An enzyme-linked immunosorbent assay using adsorbed mycobacterial sonicates for the serodiagnosis of tuberculosis

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Summary
Sera from 123 patients with confirmed tuberculosis and 576 patients with other non-mycobacterial diseases were tested for antibodies to an adsorbed mycobacterial sonicate antigen employing an enzyme-linked immunosorbent assay. The results show significant differences in IgG levels between sera from tuberculosis patients and controls. The results of delayed hypersensitivity skin-test reactions to purified protein derivative in both the patient and control groups were unrelated to antibody levels. The assay was able to detect antibodies for at least 4 months after diagnosis and treatment. Patients diagnosed as having tuberculosis 7 months or more before being tested did not demonstrate increased antibody levels. The assay could be useful not only as a screening technique for diagnosing tuberculosis in an immunised population but also as a means of monitoring antituberculosis treatment.

Tuberculosis remains a major cause of mortality and morbidity in Third World countries and is taking on new importance as a cause of serious infection in patients with the acquired immunodeficiency syndrome (AIDS). The search for reliable, reproducible and specific serological tests for the diagnosis of tuberculosis has been an active area of research for many years. Enzyme-linked immunosorbent assay (ELISA) methods are sensitive and reproducible and do not require sophisticated equipment. Recently this technique has provided encouraging results for the serodiagnosis of tuberculosis.

In this study, ELISA was developed for detecting IgG antibodies against an adsorbed fraction of mycobacterial sonicate. The results indicate that this technique is reliable and reproducible and may differentiate between patients with active pulmonary tuberculosis and those with a variety of non-mycobacterial diseases or normal healthy volunteers.

Subjects and methods

Serum samples were obtained from 123 patients with active pulmonary tuberculosis confirmed by positive identification of Mycobacterium tuberculosis in sputum samples. Control sera were obtained from 576 individuals with a variety of non-tuberculous diseases. A further study examined antibody levels in 37 patients hospitalised for a variety of non-tuberculous conditions and 17 with pulmonary tuberculosis. In this group the antibody levels were related to the result of a delayed hypersensitivity skin test employing purified protein derivative (PPD) as antigen.

Antigen preparation

Mycobacterium tuberculosis organisms were isolated as previously described. Organisms at 10 x 10^6/ml were sonicated at 4°C in a MSE Soniprep 150 sonicator (18 µm peak-to-peak) and centrifuged at 2,000 g for 15 minutes at 4°C. The resultant supernatant was then collected, and after the protein level had been determined was plated onto plastic Petri dishes that had been precoated with 25 µg/ml normal human immunoglobulin. Adsorption was allowed to continue overnight at 4°C in a humidified chamber. Mycobacterial sonicate not adhering to the plate was collected, pooled and used as antigen in subsequent ELISA.

ELISA

ELISA was performed in flat bottomed 96-well plates (Nunc) using standard ELISAs in which the wells were coated with 100 µl of 10 µg/ml antigen in sodium carbonate buffer, pH 9.6. The plates were washed 4 times with phosphate-buffered saline (PBS)-Tween, unbound sites blocked with 100 µl of 0.5% BSA, and after washing 100 µl of a 1/40 dilution of serum PBS-Tween was added and incubated for 2 hours. After washing 100 µl of peroxidase-conjugated goat antihuman IgG (γ-chain-specific) was added to the wells. After a final wash, a further 100 µl of peroxidase substrate was added and the reaction terminated after 15 minutes with 50 µl of 2,5M sulphuric acid. Each sample was read at 492 nm in a Dynatech Multiscan ELISA reader.

Results

Sera from 576 patients with a variety of non-tuberculous diseases were tested and the results compared with those of 123 proven tuberculosis patients. The results (Fig. 1) indicate that the ELISA employed clearly differentiated antibody levels in the serum of controls and of patients with tuberculosis. Fig. 2 demonstrates that the assay significantly differentiates antibody levels between patients and controls for up to 4 months after the initiation of therapy. Sera from patients who have had tuberculosis for 7 months or longer consistently showed antibody levels within the normal range. In the group of patients who had been treated for 5-7 months antibody levels were above those of normal controls (results not significant).

The assay was performed using sera from 54 patients or controls, 30 of whom were PPD-positive. The other 24, comprising 18 normal individuals and 6 patients with advanced tuberculosis, were negative on skin testing. Table 1 demonstrates that no significant difference in antibody levels could be detected between these groups. Of interest was the observation that the 6 patients...
with proven tuberculosis but with negative delayed hypersensitivity skin tests, continued to show significant antibody levels similar to those of the 12 patients with positive skin tests.

TABLE I. SEROLOGICAL MEASUREMENT OF ANTI-MYCO. TUBERCULOSIS ANTIBODIES IN THE SERUM OF DONORS WITH DIFFERENT PPD SKIN-TEST RESPONSES (OPTICAL DENSITY VALUES AT 492 nm)

<table>
<thead>
<tr>
<th>Serum source</th>
<th>PPD-positive</th>
<th>PPD-negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal donors (37)</td>
<td>0.134 ± 0.052 (19)</td>
<td>0.125 ± 0.040 (18)</td>
</tr>
<tr>
<td>Patients (17)</td>
<td>0.405 ± 0.09 (11)</td>
<td>0.391 ± 0.062 (6)</td>
</tr>
</tbody>
</table>

No. of patients in each group in parentheses.

Discussion

The evidence that Myco. tuberculosis infections result in humoral responses which can be measured against mycobacterial antigens is generally accepted. Because of the sensitivity and relative simplicity of ELISA, this technique has been favoured in recent studies to determine antibody levels to mycobacterial antigens. The serological diagnosis of tuberculosis using ELISA has depended heavily on the antigen used. Since previous investigations showed that adsorption of mycobacterial extracts with pooled normal human immunoglobulin eliminates the majority of the nonspecific reactivity observed in healthy donor sera (results not shown), this form of antigen was employed in these studies.

Reports on the relationship between the results of tuberculin reactivity and mycobacterial antibody levels are conflicting. PPD positivity in healthy individuals has been associated with higher antibody levels to mycobacteria and the studies of Kardjito et al. correlate high specific IgG levels with tuberculin positivity. Other workers, however, detected no significant differences in antibody levels between PPD-positive or negative donors. The present study confirms these latter findings and suggests that adsorption of the mycobacterial sonicate removes antigens that are otherwise recognised nonspecifically by sera from PPD-positive donors.

The data reported suggest that the ELISA could be used for the serodiagnosis of tuberculosis with a high degree of sensitivity and specificity. Significant differences in antibody levels between controls or patients with a variety of nontuberculous diseases and those with tuberculosis could be detected. This indicates that the ELISA could be useful for large-scale screening for the diagnosis of tuberculosis.

To assess the influence of therapy on the level of antitubercular antibodies, sera from patients on antituberculosis treatment were examined. The highest levels of antibody were detected in the sera of patients who had received therapy for less than 4 weeks. The mean titre subsequently decreased on therapy but remained significantly raised until 4 months after the initiation of treatment. Thereafter antibody levels diminished so that mean antibody levels after 7 months of therapy approximated those found in normal controls. In conclusion, therefore, the ELISA performed with adsorbed mycobacterial sonicates was capable of distinguishing between patients with acute tuberculous infections and control subjects and proved useful as a means of monitoring the effect of antituberculosis treatment.

REFERENCES

Evaluation of the integrated 3-hour plasma cortisol concentration as a test for Cushing's syndrome

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Summary

Twenty-four hour urinary free cortisol and mean and integrated 13h00 - 16h00 plasma cortisol levels were measured in 9 patients with proven Cushing's syndrome (5 with Cushing's disease, 2 with ectopic adrenocorticotrophic hormone production due to bronchial carcinoma and 2 with adrenal adenomas) and in 21 patients without Cushing's syndrome. The 24-hour urinary free cortisol levels and mean and integrated 13h00 - 16h00 plasma cortisol estimations clearly distinguished patients with Cushing's syndrome from those without. However, adequate suppression on dexamethasone suppression tests (false negatives) were obtained in 3 of the 9 patients with Cushing's syndrome. Since the integrated 13h00 - 16h00 plasma cortisol estimation is cheaper and simpler than the mean 13h00 - 16h00 plasma cortisol estimation, we recommend it as an adjunct in the diagnosis of Cushing's syndrome.

Subjects and methods

A cannula was inserted into a deep antecubital vein of each patient at least 30 minutes before the test began. None of the subjects had evidence of hepatic or renal dysfunction, as demonstrated by standard laboratory techniques. The mean plasma cortisol concentrations at 20-minute intervals over a 3-hour period (13h00 - 16h00) were measured in 9 patients with proven Cushing's syndrome (5 with Cushing's disease, 2 with ectopic adrenocorticotrophic hormone production due to bronchial carcinoma and 2 with adrenal adenomas) and in 21 controls: 1 with neurotic depression and 20 with obesity of whom 12 had diabetes mellitus and/or hypertension. The diagnosis was supported by established biochemical and radiological tests in the patients with Cushing's syndrome and confirmed by surgery in the 5 patients with Cushing's disease and the 2 patients with adrenal adenomas. All the controls showed normal plasma cortisol suppression with the overnight dexamethasone suppression test, and on follow-up periods exceeding 6 months none has so far developed Cushing's syndrome.

Twenty-four hour urinary free cortisol levels were also measured in all subjects. In addition, equal aliquots taken from each 20-minute sample were combined to produce a 3-hour pool (13h00 - 16h00 integrated cortisol). Cortisol levels were determined by radio-immunoassay in all plasma and urine samples. The intra-