FIELD STUDIES ON TUBERCULIN REACTION

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INTRODUCTION

Tuberculin skin test was, in former days, endowed with a great value as one of the indispensable tools for the investigation of the epidemiology of tuberculous infection. BCG vaccination, however, has been widely adopted in this country following the promulgation of BCG vaccination law on July 1st 1949. The majority of the people, therefore, are now conferred with tuberculin hypersensitivity of various intensity, and the distribution of tuberculin positive reactors in a population does not necessarily mean its density of tuberculous infection. Under these circumstances, the tuberculin test has become to bear twofold purposes, first, to detect naturally infected individuals, second, to select those who are desirable to be vaccinated with BCG. Up to present, the tuberculin test has been conducted solely with a 1:2,000 dilution of OT as a routine procedure, for which the study of Nobechi and his co-workers\textsuperscript{1, 2} had presented a theoretical foundation. The hypersensitivity conferred by BCG vaccination, however, is not always the same in its intensity and duration, but varies in each individual to a considerable extent. Therefore, the general state of tuberculin sensitivity of the people in Japan must have changed to show a more complicated figure in comparison with that of the former days when only the naturally infected individuals reacted specifically to tuberculin with marked erythema and induration. In this connection, an attempt was made to analyze the frequency distribution of the sizes of skin reaction using various dilutions of OT in naturally infected and BCG vaccinated groups in order to obtain more detailed observations on the problems suggested above.

GENERAL DESIGN OF THE STUDIES

The present investigation was carried out employing eight groups of school children who ranged in age from 7 to 15 years in several primary schools located in the suburbs of Takada city in Niigata Prefecture. In these schools, BCG vaccination has been conducted using liquid vaccine yearly in the past seven years on the tuberculin negative children in the intradermal Mantoux test with a 1:2,000 dilution of OT. This time, in each group, the examinees were injected intracutaneously with 0.1 ml of a 1:2,000 dilution of OT at the flexor surface of the forearm of one side, and simultaneously, with 0.1 ml of another dilution of OT at the corresponding site of the opposite forearm. The tuberculin solutions of seven different concentrations were prepared by diluting the standard OT maintained at the National Institute of Health in buffered saline. The erythema elicited was read at the end of 48 hours, and the longest and shortest diameters of erythema were measured. The mean value of two diameters was regarded as the size of the reaction, and with this mean value the frequency distribution curve of the intensity of the tuberculin hypersensitivity was drawn for each dilution of OT used as will be described below.
This group was arranged to consist exclusively of the naturally infected individuals, and the total number of members was 392. In this group, the frequency distribution curves of the sizes of erythema elicited by the standard (1:2,000) and a 1:25,000 dilution of OT were drawn. The graphical presentation of the data is in Fig. 1 which shows a marked difference between the results of these two cases. Roughly speaking, the relation between the size of erythema and the rate of its occurrence in the test with a 1:25,000 dilution of OT is manifested in steeply decreasing curve running from the high frequency of small reaction to the low frequency of large reaction, and no case of erythema over 25 mm was observed. On the contrary, as shown in Curve I, the frequency of the size erythema elicited by a 1:2,000 dilution of OT increases as the size grows larger and the maximum point is present in the range of 17–18 mm, after which it is likely to decrease gradually, although the frequency of the sizes over 25 mm shows a high peak as they are not divided into more subclasses. As for the occurrence rates of double-erythema and definite induration, those responded to a 1:2,000 dilution were in the percentage of 15.6 and 58 respectively and those responded to a 1:25,000 dilution were in the percentage of 0.25 and 3.3 respectively. In short, it will be easily concluded that the intracutaneous injection of 0.1 ml of a 1:25,000 dilution of OT has only a very weak
capacity to elicit skin reaction even on naturally infected individuals. Although the individuals with erythema more than 10 mm are usually regarded as positive reactors in the test with 0.1 ml of a 1:2,000 dilution, this criterion should be altered when tested with the same dose of a 1:25,000 dilution of OT. Thinking from the fact that the occurrence rate of the erythema over 10 mm in the former case is higher than that of the erythema over 5 mm in the latter case, the erythema over 5 mm may be taken as a positive reaction in the test with a 1:25,000 dilution of OT. In practice, naturally infected subjects often show a skin reaction so intense that it results in blister formation or central necrosis in the test with a 1:2,000 dilution of OT. For the purpose of avoiding such severe reaction, we had better use a more diluted OT of a 1:25,000. Then, to those who are not sensitive to this concentration, a dilution of 1:2,000 can be employed for the retest of their tuberculin hypersensitivity without causing excessively severe reaction.

![Graph of frequency distributions of diameter of erythema elicited by the 1:100 dilution of OT in a tuberculin negative group to the 1:2,000 dilution of OT.](image)

**Fig. 2** Frequency distributions of diameter of erythema elicited by the 1:100 dilution of OT in a tuberculin negative group to the 1:2,000 dilution of OT.

**Study 2** (Group 2 . . . . . . Fig. 2)

Group 2 consisted of 210 children who showed erythema less than 9 mm in the test with a 1:2,000 dilution of OT. Re-examination of their tuberculin reactivity was made using a 1:100 dilution in order to draw the frequency distribution curve of the sizes of erythema. The results are as Fig. 2, which indicates that the erythema over 10 mm to a 1:100 dilution occurred in the rate of approximately 36 percent in this group. As to the significance of the reactivity to this dilution, nothing definite can be said only from this experi-
ment. It is undeniable, however, that the occurrence of the erythema over 10 mm in the test with a 1:100 dilution is partly due to the intensified non-specific reaction and partly due to the enhancement of specific reaction. The detailed analysis of this point has been reported by Someya and his co-workers. In Fig. 2, it is shown that the occurrence rate of 7 to 10 mm erythema in the test with the standard tuberculin is approximately the same as that of erythema over 15 mm in the test with a 1:100 dilution of OT, hence these reactions may be allowed to consider as the specific reaction against the active principle of tuberculin. It will be very difficult, however, to decide whether those individuals who show the erythema less than 10 mm in the test made with a 1:2,000 dilution but react with the erythema more than 15 mm in the test made with a 1:100 dilution should be vaccinated with BCG or not. On the other hand, however, judging from the fact that induration was palpable only in 5.2 percent of those examined with a 1:100 dilution, it can be said that almost all of them were of such a low tuberculin sensitivity as BCG vaccination can be made causing any harmful effect. This view has been supported by the long years experience of BCG vaccination in this country.

Fig. 3 Frequency distributions of diameter of Mantoux reactions to the 1:2,000 and 1:100 dilution of OT in a BCG vaccinated group.

Study 3 (Group 3 ...... Fig. 3)

This group consisted of 643 subjects vaccinated with BCG. The comparison of the frequency distribution curve of the sizes of erythema elicited by a 1:2,000 dilution and that by a 1:100 dilution of OT was made in this group.
in the same way as before. The data were graphically presented in Fig. 3. Broadly viewed, these two curves run taking a similar course, but showing the move of the peak from the range of 11–12 mm in Curve I to the range of 19–20 mm in Curve II. A comparison of the frequency distribution curves of the sizes of erythema reacted to a dilution of OT in six BCG-vaccinated groups (Group 3 to Group 8) employed in these present experiments indicates that Group 3 had been most weakly sensitized among these groups. Whereas, this group responded to a 1:100 dilution with an intense reaction as represented by Curve II in Fig. 3. The low occurrence rate of the erythema in the range of 0 to 10 mm and the steep rise of the curve in the range of 5 to 13 mm, however, appear to indicate that the modification of the form of the curve is principally due to the intensifying of specific reaction, not that of nonspecific reaction. The peak in the range of 21–22 mm in Curve II appears to correspond to the peak in the range of 11–12 mm in Curve I.

Study 4 (Group 4 ....... Fig. 4)

This group consisted of 1044 individuals who had been vaccinated with BCG. In this way, a similar experiment was made with OT in a dilution of 1:1,000 and 1:2,000. Fig. 4 shows a close resemblance of the frequency distribution curve of the sizes of erythema between these two tubersulin solutions, and at
the same time, the difference in the concentration of OT is evidently manifested in the lower occurrence rate in the range of 5–12 mm and in the higher rate of more than 21 mm in Curve I than in Curve II.

Study 5 (Group 5 . . . . . Fig. 5)

This group consisted of 503 subjects who had received BCG vaccination. Using OT in a dilution of 1:2,000 and 1:5,000, the comparison of frequency distribution curves of the sizes of erythema was made. The result is as shown in Fig. 5. The frequency distribution curves for those dilutions of OT run taking a similar course except the tendency that the curve of the 1:5,000 dilution runs over the curve of the 1:2,000 dilution in the range of 0 to 12 mm, but runs below in the range of more than 13 mm. As for induration, it was palpable in 8.2 percent in the test with the 1:2,000 dilution and 0.4 percent in the test with 1:5,000 dilution.
Fig. 6 Frequency distributions of diameter of Mantoux reactions to the 1:2,000 and 10,000 dilution of OT in a BCG vaccinated group.

Study 6, 7, 8 (Group 6, 7, 8 ...... Fig. 6, 7, 8)
Fig. 7 Frequency distributions of diameter of Mantoux reactions to the 1:2,000 and 1:25,000 dilution of OT in a BCG vaccinated group.
Fig. 8 Frequency distributions of diameter of Mantoux reactions to the 1:2,000 and 50,000 dilution of OT in a BCG vaccinated group.

Three groups of BCG vaccinated subjects 676, 605 and 861 were used respectively. Frequency distribution curves of erythema were drawn of OT in a dilution of 10,000, 1:25,000 and 1:50,000 in comparison with a 1:2,000. A brief survey of the results indicates that BCG vaccinated individuals react to those high dilutions of OT in a very low percentage: In addition to this, paying considerations to the possibility that some of the reactors to those high dilutions may have been natural infections who happened to mix in those populations, it is certain that those dilutions are not adequate for the examination of BCG-induced tuberculin sensitivity. Because, approximate one
year had elapsed since the last BCG vaccination in the groups of schoolchildren subjected to the present study. During this period, therefore, some may have got the infection and the majority may have decreased in tuberculin sensitivity.

The experiments reported in this paper were conducted in 1948, when liquid BCG vaccine was used. At present, however, lyophilized BCG vaccine of high vitality is advantageously used in this country and the similar experiment as this has been made also of some groups of school children vaccinated with the dried vaccine and it was reported in Japanese by Yanagisawa and his co-workers. The rapid progress of the dried BCG vaccine which has been made in these several years is a direct cause of the contineously increasing tuberculin sensitivity of the people. The comparison of this paper with other papers reporting of more recent studies of this problem will present an interesting information regarding the change of tuberculin sensitivity of the nation.

In view of the observations in these experiments, it may be reasonable that the dose of tuberculin is decided differently in BCG vaccinated and naturally infected individuals, and if possible, two step test is made using two different dose of OT. For instance, in naturally infected individuals, tuberculin test is desirable to be conducted at first with a 1:25,000 dilution of OT, and if negative, then with a 1:2,000 dilution. In BCG vaccinated subjects, a 1:2,000 dilution will be adequate in the first test, and if negative, again with a 1:100 dilution.

A criterion to decide a reaction as positive, however, must be prudently chosen for each dilution of OT, particularly in the case of high concentration of OT. Otherwise, reliable tuberculin test and BCG vaccination would not be expected. In addition, the discovery of a simple method to differentiate BCG-induced allergy from natural infection is now urgently needed. A decisive solution of these problems will demand a further study.

SUMMARY

1) Tuberculin skin tests were conducted in eight groups of school children of natural infection or BCG vaccination.

2) In these groups, the frequency distribution curve of diameters of erythema was drawn of various dilutions of OT in comparison with a standard (1:2,000) dilution of OT.

3) In view of the results obtained, a brief discussion was made of tuberculin reaction

REFERENCES

