

Phytohemagglutinin (PHA) skin test in the diagnosis of cellular immunodeficiency

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Phytohemagglutinin (PHA) is a nonspecific activator of lymphocytes and is of value in the in vivo and in vitro assessment of cellular immunity. One hundred and four subjects were skin tested with an intradermal injection of 1 µg of PHA. Ninety-one of 94 subjects with no apparent cellular immunodeficiency gave a positive response at 24 hours, including 5 patients with antibody deficiency but normal cellular immunity. There was a correlation between the in vivo and in vitro response to PHA in 22 of 23 normals tested. Four of 10 patients with cellular immunodeficiencies had no response to a 1 µg PHA skin test; in addition, the mean response for this group was significantly reduced when compared to normals. Five of 10 patients in the cellular immunodeficiency group gave positive skin tests in the presence of an abnormal in vitro response. The PHA skin test is a simple and useful screening test for cellular immune function and is of particular value in infants and young children since it does not require prior sensitization.

Phytohemagglutinin (PHA) is an extract of Phaseolus beans which agglutinates red blood cells and transforms lymphocytes into large primitive cells.¹ Lymphocyte transformation with PHA correlates with the capacity to manifest delayed type hypersensitivity in vivo.² Normal subjects respond, without prior sensitization to an initial intradermal injection of PHA with erythema and induration at 24 hours and a histologic picture of perivascular infiltration of mononuclear cells characteristic of cutaneous delayed hypersensitivity.³

Since patients with defects in cellular immunity are unable to respond to the intradermal injection of most antigens associated with a delayed hypersensitivity response, they also may be unable to respond to PHA. We therefore evaluated the intradermal response to PHA in patients with cellular immunodeficiency and compared their responses to those of patients with humoral immunodeficiencies, normal age-matched controls, and normal adults.

MATERIALS AND METHODS

One hundred and four subjects were divided into 2 groups (Table I). Group I consisted of 89 persons with no evidence of immunodeficiency. These included 14 hospitalized patients

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with various illnesses unrelated to immunodeficiency (7 males and 7 females, mean age 14 years), 49 normal infants and children (23 males and 26 females, mean age 3 years), and 26 normal adults (16 males and 10 females, mean age 30 years).

Group II consisted of 15 patients with immunodeficiency syndromes. They included 5 patients with selective deficiency of humoral immunity, including 2 patients with dysgammaglobulinemia and 3 patients with hypogammaglobulinemia, and 10 patients with cellular immunodeficiencies, including 3 patients with cellular immunodeficiency with immunoglobulins (Nezelof's syndrome), 2 patients with chronic mucocutaneous candidiasis, 2 patients with ataxia telangiectasia, and single patients with Wiskott-Aldrich syndrome, combined immunodeficiency, and sarcoidosis. Ten were males and 5 were females. The mean age was 7 years, the youngest being 9 months and the oldest 20 years.

All subjects were tested with an intradermal injection on the volar aspect of the forearm with 1 μ g of PHA* in 0.1 ml. The diameter of erythema and induration was measured at 24 and 48 hours. Most subjects who failed to respond to a dose of 1 μ g were tested with a dose of 10 μ g. Some subjects were tested at a dose of 0.1 μ g.

Other skin tests applied in some cases included: Dermatophyton O (monilia), 1:100 dilution (Hollister-Stier Labs.), streptokinase-streptodornase (SK-SD), 100 units SK and 25 units SD (Lederle Labs.), mumps skin test antigen (Eli Lilly & Co.), purified protein derivative (PPD), 0.0001 mg./0.1 ml. (Parke, Davis & Co.), histoplasmin (Parke, Davis & Co.), coccidioidin, 1:100 dilution (Cutter Laboratories, Inc.), and trichophyton, 500 PNU per cubic centimeter (Hollister-Stier Labs.). Contactants included 2:4 dinitrofluorobenzene (DNFB), 0.05 ml. of a 0.1 per cent solution after a sensitizing application of 0.05 ml. of a 10 per cent solution (Eastman), and 10 per cent croton oil (Magnus).

A positive skin test was considered to be 5 mm. or greater diameter of induration. Erythema alone was not considered a positive response. Responses less than this were considered abnormal.

The peripheral lymphocytes of 33 individuals were assessed for in vitro reactivity to PHA. One hundred thousand lymphocytes in 0.1 ml. were cultured in the presence of PHA-M† (Wellcome purified PHA has been shown to be cytotoxic for lymphocytes in routine culture⁴) using the micromethod of Sengar and Terasaki.⁵ One hundred thousand (1×10^5) normal lymphocytes activated by 0.05 ml. PHA incorporate radioactive precursor (H^3 thymidine 0.8 μ C., specific activity 1.9 C. per millimole) with a normal response of 30,000 counts per minute with one standard deviation $\pm$ 10,000. Total counts below 15,000 are considered abnormal.

The statistical significance of r values and the means in different groups of patients were assessed by Student's t test. Correlations were considered significant if the null hypothesis had a probability (p) of 0.05 or less.

RESULTS

Eighty-six of the 89 individuals in Group I gave positive responses to 1 μ g of PHA (Table I). The mean maximum response occurred at 24 hours; this was significantly greater ($p < 0.05$) than the 48 hour response. The maximal response occurred at 24 hours in more than 90 per cent of those individuals with positive skin tests.

No individuals developed ulceration or necrosis at the injection site, only induration, erythema, and pruritus being observed. Most reactions disappeared by 72 hours, and all reactions disappeared by 5 days. Patients tested repeatedly on as many as 3 occasions showed no increase in their response.

*Wellcome purified phytohaemagglutinin, Beckenham Kent BR3 385, England.

†Difco Laboratories, Detroit, Mich.

TABLE I. Response to phytohemagglutinin skin test in various groups of subjects*

Subject group	Num- ber tested	Num- ber posi- tive	Per cent posi- tive	Diameter of response (mm.) $\pm$ standard error			
				24 hours		48 hours	
				Erythema	Induration	Erythema	Induration
I. <i>Intact immunity</i>							
A. Hospitalized patients	14	13	92	11.4 $\pm$ 1.15	7.5 $\pm$ 0.78	8.3 $\pm$ 1.29	5.6 $\pm$ 1.37
B. Normal children	49	47	96	14.1 $\pm$ 0.75	9.0 $\pm$ 0.71	8.2 $\pm$ 0.76	4.5 $\pm$ 0.47
C. Normal adults	26	26	100	22.9 $\pm$ 0.83	14.3 $\pm$ 0.76	15.4 $\pm$ 2.20	6.8 $\pm$ 1.03
II. <i>Immunodeficiency syndromes</i>							
A. Patients with intact cellular immunity	5	5	100	15.0 $\pm$ 2.34	9.8 $\pm$ 1.33	12.3 $\pm$ 4.81	5.6 $\pm$ 2.59
B. Patients with altered cellular immunity	10	6	60	6.9 $\pm$ 2.40	5.1 $\pm$ 1.57	1.6 $\pm$ 0.75	0.8 $\pm$ 0.57

*All patients were tested with 1 μ g of PHA in 0.1 ml.

Infants and younger children were less responsive to PHA than the adult group ($p < 0.05$) (Table I).

The 3 subjects in Group I who failed to respond to an intradermal injection of 1 μ g of PHA included 2 females aged 2 and 2½ years with no evidence of disease and a 14-year-old female with chronic generalized eczema. The first 2 responded normally to monilia and SK-SD in vivo, and one had a normal lymphocyte response in vitro to PHA. Repeat testing was not done on these 2 subjects. The third subject did not respond to 10 μ g of PHA or other skin test antigens including dinitrofluorobenzene challenge after an initial vesicant response. She may have had a cellular immunodeficiency, as her lymphocytes gave a decreased response to PHA in vitro.

All 5 patients with antibody deficiencies but intact cellular immunity responded normally to a 1 μ g PHA skin test (Table I).

Four patients with cellular immunodeficiencies had negative PHA skin tests. Three others had minimally positive tests, and 3 had normal responses. The mean response with standard error for this group was 6.9 mm. $\pm$ 2.40 of erythema and 5.1 mm. $\pm$ 1.57 of induration at 24 hours and 1.6 mm. $\pm$ 0.75 of erythema and 0.8 mm. $\pm$ 0.57 of induration at 48 hours (Table I), which is significantly reduced compared to the mean response observed in age-matched normal controls in Group I ($p < 0.05$).

The patients who failed to respond to 1 μ g PHA in vivo included 2 patients with Nezelof's syndrome, one patient with chronic mucocutaneous candidiasis, and one patient with Wiskott-Aldrich syndrome (Table II).

All patients in Group II who failed to respond to PHA in vivo gave negative cutaneous responses to other common delayed hypersensitivity antigens including sensitization to DNFB.

Three of the 4 patients with a negative response to 1 μ g PHA gave an equivocal response to 10 μ g PHA. One patient with the Wiskott-Aldrich syndrome had a negative response to 10 μ g PHA. His inflammatory response was intact since he manifested a vesicant response to DNFB and an erythematous response to topical croton oil.

TABLE II. Phytohemagglutinin responses in patients with cellular immunodeficiencies

Patient	Diagnosis	Response to 1 μ g PHA skin test (mm. of induration at 24 hours)	Other skin tests	In vitro PHA response incorporation of H^3 thymidine in 1×10^5 lymphocytes (c.p.m.) (normal 30,000 c.p.m. $\pm$ 10,000)
A. F. (4 yr., male)	Chronic mucocutaneous candidiasis	Negative (0)	PPD (-) Monilia (-) SK-SD (-) Mumps (-) Trichophyton (-) PHA 10 μ g ($\pm$)	Normal, 50,000
Y. A. (10 yr., female)	Chronic mucocutaneous candidiasis	Positive (8)	PPD (-) Monilia (-) SK-SD (-) Trichophyton (-)	Normal, 22,000
S. A. (14 yr., female)	Ataxia telangiectasia	Positive (17)	Monilia (+) SK-SD ($\pm$)	Decreased, 4,000
L. D. (9 yr., female)	Ataxia telangiectasia	Positive (8)	PPD (-) Monilia (+) SK-SD (-) Histo (-) Cocci (-)	Decreased, 2,000
C. T. (2 yr., male)	Wiskott-Aldrich syndrome	Negative (0)	Monilia (-) SK-SD (-) Trichophyton (-) PHA 10 μ g (-) DFNB (-) Croton oil (+)	Decreased, 5,200
M. O. (3 yr., male)	Nezelof's syndrome	Negative (0)	PPD (-) Monilia (-) SK-SD (-) PHA 10 μ g ($\pm$)	Decreased, 40
N. C. (18 mo., female)	Nezelof's syndrome	Negative (2)	Monilia (-) SK-SD (-) Trichophyton (-) PHA 10 μ g ($\pm$)	Decreased, 6,200
J. R. (4 yr., male)	Nezelof's syndrome	Positive (5)	Monilia (-) SK-SD (-)	Decreased, 4,500
D. L. (3 yr.)	Combined immunodeficiency	Positive (5)	PPD (-) Monilia (-) SK-SD (-) Trichophyton (-) PHA 10 μ g (+) DNFB (-)	Decreased, 4,100
G. B. (14 yr., male)	Sarcoidosis	Positive (6)	PPD (-) Histo (-) Cocci ($\pm$)	Decreased, 6,300

Comparison between the in vivo and in vitro response to PHA in the 23 normals tested revealed a good correlation between the qualitative responses (normal or abnormal). There was a discrepancy in one who had a reduced skin test in the presence of a normal in vitro response. However, quantitatively the degree of response in the in vitro assay and the degree of positivity of the skin test were poorly correlated ($r = -0.31$).

There was a discrepancy between the *in vivo* and *in vitro* response to PHA in 6 patients with cellular immunodeficiency (Table II). One patient with chronic mucocutaneous candidiasis had a negative PHA skin test but responded to PHA *in vitro*. The other 5 had positive PHA skin tests with suboptimal *in vitro* responses.

DISCUSSION

The mitogenic and blastogenic properties of PHA were discovered initially by Hungerford in 1959.¹ Schrek and Stefani first reported the use of PHA intradermally as a method of interpreting delayed hypersensitivity and found direct correlation with the *in vitro* response.⁶ Bonforte and associates³ have shown that the histology of the *in vivo* response is similar to that of cutaneous delayed hypersensitivity reactions obtained with sensitizing antigens and is distinct from the histologic findings of the Arthus reaction.

Burgio and associates⁷ have demonstrated normal responses to PHA *in vivo* in infants and children and have also noted an increased response with advancing age, with a maximal response in older children and adults. Airo and associates⁸ reported delayed cutaneous response to PHA in patients with chronic lymphocytic leukemia and sarcoidosis. They noted a dose-related response with local necrosis and ulceration using a dose of 0.1 mg. of PHA. In a larger series of patients, Bonforte and associates³ have shown a normal *in vivo* response to 2 μ g of PHA in 100 per cent of normal adults, children, and term and premature infants. This study also included patients with sarcoidosis, congenital rubella syndrome, thymic dysfunction, and Hodgkin's disease. All of these reacted to the PHA skin test in the presence of a generally reduced *in vitro* response except for one patient with congenital rubella and one patient with advanced Hodgkin's disease.

Skin testing with PHA is a simple procedure with no known morbidity in a dose of 1 to 10 μ g, optimal results being obtained at doses approximating 1 μ g. Since PHA reacts without prior sensitization, it is of particular value in newborns, infants, and young children who have not been exposed to the antigens commonly used as skin tests.

Over 95 per cent of normals respond to the PHA skin test; this is considerably higher than the responses to the more commonly used skin antigens, monilia and SK-SD. The reactivity to these antigens is 50 to 90 per cent in normal adults and 30 to 50 per cent in infants and young children.^{9,11} Application of contact sensitizers such as dinitrochlorobenzene (DNCB) and dinitrofluorobenzene (DNFB) can sensitize 90 per cent of normal subjects.¹² However, these agents require initial sensitization with a vesicant dose that may result in a permanent scar. Further, a period of 10 to 13 days must elapse prior to challenge.

Since a negative skin test may result from abnormalities in the inflammatory response as well as specific defects in delayed hypersensitivity, it is of particular importance to assess the inflammatory capacity in any patient who fails to manifest a delayed cutaneous response. Cutaneous irritants such as croton oil or hypertonic saline are well suited for this purpose; however, the vesicant response to DNCB or DNFB sensitization is of similar value.^{13, 14}

The in vitro response to PHA correlates well with the in vivo response, although positive PHA skin tests with abnormal in vitro stimulation have been reported.^{3, 8, 15} The in vivo response may be a less sensitive indicator of a defect in cellular immune function than the in vitro response. A positive skin test requires only a small number of normal lymphocytes, which, through the release of soluble mediators such as migration inhibition factor, may elicit a delayed cutaneous reaction.

In contrast, PHA stimulation in vitro may be abnormal if a portion of the lymphocytes are abnormal, such as in patients with a partial loss of cellular immunity. In some of these disorders, the lymphocytes do not survive well in culture; in others there are diminished surface binding sites, and in others serum antilymphocyte factors exist.^{16, 17} Current methods of preparation of lymphocytes do not permit separation of normal from unresponsive lymphocytes; thus the total response may be abnormal if a portion of the cell population is abnormal.

The PHA skin test is an aid in the assessment of cellular immune function. The results of this study indicate that some patients with deficiencies in cellular immunity are capable of responding to PHA, while in others the response is less than normal. Although there is an apparent dissociation between the in vivo and in vitro response in some cases of partial cellular immune dysfunction, we recommend the use of the 1 μ g PHA skin test as a simple and useful screening procedure in the initial work-up of patients suspected of cellular immunodeficiency. Since PHA is currently not licensed in the United States for human use, an Investigational New Drug (IND) permit must be obtained.* Also, individual informed consent is necessary prior to application.

*IND permit obtainable from the Food and Drug Administration, Rockville, Md. 20852.

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