POTENTIATION OF MITOGEN-INDUCED LYMPHOCYTE STIMULATION BY POLYETHYLENE GLYCOLS

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The effect of polyethylene glycols of different molecular weights on mitogen-induced lymphocyte stimulation was investigated. The stimulation obtained by neuraminidase/galactose oxidase treatment, and by addition of several plant lectin mitogens, was markedly enhanced in the presence of polyethylene glycols of molecular weights ranging from 6 000 to 40 000. Optimal potentiation of stimulation was found for polyethylene glycol concentrations up to about 5 %.

The hydrophilic polymer polyethylene glycol (PEG) interacts strongly with cells of different origin: it has been adepted as a cell fusing agent for higher plant protoplasts(1) and for yeast, avian, and mammalian cells (2-5). The mechanism of cell fusion occuring after cell treatment with high concentrations of PEG (30-50%) is still unexplained. It has been postulated that PEG brings about an aggregation of cell surface proteins accompanied by changes in the cell surface charge: areas of protein-free lipid layers may thus become exposed and fuse with neighboring lipid membranes (1,5). PEG in concentrations up to 5 % is not commonly used to induce cell fusion. It should, however, also in these low concentrations interact with cell surfaces: this communication shows that PEG in concentrations from 0.8 to 5 % effects a marked enhancement of the <u>in vitro</u> lymphocyte stimulation brought about by a variety of mitogens.

Abbreviations: ConA (concanavalin A), LcA (lentil lectin), LIMA (lima bean lectin), LP (lipoprotein),LPS (lipopolysaccharide), MW (molecular weight), PEG (polyethylene glycol[s]), PHA (phytohemagglutinin), PWM (pokeweed mitogen),SBL (soy bean lectin)

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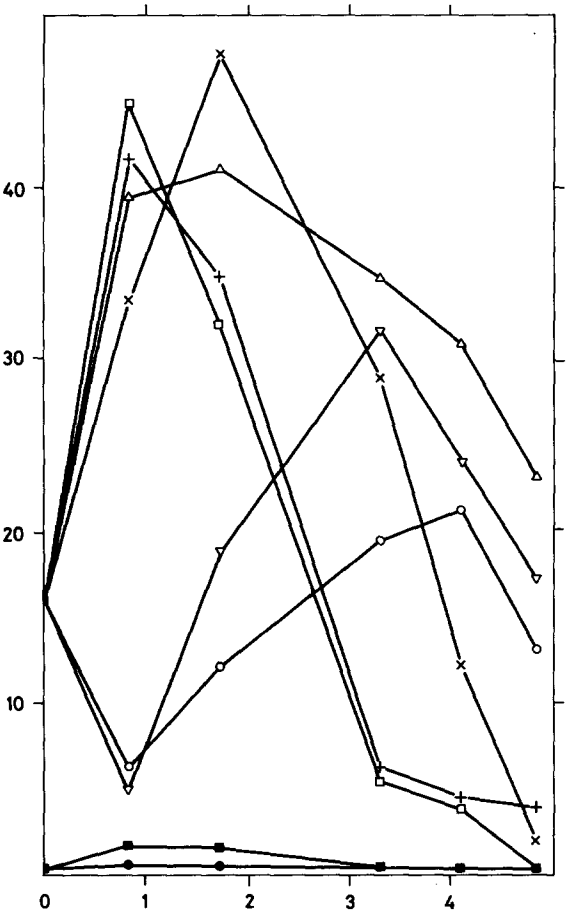

Fig. 1. Effect of PEG at different concentrations and MW on lymphocyte stimulation by neuraminidase/galactose oxidase. Abscissa: PEG concentration in % [w/v]. Ordinate: cpm of incorporated 3H-thymidine into lymphocytes [0.2 µCi/6 x 10^5 lymphocytes/0.2 ml]. Cultures were performed for 42 h in the presence of 10 % fetal calf serum. Each point represents the avarage of duplicate cultures. (●) PEG 4 000; (■) PEG 40 000; NAGO plus PEG 4 000 (○), plus PEG 6 000 (▽), plus PEG 10 000 (△), plus PEG 15 000 (X), plus PEG 20 000 (+), plus PEG 40 000 (□). Balb/c mouse spleen lymphocytes.

EXPERIMENTAL

Lymphocytes. C3H/HeJ mice were obtained from Jackson Laboratories, Bar Harbor, Maine, Balb/c mice from Dr. G. Hämmerling, Köln. Cell suspensions were prepared from mouse spleens immediately after killing, as previously described (6). Mitogens. Concanavalin A [ConA], lima bean lectin [LIMA], and lentil lectin [LcA] were prepared from the seeds as described (7,8,9). Pokeweed mitogen [PWM] was purchased from Gibco, New York, phytohemagglutinin P [PHA] from Difco, Detroit, and soy

Table 1

CELL DENSITY [CELLS/ml]	LECTIN	CONCENTRATION OF POLYETHYLENE GLYCOL (% w/v) 0	1.125	2.25	4.5
2 x 10^6	ConA	3700±600	6900±1800	16100±2200	60±25
	C*	60±30	50±20	50±40	20±20
4 x 10^6	ConA	21300±300	28400±8100	55100±8600	51900±800
	C*	936±450	780±300	770±200	400±250
8 x 10^6	ConA	65100±2600	82900±2900	63900±4800	45300±3200
	C*	2500±1300	3500±650	4100±3100	4100±1050

Effect of different cell densities and different PEG concentrations on the stimulation of C3H/HeJ mouse splenocytes by ConA [5 µg/ml].3H-thymidine incorporation into DNA [cpm per 3 x 10^5 cultured cells (cell density 2 x 10^6 per ml), 6 x 10^5 cultured cells (cell density 4 x 10^6/ml), and 12 x 10^5 cultured cells (cell density 8 x 10^6 cells/ml)]. *= controls without mitogen. For specific culture conditions compare legend to fig. 2.

bean agglutinin [SBL] from Miles, Elkhart, Indiana. Neuraminidase (vibrio cholerae, 500 U/ml) was purchased from Behringwerke, Marburg, galactose oxidase (62 U/mg) from Worthington Biochemical Corp., New Jersey. Lipopolysaccharide from Salmonella abortus equi [LPS] was a gift of Dr. C. Galanos, Freiburg, lipoprotein [LP] was prepared from E. coli B/r according to Inouye et al. (10). Polyethylene glycols of molecular weights 1 500, 4 000 and 10 000 were purchased from Roth, Karlsruhe, of molecular weights 6 000, 15 000, 20 000 and 40 000 from Serva, Heidelberg.

Stimulation experiments. Stimulation experiments with lectins, LPS and LP were performed in flat bottom Falcon 3040 microtiter plates as described (6). Mitogenicity was measured by the uptake of 3H-thymidine into DNA and by microscopic observation of blast formation (6). Lymphocyte stimulation by neuraminidase/galactose oxidase was performed in flat bottom Falcon 3040 microtiter plates using a modification of the original method (11): Neuraminidase and galactose oxidase were added simultaneously to the lymphocyte culture at 50 U/ml and 0.5 U/ml, respectively. 75 % of the enzyme containing medium was removed after 24 h of culture and replaced by fresh medium (RPMI 1640 plus 10 % fetal calf serum) containing 1 µCi/ml 3H-thymidine (Amersham, 25 Ci/mMol). After 42 h of culture the cells were processed by a cell culture harvester (Skatron, Lierby, Norway) on glass fiber filters, and counted by liquid scintillation. All experiments were done at least in duplicates.

RESULTS

Figure 1 shows the effect of polyethylene glycols in different concentrations on neuraminidase plus galactose oxidase stimulation of Balb/c mouse spleen lymphocytes. Dependent on

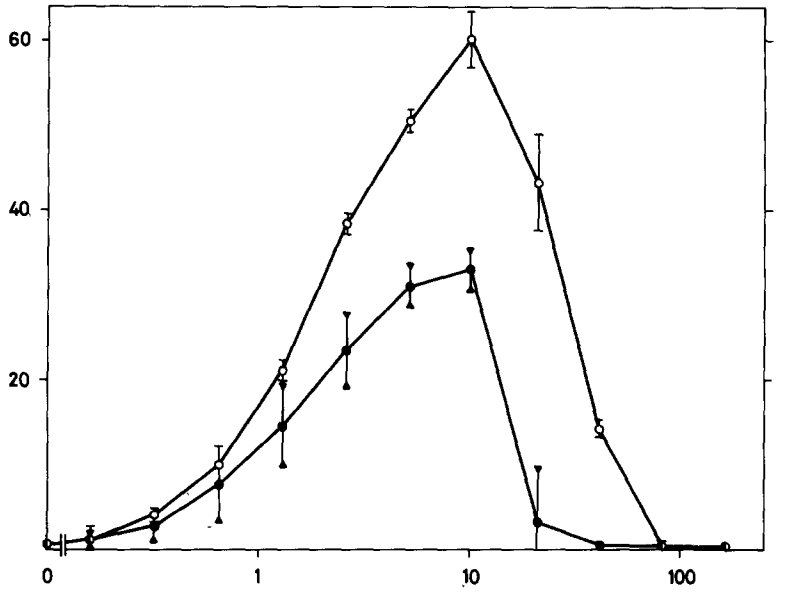

Fig. 2. Dose response curves for 3H-thymidine incorporation after lymphocyte stimulation with ConA in the presence of 4.5 % [w/v] PEG 6 000 (O) and in the absence of PEG (●). Abscissa: lectin concentration in µg/ml. Ordinate: cpm per 6 x 10^5 cultured cells. Cultures were performed for 72 h in the presence of 6.6 % human pool serum (Flow). C3H/HeJ mouse spleen lymphocytes. Each point represents the average of duplicate cultures.

the molecular weight of PEG used, an enhancement of stimulation was obtained at concentrations between 0.8 and 5 % PEG. Best results were achieved with PEG of molecular weights (MW) from 6 000 to 40 000, leading to enhancement factors of 2 to 3. For PEG 4 000 and PEG of lower MW (data not demonstrated) only marginal enhancing effects were observed. When added to unstimulated control cells (fig. 1, closed symbols), PEG of MW up to 6 000 showed only a negligible enhancement of the background activity of thymidine incorporation, whereas PEG 10 000 - 40 000 showed a slight enhancement.

Tab. 1 summarizes the effects of different concentrations of PEG 6 000 on ConA stimulation. Additionally, different cell den-

Table 2

LECTIN	CONC. IN µg/ml	WITH PEG 6 000	WITHOUT	WITH PEG 20 000	WITHOUT	WITH PEG 40 000	WITHOUT
ConA	0	30±15	110±60	80±20	110±60	60±40	110±60
	5	7900±1350	6700±750	2450±410	6700±750	3000±950	6700±750
	20	4650±400	850±200	4800±100	850±200	3650±400	850±200
	40	850±50	500±40	2850±100	500±40	800±250	500±40
LcA	0	30±15	110±60	720±100	240±40	.	.
	0.5	8600±750	3900±200	4100±200	3040±600	.	.
	5	1700±200	650±150	4500±800	1160±80	.	.
PHA	0	.	.	720±100	240±40	.	.
	10	.	.	2950±250	740±40	.	.
LIMA	0	190±40	170±40	720±100	240±40	.	.
	1	490±100	220±60	640±90	290±80	.	.
	10	840±70	470±30	660±80	220±20	.	.
	100	440±70	260±20	1240±280	160±60	.	.
PWM	0	190±40	170±40	720±100	240±40	.	.
	1	225±40	200±40	740±40	580±40	.	.
	10	480±80	250±100	980±280	660±80	.	.
	100	180±40	200±50	900±120	240±40	.	.
SBL	0	190±40	170±40	720±100	240±40	.	.
	1	180±50	175±40	780±40	660±80	.	.
	10	270±20	170±40	1020±120	420±20	.	.
LP	0	190±40	170±40	720±100	240±40	.	.
	1	810±100	790±60	1260±60	420±50	.	.
	10	1280±60	1080±110	1530±130	520±120	.	.
LPS	0	190±40	170±40	720±100	240±40	.	.
	1	880±30	310±30	900±60	310±30	.	.
	10	690±120	350±50	1220±100	460±40	.	.

Summary of several experiments performed with C3H/HeJ mouse spleen lymphocytes: stimulation of 3H-thymidine incorporation into DNA (cpm / 6 x 10^5 cultured cells) by a variety of mitogens. . = not done. For specific culture conditions compare legend to fig. 2.

sities were tested. As seen from the table, optimal enhancement was obtained at a cell density of 4 x 10^6/ml with PEG concentrations ranging from 2.25 - 4.5 %, and at 2 x 10^6/ml at PEG concentrations of 2.25 %. The dose response curve of a stimulation experiment with ConA in the presence of 4.5 % PEG 6 000 is depicted in figure 2; for comparison the results obtained without PEG are shown. As seen from the curves, enhancement of stimulation was found for all lectin concentrations tested. The effect was most obvious at ConA concentrations exceeding 10 µg/ml: by the addition of PEG the falling limb of the dose res-

ponse curve is shifted towards higher lectin concentrations. The results obtained with a further variety of lectins, LP, and LPS are summarized in tab. 2: LcA, a lectin having a sugar specificity similar to ConA, showed a comparably enhanced response. The response towards PHA is also enhanced; similarly we observed an enhancement of the stimulation induced by the weakly mitogenic lectins LIMA, PWM, and SBL. Interestingly, the stimulation obtained for the B-lymphocyte mitogen LP was enhanced only weakly. The B-mitogen LPS that has no stimulating effect towards C3H/HeJ mouse lymphocytes (12) shows a weak but significant effect in the presence of PEG.-Some ConA stimulation experiments were performed in the presence of PEG 40 000, which is a weak mitogen by itself (comp. tab. 2). The stimulation obtained by Con A was enhanced for high ConA concentrations. In all experiments, thymidine incorporation was accompanied by blast formation. In some cases, thymidine incorporation into the nucleus of stimulated blast cells was made visible by radioautography (data not shown).

DISCUSSION

The hydrophilic, polymeric PEG was able to enhance the lymphocyte stimulation obtained by a variety of mitogens. By PEG of MW 6 000 - 40 000 the stimulating activity of neuraminidase/galactose oxidase and of the strongly mitogenic plant lectins ConA and LcA is further increased. In addition, the answer of murine lymphocytes towards the weaker mitogenic substances LIMA, PWM, and SBL is enhanced. Thus, PEG could be especially useful as a tool to amplify the response towards weak mitogens. Only a weak effect of PEG can be observed for the typical B-lymphocyte mitogen LP. The weak but significant stimulation obtained by LPS in C3H/HeJ mice in the presence of PEG could suggest that LPS

under certain conditions is also able to activate these cells. However, we cannot exclude at the moment trace contaminations of LPS with LP; further experiments are in progress.

The enhancement of stimulation is dependent on cell density, on PEG concentration and MW. PEG of MW 400 - 4 500 had only a marginal effect, whereas pronounced enhancement was seen with PEG of MW 6 000 - 40 000. PEG 6 000 had only a weak influence on the controls without mitogen; PEG 20 000 and 40 000 were slightly mitogenic by themselves. In the presence of 2.25 % PEG 6 000, an enhancement of the stimulating activity of LIMA and ConA was shown in human lymphocytes (W. Bessler, unpublished). Similarly, a potentiation of weak mixed lymphocyte culture reactions by 4 - 5 % PEG 6 000 has been shown by Ben-Sasson and Henkart (13). The mechanism of the synergistic effect of PEG is unknown. Preliminary studies showed that membrane ConA receptors, as visualized by fluorescent ConA, formed aggregated spots after PEG treatment. Additionally it could be shown that the membrane potential of PEG treated cells is transiently reduced (J.H.Peters and D.F.Hülser, unpublished). Thus, PEG treatment could lead to changes of the cell surface charge, accompanied by aggregation of cell surface glycoproteins (comp. 1). These aggregated proteins might be better accessible to further crosslinking by lectins, or to the proposed (11) crosslinking process occuring after the oxidation of membrane glycoproteins with galactose oxidase, or with sodium periodate for which PEG enhancement of stimulation was also observed (data not shown).

Crosslinking could additionally occur between the surfaces of cells in contact with each other. In fact, our microscopical observations (not demonstrated) showed a rapid cell aggregation

following PEG treatment, independent of the presence of a mitogen. It has been described previously that cell contact is a prerequisite for mitogen stimulation of T-lymphocytes (14), and that intercellular communication is established between mitogen-treated lymphocytes in contact (15). We therefore suggest that PEG primarily acts as an agent to increase cell contacts between immune cells. This may explain why weak mitogens which are generally also weakly agglutinating agents are specifically enhanced in their mitogenicity when PEG is added to the cultures.

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