

# Appraisal of the total blood lymphocyte proliferation assay as a diagnostic tool in screening for tuberculosis

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**Summary.** The total blood lymphocyte proliferation assay (TLP) was evaluated as a screening test for infection with *Mycobacterium tuberculosis* and was compared with the tuberculin (Mantoux) skin test. The results of TLP assays performed on 33 patients with tuberculosis and 37 non-tuberculous subjects were compared with results of skin tests performed in the previous year. There was a high correlation between skin test responses and TLP responses to PPD which was statistically significant. The sensitivity, specificity and the predictive value of a positive test were also similar for the skin test and TLP test. These findings suggest that the TLP test is as effective in screening for *M. tuberculosis* infection as tuberculin skin testing. Future research leading to further simplification of the TLP method may lead to it replacing intradermal skin testing.

## Introduction

Although the aetiological agent of human tuberculosis was discovered by Robert Koch more than 100 years ago, early detection of infection with *Mycobacterium tuberculosis* is still difficult in spite of the tremendous advances in the study of this disease. Infection by the organism is closely associated with delayed hypersensitivity (DH) to tuberculin, perhaps the best recognised example of a cell-mediated immune response in man.<sup>1</sup> Originally, the response to *M. tuberculosis* was studied in the tuberculin skin test. However, during the past 25 years, several in-vitro techniques have become available, some of which have been found to be equally sensitive indicators of tuberculous infection.<sup>2,3</sup> Proliferation of peripheral blood lymphocytes *in vitro* in response to antigens of *M. tuberculosis* is generally correlated with the skin test response to PPD, although instances have been reported of lymphocyte proliferation in the absence of a DH response to PPD.<sup>4</sup>

Lymphocyte proliferation *in vitro* is an accepted technique for assessing cell-mediated immunity (CMI). However, because it requires quantities of blood that can be obtained only by venepuncture, and because of the need to separate the mononuclear cells, the technique has been too cumbersome for routine use. Recently, we have developed a modification of the

lymphocyte proliferation assay, which can be performed on 100- $\mu$ l samples of whole blood, a quantity that can be obtained by fingerprick. In a study of individuals exposed to infection by *Leishmania major* or vaccinated with BCG, the simplified assay yielded results comparable to those of the classical assay employing separated peripheral blood mononuclear cells.<sup>5</sup>

The objective of the present study was to determine the significance of the modified whole-blood lymphocyte proliferation assay in subjects with or without tuberculosis and to evaluate this method of screening for infection by *M. tuberculosis*.

## Materials and methods

### Subjects

Seventy patients attending two centres for chest diseases in Israel, were recruited; 33 were patients with tuberculosis who met the criteria of the Israeli Ministry of Health—i.e., clinical and radiological evidence, together with bacteriological confirmation—and 37 were patients with chest diseases other than tuberculosis. None of the subjects had a known history of BCG vaccination. In both groups, the age range was 21–93 years (median 62 years) and the numbers of males and females were approximately equal.

### Skin tests

The results of tuberculin skin tests performed during the previous year were obtained from the patients'

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records; in most cases these had been recorded simply as "positive" or "negative". In those cases in which the diameter of the skin test responses had been recorded, tests yielding diameters  $\geq 10$  mm were considered to be positive.

#### Total blood lymphocyte proliferation assay

The total blood lymphocyte proliferation assay (TLP) was performed as described previously.<sup>5</sup> Briefly, 100- $\mu$ l samples of blood obtained by fingerprick were drawn into sterile, disposable 100- $\mu$ l micropipettes (Blaubrand, intraMark, Germany), and transferred into tubes containing 1.5 ml of RPMI 1640 cell-culture medium (Ysum, Israel) supplemented with glutamine 2 mmol, streptomycin 100 mg/L, penicillin  $10^5$  U/L (Teva, Israel) and 1  $\mu$ l of heparin without preservatives (Leo, Ballerup, Denmark). The diluted blood was mixed thoroughly and distributed in 100- $\mu$ l volumes into round-bottomed 96-well plates (Cel-cult, Sterlin). Mitogen (Phytohaemagglutinin-M, PHA; Difco) 0.3  $\mu$ g/well and antigen (Tuberculin Purified Protein Derivative, PPD; Statens Serum Institut, Copenhagen, Denmark) 1 or 8  $\mu$ g/well were added in 25- $\mu$ l volumes. The plates were incubated for 6 days at 37°C in an atmosphere of air plus CO<sub>2</sub> 6%; 6 h before harvesting, 1  $\mu$ Ci of <sup>3</sup>H-thymidine in a volume of 25  $\mu$ l (Nuclear Research Center, Israel; 2 Ci/mmol) was added to each well. Every test was performed in triplicate. Cells were harvested with an automatic cell-harvester (Titertek), and the incorporated <sup>3</sup>H-thymidine was assayed by liquid scintillation spectrometry (Packard Tricarb). Results are presented as counts/min (cpm), or as stimulation index:

cpm of antigen containing wells/cpm of wells containing cells without antigen.

#### Statistical analysis

Results of the skin test and the TLP test were analysed statistically by means of  $\chi^2$ -test and Fisher's test of exact probability. Correlation was determined in terms of the strength of association between pairs of variables by calculating Odds ratios, because of the qualitative nature of the skin test results.

## Results

Cells were considered to have responded to PPD in the TLP assay if, in the presence of one of the concentrations of the antigen, the cpm/were  $\geq 3000$  and the stimulation index  $\geq 3$ .<sup>5</sup> In this study, all the specimens that yielded cpm  $\geq 3000$  also yielded a stimulation index  $\geq 3$ , therefore only the results of cpm values are presented.

Of the 33 subjects whose cells responded to PPD, 70% demonstrated higher responses to 8  $\mu$ g (average

cpm 11923 SD 8058) and 30% higher responses to 1  $\mu$ g PPD (average cpm 14392 SD 10583). The higher response was taken as that subject's response to PPD.

The responses to PPD in the TLP and the skin test are shown in the figure. These data demonstrate that 75% of those failing to react to PPD in the TLP also failed to respond in the skin test. Similarly, c. 75% of those responding in the TLP had also given a positive response to PPD in the skin test. The Odds ratios for the association of responses in the TLP and the skin test were 8.5 among the tuberculosis patients, and 12.0 among the controls ( $p < 0.01$ ). Thus, there appeared

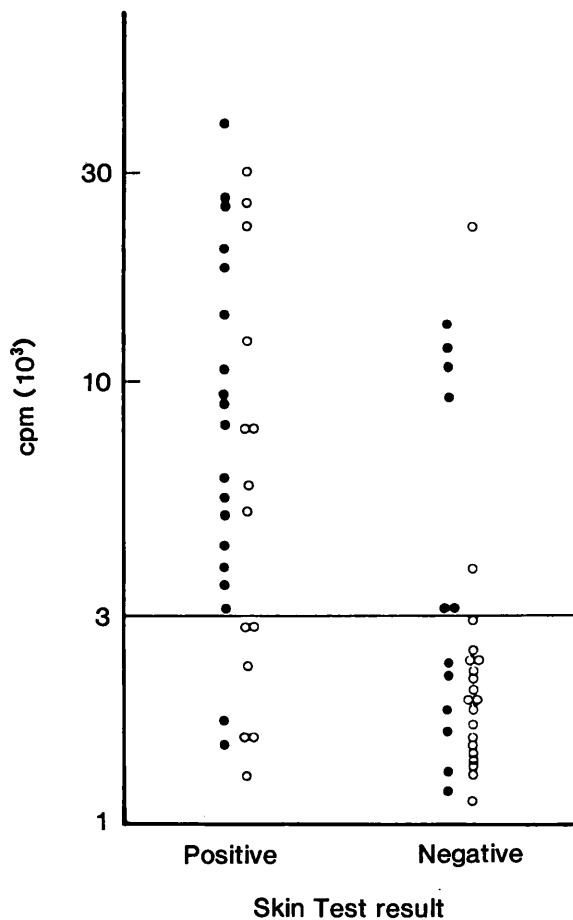


Figure. Comparison of response to PPD in the TLP assay (cpm) and in the skin test; ● cases, ○ controls. For each individual, the higher cpm values are shown.

Table. Comparison of skin test and TLP test with the diagnosis of tuberculosis by the physicians

	Number of subjects			
	Cases	Controls	Total	
Skin test result				
Positive	19	14	33	Sensitivity 61.3%. Predictive value of a positive test 57.6%. Specificity 58.8%.
Negative	12	20	32	
Total	31	34	65	
TLP test				
Positive	21	15	36	Sensitivity 63.6%. Predictive value of a positive test 58.3%. Specificity 59.5%.
Negative	12	22	34	
Total	33	37	70	

to be a close correlation between the response in the TLP assay and that in the tuberculin skin test.

Furthermore, as shown in the table, the tests appeared equally capable of distinguishing between the patients with tuberculosis and those with other non-tuberculous disease. The skin test and the TLP test were compared with clinical, laboratory and radiological diagnosis of the disease—the criterion of validity being the diagnosis of the disease by physicians at the centres. All the basic measures of criterion validity (sensitivity, specificity and predictive value of a positive test) were similar for both tests and ranged between 58% and 64%.

## Discussion

The purpose of population screening is usually to detect as many cases as possible of the disease or risk factor that is being sought. The validity of a screening test is judged by determining its sensitivity, specificity and the predictive value of a positive test.<sup>6</sup> These components are measured by reference to the results derived from a definitive diagnostic procedure. The predictive value of a positive test depends upon the prevalence of the disease or risk factor in the population tested, as well as the sensitivity and specificity of the test. The higher the prevalence, the more likely it is that a positive test will predict the disease.<sup>7</sup>

In the present study, the sensitivity and specificity of the TLP test and the skin test were similar in the population tested, and both tests had predictive values of *c.* 60%. Thus, although neither test had a high predictive value, the effectiveness of the TLP as a screening test for tuberculosis was similar to that of the tuberculin test.

Reports of the correlation between skin tests and lymphocyte proliferation assays in tuberculosis is not unanimous; in two studies,<sup>2,3</sup> the correlation between the tests was statistically significant, whereas studies

by others,<sup>8-10</sup> demonstrated at best only a rough correlation. In this study, as in some of the previous ones, there was a highly significant association between the two tests as shown by the very high Odds ratio values. However, the higher correlation among non-tuberculous subjects remains unexplained and further studies are needed to explore this finding. The discrepancies among the various studies cited may be accounted for by important differences, such as the number of subjects studied, whether or not patients with tuberculosis and normal tuberculin- or BCG-positive individuals were studied, and the nature of the antigens employed in the lymphocyte proliferation assay and the skin test.

It appears certain that the response to PPD in the skin test will remain the primary measure for screening populations for infection by *M. tuberculosis*, because of its low cost and the fact that it requires a minimum of equipment. However, the tuberculin test is difficult to standardise. The procedure is subject to variation in all its phases,<sup>11</sup> variability from reader to reader was found to be nearly as great as the biological variability from subject to subject. Also the use of the test requires that the individual be carefully identified and seen on a second occasion, usually 48 h after administration of the antigen.<sup>12,13</sup> On the contrary, in the TLP test as described, only a small quantity of blood obtainable by fingerprick is required and subjects do not have to return for reading of results. Therefore, the simplified TLP test could have a place in the clinical armamentarium available for the diagnosis of tuberculosis. Future research leading to further simplification of this method may lead to it replacing intradermal skin testing.

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