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Anchorage and lymphocyte function. Contact-induced augmentation of T-cell activation

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Summary. Beads of polyacrylamide, latex or DEAE-Sephadex markedly augmented the stimulation of unfractionated or T-enriched lymphocytes by concanavalin A (Con A) or phytohaemagglutinin (PHA). The beads were not mitogenic in the absence of Con A or PHA. A prerequisite for the bead-induced augmentation was that the stimulated lymphocytes had been depleted of phagocytic and/or adherent accessory cells. The enhancing effect of beads was most pronounced during the initial 12 h after the beginning of lymphocyte stimulation, but not limited to this early phase of the growth period. The stimulation of lymphocytes in petri dishes of adhesive tissue culture plastic and non-adhesive bacterial plastic were compared. The magnitude of the stimulation on the non-adhesive surface was 10–50% lower than on the adhesive one, this difference being most pronounced at hyperoptimal mitogen concentrations.

These results indicate that contact between some cell type and a solid surface can improve lymphocyte stimulation under experimental conditions when the number of phagocytic and adherent accessory cells is a limiting factor. The fact that cultivation on bacterial plastic, where adhesion and spreading were abolished, produced substantial stimulation (albeit reduced) demonstrates that substrate contact may be important, but is not a prerequisite, for lymphocyte activation.

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INTRODUCTION

Mitogenic lectins trigger T lymphocytes to DNA synthesis. Non-θ-bearing accessory cells seem to be required for this activation (Levis & Robbins, 1970; Schmidtke & Hatfield, 1976; Habu & Raff, 1977) which leads to the formation of growth factors and modifies the sensitivity of the T lymphocytes to such factors (Gillis & Smith, 1977; Larsson & Coutinho, 1979). Growth factors cannot activate the cells but make triggered cells go through the mitotic cycle. The mitogen-induced effects on the quiescent T cell leading to activation and the important role of accessory cells are poorly understood. In the mouse as few as 1% adherent cells can completely restore the responsiveness to T-cell mitogens (Habu & Raff, 1977). In human lymphocytes, approximately 10% adherent cells produce a restorative effect (Schmidtke & Hatfield, 1976). All concentrations of normal macrophages enhance lymphocyte transformation (Wing & Remington, 1977). Low concentrations of activated macrophages also enhance transformation whereas high concentrations are inhibitory (Wing & Remington, 1977).

In contrast to haematopoietic cells and lymphocytes, normal fibroblasts, endothelial and epithelial cells are anchorage-dependent and grow almost exclusively when spread on a surface (Stoker, O'Neill, Berryman

& Waxman, 1968). We have recently studied the interaction between human mononuclear cells and adhesive surfaces (Wanger & Sundqvist, 1980). One factor observed to influence the adhesiveness of these cells was concanavalin A (Con A). Con A facilitates adhesion of normally non-adherent mononuclear cells to the substrate and at high concentrations (100 μg/ml) provokes spreading in the majority of these cells (Wanger & Sundqvist, 1980). The present study was performed with human mononuclear cells in order to elucidate the possible role of substrate contact and spreading in T-lymphocyte activation. The results obtained demonstrate that cell–substrate interaction exerts a pronounced influence on this activation. Thus, non-mitogenic beads of polyacrylamide, latex or DEAE-Sephadex markedly augment the magnitude of T-cell triggering by Con A or phytohaemagglutinin (PHA). Although these data clearly show that lymphocyte stimulation is influenced by contact between some cell type and solid surfaces this influence is probably not exerted directly on the activated lymphocytes but mediated via accessory cells.

MATERIALS AND METHODS

Cell purification

Mononuclear cells were isolated from defibrinated venous blood from healthy adults by sedimentation in gelatin. These cells were then incubated with iron powder (400 mg/100 ml blood) at 37° for 30 min and phagocytic cells and remaining iron powder were removed by a magnet. In order to remove adherent cells, the cells were thereafter incubated in tissue culture bottles (Falcon plastics 3001) during 60 min at 37°. Erythrocytes were lysed by addition of ammonium chloride (0·83%). Separation of the mononuclear cells into T-lymphocyte enriched and depleted fractions, respectively, was performed using sheep-erythrocyte rosette sedimentation as described earlier (Natvig, Perlman & Wigzell, 1976). The viability of the purified lymphocytes was generally greater than 98%.

Cell culture and substrates

Stimulation of lymphocytes to DNA synthesis was investigated using triplicate cultures of 10^6 cells in 1 ml RPMI 1640 (Gibco) with 10% foetal calf serum in round-bottomed 10 × 75 mm glass tubes or in tissue culture petri dishes (Falcon 3001 or 1008). Con A at a concentration of 100, 25 or 10 μg/ml or PHA 1 or 0·5 μg/ml were added to the cultures. Con A and PHA were obtained from Pharmacia Chemicals, Uppsala, Sweden.

The mononuclear cells used were stimulated by mitogen in the presence and absence of different kinds of beads. Latex beads were purchased from Serva Feinbiochimica Heidelberg, Germany. Their mean diameter was 6 μm. 4×10^5 beads were added to each lymphocyte culture. Polyacrylamide beads (Biogel) were purchased from Biorad lab. The mean diameter of these beads swollen in PBS was approximately 60 μm. 10^5 beads were added to each lymphocyte culture. DEAE-Sephadex was purchased from Pharmacia, Uppsala, Sweden (Cytodex). The wet mean size of these beads swollen in PBS was 195 μm. 10^4 beads were added to each lymphocyte culture.

α-Methyl-D-mannoside (αMM) was added to cultures containing Con A (100 μg/ml) 24 h after the beginning of the culture period to a final concentration of 0·1 M. In the standard experiment the lymphocytes were cultured during three days. The culture period was terminated with an 18 h pulse of 0·2 μCi [^{14}C]-thymidine. DNA synthesis was measured as incorporation into the trichloroacetic acid insoluble fraction of the cells.

In order to study adherence and spreading, 10^6 cells respectively, of unfractionated or T-cell enriched lymphocytes were allowed to settle in petri dishes either of tissue culture plastic (Falcon 3001) or bacterial plastic (Falcon 1008) in the presence or absence of Con A (100, 25 or 10 μg/ml) or PHA (100 or 1 μg/ml) in 1 ml RPMI 1640 with 5 or 10% FCS.

RESULTS

Bead-induced enhancement of T-cell stimulation

Human lymphocytes, purified as described in the legend to Fig. 1, were stimulated by PHA or Con A in the presence or absence of beads either for varying time periods or during 3 days, the time at which stimulation was maximal (Table 1 and Fig. 1). It can be seen in Table 1 that beads of polyacrylamide (Biogel), latex or DEAE Sephadex (Cytodex) augmented the stimulation markedly above that of cells cultured without beads. Beads of polyacrylamide induced the most pronounced augmentation. The results in Fig. 1 show that the augmentation was present throughout the culture period and in some experiments persisted on day 6 when the DNA synthesis of cells without beads had decreased to the same level as that of medium controls.

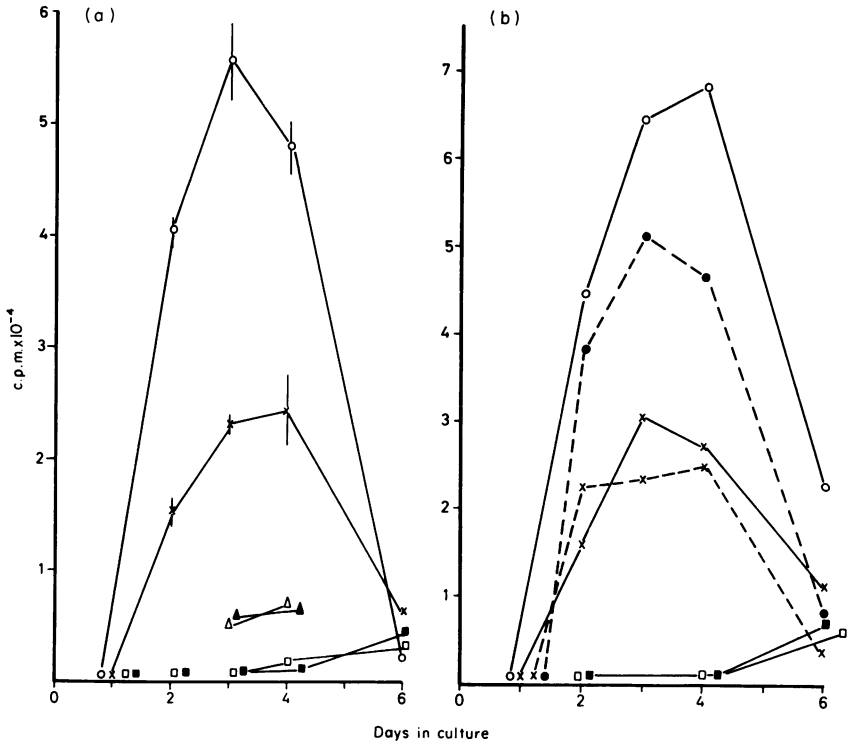

Figure 1. The influence of polyacrylamide beads on lymphocyte stimulation in relation to the length of the culture period. Human mononuclear cells were purified by sedimentation of defibrinated venous blood in gelatin and treatment with carbonyl iron. T-enriched lymphocytes were obtained using sheep-erythrocyte rosette sedimentation. (a) ×, T cells cultured with Con A 10 μg/ml alone. o, T cells cultured with Con A, 10 μg/ml, and Biogel. □, Cells kept in medium only. ■, Cells kept in medium in the presence of Biogel. △, Non-T-cells cultured with Con A 10 μg/ml. ▲, Non-T-cells cultured with Con A and Biogel. (b) Unfractionated lymphocytes cultured with Con A 10 μg/ml in the absence (×——×), and presence (o——o) of Biogel. Cells stimulated with Con A 25 μg/ml in the absence (× — — — ×) and presence (o— — —o) of Biogel. Cells cultured in medium only in the absence (□) and presence (■) of Biogel. Each value is the mean of triplicate cultures. The vertical bars in Fig. 1a represent standard deviation.

Unfractionated or T-enriched cells cultured with beads in the absence of mitogen exhibited no increase of thymidine incorporation compared with cells cultured in medium alone. This shows that the influence of beads on the stimulation required the simultaneous presence in the cultures of both beads and mitogen. The augmentation of thymidine incorporation by beads was observed virtually to the same extent in unfractionated and in T-enriched lymphocytes (Table 1 and Fig. 1). In contrast, the presence of beads did not augment stimulation of T-depleted cells by mitogen. However, a suggestive bead-induced augmentation of thymidine incorporation in non T-cells cultured without mitogen was seen (Table 1).

In order to investigate whether the influence of beads on lymphocyte stimulation was limited to a certain phase of the growth cycle polyacrylamide beads were added to cell cultures at different times after the beginning of stimulation by Con A. The results shown in Fig. 2 suggest that most of the potentiating effect of the beads was achieved during the first 12 h of the stimulation. However, the presence of beads in the cultures seemed to exert an apparent enhancing effect when added as late as 24 h after the beginning of the stimulation.

Removal of phagocytic and/or adherent cells is a prerequisite for the bead-induced augmentation

Table 2 contains information on the influence of beads

Table 1. Lymphocyte stimulation in the presence and absence of beads

Exp. no Type of beads used	Cell fraction studied	Response of human blood lymphocytes (c.p.m. × 10^{-6} cells)†					
		Cultures without beads			Cultures with beads		
		Medium control	Con A 10 μg/ml	Con A 25 μg/ml	Medium control	Con A 10 μg/ml	Con A 25 μg/ml
1 Polyacryl-amide	T-cell enriched*	533±51	21,966±1529	17,389±636	636±136	79,718±8934	43,559±3600
2 Polyacryl-amide	T-cell enriched*	600±165	20,259±2061	28,653±2380	840±100	83,353±2804	47,313±2294
	T-cell depleted*	2057±136	–	8614±328	4107±408	—	8132±592
3 Polyacryl-amide	Unfractionated	660±84	21,591±1100	24,465±979	856±126	63,663±2168	44,517±1886
	T-cell enriched*	412±89	29,167±2581	13,340±1559	811±84	60,185±4384	30,510±1395
	T-cell depleted*	1030±77	2958±193	—	1915±107	3438±604	—
4 Latex	Unfractionated	480±31	23,232±3085	20,615±1999	671±241	58,239±5451	32,157±6756
5 Latex	T-cell enriched	639±64	5251±2359	8697±1910	838±151	46,538±4829	22,714±4097
6 DEAE Sephadex	Unfractionated	554±30	14,379±1067	18,048±1977	519±43	30,848±540	28,703±1716

*The number of sheep-erythrocyte rosette forming cells in the T-enriched fractions was generally >93%, and in the T-depleted fractions it was generally <5%.

†Mean of triplicate tubes ± standard deviation.

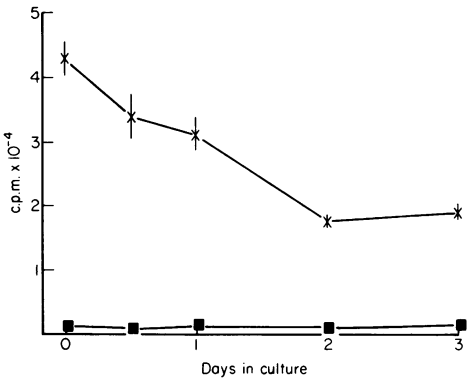

Figure 2. The influence of beads on lymphocyte stimulation in relation to the growth cycle. Human mononuclear cells purified as described in the legend to Fig. 1 were stimulated by Con A, 10 μg/ml (×). Control cells (■) were cultured in the absence of Con A. At different times (0, 12, 24 and 48 h), polyacrylamide beads were added to the cultures. Each point on the curves represents the thymidine incorporation obtained when beads were added at the corresponding time.

on lymphocyte stimulation before and after removal of phagocytic and adherent accessory cells. Before treatment with iron powder the beads usually did not affect stimulation by Con A or PHA. It is worth noting that the presence of beads in medium controls of the lymphocytes before depletion of phagocytic and adherent cells decreased the level of background stimulation. After the procedures used to remove phagocytic and adherent cells, the magnitude of the stimulation by Con A and PHA in the absence of beads decreased significantly. After this treatment of the cells the presence of beads in the lymphocyte cultures augmented the stimulation by both mitogens. Furthermore, the beads increased the magnitude of the stimulation above the level before removal of phagocytic and adherent accessory cells.

Stimulation of mononuclear cells in petri dishes of bacterial or tissue culture plastic

Bacterial plastic is relatively non-adhesive for cells in

Table 2. Stimulation of lymphocytes before and after removal of phagocytic and adherent cells

Exp. no.	Purification method	Response of human blood lymphocytes (c.p.m. × 10^{-6}) cells*					
		Cultures without beads			Cultures with beads		
		Medium control	Con A 10 μg/ml	Con A 25 μg/ml	Medium control	Con A 10 μg/ml	Con A 25 μg/ml
1	Gelatin sedimentation	1562±665	35,934±926	48,579±12,085	813±41	30,513±1457	44,621±5176
	Gelatin sedimentation and carbonyl iron treatment	661±33	19,234±666	17,606±6029	690±65	50,450±3543	27,701±3775
	Gelatin sedimentation and carbonyl iron treatment followed by adherence step	429±200	13,477±2099	10,147±1159	729±203	52,329±2334	18,725±6798
2	Gelatin sedimentation	1431±116	34,863±2352	26,851±1861	461±15	38,409±2324	29,013±4002
	Gelatin sedimentation and carbonyl iron treatment	581±111	24,801±4246	17,327±2282	730±223	47,950±3621	24,349±2444
	Gelatin sedimentation and carbonyl iron treatment followed by adherence step	320±8	13,602±1762	8751±1651	434±37	39,559±3930	12,197±1711
3	Gelatin sedimentation	1054±103	57,620±2253	40,371±4126	930±73	62,309±2244	42,563±3263
	Gelatin sedimentation and carbonyl iron treatment	710±70	20,301±2164	17,723±2024	852±27	86,179±3310	39,754±2984
	Gelatin sedimentation and carbonyl iron treatment followed by adherence step	605±101	13,915±3310	ND	611±64	72,448±4257	ND
		Medium	PHA 0·5 μg/ml	PHA1 μg/ml	Medium	PHA 0·5 μg/ml	PHA1 μg/ml
4	Gelatin sedimentation	1511±209	36,398±2617	36,714±2421	570±75	41,632±2240	45,035±2629
	Gelatin sedimentation and carbonyl iron treatment	1489±516	25,291±922	26,385±1330	914±90	55,453±3941	55,033±1711
	Gelatin sedimentation and carbonyl iron treatment followed by adherence step	687±281	17,572±4384	17,090±2363	792±81	45,994±4379	44,796±3939

*Mean of triplicate tubes ± standard deviation. ND, not done.

Human mononuclear cells purified and stimulated by mitogen in the presence and absence of polyacrylamide beads as described in the legend to Fig. 1. The cells from different individuals were stimulated as indicated in the table after (1) gelatin sedimentation, (2) gelatin sedimentation and treatment with carbonyl iron, and (3) gelatin sedimentation, carbonyl iron treatment and an adherence step. The latter was performed by incubation of the cells during 60 min in RPMI 1640 and 10% FCS at 37°C in a Falcon plastic bottle.

general whereas most cell types adhere to and spread well on tissue culture plastic (Willingham, Yamada, Yamada, Pouysségur & Pastan, 1977). Con A and PHA, at high concentrations, mediated adhesion of lymphocytes to tissue culture plastic leading to spreading of the majority of these cells (Fig. 3a) (Wanger & Sundqvist, 1980). The same concentrations of these mitogens did not provoke adhesion and spreading of lymphocytes allowed to settle on bacterial plastic (Fig. 3b). The tendency to spread on tissue culture plastic was less pronounced but yet significant at lower mitogen concentrations (Con A 10 and 25 μg/ml). These results indicated that comparative cultivation of lymphocytes in petri dishes of tissue culture plastic and

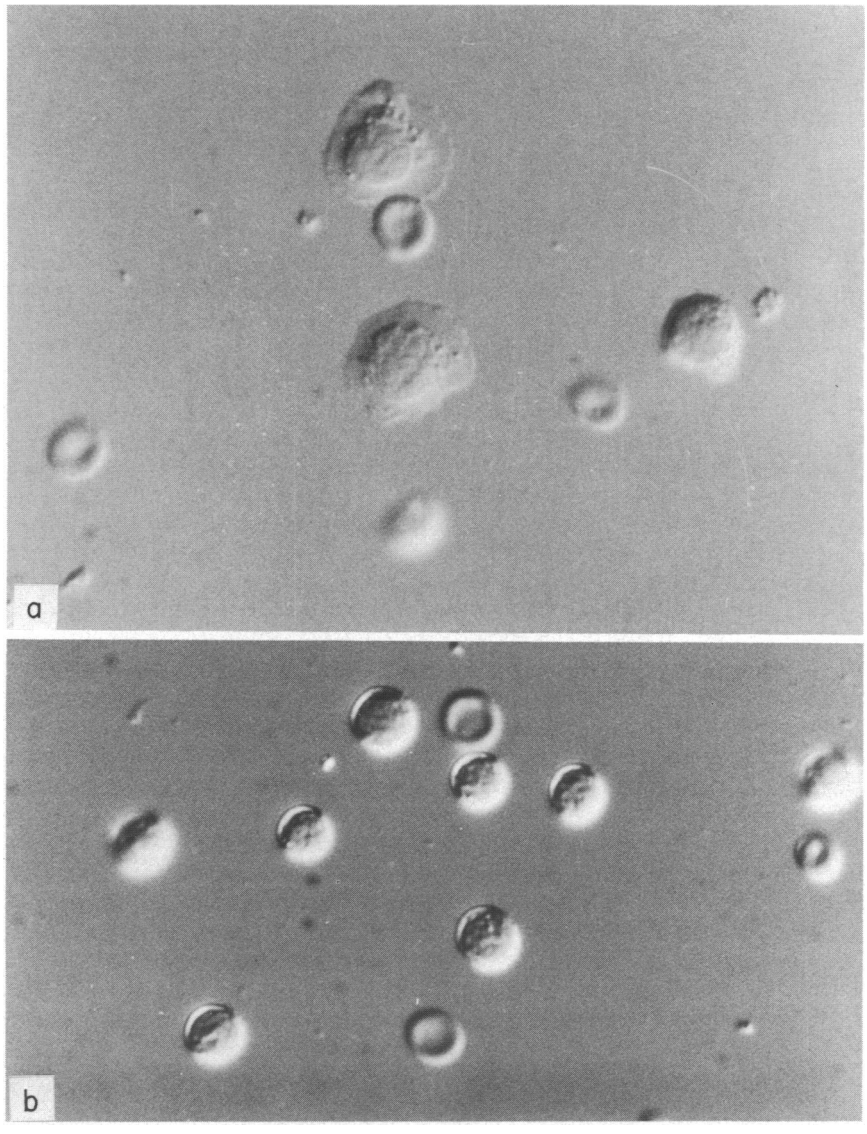

Figure 3. (a) The appearance of human T-enriched lymphocytes settled on tissue culture plastic in the presence of Con A 100 μg/ml during 60 min as revealed by Nomarski optics. The adherence was performed in RPMI with 10% FCS. Essentially the same morphological alterations were observed with PHA (not shown). (b) Experimental conditions identical to those in (a) but the cells were allowed to settle on bacterial plastic.

bacterial plastic would provide information on the influence of adhesion and spreading on lymphocyte stimulation.

It can be seen in Table 3 that stimulation of lymphocytes with Con A on bacterial plastic decreased thymidine incorporation compared to that of the same cells cultured on tissue culture plastic. The decrease varied from 10 to 50% being most pronounced at Con A 100 μg/ml. This mitogen dose, which was the most potent inducer of spreading on tissue culture plastic, caused attachment to bacterial plastic of less than 1% of all cells and no spreading was observed. Therefore, the fact that a substantial stimulation was observed on bacterial plastic (in spite of the reduction in thymidine

Table 3. Stimulation of lymphocytes on adhesive and non-adhesive plastic

Exp. no.	Response of human blood lymphocytes c.p.m./culture†					
	Cultures on bacterial plastic			Cultures on tissue culture plastic		
	Medium	Con A 100 μg/ml	Con A 10 μg/ml	Medium	Con A 100 μg/ml	Con A 10 μg/ml
1*	192±56	39,753±5858	31,422±1420	138±35	81,377±8294	36,480±2103
2*	430±89	125,035±5167	126,181±4193	407±73	192,177±1579	157,502±450
3*	598±46	-	57,140±2542	641±151	—	64,138±6169

*Experiments 1 and 3 were performed with 10^6 and Exp. 2 with 2×10^6 cells per culture.
†Mean of triplicate tubes ± standard deviation.

incorporation at a hyperoptimal Con A concentration) shows that attachment to substratum was not necessary for lymphocyte activation.

DISCUSSION

The growth of non-malignant fibroblasts and epithelial cells is anchorage-dependent (Stoker *et al.*, 1968). There is a strong relationship between multiplication of these cells and the flattened morphology or spreading caused by substrate contact (Folkman & Moscona, 1978). In contrast, lymphocytes are relatively non-adherent and consequently cannot spread on a substrate. However, we have observed that human blood lymphocytes in contact with a substrate are capable of adhesion and spreading in the presence of Con A or PHA (Wanger & Sundqvist, 1980). These ligands probably cross-link the cells to the substrate, enabling spreading to take place. The present study was initiated because of the possibility that spreading of lymphocytes on a surface might influence activation of the cells to DNA synthesis. The results obtained show that beads of polyacrylamide, latex or DEAE-Sephadex markedly augment T-cell activation by Con A or PHA. The beads were not mitogenic in the absence of Con A and PHA. Since beads of different materials all augmented the stimulation and lacked mitogenic effect, the augmentation is probably the result of surface contact *per se* rather than to hypermitogenic properties of the complex between lectin and bead. These results may seem to differ from those of others studying T- and B-cell activation using beads (Greaves & Bauminger, 1972). However, this difference is probably due to obvious experimental variations. Greaves and Bauminger used mouse lymphocytes and mitogen covalently coupled to Sepharose beads whereas we have used human cells and soluble mitogen.

Further information concerning the possible influence of substrate contact on lymphocyte activation was obtained by comparing stimulation at an adhesive and a non-adhesive surface. The result of this experiment, namely that the magnitude of the stimulation was 10–50% lower on the non-adhesive material, is crucial also for the interpretation of the bead-induced enhancement. Thus, the fact that experimental conditions which almost completely abolished adhesion and spreading merely caused a partial inhibition of stimulation, and preferentially at a hyperoptimal mitogen concentration, means that substrate contact is important but not a prerequisite for activation.

A prerequisite for the demonstration of a potentiating influence of beads on lymphocyte stimulation seems to be that the stimulated lymphocytes have been depleted of phagocytic and adherent cells. The procedures applied for removal of phagocytic and adherent cells decreased lymphocyte stimulation in the absence of beads. It follows that in these experiments the number of accessory cells was probably a limiting factor. Therefore, one likely explanation for the bead-induced augmentation is that accessory cells (other T cells or macrophages) involved in lymphocyte stimulation are more effective when attached to a surface.

It is logical to assume that optimal function of adherent accessory cells of the monocyte-macrophage series is dependent on anchorage of these cells to a substrate. The present results suggest that this anchorage-dependence is a critical factor for lymphocyte stimulation when there are relatively few phagocytic/adherent accessory cells in the cultures. In the light of this, it seems reasonable to assume that bacterial plastic exerted a direct negative effect on the function of adherent accessory cells and therefore indirectly

affected lymphocyte stimulation. Another explanation for the bead-induced augmentation of lymphocyte stimulation may be that phagocytic accessory cells internalize beads and that this endocytosis stimulates the accessory cells. However, since the size of both the polyacrylamide beads and particularly the beads of Sephadex is larger than that of the accessory cells this possibility appears unlikely.

An alternative interpretation of the potentiating influence of beads on lymphocyte activation may be that the presence of beads affects cell–cell interaction. Although it is not elucidated it is possible that contact inhibition can inhibit lymphocyte growth, particularly in large clusters of cells (see however Peters, 1972). Beads may interfere with cell agglutination and contact inhibition. However, the fact that the bead-enhanced mitogen responses were restricted to lymphocytes depleted of phagocytic and adherent cells argues against this interpretation. There is no known reason to expect that contact inhibition should be more pronounced after removal of phagocytic and adherent cells.

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