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Synergy between human T and B lymphocytes in their response to phytohaemagglutinin and pokeweed mitogen

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Summary. Human B- and T-lymphocyte preparations were isolated by separating T lymphocytes that formed rosettes with sheep erythrocytes from unrosetted B lymphocytes. Pokeweed mitogen stimulates the proliferation of both B- and T-lymphocyte preparations. In contrast, phytohaemagglutinin stimulates little or no proliferation of purified B lymphocytes although it stimulates the proliferation of T lymphocytes. Lymphoid preparations containing both T and B lymphocytes are more responsive to both mitogens than are either T- or B-lymphocyte preparations. This observation suggested synergy between T and B lymphocytes in the response of unfractionated lymphocytes to mitogens. The basis for this synergy was shown to be the capacity of T lymphocytes to facilitate the proliferation of B lymphocytes cultured with pokeweed mitogen or phytohaemagglutinin. The activity of T lymphocytes is not dependent upon their proliferation or attributable to their release of mitogenic factors.

With regard to the clinical evaluation of immune function, our results indicate that the proliferative response of human lymphocytes to phytohaemagglutinin or pokeweed mitogen cannot be directly related to the percentage of T lymphocytes in the lymphoid preparation.

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INTRODUCTION

The plant lectins, pokeweed mitogen (PWM) and phytohaemagglutinin (PHA), stimulate human lymphocytes to divide. A large body of experimental data indicates that both human B and T lymphocytes are directly stimulated by PWM while PHA directly stimulates only T-lymphocyte proliferation (Geha and Merler, 1974; Greaves, Janossy and Doenhoff, 1974; Mellstedt, 1975). Consequently, the response of human lymphocytes to PHA has been employed for the clinical evaluation of T-lymphocyte function. While it is clear that this response is a useful qualitative assay of T-lymphocyte function, it is less clear that it can be employed as a quantitative assay.

Recently, we found that PHA-induced thymidine incorporation by lymphocytes from different patients did not correlate with the number of T lymphocytes placed into culture (Weksler, Kuntz, Lockshin, Kohn and Eisenhauer, 1974). Furthermore, Phillips and Roitt (1973), Phillips and Weisrose (1974), Epstein, Kreth and Herzenberg (1974) and Chess, MacDermott and Schlossman (1974) have reported that human B lymphocytes, isolated by reaction with anti-immunoglobulin antibody, proliferate in the presence of PHA.

We have studied the response to PWM and PHA of unfractionated, B, or T human blood lymphocyte preparations. Unfractionated and isolated T lym-

phocytes responded to both mitogens. B lymphocytes, isolated by removing rosette-forming T lymphocytes, showed a modest response to PWM and little or no response to PHA. However, B lymphocytes in the presence of T lymphocytes, proliferate vigorously when cultured with either PHA or PWM. Lymphocyte preparations containing 20–40 per cent T lymphocytes incorporated more thymidine when cultured with PHA or PWM than did B or T lymphocytes cultured alone. Synergy between B and T lymphocytes in their response to mitogens was thus demonstrated.

MATERIALS AND METHODS

Preparation of lymphocyte suspensions

Fifty to 250 ml of venous blood from healthy volunteers were drawn into a plastic syringe containing 10 units of heparin (Upjohn Company, Kalamazoo, Michigan) per millilitre of blood. The blood was diluted with an equal volume of calcium and magnesium-free Hanks's balanced salt solution (HBSS, Grand Island Biological Company, Grand Island, New York). Thirty-five millilitres of the diluted blood were layered over 12 ml of a mixture of Ficoll (Pharmacia Fine Chemicals, Piscataway, New Jersey) and sodium diatrizoate (Hypaque, Winthrop Laboratories, New York, New York) in sterile 25 × 150 mm glass screw-top tubes (Corning Glass Works no. 9825, Corning, New York). Ficoll–Hypaque solution was prepared by mixing one part of 50 per cent Hypaque with four parts of 8 per cent (weight/volume) Ficoll in water. The density of the Ficoll–Hypaque mixture was adjusted to a specific gravity of 1·078–1·080 with distilled water and passed through a 0·45 μm Millipore filter. Tubes containing the diluted blood layered over Ficoll–Hypaque were centrifuged at 400 *g* for 40 min at 20°. Mononuclear cells, removed from the interface, were washed three times with HBSS and collected by centrifugation at 150 *g* for 10 min at 20°. The cells were then resuspended in HBSS at a concentration of 2 million lymphocytes per millilitre.

Preparation of sheep erythrocyte rosettes and fractionation of T and B lymphocytes

For the purposes of this paper, the term 'T lymphocytes' will be used to refer to those human lymphocytes which formed rosettes with sheep erythrocytes and the term 'B lymphocytes' will refer to human lymphocytes which failed to form sheep erythrocyte rosettes. Sheep erythrocytes were washed and resuspended in HBSS as a 5 per cent (volume/volume) suspension. The cell suspension was incubated at 37° for 30 min with 0·4 u of *Vibrio cholerae* neuraminidase to enhance binding to T lymphocytes as described by Weiner, Bianco and Nussenzweig (1973). The neuraminidase-treated sheep erythrocytes ($SRBC_N$) were washed three times with HBSS and resuspended as a 0·5 per cent suspension in RPMI 1640 containing 10 mM HEPES buffer (Grand Island Biological Company). Ten millilitre of the $SRBC_N$ suspension and 10 ml of the lymphocyte suspension were incubated for 15 min at 37°, centrifuged at 200 *g* for 10 min at 20° and finally held for 20–30 min on ice. Approximately three-quarters of the supernatant above the $SRBC_N$–lymphocyte pellet were removed and the cells gently resuspended. Twelve millilitre of the $SRBC_N$–lymphocyte suspension were layered over 3 ml of Ficoll–Hypaque in 16 × 125 mm plastic tubes. The cells were centrifuged at 400 *g* for 40 min at 4°. Unrosetted B-lymphocytes remained at the interface. They were removed and resuspended in RPMI 1640 with 100 u penicillin/ml, 100 μg streptomycin/ml and 2 mM glutamine (Grand Island Biological Company). Rosetted T lymphocytes were in the pellet beneath the Ficoll–Hypaque. They were washed twice with 0·83 per cent ammonium chloride–0·17 M Tris buffer, pH 7·2, to lyse the SRBC (Boyle, 1968), and collected by centrifugation. The unfractionated, purified B and T lymphocytes were washed three times with RPMI 1640 and resuspended at a concentration of 10^6 lymphocytes per millilitre in the final culture medium: RPMI 1640 with penicillin, streptomycin, glutamine and 10 per cent heat-inactivated human AB serum. Lymphocyte preparations irradiated with 3000 R from a ^{137}Ce source were viable but showed no significant response to either mitogen. Viability of all lymphocyte preparations used was over 96 per cent as measured by trypan blue dye exclusion.

Identification of immunoglobulin-bearing and sheep erythrocyte rosette-forming lymphocytes

Five hundred thousand lymphocytes, suspended in 0·05 ml HBSS, were incubated with 0·05 ml of a 1:10 dilution of fluorescein-conjugated rabbit anti-human immunoglobulin (Lot 112471B, Sylvana Laboratories, Millburn, New Jersey) and incubated for 15 min at room temperature. The lymphocytes

were washed twice with HBSS and resuspended in 0·5 ml of HBSS to which was added 0·5 ml of a 0·5 per cent suspension of neuraminidase-treated sheep erythrocytes. The cell mixture was incubated for 15 min at 37° in a water bath, centrifuged for 10 min at 200 *g* at room temperature and held 30–40 min on ice. Three-quarters of the supernatant was removed and the cell button gently resuspended. A drop of the suspension was examined under a nail polish-sealed cover slip with a Leitz–Ortholux microscope under phase contrast illumination with incidence light immunofluorescence employing a HBO 200 mercury lamp, BG 38 filter and Ploem optics. Each field was evaluated for immunofluorescent cells and rosetted cells. Two hundred cells from each preparation were examined.

Lymphocyte cultures

Each culture well contained 0·2 ml of final culture medium, $1–2 \times 10^5$ lymphocytes and where indicated 2 μg PHA-E-312A (Burroughs-Wellcome and Company, Research Triange, North Carolina) or 2 μl stock pokeweed mitogen (Grand Island Biological Company). Cultures were established in triplicate and routinely incubated in a 5 per cent CO_2/95 per cent humidified air environment. Sixteen hours prior to the termination of the incubation period, 2 μCi of tritiated thymidine (specific activity 2 Ci/mM) (New England Nuclear Company, Boston, Massachussetts) in 50 μl of final culture medium were added to each culture well. At the end of the incubation period the lymphocytes were aspirated from the wells, transferred to glass fibre filter paper (Reeve-Angel, Incorporated, Clifton, New Jersey) and washed with water using an apparatus based on the design of Hartzman, Segall, Bach and Bach (1971). The glass fiber discs were placed into 14 × 44 mm vials and 2½ ml of Hydromix (Yorktown Research Company, New Hyde Park, New York) were added. These vials were placed inside standard scintillation vials and counted in a Beckman ambient temperature liquid scintillation counter. The average of the counts per minute thymidine incorporated per well are given. The variability of replicate counts was less than 18 per cent in the data presented. The counting efficiency for tritium under these conditions was 34 per cent.

RESULTS

Isolation of lymphocyte subpopulations

Separation of human lymphocytes on the basis of their ability to form rosettes with sheep erythrocytes yielded preparations greatly enriched with T or B lymphocytes. The lymphoid preparations were examined using a combined assay for the identification of immunoglobulin-bearing and rosette-forming lymphocytes. Lymphocytes reacting with fluorescent antisera to human immunoglobulin represented 22 per cent of the unfractionated lymphocyte preparation, 5 per cent of the T-lymphocyte preparation and 97 per cent of the B-lymphocyte preparation (Table 1). It is recognized that the lymphoid cells so identified may represent a mixture of B and K lymphocytes. Ten to 15 per cent of the unfractionated mononuclear cells isolated by the Ficoll–Hypaque method from whole blood phagocytosed latex particles. Six per cent of the mononuclear cells in the B-lymphocyte preparation and 3 per cent of

Table 1. Characteristics of purified human blood lymphocytes*

	Percentage of lymphocytes reactive with:	
	Rabbit anti-human immunoglobulin†	Sheep erythrocytes†
Unfractionated lymphocytes	22 (18–26)	75 (68–86)
Purified T lymphocytes	5 (3–13)	94 (87–97)
Purified B lymphocytes	97 (95–98)	2 (1–5)

* Human blood lymphocytes were isolated from blood by the Ficoll–Hypaque method. T lymphocytes which formed rosettes with sheep erythrocytes were separated from non-rosetted B lymphocytes.

† Mean and range of five observations.

Table 2. Thymidine incorporation by unfractionated T or B lymphocytes in response to various concentrations or PHA or PWM*

Preparation	Mitogen							
	PHA (μg/ml)				PWM (μl/ml)			
	0	1	10	100	0	1	10	100
Unfractionated lymphocytes	0·6	82·7	141·8	1·6	0·5	10·9	11·9	2·9
Purified T lymphocytes	0·4	18·3	41·2	2·8	0·1	4·6	7·3	1·0
Purified B lymphocytes	2·2	2·4	5·3	2·1	2·3	3·0	8·6	1·6

* Lymphocytes were isolated from blood by the Ficoll–Hypaque technique. T lymphocytes which formed rosettes with sheep erythrocytes were separated from unrosetted B lymphocytes. Lymphocytes were incubated in the presence or absence of PHA for 120 h or of PWM for 168 h. The results given are the mean of three experiments and are expressed as c.p.m./culture ($\times 10^{-3}$).

the cells in the T-lymphocyte preparation were phagocytic.

Response of unfractionated, B-lymphocyte or T-lymphocyte preparations to phytohaemagglutinin or pokeweed mitogen

Unfractionated, purified B or purified T lymphocytes were incubated with or without PHA or PWM (Tables 2 and 4). In the absence of PHA or PWM, thymidine incorporation is mainly due to the metabolic activity of the B-lymphocyte. Thus, in the absence of mitogen, the B-lymphocyte preparation incorporated 2–5 times more thymidine than did the unfractionated preparation and 8–17 times more thymidine than did the T-lymphocyte preparation. The response of these lymphoid preparations to varying doses of mitogen (Table 2) or incubated for varying periods of time (Fig. 1) was studied. The lymphoid preparations respond optimally to a final concentration of 10 μg PHA/ml and of 10 μl PWM/ml and these concentrations were used routinely. The maximal response of lymphocytes to PHA was observed after 5 days and to PWM after 7 days in culture. These incubation periods were chosen for further studies. From these two studies it can also be seen that PHA and PWM stimulate thymidine incorporation by T-lymphocytes. In contrast, B-lymphocytes responded to PWM but showed little response to PHA. The observation that unfractionated lymphocytes in the presence of PHA or PWM incorporated more thymidine than did purified B- or T-lymphocyte preparations in the presence of these mitogens suggested a synergistic interaction between B- and T-lymphocytes in their response to these mitogens.

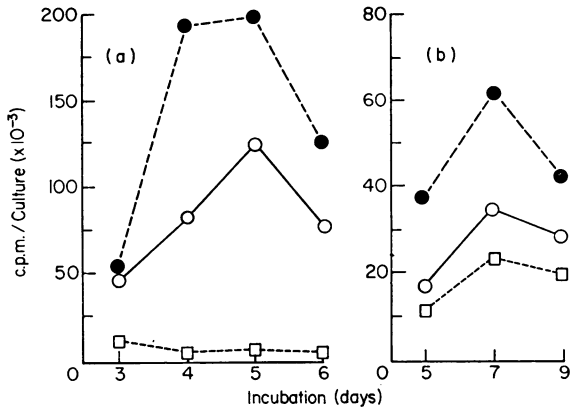

Figure 1. Lymphocyte proliferation induced by (a) phytohaemagglutinin or (b) pokeweed mitogen. Culture wells were filled with 10^5 unfractionated (●), purified T (○) or purified B (□) lymphocytes suspended in 0·1 ml of culture medium. To each well was added either 2 μg PHA or 2 μl PWM in 0·1 of culture medium. The cells were incubated from 2–5 days (PHA) or from 4 to 8 days (PWM) prior to a 24 h pulse with 1 μCi or [^{3}H]thymidine. The means of two experiments are given. The c.p.m. given represented the net counts obtained by subtracting the thymidine incorporated by lymphocytes cultured in the absence of mitogen.

Synergy between T- and B-lymphocytes in their response to PHA or PWM

To test the hypothesis that T and B lymphocytes co-operate in their response to PHA or PWM, purified B lymphocytes were mixed in varying

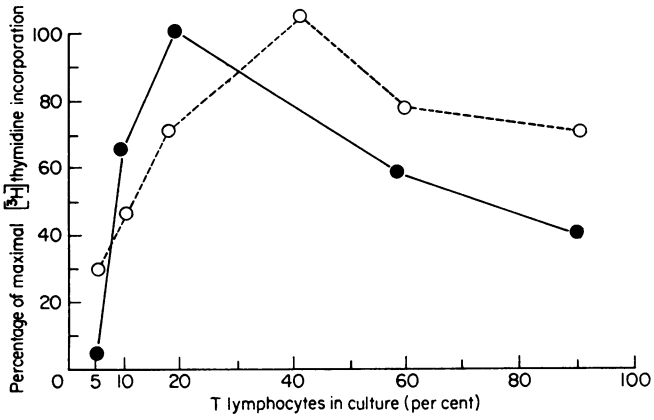

Figure 2. Proliferation of mixed B- and T-lymphocyte populations induced by phytohaemagglutinin (●) or pokeweed mitogen (○). Purified T lymphocytes (91 per cent sheep erythrocyte binding cells) and purified B lymphocytes (5 per cent sheep erythrocyte binding cells) were prepared and mixed in various proportions to yield lymphocyte preparations containing 5–91 per cent T lymphocytes. 10^5 lymphocytes in 0·1 ml of final culture medium were mixed with 0·1 ml of final culture medium containing either 2 μg of PHA or 2 μl of PWM and incubated for 120 h (PHA) or 168 h (PWM). Thymidine incorporated by lymphocytes containing various proportions of T lymphocytes is expressed as a percentage of the greatest thymidine incorporated by the optimal combination of B and T lymphocytes cultured with PHA ($106{\cdot}3\times10^3$ c.p.m.) or PWM ($46{\cdot}8\times10^3$ c.p.m.). Unfractionated lymphocytes incorporated $98{\cdot}6\times10^3$ c.p.m. when cultured with PHA and $49{\cdot}3\times10^3$ c.p.m. when cultured with PWM.

proportions with purified T lymphocytes to yield preparations containing the same total number of lymphocytes but varying percentages of T and B cells. A representative experiment correlating mitogen-induced thymidine incorporation by cultured lymphocytes, with the percentage of T lymphocytes in the lymphoid cell preparations is shown in Fig. 2. In the presence of PHA, purified B lymphocytes incorporated less than 5 per cent of the maximal thymidine incorporated by mixed B- and T-lymphocyte cultures. Addition of 10 per cent T lymphocytes to the B-lymphocyte preparation markedly augments this response. Purified B lymphocytes do respond to PWM but, again, addition of T lymphocytes augments the response. Synergy between the B and T lymphocytes is thus demonstrated by the fact that thymidine incorporation by preparations containing both B and T lymphocytes is greater than thymidine incorporation by the same number of B and T lymphocytes cultured separately. In three experiments, such as illustrated in Fig. 2, maximal thymidine incorporation by lymphocytes cultured with PHA or PWM was observed in cultures containing 20–50 per cent T lymphocytes. Thymidine incorporation by unfractionated lymphocytes ranged from 78 to 106 per cent of the maximal thymidine incorporated by the optimal combination of B- and T-lymphocyte preparations.

Stimulation of B-lymphocyte thymidine incorporation by mitogen-activated T lymphocytes

The addition of small numbers of T lymphocytes to a purified B-lymphocyte preparation markedly enhanced thymidine incorporation by the mixed B- and T-lymphocyte preparation. This suggested that activated T-lymphocytes in the presence of mitogens might facilitate thymidine incorporation by B-lymphocytes. To test this hypothesis, activated T-lymphocytes were prepared by culturing purified T-lymphocytes with PHA or PWM for 5 days. These cells were washed, irradiated (3000 R) and cultured with equal numbers of freshly prepared autologous purified B lymphocytes in the presence of PHA or PWM. The proliferative response of purified B cells cultured with PHA or PWM in the presence or absence of activated T lymphocytes is shown in Table 3. In this experiment, thymidine incorporation by purified B lymphocytes was minimally stimulated by PWM but not by PHA. In contrast, thymidine incorporation by purified B lymphocytes cultured with PHA or PWM was markedly stimulated in the presence of activated T lymphocytes. Irradiated, activated T lymphocytes did not incorporate significant amounts of thymidine. It is therefore probable that the thymidine incorporated by these cultures reflects B-lymphocyte DNA synthesis facilitated by

Table 3. Effect of activated T lymphocytes on thymidine incorporation by B lymphocytes in the presence of PHA or PWM*

Responding lymphocytes	Additions to culture	Mitogen	Thymidine incorporated (c.p.m. × 10^{-3})
B lymphocytes	—	—	1·9
B lymphocytes	—	PHA	2·0
B lymphocytes	PHA-activated supernate	PHA	2·3
B lymphocytes	PHA-activated lymphocytes (3000 R)	PHA	21·5
PHA-activated lymphocytes (3000 R)	—	PHA	1·0
B lymphocytes	—	—	2·3
B lymphocytes	—	PWM	4·1
B lymphocytes	PWM-activated supernate	PWM	2·7
B lymphocytes	PWM-activated lymphocytes (3000 R)	PWM	15·8
PWM-activated lymphocytes (3000 R)	—	PWM	0·6

* Culture wells were filled with 10^5 purified B lymphocytes suspended in 0·1 ml of culture medium with or without 2 μg PHA or 2 μl PWM. 0·1 ml of supernatant medium from cultures of activated T lymphocytes or 0·1 ml of culture medium or 0·1 ml of culture medium containing 10^5 autologous irradiated activated T lymphocytes were added to the wells. All cultures were incubated 6 days. Activated T lymphocytes were prepared by culturing purified T lymphocytes with 10 μg PHA/ml or 10 μl PWM/ml for 5 days. The activated T lymphocytes were washed, irradiated (3000 R) and resuspended in culture medium at a concentration of 10^6 lymphocytes per millilitre.

Table 4. Thymidine incorporation by purified B lymphocytes in the presence of PHA or PWM*

			Thymidine incorporated (c.p.m. × 10^{-3})			
			Experiment I		Experiment II	
			Mitogen		Mitogen	
Responding lymphocytes	Additions to culture	Mitogen	Absent	Present	Absent	Present
Unfractionated lymphocytes	—	PHA	0·6	189·4	0·4	142·5
T lymphocytes	—	PHA	0·2	154·2	0·1	173·7
B lymphocytes	—	PHA	2·3	15·4	1·3	17·4
B lymphocytes	Irradiated T lymphocytes	PHA	—	136·2	1·4	121·3
B lymphocytes	Autologous erythrocytes	PHA	—	15·5	1·9	25·0
Unfractionated lymphocytes	—	PWM	0·3	13·3	0·4	40·8
T lymphocytes	—	PWM	0·1	7·6	0·1	8·5
B lymphocytes	—	PWM	1·7	5·1	1·3	4·6
B lymphocytes	Irradiated T lymphocytes	PWM	—	13·2	1·3	24·1
B lymphocytes	Autologous erythrocytes	PWM	—	4·6	1·8	7·6
Irradiated T lymphocytes	—	PHA	—	0·6	0·1	0·4
Irradiated T lymphocytes	—	PWM	—	2·3	0·1	0·5

* Culture wells were filled with 10^5 unfractionated, T or B lymphocytes suspended in 0·1 ml of culture medium with or without 2 μg PHA or 2 μl PWM. To these wells was added either 0·1 ml culture medium or medium containing 10^5 autologous irradiated (3000 R) T lymphocytes or medium containing 10^5 autologous erythrocytes. In one set of wells, 10^5 irradiated T lymphocytes were mixed with either 2 μg PHA or 2 μl PWM in 0·2 ml of culture medium. Cultures containing PWM were incubated 168 h. Cultures containing PHA were incubated 120 h.

irradiated-activated T lymphocytes in the presence of PHA or PWM. No evidence for a soluble mitogenic factor released by cultured T lymphocytes was found. Thus, media from activated T lymphocytes did not stimulate thymidine incorporation by B lymphocytes cultured with mitogen.

Thymidine incorporation by B lymphocytes cultured with T lymphocytes in the presence of PHA or PWM

That mitogen-activated T lymphocytes in the presence of PHA or PWM could stimulate thymidine incorporation by B lymphocytes prompted several experiments to define the nature of this interaction between B and T lymphocytes. The capacity of irradiated T lymphocytes, not previously incubated with mitogen, to facilitate thymidine incorporation by B lymphocytes cultured with PHA or PWM was tested. The results of two representative experiments are shown in Table 4. Addition of purified irradiated T lymphocytes to purified B lymphocytes cultured with PHA or PWM markedly stimulated thymidine incorporation. Thus, B lymphocytes cultured with PHA in the presence of T lymphocytes incorporate 7–9 times more thymidine than they do in the absence of T lymphocytes. Similarly, B lymphocytes cultured with PWM in the presence of T lymphocytes incorporate 2–4 times more thymidine than they do in the absence of T lymphocytes. Disruption of the T lymphocytes by freezing and thawing resulted in a loss of their capacity to stimulate B-lymphocyte proliferation (data not shown).

That thymidine incorporation by B lymphocytes incubated with irradiated T lymphocytes and PHA was due to the proliferation of B lymphocytes was confirmed by the identification of surface immunoglobulin on lymphoblasts at the end of the incubation of irradiated T lymphocytes, B lymphocytes and PHA. In two experiments, at the end of the incubation period 67 and 76 per cent of the lymphoblasts, identified by phase microscopy, reacted with fluoresceinated antisera to human immunoglobulin.

As PHA bound to inert particles such as Sepharose stimulate purified murine B-lymphocyte proliferation (Greaves and Bauminger, 1972), it was possible that the irradiated T lymphocytes served as an inert mitogen-binding particle and in this manner stimulated B-lymphocyte proliferation. Human erythrocytes bind both PWM and PHA and were tested for their capacity to facilitate thymidine incorporation by B lymphocytes. As can be seen from Table 4, human erythrocytes incubated with purified B lymphocytes and PHA or PWM did not enhance thymidine incorporation by B lymphocytes.

DISCUSSION

B- and T-lymphocyte preparations were isolated from mononuclear blood leucocytes by separating lymphocytes that formed rosettes with sheep erythrocytes from unrosetted lymphocytes. The response of these preparations to PHA or PWM was measured. PWM stimulated thymidine incorporation by both B- and T-lymphocyte preparations. PHA stimulated thymidine incorporation by the T- but not the B-lymphocyte preparation.

The response of purified human B lymphocytes to PHA has been determined by several investigators with apparently conflicting results. Epstein *et al.* (1974), Chess *et al.* (1974) and Phillips and his co-workers (1973, 1974) have offered evidence that purified B-lymphocytes were stimulated to proliferate by PHA. In contrast, Geha and Merler (1974), Geha, Rosen and Merler (1974), Greaves *et al.* (1974) and Lohrmann, Novikovs and Graw (1974) found that highly purified preparations of B lymphocytes were not stimulated by PHA. These results are most likely explained by differences in the techniques used to isolate B lymphocytes and in some cases to differences in the purity of the B-lymphocyte population obtained. Every group that found purified B lymphocytes respond to PHA had selected for B-lymphocytes by their reaction with anti-human immunoglobulin or with the Fc determinant of human immunoglobulin. Such procedures may alter responses of B lymphocytes to mitogens. Reactions with the surface immunoglobulin or Fc receptor has been shown to enhance the reactivity of B lymphocytes and may augment their reactivity to PHA (Mackler, Altman, Rosenstreich and Oppenheim, 1974; Katz and Unanue, 1972). Further, except for the method of Chess *et al.* (1974), these techniques have yielded B-lymphocyte preparations containing 10–15 per cent non-immunoglobulin bearing lymphocytes. In contrast, we and the other groups who have found little or no response of B lymphocytes to PHA have prepared B lymphocytes by removing T cells which had formed rosettes with sheep erythrocytes. Such B-lymphocyte preparations usually con-

tained fewer than 5 per cent non-immunoglobulin bearing lymphocytes. The purity of the B-lymphocyte preparation is critical. Contamination of such preparations by as little as 5–10 per cent T lymphocytes permits a vigorous response to PHA.

When T lymphocytes were mixed in varying proportions with B lymphocytes and cultured with PHA or PWM, maximal thymidine incorporation was observed when T lymphocytes made up 20–50 per cent of the total lymphoid population. Such mixtures of B- and T-lymphocyte preparations were as responsive to these mitogens as were unfractionated lymphocytes. The mixed lymphocyte preparations incorporated more thymidine when cultured with PHA or PWM than did the B- and T-lymphocyte preparations cultured separately. Thus, thymidine incorporation by blood lymphocytes cultured with PHA cannot be used as a quantitative index of T-lymphocyte function. These findings also suggest synergy between B- and T-lymphocytes in their response to PHA or PWM. Evidence presented by several laboratories (Geha *et al.*, 1974; Lohrmann *et al.*, 1974; Geha and Merler, 1974; Piguet and Vassali, 1972; Winkelstein, 1971) supports a co-operative interaction between T and B lymphocytes in their response to antigens or plant lectins. On the other hand, thymidine incorporation by human tonsillar lymphocytes incubated with PHA was found to be directly related to the percentage of T lymphocytes in culture (Greaves *et al.*, 1974).

Synergy between blood lymphocytes in their response to mitogens appears to result from the capacity of T lymphocytes to facilitate thymidine incorporation by B lymphocytes cultured with PHA or PWM. The capacity of T lymphocytes to facilitate the response of B lymphocytes to PHA or PWM depends upon the structural integrity of the T lymphocytes but not upon their proliferation or their release of mitogenic factors. Thus, irradiated (3000 R) T lymphocytes but not culture supernatant augmented thymidine incorporation by B lymphocytes cultured with these mitogens and resulted in the appearance of lymphoblasts with surface immunoglobulin in such cultures. Lohrmann *et al.* (1974) showed that irradiated (5000 R) T lymphocytes facilitated B-lymphocyte proliferation, although Geha *et al.* (1974) found T lymphocytes irradiated with 7000 R did not stimulate B-lymphocyte proliferation. Recently, Elfenbein and Gelfand (1975) showed that mitomycin-inactivated murine thymocytes which had bound PHA stimulated syngeneic B-lymphocyte proliferation by 'presenting' PHA or PWM to B lymphocytes in a form that is more mitogenic than soluble PHA or PWM. Greaves and Bauminger (1972) had shown that PHA or PWM coupled to Sepharose particles was more mitogenic for murine B lymphocytes than were soluble mitogens. However, binding of PHA or PWM by a cell did not, in itself, yield a stimulant of human B-lymphocyte proliferation. Human erythrocytes which bind both mitogens did not facilitate B-lymphocyte proliferation. Further, B lymphocytes bind both mitogens and if surface binding of mitogens by any cell were a sufficient stimulus to B-lymphocyte proliferation, purified B lymphocytes should respond to these mitogens. How the interaction between mitogen and T lymphocytes facilitates B-lymphocyte proliferation is not known. Piguet and Vassali (1972) and Andersson, Möller and Sjöberg (1972) found a soluble factor produced by murine T lymphocytes that stimulated the proliferation of B lymphocytes cultured with mitogen. This factor was not mitogenic by itself. Other investigators (Geha *et al.*, 1974; Epstein *et al.*, 1974) were not able to demonstrate soluble factors released from T lymphocytes that stimulated B-lymphocyte proliferation. We have no evidence that a soluble mitogenic factor is released from T lymphocytes and conclude that mitogens bound to T lymphocytes directly stimulate B-lymphocyte proliferation.

Review of the published studies and our own results lead us to suggest: (i) that human B lymphocytes are stimulated to proliferate in the absence of T lymphocytes by PWM but not by PHA; (ii) that T lymphocytes augment the proliferation of B lymphocytes cultured with PHA or PWM; and (iii) that T and B lymphocytes demonstrate synergy in their response to PHA or PWM. Finally, the capacity of human T lymphocytes to stimulate proliferation of B lymphocytes may reflect a mechanism that underlies the co-operative interaction of B and T lymphocytes in the immune response. That is, T lymphocytes may stimulate the proliferation of B lymphocytes in the presence of antigen. This hypothesis is supported by the studies of Coutinho and Möller (1973) which suggested that T-independent antigens directly stimulate B-lymphocyte proliferation and by the fact that lipopolysaccharide, a B-lymphocyte mitogen, can replace T lymphocytes in the generation of the immune response to a T-dependent antigen (Möller, Andersson and Sjöberg, 1972).

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