Clin. exp. Immunol. (1985) **60**, 396–402.

Defective *in vitro* antibody production in response to pokeweed mitogen and influenza antigen in patients with Hodgkin's disease

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(Accepted for publication 5 December 1984)

SUMMARY

Cultures of peripheral blood mononuclear cells (PBM) from 33 patients with Hodgkin's disease, were stimulated *in vitro* with pokeweed mitogen (PWM) or influenza antigen. Impaired production of immunoglobulin (Ig) of one or more of the three main classes (IgG, IgM and IgA) in PWM stimulated cultures was found in 22 patients and in 11 patients no Ig of any class was produced. Antibody to influenza virus was detected in PWM stimulated PBM cultures in 13 of 14 normal individuals, but in only four of 25 patients with treated Hodgkin's disease though IgG was produced in 16 of 25. Influenza antigen induced anti-influenza antibody production in 10 of 12 cultures from normal individuals but in only two of 22 from patients. The results confirm our earlier report of defective antibody production *in vitro* by PBM from patients with Hodgkin's disease and indicate that polyclonally activated production of immunoglobulins of several classes is defective, though *in vivo* humoral immunity is normal.

Keywords Hodgkin's disease pokeweed mitogen influenza

INTRODUCTION

Cell-mediated immune function is defective in both untreated and treated patients with Hodgkins disease (HD) (Schier, 1954; Kelly, Good & Varco, 1985; Eltringham & Kaplan, 1973; Aisenberg, 1965; Hersch & Oppenheim, 1965; Levy & Kaplan, 1974). The basis for this has not been established but a monocyte-mediated inhibition of lymphocyte function has been described in HD (Schechter & Soehnlen, 1978) and attributed to release of prostaglandin E_2 (Goodwin *et al.*, 1977).

In contrast to this, humoral immunity has been thought to be little affected in HD. Thus, serum immunoglobulin (Ig) levels (Wagener, Van Mienster & Hannen, 1976) and *in vivo* antibody responses (Aisenberg, 1966) are usually normal. However Ig synthesis from blood mononuclear cells stimulated by pokeweed mitogen (PWM) is defective in patients with untreated HD and possibly due to suppression by adherent cells or monocytes (Twomey, 1980). We have reported defective *in vitro* antibody production by blood mononuclear cells in response to viral antigens in treated and untreated HD (Souhami, Babbage & Sigfusson, 1983). Our evidence suggested that this was due to an absent or unresponsive circulating B cell population. *In vivo* humoral immunity was normal.

We now report class specific Ig production in response to PWM, and *in vitro* production of anti-influenza antibody in response to stimulation with both PWM and influenza antigens.

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MATERIALS AND METHODS

Subjects. Heparinized blood samples were obtained from 33 patients with Hodgkin's disease (HD). All had been treated but had been taken off all treatment for at least 6 months. Thirty patients had no signs of active disease, three had relapsed and had active disease at time of testing (Table 1).

A blood sample from one of 19 healthy laboratory personnel age 20–40 was studied with each patient.

Cell preparation. Peripheral blood mononuclear cells (PBM) were prepared on a Ficoll-Hypaque (F/H) gradient, washed three times in MEM/HEPES medium and resuspended at 2×10^6 cells/ml in RPMI 1640 medium (GIBCO) supplemented with 10 mM HEPES, 10% fetal calf serum (FCS) and 10^{-5}M hydrocortisone (Sigma).

PWM stimulation. Two million PBM were placed in loose capped, round bottomed tubes. PWM (GIBCO) was added at different concentrations, range 0–80 μl/ml. The cells were then incubated at 37°C in 5% Co_2 for 7 days. All cultures were performed in triplicate. After 7 days, supernatants were harvested for measurement of Ig content.

Stimulation with influenza virus antigens. PBM from 21 patients and 12 healthy donors were cultured with a mixture of purified influenza antigens from three strains; Brazil, JAP and X31 (kindly provided by Dr J. Skehel). The final antigen concentration used was 500 ng/ml of each antigen. Culture conditions were the same as for PWM stimulation except that the medium was supplemented with 10% horse serum, instead of FCS. After 7 days in culture PBM were washed once in MEM/HEPES and resuspended in 0·5 ml of RPMI 1640, supplemented with 5% FCS for 24 h before harvesting the supernatants and assay for antibody content (Callard, 1979).

Measurement of IgG, IgA and IgM. Affinity purified rabbit antihuman IgG, IgM or IgA antibody (Dako immunoglobulins a/s) was adsorbed to flat bottomed microtitre plates by incubating the plates with 2·5 μg/ml solution of the antibody for 1 h at 37°C. After thorough washing the plates were re-incubated for 1 h with 100 μl of serial dilutions of culture supernatants or with 12 standards of known IgG, IgM or IgA concentrations. After further washing, the plates were again incubated with peroxidase conjugated rabbit anti-human IgG, M or A antibodies for 1 h. Finally, the peroxidase activity in each well was measured by incubating the plates for 10–30 min with 100 μl of 0·004% *o*-phenyldiamine in pH 5 buffer, the reaction stopped at end of incubation with 50 μl of 1 M sulphuric acid and the optical density (OD) read on a titertek Multiscan. The Ig concentration in the supernatants was calculated from the log/linear regression obtained from the standards.

Measurement of anti-influenza antibody. Supernatants from cultures stimulated with influenza antigens or PWM were assayed for anti-influenza antibodies by an ELISA technique (Callard, 1979). Influenza antigen was adsorbed onto flat bottomed microtitre plates by incubation for 1 h at 37°C. The plates were then washed and non-specific binding sites blocked with 2% FCS in medium. One hundred microlitres of undiluted culture supernatants were then added to each well for 1 h at 37°C. Bound IgG influenza antibody was detected in the same way as total IgG. Anti-influenza antibody concentrations are expressed as optical density readings.

Enumeration of B cells. The proportion of surface Ig bearing cells in the PBM preparation was counted by their ability to bind ox red blood cells (ORBC) to which affinity purified sheep anti-human κ and λ antibody had been coupled by chromic chloride (Ling, Bishop & Jefferis, 1977). After 1 h incubation at 37°C in serum free medium and two washes to remove passively adherent Ig, equal volumes of 2% ORBC suspension and PMB as 2×10^6 were mixed, pelleted by centrifugation and incubated on ice for 20 min. Fluorescein diacetate was added and the number of cells forming rosettes counted. At least 200 cells were counted and cells with three or more adherents ORBC scored as positive.

Statistical analysis. Grouped values are expressed as mean $\pm$ s.e. Comparison of Ig production in unstimulated cultures was by means of 2×2 contingency tables using the mean Ig concentration in the control cultures as the threshold value. The Mann-Whitney U-test was used to compare values in stimulated cultures. Results were analysed both as 'stimulation index' (Ig in stimulated culture/Ig in control) and as total Ig produced.

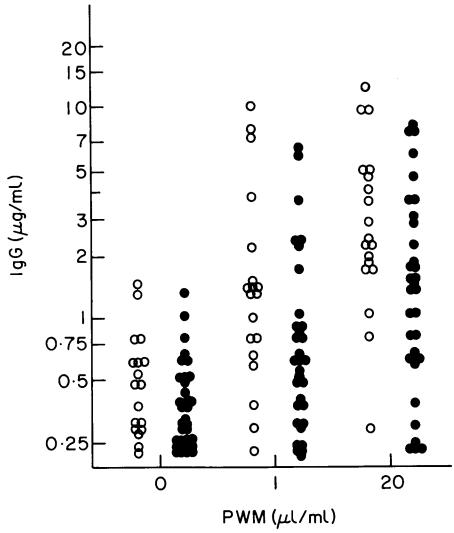

Fig. 1. Unstimulated and PWM stimulated production of IgG by PBM from patients with HD (●) and healthy controls (○). Each point is a mean of triplicate cultures (Unstimulated $P < 0{\cdot}05$; 1μl/ml PWM $P < 0{\cdot}05$; 20μl/ml PWM, $P < 0{\cdot}005$).

RESULTS

Spontaneous and PWM-induced Ig production

PBM from both normal donors and HD patients secreted a low level of each immunoglobulin class (Ig) without exogenous stimulation (Figs 1–3), those from patients with HD were significantly lower than controls except for IgA. In experiments where five different doses of PWM were used (from 1 to 80 μl/ml), 20 μl/ml (a final dilution of 1/50) was always the optimal dosage, both for patients and controls (data not shown). PWM increased production of all Ig classes in control cultures, seven-fold for IgA and IgG and 20-fold for IgM. In contrast, in cultures from 21 of the 33 HD

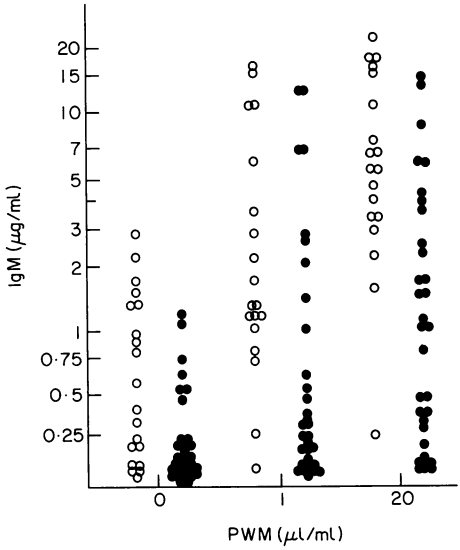

Fig. 2. As Fig. 1; IgM production. (Unstimulated $P < 0{\cdot}005$; 1μl/ml PWM $P < 0{\cdot}005$; 20μl/ml PWM $P < 0{\cdot}001$)

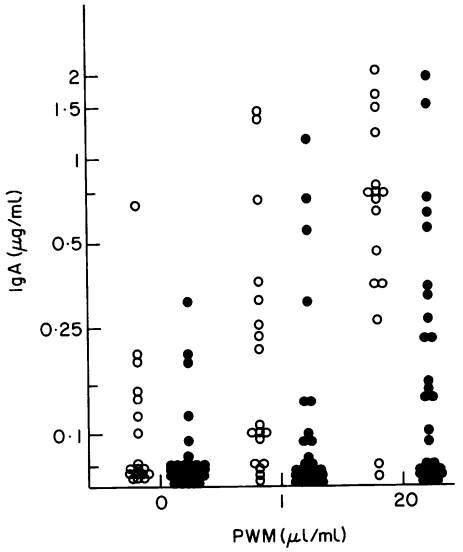

Fig. 3. As Fig. 1; IgA production. (Unstimulated, NS; 1μl/ml PWM, $P<0{\cdot}02$; 20μl/ml PWM, $P<0{\cdot}001$).

patients PWM failed to double the synthesis of at least one Ig class (IgG, $n=14$, $P<0{\cdot}05$; IgA, $n=19$, $P<0{\cdot}01$ and IgM, $n=12$, $P<0{\cdot}05$) and in 11 it did not double the Ig synthesis of any class. The amount of Ig produced by HD cultures after PWM stimulation was highly significantly less than in the controls (Fig. 1–3) (IgG, $P<0{\cdot}005$; IgA, $P<0{\cdot}001$, IgM, $P<0{\cdot}001$).

Anti-influenza antibody in PWM stimulated cultures

Supernatants from PWM stimulated PBM from 13 of 14 healthy donors contained IgG anti-influenza antibody but only four of 25 HD cultures (Fig. 4a). Of the 21 PWM stimulated cultures which did not produce anti-influenza antibody, 12 produced a good polyclonal IgG response, as did the four which produced anti-influenza antibodies (Fig. 5).

Anti-influenza antibody in influenza antigen stimulated cultures

In 10 of the 12 control cultures anti-influenza antibody was detected but none was detected in 19 of 21 patients (Fig. 4b).

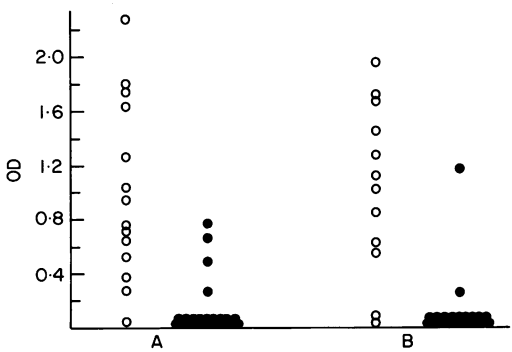

Fig. 4. Anti-influenza antibody production by PBM in response to PWM (A) or influenza antigens (B) ● = patient; ○ = controls ($P<0{\cdot}001$ both for A and B).

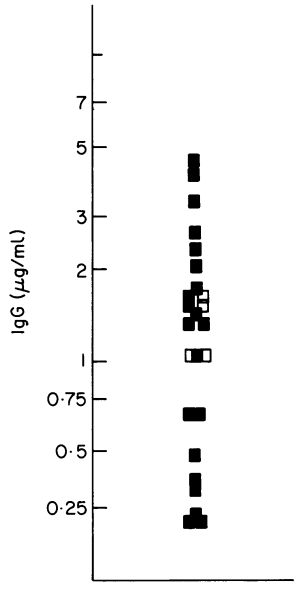

Fig. 5. Total IgG production after PWM stimulation in 25 patients with HD. □ = anti-influenza antibody produced; ■ = no anti-influenza antibody detectable.

Surface Ig bearing cells

PBM preparation from 12 healthy control donors and 19 from HD patients did not differ significantly in rosette formation with anti-human κ and λ chain coated ox red blood cells (Controls, mean 16·29; s.e. 1·13; HD mean 19·08, s.e. 1·02). There was no correlation between the number of SIg positive cells and Ig produced after PWM stimulation of HD cultures.

Associations between clinical data and PWM response

The clinical details of the 12 patients which doubled the amount of all Ig classes after stimulation

Table 1. Clinical details of the 33 patients with treated Hodgkin's disease and of those who showed poor and normal responses to PWM

	All patients	Poor PWM Responders	Normal PWM Responders
Number	33	11	12
Mean age (years) (+/−s.e.)	43·11 (2·77)	40·71 (5·98)	43·39 (4·51)
Disease stage at diagnosis			
I+II	22	5	7
III+IV	11	6	5
Splenectomized	20	8	6
Previous treatment			
Radiotherapy	19	5	6
Chemotherapy	7	3	2
Both (RT+CT)	7	3	4
Mean time since last treatment (+/−s.e.	5·02 (0·84)	2·05 (0·72)*	5·02 (1·05)*

*$P < 0·05$.

were compared with those of 11 patients that did not double any Ig class (all of whom had unstimulated Ig production below the mean of the controls). The only significant difference was a longer time from last treatment in those patients with a normal PWM response (Table 1).

DISCUSSION

This report confirms the finding of Twomey *et al.* (1980) that there was decreased *in vitro* synthesis of immunoglobulin by both unstimulated and PWM stimulated PBM in patients with Hodgkin's disease, shows it applies to the three main classes of Ig, and that patients who are unable to synthesise Ig of one class are usually unable to produce other Ig classes. We have confirmed our previous finding (Souhami *et al.*, 1983) that PBM from these patients usually do not respond to *in vitro* stimulation by viral antigens, and have shown that even when patients' PBM responded to PWM by Ig synthesis, anti-influenza antibody is not detected in the culture supernatants, though they are in supernatants from control cultures stimulated by PWM with similar Ig concentrations. These defects were present in some patients who had been off all treatment for several years and who were considered cured, although it was more commonly in those who were considered cured, although it was more commonly in those who had stopped treatment more recently.

Impaired response to PWM and antigen cannot be attributed to lack of total numbers of SIg positive cells (B cells) as they were similar in controls and patients. In our previous report (Souhami *et al.*, 1983) we showed that serum anti-influenza and anti-Varicella zoster antibody is normal in HD and that although PBM from 11 untreated patients did not produce antibody *in vitro*, spleen cell cultures did. Blood E^+ cells helped spleen E^- cells to produce antibody showing that T helper cells were present in the blood. Monocyte-mediated suppression of PHA and PWM responses have been reported in HD (Goodwin *et al.*, 1977), but not consistently confirmed (Holm *et al.*, 1982). Co-cultures of blood and spleen cells from patients with HD did not provide evidence of suppression of antibody production (Souhami *et al.*, 1983).

Functionally distinct subpopulations of B cells may be localized at different anatomical sites. For example surface μ^+ δ^- B cells localise in the marginal zone of the spleen in rats, and do not recirculate (Gray *et al.*, 1982). Human B cells which respond to viral antigen have not yet been identified, but the responding B cell in PWM stimulated cultures is surface IgD negative (δ^-) (Kuritani & Cooper, 1982). In patients with rheumatoid arthritis where a similar unresponsiveness to PWM has been described, the proportion of B cells forming rosettes with mouse red blood cells is increased (Plater-Zyberk 1983) and this is the surface δ^+ population (Lucivero, Lawton & Cooper, 1981). Therefore in HD there may be a deficiency or absence of the circulating δ^- population of B cells. However this explanation does not account for the failure of production anti-influenza antibodies in cultures from HD patients which respond to PWM.

We provide further evidence of a disordered B cell recirculation in HD. Although the nature of the defect is unresolved, the finding is a reminder that conclusions about humoral immunity in disease cannot be solely drawn by *in vitro* tests on blood.

Dr Sigfusson was supported by grants from the Wellcome Trust and the Rockefeller Foundation.

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