BRONCHIAL CHALLENGE WITH PURIFIED PROTEIN DERIVATIVE OF MYCOBACTERIUM TUBERCULOSIS IN ASHTMA

A.V. Pherwani
V.A. Patwari

ABSTRACT

Response to bronchial challenge (BC) with purified protein derivative of Mycobacterium tuberculosis (PPD), was studied in children with bronchial asthma and correlated with Mantoux test and serum immunoglobulin E (IgE) against PPD (PPD-Sp IgE).

Nearly 59% patients with bronchial asthma and 12.2% patients with pulmonary tuberculosis gave positive BC. Children with extra-pulmonary tuberculosis and normal children did not show positive BC. In asthma, 25% gave early (EAR), 50% gave late (LAR) and 25% gave both early and late (DAR) asthmatic response. Forced expiratory flow volumes in1 sec (FEV.1) of 13 age and sex matched asthmatic and normal children showed similar volumes before BC, however, the values were significantly lower in asthma at 20 min ($p<0.005$) and 24 h ($p<0.005$) after BC. There was no relationship between response to BC and the severity or chronicity of asthma.

PPD-Sp IgE was estimated by the radioimmunoassay method (Pharmacia Diagnostics). It was detected in 75% with positive BC and none of the controls. The titre was of Phadebas RAST Class III in 66.7%, Class II in 22.2% and Class I in 11.1%. The presence of early Mantoux reactions, positive BC with PPD and serum PPD-Sp IgE suggest the existence of Type I or Arthus type of reactions to PPD, which could cause hyperreactive airways in some cases of asthma.

Key words: *Ashtma, Tuberculosis, IgE, Bronchial challenge.*

Type I or IgE mediated allergic reactions have been demonstrated following Mantoux test and experimentally after injecting *Mycobacteria* in the footpads of mice(1). We hypothesized that repeated exposure to tuberculoprotein, due to high prevalence of tuberculosis in India, may be the cause of Arthus type of reactions in the lungs, manifested by symptoms of asthma. In this context, we challenged the airways with purified protein derivative (PPD) as an antigen and also determined levels of serum IgE, specifically directed against PPD (PPD-Sp IgE).

Material and Methods

Four groups of children above the age of 7 years, who could be trained to perform spirometric pulmonary function tests, were selected for the study. Group A (n = 41) were cases of bronchial asthma diagnosed clinically and by the response to bronchodilator drugs. Asthma was graded as mild when respiratory rate was between 40-45/min, rhonchi were heard at end expiration; moderate when respiratory rate was between 46-60/min, rhonchi were heard during inspiration and expiration; severe when respiratory rate was more than 60/min, heart rate more than 120/min, cyanosis and rhonchi heard without the aid of stethoscope. Asthma of less than 2 years duration was classified as recent and of more than 2

From the Department of Pediatrics, Institute of Child Health, Sir J.J. Group of Hospitals and Grant Medical College, Bombay, and the P.D. Hinduja National Hospital and Medical Research Centre, Bombay.

Reprint requests: Dr. Asha V. Pherwani, 303 Samudra Mahal, Shivsagar Estate, Worli, Bombay 400 018.

Received for publication: October 3, 1989;
Accepted: January 25, 1992

years duration as chronic onset. An attack rate of one or more per month, for a year or more was classified as perennial asthma. Seasonal and occasional asthma was classified as non-perennial.

Group B (n = 11) were patients with pulmonary tuberculosis diagnosed on the basis of cough of more than 1 month duration, fever, loss of weight and appetite, Mantoux test of more than 10 mm induration and chest roentgenogram showing hilar lymph node enlargement or parenchymal involvement not responding to antibiotics. Group C (n = 5) were cases with extra-pulmonary tuberculosis diagnosed on lymph node biopsy or cerebrospinal fluid examination. Group D were controls who were age and sex matched with Group A. The age of the children was verified from school birth certificates. Height was measured with anthropometer rod and sliding caliper. Written consent was obtained from the parents.

Spirometry was performed on Fukada Sangyo spiroanalyzer model ST-200. The procedure was fully explained and demonstrated to the child. Bronchial challenge (BC) was only performed when the child was off bronchodilator drugs for 12-36 h, did not have respiratory tract infection in the past month and had a normal FEV.1 for his age and sex(3).

The PPD solution supplied by BCG Laboratories, Guindy, Madras, contained 0.1 mg/ml PPD. The diluent of the PPD contained 1.452 g potassium dihydrogenphosphate, 7.601 g disodium hydrogen phosphate, 0.5 dl 10% between 80 and 0.5 dl 20% quinosol in 1 litre of distilled water. Wright's nebuliser (Hudson) having an output of 0.13 L/min, driven by continuous air flow of 6-8 L/min, from a compressed air source of 50 psi(4,5) was used to deliver the PPD and its diluent for BC.

Baseline spiromtry was performed in all cases and if forced expiratory flow volume in 1 sec (FEV.1) was normal, BC was performed first with the diluent and thereafter with the PPD 24 h later. As BC with PPD was only performed if FEV.1 was normal both at 20 min and 34 h post challenge with the diluent, the study could not be blind. On the day of BC with PPD, 0.1 ml of PPD was also injected intradermally and the induration read after 20 min and 24 h. An induration of more than 10 mm was considered as positive Mantoux reaction.

As we were dealing with a highly potent antigen, we did not perform BC with increasing dilutions. A drop in FEV.1 of more than 20% after 20 min, 24 h or both was considered as early (EAR), late (LAR) and dual (DAR) asthmatic reaction, respectively. Statistical analysis was done using the Students 't' test.

Blood was collected on the day of challenge with PPD, serum was separated and stored in a frozen condition. PPD-Sp IgE was estimated in 12 asthmatic and 5 normal sera in a water-bath at 56°C for 1 h, by the radioimmunoassay method using the kit and instructions supplied by the manufacturers (Pharmacia, AB, Uppsala, Sweden). The results were expressed in radioallergosorbent (RAST) Class I-IV as per the instructions. The samples were sent to Pharmacia AB, Uppsala, Sweden, for cross checking. Data was analysed on Casio personal computer model PB-410.

Results

The mean age was 11.1, 10.3, 11 and 11.6 yr in Groups A, B, C and D, respectively. Maximum number of positive BC was seen in Group A (48.5%) as compared to Group B (18.2%) and none in Groups C and D. As there was no fall in FEV.1 fol-

lowing BC with the diluent, all our positive challenges can be attributed to PPD. The response was in the form of EAR, LAR or DAR. In Group A, EAR was seen in 25% (n = 6), LAR in 50% (n = 12) and DAR in 25% (n = 6). In Group B, DAR was seen in 100% (n = 2). These responses did not correlate with Mantoux test. Out of the 6 patients with EAR, Mantoux test was positive in 4 after 20 min, in 6 after 24 h and was negative in 2. Out of the 6 patients with DAR, Mantoux was positive in 3, both after 20 min and 24 h and was negative in 3. As shown in *Table I*, mean FEV.1 was similar in asthmatic and normal children before BC, but asthmatics had significantly lower FEV.1 both 20 min ($p = <0.005$) and 24 h ($p = <0.005$) after BC. *Table II* shows that there is no relation between positive BC and the severity and chronicity of asthma.

PPD-Sp IgE was detected in 75% (9/12) asthmatic and none of the 5 normal children where it was estimated. The titre of PPD-Sp IgE was Phadebas RAST Class III in 6 (66.7%), Class II in 2 (22.2%) and Class I in 1 (11.1%). There were no Class IV reactions. There were no untoward reactions following BC and all symptoms due to BC could be controlled with usual medication within 7 days.

TABLE I—*Forced Expiratory Flow Volume in 1 sec (FEV.1) with PPD Before and After Bronchial Challenge*

Age (years)	Bronchial asthma (n = 41)	Normal patients (n = 13)
FEV.1 (L)		
Pre-challenge	1.27 ±0.77	1.29 ±0.43
Post-challenge		
20 min	0.77 ±0.33*	1.28 ±0.48*
24 h	0.8 ±0.2*	1.32 ±0.46*

*Students 't' test, $p<0.005$

TABLE II—*Relation Between Type of Asthma and Bronchial Challenge with PPD*

Type of asthma (n = 41)	Bronchial challenge with PPD			
	Positive		Negative	
	n	%	n	%
Mild	3	12.5	2	11.8
Moderate	11	45.8	9	52.9
Severe	10	41.7	6	35.3
Perennial	16	66.7	8	47.1
Non-perennial	8	33.8	9	52.9
Recent onset	9	37.5	6	35.3
Chronic	15	62.5	11	64.7

Discussion

Elevated serum immunoglobulins have been demonstrated in patients with pulmonary tuberculosis(4,6-8). Specific IgE against tuberculoprotein has been demonstrated by Gatner and Msibi(8) and Pherwani and Jayram(6). Pelletier *et al.*(1) have demonstrated delayed hypersensitivity reactions to tuberculoprotein as evidenced by deposition of immunoglobulins in the footpads of mice 6 and 24 h. We have previously shown that 20% children with asthma have radiological findings and a positive Mantoux test suggestive of tuberculosis(9). These findings suggest that IgE against tuberculoprotein may be the cause of hyperreactive airways in some cases with asthma.

Positive BC was seen in 48.5% cases of asthma as compared to 18.2% cases of pulmonary tuberculosis and none in extrapulmonary tuberculosis and normal children. Our study is hindered by the fact that PPD contains many proteins and these pure, isolated proteins were not available. Probably

a better correlation could be had with purer antigens. As explained earlier, the dose used by us is arbitrary. As only 10-20% aerosol is inhaled, we presume that, of the 1 ml dose inhaled, 0.1-0.2 ml has reached the peripheral airways, which is similar to the dose used for Mantoux test. Metzger and Zawale(10) have demonstrated that the dose required for airway response is less than that required for dermal response; accordingly, we could have used a smaller dose for inhalation.

None of the sera was positive for PPD-Sp IgE at room temperature, but when mildly heated, 75% asthmatic and none of the normal children showed the presence of PPD-Sp IgE. Heating probably frees IgE from its complex with IgG, rendering it detectable(6). As we have not measured IgE levels in bronchoalveolar lavage, we do not know whether it is produced locally or if serum levels represent spillover from the lungs. We have not observed the effect of antituberculosis therapy on these children as it was not in the initial design of the study.

The presence of early Mantoux reactions, positive BC with PPD and the detection of PPD-Sp IgE in the serum, suggest the presence of Type I, Arthus type of IgE mediated allergic reactions to PPD in the lungs, which could be the cause of hyperreactive airways in some cases of bronchial asthma. Further controlled trials are needed to confirm these observations.

REFERENCES

1. Pelletir M, Forget A, Bourassa D, Skamene E. Histological and immunopathological studies of delayed hypersensitivity reaction to tuberculin in mice. Infec Immunol 1984, 46: 873-875.
2. Gardner RM. Standardization of spirometry: A summary of recommendations from the American Thoracic Society. Ann Inter Med 1988, 108: 217-220.
3. Dockcroft DW, Killian DN, Mellon JJA, Hargreave FE. Bronchial reactivity to inhaled histamine-A method and clinical survey. Clin Allergy 1977, 7: 235-243.
4. Juniper EF, Firth PA, Dinnet C, Cockcroft DW, Hargreave FE. Reproducibility and comparison of response to inhaled histamine and methacholine. Thorax 1978, 33: 705-710.
5. Mallik SK, Jindal SK. Pulmonary function tests in healthy children. Indian Pediatr 1985, 22: 677-680.
6. Pherwani AV, Jayram L, Masood MA. Determination of serum Immunoglobulin E against PPD antigen (PPD-Sp IgE) in patients with active pulmonary tuberculosis. (In press).
7. Radin RC, Zeiss CR, Phair JP. Antibodies to purified protein derivative in different immunoglobulin classes in the diagnosis of tuberculosis in man. Arch Allergy Appl Immunol 1983, 70: 25-29.
8. Gatner EMS, Msibi V. Circulating immunoglobulin levels in patients with pulmonary tuberculosis with special reference to IgE. Tubercle 1982, 63: 113-117.
9. Pherwani AV, Shroff MM, Bhave SY. Study of conventional chest radiographs of 200 Indian children who presented with an acute episode of wheezing. Bombay Hosp J 1991, 33: 6-8.
10. Metzger WJ, Zawale D, Richardson HB. Local allergen challenge and bronchoalveolar lavage of allergic asthmatic lungs: description of model and local airway inflammation. Chest, 1986, 89: 477-483.