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QUANTIFIED DEFICIENCY OF LYMPHOCYTE RESPONSE TO PHYTOHAEMAGGLUTININ IN IMMUNE DEFICIENCY DISEASES

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SUMMARY

Using a quantitative assay of lymphocyte responsiveness to different concentrations of phytohaemagglutinin (PHA), it has been possible to demonstrate that the lymphocytes of children with ataxia telangiectasia do not respond normally. The response to PHA is nearer normal at higher concentrations of PHA.

In the same system, it has been shown that although the lymphocytes of children with probable infantile sex-linked agammaglobulinaemia (Bruton's disease) may respond normally at higher concentrations of PHA, at lower concentrations their response is subnormal.

The results reported indicate that if lymphocyte response to PHA is defective in an immune deficiency disease, then the response at lower concentrations of PHA provides the most sensitive discriminatory information.

INTRODUCTION

Since Bruton described a boy with agammaglobulinaemia in 1952 (Bruton, 1952) immune deficiency diseases have come to be recognized more commonly and in distinct clinical syndromes (Seligmann, Fudenberg & Good, 1968; Fudenberg *et al.*, 1970).

It would appear that the blast response of lymphocytes of normal persons *in vitro* to antigens such as candida, correlates with delayed hypersensitivity skin reactions (Gotoff, 1968). The situation with regard to phytohaemagglutinin (PHA) is not as clear because the basic mechanism of the action of PHA on lymphocytes has not been elucidated (Naspitz & Richter, 1968). However, a correlation has been noted between the normality of function of the thymic influenced lymphocytes and the ability of lymphocytes in peripheral blood to respond *in vitro* to stimulation with PHA. This correlation has been reported in the clinical situation in diseases like thymic aplasia (Lischner, Punnett & DiGeorge, 1967) and in experimental animal models (Inversen, 1969; Rodey & Good, 1969). Much of the work in the past on immune deficiency diseases and conclusions drawn from it, has been based on

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gross differences in response of lymphocytes to PHA. The purpose of this paper is to describe the results of investigations of children with immunological deficiencies using a densitive, reproducible, *in vitro* lymphocyte culture system. As PHA appears to produce a mitogenic response in the lymphocytes of all normal persons, it would seem to be an ideal mitogen to use in attempting to discriminate minor degrees of abnormal lymphocyte function. It is intended that the approach described will offer a more refined tool for the recognition of disorders of the immune mechanism.

MATERIALS AND METHODS

The response *in vitro* of lymphocytes to phytohaemagglutinin was determined in the following patients: six children with ataxia telangiectasia, seven children with infantile sex-linked agammaglobulinaemia, one child with non-congenital agammaglobulinaemia, three children with isolated IgA deficiency and eight children with recurrent infections who had normal or raised serum immunoglobulin levels.

The lymphocyte culture technique is that of Fitzgerald (1971). Briefly, washed buffy coat leucocytes containing one million lymphocytes per culture tube are cultured in 1 ml Medium 199 (20% calf serum). To each of a series of duplicate culture tubes is added increasing amounts of phytohaemagglutinin over the concentration range of 2–800 μg/ml and the cultures incubated at 37°C for 72 hr. To assess DNA synthesis as a measure of blast transformation, 2 μCi of tritiated thymidine is added for the last 2 hr of culture. The acid-insoluble material is solubilized, mixed with a scintillant and counted in a beta liquid scintillation counter (Packard Tricarb). The results of the patient's lymphocytes are compared with those of a normal individual taken at the same time and cultured in parallel. If the normal person's lymphocyte response is within the normal range as previously described (Fitzgerald, 1971) then the test is presumed to be technically satisfactory.

RESULTS

The case histories of seven children with infantile, sex-linked, agammaglobulinaemia are given in the Appendix and the results of serum immunoglobulin concentrations are summarized in Table 1. Also in Table 1 are the immunoglobulin levels of six children with ataxia telangiectasia whose case histories are summarized elsewhere (Dwyer *et al.*, 1971) and one child, T.D. who we believe has non-congenital agammaglobulinaemia.

As can be seen from Fig. 1 the lymphocyte response to PHA of those patients with ataxia telangiectasia did not fall within the normal limits. One point to note is that the curves in all cases are nearer normal at the concentration of 200 μg PHA per ml than at 20 μg PHA per ml. In order to utilize this fact, the dose–response ratio, which is the ratio of log (cpm 200 μg PHA-cpm 0 μg PHA) to log (cpm 20 μg PHA-cpm 0 μg PHA) has been determined. As seen in the third column of Fig. 3 these are more than 2 S.D. above the mean, for the patients with ataxia telangiectasia.

The results of lymphocyte culture of the seven children with infantile sex-linked agammaglobulinaemia (Figs 3, 4, 5 and 6) shows that the response of none of the seven is normal at all concentrations of PHA. Again there is the tendency for the response to be nearer normal at 200 μg PHA per ml than at 20 μg PHA per ml. The PHA dose–response ratio is graphed in the second column of Fig. 2 and again the majority of children have results beyond the

TABLE 1. Serum immunoglobulin levels in a group of children with immune deficiency diseases

Patient	Diagnosis	Serum immunoglobulins		
		IgG (mg/100 ml)	IgA (mg/100 ml)	IgM (mg/100 ml)
M.E.	AT	2085	< 10	85
R.E.	AT	< 10	< 10	288
L.E.	AT	1750	290	146
Ge.H.	AT	2050	178	175
Gl.H.	AT	1050	< 10	240
S.H.	AT	890	183	190
L.McL.	ISLA	*220	< 10	< 10
P.McL.	ISLA	*300	< 10	< 10
S.C.	ISLA	*270	< 10	10
J.C.	ISLA	*210	< 10	< 10
Ga.W.	ISLA	*350	< 10	< 10
Gl.W.	ISLA	*280	< 10	< 10
N.Y.	ISLA	< 10	< 10	< 10
T.D.	NCA	34	< 10	< 10

All of these levels determined when the child was over 3 years of age except T.D. who was 30 months old.

Hyland 'Immunoplates' used for all estimations.

ISLA = Probable infantile sex-linked agammaglobulinaemia.

AT = ataxia telangiectasia.

NCA = Probable non-congenital agammaglobulinaemia.

* Being treated with parenteral γ-globulin.

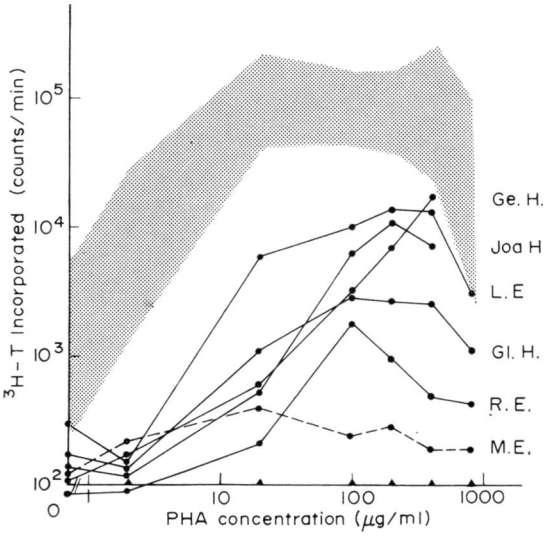

FIG. 1. The lymphocyte response to different concentrations of PHA of six children with ataxia telangiectasia. The shaded area represents ± 2 S.D. from the normal mean response. The interrupted line (M.E.) represents the response of lymphocytes taken during a leukaemic phase of lymphosarcoma from which this child subsequently died.

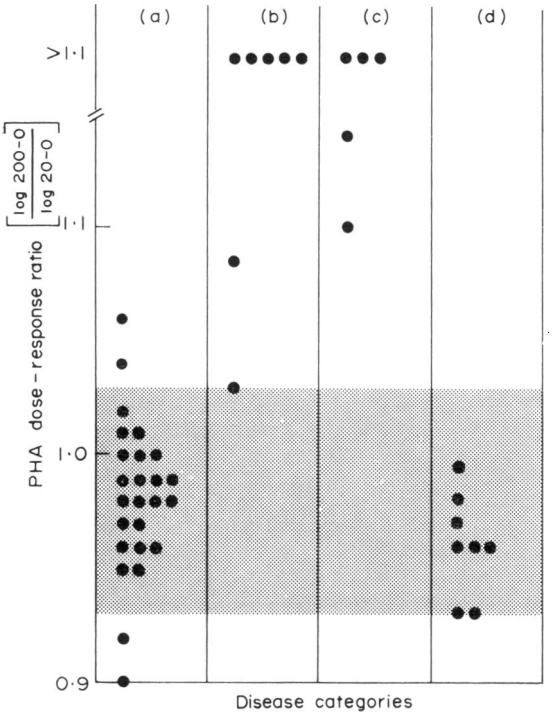

FIG. 2. The PHA dose–response ratio of a group of children with suspected immune deficiency diseases compared with normal controls. The shaded area represents ±2 S.D. from the mean. (a) Normal; (b) probable infantile sex-linked agamma-globulinaemia; (c) ataxia telangiectasia; (d) a group of children with recurrent infections but with normal serum immunoglobulin levels.

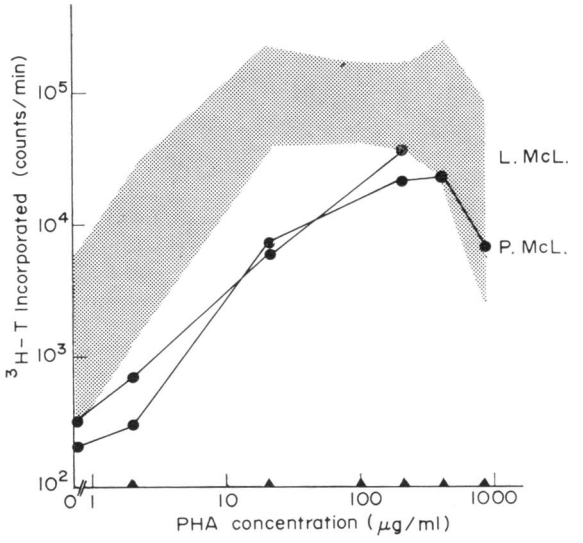

FIG. 3. The PHA dose–response curve of the lymphocytes of two brothers with probable infantile sex-linked agammaglobulinaemia.

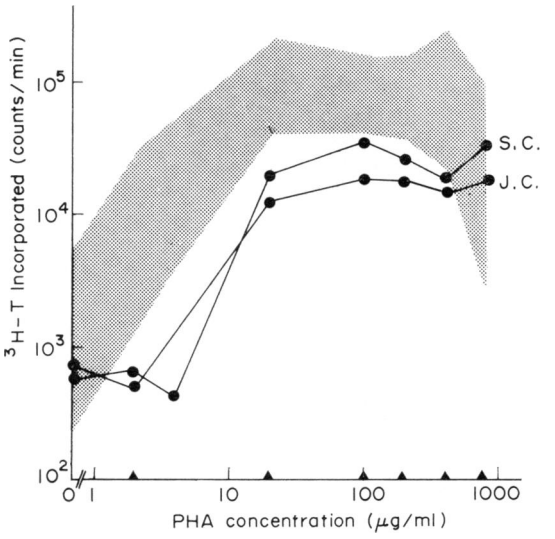

FIG. 4. The PHA dose–response curve of the lymphocytes of two boys with probable infantile sex-linked agammaglobulinaemia.

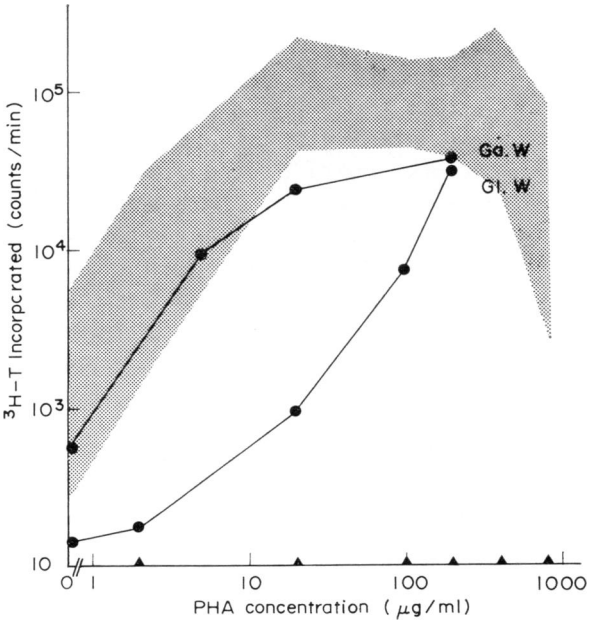

FIG. 5. The PHA dose–response curve of the lymphocytes of two brothers with probable infantile sex-linked agammaglobulinaemia.

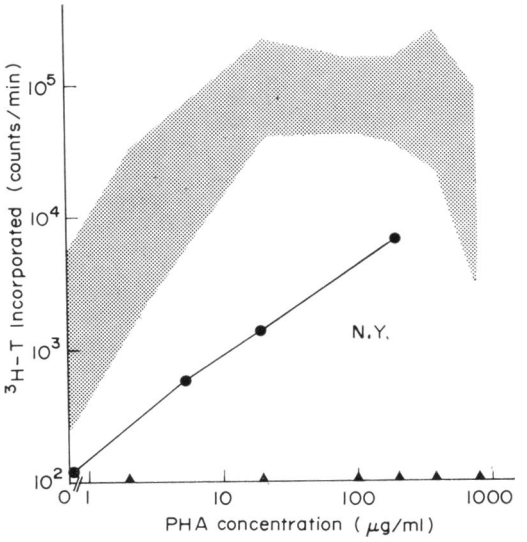

FIG. 6. The PHA dose–response curve of the lymphocytes of a boy with probable infantile sex-linked agammaglobulinaemia.

normal range. The response of the lymphocytes of one child, T.D., who has probable non-congenital agammaglobulinaemia, was normal.

As a control for this ratio and for the dose–response curve from which it is derived, the response of the lymphocytes of a number of children with recurrent infections but who have normal or raised levels of immunoglobulins were tested and found to lie within normal limits as shown in the fourth column of Fig. 2.

The response of the lymphocytes of two children with persistent isolated IgA deficiency and one who had a very low level of IgA that has subsequently risen to normal levels has been determined. The lymphocytes of two of the children were normal. The lymphocytes of the third child, one of the two with absent IgA, has been abnormal when cultured on three separate occasions. He is the only one of the three who has persistent respiratory infections and is failing to thrive, so that his abnormal lymphocyte responsiveness, compared to the other children, may signify a more severe immune deficiency.

One child who has undetectable serum IgG and IgA and normal levels of IgM has normal lymphocyte function as determined by this test.

DISCUSSION

It has been shown that it is possible to statistically define the limits of the response of lymphocytes of normal individuals at different concentrations of PHA (Fitzgerald, 1971). With these limits defined, it is feasible to compare the response of lymphocytes of patients with suspected lymphocyte abnormalities with that of normals.

Ataxia telangiectasia is a readily diagnosible clinical syndrome consisting of cerebellar ataxia, dilated blood vessels on the bulbar conjunctiva and variable immune deficiencies. While there is general agreement that both the thymus and the PHA responsiveness of

lymphocytes are abnormal in most patients with ataxia telangiectasia (Naspitz & Richter, 1968), Schuler *et al.* (1966) have reported one case where PHA responsiveness was normal. All children in our series had impaired responses at all concentrations of PHA.

The disease, infantile sex-linked agammaglobulinaemia (formerly known as Bruton's disease, Seligman *et al.*, 1968), is not as clearly delineated a syndrome as ataxia telangiectasia but the clinical definition we have used is that of Janeway (1968). All the children are males, all had an early onset of infections and there are two sets of brothers in the group. There is doubt about the diagnosis of the child T.D. He presented at 17 months, an older age than the other children, following a prolonged, vague illness terminating in aplastic anaemia which recovered in 2 weeks following a blood transfusion and no specific therapy. An electrophoretic pattern on cellulose acetate of serum taken 10 days after the blood transfusion showed a γ-globulin band estimated to contain 1·5 g/100 ml.

The amount of γ-globulin in the blood of the transfusion would not have been sufficient to produce this IgG level had he been agammaglobulinaemic prior to transfusion. His lymphocyte response to PHA is normal. For these reasons and because the behaviour of his lymphocytes in another *in vitro* system is different from the other seven boys (Dwyer & Hosking, 1971) we believe that T.D. has non-congenital agammaglobulinaemia.

In contrast to the picture in ataxia telangiectasia, there is general agreement that thymic function and lymphocyte responsiveness to PHA *in vitro* of patients with infantile sex-linked agammaglobulinaemia is normal (Gatti & Good, 1970).

The lymphocyte response in our series of children with this clinical diagnosis tends to be nearer normal than those of the children with ataxia telangiectasia and in four instances, the response at the higher concentrations of PHA falls within the normal range. However, the response at 20 μg PHA/ml is more than two standard deviations below the mean for all the children.

This finding of abnormal lymphocyte responsiveness to PHA of children with infantile sex-linked agammaglobulinaemia is at variance with those from other laboratories (Gotoff, 1968; Bradley & Oppenheim 1967; Fudenberg & Hirschhorn, 1964; Daguillard *et al.*, 1969). There are a number of possible reasons for this. Firstly our seven boys may not have this disease, i.e. the diagnosis based on clinical and laboratory findings may be incorrect. While this is possible as there is no absolute criterion for the diagnosis of this disease, the clinical and immunoglobulin findings do agree with current concepts of the syndrome, e.g. none of the children have detectable pharyngeal tonsils and there is a positive family history consistent with sex-linked recessive inheritance in two families.

It is possible that all seven boys represent a new disease or a disease variant of classical infantile sex-linked agammaglobulinaemia in that there is a deficiency of thymic function. In support of this latter hypothesis is the finding of an abnormal thymus in the child P.McL. This child did have some evidence in life of thymic dysfunction in that his peripheral blood lymphocyte count was frequently at or below the lower limit of normal. He does, however, have a brother, L.McL. whose lymphocyte count is normal but the lymphocyte response to PHA is abnormal.

Another possibility is that by defining the normal limits of response at a number of concentrations of PHA we have been able to demonstrate a definite, if relatively minor in some instances, variation in lymphocyte responsiveness from normal.

If one assumes that our children do have infantile sex-linked agammaglobulinaemia then a consistent abnormality in the response of their lymphocytes to PHA has been demonstrated.

At this stage, the exact relationship between PHA responsiveness of lymphocytes and thymic function cannot be stated with certainty.

The post-mortem findings of histologic abnormalities in the thymus and other lymphoid organs of the child P.McL. would suggest some correlation. However, during life he had a normal delayed hypersensitivity skin reaction to candida extract. One child, Ga.W. has a negative Mantoux test 1 year after BCG vaccination but had a normal delayed hypersensitivity response to candida extract. J.C. also failed to convert to Mantoux positive 1 year after BCG vaccination. The child N.Y. failed to show a positive delayed hypersensitivity reaction to candida extract 2 weeks after clinical oral candidiasis. The oral candidiasis is not a recurring or chronic problem in this child but seemed to be related to broad spectrum antibiotic therapy of a pulmonary infection. The candidiasis cleared with treatment with the antifungal agent 'mycostatin'.

At the present time it would seem wise to consider both the absolute height of the dose–response curve and its shape in assessing the results of lymphocyte culture in patients with suspected immune deficiency. While there does seem to be a correlation between a generally lowered response and raising of the PHA dose–response ratio, our sampling to date has not been broad enough to say whether or not this correlation will hold with all causes of deficient lymphocyte function.

APPENDIX

L.McL. *Male. Date of birth,* 1 *April* 1961

This boy had recurrent attacks of bronchitis from the age of 8 months. In 1964 left lower lobe collapse was noted and electrophoresis of serum proteins showed that the γ-globulin band was absent. The left lower lobe has remained collapsed and he has continued to have recurrent chest infections. He has had no detectable antibody rise following stimulation with a number of antigens and has no IgA or IgM. IgG levels are maintained with fortnightly injections of intramuscular γ-globulin. He has a normal lymphocyte count.

P.McL. *Male. Date of birth,* 5 *September* 1964. *Date of death,* 13 *August,* 1970

He was a brother of L.McL. As his brother's diagnosis was known by the time of P.McL.'s birth, serum immunoglobulin concentration estimations were performed in the first few months of life. These showed a progressive drop of IgG from birth and failure to develop detectable IgA or IgM. Throughout life he had a mild lymphopenia with lymphocyte counts between 1000 and 1500 per cu mm. Despite treatment with γ-globulin he had persistent otitis media and recurrent chest infections associated with a left lower lobe collapse. He developed thrombocytopenia at the age of $3\frac{1}{2}$ years which resulted in frequent epistaxes requiring blood transfusion. He also showed signs of malabsorption and this, together with progressive pulmonary incompetence, led to his death at the age of 5 years. Post-mortem examination showed a hypocellular thymus containing few lymphocytes and few Hassall's corpuscles. Gut associated lymphoid tissue was deficient and lymph nodes had no follicles and were generally lymphocyte depleted.

S.C. *Male. Date of birth,* 26 *June,* 1966

This boy has no siblings and there is no family history of recurrent infections. He had septic arthritis and recurrent respiratory infections prior to the age of 2 years when an electro-

phoretic pattern of his serum revealed the absence of γ-globulin. Subsequent immunochemical measurements showed that he had less than 10 mg/100 ml of IgG and IgA and 10 mg/100 ml of IgM. Since diagnosis of an underlying immune deficiency and treatment with γ-globulin, he has continued to have mainly upper respiratory infections with persistent otitis media. He does have an occasional attack of pulmonary infection.

J.C. *Male. Date of birth*, 16 *September* 1955

He commenced having respiratory infections at 6 months of age and had numerous attacks of impetigo. He has had a persistent cough since the age of 2 years and has bronchiectasis demonstrated radiographically. He was diagnosed as having agammaglobulinaemia in 1957 and has been treated with parenteral γ-globulin since then. This treatment has resulted in a considerable lessening of the frequency of attacks of acute infections but the irreversible lung damage occasionally leads to flaring of acute pulmonary infection. J.C. has one normal brother and eleven normal sisters.

Ga.W. *Male. Date of birth*, 23 *September* 1959

He had two maternal uncles who died in their first 4 years of life from infections. He had a series of infections in the first 5 years of life including an *Haemophilus influenzae* septicaemia resulting in a septic arthritis and several attacks of pneumonia, conjunctivitis and otitis media. At the age of 5 years the diagnosis of agammaglobulinaemia was made on the basis of absent γ-globulin on serum electrophoresis. Subsequently, IgG levels have been maintained with parenteral γ-globulin but IgA and IgM have not been detected in his serum by radial immunodiffusion. Despite parenteral γ-globulin he has continued to have recurrent otitis media and bronchitis leading to bronchiectasis. He has had a normal lymphocyte count at all times (2000–4000 lymphocytes per cu mm.)

Gl.W. *Male. Date of birth*, 29 *September* 1965

He was diagnosed as having agammaglobulinaemia when he failed to develop serum IgA and IgM levels during the first 6 months of life. Maternal IgG levels in his serum fell during this time. He commenced parenteral γ-globulin at 8 months and has had no serious infections. Gl.W. is the brother of Ga.W. There are two other siblings in the W. family, a boy and a girl, both normal.

N.Y. *Male. Date of birth*, 20 *June* 1964

He had a number of upper respiratory infections and one subcutaneous abscess prior to his first admission to this hospital with bullous impetigo at the age of 2 years. At this time, peripheral blood examination revealed the presence of neutropenia. Bone marrow examination showed maturation arrest of the myeloid series. However, with no specific therapy the peripheral blood neutrophils returned to normal over the subsequent 2 weeks. He was again referred to this hospital at the age of 6 years because of recurrent chest infection. Chest X-ray showed middle lobe collapse and patchy pneumonitis in the left base. Agammaglobulinaemia was diagnosed on the basis of serum immunoglobulin estimation. He had a normal neutrophil count but a mild lymphopenia (1200/cu mm). He has a normal brother and sister and there is no family history of recurrent infections or autoimmune diseases. During treatment for the chest infection he developed oral candidiasis which settled with local treatment. A candida skin test performed 2 weeks later was negative.

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