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doi: 10.15789/2220-7619-2018-4-5.11

HETEROGENEITY OF POPULATIONS OF THE FLEA *CITELLOPHILUS TESQUORUM ELBRUSENSIS* DETECTED ON THE BASIS OF ANALYSIS OF PROTEOMIC PROFILES BY THE METHOD OF MALDI-TOF MS

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The aim of the work was to analyze proteomic profiles of imago *C. t. elbrusensis*, the basic vector of the causative agent of plague in territory of the Central-Caucasian high-mountain natural focus of plague.

Proteomic profiles of 49 specimens of imago *C. t. elbrusensis* collected in populations of East and North Prielbrusye in June–August, 2017 were analyzed in the course of this work. All parasites were previously characterized by the following signs: sex, state of gastrointestinal tract, generative state of females. Each sample was studied individually by homogenization and extraction of proteins in 80% TFA. Spectra were collected and analyzed on MALDI-TOF mass spectrometer Microflex LT (Bruker, Germany) by using pre-established programs Flex Control V 3.3.5 and Flex Analysis v 3. (Bruker, Germany). The additional analysis of signal frequency and statistical processing were carried out using programs Microbe MS (Lash P., 2016).

The MSP analysis of the dendrogram constructed on the basis of super-spectra (generalized spectra of each sample) on the basis of differences in their protein composition showed clearly that *C. t. elbrusensis* was clustering into two basic geographical groups: a group of East Prielbrusye and a group of North Prielbrusye. At the same time the analysis of proteomic profiles of fleas of each of these groups revealed heterogeneity of protein composition of samples collected from points, most remote from each other in the region of 2–12 000 Da. It makes possible to differentiate some local proteotypes in populations of *C. t. elbrusensis* of basic geographical groups, each of which is characterized by certain frequency of both ribosomal and individual proteins denoting sufficiently long isolation of the given local populations of parasites — vectors of the causative agent of plague, owing to disconnection of settlements of their hosts — mountain sousliks in the conditions of mountain landscape of Prielbrusye.

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doi: 10.15789/2220-7619-2018-4-5.12

THE GRANULOCYTES PHAGOCYtic CAPACITY TO *YERSINIA PESTIS* IN BLOOD SAMPLES OF ANTI-PLAGUE VACCINATED PEOPLE ACCORDING TO FLOW CYTOMETRIC ANALYSIS DATA

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Phagocytosis is the basis of cellular immunity in bacterial infections, but there is currently no information on the phagocytic activity of human blood granulocytes for killed *Yersinia pestis* cells grown at 28°C and the effect of anti-plague vaccination on this indicator. In this work a problem of obtaining such information was solved for the first time with the help of flow-cytometric technology, which allows to evaluate objectively the indices of the phagocytic reaction in whole blood samples without allocation of phagocytes and serum from it. Heat-killed *Y. pestis* (EV NIIEG), *Escherichia coli* (25922ATS) and *Staphylococcus aureus* (209P) cells, stained with FITC, were used in the experiments. Individual phagocytic reaction indicators were

determined in blood samples of people, living in the territory of the Caspian sandy natural plague focus (130 persons), before and one month after anti-plague vaccination with respect to three types of bacteria after 15 min of incubation *in vitro* by the method of Hasui M. et al. (1989), modified by us according to recommendations of White-Owen C. et al. (1992). The results were taken into account on the CyAn ADP™ Dako Cytomation flow cytometer using the Summit v.4.3 Built 2445 software. Against the background of high phagocytic indices for *E. coli* and *S. aureus*, respectively 97.3±0.24 and 98.5±0.13%, in relation to *Y. pestis* were recorded the reduced phagocytic activity of granulocytes 55.6±2.1% in blood samples before vaccination. The phagocytic numbers measured in the FITC fluorescence intensity units for blood granulocytes that absorbed *Y. pestis* cells were on average (Mean) twice lower than for *E. coli* and *S. aureus* at significantly higher coefficients of variation on this parameter (CV = 169±3.7%) in comparison with CV for *E. coli* (68.8±1.6%) and *S. aureus* (66.1±0.9%). A month after the anti-plague vaccination, the blood granulocyte phagocytic activity to *Y. pestis* increased to 82.4±2.8 (p < 0.001), indicating that a new cellular test for anti-plague immunity evaluation in humans may be developed on the basis of the rapid whole blood granulocyte phagocytic activity to *Y. pestis* cells determination *in vitro*.

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doi: 10.15789/2220-7619-2018-4-5.13

ANTI-PLAGUE VACCINATION STIMULATES THE NEUTROPHIL EXTRACELLULAR TRAPS FORMATION TO INCREASE THE *YERSINIA PESTIS* KILLING EFFICIENCY *IN VIVO*

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Neutrophil extracellular traps (NETs) formation is a recently described anti-microbial mechanism of neutrophils which involves the release of chromatin decorated with granular proteins in order to bind extracellularly and kill microorganisms. However, the role of NETs in anti-plague immunity is unknown. Our aim was to show that NETs participate in *Yersinia pestis* killing and significantly increase the bacterial clearance *in vivo*, when post-vaccination anti-plague immunity in mice is created. BALB/c mice were immunized subcutaneously by protective dose of live *Y. pestis* EV NIIEG cells (2.5×10^4) and results were recorded on the 21st day after vaccination. Contribution of NETs to bacterial killing was determined by intraperitoneal (i.p.) inoculation of 150 U/mouse micrococcal nuclease (MCN) or EDTA-inactivated MCN to vaccinated and control mice 10 min before i.p. challenge of 10^8 live *Y. pestis* EV cells, grown 48 h at 28°C. After 4 h, animals were killed and the collected peritoneal lavage (PL) were seeded on polylysine pretreated coverslips, where the percentage of NET-forming neutrophils (NFN) were determined by fluorescence microscopy using DNA staining with propidium iodide. Colony-forming units (CFU) in PL were evaluated using Hottinger agar after 72 h of bacterial grown at 28°C. Phagocytic capacity of neutrophils to i.p. injected FITC-labeled *Y. pestis* cells was measured in PL samples by flow cytometry. Vaccination stimulated NETs formation in response to live *Y. pestis* cells (from control NFN values 8.3±0.9 to 41.5±2.3%, p < 0.001 for n = 6) and this accompanied the increased bacterial killing, reflected in 10-fold decreasing of CFU in PL of vaccinated animals, against the background of the absence