# IMMUNOLOGICAL MEMORY FORMED IN RESPONSE TO ADMINISTRATION OF GamTBvac RECOMBINANT TUBERCULOSIS VACCINE CANDIDATE: CLINICAL TRIALS IN HEALTHY VOLUNTEERS

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So far BCG, a live attenuated *Mycobacterium bovis* strain remains the only available vaccine for tuberculosis prevention and control. Although BCG is effective against miliary tuberculosis and tuberculous meningitis in children, it barely protects adults and adolescents from the pulmonary form of the disease or reactivation of the latent infection. Still, its effectiveness can be increased by using recombinant booster vaccines containing both *M. bovis* and *M. tuberculosis* antigens. This article reports preliminary data on the safety and immunogenicity of a recombinant vaccine candidate, GamTBvac, developed for tuberculosis prevention. Its immunogenicity was studied in 12 volunteers. Over the course of 20 weeks following GamTBvac administration, we measured cell-mediated and humoral immune responses using interferon-gamma release assays and multiplex xMAP-based immunoassays. On day 140 after the first administration of the vaccine, 10 participants of the study (83 %) still showed a positive cellular response to all antigens contained in the vaccine. Both sense antigens CFP10 and ESAT6 induced production of IgG antibodies between days 98 and 140 of the observation. The Ag85 antigen induced a relatively weak humoral response. On the whole, the recombinant GamTBvac is safe and activates cell-mediated and humoral components of the adaptive immunity, forming immunological memory.

**Keywords:** immunological memory, humoral immunity, cell-mediated immunity, tuberculosis, BCG, *M. bovis*, *M. tuberculosis*, IGRA, suspension immunoassay, ESAT6, CFP10, Ag85

**Funding:** this work was supported by the Russian Ministry of Health and the Federal Agency for Scientific Organizations (state task no. 0574-2014-0023)

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**Received:** 20.09.2017 **Accepted:** 20.10.2017

# ИММУНОЛОГИЧЕСКАЯ ПАМЯТЬ, ФОРМИРУЕМАЯ В ОТВЕТ НА ВАКЦИНАЦИЮ ПРОТИВОТУБЕРКУЛЕЗНОЙ РЕКОМБИНАНТНОЙ ВАКЦИНОЙ «ГамТБвак»: КЛИНИЧЕСКИЕ ИССЛЕДОВАНИЯ ВАКЦИНЫ НА ЗДОРОВЫХ ДОБРОВОЛЬЦАХ

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В настоящее время единственной применяемой в мире вакциной против туберкулеза является БЦЖ — живой аттенуированный штамм *Mycobacterium bovis*. Она защищает детей от милиарного туберкулеза и туберкулезного менингита, но не уберегает взрослых от легочного туберкулеза и реактивации латентной формы инфекции. Для повышения эффективности вакцины БЦЖ у взрослых и подростков разрабатываются рекомбинантные бустерные вакцины, несущие антигены как *M. bovis*, так и *M. tuberculosis*. В статье приводятся первые данные о безопасности и иммуногенности кандидатной рекомбинантной вакцины для профилактики туберкулеза «ГамТБвак». Изучали иммуногенность препарата на 12 добровольцах. Оценку проводили по изменению у испытуемых параметров клеточного и гуморального иммунитета (методами IGRA-тест и мультиплексный иммунологический xMAP анализ соответственно) в течение 20 нед. после введения препарата. На 140-й день с момента первой вакцинации у 10 (83 %) из 12 иммунизированных «ГамТБвак» добровольцев сохранялся положительный клеточный ответ на все антигены, входящие в состав вакцины, по сравнению с уровнем до вакцинации. Оба смысловых антигена CFP10 и ESAT6 индуцировали достоверную выработку IgG антител с 98-го и 140-го дней наблюдения соответственно. Антиген Ag85A вызывал сравнительно низкий гуморальный ответ. В целом, изучаемая рекомбинантная вакцина «ГамТБвак» обладает необходимым уровнем безопасности и активирует клеточное и гуморальное звенья адаптивного иммунитета, формирует клеточную память.

**Ключевые слова:** иммунологическая память, гуморальный иммунитет, клеточный иммунитет, туберкулез, БЦЖ, *M. bovis*, *M. tuberculosis*, IGRA, суспензионный иммунологический анализ, ESAT6, CFP10, Ag85

**Финансирование:** работа выполнена при поддержке Министерства здравоохранения РФ и ФАНО России (государственное задание № 0574-2014-0023).

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**Статья получена:** 20.09.2017 **Статья принята к печати:** 20.10.2017

According to the estimates in [1], tuberculosis has killed over 1 billion people in the past 200 years — more than smallpox, plague, malaria, influenza, cholera and AIDS combined. By the end of the 19th century, one in every five deaths was tuberculosis-related [1]. The situation improved after the BCG (Bacillus Calmette–Guérin) vaccine was introduced in the 1920s. It was hoped that tuberculosis would be completely eradicated, given a drastic decrease in incidence rates in Europe and the US [2]. But that did not happen, largely due to the properties of the BCG vaccine itself. For example, it was discovered that although BCG does protect children from miliary tuberculosis and tuberculous meningitis, it still offers limited protection from the aerosol infection to teenagers and adults. Besides, effectiveness of vaccination varies across regions, from high in Europe and North America to almost negligible in the equatorial areas [2]. Thereby, although tuberculosis is curable and preventable, it still remains one of the top three causes of death from infection. In 2015, 10.4 million new cases of active TB were reported, and at least 1.8 million people died from this disease [3].

One of the troubling aspects of tuberculosis is the increasing incidence of its multidrug-resistant forms. In 2015 about half a million new cases of multidrug-resistant tuberculosis (MDR TB) were reported. Unfortunately, therapies for MDR TB are very expensive (over $ 10,000 per treatment course), with successful treatment outcomes of only 50 %. Experts estimate, that today about 50 million people all over the world have latent MDR TB. The probability of its reactivation throughout life is over 10 % [4]. MDR tuberculosis is a pressing issue in Russia. A joint contribution of India, China and Russia to the global incidence rate is 45 % [3]. In Russia alone incidence remains as high as 115 cases per 100,000 people, going up to 160 cases in some regions [5].

WHO's global plan is to reduce TB incidence and mortality by 90 % and 95 %, respectively, by 2035 [3]. This ambitious goal cannot be achieved without novel effective vaccines. Recombinant vaccines containing *Mycobacterium tuberculosis* antigens, some of which are present in BCG, make up the large proportion of all vaccines developed today [6]. These vaccines are boosters aimed to reinforce the immune response previously induced by BCG and are not intended for primary immunization of neonates, which determines their antigen composition [7]. One of such vaccines is the recombinant GamTBvac containing two mycobacterial antigen fusions (Ag85A and ESAT6-CFP10) with dextran-binding domain (DBD) immobilized on dextran. The adjuvant is represented by DEAE-dextran core and CpG oligonucleotides (TLR9 antagonist). Rationale for vaccine composition and formulation can be found in our previous work [8].

Preclinical studies have demonstrated strong immunogenicity and efficacy of GamTBvac in mice and guinea pigs [8]. GamTBvac has been shown to confer protection against the H37Rv strain of *M. tuberculosis* under aerosol and intravenous challenges. As anticipated, GamTBvac was particularly effective when used as a booster in animals who received a BCG prime. Following the successful completion of the preclinical trial, we obtained an approval from the Ministry of Healthcare of the Russian Federation (authorization ID 179 dated April 10, 2015) to initiate a clinical study (CS) of GamTBvac's safety and immunogenicity in BCG-vaccinated healthy volunteers. The protocol of the study is available in the international database NIH [9]. Below we report the first results of this study.

## METHODS

### *Clinical study design and protocol*

The clinical study was conducted in compliance with the laws of the Russian Federation and in accordance with domestic and international regulations and ethical standards [10–13]. Our phase I/IIA study of immunogenicity and safety recruited 60 healthy BCG-vaccinated male and female volunteers aged 18 to 49 years. The study was designed to have three stages (Fig. 1). Among the exclusion criteria at screening (besides ongoing drug therapies and acute conditions) was a positive QuantiFERON-TB Gold (Qiagen, USA) test for latent tuberculosis [14, 15]. In the first stage, we studied safety of a single GamTBvac dose in 24 volunteers; of them 12 participants received a placebo, and 12 other — 1/4 of the anticipated dose. To evaluate vaccine safety, participants were followed for adverse effects (according to WHO's classification [16]) for 20 weeks after the injection. Medical checkups included physical examination, ECG, blood and urine tests, chest X-ray, etc. In the second stage of the CS, we evaluated immunogenicity of GamTBvac in 12 volunteers who received two injections of the vaccine (1/4 of the anticipated dose each) separated by a 2-month interval. Dynamics of humoral and cell-mediated immune responses was monitored for 20 weeks following vaccination. The third stage of the CS aiming to determine the optimal dosage is still ongoing, and its results are not included in this work. It is being carried out in 24 other volunteers, of whom 12 have already received half of the anticipated dose and 12 other — the maximal (full) dose of the vaccine.

### *Expression and cloning of recombinant M. tuberculosis antigens*

To induce expression of Ag85A and ESAT6-CFP10 antigens fused with dextran-binding domain [8], we used BL21(DE3) pLysS cells of *Escherichia coli*. The agitated cultures were grown in Lysogeny Broth (LB) containing 100 μg/ml ampicillin and 25 μg/ml chloramphenicol, at 37 °C. Once the cultures reached the optical density of 0.7–1 (at 600 nm), antigen expression was induced by adding to the suspension isopropyl β-D-1-thiogalactopyranoside (IPTG) at a final concentration of 0.5 mM and incubating the cultures at 30 °C for 4 hours, with agitation. Then the *E. coli* culture was centrifuged for 20 min at 6,000 g and 4 °C. The bacterial pellet was lysed in a buffer

Concentrations of antigens used for coupling to microspheres

| Antigen | Selected concentration for coupling, μg per $10^6$ microspheres | Microsphere region |
|---|---|---|
| DBD-ESAT6-CFP10 | 5 | 61 |
| CFP-10 | 20 | 33 |
| ESAT-6 | 10 | 25 |
| DBD | 20 | 55 |
| DBD-Ag85A | 20 | 67 |
| Ag85A | 20 | 42 |

containing 20 mM Tris-HCl pH 8, 200 mM NaCl, Triton-X100 0.1 %; after the cells were lysed, lysozyme was added to the buffer at a final concentration of 25 μg/ml; the mixture was incubated for 30 min at RT and then sonicated. Recombinant antigens were isolated either from the pellet after the lysis step or from supernatant. The lysate was centrifuged at 17,000 g for 20 min.

Ag85A-his8, DBD-his8, and ESAT6-his8 antigens were isolated from lysed bacterial cultures carrying the expression vector pET42b coding for *M. tuberculosis* proteins. The expressed proteins aggregated into insoluble inclusion bodies. Before dissolving the bodies in the buffer containing 8 M urea, we purified them three times in a lysing buffer to remove soluble and insoluble admixtures and pelleted by centrifugation. The micobacterial antigens dissolved in 8M urea were pulse-renaturated and run through the affinity column HisPrep FF 16/10 (GE, USA) for purification according to the manufacturer's protocol.

The antigen CFP10 was isolated from the lysates of bacterial cultures carrying the expression vector pTXB1 coding for CFP10. The soluble CFP10 protein was purified using the affinity matrix Chitin Resin (NEB, USA) according to the manufacturer's protocol.

*Evaluation of cell-mediated immunity*

To quantify the T-cells sensitive to M. tuberculosis antigens, the interferon-gamma release assay (IGRA) was performed [17, 18] modified as described in [19]. Briefly, 100 μl of whole blood were collected into a vacuum tube containing lithium heparin anticoagulant and the leukocyte fraction and introduced to 600 μl of the complete growth medium (90 % of medium 199, 10 % of fetal bovine serum, 2 mM L-glutamine, 10 mM HEPES, 50 μg/ml gentamicin sulfate (all ingredients by PanEco, Russia). The resulting sample was divided into 4 aliquots. The recombinant antigens DBD-Ag85A and DBD-ESAT6-CFP10 contained in GamTBvac were added to 2 of the 4 aliquots at a final concentration of 50 μg/ml. One of the remaining aliquots was supplemented with concanavalin A (extracted from *Canavalia ensiformis*; Sigma-Aldrich, Germany) which non-specifically induces production of IFN by lymphocytes. This sample was used as a positive stimulation control. In the last aliquot 20 mM sterile salt-free TRIS, pH 7.5 was placed, and used as a negative control. In total, 4 stimulation reactions were run in separate tubes per blood sample: with antigens DBD-Ag85A and DBD-ESAT6-CFP10, and positive/negative controls. Blood-containing growth media with live lymphocytes, stimulating antigens and controls were incubated for 72 hours under sterile humid conditions at +37 °C. Upon incubation, interferon gamma was quantified using A-8752 gamma-interferon-ELISA-BEST kit (Vector-Best, Russia). Increased levels of interferon gamma as compared to the levels of its spontaneous production in the negative control were regarded as a positive response to stimulation [15, 19]. For each of 12 volunteers who participated in this stage of our study, blood was collected before vaccination (day 0) and on days 1, 42, 63, 98 and 140 after it.

*Suspension immunoassay for measuring humoral immunity*

For serology-based quantification of antibodies to Ag85A, ESAT6, CFP10 and DBD and their fusions DBD-Ag85A and DBD-ESAT6-CFP10, we used the obtained recombinant proteins and 6 xMAP-based monoplex assays (Luminex Corporation, USA) [20].

The optimal quantities of (fusion) antigens (5 to 20 μg per$10^6$ microspheres) were coupled to 6 microsphere sets (see the Table) through carbodiimide reactions according to the protocol described in The xMAP *Cookbook*, *3rd ed*. by Luminex [20].

Microspheres ($1 \times 10^6$) were activated in 80 μl of the activation buffer (0.1 M $NaH_2PO_4$, pH 6.2) containing 10 μl of 50 μg/ml (in $dH_2O$) 1-ethyl-3-[3-dimethylaminopropyl carbodiimide hydrochloride (EDC) and 10 μl of 50 μg/ml (in dH2O) N-hydroxysulfosuccinimide sodium salt (s-NHS) for 20 min at 25 °C by rotation at 20 rpm. The activated microspheres were washed twice and resuspended in 500 μl of the coupling buffer (50 mM MES, pH 5.0), and the target antigens were added to the suspension. The microspheres were further incubated for 2 h in the dark at 25 °C, with mixing by rotation, and washed three times in the blocking buffer, then resuspended in 1 ml of the storage buffer and stored for at least 16 h until further analysis. The microspheres were counted using the automated cell counter TC20 (Bio-Rad Laboratories, USA). The blocking/storage buffer was PBS-TBN (PBS, 0.1 % BSA, 0.02 % Tween-20, 0.05 % $NaN_3$).

The indirect serological assay was run as recommended in [20]. Fifteen μl of PBS-TBN with 2,500 microspheres per region and 50 μl serum prediluted with PBS-TBN 50-fold (to a final concentration of 1 : 100) were placed into a well of a 96-well Microlon flat bottom clear polystyrene plate (Greiner, Austria). The mixture was incubated in the thremoshaker PST-60HL-4 (Biosan, Latvia) for 60 min at +25 °C and 800 rpm and then washed using the handheld magnetic separator MILLIPLEX (Merck Millipore, Germany). Briefly, 100 μl PBS-TBN were added into each well and the plate was left in the shaker for 30 seconds at 800 rpm for separation; 2 washing cycles were run (washing steps were the same throughout this part of the experiment). Then microspheres were resuspended in 50 μl PBS-TBN and combined with 50 μl of 5 μg/ml (in PBS-TBN) anti-human IgG goat antibodies conjugated with phycoerythrin (One Lambda/Thermo Fisher Scientific, USA). The final dilution of the conjugate in each well was 2.5 μg/ml. The suspension was incubated in the thermoshaker for 30 min at +25 °C and 800 rpm and washed. The washed microspheres were resuspended in 100 μl PBS-TBN. Results were processed using MAGPIX (Luminex, USA). For the analysis, we used a minimum of 100 microspheres of the same region per well.

Results of antibody quantification were expressed as MFI (median fluorescence intensity). For each sample, negative control (normal rabbit serum) was subtracted from the raw value. For each of 12 volunteers who participated in this stage of our study, blood was collected and antibodies to all studied antigens were quantified before vaccination (day 0) and on days 1, 42, 63, 98 and 140 after it.

*Statistical processing*

Data were statistically processed in MS Excel and GraphPad Prism 6. Significance of differences for humoral and cell-mediated responses was determined using the Wilcoxon matched-pairs test. Difference was considered significant at $p < 0.05$.

RESULTS

*Safety of GamTBvac administered in the minimal dose*

No (serious) adverse effects were registered in the course of our clinical study (Fig. 1). All abnormalities in lab tests and

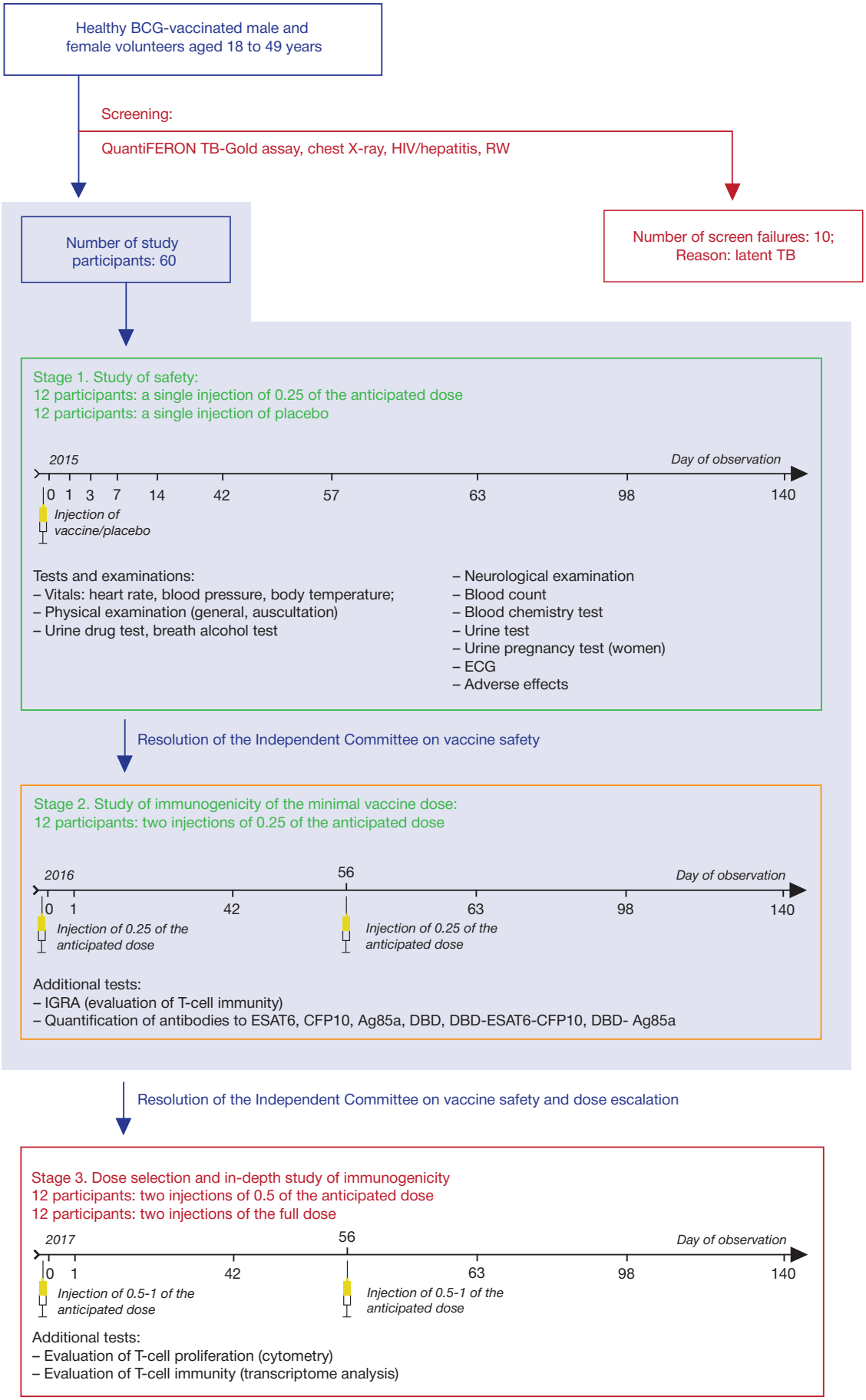


**Fig. 1.** The schematic of the study design. Stages 1 and 2 of the study reported in this article are shown in bold

ECG were found clinically insignificant by the experts of the authorized clinical facility [9]. Six participants reported post-vaccination redness at the injection site 24 h after the injection, persisting for no longer than 3 days. This adverse effect is described in the vaccine information leaflet and classified as mild by WHO [16]; therefore, it was regarded as clinically insignificant. In the light of the above, we conclude that ¼ of the anticipated GamTBvac dose [8] containing 0.006 mg of the antigen protein DBD-Ag85A (the mycobacterial protein Ag85A fused with dextran-binding domain), 0.006 mg of the antigen protein DBD-ESAT6-CFP10 (the mycobacterial protein ESAT6 fused with the mycobacterial CFP10, fused with dextran-binding domain), 2.5 mg of 500 kDa dextran; 0.125 mg of 500 kDa DEAE-dextran and 0.0375 mg of CpG-oligonucleotides (5'-ggGGGACGA:TCGTCgggggg-3') has a high degree of safety.

*Cell-mediated immunity*

Cell-mediated immunity plays a key role in protecting the host from tuberculosis, therefore, evaluation of vaccine immunogenicity should be in the first place account for the cell-mediated immune response to a vaccine [6, 21, 22]. Normally, immunogenicity is evaluated by measuring secretion of INF-γ in response to its stimulation with antigens contained in the vaccine. The most common methods used for this purpose are IGRA, ELISPOT (Enzyme-Linked ImmunoSpot) and flow cytometry. In this study we used a modified IGRA.

While studying INFγ secretion by T lymphocytes in response to the stimulation with recombinant *M. tuberculosis* antigens, we discovered that both antigens contained in the vaccine (DBD-Ag85A and DBD-ESAT6-CFP10) induce significantly increased production of INFγ (Fig. 2, A, B). A reliably increased response to DBD-Ag85A was observed after the first immunization, starting form day 42. Because Ag85A is expressed by *M. bovis* and all volunteers were previously vaccinated with BCG as recommended by the national immunization schedule, early cell-mediated response to Ag85A can be explained by a booster effect of GamTBvac [9]. The cell-mediated response to DBD-ESAT6-CFP10 became more pronounced a month after the second dose of GamTBvac, starting from day 98. On day 140 following the first vaccination, 10 (83 %) of 12 GamTBvac-vaccinated participants demonstrated a sustained cell-mediated response to all antigens contained in the vaccine, in comparison with that measured before the experiment.

Therefore, we conclude that the antigen composition of GamTBvac is immunogenic and induces T-cell response. Both antigen fusions have pronounced immunogenicity, although the temporal patterns of the immune response are different.

*Humoral immunity*

The temporal pattern of accumulation of class G antibodies to the antigens contained in GamTBvac (DBD-Ag85A and DBD-ESAT6-CFP10) reveals that both antigen fusions (to a greater or lesser extent) induce secretion of antibodies (Fig. 2, C–H), but the fusion DBD-ESAT6-CFP10 triggers a stronger humoral response (Fig. 2, C). Starting from day 63 after the first dose was administered, the antibody levels increased significantly. On the final day of the experiment (day 140), 11 (92 %) of 12 volunteers still had antibodies to the DBD-ESAT6-CFP10 fusion. Analysis of immunogenicity of its components revealed that both sense antigens CFP10 (Fig. 2, D) and ESAT6 (Fig. 2, E) stimulated production of IgG antibodies between days 98 and 140 of the experiment, respectively ($p^{**} = 0.0022$ and $p^{*} = 0.034$). Interestingly, DBD was also immunogenic, inducing an earlier response than the sense antigens, starting from day 63 (Fig. 2, F) after immunization.

Another fusion antigen DBD-Ag85A demonstrated a relatively low immunogenicity (Fig. 2, G). For this antigen, significant differences were observed staring from day 98 ($p^{*} = 0.041$) following the immunization. However, no significant associations ($p > 0.05$) between vaccination and production of IgG antibodies to Ag85A were observed (Fig. 2, H).

DISCUSSION

Phase I of the clinical study of GamTBvac has demonstrated that the vaccine has an acceptable safety profile when administered in the studied doses twice to healthy volunteers without latent tuberculosis previously vaccinated with BCG. No serious adverse effects were reported. Redness was observed in 6 participants at the injection site 24 hours after the injection, persisting for no longer than 3 days. This adverse effect is described in the vaccine information leaflet and is classified as mild by WHO [16]; therefore, it was regarded as clinically insignificant.

Our study demonstrates that humoral response was induced in 10 (83 %) of 12 volunteers who received the GamTBvac vaccine. The fusion DBD-Ag85A enhanced the booster effect already observed after the first injection (Fig. 2, A). This effect is apparently associated with Ag85A, because the second antigen DBD-ESAT6-CFP10 elicited the immune response only after the second injection (Fig. 2, B). In our study we did not evaluate separate contributions of each fusion component to the cell-mediated immune response. However, these contributions were studied in depth in our previous experiments conducted in mice [8]. We showed that Ag85A and ESAT6 were the most immunogenic, while CFP10 induced a less pronounced response, although still significantly contributing to the booster effect of the vaccine. We also demonstrated that DBD did not make a significant contribution to the activation of the cell-mediated immunity [8]. This is consistent with the results obtained by other researchers. For example, a few clinical trials of recombinant vaccines H1, H4 and H56 conducted by the State Serum Institute (Copenhagen, Denmark) showed that Ag85 and ESAT6 are highly immunogenic [21–23], triggering a sustained cell-mediated response that was observed half a year after the first immunization. Our study demonstrates that GamTBvac also induces formation of cell memory observed through day 140 of the study.

Cell-mediated immunity has been conventionally thought to have a primary role in protection against tuberculosis, but recently evidence has started to emerge of a more significant role of humoral immunity [6, 24]. In this light, studies of the activation of humoral immunity by TB vaccines are becoming increasingly important. GamTBvac induced humoral response to all components of the antigen fusion DBD-ESAT6-CFP10 (Fig. 2, C–F). The earliest and strongest response was observed for antigen CFP10 (Fig. 2, D), indicating that CFP10 induces both cell-mediated and humoral immunity. A statistically reliable response to ESAT6 occurs later and is less intense, but it appears to make its own contribution to the total immunogenicity of DBD-ESAT6-CFP10 (Fig. 2, C, E).

Obviously, a sustained strong humoral response to the "auxiliary" antigen DBD (Fig. 2, F) cannot associated with protection against TB, but anti-DBD immunoglobulins are potential candidate serologic markers that can be employed by population studies or precision medicine, if patient's immunization status be unknown.

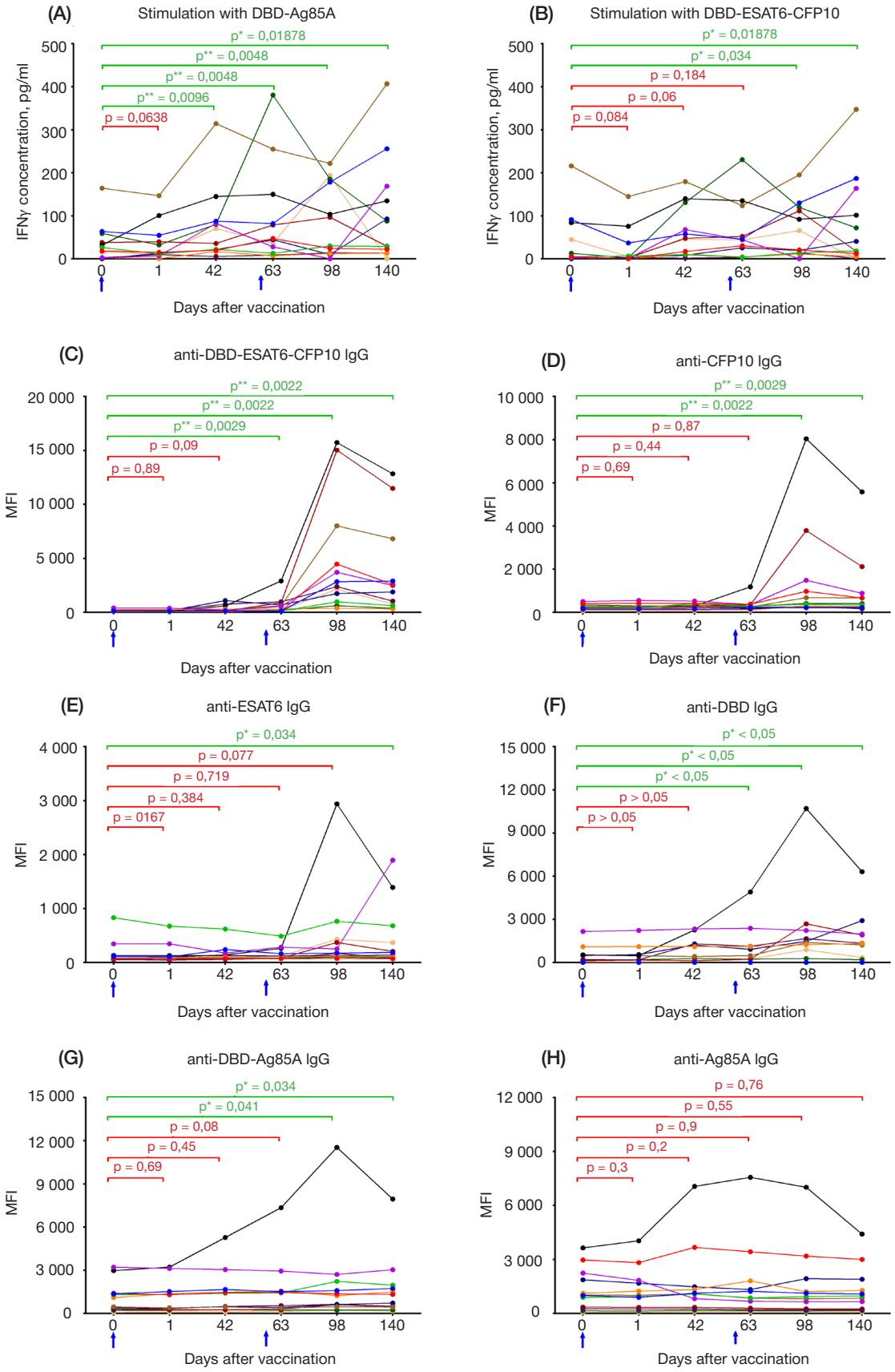


**Fig. 2.** Immunogenicity of the GamTBvac vaccine in 12 volunteers.
**(A)**, **(B)** Evaluation of cell-mediated immunity. For the analysis we employed a modification of IGRA [19] that measures INFγ secretion by whole blood lymphocytes in response to stimulation with antigens DBD-Ag85A **(A)** and DBD-ESAT6-CFP10 **(B)**. The value of spontaneous IFNγ secretion was subtracted from the raw value.
**(C)**–**(H)** x-MAP-based quantification of antibodies to the antigen fusion **(C)** and its components: CFP10 **(D)**, ESAT6 **(E)**, DBD **(F)**, and DBD-Ag85A **(G)** and its main component Ag85A **(H)**. Statistical significance was calculated using the Wilcoxon matched-pairs test. Significant differences over the observation period (*p < 0.05 and **p < 0.01) are shown in green, in comparison with day 0. Red p-values indicate the absence of significant differences. Vaccination days are represented by blue arrows

Surprisingly, Ag85A had a low humoral immunogenicity (Fig. 2, 3). This antigen seems to make a zero contribution to the total immunogenicity of the fusion DBD-Ag85A (Fig. 2, G). Previously, Ag85A was shown to induce high antibody titers both in BCG-unvaccinated and vaccinated animals [8]. The absence of humoral response may be explained by the incorrect folding of Ag85A used for coupling with xMAP microspheres. On the other hand, some of the volunteers had stably high antibody titers throughout the study, which may disprove this supposition. Possibly, increased antibody titers induced by BCG vaccination rapidly eliminate small amounts of antigen proteins without triggering the booster effect. Each of these hypotheses needs to be tested further.

As the role of humoral immunity in patients with tuberculosis is becoming increasingly acknowledged, preclinical and clinical trials of TB vaccines should focus more on the contribution of each vaccine component to the elicited humoral response [24]. Unfortunately, the majority of studies of TB vaccines do not report any findings about antibody response [22, 23] or do not differentiate between contributions made by individual antigens [21]. Our results demonstrate that effects of antigens contained in GamTBvac can be directly opposite in terms of establishing the cell-mediated and humoral immunological memory.

## CONCLUSIONS

The studied recombinant vaccine GamTBvac has a sufficient degree of safety and activates humoral and cell components of the immune system leading to the establishment of immunological memory. Our study demonstrates that 1/4 of the anticipated vaccine dose induces sustained cellular and humoral responses. We anticipate that stage 3 of our study, which is still ongoing, will be successful and that we will be able to demonstrate the strong immunogenicity of the vaccine using the most advanced techniques, including flow cytometry and transcriptome analysis. We also hope to carry out Phase IIb to confirm vaccine effectiveness.

**References**


1. Kaufmann SHE, Weiner J, von Reyn CF. Novel approaches to tuberculosis vaccine development. Int J Infect Dis. 2017 Mar; 56: 263–7. DOI^: 10.1016/j.ijid.2016.10.018
2. Nguipdop-Djomo P, Heldal E, Rodrigues LC, Abubakar I, Mangtani P. Duration of BCG protection against tuberculosis and change in effectiveness with time since vaccination in Norway: A retrospective population-based cohort study. Lancet Infect Dis. 2016 Feb; 16 (2): 219–26. DOI^: 10.1016/S1473-3099(15)00400-4
3. World Health Organization. Global Tuberculosis Report 2016. 21th ed. Geneva: World Health Organization; 2016. 214 p.
4. Glaziou P, Sismanidis C, Floyd K, Raviglione M. Global Epidemiology of Tuberculosis. Cold Spring Harb Perspect Med. 2015 Feb; 5 (2): a017798. DOI^: 10.1101/cshperspect.a017798
5. O sostoyanii sanitarno-epidemiologicheskogo blagopoluchiya naseleniya v Rossiyskoy Federatsii v 2016 godu. National Report. Moscow: Russian Federal Service for Surveillance on Consumer Rights Protection and Human Wellbeing (Rospotrebnadzor); 2017. 220 p. Russian.
6. Karp CL, Wilson CB, Stuart LM. Tuberculosis vaccines: Barriers and prospects on the quest for a transformative tool. Immunol Rev. 2015 Mar; 264 (1): 363–81. DOI^: 10.1111/imr.12270. PubMed PMID: 25703572.
7. Frick M. The Tuberculosis Vaccines Pipeline: A New Path to the Same Destination? [Internet]. 2015 Pipeline Report. HIV, HCV, and TB. London, NY: HIV i-Base/Treatment Action Group; July 2015 [cited 2017 Oct 9]. Available from: http://www.pipelinereport.org/2015/tb-vaccines
8. Tkachuk AP, Gushchin VA, Potapov VD, Demidenko AV, Lunin VG, Gintsburg AL. Multi-subunit BCG booster vaccine GamTBvac: Assessment of immunogenicity and protective efficacy in murine and guinea pig TB models. PLoS One. 2017; 12 (4): e0176784. DOI^: 10.1371/journal.pone.0176784
9. Evaluation of the Safety and Immunogenicity of the Recombinant Subunit Vaccine "GamTBvac" Against the Tuberculosis [Internet]. U.S. National Library of Medicine. ClinicalTrials.gov Identifier: NCT03255278 [cited 2017 Oct 9]. Available from: https://clinicaltrials.gov/ct2/show/NCT03255278
10. ICH Harmonised Tripartite Guideline. Guideline for good clinical practice E6(R1). Current Step 4 version. Dated 10 June 1996 [Internet]; [cited 2017 Oct 9]: [59 p.]. Available from: http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E6/E6_R1_Guideline.pdf
11. Federal'nyy zakon ot 12.04.2010 № 61-FZ (red. ot 03.07.2016) «Ob obrashchenii lekarstvennykh sredstv». Federal law of the Russian Federation. Russian.
12. GOST 33044-2014 Printsipy nadlezhashchey laboratornoy praktiki. Mezhgosudarstvennyy standart. Data vvedeniya: 1 avgusta 2015 g. State Standard. Identical to OECD Guide 1:1998 OECD Principles of good laboratory practice. Russian.
13. Prikaz Ministerstva zdravookhraneniya RF ot 1 aprelya 2016 g. № 200n «Ob utverzhdenii pravil nadlezhashchey klinicheskoy praktiki». Order of the Ministry of Healthcare of the Russian Federation. Russian.
14. Mazurek GH, Jereb J, Lobue P, Iademarco MF, Metchock B, Vernon A, et al. Guidelines for using the QuantiFERON-TB Gold test for detecting Mycobacterium tuberculosis infection, United States. MMWR Recomm Rep. 2005 Dec 16; 54 (RR-15): 49–55.
15. QuantiFERON-TB Gold Plus (QFT-Plus) ELISA Package Insert. Rev. 04. Hilden: QIAGEN GmbH; Feb 2016.
16. Melikhov OG, Atarshchikov DS. Nezhelatel'nye yavleniya v klinicheskikh issledovaniyakh lekarstvennykh sredstv. Kachestvennaya klinicheskaya praktika. 2003; (2): 11–6. Russian.
17. Lalvani A, Brookes R, Wilkinson RJ, Malin AS, Pathan AA, Andersen P, et al. Human cytolytic and interferon gamma-secreting CD8+ T lymphocytes specific for Mycobacterium tuberculosis. Proc Natl Acad Sci U S A. 1998 Jan 6; 95 (1): 270–5. PubMed PMID: 9419365.
18. Dewan PK, Grinsdale J, Liska S, Wong E, Fallstad R, Kawamura LM. Feasibility, acceptability, and cost of tuberculosis testing by whole-blood interferon-gamma assay. BMC Infect Dis. 2006 Mar 15; 6: 47. PubMed PMID: 16539718.
19. Plekhanova MA, Aksenova VA, Tkachuk AP, Patsula YI, Krivtsova LA, Kolomeets AN. Evaluation of specific antigens at the early stage of tuberculous infection in children. Tuberkulez i bolezni legkikh. 2017; 95 (1): 27–33. Russian.
20. Luminex. The xMAP Cookbook. A collection of methods and protocols for developing multiplex assays with xMAP Technology. 3rd ed. [Internet]; [cited 2017 May 5]. Available from: http://info.luminexcorp.com/en-us/download-the-xmap-cookbook
21. Luabeya AKK, Kagina BMN, Tameris MD, Geldenhuys H, Hoff ST, Shi Z, et al. First-in-human trial of the post-exposure tuberculosis vaccine H56:IC31 in Mycobacterium tuberculosis infected and non-infected healthy adults. Vaccine. 2015 Aug 7; 33 (33): 4130–40. DOI^: 10.1016/j.vaccine.2015.06.051
22. Geldenhuys H, Mearns H, Miles DJC, Tameris M, Hokey D, Shi Z, et al. The tuberculosis vaccine H4:IC31 is safe and induces a persistent polyfunctional CD4 T cell response in South African adults: A randomized controlled trial. Vaccine. 2015 Jul 9; 33 (30): 3592–9. DOI^: 10.1016/j.vaccine.2015.05.036
23. Reither K, Katsoulis L, Beattie T, Gardiner N, Lenz N, Said K, et al. Safety and immunogenicity of H1/IC31H®, an adjuvanted TB subunit vaccine, in HIV-infected adults with CD4+ lymphocyte counts greater than 350 cells/mm3: A phase II, multi-centre, double-blind, randomized, placebo-controlled trial. PLoS One.

2014; 9 (12): e114602. DOI^: 10.1371/journal.pone.0114602
24. Lu LL, Chung AW, Rosebrock TR, Ghebremichael M, Yu WH, Grace PS, et al. A Functional Role for Antibodies in Tuberculosis. Cell. 2016 Oct 6; 167 (2): 433–43.e14. DOI^: 10.1016/j.cell.2016.08.072

**Литература**

1. Kaufmann SHE, Weiner J, von Reyn CF. Novel approaches to tuberculosis vaccine development. Int J Infect Dis. 2017 Mar; 56: 263–7. DOI^: 10.1016/j.ijid.2016.10.018
2. Nguipdop-Djomo P, Heldal E, Rodrigues LC, Abubakar I, Mangtani P. Duration of BCG protection against tuberculosis and change in effectiveness with time since vaccination in Norway: A retrospective population-based cohort study. Lancet Infect Dis. 2016 Feb; 16 (2): 219–26. DOI^: 10.1016/S1473-3099(15)00400-4
3. World Health Organization. Global Tuberculosis Report 2016. 21th ed. Geneva: World Health Organization; 2016. 214 p.
4. Glaziou P, Sismanidis C, Floyd K, Raviglione M. Global Epidemiology of Tuberculosis. Cold Spring Harb Perspect Med. 2015 Feb; 5 (2): a017798. DOI^: 10.1101/cshperspect.a017798
5. О состоянии санитарно-эпидемиологического благополучия населения в Российской Федерации в 2016 году: Государственный доклад. М.: Федеральная служба по надзору в сфере защиты прав потребителей и благополучия человека; 2017. 220 с.
6. Karp CL, Wilson CB, Stuart LM. Tuberculosis vaccines: Barriers and prospects on the quest for a transformative tool. Immunol Rev. 2015 Mar; 264 (1): 363–81. DOI^: 10.1111/imr.12270. PubMed PMID: 25703572.
7. Frick M. The Tuberculosis Vaccines Pipeline: A New Path to the Same Destination? [Интернет]. 2015 Pipeline Report. HIV, HCV, and TB. London, NY: HIV i-Base/Treatment Action Group; July 2015 [дата обращения: 9 октября 2017 г.]. Доступно по: http://www.pipelinereport.org/2015/tb-vaccines
8. Tkachuk AP, Gushchin VA, Potapov VD, Demidenko AV, Lunin VG, Gintsburg AL. Multi-subunit BCG booster vaccine GamTBvac: Assessment of immunogenicity and protective efficacy in murine and guinea pig TB models. PLoS One. 2017; 12 (4): e0176784. DOI^: 10.1371/journal.pone.0176784
9. Evaluation of the Safety and Immunogenicity of the Recombinant Subunit Vaccine "GamTBvac" Against the Tuberculosis [Интернет]. U. S. National Library of Medicine. ClinicalTrials.gov Identifier: NCT03255278 [дата обращения: 9 октября 2017 г.]. Доступно по: https://clinicaltrials.gov/ct2/show/NCT03255278
10. ICH Harmonised Tripartite Guideline. Guideline for good clinical practice E6(R1). Current Step 4 version. Dated 10 June 1996 [Интернет]; [дата обращения: 9 октября 2017 г.]: [59 с.]. Доступно по: http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E6/E6_R1_Guideline.pdf
11. Федеральный закон от 12.04.2010 № 61-ФЗ (ред. от 03.07.2016) «Об обращении лекарственных средств».
12. ГОСТ 33044-2014 Принципы надлежащей лабораторной практики. Межгосударственный стандарт. Дата введения: 1 августа 2015 г.
13. Приказ Министерства здравоохранения РФ от 1 апреля 2016 г. № 200н «Об утверждении правил надлежащей клинической практики».
14. Mazurek GH, Jereb J, Lobue P, Iademarco MF, Metchock B, Vernon A, et al. Guidelines for using the QuantiFERON-TB Gold test for detecting Mycobacterium tuberculosis infection, United States. MMWR Recomm Rep. 2005 Dec 16; 54 (RR-15): 49–55.
15. QuantiFERON-TB Gold Plus (QFT-Plus) ELISA Package Insert. Rev. 04. Hilden: QIAGEN GmbH; Feb 2016.
16. Мелихов О. Г., Атарщиков Д. С. Нежелательные явления в клинических исследованиях лекарственных средств. Кач. клин. практ. 2003; (2): 11–6.
17. Lalvani A, Brookes R, Wilkinson RJ, Malin AS, Pathan AA, Andersen P, et al. Human cytolytic and interferon gamma-secreting CD8+ T lymphocytes specific for Mycobacterium tuberculosis. Proc Natl Acad Sci U S A. 1998 Jan 6; 95 (1): 270–5. PubMed PMID: 9419365.
18. Dewan PK, Grinsdale J, Liska S, Wong E, Fallstad R, Kawamura LM. Feasibility, acceptability, and cost of tuberculosis testing by whole-blood interferon-gamma assay. BMC Infect Dis. 2006 Mar 15; 6: 47. PubMed PMID: 16539718.
19. Плеханова М. А., Аксенова В. А., Ткачук А. П., Пацула Ю. И., Кривцова Л. А., Коломеец А. Н. Оценка специфических антигенов ранней стадии туберкулезной инфекции у детей. Туберк. и болезни легких. 2017; 95 (1): 27–33.
20. Luminex. The xMAP Cookbook. A collection of methods and protocols for developing multiplex assays with xMAP Technology. 3rd ed. [Интернет]; [дата обращения: 5 мая 2017 г.]. Доступно по: http://info.luminexcorp.com/en-us/download-the-xmap-cookbook
21. Luabeya AKK, Kagina BMN, Tameris MD, Geldenhuys H, Hoff ST, Shi Z, et al. First-in-human trial of the post-exposure tuberculosis vaccine H56:IC31 in Mycobacterium tuberculosis infected and non-infected healthy adults. Vaccine. 2015 Aug 7; 33 (33): 4130–40. DOI^: 10.1016/j.vaccine.2015.06.051
22. Geldenhuys H, Mearns H, Miles DJC, Tameris M, Hokey D, Shi Z, et al. The tuberculosis vaccine H4:IC31 is safe and induces a persistent polyfunctional CD4 T cell response in South African adults: A randomized controlled trial. Vaccine. 2015 Jul 9; 33 (30): 3592–9. DOI^: 10.1016/j.vaccine.2015.05.036
23. Reither K, Katsoulis L, Beattie T, Gardiner N, Lenz N, Said K, et al. Safety and immunogenicity of H1/IC31H®, an adjuvanted TB subunit vaccine, in HIV-infected adults with CD4+ lymphocyte counts greater than 350 cells/mm3: A phase II, multi-centre, double-blind, randomized, placebo-controlled trial. PLoS One. 2014; 9 (12): e114602. DOI^: 10.1371/journal.pone.0114602
24. Lu LL, Chung AW, Rosebrock TR, Ghebremichael M, Yu WH, Grace PS, et al. A Functional Role for Antibodies in Tuberculosis. Cell. 2016 Oct 6; 167 (2): 433–43.e14. DOI^: 10.1016/j.cell.2016.08.072