Influence of pathogenic and atypical mycobacteria on immune status of guinea pigs

V. S. Boiko*, L. V. Kovalenko*, A. I. Zavgorodniy*, A. P. Paliy*, A. O. Bondarchuk**

* National Scientific Center "Institute of Experimental and Clinical Veterinary Medicine", Kharkiv, Ukraine
** Kharkiv State Zooveterinary Academy, Kharkiv, Ukraine

Abstract. In recent years a diversity of researchings both native and foreign scientists has been devoted to the investigation of animal tuberculosis. However, there are a number of questions required more detailed studying and scientific justification. Among involved questions specific place is occupied by the problem of animal immunity in cases of infection by pathogenic and atypical mycobacteria. The aim of our work was to study the influence of pathogenic and atypical mycobacteria on the immune status of laboratory animals. The influence of pathogenic and atypical mycobacteria on the immune status of guinea pigs was studied by infecting them with a suspension from the bacterial mass of each individual mycobacterial culture (M. bovis, M. avium and M. fortuitum). The suspension was prepared at the rate of 1.0 mg / ml of sterile NaCl and injected subcutaneously into animals at a dose of 1.0 ml. Blood samples were taken on the 7th, 14th, 30th and 60th days after the injection of mycobacterial cultures to determine the most informative indicators of the immunological status of animals. Amount of neutrophils and phagocytic activity were determined in the blood of experimental animals. The total protein level was determined spectrophotometrically in the serum of guinea pigs, the level of circulating immune complexes (CIC) of average molecular weight – by the method of Grinevich & Alferov, seromukoida – by Weimer & Moshin. The data obtained that M. bovis caused the biggest growth in some cellular and humoral immunity indicate (the percentage of neutrophils by 28.6% and their phagocytic activity by 15.8%, the level of total protein is 76.13 g/l, circulating immune complexes 0.190 mg/ml and seromukoids per day 7 (0.099 mg/ml) on the 30th day after infection in comparison with animals of the control group. Animals infected with the culture of atypical mycobacteria M. fortuitum showed less noticeable changes in immunity, compared to animals infected with the pathogen of tuberculosis.

Keywords: mycobacteria; M. bovis; M. avium; M. fortuitum; immunobiological parameters; guinea pigs.

Уплив патогенних і атипових мікобактерій на імунний статус мурчаків

В. С. Бойко*, Л. В. Коваленко*, А. І. Загородній*, А. П. Палій*, А. О. Бондарчук**

* Національний науковий центр "Інститут експериментальної та клінічної ветеринарної медицини", Харків, Україна
** Харківська державна зооветеринарна академія, Харків, Україна

Анотація. Вивчення туберкульозу у тварин присвячено цілій ряд наукових робіт як вітчизняних, так і зародкових учених. Проте нині залишається низка питань, які потребують більш детального вивчення та наукового обґрунтування. Серед них особливе місце посідає проблема імунітету тварин за інфікування патогенними та атиповими мікобактеріями. Метою роботи було вивчити вплив патогенних і атипових мікобактерій на імунний стан лабораторних тварин. Уплив патогенних і атипових мікобактерій на імунний статус мурчаків вивчали методом інфікування їх забарвлення із бактеріальної маси кожного окремого культури мікобактерій (M. bovis, M. avium i M. fortuitum). Зависілись витрати на готують з розрахунку 1,0 мг/мл стерильного фізіологічного розчину та вводили тваринам підкожно в дозі 1,0 мл. Для дослідження найбільш інформативних показників імунологічного стану тварин на 7, 14, 30 і 60 дійсність введення культур мікобактерій відбирали проби крові. В крові дослідних тварин визначали кількість нейтрофілів і фагоцитарну активність згідно методичних рекомендацій "Методи дослідження маркерів функціонального стану клітин периферичної крові та кісткового мозку тварин" (2013). У сироватці крові мурчаків спектрофотометрично визначали рівень загального білка; методом Гриневича та Алферова (1985) – рівень циркулюючих імунних комплексів середньої молекулярної маси; методом Веймера та Мошин (1952) – серомукоїдів. Результати вивчення впливу культур M. bovis, M. avium і M. fortuitum на імунний статус мурчаків свідчать про те, що збудник туберкульозу M. bovis на 30 дійсність зараження викликає у тварин найбільше підвищення деяких показників клітинного та гуморального імунітету (відсоток нейтрофілів на 28,6%, їх фагоцитарна активність на 15,8%, рівень загального білка до 76,13 г/л, циркулюючих імунних комплексів до 0,190 мг/мл, серомукоїдів на 7 добу до 0,099 мг/мл) порівняно з тваринами контрольної групи. У тварин, інфікуваних культурою атипових мікобактерій M. fortuitum, відмічали менш виражені зміни показників імунітету, порівняно з тваринами, зараженими збудником туберкульозу.
**Introduction**

Despite the achieved success in the control of animals’ infection diseases today the danger is represented by infectious diseases of bacterial etiology, which are characterized by high contagiousness. Tuberculosis – it is the main economically significant bacterial disease (LoBue et al., 2010; Paliy et al., 2015; Bouchez-Zeria et al., 2018; Borja et al., 2018).

Directly under production conditions, questions arise about the non-specific response of animals to tuberculin injection. This is due to many factors that directly affect the immune status of the animal (Jenkins et al., 2018).

The genus *Mycobacterium* includes more than one hundred and fifty recognized species, most of which are in the environment, and many of which may be pathogenic for mammals (King et al., 2017). A *Mycobacterium bovis* causes animals and sometimes humans tuberculosis. In epidemiological terms, the disease can be recorded in some species of wild animals, creating a reservoir of infection (Palmer, 2013). There are exceptional strains of *M. bovis*, which have some features of *M. tuberculosis*. Strains were exposed from 8 patients who lived in Kazakhstan. Though molecular markers were typical for *M. bovis*, patterns of growth and biochemical test results were interstitial between *M. bovis* and *M. tuberculosis* (Kubica et al., 2006). The pathogens of tuberculosis are often isolated from caseous lesions (68%), as well as milk of infected animals (18%), which is a risk to consumers of livestock products (Leite et al., 2003). Mycobacteria of *M. bovis* are straight, or slightly curved, short, thin, with rounded ends of a stick 0.3–1.6 microns wide and 1.5–2 microns long. Inside the cells there are sometimes fills (“Grains Fly”), which are located at the ends of mycobacteria. The size of mycobacteria and the number of inclusions depend on the duration and conditions of cultivation, and the ability to polymorphism is observed both in the cultivated in nutrient media, and in the allocation of them from pathological material, where there are coccoïd forms and rods (Brennan & Nikaido, 1995).

*Mycobacterium M. avium* – thin, oblong, curved, immobile, acid resistant rods with rounded edges, stained by the Ziehl-Neelsen method in a bright red color. *M. avium* is characterized by a strong polymorphism, as a result of which colored smears reveal forms of coccoïd, long-grained and non-granular rods, while coccoïd forms or branched forms predominate in old cultures (Runyon, 1965). *Mycobacterium M. avium* is a major pathogen of domestic, synanthropic, zoo and wild bird tuberculosis. An infected bird dies from a generalized form of tuberculosis in 30–60 days (Mutalib & Riddell, 1988).

Mycobacterioses caused by atypical mycobacteria of *M. fortuitum*, the clinical manifestations of which are characterized by pneumonia and damage to the digestive tract (Okamori et al., 2018), are quite common among people. This type of mycobacterium is widespread in livestock farms in Ukraine, but it does not represent an epizootological risk for animal husbandry, but only predetermines the state of increased sensitivity to tuberculin and allergen from atypical mycobacteria (Kotlyar, 2016).

The aim of our work was to study the effect of pathogenic and atypical mycobacteria on the immune condition of laboratory animals.

**Materials and methods**

Experimental researches were carried out in the laboratory for the study of tuberculosis and the laboratory of clinical biochemistry of the National Scientific Center “Institute of Experimental and Clinical Veterinary Medicine”. The effect of pathogenic and atypical mycobacteria on the immune status of guinea pigs was studied by infecting them with a suspension from the bacterial mass of each individual mycobacterial culture *M. bovis* (strain Vallee), *M. avium* (strain IEKVM UAAN) and *M. fortuitum* (strain № 122). The suspension was prepared at the rate of 1.0 mg/ml of sterile NaCl and injected subcutaneously into animals at a dose of 1.0 ml. To research the most informative indicators of the immunological state of animals on the 7th, 14th, 30th and 60th days after the injection of mycobacterial cultures blood samples were taken. In the blood of experimental animals the amount of neutrophils and phagocytic activity were determined according to the methodological recommendations “Methods of researches markers of the functional state of the cells of peripheral blood and bone marrow of animals”.

In the serum of guinea pigs the total protein level was determined spectrophotometrically, the level of circulating immune complexes (CIC) of average molecular weight — by the method of Grinevich and Allerov (1985), seromucoida — by Weimer and Moshin (1952).

**Results**

The results of conducted scientific experiments to determine the immunobiological parameters of experimental guinea pigs for infection with various types of mycobacteria are shown in Table 1. For the analysis of data in the Table 1, two-way analysis of Fisher variance was used (the first one was the type of culture, the second one – time or age) with the help of “Two-way analysis of variance without repetition” due to “Data mining” ASP MS

<table>
<thead>
<tr>
<th>Cultures</th>
<th>Biomarkers of nonspecific immunity</th>
<th>Blood analysis through the days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>7</td>
</tr>
<tr>
<td>Total protein, gr/l</td>
<td>78.23 ± 1.25</td>
<td>74.31 ± 2.03</td>
</tr>
<tr>
<td>CIC, mg/ml</td>
<td>0.161 ± 0.070</td>
<td>0.172 ± 0.060</td>
</tr>
<tr>
<td>Seromucoids, mg/ml</td>
<td>0.099 ± 0.012</td>
<td>0.097 ± 0.016</td>
</tr>
<tr>
<td><strong>M. avium</strong></td>
<td>Total protein, gr/l</td>
<td>75.24 ± 1.24</td>
</tr>
<tr>
<td>CIC, mg/ml</td>
<td>0.144 ± 0.040</td>
<td>0.149 ± 0.070</td>
</tr>
<tr>
<td>Seromucoids, mg/ml</td>
<td>0.096 ± 0.018</td>
<td>0.094 ± 0.021</td>
</tr>
<tr>
<td><strong>M. fortuitum</strong></td>
<td>Total protein, gr/l</td>
<td>73.54 ± 1.31</td>
</tr>
<tr>
<td>CIC, mg/ml</td>
<td>0.132 ± 0.030</td>
<td>0.138 ± 0.050</td>
</tr>
<tr>
<td>Seromucoids, mg/ml</td>
<td>0.095 ± 0.013</td>
<td>0.094 ± 0.015</td>
</tr>
<tr>
<td><strong>Control</strong></td>
<td>Total protein, gr/l</td>
<td>66.25 ± 1.34</td>
</tr>
<tr>
<td>CIC, mg/ml</td>
<td>0.121 ± 0.060</td>
<td>0.122 ± 0.070</td>
</tr>
<tr>
<td>Seromucoids, mg/ml</td>
<td>0.092 ± 0.013</td>
<td>0.091 ± 0.018</td>
</tr>
</tbody>
</table>
The level of seromucoids, which belong to suppressor proteins, within 30 days of the research, was increased in all experimental groups of animals with the greatest distinction of 7.6% on the 7th day in the serum of guinea pigs infected with M. bovis against control. On the 60th day, an index decrease in the serum of animals of all groups was recorded with a maximum difference up to 6.5% in the group of guinea pigs infected with M. fortuitum.

One of the types of leukocytes that are involved in the protective reactions of the organism, in particular phagocytosis, are neutrophils. Consequently, the dynamic of changing in the number of neutrophils in the blood of laboratory animals infected with various types of mycobacteria was determined (Fig. 1). The data submitted in the 1st Figure shows that under the influence of all three species of mycobacteria, a percentage increase of neutrophils in the blood of guinea pigs was recorded during a 30-day study with a maximum difference by 28.6% animals infected with M. bovis as compared with control. It is also important to note that on the 60th day the decrease of these cells by 10.7% in the blood of animals infected with M. fortuitum was observed, it might be connected to the increased destruction of neutrophils affected by the infection development.

Each neutrophil cell is capable for only one phagocytic event; therefore, their number reflects and agrees upon the data on phagocytic activity (Fig. 2).

The Figure 2 shows the percentage increase of phagocytic neutrophils activity in the blood of animals infected with all three types of mycobacteria studied in comparison with tested animals, but the greatest deviation by 15.8% on the 30th day was noted in the blood of animals affected by M. bovis. The increase number of active phagocytes in the blood, which retain a high phagocytic activity, indicates an increased immune response. During the latter stages of research, a 3.2% decrease of FA in the blood of animals that were infected with M. fortuitum was recorded. Reduction of FA might be connected to excessive formation of immature neutrophils in the bone marrow.

### Discussion

Other studies have found that infection of cattle apart with such cultures as M. bovis, M. tuberculosis and M. kansasi also shows a different immune response of animals. The delayed-type hypersensitivity was revealed by inoculation of each culture of mycobacteria; however, the response to the injection of M. bovis and M. tuberculosis exceeded the response to infection with M. kansasi.

Specific responses were given by all animals due to injection of M. tuberculosis and M. bovis in 3 weeks after inoculation. After 6 weeks animals infected with M. tuberculosis had a decreased immune response, whereas animals with M. bovis remained the same. When inoculating M. kansasi, early initial antibody responses decreased 10 weeks after the start of the experiment. These results show that the immune response of animals is due to antigenic load, and not the development of the pathological process (Waters et al., 2010).

Various natural mechanisms of different macro organisms for inactivation of the pathogen of M. tuberculosis reported, which in turn causes the induction of adaptive immunity (Crevel et al., 2002).
A detailed study of the immune response of the macro organism to its infection with various types of mycobacteria, including those with natural and artificial mutations, opens the way to the creation of effective vaccines (Blanco et al., 2012).

Conclusions

Comparative studies about the effect of the cultures *M. bovis*, *M. avium* and *M. fortuitum* on the immune status of guinea pigs indicate that *M. bovis*, the pathogen of tuberculosis, on the 30th day after infection, caused the animals the greatest increase in some indicators of cellular and humoral immunity (neutrophils by 28.6% and their phagocytic activity by 15.8%, the level of total protein was 76.13 g/l, circulating immune complexes 0.190 mg/ml and seromukoids per day 7 (0.099 mg/ml) in comparison with animals of the control group.

Animals infected with the culture of atypical mycobacteria *M. fortuitum* showed less noticeable changes in immunity, compared to animals infected with the pathogen of tuberculosis.

References


