S.A.Bugorkova[1], T.N.Shchukovskaya[1], N.I.Mikshis[1], S.A.Shcherbakova[1], O.M.Kudryavtseva[1], E.V.Kuklev[1], V.I.Dubrovina[2], A.K.Noskov[2], K.M.Korytov[2], S.V.Balakhonov[2], D.N.Sandzhiev[3], S.V.Konusheva[3], S.P.Savchenko[3], G.B.Matsakova[3], L.V.Shchuchinov[4], E.P.Mikhailov[5], B.L.Agapov[6], K.B.Iashkulov[7], T.B.Kaliaeva[7], V.V.Kutyrev[1]

Scientific and Methodological Support of Activities on Carrying Out Immunological Monitoring of Vaccinated Against Plague Persons Residing in the Territories of Natural Foci of the Infection

[1]Russian Research Anti-Plague Institute «Microbe», Saratov, Russian Federation; [2]Irkutsk Research Anti-Plague Institute of Siberia and Far East, Irkutsk, Russian Federation; [3]Rospotrebnadzor Administration in the Republic of Kalmykia, Elista, Russian Federation; [4]Rospotrebnadzor Administration in the Republic of Altai, Gorno-Altaisk, Russian Federation; [5]Altai Plague Control Station, Gorno-Altaisk, Russian Federation; [6]Astrakhan Plague Control Station, Astrakhan, Russian Federation; [7]Elista Plague Control Station, Elista, Russian Federation

The article covers the issues related to the scientific substantiation and methodological support of immunological monitoring of persons vaccinated against plague upon epidemic indications. The problematic issues of the methodology for the assessment of immunological efficiency (efficacy) of plague live vaccine (PLV) are noted. The current tasks and possible prospects for the introduction of immunological monitoring of persons vaccinated against plague upon epidemic indications have been defined. The algorithm of efficacy estimation of plague live vaccine in vaccinated (revaccinated) persons has been tested under real conditions. Analysis of the results of efficacy evaluation of plague live vaccine among vaccinated (revaccinated) people against plague living in the territories of natural foci of this infection has been performed. Demonstrated is the possibility of using immunological monitoring results in creating an objective basis for improving the strategy of specific plague prevention in natural foci of this infection. The priority areas for further optimization of the specific plague prevention in the territories of natural foci of the infection, including those related to the formation of individual regimen revaccination tactics, taking into account the possibilities of creating modern and effective vaccines, are outlined.

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Corresponding author: Svetlana A. Bugorkova, e-mail: rusrapi@microbe.ru.

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11 natural plague foci are located in the territory of the Russian Federation with a total area of more than 221 thousand km^2. Since 2014 plague epizootics have been registered in some regions of Russia (the Republics of Altai, Tuva, Kalmykia, Dagestan, Astrakhan Region) [15]. Over the past three years (2014–2016) the unfavorable epidemiological situation has developed in the territory of Gorny Altai high-mountain natural plague focus, where human cases of bubonic form were recorded [1].

Based on a long-term unfavorable epizootiological forecast on plague for a number of natural foci (Gorny Altai highland, Pre-Caspian sandy, Tuvinian mountainous) and taking into account the data of the current strategic investigation, the organizational work was intensified to ensure the sanitary-epidemiological safety of the population living in these territories. Sanitary regulations (SR 3.1.7.3465-17 "Prevention of plague") provide for enhancement of the measures for specific prevention in the comprehensive set of measures concerning assurance of epidemiological surveillance and prevention of plague.

Vaccination is one of the types of medical intervention and refers to the activities that require significant material costs, since it is arranged for vaccine coverage of wide sections of the population. In this regard, it is important to have an actual understanding of the efficacy of the applied vaccine and of relevance of specific prophylaxis in each particular case.

The legislative framework for vaccination

in our country is based on the Federal Law No. 368-ФЗ dated December 21, 2013 "On Immunoprophylaxis of Infectious Diseases" and the Order of the Ministry of Health of the Russian Federation dated March 21, 2014 No. 125n "On the National Immunization Schedule and the preventive vaccination schedule upon epidemic indications". Immunoprophylaxis is a complex of measures taken to prevent, localize and eliminate infectious diseases through preventive vaccination. According to these documents, the population living in plague enzootic territory is subject to vaccination upon epidemic indications.

Specific prophylaxis of plague in Russia applies dry live plague vaccine – lyophilized live culture of vaccine strain of the plague microbe *Yersinia pestis* EV of the NIIEG line manufactured by the Federal Government Health Institution Stavropol Research Anti-Plague Institute. Vaccination with the live plague vaccine (LPV) is performed via the epicutaneous method, which is characterized as weakly reactogenic. According to the instruction on the use of the LPV, vaccination with this preparation develops a tense immunity with a duration of 6–12 months, and it requires annual revaccination of people.

Many years of experience in the use of LPV shows the efficacy of vaccination, nevertheless, the opinions of specialists around the world on this issue are ambiguous. Most of concerns refer to the possible reversion to virulence of live vaccines [21, 30, 38] and their high reactogenicity [31]. Whole genome sequencing of *Yersinia pestis* EV strain of the NIIEG line detected the presence of an extensive deletion of the *pmg* zone and of the high pathogenicity island HPI, which confirmed the impossibility of its reversion to virulence in the macroorganism [12], and the immunogenicity and safety of the *Y. pestis* EV vaccine strain was defined in experimental studies and on volunteers [10].

More than 10 million people were vaccinated in the USSR, but targeted studies on assess of epidemiological efficacy of vaccination were not conducted in view of the absence of human infection cases. A generalized analysis of the results of vaccination with live EV vaccine (manufactured by "Saigon") of a population of more than 2 million people (2089388) in six provinces of South Vietnam demonstrated that vaccination did not significantly affect the reduction in incidence among vaccinated people, but the course of disease was easier and with less frequent cases of complications in the form of secondary pneumonia [39, 40]. However, according to the data by N.I. Nikolayev [14], in Vanemyao (Inner Mongolia) during the period of aggravation of the epidemiological situation in 1945, the incidence rate in the vaccinated group (0.25 per 1000 people) was reduced 100 times in comparison to not vaccinated (28.8 per 1000 people) as a result of the use of LPV. High efficacy was as well demonstrated in using the *Y. pestis* EV vaccine strain in the Belgian Congo and South Africa [24, 25].

The most accessible way to assess specific immunity in vaccinated individuals is to determine the level of specific antibodies. A commercial ELISA test system for detection of antibodies to the plague microbe (ELISA-Ab-F1 *Yersinia pestis* according to TU 9388-041-01898109-2011) produced by Federal Government Health Institution Russian Research Anti-Plague Institute "Microbe" is used to evaluate the specific anti-plague immunity.

But the presence of specific antibodies to the F1 of plague microbe does not correlate with protection against infection [3, 4, 5, 41], since cellular factors of the immune system play a leading role in the formation of anti-plague immunity [26, 37].

Currently there is no data on the required level of protective cellular reactions of the organism to LPV and as well no data on the correlation of these characteristics with the indices of a specific humoral response. There is no approved standard for evaluation of the level of anti-plague immunity in humans. So-called protective titers (minimum antibody content in the blood) that ensure protection against infection have been identified for a number of vaccine-preventable infections (measles, tetanus, diphtheria, poliomyelitis, hepatitis B). Their detection with the use of certified test systems, allows evaluation of the state of specific protection [13], and the Methodical Instructions MI 3.1.2943-11 "The organization and performance of serological monitoring of the state of collective immunity against controlled infections (diphtheria, tetanus, measles, rubella, mumps, poliomyeli-

tis)" prescribes the procedure for conducting serological monitoring of the persons vaccinated against these infections. There is no single opinion on formation of changes in individual immunological parameters in the development of a specific anti-plague response, there is no information about the required amount of laboratory research, no defined diagnostic value of identification of a number of immunological parameters and the period of evaluation. All the above became the **objective** of this present work.

The cell-mediated anti-plague immune response develops along the dominant Th1 path, which is characterized by the appearance of pathogen-specific T-lymphocytes that synthesize IFN-γ, TNF-α [22, 37]. A special role in enhancing the bactericidal activity of macrophages, besides IFN-γ, TNF-α [29], belongs to IL-17 [20, 27, 33], which has a great importance in formation of mucosal anti-plague immunity [28]. It has been experimentally proved on the models of bubonic and pulmonary forms of plague, that the presence of high titers of antibodies to *Y. pestis* antigens in case of low synthesis activity of cytokines IFN-γ, TNF-α, IL-17 in biomodel animals (inbred mice, great apes and nonhuman primates) does not protect them from death from the plague infection [23, 36].

A methodical approach for indirect evaluation of anti-plague immunity has been defined resulting from a long-term search for biomarkers, indicating the presence of intense specific immunity [32, 42]. The approach is based on the nature of the redistribution of T-and B-lymphocytes and regulatory subpopulations of T-lymphocytes carrying markers: CD3 (common for all T-lymphocytes), CD4 (T-helpers), CD8 (T-killers), CD45/CD4/CD45RA/CD4RO (memory T-cells), CD19 (B-lymphocytes) [2], CD69 (marker of early cell activation) [17, 18] and changes in the spontaneous and induced production of marker Th1- and Th2-cytokines [19].

The studies of many years conducted by the Federal Government Health Institution Russian Research Anti-Plague Institute "Microbe" on investigation of the processes occurring with the immune system cells of persons vaccinated against plague allowed to put forward comprehensive methodological approach to assess the actual immunity of persons vaccinated/revaccinated against plague and the protection properties of anti-plague vaccination. The proposed approach included a sequential evaluation of activity of cellular and humoral elements of innate and adaptive immune systems of individuals vaccinated/revaccinated against plague. That allowed to characterize in blood cell cultures the functional activity of phagocytic cells and lymphocytes by spontaneous and ligand TLR2-induced– conconovaline A (KonA) production of marker cytokines (IFN-γ,TNF-α, IL-4, IL-17) and determine the immunophenotype of lymphocytes ($CD3^+$, $CD4^+$, $CD8^+$, $CD16^+$, $CD19^+$, $CD69^+$, $CD95^+$), and levels of: general IgE, circulating immune complexes, main classes IgG, IgA, IgM and subclasses IgG1, IgG2, titers of specific antibodies to the capsular antigen of plague microbe.

The Federal Government Health Institution Russian Research Anti-Plague Institute "Microbe" also leads an experimental work on the search for antigen-specific cell tests including detection of markers of early (CD45/CD3/CD25) and late (CD45/CD3/HLA-DR) lymphocyte activation to characterize the intensity of anti-plague immunity [11]. A number of authors are working on improvement of the evaluation strategy of post-vaccination immunity against plague and tularemia [7], others point out the effect of frequency of vaccinations with LPV on the dynamics and content of lymphocytes with receptors for *Y. pestis* antigens [6].

In 2016–2017 employees of anti-plague institutions (Federal Government Health Institutions: Russian Research Anti-Plague Institute "Microbe", Irkutsk Research Anti-Plague Institute of Siberia and Far East, Astrakhan Plague Control Station, Elista Plague Control Station, Altai Plague Control Station) carried out a multicenter immunological study in close cooperation with the regional offices and bodies of the Federal Service on Customers' Rights Protection and Human Well-being Surveillance (Rospotrebnadzor Administration in the Republic of Kalmykia, Sanitary and Epidemiological Center in the Republic of Kalmykia, Rospotrebnadzor Administration in the Republic of Altai) and the Ministries of Health of the Republics of Kalmykia and Altai.

The preparatory stage of the work included development of a research program that would

clearly administrate the procedures of collection, transportation and storage of biological material. An essential requirement for this kind of research was the preliminary questioning of people in order to form indicator groups and obtaining of a written consent of people to participate in the study. An authorization for this work was issued by the ethics committee of the FSBEI of Higher Education Saratov State Medical University n.a. V.I. Razumovsky (report No. 5 of 02.02.2016).

Immunological research was performed in the territories of two natural plague foci – the Pre-Caspian sandy (Astrakhan region, Lagan and Black Earth regions of the Kalmykia Republic) and the Gorny Altai highland (Kosh Agach region of the Altai Republic) using a uniform algorithm that includes application of a complex of modern tests, that allow characterization of cell and humoral immunity in response to anti-plague vaccination and immunological protection of LPV. More than 300 vaccinated / revaccinated persons were examined during the reporting period. The LPV manufactured by Federal Government Health Institution Stavropol Research Anti-Plague Institute (batch No 1-15, 12.03.2015–12.03.2018) was used in this project. Vaccination was made by the epicutaneous method at a dose of $3 \cdot 10^9$ m.c. in 0.15 ml in accordance with the instructions for use of the preparation.

This study showed a relative immunological protection of LPV, including the cases of annual use by employees of anti-plague institutions. During the specified timeframe, among more than 17,000 vaccinated people living in the Kosh-Agach district of the Altai Republic, and about 2,000 respondents annually vaccinated in the Republic of Kalmykia, there was not a single case of applying to medical facilities for local or general reactions to epicutaneous vaccination with LPV.

The immunological study of the persons vaccinated/revaccinated with the LPV demonstrated the absence of accumulation of circulating immune complexes (cic) and an increase in the proportion of cells bearing the marker of early apoptosis (CD95). There was also no correlation between another revaccination and the increase in the level of IgE. At the same time, among all the examined persons, in 20 % of cases, a high level of Ige (more than 180 IU/ml) was noted before VPL vaccination. The following vaccination did not significantly effect the change in the IgE concentration in the blood serum of these people and was not accompanied by a leap in the IL-4 index that participates in the launch of its synthesis. Nevertheless, it would be reasonable to designate potential risk groups as regards development of allergic complications of the vaccination among individuals with a high IgE content in serum.

When evaluating the immune status of the vaccinated group based on the cumulative analysis of laboratory parameters that characterize the quantitative and functional activity of the cells of the immune system, it should be taken to consideration that only about 85 % of the vaccinated react to the antigen by activating immunocompetent cells, and in 5–15 % of the vaccinated the immune system either does not significantly respond to vaccination, or may even react with a certain hyper-reactivity. Thus, it is possible that no production of antibodies against the antigen will occur even after multiple vaccinations (primary vaccinal deficiency) due to the absence of class HLA II molecules that recognize specific antigen. At the same time, in the course of multiple vaccinations, the secondary immune response develops quickly, and in a few months, the titers of specific antibodies decrease sharply (secondary vaccinal insufficiency), though the immunity does not necessarily disappear [8]. Therewith, the absence of antibodies does not always indicate a loss of immunity, and on the contrary, the presence of antibodies after vaccination does not guarantee protection against infection.

According to the received data, during the complete follow-up period (one year after vaccination or revaccination), the proportion of people with specific antibody titers above the diagnostic level (1:80) of the ELISA test system for the detection of antibodies to the plague microbe (ELISA-Ab-F1 *Yersinia pestis*) varied from 4 to 85 %. The maximum accumulation of antibodies was recorded 3 months after the initial vaccination or after the first revaccination, and 1 month after the second revaccination with the LPV. Correlation analysis did not determine any reliable interconnection between

the dynamics of antibody titers to fraction 1 (F1) of plague microbe and the changes in the concentration of IgG, IgM and IgA, as well as the indicators of the functional activity of cellular immunity factors. Thus, the stimulation of the humoral response occurred in not less than 85 % of the vaccinated group and remained in the persons during the year of follow-up.

Analysis of the cellular component of immune system was performed based on results of lymphocyte phenotyping and changes in spontaneous and mitogen-induced cytokine production, using only commercial kits and test systems of domestic and foreign production. The most accurate data about the potential functional capabilities of activated cells can be obtained by applying tests for *ex vivo* assessment of induced cytokine production by blood cells [16]. In addition, the use of commercial inductors with characterized properties, for example, concanavalin A – lectin, used in immunology as a specific T-cell mitogen (TLR2 agonist), makes this assessment informative and standardized [9]. The key problem of further enhancement of the specificity of *ex vivo* tests for assessing the functional capability of LPV-activated blood cells is the lack of purified and characterized antigenic preparations of plague microbe of domestic production, the production of which remains a crucial task of our time.

Application of a cytofluorimetric analysis revealed an increase of the average value of the percentage of T-helper cells ($CD4^+$) in the blood and an increase of the immunoregulatory index as the ratio of $CD4^+$ to $CD8^+$ after vaccination/revaccination with LPV within 1 to 6 months period and retention of the immunoregulatory index throughout 12 months of follow-up of vaccinated persons. At the same time, it was noted that people with low immunoregulatory index (less than 1.0) before vaccination had poor reaction to LPV, this reaction was short-term (up to 6 months). Among the examined group, the proportion of such people was about 5 %, which indicates the importance of monitoring the immune status of the population living in plague enzootic territory.

Based on the study of biomarker cytokines, it was concluded that the proportion of individuals with immune response developed predominantly on the cell type increased significantly 6 months after the first revaccination of LPV (50–80 %), reached a maximum before and 1 month after the second revaccination (90–100 %), but decreased to zero 6 months after the second revaccination, remaining at the average level after the following revaccinations. Nevertheless, despite the wide range of tests used to assess the cellular component of the immune system, there was no correlation found between the dynamics of specific antibody titers after regular vaccination (revaccination) and the changes in biomarker cytokines.

During the spontaneous and induced test of nitro blue tetrazolium recovery by phagocytic cells (NBT test) in response to vaccination (revaccination), the increase in the functional activity of blood phagocytes was noted with maintaining high values of this index during the entire observation period.

Thus, the results of immunological monitoring of people vaccinated against plague in two natural foci of plague indicate the activation of the humoral and cellular immunity components in the examined people and the absence of damaging effect of the vaccine on the cells of immune system.

Another key aspect of the study was an explanation of the reasons of detection of certain individual and territorial features in the reaction of residents of the surveyed territories to vaccination.

The reference provision in this matter was the organizational principle of the human organism protection systems, based on the genetic control of the variability of functioning, in particular, of the immune system [34, 35]. Allelic variants of the HLA-DQA1, HLA-DQв1 and HLA-DRB1 class II haplotypes of the main histocompatibility complex among the residents of the Pre-Caspian sandy and Gorny Altai highland natural plague foci were identified as well as a statistically significant association between allelic variants of HLA-DRB1, HLA-DQA1 and HLA-DQB1 and the intensity of production of Th1- and Th2-associated cytokines (INF-γ, TNF-α, IL-4 and IL-10). Further extension of the area of research interests related to the monitoring of the native population inhabiting the territory of the Tuva mountain natural plague focus will enhance the general understanding of the correlation between the characteristics of

the HLA haplotype and the intensity of specific anti-plague immunity.

The actual opportunity of application of the results of immunological monitoring carried out in natural foci of plague is the ability to predict the nature and intensity of the immune response in individuals vaccinated with LPV.

After performance of the evaluation of immunological efficacy (actual vaccinated status) with LPV based on a number of indirect indicators, a question about the efficiency of immunization as a preventive measure remains open. In this aspect, the general mission of the planned long-term immunological monitoring of the population vaccinated against plague inhabiting the territories of natural foci is to determine the "protective level of cellular reactions". The actual opportunity of application of the results of immunological monitoring carried out in natural foci of plague is the ability to predict the nature and intensity of the immune response in individuals vaccinated with LPV.

From methodological point of view, evaluation of the immunological efficacy of anti-plague vaccination can be performed selectively (among various groups of the population living in the territory of natural foci of plague) or targetedly, focusing on indicator groups, or groups of risk (medical personnel; specialized laboratories employees; people engaged in certain activities with the risk of plague infection – hunters, shepherds, etc.). However the major remaining issue is the administrative regulation of the relevancy for immunological monitoring (along with epizootiological and microbiological ones) in the territories of natural foci of plague, as well as its further methodological improvement. Authorization of draft documents on regulatory administration of the procedures of vaccinated people examination (Methodological Recommendations "Evaluating the level of immunity in persons vaccinated (revaccinated) against plague") and the procedure of organizing and conducting their monitoring (Methodical Instructions "Organizing and conducting immunological monitoring of individuals vaccinated against the plague upon epidemic indications") will facilitate coordination of work and standardization of the results of immunological monitoring. This will allow timely adjustments of vaccination according to epidemic indications and improve tactical approaches to the use of LPV in the framework of measures for the specific prevention of plague in natural foci of infection.

Therefore, the priority task of specific prevention of plague is to achieve effective protection of the population inhabiting the territory of natural plague foci for the period of increased epizootic activity. Consequently the need for immunological monitoring in combination with epizootiological and microbiological monitoring performed in plague foci is indubitable. It will certainly allow to bring the evaluation of the epidemiological situation in the natural focus of the plague to a new ecosystem level.

Immunological monitoring of vaccinated people is not only the new knowledge, but also the new opportunities for making management decisions to ensure the sanitary and epidemiological protection of the population living in the territories of natural foci of the plague. It is a source of objective information for further improvement of the strategy for applying LPV and developing individual revaccination tactics at the real incentive to develop modern and effective means of specific prevention of plague.

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Authors:

Bugorkova S.A., Shchukovskaya T.N., Mikshis N.I., Shcherbakova S.A., Kudryavtseva O.M., Kuklev E.V., Kutyrev V.V. Russian Research Anti-Plague Institute "Microbe". 46, Universitetskaya St., Saratov, 410005, Russian Federation. E-mail: rusrapi@microbe.ru.

Dubrovina V.I., Noskov A.K., Korytov K.M., Balakhonov S.V. Irkutsk Research Anti-Plague Institute of Siberia and Far East. 78, Trilissera St., Irkutsk, 664047, Russian Federation. E-mail: adm@chumin.irkutsk.ru.

Sandzhiev D.N., Konusheva S.V., Savchenko S.P., Matsakova G.B. Rospotrebnadzor Administration in the Republic of Kalmykia. 8, Balakaeva St., Elista, 358000, Russian Federation. E-mail: rpnrk1@yandex.ru.

Shchuchinov L.V. Rospotrebnadzor Administration in the Republic of Altai. 173, Kommunistichesky Avenue, Gorno-Altaisk, 649002, Russian Federation. E-mail: rpn_ra@mail.gorny.ru.

Mikhailov E.P. Altai Plague Control Station. 2, Zavodskaya St., Gorno-Altaisk, 649002, Russian Federation. E-mail: chuma@mail.gorny.ru.

Agapov B.L. Astrakhan Plague Control Station. 3, Kubanskaya St., Astrakhan, 414000, Russian Federation. E-mail: antichum@astranet.ru.

Iashkulov K.B., Kaliaeva T.B. Elista Plague Control Station. Elista, 358000, Russian Federation. E-mail: pestis-kalmykia@yandex.ru.

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