An Investigation of Takahashi's Antitubercle Phosphatide Kaolin Agglutination Test (KAT) in Leprosy[1]

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The immune response in leprosy has been the subject of much recent investigation. Leprosy may present in two well-defined polar forms at either end of the immunologic spectrum, with intermediate less well-defined forms lying in between. These two forms, the tuberculoid and lepromatous types are each different from the other also in their clinical presentation, and the distinction between them can most often be made on clinical grounds alone. Tuberculoid leprosy is said to be characterized by a well-developed cell-mediated immune response to the antigens of *Mycobacterium leprae,* while in lepromatous leprosy the cell-mediated response is said to be diminished to a variable extent. In contrast the humoral component of the immune response is said to be well-developed in lepromatous leprosy ([1, 3]).

Many studies have been made of the antibodies in leprosy. Some antibodies have also been demonstrated against antigens from nonmycobacterial sources in lepromatous leprosy. These antibodies may lead to false positive results in serological procedures, a well-known example being in serologic tests for syphilis ([4]).

The antitubercle phosphatide kaolin agglutination test was developed by Takahashi ([7]) as a technic for use in serodiagnosis of tuberculosis. Its value as a diagnostic procedure has been variously assessed by different workers ([5, 7, 8, 9]). Takahashi ([7]) found the test to be negative in diagnostic titers in leprosy patients. On the other hand, Weber *et al* ([8]) reported that in their hands the test was positive in 67% of leprosy patients. The present paper is a report of the findings in leprosy patients (both tuberculoid and lepromatous) of an investigation using the KAT in Sri Lanka (Ceylon).

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MATERIALS AND METHODS

The leprosy patients investigated in this study were individuals undergoing either institutional or domiciliary treatment for leprosy and considered to have active disease. For the purpose of the study all patients were clinically divided into tuberculoid and lepromatous groups, the diagnosis and classification of type of disease being as made by the Antileprosy Campaign of Ceylon; in relevant cases the clinical diagnosis made was supported by examination of smears and tissue biopsies. A total of 118 patients with lepromatous disease and 135 with tuberculoid disease were studied. Twenty-three contacts of leprosy patients, not considered to have leprosy, were also investigated at the same time.

The blood samples collected were all "spot specimens," no special precautions (such as fasting of patient) having been taken, the sample being collected at any time of the day whenever the patient was available using sterile dry equipment. The sera were separated and tested either within 24 hours of blood collection ("fresh" sera) or quick frozen at -70°C ("frozen" sera) and thawed immediately before being tested. The tests were done in mixed batches of 15 sera each. These batches consisted of sera from tuberculosis patients, leprosy patients, contacts of leprosy patients, patients with nonmycobacterial disorders and "normal" blood bank donors; the sera being coded in such a manner that the investigator did not know the identity of the serum being tested.

The materials used in the test were obtained from commercial sources (Messrs. Daichi Seiyaku and Company, Tokyo, Japan) and the method of the test was that described in the literature supplied with the test kits. Briefly, the method of the test was as follows. Tris (hydroxymethyl) aminomethane (TME) buffer was prepared by the addition of one part of the TME mixture in the kit to nine parts of physiological saline prepared with deionized water ($\geqslant$5,000,000Ωcm). The

buffer solution, the kaolin suspension to be used, and the phosphatide methanol antigen were all warmed at approximately 40°C for ten minutes in a water bath. Thereafter, 19 parts of TME buffered saline were placed in a small beaker with a magnetic stirrer and one part of the phosphatide antigen was added to it. While constantly stirring gently, ten parts of the kaolin suspension were added using a pipette. Stirring was continued for another two minutes and the sensitized kaolin suspension was then incubated at 37°C (in a water bath) for 30 minutes, the suspension being shaken at 15 minutes, and at the end of 30 minutes. It was used immediately following preparation.

Doubling dilutions of serum were prepared ranging from 0 through 1/8, 1/16, 1/32, 1/64 to 1/1024, using the TME buffered saline. To each tube of the set of dilutions was added 0.1 ml of the sensitized kaolin suspension and shaken thoroughly. The sera were then incubated at 37°C in a water bath for 30 minutes, being again shaken at 15 minutes and at the end of incubation. After incubation the tubes were allowed to stand at room temperature for another 30 minutes and then centrifuged at 2,000 rpm for 5 minutes. Two to three tubes containing only buffered saline as controls were set up together with each batch of sera.

The results were recorded as the titers of antibody, the latter being considered to be the highest dilution at which definite clumps of kaolin particles which could not be broken up by gentle agitation were observed with the unaided eye against a black background. The control tubes always showed no clumping.

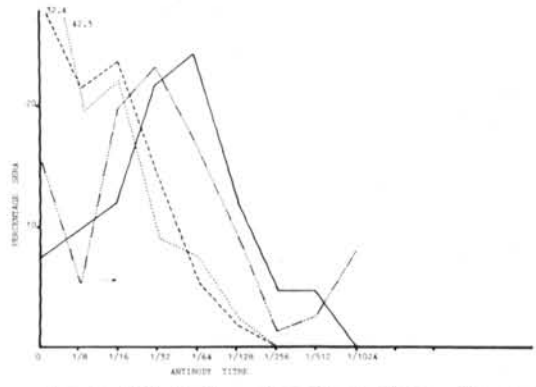

FIG. 1. Distribution of antibody titers of frozen and fresh sera in leprosy patients: tuberculoid leprosy-frozen sera (·····), and fresh sera (------); lepromatous leprosy-frozen sera (.—.....—.), and fresh sera (———).

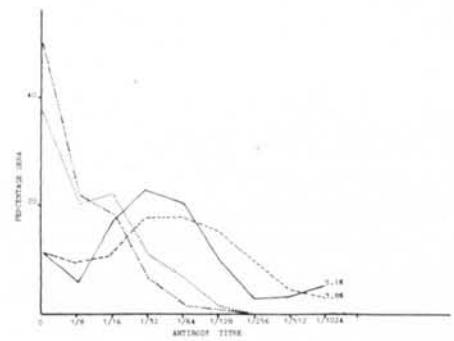

FIG. 2. Distribution of antibody titers in different groups of subjects: tuberculoid patients (.....), lepromatous leprosy patients (———), blood bank donors (—.....—..), and tuberculosis patients (--------).

RESULTS

Comparison of the distribution of antibody of frozen and fresh sera showed that no significant fall of antibody titer occurred as a result of freezing sera (Fig. 1). This conclusion was also supported by a similar finding in the results of frozen and fresh sera in tuberculosis patients ([5]). Hence, for the purposes of this study, the results of sera, both frozen and fresh, are pooled together.

Figure 2 presents the distribution of titers in the two polar types of disease; also presented in the same figure are the distributions of titers shown by blood bank donors and tuberculosis patients ([5]). It is seen that the distribution of titers in tuberculoid patients resembles that of blood bank donors while that of lepromatous patients resembles that of tuberculosis patients.

The correlation between such factors as age, duration of treatment and Bacillary Index (BI) in lepromatous patients is presented in Tables 1, 2 and 3, respectively. No valid conclusions could be drawn from this data.

It was found that two of three contacts of lepromatous patients tested thought to be clinically normal, had high antibody titers (1/64 and 1/128 respectively) considered to be diagnostic in tuberculosis ([5, 7, 8]), while only one of twenty tuberculoid leprosy contacts had a high titer of 1/64, the majority

TABLE 1. *Relationship between duration of treatment and KAT titer in lepromatous leprosy. Approximate percentage of cases.*

Duration of treatment (in years)	Titer		
	0-1/8	1/16-1/32	⩾1/64
New cases and those up to 2 years of treatment	1	11	15
3-5	4	9	4
5-10	5	8	3
⩾11	8	13	22

TABLE 2. *Relationship between Bacillary Index and KAT titer in lepromatous leprosy. Approximate percentage of cases.*

Bacillary Index	Titer		
	0-1/8	1/16-1/32	⩾1/64
0	19	11	9
1	2	4	9
2	2	13	4
3	2	9	11
4	—	2	—

TABLE 3. *Relationship between age and KAT titer in lepromatous leprosy. Approximate percentage of cases.*

Age (in years)	Titer		
	0-1/8	1/16-1/32	⩾1/64
⩽20	1	2	3
21-40	4	13	17
⩾41	13	25	22

being negative while the others showed titers of 1/8 and 1/16. These findings in contacts of leprosy patients merit further investigation.

DISCUSSION

It is well-established that mycobacteria share antigens ([2, 6]). The findings of antiphosphatide antibodies to the same phosphatide antigens in both leprosy and tuberculosis patients suggest that phosphatide antigens are probably shared by *M. tuberculosis* and *M. leprae*.

The findings of this study differ completely from those of Takahashi in Japan ([7]), and appear to agree with those of Weber *et al* in Israel ([8]). The marked difference in the pattern of distribution of antibody titers in tuberculoid and lepromatous patients, is similar to that described with other types of antibodies to mycobacterial antigens in leprosy patients. The determination of antibodies could provide another means of distinguishing tuberculoid from lepromatous patients. One of the main difficulties associated with the use of serological methods in diagnostic work has been the nonavailability of easily performed technics that can be adopted for clinical laboratory use. In this respect the KAT affords an easily performed method of serological testing for use on a large scale, even under field conditions ([9]).

SUMMARY

Takahashi's antitubercle phosphatide kaolin agglutination test is an easily performed serological technic, found to have diagnostic potential in tuberculosis. The test was performed on sera from leprosy patients. It was found that lepromatous patients showed a distribution of titers similar to that of tuberculous patients, while in tuberculoid disease, the distribution resembled that of sera from normal persons. The occurrence of antibodies to phosphatide antigens in both lepromatous disease and tuberculosis suggests that these antigens may be shared by *M. leprae* and *M. tuberculosis*.

RESUMEN

La prueba de aglutinación antitubercular fosfátido caolín de Takahashi es una técnica serológica fácilmente realizable, que se ha encontrado tiene un potencial diagnóstico en la enfermedad tuberculosa. La prueba fué realizada con sueros de pacientes leprosos: se encontró que los pacientes lepromatosos mostraron una distribución de títulos similar a la de los pacientes tuberculosos, mientras que en los enfermos tuberculoides la distribución fué similar a la de los sueros de personas normales. La presencia de anticuerpos contra antígenos fosfatídicos tanto en la enfermedad lepromatosa como en la tuberculosis sugiere que estos antígenos pueden ser compartidos por el *M. leprae* y el *M. tuberculosis*.

RÉSUMÉ

L'épreuve d'agglutination sur kaolin du phosphatide antituberculeux, mis au point par Takahashi, est une technique sérologique facile à utiliser, et dont on a constaté l'intérêt pour le diagnostic éventuel de la maladie tuberculeuse. L'épreuve est effectuée sur du sérum provenant de malades de la lèpre. On a observé que les malades lépro-

mateux présentaient une distribution de titres semblables à celles que l'on constate sur les malades tuberculeux, alors que dans l'affection du type tuberculoïde, la distribution ressemble à celle que l'on trouve chez des personnes normales. La présence d'anticorps aux antigènes phosphatidiques, tant dans la lèpre de type lépromateux que dans la tuberculose, suggère que ces antigènes peuvent être détenus en commun par *M. leprae* et par *M. tuberculosis.*

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