

ANNOTATION

to a dissertation for the degree of Doctor of Philosophy (PhD) in the specialty 6D110100 - Medicine Arailym Assylkhanovna Abilbayeva on the theme
"The Identification of diagnostically significant antigenic components of *M. tuberculosis* for use in early immunodiagnosis of tuberculosis"

Relevance of the research topic

Tuberculosis (TB) is one of the persistent global health problems of the population in the world. Despite to a large-scale vaccination, the usage of modern drugs and the implementation of national and international tuberculosis control programs, about 10 million people with tuberculosis and 1.5 million deaths are registered every year in the world (Global tuberculosis report // WHO, 2019).

The fight against tuberculosis in Kazakhstan remains a priority due to its particular relevance and social significance. Programs of anti-tuberculosis measures are being developed, significant funds are allocated for the treatment and rehabilitation of patients with tuberculosis, as well as for the prevention of the disease (Decree of the Government of the Republic of Kazakhstan dated December 21, 2007 No. 1263 "On measures to protect the population from tuberculosis in the Republic of Kazakhstan; Health Development Program -" Densaulyk "on 2016-2020; Order of the Ministry of Health No. ҚРДСМ-108/2020 dated 23.09.2020). The diagnostic capabilities of medical institutions in our country have been expanded – the various methods for diagnosing tuberculosis are used - bacteriological, radiological, molecular genetics, as well as immunological methods - tuberculin skin test (TST), quantiferon test (IGRA), TB-SPOT.

At the same time, despite of the measures were taken, the prevalence of tuberculosis in the Republic of Kazakhstan remains high (World Health Organization (WHO) estimates of the incidence of tuberculosis by country, 2019). One of the ways to solve this problem is to improve the diagnostic methods used today and the introduction of new methods for early diagnosis of tuberculosis, since the used arsenal of diagnostic methods is not effective enough for the diagnosis of all kinds of tuberculosis.

The above methods have both advantages and disadvantages. The bacteriological, X-ray and molecular genetic methods are limited in the diagnosis of early, latent and extrapulmonary forms of TB. Immunological methods also have a number of problems such as false-positive reactions (TST, TB-SPOT), low sensitivity in detecting latent tuberculosis (TST), the inability to predict the risk of developing active disease in infected individuals (IGRA) (Khairunisa Suleiman, 2017). In this regard, there is a need to develop diagnostic approaches that do not have the above disadvantages.

One of the promising areas of modern scientific research in the field of improving early diagnosis of tuberculosis is the creation of effective test systems based on mycobacterial antigens. To solve this problem, the diagnostic efficacy of various *M. tuberculosis* antigens is being investigated all over the world, which make it possible to detect the disease at different stages of development, as well as to carry out differential diagnosis and control the effectiveness of tuberculosis treatment.

As you know, *M. tuberculosis* has a large number of antigens, among which there are secretory, structural and phase-specific antigens. The most diagnostically significant in the immunodiagnosis of tuberculosis were ESAT6 (Rv3875), CFP10 (Rv3874), TB7.7 (Rv2654c), AlaDH (Rv0512), Ag85, LAM (Luo W., Qu Z.-L., 2015; Lalvani A., 2001; Bahk YY, 2004; Chiara DB, 2018; Kumar G., 2008; Broger T., 2019). These antigens are also called immunodominant.

Currently, there is a need to develop diagnostic kits for early diagnosis of tuberculosis, based on the use of immunodominant antigens *M. tuberculosis*, which have shown high sensitivity and specificity. In addition, it is very important that these diagnostics be tested in different geographic conditions, in different populations that have differences in genetic background, immunological responsiveness and social environment.

Purpose of research:

Comparative analysis of the diagnostic potential of immunodominant antigens of *M. tuberculosis* and their combinations for the creation of effective diagnostics

Tasks of research:

1. To study the specific production of pathogenetically significant cytokines upon stimulation with a complex of secretory antigens of *M. tuberculosis* (Rv3875, Rv3874, Rv2654c), Rv0512 and a combination of these antigens
2. To study the diagnostic efficiency of *M. tuberculosis* antigens (Rv3875, Rv3874, Rv2654c), Rv0512 and their combinations for the production of specific antibodies
3. To assess the influence of different types of immune response on the efficiency of antigen-specific cytokine production and antibody production upon stimulation with *M. tuberculosis* antigens (Rv3875, Rv3874, Rv2654c) and Rv0512
4. To analyze the influence of various factors (gender, constitutional, lifestyle, concomitant diseases) on antigen-specific production of cytokines and specific antibodies upon stimulation with *M. tuberculosis* antigens (Rv3875, Rv3874, Rv2654c) and Rv0512.

Objects of research: The blood samples were taken from 280 people aged 18-60 years: 131 patients with tuberculosis, with clinical signs and a confirmed diagnosis based on bacteriological and molecular genetic analyzes, as well as 149 apparently healthy individuals, blood donors, with no clinical symptoms and no signs of pathology. fluorographic image (Order No. 680 of November 10, 2009 (as amended on August 2, 2012)).

Subject of research: cytokines produced by *M. tuberculosis* sensitized T cells and specific antibodies

Research methods: antigen-specific stimulation followed by ELISA analysis, immunochromatographic analysis and statistical methods

Scientific novelty:

In the experiment, the diagnostic potential of synthetic *M. tuberculosis* antigens and their new combination (Rv3875, Rv3874, Rv2654c and Rv0512) was assessed for the specific production of IL-2 and IFN- γ produced by immunocompetent cells.

In addition, in our work, the diagnostic efficiency of *M. tuberculosis* antigens (Rv3875, Rv3874, Rv2654c, and Rv0512) was assessed based on the specific production of antibodies by immunochromatographic analysis.

The diagnostic efficiency of assessing the antigen-specific production of IL-2 upon stimulation with *M. tuberculosis* antigens (Rv3875, Rv3874, Rv2654c and Rv0512) was shown.

The previously unused combination of Rv3875, Rv3874, Rv2654c and Rv0512 antigens has shown high diagnostic efficiency for detecting tuberculosis infection.

The main provisions of the dissertation research submitted for defense:

1. The diagnostic potential of the complex of immunodominant antigens of *M. tuberculosis* Rv3875, Rv3874, Rv2654c is higher than Rv0512 antigen. At the same time, the maximum diagnostic efficiency was demonstrated by the combined use of all the above antigens in tests based on the specific production of pathogenetically significant cytokines and specific antibodies in tuberculosis infection.

2. Under conditions of stimulation with *M. tuberculosis* Rv3875, Rv3874, Rv2654c and Rv0512 antigens, the method for assessing the antigen-specific production of IL-2 showed a higher diagnostic efficiency in comparison with the analogous method using IFN-gamma and the immunochromatographic method with the assessment of the production of specific antibodies.

Practical significance of research:

As a result of the study, an optimal set of synthetic *M. tuberculosis* antigens was proposed, as well as optimal diagnostic methods for the further development of diagnostic kits for early immunodiagnosis of active tuberculosis.

The research results are applicable in immunobiotechnology in the development of test systems for the diagnosis of tuberculosis, as well as in the educational process for students of the specialty "General Medicine", interns, residents, undergraduates and PhD doctoral students, at advanced training courses for medical workers.

Target consumers - immunobiotechnologists, phthisiatricians, GPs, clinical immunologists, molecular biologists.

The results of scientific work were reported at international conferences. According to the results of the study, articles and abstracts were published in medical journals recommended by the Committee for Control in the Sphere of Education and Science of the Ministry of Education and Science of the Republic of Kazakhstan, as well as in a journal peer-reviewed in Clarivate Analytics and Scopus (IF - 3.2).

Personal contribution of the doctoral student:

All the results presented in the thesis and having scientific novelty were obtained by the author personally. The author on the topic of the dissertation conducted a literary search, collection of a database, primary processing of materials. In addition, the author personally conducted an immunological study, interpretation of the results, and statistical analysis of the data obtained.

Approbation of the dissertation results:

The main results of the dissertation were reported on:

1. VII International scientific-practical conference "New concepts of mechanisms of inflammation, autoimmune response and tumor", Kazan, Republic of Tatarstan, June 6-8, 2019. (oral presentation)

2. The Joint Immunological Forum of the VIII Conference of the Russian Cytokine Society: "Cytokines in the diagnosis, pathogenesis and treatment of human diseases", Novosibirsk, Russian Federation, June 24-29, 2019. (oral presentation)

3. International scientific-practical conference "Actual issues of clinical immunology" II Congress of immunologists of the Republic of Kazakhstan, Nur-Sultan, Republic of Kazakhstan, October 4-6, 2019. (oral presentation)

4. International Scientific Conference of Young Scientists "Fundamental Research and Innovations in Molecular Biology, Biotechnology, Biochemistry" to the 80th anniversary of the birth of Academician Murat Abenovich Aitkhozhin, Almaty, Republic of Kazakhstan, November 28-29, 2019. (oral presentation)

5. On the extended interdepartmental meeting of "S.D. Asfendiyarov KazNMU" (Protocol №1, 04.06.2021)

Publications:

According to the research results, 7 scientific papers were published. Of them:

- in a journal included in the international database Web of Science Core Collection (Clarivate Analytics, Impact factor - 3.2) and Scopus - 1
- in journals recommended by the Committee for Control in the Sphere of Education and Science of the Ministry of Education and Science of the Republic of Kazakhstan -4
- in materials of international conferences – 2

The volume and structure of dissertation:

The dissertation work is presented on 125 pages of computer text (font - Times New Roman, - 14 pt), consists of an introduction, a literature review, a description of the material and research methods, own research results, discussion, conclusions, a list of references from 205 sources, applications. The work is illustrated with 54 figures and 22 tables.