THE EFFECT OF THE HUMORAL FACTOR OF IMMUNIZED GUINEA PIGS ON THE VIRULENCE AND CATALASE ACTIVITY OF Bacillus tuberculosis

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It has been established [3] that during the first month after vaccination the serum of immune guinea pigs acquires the properties of suppressing multiplication of the tubercle bacilli and of altering microbial catalase activity. In addition, the modern literature includes a significant number of reports devoted to studies of altered virulence of the tubercle bacilli as a result of action of antibacterial preparations. The data [10] pertaining to decrease in virulence of tuberculosis microbes that have acquired a resistance to isoniozid were confirmed in studies carried out in vitro and in vivo [4, 6-9, 11, 12, 15, 16, 19, 20]. Strains of tuberculosis mycobacteria resistant to phthivazid also exhibit a decreased virulence to guinea pigs [1, 2]. A decrease in virulence in mycobacteria resistant to antibacterial preparations is accompanied by lower catalase activity. However, certain investigators [13, 21] observed an opposite relationship between the degree of virulence and the catalase activity of the tubercle bacillus, and a number of investigators [5, 18] found no relationship between these properties.

In this investigation we studied the effect of the immune guinea pig sera on the virulence of tuberculosis mycobacteria and established the nature of the relationship between virulence and the catalase activity of these microbes.

EXPERIMENTAL METHODS

A strain of bovine tubercle bacillus (No. 109 of the Leningrad Institute of Tuberculosis) was grown on Sutton's medium. On the 30-35th day after inoculation, 0.25 volume of guinea pig serum, obtained at different time intervals after immunization (1 mg of dried BCG vaccine in 0.2 ml of physiological solution intradermally) was introduced under the surface growth. To remove contaminants, 1 ml of physiological solution containing 300 units of penicillin and 300 units of tetracycline was added to the serum [14]. On the following day guinea pigs were inoculated with the treated culture (0.06 mg of raw culture in 0.5 ml of physiological solution, subcutaneously). Animals were sacrificed after 45-50 days and autopsied; macroscopically observable organ pathology was studied to evaluate the virulence of tuberculosis mycobacteria (right ventral lung region, liver, spleen, regional, and mediastinal lymph nodes). Four groups of guinea pigs (4 animals per group) were used in the study. Animals infected with tubercle bacillus treated for 1 day with antibiotics served as controls. The number of infected control animals was equal to that of the experimental and both groups were sacrificed simultaneously.

The catalase activity of the causative agent of tuberculosis was determined in each experiment by the iodometric method.

RESULTS

The destruction of organs, observed in the controls, was clearly evident at all times. Mediastinal nodes were definitely denser. The lung region examined contained from 30 to 50 medium caseous tubercles (9 cases); in 7 cases numerous confluent tubercles were observed. Large confluent areas reaching 6 mm in diameter (in 8 cases) or numerous small caseous tubercles (8 cases) were seen in the liver. Numerous massive caseous areas were found in the spleen; the organ was greatly enlarged—to 56 × 22 mm, the average weight was 5 g (3.2 - 6.7 g). Regional lymph nodes were 10 to 18 mm in diameter.

Macroscopic organ destruction in the animals of the experimental group was less pronounced, which is explained primarily by the decreased virulence of the tuberculosis mycobacteria and not by their inadequate concentration, since guinea pig serum did not suppress mycobacterial multiplication after the prolonged post-immunization period [3]. By the 4th day after vaccination guinea pig serum decreased the virulence of mycobacteria; one lung region had 20 tubercles, the liver and spleen showed extensive changes, but less so than in the controls. The effect of serum removed from animals on the 15th post-immunization day was more pronounced, and guinea pig serum obtained on the 30th post-vaccination day was most active. The culture treated with this serum did not produce an enlargement of mediastinal nodes in the infected animals; one lung region contained 10-12 caseous tubercles and no macroscopic changes were observed in the liver. The spleen was of normal size and weighed 1.03 to 1.5 g; no macroscopic damage was detected. After prolonged post-immunization period—on the 8th day—the serum still suppressed significantly the virulence of the tuberculosis mycobacteria: mediastinal lymph nodes were not enlarged, the lung region contained 22-25 small tubercles, liver showed no changes, the spleen had normal dimensions (28 × 15—32 × 16 mm in area, 1.1—1.4 g in weight). No damage was noted in 2 cases and in 2 cases there were individual tubercles. The regional lymph nodes of all experimental animals were enlarged similar to that in the controls: their diameter varied from 7 to 18 mm.

Immune guinea pig serum always lowered the catalase activity of the tubercle bacillus: on the 4th day after immunization the activity was 80% that of the control, on the 15th day—69.5%, on the 30th day—90.2% and on the 80th day—58%. However, there was no correspondence between the degree of suppression of enzymatic activity and the degree of decrease in virulence of the tuberculosis mycobacteria.

These results confirm that, in the course of developing anti-tuberculosis immunity, guinea pig serum really acquires the property to act on the tubercle bacillus; it not only suppresses the multiplication and lowers the catalase activity, but also noticeably decreases the virulence. It should be pointed out that the absence observed [3] in the animal serum during the late immunization period of a factor suppressing mycobacterial multiplication does not indicate the loss of ability to affect the virulence of the tubercle bacillus.

The decrease in virulence of the tubercle bacillus is accompanied by a decrease in the microbial catalase activity, but complete correspondence in these properties was lacking. Thus, the more significant decrease in virulence was observed in the cases showing the least decrease in the catalase activity (a culture treated with serum obtained on the 30th day post-immunization day). Evidently, one should support the opinions of the few investigators [5, 18] who deny the relation of catalase activity of the tubercle bacillus to the degree of its virulence, inasmuch as these properties are, evidently, independent of each other, not related directly to each other by physiological manifestations of the etiologic agent of tuberculosis.

SUMMARY

It was found that the serum of immunized guinea pigs acquired the capacity to reduce the virulence of tuberculosis mycobacteria. There was also an attendant reduction in the catalase activity of tuberculosis bacilli although no parallelism in the changes of these properties was observed. The catalase activity and the extent of virulence do not seem to be directly associated with each other, insofar as manifestations of the vital activity of tuberculosis bacilli are concerned.

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