LATENT TUBERCULOSIS IN WHITE MICE

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V. S. Libenson, V. I. Braude, V. F. Chernyshev, and V. K. Vasil'ev

Department of Experimental Pathology and Therapy (Head—I. M. Bondarev, Doctor of Medical Sciences), Moscow Scientific Research Institute for Tuberculosis (Dir.—T. P. Mochalova, Candidate of Medical Sciences), Ministry of Health, RFSFR
Presented by P. D. Gorizontov, Active Member, Academy of Medical Sciences, USSR
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White mice have recently come into ever wider use in studying various problems of the therapy and prophylaxis of tuberculosis [1-7]. All authors note that these animals have a high natural resistance and that it is consequently necessary to administer large doses of Mycobacterium tuberculosis in order to induce the disease. For example: mice die of tuberculosis 30-40 days after intravenous injection of 0.1 mg of a high-virulence culture of the bovine type [3].

The present work was devoted to a study of the fate of Mycobacterium tuberculosis administered to mice in minimal doses and of the reactions of these animals to such administration.

EXPERIMENTAL METHOD AND RESULTS

In the 1st series of experiments 170 mice were inoculated intravenously with various doses of bovine Mycobacterium tuberculosis, of attenenuated-virulence strain No. 8. Doses of 0.1 and 0.01 mg caused tuberculosis in all the mice. Of the 30 mice inoculated with 0.0001 mg of the culture 3 survived for 10-14 months without contracting tuberculosis. Of the 23 mice which received 0.00001 mg of the culture only, 2 contracted tuberculosis, 17 surviving for from 6-14 months after inoculation.

A dose of 0.000001 mg of the culture, administered to 70 mice, did not cause a single case of macroscopically detectable tuberculosis. More than half the animals survived for from 6-16 months after inoculation. Histological examination of the lungs, liver, and spleen revealed no tuberculous changes in the majority of the animals. Isolated foci were detected in the lungs of only 3 of the 7 mice killed after 16 months. Mycobacterium tuberculosis was usually not detected on microscopic examination of smears (stained by Ziehl-Neelsen's method and investigated by fluorescence microscopy). However, cultures of internal-organ homogenates yielded the initial microorganism in 32 of the 70 mice. In 15 cases cultures were obtained from the organs of mice inoculated 6-14 months previously.

We conducted the following experiment to study the dynamics of the histological changes and multiplication of Mycobacterium tuberculosis in the internal organs of white mice inoculated with small doses of this bacterium.

In the 1st group 71 males with an average weight of 16-18 g received 0.000001 mg of a semidry culture of Mycobacterium tuberculosis, strain No. 8. As a control 62 mice were inoculated with the same culture in a dose of 0.001 mg (2nd group). Before the experiment began the culture was passed through a rabbit and a mouse, which increased its virulence. However, the maximum possible virulence was not achieved, as is indicated by the results of intravenous injection of 14 mice with 0.1 mg of this culture: half of them died of tuberculosis only after an average of 3.5 months. Five animals from each group were killed after 3 days, 1, 2, and 3 weeks, and every month thereafter. Four mice from the 1st group were killed after 10 months and the last two after 11 months; the last 2 mice in the control group were killed after 9 months. The lungs, liver, and spleen were subjected to histological investigation. Cultures were made from the organs of 3 mice of the 1st group and 2 mice of the 2nd group on Hel'berg's medium.

Tuberculous foci were detected in the lungs of almost all the mice in the 2nd group 3 months after inoculation. Such foci were noted in only 6 of the 11 mice inoculated with 0.000001 mg of the culture, which survived for 9-11 months. We were unable to isolate Mycobacterium tuberculosis from the organs of any of the animals in this experi-

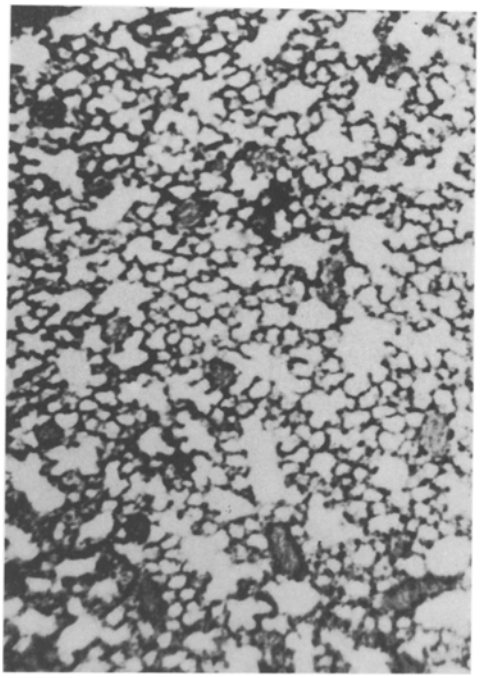

Fig. 1. Severe infection of the pulmonary vessels and acinous edema of the alveoli. Microphotograph. Hematoxylin-eosin staining. 10 × ocular, 8 × objective.

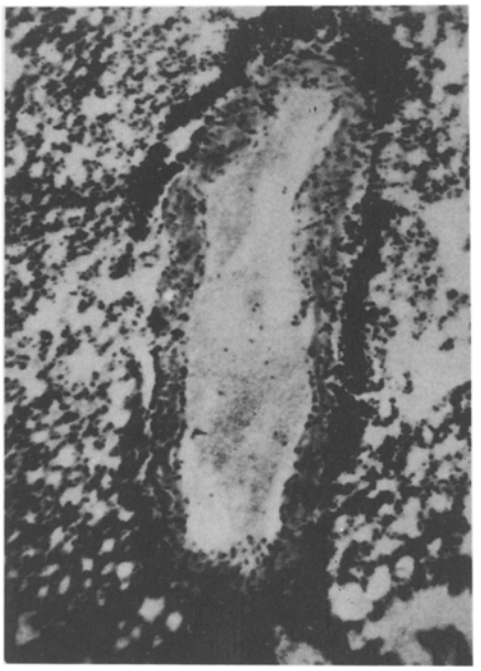

Fig. 2. Perivascular and interstitial lymphoid infiltration of the lung. Microphotograph. Hematoxylin-eosin staining. 10 × ocular, 8 × objective.

ment. In the animals of the 2nd group the extent to which Mycobacterium tuberculosis could be cultured reached its maximum 3 weeks after inoculation and remained at this level until the end of the observation period. Isolated colonies of this microorganism were cultured from the organs of the mice of the 1st group from the 1st few days after inoculation onward. Intensive multiplication of the causative agent was detected in the tissues, especially the spleen, from the 1st month onward. Throughout the entire experiment fewer bacteria were cultured from the liver and, especially, the lungs of the mice of the 1st group than from their spleens; it was only after 9-11 months, when some of the mice exhibited macroscopically detectible tuberculous lesions in the lungs, that the quantity of bacteria cultured from the lungs became greater than the quantity cultured from the spleen and liver. It was maximal throughout the entire observation period.

The appearance of macroscopically detectible tuberculous lesions in the lungs of the mice thus coincided with a sharp increase in the quantity of Mycobacterium tuberculosis present. This is also confirmed by a comparison of the extent to which this microorganism was cultured from the lungs of 4 simultaneously-killed mice with macroscopically detectible tuberculosis and from the lungs of 4 mice which appeared healthy (all from the 1st group). While the mean culturability index for the former was 3.5 ± 0.4, for the latter it was 1.8 ± 0.3 ($P < 0.02$).

As histological investigations showed, the Mycobacterium tuberculosis culture failed to cause the morphological reactions specific to tuberculosis in any of the animals before 6 months and in almost half the animals which survived for from 6-11 months after inoculation. This means that the tuberculous infection took a latent course. However, this latency was relative. Three days after administration of 0.000001 mg of the Mycobacterium tuberculosis culture we noted circulatory disturbances, in the form of arterial hyperemia and pulmonary edema; we believe these to be a primary irritation reaction to infection (Fig. 1). This reaction is quite transient; it disappears within several days. From the 3rd week onward there are widespread changes in the local lymphatic apparatus: the lymph nodes undergo hyperplasia and annular lymph-cell infiltrations are formed about the vessels. The pulmonary lymphatic hyperplasia develops earliest, is most marked, and lasts longest (Fig. 2). These hyperplastic changes in the lymphoid and reticular tissue may apparently be regarded as a morphological manifestation of immunogenesis.

Intravenous administration of minimal doses of Mycobacterium tuberculosis to white mice may thus lead to the development of a latent infection. Its duration (at identical doses) depends on the virulence of the causative agent and the individual resistance of the subject; under certain conditions animals may apparently remain infected

without contracting tuberculosis for the greater part of their lives or even their entire lifetimes (the average life-span of a white mouse is 2 years).

The latent-infection model may, in our opinion, be used to study certain problems of the immunobiology of tuberculosis and for investigating the influence of vaccination, chemoprophylaxis, and other factors on the infected organism.

SUMMARY

Following intravenous injection of 0.000001 mg of tuberculosis bovine type culture with attenuated virulence, albino mice may become mycobacteria carriers for more than a year without developing the disease.

In the authors' opinion, this model latent tuberculosis infection affords a favorable opportunity for studying some problems of tuberculosis immunobiology, notably the factors either conducive or preventive to the development of the infection into the disease.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
