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Stability attenuation of BCG vaccine strain

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As the basis for prevention of tuberculosis is the use of BCG vaccine. The experience of using the tuberculous vaccine has shown quite positive results. *Mycobacterium bovis* of BCG vaccine strain, which are preserved in laboratories from different countries may differ slightly in their biological properties, including virulence and immunogenicity. Objective of work is to investigate the stability attenuation of BCG vaccine strain. BCG vaccine strain (BCG-Russia) was used for investigations. The morphology, cultural and biochemical properties of mycobacteria determined by means of agreed-upon methods. We used amplifier iCycler iQ5 and a set of reagents for PCR-amplification of DNA with real-time detection for carrying out PCR. The determination of the virulence and sensitizing properties of mycobacteria was carried out using the biological test. The pieces of the spleen and lungs were taken for histological examination. It was found that *Mycobacterium bovis* BCG strains formed on the nutrient medium by Mordovskiy the matted colonies of ivory color with a hilly surface and uneven edges (R-forms). In the smears from colonies, after staining by Ziehl-Neelsen, acid-resistant and non-acid resistant sticks were observed. Mycobacteria BCG strain in the medium of Shkolnykova formed microcolonies, which morphologically resembled «cord». Gene-molecular studies have established the presence of the DNA-target in the mycobacteria of the vaccinal strain. In infected Guinea pigs 3–4 weeks later, an ulcer was formed at the place of introduction of the suspension. Mycobacteria did not cause in laboratory animals the death and development of an infectious process characteristic of tuberculosis. At the autopsy of Guinea pigs characteristic macroscopic changes (tubercular nodes) were not observed. In the spleen of all animals, moderate hyperemia, red pulp hyperplasia were observed. Lymphoid follicles had signs of hyperplasia. At the intersection of laboratory animals of the second and third passages macroscopic pathoanatomical changes were not found. Conclusion: BCG strain do not cause macroscopic pathoanatomical changes in Guinea pigs during three «blind» passages, indicating the stability of his attenuation.

Key words: biological activity, mycobacteria, BCG strain, virulence, stability of attenuation, Guinea pigs.

Стабільність атенуації вакцинного штаму BCG

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Основа профілактики туберкульозу людей полягає у використанні вакцини БЦЖ. Досвід використання протитуберкульозної вакцини показав достатньо позитивні результати. *Mycobacterium bovis* вакцинного штаму BCG, які зберігаються в лабораторіях різних країн, можуть децю відрізнятися за своїми біологічними властивостями, в тому числі вірулентністю та імуногенністю. Мета роботи: дослідити стабільність атенуації вакцинного штаму BCG. Для досліджень використовували вакцинний штам BCG (BCG-Russia). У мікобактерій визначали морфологію, культуральні та біохімічні властивості загальноприйнятими методами. Для проведення ПЛР використовували ампліфікатор iCycler iQ5 та комплект реагентів для ПЛР-ампліфікації ДНК з детекцією в режимі реального часу. Визначення вірулентності мікобактерій проводили за допомогою біологічної проби. Для гістологічного дослідження відбирали шматочки селезінки та легень. Встановлено, що *Mycobacterium bovis* штаму BCG утворювали на живильному середовищі «Нове» Мордовського матові колонії кольору слонової кістки з горбкуватою поверхнею та нерівними краями (R-форми). У мазках із колоній, після фарбування за Ціль-Нільсеном, спостерігалися кислотостійкі та некислотостійкі палички. Мікобактерії штаму BCG у середовищі Школьникової утворювали мікроколонії, які морфологічно нагадували «косички». Генно-молекулярними дослідженнями встановлено наявність ДНК-мішені у мікобактерій вакцинного штаму. У заражених мурчаків

через 3–4 тижня утворювалася виразка на місці введення суспензії. На розтині морських свинок характерних макроскопічних змін (туберкульозних вузликів) не спостерігалось. У селезінці всіх тварин відмічено помірне повнокров'я, гіперплазію червоної пульпи. Лімфодні фолікули мали ознаки гіперплазії. На розтині лабораторних тварин другого та третього пасажів макроскопічних патологоанатомічних змін не знайдено. Висновок: штам BCG не спричинює макроскопічних патологоанатомічних змін у морських свинок упродовж трьох «сліпих» пасажів, що свідчить про стабільність його атенуації.

Ключові слова: біологічна активність, мікобактерії, штам BCG, вірулентність, стабільність атенуації, морські свинки.

Introduction

As the basis for prevention of tuberculosis is the use of BCG (Bacille Calmette–Guérin) vaccine. The experience of using the tuberculous vaccine has shown quite positive results (Koreckaja, 2011).

For the last time there is a growth of postvaccinal complications (Pasechnik et al., 2014; Lepshina et al., 2015). Growth in the development of complications may be associated with under-studied mechanisms for the formation of antituberculous immunity. Also, the data of the biological cycle of development, the presence of altered (filtered, uncultivated) forms of the causative agent and other are not taken into account (Gizatullin et al., 1980; Chernushenko et al., 2002; Vlasenko et al., 2007; Tkachenko, 2014).

The effectiveness of the vaccination depends on the vaccine fabricated by different producers. *Mycobacterium bovis* of BCG vaccine strain which are preserved in laboratories from different countries may differ slightly in their biological properties, including virulence and immunogenicity (Vijayalakshmi et al., 1995; Ritz et al., 2008; Luca and Mihaescu, 2013; Zhang et al., 2016; Kuljaba et al., 2016).

Objective of this review: to investigate the stability attenuation of BCG vaccine strain.

Materials and methods

For investigations was used of BCG vaccine strain (BCG-Russia strain taken from the BCG vaccine, the producer is «Research and Production Association Microgene» of Ministry of Public Health of the Russian Federation, Stavropol city).

The morphology, cultural and biochemical properties of mycobacteria determined by means of agreed-upon methods (Kassich et al., 1990).

The presence of the cord-factor was investigated by the ability of mycobacteria to form a microcolonies in the form of «plaits», «cord» on 15 day cultivation them in the semisynthetic medium in accordance with Shkolnykova.

For carrying out PCR we used amplifier iCycler iQ5 (producer Bio-Rad, USA) and a set of reagents for PCR-amplification of DNA (*Mycobacterium tuberculosis-Mycobacterium bovis* complex, including BCG strain) with real-time detection («MIKO-GEN»), the producer is Non-governmental organization/ NGO/ DNA-technology, Russian Federation).

The determination of the virulence and sensitizing properties of mycobacteria was carried out using the biological test (Manchenko et al., 1994). To do this, 3 Guinea pigs was infected subcutaneously in the inguinal region by means of suspensions of studied mycobacteria

at the concentration of 10 mg/ml. Non-infected animals were as control.

The Guinea pigs were observed for 90 days. At the end of the experiment animals were euthanized. The pieces of the spleen and lungs were taken for histological examination.

The material was fixed with 10% neutral formalin solution. Paraffin sections were made on a sledge microtome and stained with hematoxylin-eosin (Horalskyi et al., 2005).

To check the stability of mycobacterium virulence, the second and third «blind» biological passages were carried out using Guinea pigs in accordance with the conventional method (Kassich et al., 1990).

Results and discussion

As a result of the research, it was found that *Mycobacterium bovis* BCG strains formed on the nutrient medium by Mordovskyi the matted colonies of ivory color with a hilly surface and uneven edges (R-forms). In the smears from colonies, after staining by Ziehl-Neelsen, acid-resistant and non-acid resistant sticks were observed.

Mycobacteria culture did not grow at room temperature and at temperature 45°C, in meat-peptone agar, in an egg medium with 5% sodium chloride and in the medium with sodium salicylate. It had peroxidase and catalase activity, did not hydrolyze TWEEN-80.

Mycobacteria BCG strain in the medium of Shkolnykova formed microcolonies, which morphologically resembled «cord» (Fig. 1). Such fluffy clumps «resembling on plaits» were 5–10 microns in width and up to 100 microns in length.

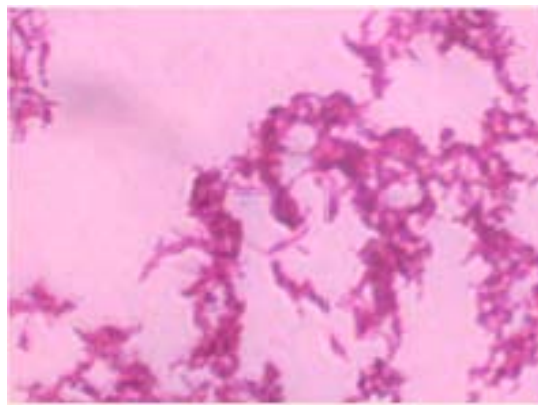


Fig. 1. Microscopical appearance of mycobacterial culture, presence of cording. Staining by Ziehl-Neelsen. $\times 1000$

Gene-molecular studies have established the presence of the DNA-target in the mycobacteria of the vaccinal strain.

In infected Guinea pigs 3–4 weeks later, an ulcer was formed at the place of introduction of the suspension. Animals responded during the experiment to the introduction of tuberculin (PPD) for mammals.

Mycobacteria did not cause in laboratory animals the death and development of an infectious process characteristic of tuberculosis. At the autopsy of Guinea pigs characteristic macroscopic changes (tubercular nodes) were not observed.

As a result of the histological examination of lung specimens of infected animals, alternation of dystelectasis and emphysema sites, hyperemia was established. In sites of reduced airiness, some alveoli contained swollen fluid (Fig. 2). Inflammatory infiltration was weakly inflamed, had a diffuse nature.

In the spleen of all animals, moderate hyperemia, red pulp hyperplasia were observed. Lymphoid follicles had signs of hyperplasia in the form of enlargement at the expense of the expansion of reproductive centers (Fig. 3). The strenuous elements were swollen.

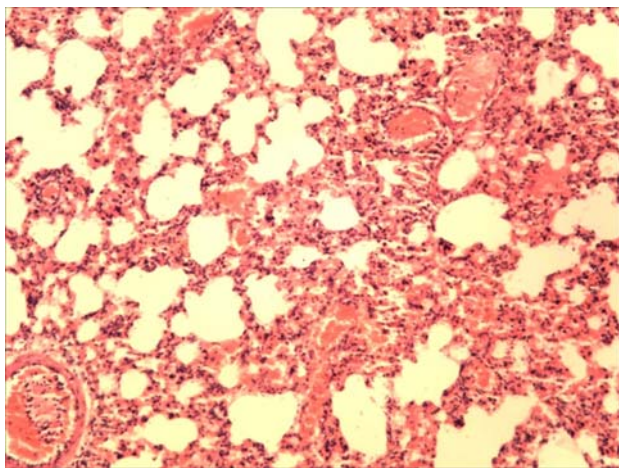


Fig. 2. Morphological changes in the lung: intra-alveolar edema. Stain with haematoxylin and eosin. ×100

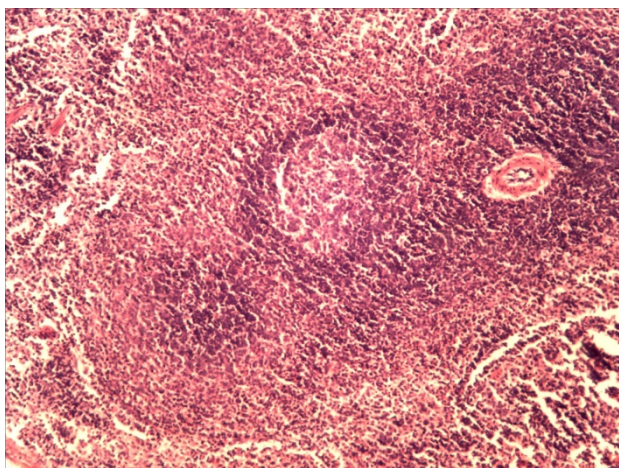


Fig. 3. Morphological changes in the spleen: hyperplasia of lymph follicle. Stain with haematoxylin and eosin. ×100

In our previous works we obtained identical results (Tkachenko et al., 2007; 2012; 2016).

At the intersection of laboratory animals of the second and third passages macroscopic pathoanatomical changes were not found.

Conclusion

BCGvaccine strain do not cause macroscopic pathoanatomical changes in Guinea pigs during three «blind» passages, indicating the stability of his attenuation.

Prospects for further research are to determine the effectiveness of the BCG vaccine in an experiment on laboratory animals of various species.

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