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Relationship Between Enhanced Turnover of Phosphatidylinositol and Lymphocyte Activation by Mitogens

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1. Various lectins [*Phaseolus vulgaris* phytohaemagglutinin, *Glycine max* (soy-bean) agglutinin, *Triticum vulgaris* (wheat-germ) agglutinin and *Axinella polyploides* agglutinin] and antibodies to pig Ig (immunoglobulin) that are bound by pig lymphocytes were assessed in terms of their capacities to stimulate lymphocyte transformation and to enhance phosphatidylinositol turnover. Transformation was measured after 45h of culture by incorporation of [6-^{3}H]thymidine into DNA, whereas phosphatidylinositol metabolism was assessed after 1h of culture by incorporation of $^{32}P_i$. 2. Transforming agents (*P. vulgaris* and *G. max* agglutinins and rabbit antibodies to pig Ig) increased phosphatidylinositol turnover, but non-transforming agents (*T. vulgaris* and *A. polyploides* agglutinins and Fab fragments of rabbit antibodies to pig Ig) failed to induce any significant enhancement. Subsequent cross-linkage of the bound, non-transforming Fab fragments with a goat antiserum to rabbit Ig stimulated transformation and phosphatidylinositol turnover. 3. Each transforming agent gave characteristic optimal dose responses that were similar for both phosphatidylinositol turnover and transformation. 4. The results indicate that activation of T- and B-lymphocytes is accompanied by enhanced phosphatidylinositol turnover and that in the case of B-cells this enhancement depends on the cross-linkage of surface receptors. They are consistent with the proposal that turnover represents an essential early step in the transformation process.

It is generally accepted that cellular activation is frequently initiated by the interaction of extracellular 'messengers' with specific cell-surface 'receptors'. The molecular mechanism of activation, particularly the transmission of information across the surface membrane, has, however, yet to be elucidated. Studies of various systems have revealed that activation in response to extracellular stimuli is frequently associated with an increase in plasma-membrane, phospholipid metabolism, especially of phosphatidylinositol (Hokin, 1968; Michell & Lapetina, 1972; Pasternak, 1972; Ferber, 1973; Lapetina & Michell, 1973). For example, stimulation of lymphocyte transformation by phytomitogens and by anti-Ig‡ and anti-lymphocyte sera is accompanied by the rapid selective incorporation of long-chain fatty acids into the plasma-membrane phospholipids (Resch *et al.*, 1971; Resch & Ferber, 1972), and of P_i and *myo*-inositol into phosphatidylinositol (Fisher & Mueller, 1968, 1971; Masuzawa *et al.*, 1973). The enhanced incorporation of P_i into phosphatidylinositol exhibits a number of features which would be expected to be characteristic of an early event in cellular activation. Thus it is specific, is detected within a few minutes of the stimulus and precedes other biochemical responses such as nucleic acid and protein synthesis. The role of the increased phospholipid metabolism has, however, not been defined. Most workers have emphasized the possible direct effects on the structure and function of the cell membrane, especially the regulation of the influx and efflux of macromolecules (e.g. phagocytosis and pinocytosis; Karnovsky & Wallach, 1961), metabolites and ions (Resch & Ferber, 1972). Alternatively, Michell & Lapetina (1972) have suggested that enhanced phosphatidylinositol turnover reflects the increased production of *myo*-inositol 1:2-cyclic phosphate, to which they ascribed the role of a 'second messenger'; evidence in support of this suggestion is, however, presently lacking (Freinkel & Dawson, 1973). In spite of the above attractive arguments the significance of increased phosphatidylinositol metabolism and its relationship to cell activation remain uncertain. The following experiments were designed to determine the degree of specificity of this relationship for lymphocytes activated by various mitogens.

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‡ Abbreviation: Ig, immunoglobulin.

The stimulation of T-lymphocytes by certain lectins (Sharon & Lis, 1972), especially *P. vulgaris* phytohaemagglutinin, to transform into blast cells that subsequently undergo mitosis represents an excellent experimental model for gene activation in a mammalian cell (Greaves & Janossy, 1972). The initial event in the transformation process is the binding of the lectin by surface glycoproteins (Allan *et al.*, 1971, 1972). This interaction is rapidly followed by lymphocyte agglutination, and by the redistribution ('patching' and 'capping') and endocytosis of the glycoprotein receptors (Loor, 1973). However, some lectins, notably *T. vulgaris* and *A. polyploides* agglutinins, although causing agglutination, capping and endocytosis, fail to stimulate transformation as judged by the incorporation of thymidine into DNA (Hayman, 1973). The lack of transformation is probably due to the failure of these lectins to bind and/or cross-link the specific surface glycoprotein(s) which mediates transformation (M. J. Hayman & M. J. Crumpton, unpublished work). B-lymphocytes can be stimulated to transform by antibodies to Ig (Sell *et al.*, 1965), but not by the Fab fragments of these antibodies, although subsequent cross-linkage of the bound Fab fragments by a homologous antiserum induced transformation (Fanger *et al.*, 1970). The above distinctions between transforming and non-transforming lectins, and between bivalent antibodies to Ig and their monovalent Fab fragments, made it possible to determine whether enhanced phosphatidylinositol turnover is initiated by mere binding and redistribution of cell-surface components or whether it is directly related to the cross-linkage of the specific membrane component mediating transformation. The results indicate that increased phosphatidylinositol turnover is intimately related to lymphocyte activation and that, at least in B-lymphocytes, the stimulation of the increase depends on the cross-linkage of the receptor.

Materials and Methods

Materials

Purified *P. vulgaris* phytohaemagglutinin was purchased from Wellcome Reagents Ltd., Beckenham, Kent, U.K., and purified preparations of *T. vulgaris* agglutinin (Allen *et al.*, 1973) and *G. max* agglutinin (Gordon *et al.*, 1972) were given by Dr. A. K. Allen (Charing Cross Hospital Medical School, London, U.K.) and Professor N. Sharon (Weizmann Institute, Rehovet, Israel) respectively. *P. vulgaris* phytohaemagglutinin and *G. max* agglutinin have an antibody-like specificity for *N*-acetylgalactosamine residues, whereas *T. vulgaris* agglutinin binds *N*-acetylglucosamine residues, especially *NN'*-diacetylchitobiose and higher oligomers (Sharon & Lis, 1972; Allen *et al.*, 1973). *Axinella* agglutinin, which binds D-galactose residues, was isolated from *A. polyploides* (provided by the Marine Biological Laboratory, Plymouth, U.K.). The sponge (100g wet wt.) was homogenized (Sylverson emulsifier) in 500ml of water at 2°C and the soluble fraction was separated by centrifuging (Sorvall RC2-B centrifuge; 20000rev./min for 1h). The agglutinin was isolated from the supernatant by adsorption to a column (90cm×1.5cm) of Sepharose 6B in 75mM-sodium phosphate buffer–75mM-NaCl, pH7.3, and subsequent elution with 1% (w/v) galactose dissolved in the same solvent. The yield of purified agglutinin was about 25mg dry wt. (M. J. Crumpton & A. P. MacLennan, unpublished work). Fluorescent *T. vulgaris* agglutinin was prepared by treating the agglutinin [10mg/ml of 0.1M-Na_2CO_3 buffer, pH9.0, containing 10% (v/v) dioxan] with fluorescein isothiocyanate (0.1vol. of a 5mg/ml solution in acetone) at 4°C overnight; dye that had not reacted was removed by filtration through Sephadex G-25.

Purified samples of phosphatidylinositol were given by Professor J. N. Hawthorne, University of Nottingham, or were purchased from Koch–Light Laboratories Ltd., Colnbrook, Bucks., U.K. [6-^{3}H]-Thymidine (5Ci/mmol) and $^{32}P_i$ (5Ci/mmol) were obtained from The Radiochemical Centre, Amersham, Bucks., U.K. Pig Ig was prepared from serum by precipitation with 14% (w/v) Na_2SO_4 and subsequent elution from DEAE-Sephadex A-50 equilibrated with 10mM-sodium phosphate buffer, pH7.0. All chemicals were of analytical grade and glass-distilled water was used in all experiments.

Anti-(pig Ig)sera were raised in rabbits by two injections, separated by an interval of 6 weeks, of 1mg of pig Ig emulsified in 1ml of Freund's complete adjuvant; serum was collected about 3 weeks after the second injection. Fab fragments were prepared by digesting the Ig fraction of the above antisera with papain as described by Porter (1959). The digest was dialysed against 10mM-sodium phosphate buffer, pH7.2, and the crystalline Fc fragment was separated by centrifuging. Undigested Ig and aggregated Fab fragments were removed from the supernatant immediately before use by elution from a column (95cm×1.5cm) of Bio-Gel P150 equilibrated with 75mM-sodium phosphate buffer–75mM-NaCl, pH7.3. The goat antiserum to rabbit Ig was given by Dr. R. M. E. Parkhouse of this Institute.

Pig lymphocytes were prepared as previously described (Allan *et al.*, 1971) by gently teasing small fragments of mesenteric lymph node into Eagle's medium [BHK; twice the usual concentration of amino acids and vitamins (Stoker & Macpherson, 1964)], filtering through a small plug of absorbent cotton wool and washing three times with medium. Approximately 15% of the cells were stained by a

fluorescein conjugate of rabbit antibodies to pig Ig and on this basis were judged to be B-lymphocytes.

Lymphocyte transformation assay

Lymphocyte transformation was assayed as previously described (Allan *et al.*, 1971). The mitogen (lectin or antibody in 10μl) was added to 10^6 lymphocytes in 1ml of Eagle's medium containing 20% (v/v) foetal calf serum (Flow Laboratories Ltd., Irvine, Ayrshire, U.K.). DNA synthesis was measured after 45h of culture by adding 1μCi of [6-^{3}H]thymidine and incubating for a further 4–6h. Transformation was also estimated by using the same cell density and total cell number as those used for measuring phosphatidylinositol turnover. In this case 5×10^7 lymphocytes were cultured in 5ml of medium for 45h and for a further 4h with 2μCi of [6-^{3}H]thymidine.

Enhanced phosphatidylinositol turnover

Lymphocytes (5×10^7) were preincubated at 37°C for 16h in round-bottomed glass centrifuge tubes (11cm×2.4cm) with 5ml of phosphate-free Eagle's medium containing 5% (v/v) of foetal calf serum in an atmosphere of air supplemented with 5% (v/v) of CO_2. The cells were recovered by centrifuging (800*g* for 5min), washed once with phosphate-free medium, resuspended in 5ml of phosphate-free medium containing 10% (v/v) of dialysed foetal calf serum and incubated at 37°C for 15min with the mitogen (lectins or antibody in 10μl) before the addition of 30–50μCi of $^{32}P_i$. The effect of the valency of the mitogen on turnover was determined in an identical manner, except that the cells were incubated for 20min with the Fab fragments, followed by 20min incubation with 20μl of a 1:10 dilution of a goat anti-(rabbit Ig)serum before addition of the $^{32}P_i$. Although significant enhanced incorporation of $^{32}P_i$ into phosphatidylinositol, relative to the control value, was observed with all transforming agents after 10min incubation at 37°C, a period of 1h was adopted as a routine since increased enhancement was obtained (3-fold at 10min, increasing to about 10-fold at 1h for *P. vulgaris* phytohaemagglutinin; see also Fig. 2A of Fisher & Mueller, 1968). The cells were recovered by centrifuging, washed twice with 0.9% NaCl and were resuspended in 1ml of 0.9% NaCl.

Phospholipids were extracted from the cell suspensions with chloroform–methanol (1:2, v/v) by the method of Kates (1972), were separated by chromatography on Wagner papers as described by Kai & Hawthorne (1966) and were detected by exposure to I_2 vapour. Radioactive spots were revealed by exposing the chromatograms to X-ray film for 1–2 days. ^{32}P-labelled phosphatidylinositol was identified as a radioactive spot occupying an identical position with an authentic sample of phosphatidylinositol. Radioactivity incorporated into phosphatidylinositol was determined by removing the area of the phosphatidylinositol spot into a scintillation vial with 10ml of scintillation fluid (Kinard, 1957) and counting for 5min in a Packard Tri-Carb liquid-scintillation spectrometer. Cultures were set up in duplicate or triplicate and controls comprising cells that were treated in an identical manner except for the absence of mitogen were included in each test.

Results and Discussion

Stimulation of transformation and phosphatidylinositol turnover by lectins

Fig. 1 shows that various amounts of *T. vulgaris* and *A. polyploides* agglutinins, within the range 1–100μg, failed to stimulate pig lymphocyte transformation as judged by the absence of any enhanced incorporation of thymidine into DNA. Under the same conditions 1–10μg of *P. vulgaris* phytohaemagglutinin induced considerable DNA synthesis. It is also apparent (Fig. 1) that *P. vulgaris* phytohaemagglutinin-induced transformation was not inhibited by the addition of *T. vulgaris* agglutinin (20μg/culture; equivalent to a 10–200-fold molar excess relative to *P. vulgaris* phytohaemagglutinin). The latter result indicates that *T. vulgaris* agglutinin

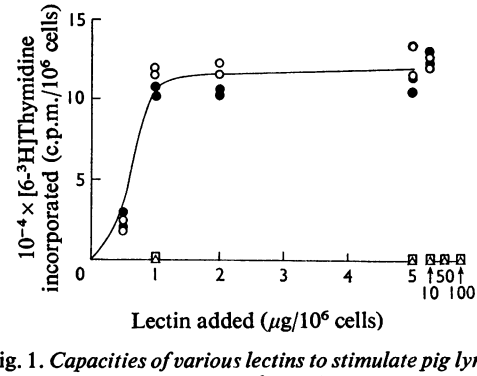

Fig. 1. *Capacities of various lectins to stimulate pig lymphocyte transformation*

Transformation was assessed in terms of incorporation of [6-^{3}H]thymidine into DNA after 45h of culture of 10^6 lymphocytes in 1ml of medium with increasing amounts of *P. vulgaris* phytohaemagglutinin (○), *T. vulgaris* agglutinin (□) and *A. polyploides* agglutinin (△). The effect of a constant amount of *T. vulgaris* agglutinin (20μg) on the enhanced incorporation induced by *P. vulgaris* phytohaemagglutinin (●) is also shown. Control cultures comprising cells incubated in the absence of lectin incorporated about 400c.p.m.

is not toxic to lymphocytes and that it does not compete with *P. vulgaris* phytohaemagglutinin for the lymphocyte glycoprotein(s) mediating transformation. The results of other studies indicate that the failure of *T. vulgaris* and *A. polyploides* agglutinins to stimulate lymphocyte transformation is not due to lack of binding. Thus *P. vulgaris* phytohaemagglutinin, *T. vulgaris* agglutinin and *A. polyploides* agglutinin had similar agglutination titres for pig lymphocytes (1:32, 1:16 and 1:128 dilution of 100 μg/ml solution respectively), whereas a fluorescein conjugate of *T. vulgaris* agglutinin stained about 90% of the cells and induced a similar degree of capping (about 10% of the cells) to that elicited by a transforming lectin (*Lens culinaris* phytohaemagglutinin; Young *et al.*, 1971). Also, as judged from the analysis of binding curves obtained by using ^{125}I-labelled lectins, pig lymphocytes possessed similar numbers of binding sites for *P. vulgaris* phytohaemagglutinin, *G. max*, *T. vulgaris* and *A. polyploides* agglutinins (3×10^6, 2×10^6, 3×10^6 and 4×10^5 respectively) with equilibrium constants for dissociation greater than 2×10^{-6} M (V. C. Maino, unpublished work). The most plausible explanation for the lack of transformation by *T. vulgaris* agglutinin is that *P. vulgaris* phytohaemagglutinin stimulation is mediated by a specific cell-surface glycoprotein(s) which fails to bind *T. vulgaris* agglutinin (Hayman, 1973). The situation with *A. polyploides* agglutinin appears to be more complicated and the reason for the lack of transformation has not as yet been clearly defined. The above lack of correlation between capping and transformation is consistent with the suggestion (Elson *et al.*, 1973) that capping is not in itself a sufficient stimulus to initiate lymphocyte DNA synthesis.

Table 1. *Effect of various lectins on the incorporation of $^{32}P_i$ into phosphatidylinositol of pig lymphocytes*

The reported values were taken from a typical experiment and represent the means of duplicate determinations. Other experiments gave similar results (*P. vulgaris* phytohaemagglutinin-induced stimulation after 1 h of culture within the range 8–12-fold of the control value).

Lectin	Concn. (μg/ml per 10^7 lymphocytes)	$^{32}P_i$ incorporated into phosphatidylinositol (c.p.m.)
None	—	532
P. vulgaris phytohaemagglutinin	10	5183
G. max agglutinin	40	2660
T. vulgaris agglutinin	4	588
T. vulgaris agglutinin	40	430
P. vulgaris phytohaemagglutinin + *T. vulgaris* agglutinin	10 + 10	4890
A. polyploides agglutinin	4	574

The effect of *P. vulgaris* phytohaemagglutinin on the incorporation of P_i into the phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol of pig lymphocytes was assessed after 1 h of culture. The results revealed an enhanced incorporation into phosphatidylinositol whereas the incorporation into the other phospholipids showed little enhancement. The selective stimulation of phosphatidylinositol turnover agrees with results published previously by Fisher & Mueller (1968) and Masuzawa *et al.* (1973). The capacities of the various lectins to enhance the incorporation of P_i into phosphatidylinositol are summarized in Table 1. The results show that 10 μg of *P. vulgaris* phytohaemagglutinin elicited a 10-fold enhancement and that 40 μg of *G. max* agglutinin gave a 5-fold stimulation; similar enhancements have been reported previously for *P. vulgaris* phytohaemagglutinin (about 7-fold during a period of 30 min; see Fig. 2A of Fisher & Mueller, 1968). In contrast, similar amounts of *T. vulgaris* and *A. polyploides* agglutinins caused no significant stimulation relative to the control value and, further, the enhancement by *P. vulgaris* phytohaemagglutinin was not decreased by the addition of a 5-fold molar excess of *T. vulgaris* agglutinin. It was concluded that the non-transforming lectins, *T. vulgaris* and *A. polyploides* agglutinin, fail to stimulate phosphatidylinositol metabolism. These results rule out the possibility that phosphatidylinositol turnover is enhanced by the mere binding of lectin to the lymphocyte surface and/or the subsequent perturbation of the membrane arising from the redistribution of membrane components. Further, since *T. vulgaris* agglutinin induces capping and subsequent internalization of surface membrane components, it appears unlikely that enhanced phosphatidylinositol metabolism is related to pinocytosis, as has been previously suggested (Karnovsky & Wallach, 1961).

One characteristic feature of mitogenic lectins is the amount required to induce maximal transformation as judged by thymidine incorporation into DNA (Greaves & Janossy, 1972). For example, maximal stimulation by using the conventional assay (i.e. 10^6 lymphocytes in 1 ml) required about 2 μg of *P. vulgaris* phytohaemagglutinin or about 50 μg of *G. max* agglutinin; the different optimal lectin concentrations are believed to reflect the relative affinities of the lectins for the glycoprotein(s) mediating transformation (M. J. Hayman & M. J. Crumpton, unpublished work). Given the above distinction it was decided to determine whether a similar relationship between lectin concentration and degree of stimulation exists for phosphatidylinositol metabolism. Fig. 2 compares the dose–responses of *P. vulgaris* phytohaemagglutinin, *G. max* agglutinin and *T. vulgaris* agglutinin for transformation and

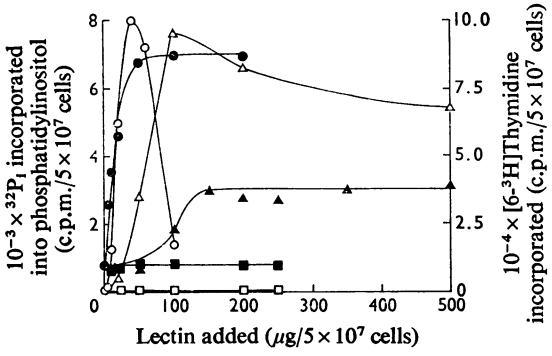

Fig. 2. *Dose–response curves for stimulation of enhanced phosphatidylinositol turnover and transformation by various lectins*

Increasing amounts of *P. vulgaris* phytohaemagglutinin (○, ●), *G. max* agglutinin (△, ▲) and *T. vulgaris* agglutinin (□, ■) were incubated with 5×10^7 pig lymphocytes in 5ml of medium under identical conditions except that phosphate free medium was used for the assay of phosphatidylinositol turnover. Incorporation of $^{32}P_i$ into phosphatidylinositol (closed symbols) was assayed after 1h of culture and incorporation of [6-^{3}H]thymidine into DNA (open symbols) after 45h of culture.

phosphatidylinositol turnover under identical culture conditions (5×10^7 lymphocytes in 5ml), except that phosphate-free medium was used for the assay of phosphatidylinositol turnover. As noted above, *T. vulgaris* agglutinin failed to promote either transformation or phosphatidylinositol turnover at all concentrations examined. The results indicate that approximately the same amounts of *P. vulgaris* phytohaemagglutinin or *G. max* agglutinin were required to elicit maximal phosphatidylinositol turnover at 1h and maximal thymidine incorporation at 45h. The apparent close relationship between the amounts required for the optimal responses is consistent with the proposal that enhanced phosphatidylinositol metabolism represents an integral step in the transformation process. The reason for the marked differences between the phosphatidylinositol and thymidine responses initiated by amounts of lectins greater than those required for the maximal effect (see Fig. 2) and, in particular, for the decrease in thymidine incorporation is not known. Various explanations are, however, possible. For example, high concentrations of lectins may be toxic after 45h of culture, but their toxicities may not be sufficiently marked after 1h to cause a significant decrease in phosphatidylinositol turnover.

Although Fig. 2 shows that *P. vulgaris* phytohaemagglutinin and *G. max* agglutinin stimulated similar optimal incorporations of [6-^{3}H]thymidine, under the conditions of the conventional assay *G. max* agglutinin always induced lower incorporation than *P. vulgaris* phytohaemagglutinin (about 40%; Hayman, 1973). The similarity revealed in Fig. 2 probably represents an artifact due to the use of non-ideal culture conditions (e.g. cell density, dialysed foetal calf serum) that failed to support optimal thymidine incorporation. On the other hand the difference in enhancement of phosphatidylinositol turnover induced by *G. max* agglutinin relative to *P. vulgaris* phytohaemagglutinin (about 4-fold versus about 9-fold) has been consistently obtained (see also Table 1). The reason for this difference is not known. However, by analogy with the different degrees of transformation induced by different phytohaemagglutinins (Jones, 1973), it seems possible that *P. vulgaris* phytohaemagglutinin stimulates a larger proportion of the lymphocytes than *G. max* agglutinin. Further, if *P. vulgaris* phytohaemagglutinin stimulates only a portion of the T-lymphocyte population as is suggested by Jones' (1973) results, then higher orders of enhancement of phosphatidylinositol turnover than those currently reported should be obtained given a pure population of responding cells.

Table 2. *Effect of valency of rabbit antibodies to pig Ig on stimulation of phosphatidylinositol turnover and transformation of pig lymphocytes*

Phosphatidylinositol turnover was measured by using 5×10^7 lymphocytes in 5ml of medium and 10μl of a 1:10 dilution of the Ig fraction of a rabbit anti-(pig Ig)serum or the equivalent amount of the Fab fragments. Transformation was assessed by using 10^6 lymphocytes in 1ml of medium and the concentration of rabbit antibodies to pig Ig that induced maximal incorporation of thymidine (10μl of a 1:100 dilution). Experimental details are given in the Materials and Methods section.

Serum	Valency	$^{32}P_i$ incorporated into phosphatidylinositol (c.p.m.)	[6-^{3}H]Thymidine incorporated (c.p.m.)
Rabbit anti-(pig Ig)	Bivalent	3342	12000
Fab fragments of rabbit anti-(pig Ig)	Monovalent	830	1200
Fab fragments+goat anti-(rabbit Ig)	Cross-linked	3770	13000
Goat anti-(rabbit Ig)	Bivalent	920	460
None	—	853	400

Stimulation of B-lymphocytes and effect of valency of the ligand on enhancement of phosphatidylinositol turnover

Various studies have suggested that cross-linkage of surface-membrane components is an essential prerequisite for lymphocyte activation (Greaves & Janossy, 1972). Unfortunately, it was not feasible to determine whether cross-linkage by lectins was also essential for the enhancement of phosphatidylinositol turnover owing to the unavailability of monovalent reagents. This question can, however, be answered by using B-lymphocytes and antibody to Ig as the mitogen. The results of these experiments are summarized in Table 2. Bivalent rabbit antibodies to pig Ig increased the phosphatidylinositol turnover of pig lymphocytes about 4-fold, whereas an equivalent amount of the monovalent Fab fragments of these antibodies failed to induce significant enhancement. However, cross-linkage of the bound Fab fragments by a goat anti-(rabbit Ig) serum restored the increased phosphatidylinositol turnover to the same value as that obtained with the bivalent antibodies, whereas the goat antiserum alone caused no enhancement. These results indicate that stimulation of phosphatidylinositol turnover by antibodies to Ig is accomplished through cross-linkage. Table 2 also shows that the above reagents had the same relative effects on the incorporation of thymidine into DNA after culture for 45h. This parallelism again emphasizes the close relationship between the two responses. Incidentally, since the lymphocyte population contained about 15% of B-cells only (see the Materials and Methods section) it seems likely that a pure population of B-lymphocytes would have given about a 25-fold enhancement in phosphatidylinositol turnover. It is at present not possible to judge whether the response in phosphatidylinositol turnover accompanying activation is greater in B cells than in T cells owing to lack of knowledge of the proportion of lymphocytes activated by *P. vulgaris* phytohaemagglutinin.

Although previous results (Masuzawa *et al.*, 1973) obtained by using pokeweed mitogen suggested that transformation of B-lymphocytes may not be accompanied by enhanced phosphatidylinositol turnover, the above results indicated that this enhancement is a characteristic feature of lymphocyte activation in general.

Conclusions

These results support the hypothesis that the stimulation of enhanced phosphatidylinositol turnover by lymphocyte surface-binding ligands represents an essential early step in the mechanism of activation of T- and B-lymphocytes and that at least in B-lymphocytes it is initiated by the cross-linkage of specific membrane components.

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