THE CANDIDATE ANTI-TUBERCULOSIS mRNA VACCINE IMMUNOGENICITY AND REACTOGENICITY DEPENDENCY ON THE ANIMAL'S SEX AND THE VACCINE DOSE

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mRNA vaccines turned out to be highly effective in combating the COVID-19 pandemic and other viral infections. Despite extensive study of mRNA vaccines in the last five years, the issue of safety of their use is still relevant. The study aimed to assess immunogenicity of two anti-tuberculosis mRNA vaccine doses in female and male rats 2 and 4 weeks after vaccination. Hematological and biochemical parameters of blood were determined within the same timeframe. The dose-dependent nature of mRNA vaccine immunogenicity was confirmed in both females and males. Vaccination led to moderate lymphopenia and neutrophilia in male rats, as well as to apparent dose-dependent and sex-related changes in blood biochemistry parameters at various time points. The findings suggest moderate toxicity of the anti-tuberculosis mRNA vaccine and the importance of assessing its toxic effects at various time points in animals of both sexes.

Keywords: mRNA vaccine, tuberculosis, immunogenicity, toxicity

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ЗАВИСИМОСТЬ ИММУНОГЕННОСТИ И РЕАКТОГЕННОСТИ КАНДИДАТНОЙ мРНК-ВАКЦИНЫ ПРОТИВ ТУБЕРКУЛЕЗА ОТ ПОЛА ЖИВОТНЫХ И ДОЗЫ ПРЕПАРАТА

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мРНК-вакцины показали высокую эффективность в борьбе с пандемией COVID-19 и другими вирусными инфекциями. Несмотря на интенсивное изучение мРНК-вакцин в последние пять лет, вопрос о безопасности их применения все еще остается актуальным. Целью работы было оценить иммуногенность противотуберкулезной мРНК вакцины в двух дозах у самок и самцов крыс через 2 и 4 недели после вакцинации. В эти же сроки определяли гематологические и биохимические показатели крови. Подтверждена дозозависимость иммуногенности мРНК вакцин как у самок, так и у самцов. Вакцинация привела к умеренной лимфоцитопении и нейтрофилии у самцов крыс, а также к выраженным дозо- и гендерзависимым изменениям в биохимических параметрах крови в различных временных точках. Полученные результаты свидетельствуют об умеренной токсичности противотуберкулезной мРНК вакцины и важности исследования ее токсических действий в различных временных точках у животных обоих полов.

Ключевые слова: мРНК-вакцина, туберкулез, иммуногенность, токсичность

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Currently, new mRNA vaccines against various viral and bacterial infections, including tuberculosis, the previously developed live, attenuated, recombinant, and DNA vaccines against which have not shown acceptable effectiveness, are being actively developed [1, 2]. However, there are many gaps in our understanding of the biology of mRNA vaccines delivered using lipid nanoparticles (LNPs). It is well known, that the mRNA-LNPs show broad biodistribution, are found in most tissues [3, 4], cause inflammatory response at the injection site, and can be associated with neuroinflammation [5]. There are plenty of reports that the use of mRNA-based vaccines is sometimes associated with allergy, myocarditis, and pulmonary hemorrhage [6]. In some cases, vaccination with the mRNA-LNPs leads to the delayed transcriptome alterations and moderate CpG island methylation level changes in peripheral blood monocytes [7]. Furthermore, there is insufficient information about the biodistribution and clearance time of mRNA vaccines and antigens encoded by these vaccines [4].

The pre-clinical trials of the RNA-1273 and BNT162b2 vaccines against COVID-19 involving rats showed a moderate decrease in the animals' body weight and the body temperature increase in the first 24 h after vaccination. Pro-inflammatory hematological and biochemical alterations, as well as the cytokine level increase were also revealed [8, 9]. Macroscopic alterations included the increase in the weight of the spleen, liver, and adrenal glands, while microscopic alterations included moderate inflammation at the injection site, in the groin, in the iliac and popliteal lymph nodes, along with the signs of inflammation in the liver and spleen. All alterations were dose-dependent, the majority of indicators were back to normal 2–3 weeks after administration of the last vaccine dose [8, 9]. Clinical trials of the RNA-1273, BNT162b2, and CS-2034 mRNA vaccines [10–12] also showed the dose-dependent, local irritant, and systemic adverse effects, including inflammation at the injection site, fatigue, headache, fever, muscular and joint pain, changes in the hematological and biochemical parameters of blood. The mRNA vaccine adverse effects can be associated with both active substance of the vaccine, the mRNA molecule, and the antigens encoded by this molecule or lipid nanoparticles, in which RNA is encapsulated.

We have previously shown that the mRNA vaccine against tuberculosis we have developed yields adaptive and protective immune responses [13]. However, safety of its use was poorly understood. The study aimed to assess toxic effects of two anti-tuberculosis mRNA vaccine doses based on the hematological and biochemical parameters of blood in male and female rats 2 and 4 weeks after vaccination.

METHODS

Animals

The experiment involved 30 female (126–149 g) and 30 male (154–180 g) SPF Wistar rats aged 8–12 weeks. The tests were performed at the breeding nursery of the National Research Centre "Kurchatov Institute"; the animals were kept under the SPF conditions with the fixed 12.00 : 12.00 h light/dark cycle and ad libitum access to food and water.

Experimental design

Three experimental groups, 10 males and 10 females per group, were formed for the study:

group I: the group administered the MTB-mEp5-1 mRNA vaccine in a dose of 5 μg/animal;

group II: the group administered the MTB-mEp5-1 mRNA vaccine in a dose of 15 μg/animal;

group III: the group administered the phosphate buffered saline (PBS).

The mRNA vaccine or phosphate buffered saline was administered to the experimental animals twice with a 14-day interval: 200 μL intramuscularly into the thigh using a 3-piece insulin syringe with the 26G needle (Fig. 1A). The animals were euthanized using the tiletamine-zolazepam plus xylazine anesthesia in a dose of 15 mg/kg and 6 mg/kg, respectively, with subsequent drainage of blood from the heart cavities and collection of the spleen and inguinal lymph nodes. Five males and five females per group were euthanized on day 16 of the experiment (24 h after administration of the second vaccine dose), other animals were euthanized on day 29 (two weeks after administration of the second vaccine dose). After euthanasia, blood was collected from the heart cavities of all animals for further clinical and biochemical testing. Furthermore, inguinal lymph nodes and the spleen were collected to access the mRNA vaccine immunogenicity.

The mRNA vaccine doses used correspond to the dose of the CVnCoV mRNA vaccine against COVID-19 that is currently undergoing clinical trials [NCT04652102]. MTB-mEp5-1 is a vaccine with the unmodified nucleotide composition (without any uridine analogues). The dose of the CVnCoV mRNA vaccine corresponds to 12 μg. The doses of the tested candidate vaccine we have selected (5 and 15 μg) are similar to the doses used in the clinical trial, but not adjusted to the animal's weight.

MTB-mEp5-1 vaccine

The MTB-mEp5-1 multi-epitope mRNA vaccine matches the 5′-TPL-mEpitope-mRNA1273-3′ vaccine that has been reported before, with minor modifications [13]. During *in vitro* transcription, m7G(3'OMe)pppA(2'OMe)pG (Biolabmix; Russia) at a concentration of 2.4 mm was used as a cap analogue. One more adenine was added to the plasmid DNA sequence after the T7 promoter sequence in order to ensure specific insertion of this cap analogue during the co-transcriptional capping. The MTB-mEp5-1 mRNA sequence is provided in the Appendix 1.

The other previously reported [13] stages of the mRNA vaccine development remained unchanged. mRNA was formulated into lipid nanoparticles using the microfluid cartridge in the NanoAssemblr™ Benchtop system (Precision NanoSystems Inc.; Canada). Particles were concentrated and sterilized using the PES membrane filter with the 0.22 μm pore size. The lipid nanoparticles were stored at +4 °C for no longer than 3 weeks before administration of the vaccine.

Analytical characterization of the particles obtained was performed as previously reported [13]; it included estimation of the particle size, polydispersity index, zeta potential, mRNA encapsulation percentage and integrity. Particle size was within the range of 86–88 nm in all experimental groups, zeta potential was between –3 and –2 mV, polydispersity index did not exceed 0.1. The mRNA encapsulation percentage exceeded 90%, and RNA integrity was above 85% according to the capillary electrophoresis data.

Blood hematology and biochemistry tests

Blood was collected from the heart cavities into the test tubes containing anticoagulant (K3-EDTA) for hematology testing; the DIATRON Abacus Junior 22.5 hematology analyzer was used for testing. Red blood cell counts, hemoglobin levels, hematocrit, white blood cell counts, platelet counts, and white blood cell differential were determined in the whole blood samples.

Blood samples were collected into the separator gel test tubes and centrifuged at 3000 g to obtain serum for further blood biochemistry testing. Serum concentrations of alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea, alkaline phosphatase, total bilirubin, total protein, glucose were determined using the Architect c8000 chemistry analyzer (Abbott; USA).

Determination of the number of IFNγ-producing cells

The protective T-cell immune response level was estimated based on the number of cells extracted from the spleen and inguinal lymph nodes that secreted IFNγ in response to stimulation with the mycobacterial antigens (*M. tuberculosis* sonicate, 10 μg/mL) or ESAT6 recombinant protein at a concentration of 10 μg/mL (the ESAT6 production method was reported earlier [14]) by the ELISPOT method using the Mouse IFNγ ELISpot Set (BD; USA) and AEC Substrate Set (BD; USA) in accordance with the manufacturer's guidelines.

Statistical analysis

All data were tested for normality using the Shapiro–Wilk (W) test. The median and interquartile range (Q_{25}–Q_{75}) were calculated; the intergroup comparisons (post hoc analysis) were carried out using the Tukey`s test. One-way analysis of variance (ANOVA) was used for comprehensive assessment of the data of independent groups at the control points. The results were considered significant at $p < 0.05$. Statistical analysis was performed using the Statistica 6.0 software (StatSoft; USA).

RESULTS

Immunogenicity assessment

We assessed the effects of the vaccine dose on the adaptive immune response formation in female and male rats at two time points. A significant impact of immunization on the levels of IFNγ-producing cells (IPCs) in the spleen and the inguinal lymph node after specific stimulation was revealed (Fig. 1B). However, the number of IPCs in the spleen was significantly higher. Large differences in the IPC number per well depending on the specific stimulation type (ESAT6 protein or *M.tb* sonicate) were also reported for both lymph node cells and cells of the spleen at both time points. It is most likely that these effects are explained by different target antigen concentrations in the formulation used for specific stimulation.

Estimation of the adaptive immune response dynamics showed that the most prominent response was observed on day 29 of the experiment, two weeks after the second vaccination, which is in line with the literature data.

The dose-dependent effects were observed after stimulation with ESAT6: IPC levels in the lymph node cells were higher in group I administered the dose of 5 μg, than in group II on day 16 of the experiment, and on day 29 of the experiment IPC levels in the cells of the spleen were almost twice higher in group II, than in group I. No significant differences in the adaptive immune response levels between males and females were observed ($p > 0.05$).

Thus, our findings suggest that administration of two MTB-mEp5-1 mRNA vaccine doses to rats results in the

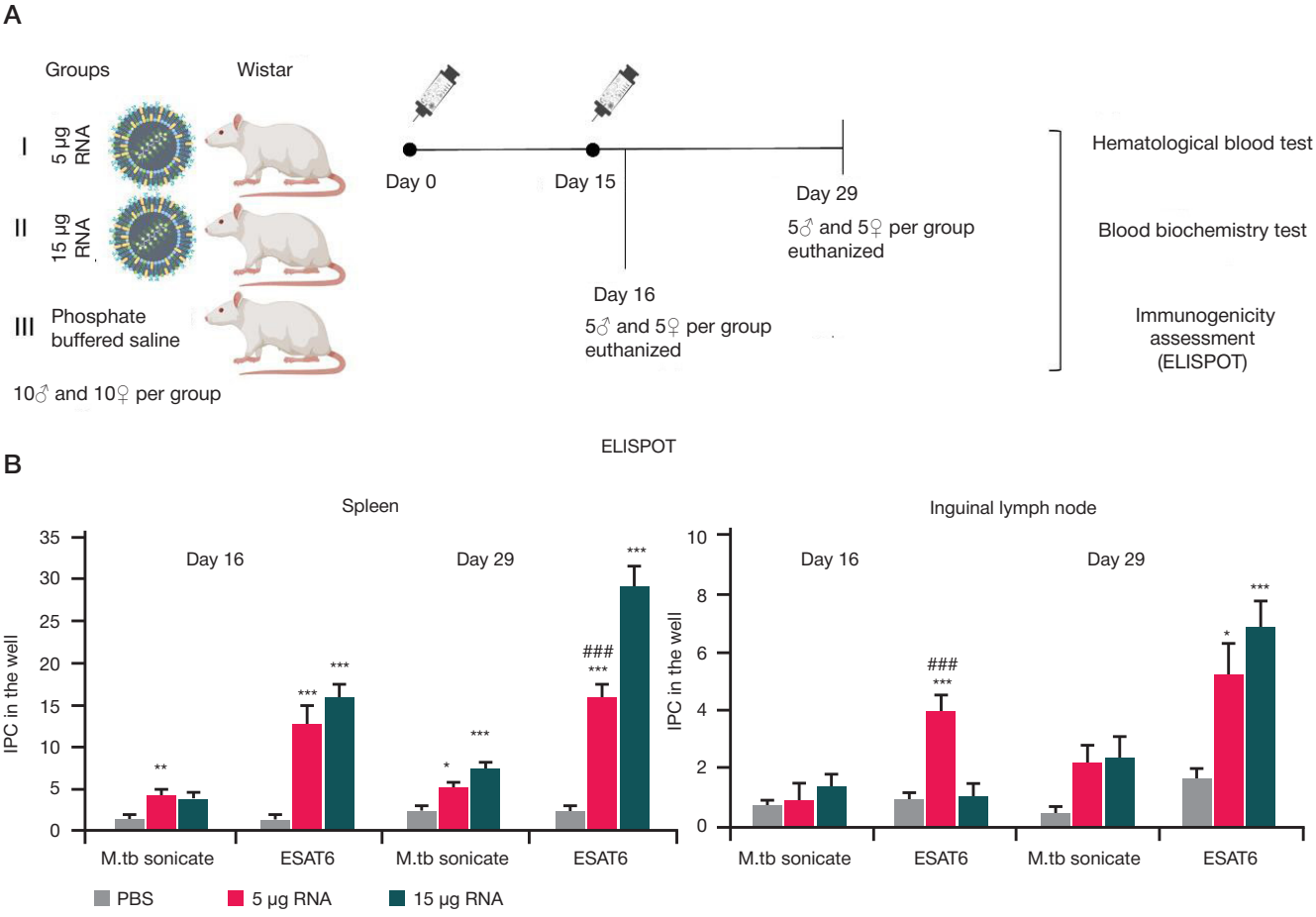

Fig. 1. mRNA vaccine immunogenicity assessment. **A**. Experimental design. **B**. ELISPOT analysis data for cells of the spleen and inguinal lymph nodes. IPC – IFNγ-producing cells. The data are provided as mean ± error of the mean. * — $p < 0.05$; ** — $p < 0.01$; *** — $p < 0.001$ compared to appropriate control group (PBS); ### — $p < 0.001$ compared to the group administered 15 μg of RNA

Table 1. Hematological parameters of blood

Group	Sex		WBC 10^9/L	Neu%	Lym%	Mon%	Eos%	Bas%	RBC 10^{12}/L	HGB g/L	HCT	PLT 10^{12}/L
Day 16 of the study												
Phosphate buffered saline (PBS)	♂	Median	5.86	24.9	67.9	5.9	1.1	0.1	6.3	146	38.4	684
		Q25–Q75	5.74–5.87	24 25.4	67.5–68.9	4.6–6.5	1–1.5	0.1–0.1	6.1–6.4	144–146	38.2–38.4	678–936
	♀	Median	4.06	21.1	72.5	4.6	2	0.1	6.03	137	36.7	1197
		Q25–Q75	3.67–4.45	20.0–21.6	71.7–72.5	3.9–4.6	1.1–2.6	0.00–0.10	5.99–6.33	135–144	36.5–38.9	925–1311
MTB–mEp5-1 5 μg	♂	Median	5.78	33.6	57.6	6.3	1.4	0.2	6	137	36.3	1054
		Q25–Q75	5.55–6.44	29.4–34.5	55.9–63.6	5.7–6.3	1.3–1.4	0.1–0.2	5.9–6.1	136–137	35.9–37.2	987–1079
	♀	MMedian	5.01	32.3	59.5	6.4	1.4	0.1	6.1	138	37.2	990
		Q25–Q75	4.6–5.63	28.4–37.4	54.5–68.0	4.3–6.5	1.2–1.5	0.10–0.20	5.99–6.20	138–139	37.1–38.1	951–1011
MTB-mEp5-1 15 μg	♂	Median	4.69	51.1*	38.8*	7.3	1.5	0.2	5.9	132	35	978
		Q25–Q75	3.67–6.19	48.6–54.9	38–42.4	5.6–8.7	1.3–1.6	0.1–0.2	5.8–6.0	131 134	34.2–35.3	908–985
	♀	Median	3.51	31.1	64	4.6	1.7	0.1	6.07	139	38	867
		Q25–Q75	2.87–4.29	26.4–35.7	56.3–66.6	3.9–4.8	1.6–2.1	0.10–0.10	6.07–6.21	139–145	37.5–38.2	845–906
Day 29 of the study												
Phosphate buffered saline (PBS)	♂	Median	5.7	42.2	51	5.1	1.5	0.1	6.39	143	36.5	780
		Q25–Q75	5.63–6.03	37.3–44.1	48.9–56.5	4.6–5.6	1.2–1.6	0.00–0.10	6.34–6.45	141–147	35.7–36.7	774–850
	♀	Median	3.39	31.3	61.3	5.1	1.7	0.1	6.38	139	36.4	734
		Q25–Q75	3.02–3.44	30.8–32.7	60.7–61.3	4.5–5.3	1.4–2	0.1–0.2	6.16–6.44	139–141	36.2–36.5	696–781
MTB-mEp5-1 5 μg	♂	Median	6.38	25	69.6	4.2	1.2	0.1	6.58	142	37.5	738
		Q25–Q75	6.13–7.56	24.8–25.5	65.2–69.7	4.1–5.3	1.1–1.3	0–0.1	6.45–6.92	142–148	37–40.4	711–796
	♀	Median	4.1	22.9	70.7	4.5	1.4	0.1	6.91	156	39.9	780
		Q25–Q75	3.88–4.71	22.7–25.4	69.5–71.7	4–4.8	1.2–1.6	0.1–0.2	6.76–7.01	151–156	39.3–40.3	737–852
MTB-mEp5-1 15 μg	♂	Median	6.9	26.2	66.1	5	1.1	0.1	6.53	145	36.8	797
		Q25–Q75	6.19–7.41	25.9–27.6	65.5–67.4	4.6–5.8	0.9–1.3	0.1–0.2	6.42–6.66	143–149	36.6–38.1	791–845
	♀	Median	5.05	23.1	72.6	4.2	1	0.1	6.18	135	35.2	834
		Q25–Q75	4.41–6.49	22.1–24.9	69–74.2	1.7–4.6	0.9–1.1	0–0.1	6.15–6.19	135–136	34.9–35.6	782–922

Note: the data are presented as median and interquartile range (Q_{25}–Q_{75}). The data on the absolute lymphocyte and neutrophil counts are not provided, but significance of intergroup differences is the same when comparing absolute values. * — the difference from control values is significant at the selected significance level ($p < 0.05$). WBC (white blood cells) — white blood cell count; Neu% — relative neutrophil count; Lym% — relative lymphocyte count; Mon% — relative monocyte count; Eos% — relative eosinophil count; Bas% — relative basophil count; RBC (red blood cells) — absolute red blood cell count; HGB (hemoglobin) — hemoglobin concentration; HCT — hematocrit; PLT (platelets) — absolute platelet count.

development of adaptive immune response detected at two studied time points, and the response level depends on the dose, not on the animal's sex

Evaluation of hematological and biochemical alterations in blood

We found no significant effect of vaccination on the studied experimental animals' blood parameters. No significant changes were observed in females of all experimental groups at both time points (Table 1). Only males immunized with the MTB-mEp5-1 vaccine dose of 15 μg showed a significant decrease in the median absolute (1.97×10^9/L vs. 3.78×10^9/L in the PBS group) and relative lymphocyte counts (lymphopenia), along with the increase in the median absolute (2.64×10^9/L vs. 0.89×10^9/L in the PBS group) and relative neutrophil counts (neutrophilia) on day 16 of the study. However, these parameters were back to normal two weeks after administration of the second vaccine dose, on day 29 of the experiment.

When performing analysis of the main blood biochemistry indicators, significant blood biochemistry alterations were reported in the experimental animals receiving the test formulation on days 16 and 29 of the study. In female rats, vaccination affected blood levels of ALT, AST, and total protein on day 16 of the experiment (ANOVA: $F(2.12) = 4.03$, $p = 0.046$; $F(2.12) = 8.58$, $p = 0.005$; $F(2.12) = 9.05$, $p = 0.004$, respectively). Females immunized with the MTB-mEp5-1 dose of 15 μg showed a significant increase in blood levels of ALT and AST, along with the decrease in total protein levels. Females immunized with the MTB-mEp5-1 dose of 5 μg showed the increase in AST levels only. At the same time, no such blood biochemistry indicator changes were found in males.

On day 29 of the experiment, in female rats of the experimental groups, alterations of most biochemical parameters were leveled, except for ALT [ANOVA: $F(2.12) = 14.12$, $p = 0.001$], the levels of which were still high in the MTB-mEp5-1 15 μg group. On the contrary, in males, vaccination led to alteration of most blood biochemistry parameters on day 29 of the experiment: ALT ($F(2.12) = 7.65$, $p = 0.007$], AST ($F(2.12) = 8.13$, $p = 0.006$), urea ($F(2.12) = 6.5$, $p = 0.012$], and total protein levels ($F(2.12) = 5.2$, $p = 0.024$]. Our findings showed that the urea, ALT and AST levels were elevated in both experimental groups, regardless of the mRNA vaccine dose. At the same time, the administered mRNA vaccine dose had a great effect on the increase/decrease in total protein levels reported for the experimental groups.

Thus, immunization with the MTB-mEp5-1 mRNA vaccine led to apparent blood biochemistry alterations depending on the animals' sex and the vaccine dose at different time points in female and male rats. At the same time, vaccination with MTB-mEp5-1 had a moderate effect on hematological parameters of blood (Table 2).

Table 2. Biochemical parameters of blood

Group	Sex	Assessed indicators						
		ALT, U/L	AST, U/L	Urea, mM/L	Alkaline phosphatase, U/L	Total bilirubin, μM/L	Total proteing/L	Glucose, mM/L
Day 16 of the study								
Phosphate buffered saline (PBS)	♂	78; 66–80	72; 69–94	4,6; 4,3–5,1	353; 339–362	18,9; 16,6–21,1	59; 58–62	4,9; 4,8–5,0
	♀	73; 71–90	66; 64–73	5,0; 4,8–5,3	382; 350–402	17,1; 15,5–20,3	61; 59–66	4,7; 4,7–5,0
MTB-mEp5-1 5 μg	♂	78; 75–101	82; 70–92	4,8; 4,1–5,2	381; 377–420	18,0; 16,6–19,4	54; 51–59	5,0; 4,9–5,2
	♀	82; 70–96	92*; 65–95	4,4; 4,4–4,7	390; 382–408	18,0; 15,2–18,4	57; 55–59	4,3; 4,1–4,5
MTB-mEp5-1 15 μg	♂	99; 81–114	98; 87–110	4,9; 4,9–4,9	364; 361–400	18,8; 17,2–20,0	53; 51–54	4,6; 4,2–4,8
	♀	104*; 93–110	106*; 101–111	5,1; 4,0–5,2	401; 385–414	18,0; 16,2–18,9	50*; 49–52	4,3; 4,3–4,5
Day 29 of the study								
Phosphate buffered saline (PBS)	♂	73; 69–77	78; 71–80	4,2; 4,2–4,5	370; 364–402	19,5; 17,5–19,5	57; 55–60	5,0; 4,8–5,1
	♀	77; 75–78	83; 75–83	4,8; 4,5–4,9	361; 351–380	18,3; 18,2–19,1	56; 54–57	5,0; 5,0–5,3
MTB-mEp5-1 5 μg	♂	85*; 80–88	96*; 90–97	5,2*; 5,0–5,4	401; 400–414	20,4; 19,2–20,5	55; 53–55	5,1; 4,9–5,5
	♀	82; 82–90	90; 83–101	5,2; 4,4–5,3	388; 383–397	20,1; 19,0–21,0	52; 50–53	4,3; 4,2–4,7
MTB-mEp5-1 15 μg	♂	99*; 92–101	101*; 93–107	5,3*; 5,1–5,6	387; 385–410	20,0; 18,3–20,6	52*; 50–52	5,0; 4,1–5,0
	♀	105*; 96–110	102; 97–114	5,2; 4,8–5,5	415; 402–416	20,9; 19,7–21,1	50; 50–52	4,9; 4,8–5,2

Note: the data are presented as median and interquartile range (Q_{25}–Q_{75}). * — the difference from control values is significant at the selected significance level ($p < 0.05$)

DISCUSSION

Vaccination often leads to side effects of inflammatory genesis that can include pain, redness or swelling at the injection site, as well as to systemic symptoms, such as body temperature increase and altered cellular composition of blood [15]. mRNA vaccines have immunostimulatory properties due to both RNA molecule itself and the lipid nanoparticle components [16]. When entering the cell, RNA can be recognized by intracellular receptors, including toll-like receptors 3 and 7, which results in activation of the innate immunity signaling pathways [16, 17].

We have shown that male rats demonstrate lymphocytopenia and neutrophilia that level out two weeks after the mRNA vaccine administration 24 h after the second injection of the larger vaccine dose. These data are consistent with the data of pre-clinical trials of other mRNA vaccine formulations, such as RNA-1273, BNT162b2 [8 ,9], in which elevated blood cytokine levels, elevated white blood cell, neutrophil, and eosinophil counts, decreased lymphocyte counts were observed in the first 24 h after injection. It is well known that moderate inflammatory response is essential for adaptive immunity formation. The recent study [18] has also shown that the more prominent inflammatory responses to vaccine administration correlate with the development of stronger adaptive immune response. In our study, administration of the elevated vaccine dose (15 μg) resulted in the more prominent adaptive immune response, along with the altered cellular composition of blood.

In contrast to the short-term changes in hematological parameters, biochemistry alterations associated with inflammation were more stable. According to our data, females had elevated ALT levels even two weeks after administration of the second vaccine dose, while males had elevated ALT, AST, urea levels and decreased total protein levels. Alterations of these biochemical parameters can be associated with the liver and kidney function impairment. We conducted histological assessment of the animal's organs (data not shown) and found microscopic signs of inflammation in the liver (dose-dependent progression of the productive vasculitis signs: vessel wall thickening, lymphocyte infiltration, focal accumulation of lymphocytes and macrophages). At the same time, no microscopic signs of inflammation were found in the kidneys.

Blood biochemistry alterations and microscopic alterations in the liver were also reported in the pre-clinical trials of mRNA vaccines conducted by BioNTech and Moderna [8, 9]. In these trials some animals also showed elevated levels of AST, urea, alkaline phosphatase, triglycerides, cholesterol, bilirubin, along with the decreased total protein levels. These changes were accompanied by structural alterations of the liver represented by the increase in organ weight, hepatocyte vacuolation, Kupffer cell hypertrophy, single cell necrosis or hepatocyte degeneration. However, the authors do not specify, when biochemical and histological indicators return to normal after vaccination, in their pre-clinical report.

In the pre-clinical trial of the BNT162b2 mRNA vaccine, the authors assume that hepatocyte vacuolation can be associated with specific accumulation of the ALC-0315 ionizable lipids [9]. Other lipid components, such as SM102, ALC-0159 or PEG2000-DMG, can also be toxic at high doses, however, the much lower doses used in the vaccine are supposed to have no toxic effects [8, 9, 19].

In our studies, we also used lipids ALC-0315, SM-102, which can activate the innate immunity response, as earlier

reported [16]. The use of unmodified uridine in the RNA sequence represents a significant MTB-mEp5-1 vaccine difference from the RNA-1273 and BNT162b2 vaccines. The unmodified uridine is a potent innate immunity stimulator, in contrast to N1-methylpseudouridine used in the RNA-1273 and BNT162b2 vaccines [16, 17].

It is most likely that excessive innate immunity activation due to both unmodified RNA and lipid components can result in the more severe and prolonged inflammation. It should be noted that excessive immune activation capable of causing liver tissue damage can be accompanied by changes in biochemical parameters of blood. However, when using various vaccines, including RNA-1273 and BNT162b2, such extremely rare cases (one per 14 million cases) are associated with autoimmune processes [20].

The differences in the strength of the MTB-mEp5-1 vaccination effect on biochemical parameters of blood in females and males are worth special attention. Our findings show that the differences in biochemical parameters of blood 24 h after the booster mRNA vaccine dose were detected in females only. At the same time, the more pronounced alterations were detected in males two weeks after the second vaccine dose. Such differences can result from the differences in physiological vaccine concentrations related to the animals' weight (the weight of males was 15–20% higher) and other physiological features (sex hormone levels), genetic differences (including X-linked gene expression) [21]. The differences in the sex steroid hormone levels affect the immune cell functioning, which leads to differences in the immune response activity [21]. In particular, the clinical trial results suggest that women show more intense antibody production and more pronounced cellular response after vaccination [21, 22]. Vaccination of women with the mRNA-based and other vaccines results in more frequent side effects, such as body temperature increase, pain, and local inflammation [22, 23]. Thus, the literature data on the gender-specific vaccination effects are consistent with our results showing that the MTB-mEp5-1 administration causes faster changes in biochemical parameters of blood in females. These changes are likely to be associated with the higher female rats' immune system responsiveness to the mRNA vaccine components.

CONCLUSIONS

Our findings suggest high reactogenicity of the unmodified RNA-based MTB-mEp5-1 vaccine. Signs of inflammation in the studied organs and steady changes in the animals' blood biochemistry test parameters provide the basis for the extended study of the tested formulation safety and the mechanisms underlying its specific pharmacological activity.

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