IMMUNOLOGY AND MICROBIOLOGY

Postvaccinal Changes in the Nitric Oxide System after Immunization with Live Dry Tularemia Vaccine

N. P. Khirina, I. A. Vasil'eva, and A. V. Stepanov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 151, No. 1, pp. 63-66, January, 2011
Original article submitted November 17, 2009

Immunization of outbred male albino mice with live dry tularemia vaccine in a dose of 50 CFU/mouse was associated with stimulation of the NO system and accumulation of NO metabolites (nitrites and nitrates) in splenic and hepatic tissues. High levels of these metabolites persisted by day 14 after the initial and repeated immunization. These results suggest that the immunotropic effect of live dry tularemia vaccine manifested by not only modulation of the functions of immunocompetent T and B cells, NK and K cells, micro- and macrophages, but also by stimulation of intracellular anti-infection defense at the tissue level via intensification of NO synthesis.

Key Words: *nitric oxide; tularemia vaccine; tissues; immunity*

The fact that NO (a transmitter involved in the maintenance of vascular tone and nervous and immune systems functions) is one of the active biological substrates produced and released by cells of the macroorganism is beyond doubts. NO participates in rapid redox reactions with the formation of nitro- and nitroso compounds, which determines its cyclic generation and destruction. NO is continuously synthesized in the body in enzymatic reactions from L-arginine under the effects of specific NO synthases (NOS) with participation of NADPH and other bioactive substances, and under conditions of O_2 deficit with participation of nitrite reductase systems components [8,9]. The effects of NO at the organism level are largely due to its capacity to bind its main target, the heme group of regulatory enzymes cytochrome P-450, guanylate cyclase, *etc.*, formation of nitro- and nitrose compounds, and regulation of the transcription and translation of some important proteins and enzymes [5,7,13].

Immunotropic effects of NO observed after stimulation of inducible non-Ca-dependent NOS consist in induction of macrophage and lymphocyte cytotoxicity via either direct modulation of the antigenic stimulus (*e.g.* infection agent) or mediation of its effect through the cytokine system, thus promoting stimulation of immunocompetent cells and strengthening of the defense potential of the organism [1,3,10,12].

Since the immune system cells (mainly micro- and macrophages, Kupffer's cells, dendritic cells, *etc.*) are the main NO producers, measurement of its concentration is extremely important not only for characterization of their functioning, but also for understanding of the pathogenetic mechanisms of this or that disease, associated with its hyperproduction, for example, side effects of drugs, autoimmune processes, infectious and noninfectious diseases [11,14]. We think that this approach is important for characterization of the safety of modern means for specific prevention of infections (vaccines, antitoxins, immunoglobulins, *etc.*).

State Research Testing Institute of Military Medicine, Ministry of Defense of the Russian Federation, St. Petersburg, Russia. ***Address for correspondence:*** tigrandt@yandex.ru. I. A. Vasilijeva

Our aim was to obtain more ample data on the safety of vaccines by evaluating their effects on the NO system. We focused our attention on changes in the levels of nitrite and nitrate metabolites (NOx) in body tissues under the effect of immunization with an immunobiological preparation most often used in practical epidemic control: live dry tularemia vaccine. The data on changes in the functioning of immunocompetent cells and nonspecific resistance components after immunization with this vaccine and the results of studies demonstrating the formation of NO not only in immune organs, but in other host organs and systems under the effects of antigenic challenge of different nature [2,6] prompted us to undertake this research.

MATERIALS AND METHODS

Experiments were carried out on outbred male albino mice (18-20 g, n=190) from Rappolovo Breeding Center after a one-week quarantine at Clinic of Experimental Biological Models of our Institute. Experimental animals (n=160) were divided into subgroups A and B (80 per group), control group consisted of 30 mice.

The animals were immunized with commercial live dry tularemia vaccine (Plant for Production of Bacterial Preparations, Irkutsk; lot 20-1, contr. No. 110). Before immunization, the content of the ampoules with the vaccine was inoculated in MacCoy medium, incubated for 2 days at 37°C, after which suspension (50 CFU/ml) was prepared and injected subcutaneously to experimental animals, 0.5 ml per animal. Controls were injected with 0.5 ml isotonic NaCl simultaneously with immunization of the experimental animals.

Animals of subgroup A and controls were decapitated on days 3, 7, 10, and 14 after vaccination and specimens of the serum, brain, spleen, and liver were collected. Specimens of the material from 20 experimental and 9, 7, 7, and 7 control animals per term, respectively, were collected. All specimens were frozen until the study [15].

Subgroup B animals were repeatedly immunized 21 days after the first immunization. The material was collected on days 3, 7, 10, and 14 after repeated vaccination from 20 animals per term. There was no control group for experimental subgroup B, because animals of the same outbred strain kept under the same conditions (season, temperature, ration) were used. The material from vaccinated animals was defrosted before the study and NOx content was measured as described previously [15].

The data were statistically processed on a PC using traditional statistical software. The significance of differences between the samples was evaluated using Student's t test [4]. The differences were considered significant at $p<0.05$.

RESULTS

The levels of NOx metabolites in the serum and studied organs were changed in animals immunized with live dry tularemia vaccine. It is noteworthy that these changes were more pronounced in the immune system tissues (spleen, liver). The levels of NOx metabolites in the spleen and liver decreased 3, 7, and 10 days after immunization, while by day 14 they surpassed the control. The results were compared with the control pooled values for group A, because the groups virtually did not differ during the corresponding periods. According to published reports, creation of lasting strong immunity in vaccinated animals by means of immunobiological preparations implies regular repeated revaccinations [2]. The same is true for live dry tularemia vaccine. We therefore studied the effect of vaccination on the NO system not only after initial, but also after repeated immunization. Revaccination was carried out 21 days after the first immunization, in accordance with our previous experimental findings [2].

The data showed (Table 2) that, in contrast to animals receiving single immunization, high levels of nitrites and nitrates persisted in reimmunized animals during the same periods as after a single vaccination. In addition, comparative analysis of the levels of NOx metabolites during the same periods after single and repeated vaccinations showed significant differences between NOx metabolite levels in all the studied tissues ($p<0.05$-0.001). The differences were significant ($p<0.001$) for the spleen and liver on days 3, 7, and 10 after vaccination and for the serum on days 3 and 7. Significant ($p<0.05$) differences in the levels of NOx metabolites in the serum and liver were observed on day 14 and in the brain on day 7 after vaccination.

In the brain separated from the immune system, NO formation is regulated by Ca-dependent NOS and changes in the levels of NOx metabolites were less pronounced than in the serum, liver, and spleen. After the initial (single) immunization, the specific and summary levels of NOx metabolites decreased only on day 3, presumably as a result of the stress (immunization) and stimulation of the neuron processes leading to reduction of neurogenic NOS. After revaccination, significant opposite changes in NOx content in the brain, differing from the direction of changes in the immune system organs, were observed.

As for serum levels of NOx metabolites reflecting the summary changes in the oxide system in many tissues/systems, the results indicated that single immunization with live tularemia vaccine (Table 1) led to reduction of the levels thereof on days 3 and 10

TABLE 1. Content of NOx Metabolites in Tissues of Subgroup A Mice after Single Immunization (*M*±*m*)

Day after injection	NOx metabolites			
	serum, μmol/liter	spleen, pmol/mg	liver, pmol/mg	brain, pmol/mg
Control (*n*=30)	5.50±0.46	6.60±0.28	8.50±0.41	3.40±0.20
3 (*n*=20)	2.30±0.40*	4.90±0.70*	5.50±0.73***	1.40±0.23***
7 (*n*=20)	6.30±0.38	5.30±0.57*	5.80±0.47***	3.20±0.33
10 (*n*=20)	3.40±0.45*	4.70±0.44***	6.50±0.40***	3.10±0.27
14 (*n*=16)	5.70±0.80	13.90±1.65***	9.70±0.57	3.70±0.21

Note. Here and in Table 2: *$p<0.05$, ***$p<0.001$ compared to the control.

TABLE 2. Content of NOx Metabolites in Tissues of Subgroup B Mice after Single Immunization (*M*±*m*)

Day after injection	NOx metabolites			
	serum, μmol/liter	spleen, pmol/mg	liver, pmol/mg	brain, pmol/mg
Control (*n*=30)	5.50±0.46	6.60±0.28	8.50±0.41	3.40±0.20
3 (*n*=15)	8.50±1.60	16.50±2.08***	9.70±1.11	1.90±0.40***
7 (*n*=15)	2.20±0.95***	14.10±1.54***	14.00±1.32***	4.90±0.56*
10 (*n*=18)	3.50±0.23***	8.80±0.59***	13.90±0.92***	2.80±0.25
14 (*n*=17)	2.60±0.51***	11.10±0.99***	12.90±0.98***	3.20±0.19

postvaccination coinciding with changes in the parameters in hepatic and splenic tissues and on days 7, 10, and 14 in revaccinated animals (Table 2). This disagreement between serum levels of NOx and NOx levels in splenic and hepatic tissues after repeated immunization was presumably caused by stimulation of the nitrite reductase processes.

Hence, immunization with immunobiological preparations, specifically, with tularemia vaccine, was associated with well-known changes in the functioning of not only immune system components (T and B lymphocytes, macrophages, NK and K cells, phagocytes), but also with the formation of intracellular antiinfection defense at the tissue level at the expense of more intense NO synthesis characterized by increase in the levels of its metabolites in the spleen and liver, organs essential for the immune system functioning. Importantly, the changes in the NO system during vaccination were observed after single and repeated injections of the immunobiological preparation.

Our results are, no doubt, just pilot data characterizing only one of the numerous vaccines widely used in epidemic control. Presumably, such effects are intrinsic of other immunobiological preparations, but this conclusion can be made only after their appropriate evaluation.

REFERENCES

1. B. Brune, K. Sandau, and A. Von Kisten, *Biokhimiya*, **63**, No. 7, 966-975 (1998).
2. A. Zh. Vasilenko, R. Sh. Magazov, O. P. Misnikova, *et al.*, *Associated and Complex Immunization (Formation of the Host Immune Response)* [in Russian], Ufa (2000).
3. N. A. Vinogradov, *Antibiotiki Khimioter.*, **43**, No. 2, 24-29 (1998).
4. V. Ya. Gelman, *Medical Informatics: Practicum* [in Russian], 2nd ed., St. Petersburg (2002).
5. E. I. Kovalyov and V. L. Kovalyova, *Khim.-Farm. Zh.*, **37**, No. 9, 3-5 (2003).
6. E. B. Men'shikova, N. K. Zenkov, V. P. Reutov, *et al.*, **65**, No. 4, 485-503 (2000).
7. N. G. Plekhova, L. M. Somova, D. V. Zavorueva, *et al.*, *Byull. Eksp. Biol. Med.*, **145**, No. 3, 316-376 (2008).
8. V. P. Reutov, *Biokhimiya*, **67**, No. 3, 353-376 (2002).
9. V. N. Titov, *Klin. Lab. Diagnost.*, No. 2, 3-16 (2009).
10. K. S. Christopherson and D. S. Bredt, *J. Clin. Invest.*, **100**, No. 10, 2424-2429 (1997).
11. T. K. Eisenstein, D. Huang, J. J. Meisser Jr., and B. al-Ramadi, *Immunobiology*, **191**, Nos. 4-5, 493-502 (1994).
12. P. D. Freeswick, Y. Wan, A. D. Geller, *et al.*, *J. Surg. Res.*, **57**, No. 1, 205-209 (1994).
13. A. J. Gow and J. S. Stamler, *Nature*, **391**, 169-173 (1998).
14. S. J. Green, L. F. Schller, M. A. Marletta, *et al.*, *Immunol. Lett*, **43**, Nos. 1-2, 87-94 (1994).
15. H. Mohsage, B. Kok, J. R. Huizenga, and P. L. Jansen, *Clin. Chem.*, **41**, No. 6, Pt. 1, 892-896 (1995).