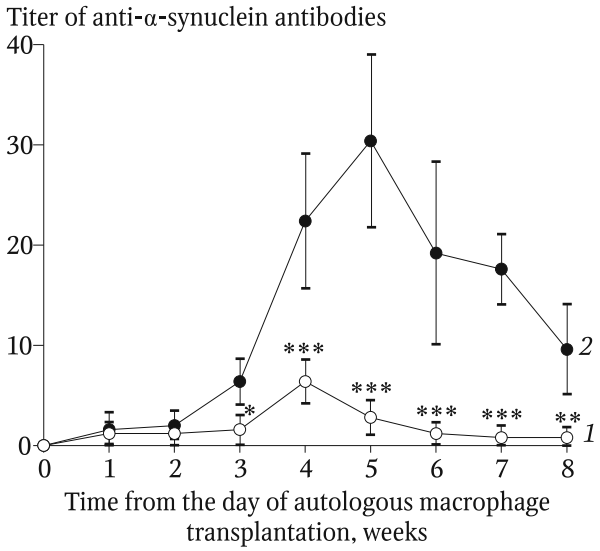

Fig. 1. Kinetics of anti-α-synuclein antibody formation in rat blood following transplantation of autologous macrophages, stimulated (*1*) or non-stimulated (*2*) *in vitro* by LPS. Each point represents the mean value for 5 rats. **p*<0.05, ***p*<0.01, ****p*<0.001 in comparison with non-stimulated macrophages.

RESULTS

Administration of autologous peritoneal macrophages cultured in the presence of LPS to rats increased the titer of antibody against α-synuclein starting from week 3 of the experiment. Antibody concentration reached its maximum on week 5 and remained high up to the end of observation (Fig. 1). Administration of non-stimulated macrophages induced significant changes in blood concentration of α-synuclein compared to the control. Obtained results indicate that direct activation of intraperitoneal macrophages with bacterial endotoxin *in vitro* followed by their re-transplantation can induce specific humoral immune response to endogenous macrophage protein.

Immunohistochemical study of brain sections from rats of the specified groups and from animals receiving single injection of high LPS dose 4 weeks after administration of non-stimulated macrophages did not reveal reduction of dopaminergic neuron quantity or accumulation of immunoreactive α-synuclein in them in the form of cytoplasmic inclusions. However, in rats receiving high LPS dose after administration

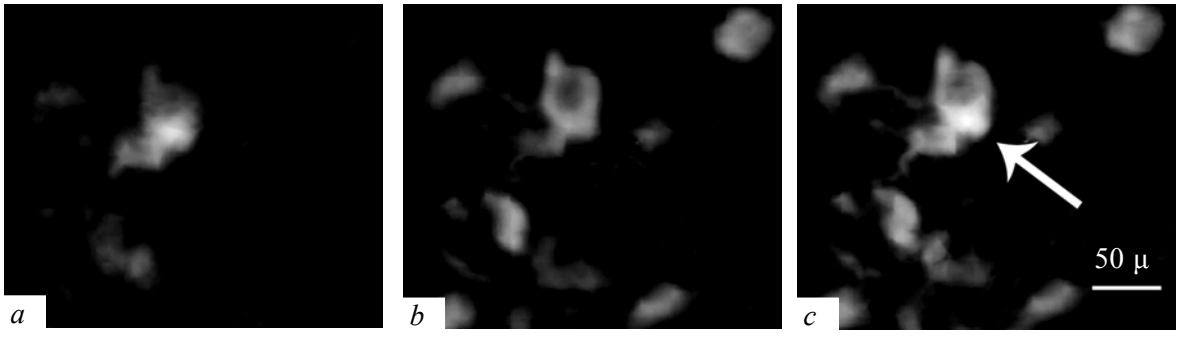

Fig. 2. Expression of immunoreactive α-synuclein (*a*), tyrosine hydroxylase (*b*) and their colocalization (*c*) in substantia nigra neurons of rats stimulated with a high dose of LPS 4 weeks after transfer of autologous LPS-stimulated macrophages. Arrow shows a neuron with double immune-positive label.

of activated macrophages, the inclusions of immunoreactive α-synuclein were found in the cytoplasm of some dopaminergic neurons of the substantia nigra (9.4±3.2% of total amount, Fig. 2). In addition, the total number of dopaminergic neurons in those animals did not differ from that in the control. High LPS doses are known to increase the permeability of the blood-brain barrier [6]. In that situation, circulating anti-α-synuclein antibodies can be logically assumed to penetrate into the nervous tissue and affect α-synuclein metabolism in neurons, which could be evaluated by accumulation of this protein in the form of cytoplasmic inclusions. Detection of the morphological sign of Parkinson-like conditions in some neurons suggests that we modeled conditions that initiate neurodegeneration, which one would expect to become apparent at longer observation.

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REFERENCES

1. A. Abeliovich, Y. Schmitz, I. Farinas, *et al.*, *Neuron*, **25**, No. 1, 239-252 (2000).
2. A. Chen, S. M. Zhang, M. A. Hernan, *et al.*, *Arch. Neurol.*, **60**, No. 8, 1059-1064 (2003).
3. E. Maries, B. Dass, T. J. Collier, *et al.*, Nat. Rev. Neurosci., 4, No. 9, 727-738 (2003).
4. A. Mcenerney-Leo, D. W. Hadley, K. Gwinn-Hardy, J. Hardy, Mov. Disord., 20, No. 1, 1-10 (2005).
5. C. F. Orr, D. B. Rowe, Y. Mizuno, *et al.*, *Brain*, **128**, No. 11, 2665-2674 (2005).
6. M. A. Salkeni, J. L. Lynch, T. Otamis-Price, W. A. Banks, *J. Neuroimmune Pharmacol.*, **4**, No. 2, 276-282 (2009).
7. K. Tanji, F. Mori, T. Imaizumi, *et al.*, *Pathol. Int.*, **52**, No. 9, 572-577 (2002).