Clin. exp. Immunol. (1976) 26, 108–117.

The effects of purified mitogenic proteins (Pa-1 and Pa-2) from pokeweed on human T and B lymphocytes *in vitro*

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(*Received* 28 *January* 1976)

SUMMARY

Purified proteins (Pa-1 and Pa-2) from pokeweed have been compared with commercial pokeweed mitogen (PWM-G) and other mitogens in their ability to stimulate human lymphocytes. With cultures of T and B cells separated from tonsil lymphocytes, thymidine uptake, blast transformation and immunoglobulin (Ig) synthesis have been measured. IgM and IgG was measured in supernates of stimulated cultures by radioimmunoassay. Pa-1, Pa-2 and PWM-G were found to be potent mitogens for unseparated tonsil lymphocytes or nylon column purified T cells. Pa-2 was found to be active at lower concentrations than Pa-1, and PWM-G was less potent than the purified mitogens. These three mitogens all stimulated unseparated lymphocytes to secrete large quantities of Ig (20–100 μg/ml) during 7 days in culture. With increasing amounts of mitogens severe decreases in immunoglobulin synthesis were observed at day 6 even with doses which were still optimal for stimulation of thymidine uptake at days 3 and 6.

With purified B cells (<2% T cells) Pa-1 was the best mitogen for thymidine incorporation. However, the secretory response was very variable. In some experiments B cells did not secrete Ig in response to mitogens; in others Pa-1 was clearly more effective at stimulating secretion than Pa-2 or PWM-G and in some experiments B cells were stimulated by all three. In one experiment Pa-1 stimulated prolymphocytic leukaemia cells to blast transformation and the secretion of IgM. It is concluded that Pa-1, Pa-2 and PWM-G are much better activators of Ig synthesis in human cultures than either PHA or LPS and that Pa-1 is the most reliable B-cell stimulant of the three.

INTRODUCTION

Unpurified salt extract of pokeweed (*Phytolacca americana*) (PWM) stimulates both T and B cells in a variety of species (Chessin *et al.*, 1966; Douglas *et al.*, 1967; Weber, 1973; Janossy & Greaves, 1975). This stimulation has been measured as thymidine incorporation or lymphoblast and plasmablast development (Douglas *et al.*, 1967). In addition increased secretion of immunoglobulin (Ig) has been measured from both mouse (Parkhouse, Janossy & Greaves, 1972) and human lymphocytes (Wu, Lawton & Cooper, 1973) stimulated with PWM. This ability of PWM to stimulate secretion of Ig has led to its use in the analysis of cellular defects in hypogammaglobulinaemia (Wu *et al.*, 1973; Waldmann *et al.*, 1974; Cooper, Keightley & Lawton, 1975; Broom *et al.*, 1975). Stimulation of B cells with PWM is, however, not always reproducible (Jones, 1972; Douglas, Kamin & Fudenberg, 1969). One of the major causes of this variability is that pokeweed extracts contain a number of mitogenic proteins (Waxdal, 1974). The proteins have been separated by gel filtration and have been designated Pa-1–Pa-5

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(Waxdal, 1974). Each of the five fractions was found to be mitogenic for spleen cells from normal mice (Waxdal, 1974). However, it appeared that Pa-1 had a special ability to stimulate B cells. Thus, only Pa-1 was mitogenic for spleen cells from nude mice (Waxdal & Basham, 1974) or was able to stimulate secretion of Ig (Basham & Waxdal, 1975). By contrast the most potent mitogen for normal mouse spleen cells was Pa-2 but this protein did not appear to stimulate Ig secretion and was non-mitogenic for spleen cells from nude mice. The present report describes the effects of Pa-1 and Pa-2 on T and B lymphocytes from human tonsil.

MATERIALS AND METHODS

Stimulants used. The purification of Pa-1 and Pa-2 has been described (Waxdal, 1974). PWM, purchased from GIBCO (PWM-G) was selected from several batches for optimal stimulation of Ig synthesis (Batch no. A641714). PHA (purified phytohaemagglutinin, Wellcome, cat. no. HA16, batch no. K6617) and LPS (Salm. marcescent, cat. no. 3130–35–8) were used at different doses. Results at optimal doses (2 μg/ml and 100–200 μg/ml respectively) are shown.

Identification of T and B cells. Cells from tonsils were prepared as described (Greaves, Janossy & Doenhoff, 1974a). E-rosette test (E) with sheep red blood cells (SRBC) was used as a T-cell marker (WHO workshop on human B and T cells, 1974). SRBC (2%) were treated with 15 u/ml of neuraminidase (Behringwerke, cat. no. ORKD 04) for 30 min at 37°C and washed (N-SRBC) before being used for E rosette formation. B cells were identified by surface immunoglobulins (smIg) using goat antihuman Ig-FITC, 1:10 dilution (Behringwerke, cat. no. OTKG 05).

Small and large cells in cultures were counted after 3 days stimulation. The proportion of T and B blast cells were analysed by E-rosette test and anti-Ig (smIg) staining. After 7 days many stimulated B cells (65%, mostly plasma blasts and plasma cells) were $smIg^-$. For this reason the cells were first stained for smIg (goat anti-Ig-TRITC, Capel Laboratories batch no. 6638) and then smeared in a cytocentrifuge and stained for intracellular Ig with anti-Ig-FITC (Hijmans, Shuit & Klein, 1969). Only 10% of $smIg^+$ cells were negative for intracellular Ig (in PWM cultures).

Purification of T and B cells. B lymphocytes were purified by eliminating E-rosettes on Ficoll-Triosil gradient (Greaves &

TABLE 1. Cell populations used

	Percentage of reactive cells			Yield of separation (%)
	E^+/Ig^-	E^-/Ig^+	E^-/Ig^- ('null')	
Tonsil T+B unseparated	45 (a)	46 (a)	9 (a)	—
	49 (b)	54 (b)	6 (b)	
Nylon fibre column filtration (T)	88 (a)	2 (a)	10 (a)	90 (a)
	85 (b)	9 (b)	6 (b)	60 (b)
Elimination of E-rosettes (B)	< 1 (a)	85 (a)	14 (a)	50 (a)
	< 1 (b)	95 (b)	5 (b)	35 (b)

(a) and (b) refer to two representative experiments.

Brown, 1974; Galili & Schlesinger, 1974). Initially $2{\cdot}5 \times 10^7$ lymphocytes were mixed with 2·5 ml FCS and 5 ml 2% N-SRBC and centrifuged at 700 rev/min. The rosetted pellet was gently resuspended after 20 min and pelletted again. After a further 60 min the cells were resuspended, layered upon Ficoll–Triosil and centrifuged. Cells at the interphase were harvested and used as non-rosette-forming B cells. T cells were purified by filtration through a nylon column (Greaves & Brown, 1974). Preparations of cells were passed through FCS to eliminate bacteria. The proportions of T (E^+) and B (smIg) cells in the purified T and B cell populations are shown in Table 1.

Culture and labelling conditions. Cells (1×10^6/ml) were incubated in RPMI medium supplemented with 10% serum, 200 mM glutamine, 200 u/ml penicillin and 100 μg/ml streptomycin. Two different FCS were used; FCS-S (Gibco, batch no. 44415) was selected on the basis of secondary antibody response to diphtheria-toxoid in the Marbrook system (Platts-Mills & Ishizaka, 1975; FCS-O (Gibco, Batch no. K 49101) was selected on the basis of [^{3}H]thymidine uptake in PHA and PWM stimulated cultures. Neither FCS was mitogenic (< 4% blast cells at day 3).

Culture vessels with flat bottoms were used. [^{3}H]thymidine uptake was measured after 3 days incubation in microplate (0·25 ml). One μCi isotope (200 mCi/mmol, specific activity, Radiochemical Centre, Amersham) was added at 64 hr. Cultures were harvested 16 hr later. Analysis of Ig synthesis was performed in microplate or bijou cultures. The latter (Sterilin, cat. no. 118/S) contained 1·5 ml culture. After 4 days half the medium was replaced with fresh medium but no stimulants were added.

Analysis of Ig synthesis. After 6–7 days (156–180 hr incubation) the absolute numbers of intermediate and large cells were

determined. The proportions of intracellular IgM, IgG and IgA positive cells within the activated populations was counted in cytocentrifuge smears. The reagents (sheep anti-human IgM-FITC, Wellcome, batch no. 6506; goat anti-human IgG-TRITC, Nordic, batch no. 27–274; sheep anti-human IgA-FITC, Wellcome, batch no. K 7727) were tested for specificity and used in combinations. Strongly and weakly positive cells were counted. The analysis of secreted IgG by radioimmunoassay has been described previously (Platts-Mills & Ishizaka, 1975). The radioimmunoassay for IgM was carried out by a similar technique using rabbit anti-IgM and purified IgM radiolabelled with ^{125}I. Immunodiffusion was carried out using 1% Agarose in 0·05 M barbital buffer pH 8·0.

RESULTS

We have reported previously that thymidine incorporation and blast transformation responses to PWM are optimal after 3 days stimulation (Greaves *et al.*, 1974a). However, in preliminary experiments we found little stimulation of Ig secretion by PWM at day 3–4 but rapid increases in secretion of Ig up to day 7. Therefore, human lymphocyte populations cultured with PWM were assessed after 3 and 6 days. After 3 days thymidine incorporation and the formation of blast cells were analysed while after 6 days secretion of Ig was also measured.

Analysis of stimulated cultures at day 3 (Figs 1–3)

Eight experiments were performed. The results seen were effected by the behaviour of the purified B-cell population in each experiment. In five experiments purified B cells responded poorly to any of the mitogens tested. A representative of these experiments is shown (expt A, Fig. 1). On the other hand in three experiments purified B cells responded to mitogens although generally less strongly than unseparated cells and one of these experiments is shown (expt B, Fig. 1). In the mixtures of T and B cells Pa-1 stimulated higher proportions of B-blast cells (39–51%) than Pa-2 (21–32%) and PWM-G (27%). Lower numbers of B blasts were seen in PHA cultures (8–14%) while LPS was almost totally ineffective. The dose–response curves of Pa-1 and Pa-2 for thymidine uptake were different (Fig. 2).

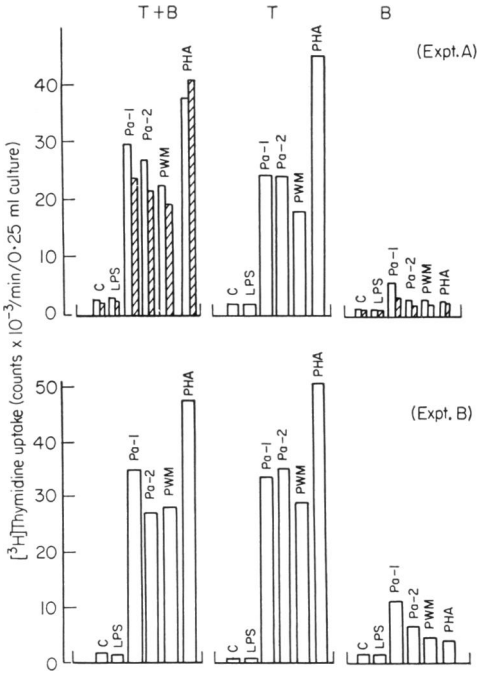

FIG. 1. Thymidine incorporation in T+B, T- and B-lymphocyte cultures after 80 hr stimulation. Controls (C) and optimal stimulating mitogen concentrations are shown (LPS: 100 μg/ml, Pa-1: 30 μg/ml; Pa-2: 1–5 μg/ml; PWM-G: 10 μl/ml; PHA: 2 μg/ml). In two representative experiments ('A' and 'B') FCS-S (open columns) was used. In expt A values with FCS-O (hatched columns) are also shown (see the Materials and Methods section).

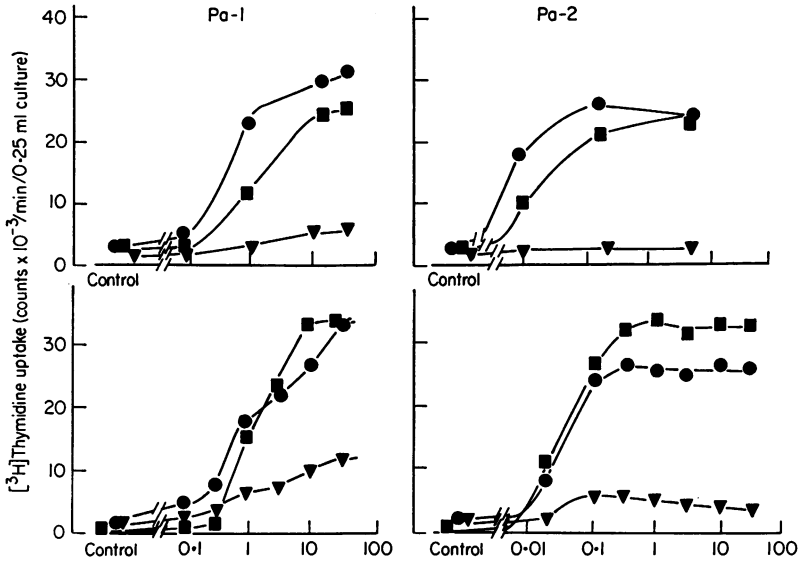

FIG. 2. Stimulation of [^{3}H]thymidine uptake (between 64 and 80 hr) with various doses of Pa-1 and Pa-2: (●) T+B; (■) T; (▲) B lymphocyte cultures. Two representative experiments are shown. The dose–response curve of PWM-G is similar to that of Pa-2. It reaches a plateau (20–27 ct/min × 10^3/min) at 10 μl/ml concentration.

Pa-2 was more potent at low concentrations (optimal range: 0·1–100 μg/ml) than Pa-1 (optimal range: 3–100 μg/ml). In T+B cultures over the whole Pa-1 dose range the proportions of B- versus T-blast cells were higher than in Pa-2 stimulated cultures (Fig. 3).

In purified B-cell populations Pa-1 was the strongest mitogen on the basis of thymidine incorporation (Fig. 1) and the number of B-blast cells present (Fig. 3). Pa-2 induced a much smaller increase of thymidine uptake. Most blast cells in Pa-1 and Pa-2 stimulated B-cell cultures carried smIg (Fig. 3). The small amount of thymidine uptake seen when B cells were cultured with PHA was attributed to the stimulation of residual T cells, and the blast cells seen formed rosettes with N-SRBC.

To some purified B-cell cultures supernatants of cultured T cells were added. T cells (6×10^6/ml)

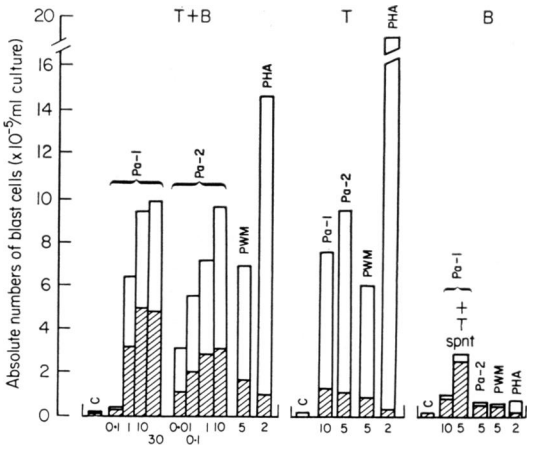

FIG. 3. Absolute numbers of T (E^+; open columns) and B ($SmIg^+$; hatched columns) blast cells T+B, T, and B cell cultures after 3 days stimulation. Stimulating mitogenic doses of Pa-1, Pa-2 and PHA are given (μg/ml). The dose of PWM-G is given as μl/ml culture. The results in the figure refer to expt A (see Fig. 1). Similar values were observed in expt B except that in Pa-1-stimulated purified B-cell cultures higher numbers of B ($SMIg^+$) blast cells were seen ($2{\cdot}5 \times 10^5$/ml).

TABLE 2. Stimulation of intracellular immunoglobulin synthesis and secretion in T+B and purified B-tonsil cell cultures during 6 days stimulation*

Cells	Stimulants	Total no. of cells recovered ($\times 10^5$/ml)	T-blast cells recovered ($\times 10^5$/ml)	Total nos of B(Ig^+) blasts† recovered ($\times 10^5$/ml)				Immunoglobulin secreted (μg/ml)	
				Ig^+	IgM^+	IgG^+	IgA^+	IgM	IgG
T+B	Control	8·0	0·2	0·5 (0·1)		n.t.		0·04	0·48
	PHA	20	14	1·5 (0·1)		n.t.		0·02	0·23
	Pa-1 (1 μg/ml)	17	5·8	6·2 (4·8)	4·0 (3·3)	1·6 (0·4)	1·2 (1·0)	15·0	4·75
	Pa-1 (5 μg/ml)	19	5·8	6·7 (5·0)	3·6 (2·8)	1·8 (0·9)	1·4 (1·0)	15·0	5·25
	Pa-2 (1 μg/ml)	19	7·0	5·5 (3·6)	3·1 (2·4)	1·7 (0·9)	0·8 (0·4)	8·8	6·0
	Pa-2 (5 μg/ml)	19	7·5	2·9 (1·5)	1·2 (0·8)	0·8 (0·4)	0·3 (0·2)	2·6	2·75
	PWM-G (10 μl/ml)	14	4·5	3·0 (2·5)		n.t.		9·0	4·65
	LPS (200 μg/ml)	6	0·8	0·6 (0·1)		n.t.		0·25	0·25
B	Control	3	0	0·4 (0·1)		n.t.		0·01	0·40
	PHA	7·5	3·8	1·0 (0·1)		n.t.		0·05	0·28
	Pa-1 (1 μg/ml)	8	0·5	2·3 (1·7)	1·8 (1·4)	0·9 (0·3)	0·2 (0·1)	2·25	1·4
	Pa-1 (5 μg/ml)	8	0·5	2·8 (2·1)	1·8 (1·6)	0·8 (0·3)	0·1 (0·1)	5·8	2·4
	Pa-2 (1 μg/ml)	7	0·8	1·5 (0·3)		n.t.		0·75	0·58
	Pa-2 (5 μg/ml)	6	0·8	1·5 (0·3)	1·1 (0·3)	0·6 (0·2)	0·1 (0·1)	0·8	0·62
	PWM-G (10 μl/ml)	6	0·3	1·2 (0·3)		n.t.		n.t.	n.t.
	LPS (200 μg/ml)	5	n.t.	0·8 (0·1)		n.t.		0·08	0·60

* Bijou cultures (1·5 ml, 10×10^5/ml cells at the beginning of culture, see the Materials and Methods section).

† The numbers in parantheses represent blast cells strongly stained for intracellular Ig. The total numbers of Ig+ blast cells are calculated values (see the Materials and Methods section).

were stimulated for 2½ days with 2 μg/ml Pa-1. The supernatants were diluted with equal amounts of medium. Additional stimulants were provided. Higher thymidine incorporation was observed when additional Pa-1 was added (2–4 μg/ml: 8400 ct/min) rather than Pa-2 (1–2 μg/ml: 4750 ct/min: unstimulated controls: 900 ct/min).

In T-cell cultures Pa-2 was more effective than Pa-1. Higher numbers of T-blast cells were seen in Pa-2 than in Pa-1 stimulated cultures (Fig. 3). Furthermore, Pa-2 was active at much lower concentration, although thymidine incorporation at optimal doses was only slightly higher than was observed in Pa-1 stimulated cultures (Fig. 2). PWM-G was a less effective T-cell stimulant than either of the purified mitogens.

Analysis of stimulated cultures after 6 days' stimulation (Table 2, Fig. 4)

Large numbers of blast cells were seen in mixtures of T and B lymphocytes cultures with Pa-1, Pa-2 or PWM-G for 6 days. Forty to 50% of these blasts were T blasts since they made rosettes with N-SRBC while the remaining 50% were positive for intracellular Ig. The absolute numbers of IgM- and IgG-containing cells are shown in Table 2. In order to assess the amount of Ig secreted by these cells, supernates from the 6-day cultures were assayed for IgG and IgM by double antibody inhibition radioimmunoassay. The results (Table 2) show that all three preparations of pokeweed were effective in stimulating Ig secretion. Even at low mitogen concentrations (Pa-1: 0·3 μg/ml, Pa-2: 0·01 μg/ml: and

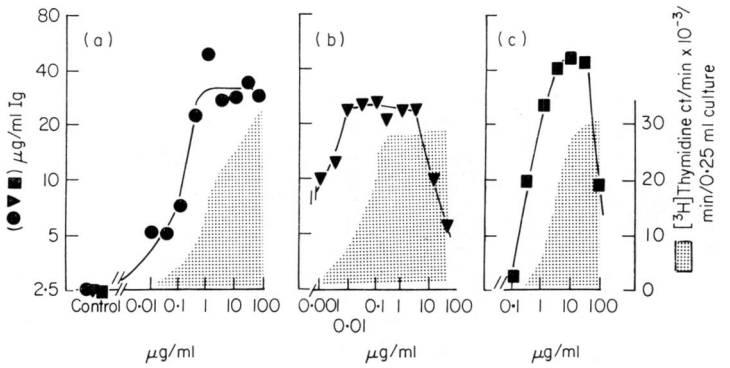

FIG. 4. Stimulation of Ig secretion in cultures of T+B cells with various doses of (a) Pa-1, (b) Pa-2 and (c) PWM-G. Cultures were stimulated for 7 days (180 hr) and IgM+IgG in supernatants was measured by radioimmunoassay. Thymidine incorporation in cultures during 64–80 hr is shown for comparison (stippled areas). Additional experiments were carried out, where in parallel cultures, thymidine incorporation was observed between 140 and 156 hr and essentially similar results were obtained.

PWM-G 1 μg/ml) considerable amounts of Ig were secreted (Fig. 4). Indeed, good secretion of Ig was observed with concentrations of mitogens which were suboptimal for stimulation of thymidine uptake at any time tested between days 3 and 6. At large doses, particularly when Pa-2 or PWM-G was used (Pa-2: above 3 μg/ml; PWM-G above 30 μg/ml) marked decreases in Ig synthesis were observed although these large doses were well within the optimal range for stimulation of thymidine incorporation at days 3 and 6.

The amounts of IgG and IgM produced in some of these cultures were very high, in several supernates IgG was 30 μg/ml or IgM was 40 μg/ml. Immunodiffusion with anti-IgG or anti-IgM respectively gave a precipitin line while control cultures were always negative (Janossy *et al.*, 1976). The few blast cells which stained for intracellular Ig in PHA and LPS stimulated cultures were mostly only weakly positive (Table 2). The inability of PHA and LPS to fully activate Ig synthesis in human B cells was reflected in the small amounts of Ig secreted.

In purified B-cell cultures the total numbers of B-blast cells were mostly low (Table 2). In the four experiments where full data were obtained the highest numbers of blast cells were observed in cultures stimulated with Pa-1 at 5 μg/ml. In these cultures 70–80% of blasts contained intracellular Ig of which

28–42% were IgM^+ and 30–40% were IgG^+. The proportion of blasts strongly positive for IgM was higher (16–17%) than for IgG (8–10%). We assume that the cells which stain strongly for intracellular Ig are secreting the bulk of the Ig in the supernates and in general we have found very good correlation between the numbers of strongly stained plasmablasts and the Ig secreted (Table 2).

TABLE 3. Stimulation of prolymphocytic leukaemia cells by Pa-1*

Cells	Stimulant	Absolute nos of cells ($\times 10^5$/ml)			Immunoglobulin secreted (μg/ml)	
		Total	T(E+)blasts	B(i.c.Ig+)blasts	IgM	IgG
Normal tonsil (T+B)	Control	10	1	1	1·5	1
	Pa-1	23	11	10	30·0	20
Prolymphocytic leukaemia (Ig+, B cells)	Control	2·5	0	0	0·12	0·24
	Pa-1	12	5·5	4·8†	5·85	0·44
	PWM-G	1·5	0·5	0·2	0·5	0·37

* Cultures were stimulated for 7 days. Stimulant doses: Pa-1 5 μg/ml; PWM-G 5 μl/ml.

† The number of blast cells strongly stained for intracellular IgM was $2{\cdot}1 \times 10^5$ cells/ml culture. Multinucleated and dividing cells were seen in this population. Other Ig+ cells had 'foamy' weakly Ig+ cytoplasmic staining (see Fig. 5).

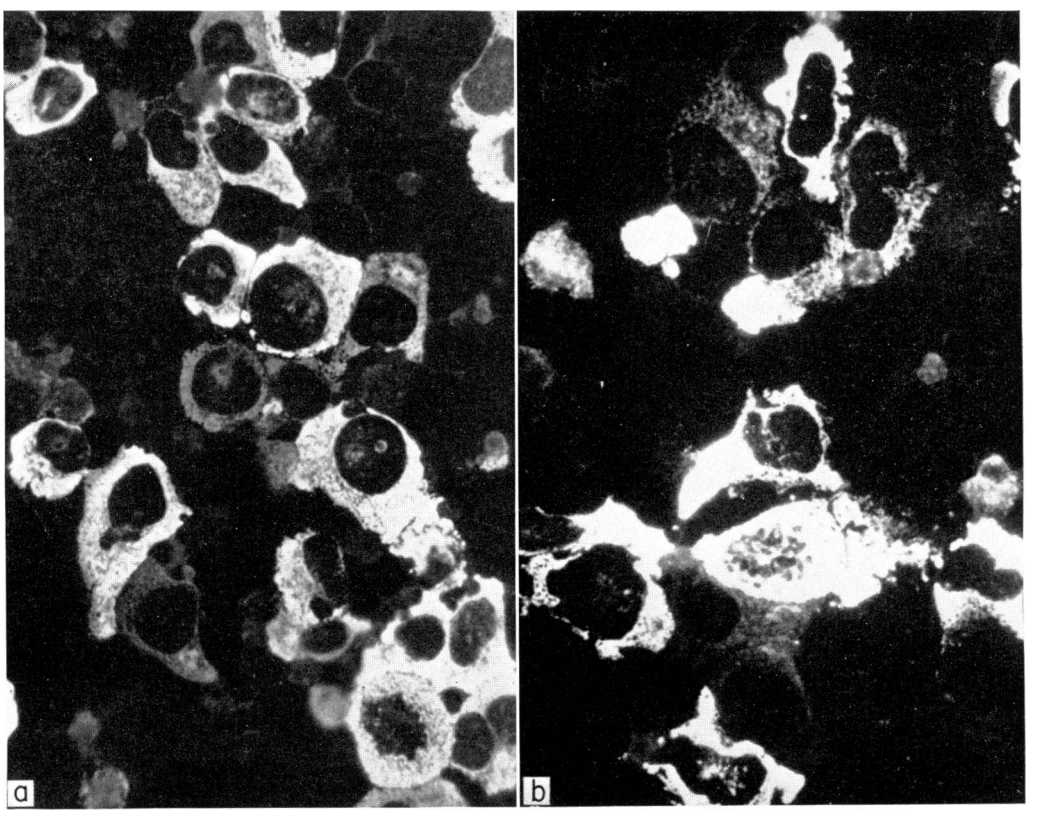

FIG. 5. Stimulation of intracellular immunoglobulin synthesis by Pa-1 in normal tonsil (T+B) lymphocyte cultures (a) and in prolymphocytic leukaemia cell cultures (b). The stimulated cultures were incubated for 7 days, and cytocentrifuge smears were stained for intracellular immunoglobulin (goat anti-human Ig-FITC; see 'Methods'). (Incident uv-light illumination; magnification × 400.)

Activation of prolymphocytic leukaemia cells by Pa-1

Peripheral blood lymphocytes from a patient with prolymphocytic leukaemia (92% B cells: E^-, $SmIg^+$, κ^+, μ^+; 6% T cells: $SmIg^-$) were incubated with PHA, Pa-1 and PWM-G for 7 days. PHA stimulated the few residual T cells to proliferate but only 1% of the blast cells in PHA stimulated cultures stained for intracellular Ig. Pa-1 also stimulated T cells; for 44% of the blast cells at day 7 were E^+/Ig^- (Table 3). The rest of blast cells (50–60%) stained intracellular with anti-μ and anti-κ but were negative with anti-δ, anti-γ and anti-λ. Some stimulated blast cells exhibited a 'foamy' network of Ig^+ material; others were atypical plasma blasts (Fig. 5) while a number of these cells had bizarre multinuclear appearance. Dividing cells with cytoplasmic Ig were also seen. The abnormal morphology and the lack of γ or λ positive cells suggested that leukaemia cells and not residual normal B lymphocytes had been activated. PWM-G failed to activate, prolymphocytic leukaemia cells in this experiment. Not only were very few large cells seen, but the secreted IgM in these cultures was much lower (1·2 μg/ml) than in Pa-1 stimulated cultures (11·7 μg/ml).

DISCUSSION

The results presented here show that Pa-1 and Pa-2 are both potent mitogens for human T and B lymphocytes. Both mitogens can stimulate the production of large quantities of IgM and IgG (40–60 μg/ml culture) during a 6-day culture. The production of very large quantities of both IgM and IgG classes has been confirmed by immunodiffusion. The choice of serum supplement, medium, the appropriate cell density and feeding of cultures at day 4 are all important for the optimal development of plasma cells in these cultures.

Pa-1, Pa-2 and PWM-G were quite different in respect of stimulating Ig production from other mitogens tested. It has been previously reported that PHA (Phillips & Roitt, 1973; Chess, MacDermott & Schlossman, 1974) and LPS; Gronowitz & Coutinho, 1975) can stimulate a few human B cells to blast transformation. We have observed the same phenomenon although in LPS-stimulated cultures the proportions of B-blast cells were hardly above the low background. Most of these B-blast cells were, however, only stained weekly for cytoplasmic Ig and radioimmunoassay showed very little secreted Ig. It is concluded that the mitogenic proteins from pokeweed are the most suitable stimulants for analysis of human B-lymphocyte activation *in vitro*.

The differences we have observed between Pa-1, Pa-2 and PWM-G are more quantitative than qualitative. Pa-2 is a more potent T-cell mitogen and is active at lower concentrations. In cultures of purified B cells, however, Pa-1 was invariably more effective at stimulating thymidine incorporation, blast transformation and Ig production than Pa-2. Also in cultures with unseparated (T+B) cells Pa-1 stimulated higher proportions of $SmIg^+$ blasts at day 3 than Pa-2 or PWM-G. These observations are similar to the observation that Pa-1 is a more potent mitogen for murine B lymphocytes than Pa-2 or PWM (Waxdal & Basham, 1974; see below).

It was also interesting that prolymphocytic leukaemia cells were stimulated by Pa-1. This observation shows that in some forms of chronic lymphocytic leukaemia the malignant cells are not blocked irreversibly at the lymphoid maturational stage. Previous investigators failed to find unequivocal evidence for blast transformation and stimulation of Ig synthesis in CLL cells by B-cell mitogens (Smith, Cowling & Barker, 1972; Cohnen *et al.*, 1973; Schweitzer, Melief & Eijsvoogel, 1973). Although not all CLL-s are responsive to Pa-1, we attribute our positive observation to the use of Pa-1 since PWM-G was far less effective in parallel cultures.

In many respects, however, our observations are at variance to those seen in mouse spleen cell cultures where only Pa-1 appears to stimulate purified B cells (Waxdal & Basham, 1974; Basham & Waxdal, 1975). The differences between Pa-1 and Pa-2 in human cultures were subtle; activation of T cells by Pa-1 and activation of B cells by Pa-2 were more apparent than in mouse cultures. These effects may be attributable to known species differences. First, in the human, T cells respond earlier than B cells (Doenhoff *et al.*, 1974; Greaves *et al.*, 1974a). Second, the activation of B cells by mitogens in human cultures is more T-cell dependent (Janossy *et al.*, 1976; Cooper *et al.*, 1975) than in cultures

of B cells from the mouse spleen (Sher *et al.*, 1973; Greaves *et al.*, 1974b). This might account for the fact that in some Pa-2-stimulated cultures of human T+B cells more Ig was produced than in Pa-1 cultures (Fig. 4). Thus, Pa-2, a potent T-cell mitogen, may be providing a strong stimulus to B cells via 'activated' T cells in addition to a weak direct effect on the B cells. Viewed as a whole it seems that activation of Ig synthesis by Pa-2 and commercial PWM was more T-cell dependent than activation by Pa-1, although in the majority of tonsils Ig secretion in Pa-1-stimulated cultures was also at least partially T-cell dependent (Janossy *et al.*, 1976).

The present study also clearly shows that the optimal dose ranges of PWM preparations for the stimulation of Ig synthesis in B cells during a 7-day culture period and for the stimulation of thymidine incorporation at days 3 and 6 are not identical. Counting of E^+ (T) blast cells in stimulated cultures after 3–6 days incubation indicated that T cells multiplied and therefore thymidine uptake in these cultures reflects, at least partially, a T-cell function. Separate standardization of conditions for Ig synthesis (a B-cell function) seems to be essential. Taken at face value, these results are in line with those of Mellstedt, Jondal & Holm (1973) which show that low PWM doses preferentially stimulate B cells, although this preferential activation in our study was manifested only in the Ig synthetic response at day 6 and not in the relative proportions of activated T- versus B-blast cells at day 3.

In conclusion, all PWM preparations tested (including the crude extract PWM-G) are suitable to elicit B-cell responses and synthesis of large amounts of Ig in human lymphocytes. Pa-1 provides stronger B-cell signals in addition to T-cell activation and this may well prove of advantage in analysing the cellular defects in hypogammaglobulinaemias and other immunological and leukaemic disorders. Conversely, Pa-2 may prove to be more useful in analysing the complex T-cell regulation of B-cell responses by human lymphocytes.

G.J. was supported by the Imperial Cancer Research Fund of Great Britain. E.G.G. was supported by the Juan March Foundation of Spain. M.W. was supported by a National Institute of Health Grant (U.S.A.) and T.P.M. was supported by the Medical Research Council of Great Britain. We are grateful to Miss Jacqui Snajdr for expert technical help. We would also like to thank Dr D. Catovsky of Hammersmith Hospital for CLL cells and the staff of Colindale Hospital for tonsilectomy specimens.

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