STUDIES ON THE REINFECTION IN EXPERIMENTAL TUBERCULOSIS OF RATS

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INTRODUCTION

In the previous paper1), the reinfection in the experimental tuberculosis of guinea pigs was studied in various aspects. In the present paper, the reinfection experiment conducted similarly in rats was reported. It has been well demonstrated that rats are congenitally resistant to the infection due to tubercle bacilli, by the investigations of Ornstein et al2), Boquet et Nègre3), Goldenberg4), Kato5) and so on6)7). Basing upon the data reported by them that tubercle bacilli can multiply considerably in those resistant rats, Lurie8) speculated that the host-parasite relationship between rats and tubercle bacilli may be symbiotic one. More recently, Wessels9) pursued the development of the experimental tuberculosis in rats through both histological observation and quantitative bacterial cultivation of various tissue. And he explained the antituberculous resistance of rats from his findings that tuberculin allergy did not develop and caseous lesions were not formed in those animals.

On the other hand, however, the investigations to have shown the antimycobacterial immunity of rats in the reinfection experiment have not yet been reported hitherto, possibly due to the technical difficulties to differentiate the bacilli of primary from secondary infections. In this connection, experiments were undertaken in an attempt to determine whether or not an anti-mycobacterial power can be shown in tuberculous rats against secondly injected tubercle bacilli. The basic principle of the experimental procedure was just the same as that of the previous paper1).

MATERIALS AND METHODS

1) Microorganisms: Three strains of tubercle bacilli, H37Rv, H37RvR-SM and 386R-INAH were used. The first two were sent from Dr. Karlson of the Mayo Foundation. The last was a pure isoniazid-resistant substrain obtained from the parent strain 386 which had been isolated in this laboratory from the sputum of a tuberculous patient.

2) The preparation of bacillary suspension, the method to evaluate the viability of those suspensions and the quantitative enumeration of viable organisms living in various tissue were described in detail in the previous paper.

3) Animals: Normal albino rats weighing approximately 100 g were used.

EXPERIMENTAL

1) The fate of tubercle bacilli in rats when they are administered intra-
venously in a large or small amount: The multiply and survival of tubercle bacilli in animal body are principally determined by the virulence of the bacilli and the intensity of the immunity produced in response to the bacillary multiplying during the infectious process. Therefore, the analysis of the proliferation curve of the bacilli in rats may shed some light on the anti-mycobacterial immunity in these animals. With this point of view, an experiment was made as follows. Two groups of rats were used, each consisting of ten. The animals of one group were injected intravenously in the caudal vein with 0.1 mg of H37Rv \(36 \times 10^5\) viable units) and those of the other group were treated in the same way with 0.001 mg \(36 \times 10^3\) v. u.) of H37Rv. The animals of each group were sacrificed two by two each one, fourteen, twenty-eight, forty-two and fifty-six days after injection, to conduct a quantitative enumeration of viable bacilli surviving in the spleen, liver and lung. The data were summarized in Fig. 1.

Fig. 1. The fate of intravenously-injected tubercle bacilli in rats

2) Comparative study of the fate of primary and secondly introduced tubercle bacilli in rats: It is a fundamental principle of tuberculous immunity that once infected individuals show a definite resistance against a subsequent infection. In this connection, an experiment was undertaken as follows in order
to examine this kind of immunity in rats. Two groups of rats were arranged, each consisting of ten. The animals of one group were infected with $25 \times 10^3$ viable organisms of the strain 386R-INAH. Three weeks after, all the animals of these two groups were infected with 4,500 viable cells of H37RvR-SM. Then, in just the same manner as before, the isolation of the primarily or secondly introduced tubercle bacilli was made at intervals of two weeks by sacrificing two animals of both groups each time. The differential isolation was possible using simultaneously Ogawa medium containing streptomycin or isoniazid in the concentration of 100 μg/ml. The result was demonstrated in Fig. 2.

**Fig. 2.** The fate of reintroduced tubercle bacilli in rats

![Graph showing the fate of reintroduced tubercle bacilli in rats](image)

**RESULTS AND DISCUSSION**

In Fig. 1, it is learned that the behaviour of tubercle bacilli to multiply in rats was different between in the lung and in the spleen or in the liver. During the observation period of eight weeks, the bacilli continued to multiply in the lung, but they ceased to increase in number in the spleen and liver in two or four weeks showing the almost horizontal growth curve thereafter. This tendency was the same as that reported by Wessels\(^9\). Thinking from the standpoint described just previously, the anti-mycobacterial immunity is considered to be manifested more remarkably in the spleen and liver than in the lung. This
is quite in contrast with the findings of Hashimoto in guinea pigs that the antibacterial immunity was most remarkably shown in the lung. In addition, macroscopical tubercles were found only in the lung, but they did not show any sign of caseation. The spleen was characterized with hyperaemia and swelling, but no tubercle was found. The spleen weight reached approximately 0.8 g in the animals inoculated with 0.1 mg of the bacilli. All the animals maintained the tendency of body weight to increase during the experimental period, but they did not react to a 1:10 dilution of OT in the examinations at any time. In Fig. 2, it is evidently shown that the reintroduced tubercle bacilli were markedly inhibited of their multiplication in the tuberculous rats, particularly in the earliar period of reinfection. Of utmost interest was, however, the fact that this antibacterial immunity against reintroduced tubercle bacilli was manifested most intensely in the lung. Recently Dubos and his co-workers and Abe have also shown this type of immunity in the experimental tuberculosis of mice in which tuberculin allergy does not develop, claiming as an evidence for the view that the tuberculin allergy is one thing and the immunity is another thing. On the other hand, however, there has been no experimental evidence that the acquired immunity against tubercle bacilli develops in mice and rats in the same mechanisms as that in guinea pigs. Both tuberculin allergy and immunity develop on the basis of tuberculous infection. Therefore, if the host-parasite relationship between tubercle bacilli and the host differs, then the nature of the immunity may be different. Particularly in tuberculosis, the infectious process has been said to develop on the relationship between mononuclear phagocytes and tubercle bacilli. Recent investigations made by Brieger, Suter, Mackaness have attached further significance to this view. In this country, reported recently from his histopathological study that in guinea pigs the number of the monocytes which appear in response to the introduction of tubercle bacilli is large but the bacilli ingested by those cells are not so numerous. Contrary to this, the appearance of the monocytes is not so large in rats as in guinea pigs but the bacilli ingested are numerous. As well known comparing with our previous paper, the rats maintained the healthy condition against the vigorous proliferation of tubercle bacilli in their bodies to such an extent as it killed the host in the case of guinea pigs. Wessels considered that the resistance of rats to tuberculous infection may be due to the fact that the cells ingesting tubercle bacilli do not come to necrosis and caseation. In this connection, the report that tuberculous rats take fatal outcome when they are treated with cortisone is of particular interest. Lastly, the author should like to think about the nature of the immunity in rats. The term “immunity” usually suggests a kind of biological phenomenon in which the antigen-antibody reaction is taking a part in a direct or indirect way. On the other hand, the phenomenon that bacterial cells multiply and then decrease in the number of viable cells occurs not only animal body but also in culture.
medium. And in the latter case, the change of pH and the accumulation of metabolites in the medium may be principal factors responsible for the phenomenon. If we consider the interior of mononuclear phagocytes as culture medium, the fate of the bacilli ingested by them may be understood without paying any particular considerations to the antigen-antibody reaction in vivo. Recently, Dubos\textsuperscript{19)}\textsuperscript{20}) pointed out it important to think the infectious process from the physicochemical standpoint. At present, we should like to present a way of explanation for rat tuberculosis that the host-parasite relationship between rats and tubercle bacilli should be called “mutually indifferent”, even though it is not symbiotic, and the immunity-like phenomenon against tuberculous infection may be of non-antibody nature.

**SUMMARY**

1) An antituberculous immunity was demonstrated in rats.

2) The nature of this immune power was discussed apart from the traditional antigen-antibody concept.

**REFERENCES**


