

## RECOGNITION OF *Mycobacterium tuberculosis* ANTIGENS MPT63 AND MPT83 WITH MURINE POLYCLONAL AND scFv ANTIBODIES

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The goal of this study was to characterize serum immunoglobulin G (IgG) antibody responses during experimental immunization of laboratory mice by purified recombinant proteins MPT63, MPT83 of *Mycobacterium tuberculosis* and artificial fusion protein MPT83-MPT63 and obtain the recombinant single chain variable fragments of antibodies (scFv) against these antigens.

This study demonstrates that the humoral immune response to MPT63, MPT83, MPT83-MPT63 fusion protein and equimolar set of MPT63 and MPT83 was highly different. For each antigen, serum antibody levels were evaluated by a cutoff value based on optical density index. A crucial role of MPT83 for immunogenicity of chimeric protein and/or cocktail of individual antigens under conditions of immunization of laboratory animals.

We obtained also specific scFv antibodies against MPT63 and MPT83. These antibodies can be used for the development of the system for quantitative determination of antigens as well as for their biological properties investigation.

Thereby, based on the results of the immune response and mycobacterial proteins antigenicity we showed highly immunogenicity properties of N-terminal part of MPT83 antigen for enhancement of ELISA sensitivity. We suggest MPT83-MPT63 fusion protein as a potential candidate on the role of antigenic substance for the serological diagnosis of tuberculosis.

**Key words:** tMPT63, MPT83, antigens, polyclonal antibodies, scFv, diagnostic.

Tuberculosis (TB) is the widespread infectious disease of human being and animals. In 2015, there were an estimated 10.4 million new (incident) TB cases worldwide and an approximately 1.6 million death [1]. Early diagnosis of TB is crucial to prevent the spread of the disease in the community. Immunological methods measure specific cellular or humoral responses of the host to detect presence of infection or disease. They do not require a specimen from the site of infection unlike reference standard in TB diagnosis such as bacteria culture. Moreover, it can take up to 6–8 weeks to isolate *Mycobacterium tuberculosis* [2], while serological tests take a few hours.

An important step for constructing of new diagnostic kits based on ELISA technique is the selection of the optimal high immunogenic substances for specific, accurate and sensitive determination of antibodies to the pathogen.

Difficulties in tuberculin skin test administration and interpretation often lead to false results [3]. An accurate serological test could provide rapid diagnosis of TB and in a suitable format would be particularly useful both as a replacement for laboratory based tests and for extending TB diagnosis to lower levels of health services, especially those without on-site laboratories [4]. Serological tests that use various *M. tuberculosis* antigens such as secretory proteins, heat shock proteins, lipopolysaccharide and peptides have been developed [5]. But despite numerous studies of the genome and proteomics of *Mycobacteria* only few ELISA kits are available for wide clinical practice use. This is most often due to negligent attitude to the choice of an antigenic substance or even with the use of total lysates of *M. tuberculosis* or protein purified derivates (PPD). As a consequence, significant variation of

sensitivity and specificity indicators, which will not allow the developed product to be used in practice or/and false positive results will be observed in antibody-based diagnostic tests due to the exposure to environmental non-tuberculous *Mycobacteria* (NTM) or prior Calmette-Gu rin bacillus (BCG) vaccination [6].

Recombinant antigens of *M. tuberculosis* and *M. bovis* strains MPT63 and MPT83 obtained from *E. coli* expression system do not differ from serologic characteristics from such own proteins of the causative agents [7]. In addition, these antigens were found only in the representatives of *M. tuberculosis complex*, and not found or expressed in of atypical mycobacteria strains [7]. This is important to overcome the false positive results caused by NTM.

MPT63, a major secreted 16 kDa protein from *M. tuberculosis*, has been shown to have immunogenic properties and has been implicated in virulence. According to literature data MPT63 cause inflammatory processes due to degranulation of mast cell with following release histamine and hexaminidase [8].

MPT83 is a lipoprotein which undergoes acylation and glycosylation and associated with bacilli cell wall by myristyl tail [9]. MPT83 is one of the ligands of toll like receptor 2 (TLR-2) [10]; also, it was described as an adhesion factor [11]; as inducer of apoptosis of infected macrophages by activating the TLR2/p38/COX-2 signaling pathway [12].

Classic BCG vaccine has been used worldwide to prevent TB disease in infants and children, but it has demonstrated limited and variable effectiveness in preventing pulmonary TB in adolescents and adults. Immunisation studies in mice indicated that MPT83-MPT83 are highly immunogenic with adjuvant. The use of alternative antigens and additional approaches for vaccine development urgently needed to protect against TB. Nevertheless, these results establish a novel platform for development of antigenic substances with inherent immunogenic characteristics that are desirable in vaccines.

It follows that the antigens chosen by the researchers are serologically valuable for use as an antigenic substance to create new diagnostic ELISA kit. In the present study, we have evaluated humoral responses in mice, TB suitable animals, to immunodominant antigens of *M. tuberculosis* MPT63 and MPT83, their cocktails and chimeric protein MPT83-MPT63 obtained as a result of the fusion of genetic sequences of individual

antigens in one open reading frame. Furthermore, in this study we obtained the recombinant murine scFv to MPT63 and MPT83. Antibodies are an important tool both for studying of biological activity of mycobacterial antigens and developing of test-systems for quantitative measurement of proteins in fluids as biomarkers of TB pathogenesis. Also the recombinant antibodies may be used for the affine purification of these antigens. Thus, it has been received polyclonal and scFv antibodies, which resemble the characteristics of monoclonal antibodies and may be used for further improve existing TB diagnostics.

Statistical data analysis. The data were statistically treated using standard MO Excel and Origin 8.0 software. To compare the data in two groups we used Student's *t*-criterion test. The difference was considered statistically significant for  $P < 0.05$ .

## Materials and Methods

*Materials and reagents:* bovine serum albumin (BSA), Complete and Incomplete Freund's Adjuvants (Sigma, USA), Ni-NTA agarose (Qiagen, Germany), imidazole, isopropyl- $\beta$ -D-1-thiogalactopyranoside (IPTG) (Thermo Scientific, Lithuania), bacterial culture medium LB (Sigma, USA), skim milk powder (Fluka, Switzerland), coomassie G250, ammonium persulfate (APS), urea, acrylamide, N,N'-Metilenbisakrilamid, 3,3',5,5'-tetramethylbenzidine (TMB) (Sigma, USA), conjugate of IgG horseradish peroxidase (HRP) (Thermo Scientific, Lithuania), tris (hydroxymethyl) aminomethane (Sigma, USA), tricine, molecular weight markers for protein gel electrophoresis (Thermo Scientific, Lithuania),  $\beta$ -mercaptoethanol, Tween-20 (Helicon, Russia), kanamycin, chloramphenicol, H<sub>2</sub>O<sub>2</sub> ("Kyivmedpreparat", Ukraine), glycerol, KCl, NaCl, Na<sub>2</sub>HPO<sub>4</sub>, NaOH, KH<sub>2</sub>PO<sub>4</sub>, ("Miranda-C", Ukraine), sodium dodecyl sulfate (SDS) (Sigma, USA). A 96-well microtiter plates for enzyme-linked immunosorbent assay (Greiner Bio One, Great Britain) were used.

### *Recombinant proteins expression*

Bacterial cultures with recombinant proteins expressed *E. coli* cells were grown at 37 °C under aeration conditions (250 rpm) up to A<sub>600</sub> — 0.3–0.5 in the LB medium with 50 mg/l of kanamycin, 170 mg/l of chloramphenicol and 1% glucose. After this, cells were precipitated and resuspended in fresh LB medium with kanamycin (50  $\mu$ g/ml)

and an inducer of expression of IPTG in a concentration of 1 mM. Target proteins expression has been performed during 3–4 hours at 30 °C under strong aeration conditions (250 rpm), after what cells have been precipitated by centrifugation at 3300 g during 15 min.

#### *Immobilized-metal affinity on-column chromatography of polyHis-tag proteins*

Column containing affine sorbent has been equilibrated with wash buffer (50 mM Na<sub>2</sub>HPO<sub>4</sub>, (pH 8.0), 0.5 M NaCl) with 6 or 8 M urea. Cell precipitates were resuspended in a same buffer (1 ml buffer solution per precipitate from 50 ml of bacterial culture). Samples have been sonicated by ultrasonic homogenizer LabsonicM (Sartorius, Germany). Cell's wall residues have been precipitated by centrifugation under 10 000 g during 20 min, and a preequilibrated Ni<sup>2+</sup>-NTA agarose column has been filled by supernatant.

Renaturation by washing the column with step by step decrease of urea concentration (8 M → 6M → 4 M → 2 M → 0 M) in wash buffer (50 mM Na<sub>2</sub>HPO<sub>4</sub>, pH 8.0; 0.5 M NaCl) has been performed for obtaining soluble recombinant proteins. Proteins were eluted by wash buffer containing 250 mM imidazole without urea. Protein for further procedures was dialyzed against PBS (0.14 M NaCl, 0.03 M KCl, 0.011M Na<sub>2</sub>HPO<sub>4</sub>, 0.002 M KH<sub>2</sub>PO<sub>4</sub>, pH 7.2).

MPT83 expressed *E. coli* cells were resuspended in PBS or urea free wash buffer and were treated and centrifuged as described above. MPT83 protein extraction was carried out in nondenaturing conditions. For higher protein yield, the technique has been modified through use DNase (10 U/ml), 1% Triton X-100 and lysozyme for better *E. coli* cell's walls and nucleoids dissociation as performed according to [13].

#### *Electrophoretic separation of proteins*

Determination of protein concentration by tricine SDS-PAGE analysis with TotalLab TL120 software were performed according to [14, 15].

#### *Immunization of experimental animals*

BALB/c 4-month female white mice (30–35 g of body mass) were used in the experiments. All animals had unlimited access to animal chow and tap water throughout the study.

The experiments are consistent with the requirements of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1986) and with ethical norms as laid down in the laws of Ukraine.

Primary and booster injections of antigens were intraperitoneal as emulsions in CFA (1<sup>st</sup>) or IFA (2<sup>nd</sup>, 3<sup>rd</sup>) administered in dose 1,52 nmol per animal ( $n = 8–10$  for each group) at intervals of 2 weeks. The level of specific antibodies to mycobacterial antigens in the sera of immunized mice was determined by ELISA after 7<sup>th</sup> day since last immunization.

#### *Enzyme-linked immunosorbent assay (ELISA)*

As antigens were used appropriate recombinant proteins of *M.tuberculosis* MPT63, MPT83, artificial fusion protein and mix set of separate component and BSA (negative control). Monoclonal antibodies against marker sequence fused with scFv (E-tag) (Amersham Bioscience, USA) were used to determine scFv antibodies; and anti-mouse IgG HRP were used as the secondary antibodies. TMB was used as chromogenic substrate. The color reaction was quantified by measuring the absorbency at 490 nm.

#### *Construction of immune phage library*

Total RNA, which was used as a matrix in cDNA synthesis, was isolated from the spleen tissue of immunized with MPT63 or MPT83 mice by TRI Reagent (Sigma, USA). The sequences encoding the variable domains of the light and heavy immunoglobulin chains (V<sub>H</sub> and V<sub>L</sub>) were amplified with the set of specific primers designed according to [16]. The high-fidelity polymerase AccuTaq LA DNA Polymerase (Sigma, USA) was used for amplification. Nucleotide sequences V<sub>H</sub> and V<sub>L</sub> were assembled by SOE-PCR (splicing by overlap extension PCR). DNA sequences of scFv were inserted into the phagemid vector pCANTAB-5E by *Sfi*I and *Not*I restriction sites. The ligase mixture was used for transformation of *E. coli* XL1-blue by the electroporation.

#### *Isolation of phage particles*

The cells transformed with scFv phagemids were infected with phage-helper M13K07 and incubated overnight. The phage cells were precipitated with a solution of PEG/NaCl (20% PEG-6000, 2.5 M NaCl) as described previously [16].

#### *Selection of scFv antibodies*

The positive clones against target antigens MPT63 and MPT83 were selected by the phage display method as described before [17].

#### *Colonies lift assay*

For pre-selection of positive clones, the colonies were transplanted into Petri dishes with a 2YT agar medium containing 2% glucose and ampicillin to a final concentration of 100 µg/ml. The prints of the colonies were

carried over on a nitrocellulose membrane (Amersham Bioscience, USA), which was previously incubated in a 5% non fat milk in PBS 60 min at 37 °C.

At the same time on the membranes of the identical size were immobilized with mycobacterial proteins MPT63 and MPT83 (incubation overnight in 10 ml of a solution in PBS, an antigen concentration of 10 µg/ml, with next blocking in a 5% solution of skim milk during 60 min). The immobilized by antigens nitrocellulose membranes were transferred to 2YT agar medium containing 100 µg/ml ampicillin and 1 mM IPTG and incubated overnight after 30 °C. After that, the membranes were treated by classic Western blot analysis using anti-E-tag and anti-mouse IgG-HRP conjugates. Inprints have been detected by 3,3'-Diaminobenzidine tetrahydrochloride (DAB) chromogenic substrate.

## Results and Discussion

One of the most important characteristic of different type antibodies is their specificity, i. e., the capacity to bind with target proteins avoiding cross-reaction with other antigens. Moreover, for ELISA test diagnostics is ultimate need the use of antigens which have a number of serologically important epitopes that would provide interaction with antibodies and increase the sensitivity of the method.

### Obtaining of recombinant proteins MPT63, MPT83 and MPT83-MPT63 fusion

Proteins expression was performed in the culture *E. coli* BL21 (DE3) Rosetta (Novagen, USA), transformed by pET24a- or pET28a-based (Novagen, USA) genetic constructs. Purification of recombinant proteins MPT63, MPT83 and MPT83-MPT63 fusion performed with metal affinity chromatography on Ni<sup>2+</sup>-NTA column. Taking into the account that MPT63 and MPT83-MPT63 fusion were insoluble, procedure of refolding was performed. Analysis of protein fractions after the refolding was performed on 10% SDS-PAGE (Fig. 1).

### Characterization of antigenicity

This study demonstrates that the humoral immune response to MPT63, MPT83, MPT83-MPT63 fusion protein and equimolar set of MPT63 and MPT83 is highly distinguished. ELISA measurements of mice serum polyclonal antibodies against differently administrated antigens are shown in Fig. 2. It was shown absence of specific antibodies titers to

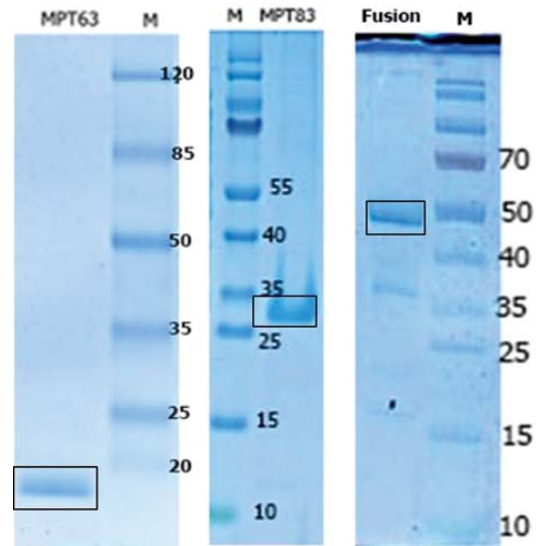


Fig. 1. Electrophoregram of used proteins: MPT63, MPT83 and fusion (MPT83-MPT63); M — molecular mass markers, kDa. Bands corresponding to the target protein are indicated with a rectangular frame

recombinant antigens of *M. tuberculosis* in mice before immunization (Fig. 2).

For each antigen, serum antibody levels were evaluated by using a cutoff value based on optical density index (ODI), a ratio between OD obtained for a test serum samples collected after immunization and OD obtained for a serum samples collected from the same animal at an initial, preimmunization time point [18]. Optimal serum dilution was chosen 1:16 000. Lower antibody titers was found against MPT63 (ODI  $\geq$  15,6), higher — against MPT83-MPT63 fusion (ODI  $\geq$  66,1). Anti-MPT83 and anti-(MPT63+MPT83 mixture) sera demonstrate practically the same (ODI  $\geq$  55,4) (Fig. 3).

### Crossreactivity of polyclonal IgG among *M. tuberculosis* antigens

Mycobacterial antigens MPT63 is secretory protein and MPT83 is myristylited and in bacilli associate with cell wall. Obtained by us recombinant analogues of these antigens potentially could show cross-reactivity due to same *E. coli* expression system and encoding polyHis tag in genetic constructs for affinity isolation and purification of antigens. It was considered that as a result of protein eluates contamination with *Escherichia* components, recombinant antigens are able show cross-reactivity to different types of antisera. MPT83 and MPT63 antigens not recognized with anti-MPT63 (OD 0,056 $\pm$ 0,02) and anti-MPT83 (OD 0,027 $\pm$ 0,014) sera respectively (Fig. 4).

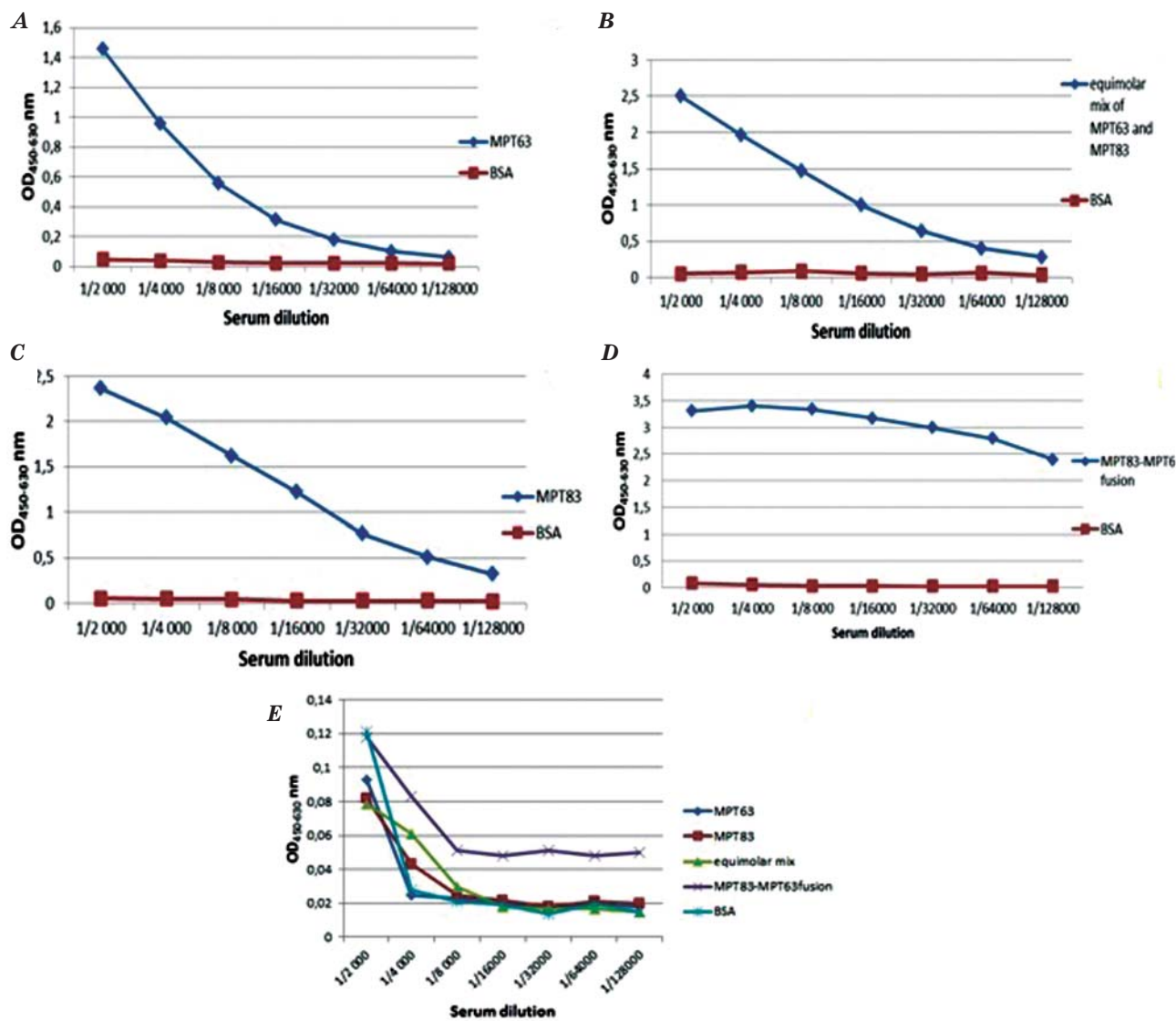


Fig. 2. IgG level to the target recombinant proteins MPT63 (A), cocktail of MPT63+MPT83 (B), MPT83 (C), MPT83-MPT63 fusion (D) in the sera of immunized mice and pre-immunized sera (E)

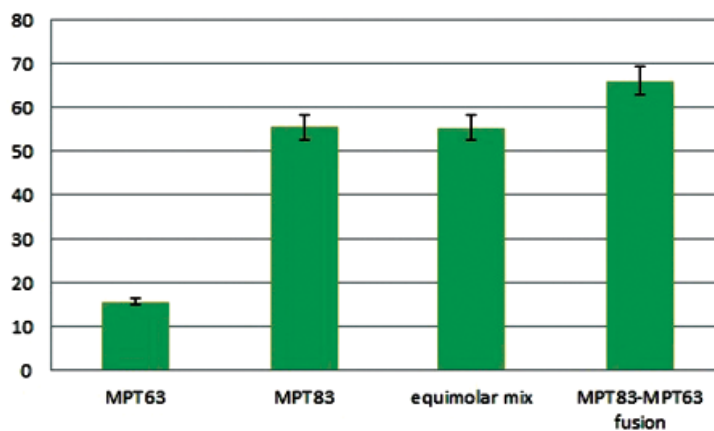


Fig. 3. Optical density index for each antigen substance  
 $ODI = OD_{(postimmunization)} / OD_{(preimmunization)}$

Moreover, we showed a crucial role of MPT83 for the chimeric protein MPT83-MPT63 and heterogeneous set of proteins in immune response (Fig. 4): anti-MPT83-MPT63 fusion (OD  $3,96 \pm 0,037$ ) and anti-(MPT+63MPT83) (OD  $0,049 \pm 3,123$ ) sera characterizes by a high level of IgG against MPT83 antigen than to MPT63 (OD  $0,05 \pm 2,536$  and  $0,011 \pm 0,487$  for anti-MPT-83 MPT63 fusion and anti-(MPT+63MPT83) sera respectively). Similar experiments were conducted to recognize all types of antigens and their combinations with different sera (Fig. 4).

Chimeric protein based on Fascycline-like domain of MPT83 (MPT83 FLD<sub>115-220aa</sub>) and MPT63 was obtained previously [19] and was used for development of efficient TB diagnostic for cattle [20]. Unlike MPT83(full)-MPT63 fusion which was used for this experiments it incomplete analogue did not retain the primary protein structure and as a result restricted sterically range of

serologically important determinants of both antigens. As a result, Fascycline-like domain of MPT83 fused to MPT63 did not contain all serologically important determinants of new synthesized MPT83(full)-MPT63 protein. As we can see (Fig. 5, A) this antigen, as in the case with full-size MPT63-MPT83 (Fig. 5, B), was better recognized by anti-MPT83-MPT63 serum, however anti-MPT63 antiserum was better recognized short MPT83-MPT63, while antiserum anti-MPT83 was better recognized the full-size MPT63-MPT83. Thus, it has been proven a special role of MPT83 for immunogenicity of fusion antigens and significance of spatial organization new antigenic composition (MPT83-MPT63) and its plasticity of serologically important epitopes exposure.

*Immune library of murine recombinant antibodies*

Total RNA ( $2-5 \mu\text{g}/\mu\text{l}$ ,  $A_{260}/A_{280} \geq 1,6$ ) from splenocytes of immunized mice which were characterized by highest immune

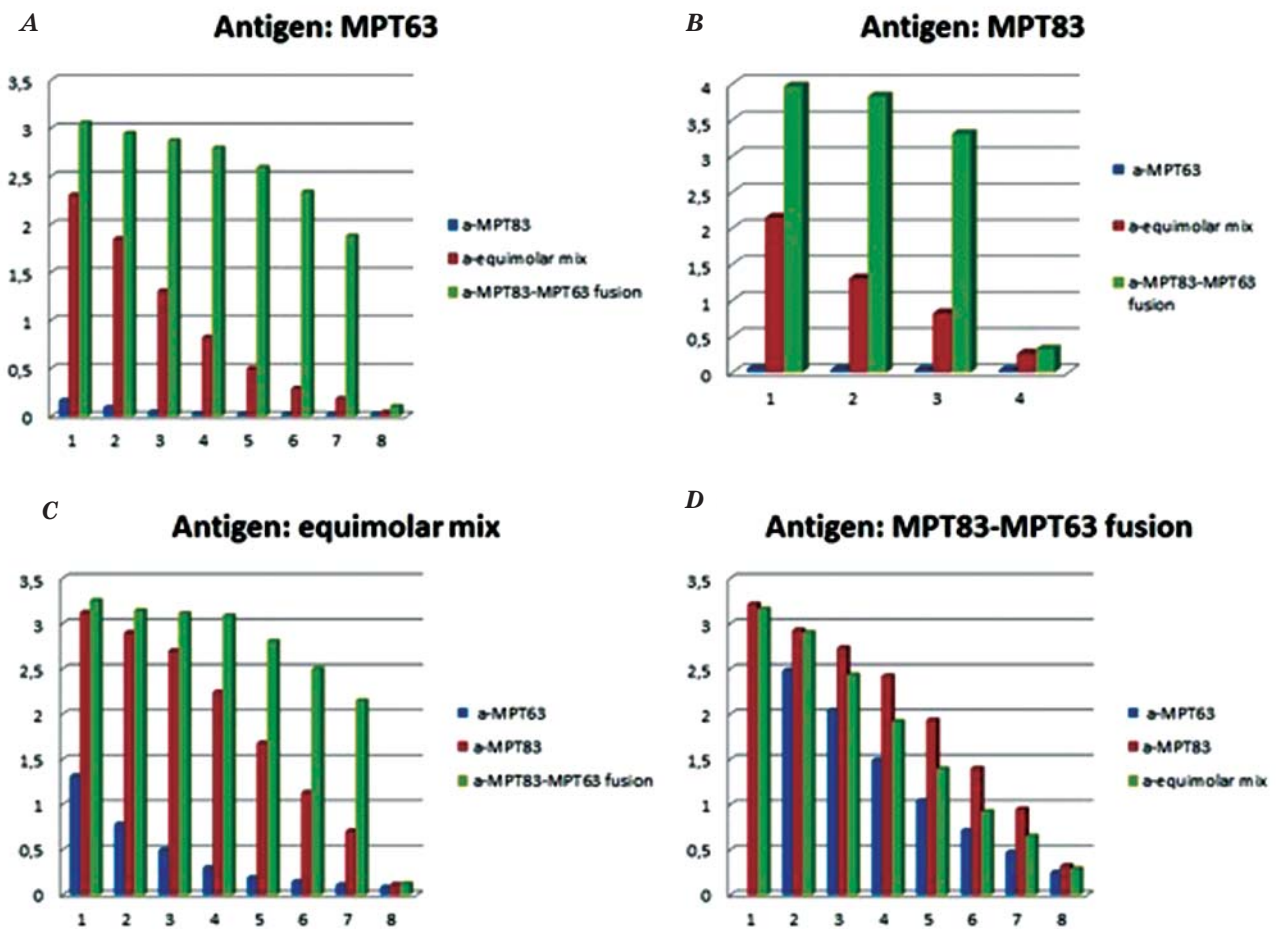
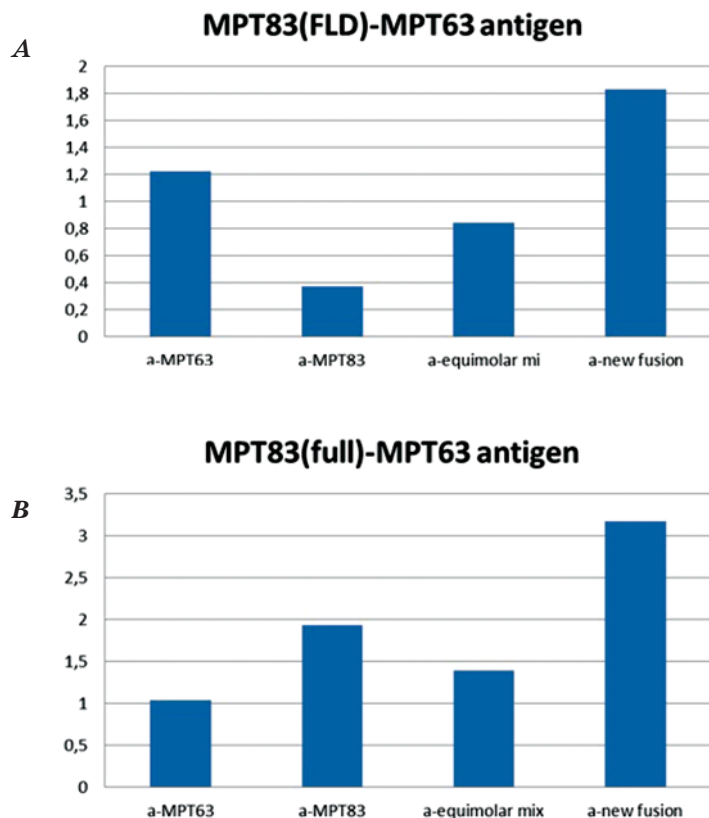


Fig. 4. Results of cross reactivity among four types of antigen substances: A — MPT63; B — MPT83; C — (MPT63+MPT83 mix); D — MPT83-MPT63 fusion with all types of antisera without of own



*Fig. 5. IgG recognition results of MPT83(FLD)-MPT63 fusion (A) or MPT83(full)-MPT63 fusion (B) antigens with 4 types of antisera*

response to MPT63 and MPT83 were used as the matrix for reverse transcriptase reaction for cDNA obtaining with next amplification of sequences encoding the variable domains of the heavy and light immunoglobulin chains. Electrophoresis on agarose gel showed 380 and 400 b.p. PCR products corresponding in size to  $V_L$  and  $V_H$  respectively (Fig. 6, A). The purified  $V_H$  and  $V_L$  were assembled in a single step of assembly PCR as described in [16]. Genes encoding scFv and phagmide pCANTAB-5E vector were treated by restriction endonucleases *SfiI* and *NotI* with next fusion by T4 DNA lygase. *E. coli* XL1-blue was chosen as a host cells.

#### *Selection and characteristic of MPT63 and MPT83 specific scFvs*

For isolation of high specific scFv against mycobacterial antigens we have developed a modified selection scheme described in [17] (we specifically do not focused at the routine antibody selection scheme that has been repeatedly and in detail described previously). After selection of library against MPT63 and MPT83 few obtained colonies studied by the method of Lift Assay. At the same time, most of the colonies were negative (Fig. 6, B). Also, several of the positive clones were tested by immunoenzyme assay. In order to

confirm that the isolated clones were highly specific to MPT63 or MPT83 we analyzed their interaction with a number of control antigens. It has been confirmed that appropriate scFv antibodies recognize MPT63 or MPT83 and do not cross-react among themselves and did not react with BSA or milk casein (Fig. 6, C).

In spite of the fact that the obtaining antibodies were not characterized by a high affinity constant unlike polyclonal antibodies obtained due mice immunization, they are more specific and reminiscent monoclonal antibodies. Our next step will be a more detailed selection of antibodies that can be used as research tools for mycobacterial antigens or even as components of test systems as positive controls.

Improved tools for TB detecting are urgently needed. In this prospective study, efforts were made to evaluate the immunodiagnostic potential of the secretory protein MPT63 and MPT83 lipoprotein of *M. tuberculosis* for developing a novel ELISA-based serodiagnostic test employing an individual, fusion or cocktail of two (16 and 22.6 kDa) recombinant proteins to enhance the sensitivity of the immunoassay, and attempts were also made to check the specificity of the all variants of antigens substances.

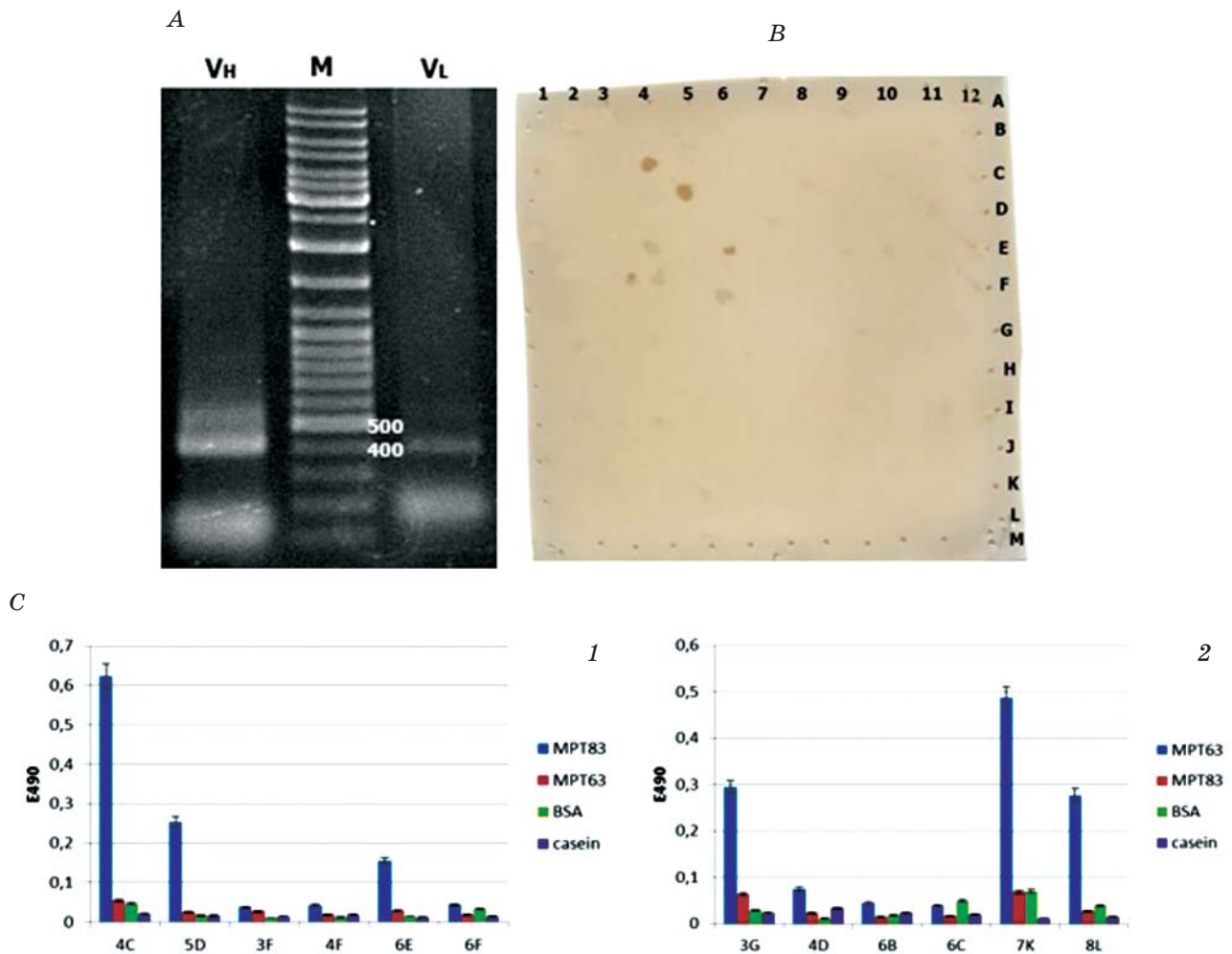


Fig. 6. A — PCR-amplification of VH- and VL-domains; B — Analysis of *E. coli* expressed scFv against MPT83 colonies with Lift Assay method; C — ELISA results of periplasmic extracts containing scFv antibodies against MPT83 (1) and MPT63 (2) from positive (Lift Assay) *E. coli* clones

Our findings clearly demonstrate that for new TB diagnosis ELISA test MPT83-MPT63 fusion protein as plate coating antigen was the best candidate among tested antigens. This chimeric antigen as a cocktail of individual MPT63 and MPT83 characterized by higher immunogenic and antigenic properties than MPT63 or MPT83 individual immune response. But unlike serological diagnostics based on a mixture of antigens or mycobacterial lysate, our proposed MPT83-MPT63 fusion antigen guarantees the reproducibility of the results, since it uses as a homogeneous antigenic substance on a solid-state carrier. Moreover, for antigens mixture, each antigen must be tested separately, which leads to an increase in the cost of the analysis, the timing of its implementation and it's not very adequate evaluation of the results.

It should be noted that due to high level of antigenicity and immunogenicity of fusion protein, it can detect trace amounts of antibodies to the pathogen. Also, the use of two completely non-homologous proteins MPT63 and MPT83 with different effects and hitherto known functions increases its value for the identification of biomarkers of TB infection. The absence of *mpt63* and *mpt83* genes in NTM strains allows the use of this antigen for the diagnosis both *M. tuberculosis* and *M. bovis* (strains that cause pulmonary TB) infected patients.

Thus, based on the results of anti-serum recognition of various proteins and their cocktails, we have chosen a fusion protein MPT83-MPT63 for TB and health patients screening by ELISA for further offer of new diagnostic kit.



**Conflicts of interest**

No potential conflict of interest relevant to this article was reported.

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**РОЗПІЗНАВАННЯ АНТИГЕНІВ  
*Mycobacterium tuberculosis*  
MPT63 ТА MPT83 ПОЛІКЛОНАЛЬНИМИ  
I scFv АНТИТІЛАМИ МИШІ**

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Метою дослідження було охарактеризувати імуноглобулін G (IgG) опосередковану відповідь за умов експериментальної імунізації лабораторних мишей очищеними рекомбінантними протеїновими препаратами *Mycobacterium tuberculosis* MPT63, MPT83, еквімолярним коктейлем протеїнів та штучним злитим протеїном MPT83-MPT63, а також отримати рекомбінантні одноланцюгові варіабельні фрагменти антитіл scFv до MPT63 та MPT83 проти цих антигенів.

Дослідження показало, що гуморальна імунна відповідь до MPT63, MPT83, химерного протеїну MPT83-MPT63 та еквімолярної суміші MPT63 і MPT83 істотно відрізняється. Для кожного антигену рівні сироваткових антитіл оцінювали, використовуючи значення обрізання на основі індексу оптичної щільності. Доведено вирішальну роль MPT83 для імуногенності химерного протеїну та/або коктейлю окремих антигенів за умов імунізації лабораторних тварин.

Отримано специфічні scFv антитіла проти MPT63 та MPT83, які можуть бути використані для розроблення системи для кількісного визначення антигенів, а також вивчення їхніх біологічних властивостей.

Було показано високоімуногенні властивості N-кінцевої ділянки MPT83, що підвищує чутливість ELISA, й запропоновано використовувати химерний протеїн MPT83-MPT63 як перспективний кандидат на роль антигенної субстанції для серологічної діагностики туберкульозу.

**Ключові слова:** антигени MPT63, MPT83, поліклональні антитіла, scFv, діагностика.

**РАСПОЗНАВАНИЕ АНТИГЕНОВ  
*Mycobacterium tuberculosis*  
MPT63 И MPT83 ПОЛІКЛОНАЛЬНЫМИ  
И scFv-АНТИТЕЛАМИ МЫШИ**

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Целью исследования было охарактеризовать иммуноглобулин G (IgG) опосредованный ответ в условиях экспериментальной иммунизации лабораторных мышей очищенными рекомбінантными протеїновими препаратами *Mycobacterium tuberculosis* MPT63, MPT83, эквиволярным коктейлем протеинов и искусственным слитым протеином MPT83-MPT63, а также получить рекомбінантные одноцепочечные варіабельные фрагменты антител scFv к MPT63 и MPT83 против этих антигенов.

Исследование показало, что гуморальный иммунный ответ к MPT63, MPT83, слитому протеину MPT83-MPT63 и эквиволярной смеси MPT63 и MPT83 существенно отличается. Для каждого антигена уровни сывороточных антител оценивали, используя значения обреза на основе индекса оптической плотности. Доказана решающая роль MPT83 для иммуногенности химерного протеина и/или коктейля отдельных антигенов при иммунизации лабораторных животных.

Получены специфические scFv антитела против MPT63 и MPT83, которые могут быть использованы для разработки системы для количественного определения антигенов, а также для изучения их биологических свойств.

Показаны высокоиммуногенные свойства N-концевого участка MPT83, что повышает чувствительность ELISA, и было предложено использовать химерный антиген MPT83-MPT63 в качестве перспективного кандидата на роль антигенной субстанции для серологической диагностики туберкулеза.

**Ключевые слова:** антигены MPT63, MPT83, поликлональные антитела, scFv, диагностика.