Immediate Hypersensitivity to Tuberculin* 

In Vivo and In Vitro Studies


Immediate responses of hypersensitivity to skin testing with purified derivative of tuberculin (PPD) were observed in 2.3 percent of 3,248 patients seen in an allergy clinic, and the relationship to delayed responses was questioned. Immediate cutaneous reactions to testing with PPD appeared in all age groups and occurred in nonatopic patients but were more common in atopic patients (P < 0.005). Delayed cutaneous reactions to testing with PPD occurred in only three out of 76 patients with immediate reactivity. Antihistaminic suppression of immediate reactivity was not followed by evidence of delayed cutaneous reactivity. In vitro tests of lymphocytic stimulation revealed indices of stimulation with PPD to be similar both in patients with immediate and delayed cutaneous reactivity. Failure to manifest delayed cutaneous reactivity following immediate cutaneous reactions alone may be explained by antigen-antibody binding and phagocytosis, by suppressor T-lymphocytes, or by impaired release or lack of response to T-lymphocytic mediators. Adverse reactions to administration of BCG vaccine in patients with immediate cutaneous reactivity might be anticipated.

The tuberculin skin test is widely used in assessment of immunity to tuberculosis and is a measure of cell-mediated immunity. Interpretation of this test is made 48 to 72 hours after injection, as recommended by the American Thoracic Society.† Usually, no attention is directed to the immediate cutaneous response.

Immediate cutaneous hypersensitivity to purified protein derivative of tuberculin (PPD) was reported to develop in one man following seven to eight tuberculin tests over a period of two years,‡ while immediate cutaneous reactivity in guinea pigs was induced after approximately six alternate-day injections of PPD.§ Significant immediate wheal and flare responses to an intradermal injection of 0.1 ml of PPD (10 tuberculin units [TU]) have been observed in a group of patients as part of routine testing with tuberculin in the Allergy Clinic at Kingston General Hospital between 1968 and 1974. These reactions were recorded in addition to the delayed response at 24 and 48 hours. The relationship of the immediate and delayed responses was questioned and then investigated by skin tests and

Materials and Methods

In Vivo Methods

Ten tuberculin units of PPD (Connaught Laboratories Ltd) was injected intradermally into the flexor surface of the left forearm of patients aged eight years or more seen at the Allergy Clinic of Kingston General Hospital between 1968 and 1974. Immediate cutaneous reactivity was assessed 20 minutes after injection and graded on a scale ranging from zero to +++.§ Delayed reactivity was assessed at 48 and 72 hours according to the criteria of the American Thoracic Society.† As other indicators of delayed cutaneous reactivity, 0.1 ml of histoplasmin (Parke-Davis stock 4-498-1-70) reconstituted as directed in 1 ml of phosphate-buffered diluent and 0.1 ml of an extract of Candida albicans (Hollister-Stier Laboratories) diluted in buffered saline solution with phenol as a preservative to 10,000 ppm/ml were also injected intradermally and were assessed in the same manner as the tuberculin test.

The assessment of atopy was based on skin testing. The presence of three or more significant immediate wheal and flare responses to any major antigenic group was interpreted as an atopic reaction in the presence of negative reactions to control injections of preservative and normal responses to intradermal injection of histamine.

A full history of previous exposure to BCG vaccine and tuberculosis was obtained where possible. In order to determine whether suppressing the immediate reaction would have any effect on the delayed response, randomly selected patients with positive immediate reactivity and negative delayed reactivity were given an antihistamine (25 mg of hydroxyzine) orally 12 hours and two hours prior to retesting with 10 TU of PPD, and the cutaneous response was observed. The results were analyzed by means of the x2 test using Yates'
correction, and values of P less than 0.05 were taken as being significant.

In Vitro Methods (Lymphocyte Cultures)

Cell Source. Blood was defibrinated by shaking with glass beads, was then gently mixed with an equal volume of a 3 percent solution of gelatin in isotonic saline solution, and was allowed to sediment at a 45° angle for five minutes and then vertically for 20 minutes at a temperature of 37°C. The supernatant was centrifuged, and the pellet of cells was resuspended in a cold 0.85-percent solution of ammonium chloride to lyse the red blood cells. The pellet was washed three times in physiologic saline solution before counting with Türk's solution in a hemacytometer. The lymphocytes were resuspended in culture medium to a concentration of 2 to 2.5 x 10^6 lymphocytes per milliliter. The suspension of cells was dispensed in 200 μl aliquots into flat-bottom wells of microwells (Linbro 6-mm Disposable-trays, 15 FB-96-TC) containing 72 wells each. The stimulating agents were added from the stock solutions in 10 μl aliquots. All determinations were set up in triplicate on each plate, and each plate was duplicated. Control cultures without stimulating agents were included in each plate. Cultures were incubated at 37°C in a water-saturated atmosphere containing 95 percent air and 5 percent carbon dioxide.

Stimulating Agents and Culture Media. Preservative-free PPD (Connaught Laboratories Ltd., CT88, lot A14) supplied at a concentration of 2 μg/ml was diluted in sterile water to make stock solutions of 2,000 μg/ml, 200 μg/ml, 20 μg/ml, 2 μg/ml, 0.2 μg/ml, and 0.02 μg/ml.

Lyophilized phytohemagglutinin in the M form (Grand Island Biological Co. control number A443015) was reconstituted with 10 ml of sterile water, as recommended by the manufacturer, and was further diluted to stock solutions of 2,000 μg/ml, 1,000 μg/ml, 500 μg/ml, 250 μg/ml, and 25 μg/ml.

Pokeweed mitogen (Grand Island Biological Co. control number C-8415151) was reconstituted with 5 ml of sterile distilled water and diluted to stock solutions of 1,000 μg/ml, 500 μg/ml, 250 μg/ml, 25 μg/ml, and 2.5 μg/ml.

The culture medium (Difco NCTC 109) was supplemented with penicillin (10^6 units/liter). Some aliquots of each cell suspension were cultured with 10 percent autologous serum, while others were cultured with 10 percent AB serum. Both sera had been inactivated by heat at 56°C for 20 minutes.

Measurement of Response. Lymphocytic transformation was estimated by uptake of tritiated thymidine following the addition of 50 μl of a 4-microcurie/ml solution into each well. Half of the plates received the dose of tritiated thymidine at 48 hours and were harvested at 72 hours, and the duplicate plates received the dose of tritiated thymidine at 96 hours and were harvested at 120 hours.

Cells were harvested using a semiautomatic multiple-sampler precipitator (Otto Hiller Co.). Samples were drawn onto glass filters and were washed with physiologic saline solution, then with a 5-percent solution of trichloroacetic acid (Fisher Scientific Company) and finally with methanol. The filters were left to dry and then were placed in scintillation vials. Ten milliliters of scintillation fluid was added (Fisher Scientific Verse), and the vials were counted in a liquid scintillation system (Packard Tricarb. Liquid Scintillation Spectrometer, model 3390-544).

The results were expressed as the stimulation index, i.e., the ratio of the radioactivity incorporated by the stimulated cultures to that incorporated by the control cultures. A stimulation index of two or more was taken as being positive.

Dose-response curves were plotted showing the response to PPD in concentrations of 100 μg/ml, 10 μg/ml, 1 μg/ml, 0.1 μg/ml, 0.01 μg/ml and 0.001 μg/ml of culture medium, the response to phytohemagglutinin in concentrations of 100 μg/ml, 50 μg/ml, 25 μg/ml, 12.5 μg/ml, and 1.25 μg/ml, and the response to pokeweed mitogen in concentrations of 50 μg/ml, 25 μg/ml, 12.5 μg/ml, 1.25 μg/ml, and 0.125 μg/ml.

Results

A total of 3,248 patients underwent skin tests between 1968 and 1974. Of these patients, 2,137 were atopic, and 1,111 were nonatopic according to the criteria listed previously. The incidence of atopy in this population of patients at the time of first testing fell with increasing age (Table 1).

A total of 473 patients exhibited delayed cutaneous reactions to testing with PPD. Positive delayed cutaneous reactions to tuberculin tests were present mainly in patients over the age of 30 years. The incidence of atopy in this group was not significantly different from the incidence of atopy in the total population studied.

A total of 76 patients had positive immediate reactions to tuberculin testing. The age distribution is shown in Table 1, indicating that immediate reactivity to testing with PPD occurred in all age groups, with an age distribution similar to that of the total population tested. Eighty-seven percent (66) of those 76 patients with positive immediate cutaneous reactivity were atopic, while only 65 percent of those patients with no immediate cutaneous reactivity to testing with PPD were atopic (P < 0.005). This difference was not confined to any age group. There was no significant difference in the incidence of positive delayed responses to histoplasmin and C. albicans. Of the 76 patients with immediate cutaneous responses testing with PPD, positive delayed reactions to histoplasmin occurred

Table 1—Age and Response to Skin Testing with PPD in Total Population of Patients

<table>
<thead>
<tr>
<th>Age, yr</th>
<th>Total</th>
<th>Positive Delayed Tuberculin Test</th>
<th>Positive Immediate Response to Tuberculin</th>
</tr>
</thead>
<tbody>
<tr>
<td>8-10</td>
<td>133 (105)</td>
<td>2 (1)</td>
<td>4 (4)</td>
</tr>
<tr>
<td>11-20</td>
<td>837 (642)</td>
<td>41 (31)</td>
<td>25 (22)</td>
</tr>
<tr>
<td>21-30</td>
<td>964 (703)</td>
<td>120 (93)</td>
<td>21 (20)</td>
</tr>
<tr>
<td>31-40</td>
<td>587 (368)</td>
<td>120 (75)</td>
<td>15 (12)</td>
</tr>
<tr>
<td>41-50</td>
<td>401 (209)</td>
<td>109 (59)</td>
<td>2 (1)</td>
</tr>
<tr>
<td>51-60</td>
<td>202 (75)</td>
<td>50 (20)</td>
<td>4 (3)</td>
</tr>
<tr>
<td>61-70</td>
<td>92 (28)</td>
<td>25 (9)</td>
<td>4 (3)</td>
</tr>
<tr>
<td>71-80</td>
<td>31 (7)</td>
<td>6 (2)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>81-90</td>
<td>1 (0)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>3,248 (2,137)</td>
<td>473 (290)</td>
<td>76 (66)</td>
</tr>
</tbody>
</table>

*Numbers within parentheses indicate number of atopic patients. Skin testing consisted of intradermal injection of 10 TU of PPD.
in ten patients (13 percent) and positive delayed reactions to C. albicans in 17 patients (22 percent); these incidences were comparable to the incidences in those with no immediate reaction to testing with PPD (10 and 20 percent, respectively). Noteworthy was the fact that the incidence of positive delayed responses to tuberculin testing in patients with immediate responses was much less than expected. Of the total population of patients, 14 percent exhibited delayed reactions to testing with PPD, but only three (4 percent) out of the 76 patients with immediate responses to tuberculin testing also had delayed responses (P < 0.025). All three patients were nonatopic.

Detailed histories of prior tuberculin skin testing and exposure to tuberculosis were available from 29 patients with immediate cutaneous reactivity. Previous exposure varied from no known prior testing or exposure in eight patients to six to seven previous skin tests over several years in three patients, and previous BCG vaccination in four patients. There was no significant difference in the history of sensitization between atopic and nonatopic patients.

In six patients the immediate cutaneous reaction was suppressed by oral administration of 25 mg of hydroxyzine 12 hours and two hours prior to testing. None of the patients exhibited delayed reactivity despite inhibition of the immediate response.

Studies of lymphocyte stimulation with incorporation of tritiated thymidine were performed in 28 patients, of whom four had only immediate cutaneous reactivity to tuberculin skin tests, three had both immediate and delayed reactions, and 11 had delayed reactions only. The results were compared with those of ten patients with no cutaneous reaction to testing with PPD.

Patients with immediate cutaneous reactivity showed similar levels of stimulation by PPD as did patients with delayed cutaneous reactivity only. All but one of the negative tests for lymphocytic stimu-
lation occurred in patients with no cutaneous reactivity to tuberculin skin tests, although some patients with negative skin tests did show positive in vitro stimulation with PPD, as would be anticipated (Fig 1). The indices of stimulation after incubation with phytohemagglutinin and pokeweed mitogen were positive in all patients, as an indication of their capacity to respond to nonspecific T-lymphocyte and B-lymphocyte mitogens.6,4

**Discussion**

Local immediate hypersensitivity to tuberculin skin tests occurred in 76 (2.3 percent) out of 3,248 patients who were tested with 10 TU of PPD. Of the 29 patients from whom a detailed history was available, eight gave no history of prior sensitization with tuberculin. To our knowledge, only one patient with local immediate reactivity to tuberculin has been previously reported, and this occurred only as a consequence of seven or eight prior injections of tuberculin over a period of two years.6

Immediate hypersensitivity to tuberculin, which is observed in all age groups, occurs in both atopic and nonatopic patients but more commonly in atopic patients. Only three patients (all nonatopic) of the 76 with immediate cutaneous reactivity to tuberculin skin tests exhibited coexistent delayed cutaneous reactivity. This suggests that the observed reduced association of immediate with delayed cutaneous reactions to tuberculin skin testing is a function of the atopic state or of a specific IgE-mediated response to tuberculin, or of both. The possibility that the immediate reaction may interfere with a delayed cutaneous reactivity as a consequence of local vasodilatation during the immediate reaction and the dissemination of antigen was ruled out by the results of suppressing the immediate reaction with administration of antihistamines in six of the patients studied in our series. In another study, Brostoff and Roitt5 have shown that with antihistaminic suppression of immediate cutaneous reactivity in pollen-sensitive patients, a delayed cutaneous response was seen in four out of six patients.

We were surprised to observe that in vitro studies of lymphocytic stimulation revealed a pattern of positive tuberculin stimulation in those patients with immediate cutaneous reactivity similar to the pattern in those with delayed cutaneous reactivity alone. On the other hand, patients with negative skin tests but with leukocytes stimulated in vitro by tuberculin have been considered by Matsaniotis et al,10 who demonstrated that after administration of BCG vaccine, in vitro testing of lymphocytic stimulation was a more sensitive indication of responsiveness than cutaneous testing. Tuberculin has been shown to be predominantly a B-lymphocytic mitogen in studies of animals.11-13 The presence of in vitro lymphocytic stimulation with PPD therefore may be expected to correlate better with production of antibodies than with manifestations of delayed hypersensitivity. Similar findings of in vitro lymphocytic stimulation in response to specific antigens has been reported in subjects with immediate cutaneous reactivity to ragweed, Alternaria, and penicillin4,12,13 in the absence of manifest delayed hypersensitivity.

Thus, a population of patients exists who are generally atopic and who, in turn, exhibit immediate cutaneous reactivity to tuberculin. These people have normal in vitro tests of lymphocyte function in response to tuberculin and nonspecific mitogens but fail to manifest in vivo delayed cutaneous responses to tuberculin skin tests. Since these patients respond normally to other antigens, such as C albicans, in producing a delayed cutaneous response, it would appear that the lack of manifest response to tuberculin is antigen-specific.

The mechanism of this failure to respond is unknown. Binding of antigen to antibody at the site of injection with possible fixation of complement may facilitate ingestion and disposal by macrophages. The immediate reaction has been shown by Cluff5 to be passively transferrable. It is possible that immunoglobulins other than IgE may bind to the antigen and prevent a T-lymphocyte response by means of antigen-antibody complexes.15

Alternative mechanisms to be considered are that tuberculin-sensitive T-lymphocytes in these patients fail to release mediators or that there is a failure of response to these mediators by monocytes and macrophages. Finally, the apparent lack of an in vivo delayed cutaneous response may be due to the action of suppressor T-lymphocytes, as suggested by Kantor.17 Since there was no evidence of general anergy, this would have to be considered to be an effect of specific tuberculin-sensitive suppressor T-lymphocytes in these patients.

The clinical significance of these findings is as yet uncertain. No patients in the study have developed overt tuberculosis, but they are being followed to determine the occurrence of this or other disease, along with evidence of impaired defense. The response to BCG vaccination following demonstration of immediate cutaneous reactivity in these patients has not been observed, but the possibility of anaphylactic-type reactions in such patients is raised by our findings and may be a warning to exercise caution in the administration of BCG vaccine. The possibility that such patients may have an enhanced response to BCG vaccine when it is administered as immunotherapy for leukemia and solid tumors may also merit further investigation.
REFERENCES


Renaissance Humanists

The Renaissance humanists, the first secular scholars of the modern era, did not stand for scientific progress, since with few exceptions (Benedetto Accolti, Pico della Mirandola) they considered the classical authors unsurpassable. Viewed sociologically, the humanists were dispensers of prestige who sought by their polished style and their erudition to make their protectors, and themselves, famous. The open admission that fame is the goal of all literary activity appears even more frequently in the humanists than in the classical literati, either because the purely individualistic conception of the literary profession was actually more developed in the Renaissance or only because more literary testaments are extant. On the other hand, in the humanist literature of the Renaissance there seems to be no case in which an author states that he is publishing his treatise in order to make further investigations possible.