EFFECT OF POKEWEED MITOGEN (PWM) ON LYMPHOCYTE LYSOSOMES

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INTRODUCTION

Resting blood lymphocytes in vitro undergo transformation into large, blastlike, dividing cells following incubation with phytohemagglutinin (PHA), a plant lectin from *Phaseolus vulgaris* (1). Farnes et al. (2) found another phytomitogen in saline extracts of the plant *Phytolacca americana* (pokeweed). Although both PHA and the pokeweed mitogen (PWM) induce *nonspecific* transformation of lymphocytes which differs markedly from *specific* transformation by antigens (3), the chemical composition and the biological effects of these agents are by no means entirely identical. PWM has been shown to possess a molecular weight much lower than that of PHA (4, 10), and to induce in vitro the formation not only of blasts like PHA-induced ones but also of a unique intermediate-sized cell type with cytoarchitectural features of plasmablasts (5).

In previous studies (6, 7), we have demonstrated the presence in human peripheral blood lymphocytes of acid hydrolase-rich granules which display many of the biochemical and structural characteristics of lysosomes in other tissues. Between 30 and 120 min after addition of PHA to intact, purified lymphocytes, redistribution of acid hydrolases (beta-glucuronidase, acid phosphatase) from a 20,000 $g \times 20$ min granular fraction into the corresponding supernatant was observed. These changes were associated with increased fragility of hydrolase-rich granules derived from PHA-stimulated lymphocytes upon subsequent incubation with membrane-disruptive agents in vitro. For these, and other reasons, it was suggested that redistribution of acid hydrolases may play a role in remodeling processes of lymphocytes stimulated by PHA (6, 7). As a necessary test of this hypothesis, we have, therefore, determined whether pokeweed mitogen could also produce changes in enzyme distribution among subcellular fractions of human lymphocytes.

MATERIALS AND METHODS

The experiments were performed as previously described (6, 7). In brief, leukocyte-rich human plasma was obtained from heparinized human blood after spontaneous sedimentation of red cells and removal of platelets. The lymphocytes were separated by passage of plasma through a column of washed nylon wool. The purified cells, more than 99% of which were lymphocytes, were washed, spun, and cultured as before (6, 7). After preincubation of the cells for 3 hr at 37°C, test aliquots were added: either 0.8 ml of NaCl (0.9%) solution, or 0.8 ml of a solution of purified PWM (trichloracetic acid precipitate, step III b according to the procedure described by Börjeson et al. (8), 25 μg dissolved in 0.8 ml of NaCl (0.9%) solution). The cultures were then incubated for a further 2 hr at 37°C. After harvesting, cell suspensions were cooled, centrifuged, subjected to hypotonic lysis of red cells, and resuspended in 0.34 M sucrose containing 0.01 M EDTA (adjusted to pH 7.0 with 1 N NaOH) and 50 units of heparin per 1 ml.

Homogenization was carried out as before (6, 7), and the following fractions were obtained: 56 $g \times 1$ min = debris; 500 $g \times 10$ min = nuclear fraction; 20,000 $g \times 20$ min = granular fraction; as well as the final 20,000 $g \times 20$ min supernatant. To release enzyme activity from the particulate phase, whole homogenates and subcellular fractions were treated with Triton X-100 (0.1% v/v).

Beta-glucuronidase, acid phosphatase, malate dehydrogenase, and protein were determined as described previously (6, 7, 9). For determining the release of sedimentable enzyme activity from lymphocyte granules in vitro, PWM was dissolved in NaCl (0.9%) solution and added in 0.04 ml aliquots to 0.76 ml aliquots of postnuclear supernatant containing both the granular fraction and the cell supernatant derived from unstimulated lymphocytes. After incubation for 1 hr at 37°C, the mixture was centrifuged at 20,000 g for 20 min, the supernatant was removed, and the pellets were adjusted to original volume with heparinized EDTA-sucrose solution containing 0.1% Triton X-100. Both supernatants and pellet suspensions were assayed for enzyme ac-

TABLE I

Total Recovered Enzyme Activity and Protein Content in Homogenates Derived from Lymphocyte Cultures Incubated with and without PWM for 2 Hr at 37°C

Enzyme	No. of Exp.		Activity units ± SEM*	SED‡	%Recovery ± SEM
Beta-glucuronidase	6	Control	19.68 ± 1.71	± 0.57	88.4 ± 1.4
		PWM	18.90 ± 1.79		86.4 ± 1.7
Acid phosphatase	6	Control	162.37 ± 17.60	± 4.32	98.2 ± 3.9
		PWM	157.15 ± 17.47		93.6 ± 3.6
Malate dehydrogenase	6	Control	438.69 ± 32.35	± 6.11	100.4 ± 3.9
		PWM	424.13 ± 29.42		92.6 ± 4.2
Protein	6	Control	566.64 ± 33.54	± 17.07	115.3 ± 5.0
		PWM	551.43 ± 32.22		118.6 ± 9.6

* Activity units (± Standard Error of Mean):
Beta-glucuronidase: mμmoles phenolphthalein released/10^7 cells/hr.
Acid phosphatase: mμmoles phosphorus released/10^7 cells/hr.
Malate dehydrogenase: mμmoles $NADH_2$ oxidized/10^7 cells/min.
Protein: μg/10^7 cells.
‡ Standard Error of Difference; Control vs. PWM.

tivity. Release of enzyme activity by PWM was expressed as (enzyme activity present in the 20,000 *g* × 20 min supernatant after incubation with PWM)/(enzyme activity present in the 20,000 *g* × 20 min supernatant after incubation with Triton X-100) (× 100).

RESULTS AND DISCUSSION

After lymphocyte cultures were incubated with PWM for 2 hr, the total activities of beta-glucuronidase, acid phosphatase, and malate dehydrogenase, as well as the total content of protein, remained unchanged compared to controls (Table I). The distribution of enzymes among subcellular fractions is summarized in Table II. In homogenates derived from lymphocyte cultures incubated with PWM, there was a marked increase of recovered beta-glucuronidase activity in the 20,000 *g* × 20 min supernatant ($P < 0.001$) with a corresponding decrease of recovered beta-glucuronidase activity in the granular fraction ($P < 0.001$), whereas nuclear and debris fractions remained unaffected. Subcellular distribution of acid phosphatase activity paralleled that of beta-glucuronidase activity. Differences in acid phosphatase activity between PWM-treated and control homogenates were significant at the 0.005 level for the 20,000 *g* × 20 min supernatant, and at the 0.025 level for the granular fraction. Recovered malate dehydrogenase activities and content of protein were unchanged in all fractions of PWM-treated homogenates, compared to controls. As shown in Table III, PWM did not cause any significant release of sedimentable beta-glucuronidase activity if directly added in vitro to granules present in a postnuclear supernatant originating from unstimulated lymphocytes. The data suggest that, during the early phase of stimulation by PWM, lysosomal hydrolases underwent redistribution from 20,000 *g* × 20 min granular fractions into corresponding supernatants. Since the subcellular distribution of mitochondrial malate dehydrogenase was unaffected, the process appeared limited to acid hydrolases.

The results show that changes in the subcellular distribution of lysosomal enzymes of lymphocytes brought about by PWM are directly comparable to those observed after stimulation with PHA, and, therefore, clearly suggest that similar mechanisms might underlie several forms of lymphocyte transformation. As previously demonstrated with PHA (7), new synthesis of acid hydrolases seems unlikely as an explanation for enzyme redistribution since there was no increase of total enzyme activity after 2 hr of incubation with PWM. Therefore, the increases of acid hydrolase activity in the 20,000 *g* × 20 min supernatant can be attributed

TABLE II

*Distribution of Enzymes among Subcellular Fractions Derived from Lymphocyte Cultures Incubated with and without PWM for 2 Hr at 37°C**

Enzyme	Treatment	No. of Exp.	Fraction					
			Debris	Nuclei	Granules	P‡ ± SED	Supernatant	P‡ ± SED
Beta-glucuronidase	Control	6	1.8 ± 0.3	9.5 ± 0.9	69.3 ± 2.1	$P < 0.001$	19.4 ± 1.5	$P < 0.001$
	PWM	6	1.4 ± 0.1	11.4 ± 1.3	61.5 ± 2.4	± 0.93	25.7 ± 1.6	± 0.53
Acid phosphatase	Control	5	3.3 ± 0.7	12.5 ± 1.3	59.8 ± 3.1	$P < 0.025$	24.4 ± 2.0	$P < 0.005$
	PWM	5	4.0 ± 0.7	14.2 ± 2.1	52.3 ± 3.6	± 1.92	29.4 ± 1.9	± 0.71
Malate dehydrogenase	Control	6	1.8 ± 0.3	8.1 ± 0.9	21.9 ± 1.5	Not sig.	68.3 ± 1.1	Not sig.
	PWM	6	1.6 ± 0.3	7.9 ± 1.0	22.3 ± 1.3	± 1.83	68.2 ± 1.1	± 1.58
Protein	Control	6	5.5 ± 1.7	7.7 ± 1.4	13.7 ± 1.0	Not sig.	73.1 ± 2.7	Not sig.
	PWM	6	5.7 ± 1.1	8.8 ± 1.6	13.7 ± 1.7	± 2.10	71.8 ± 3.3	± 1.23

* Values are given as per cent of the total recovered activity ± SEM.

‡ Paired *t*-test, ± Standard Error of Differences between paired samples.

TABLE III

*Influence of Purified Pokeweed Mitogen on Sedimentable Enzyme Activity in Postnuclear Supernatant Derived from Untreated Lymphocyte Cultures**

Agent	Dose	Beta-Glucuronidase
	μg/ml	
PWM	0.06	20.3
	0.63	19.4
	1.56	20.7
	3.13	19.3
	6.25	18.8
Solvent	0.05 (*ml/ml*)	19.3

* Samples were incubated for 1 hr at 37°C. Solvent: 0.9% NaCl. Calculation see text. n (number of experiments) = 3.

to release of sedimentable enzyme activity from hydrolase-rich granules present in lymphocytes. However, it is impossible to decide at present whether granules are actually disrupted in the intact cell or whether they burst during the preparation and fractionation of the homogenate. As in the case of PHA, no evidence was obtained for a direct action of PWM upon hydrolase-rich granules. When the data obtained with PWM and PHA were compared, the increases of acid hydrolase activity recovered in the supernatants were somewhat lower in the PWM experiments. This difference might be explained by the fact that in lymphocyte cultures stimulated by PWM the percentage of transformed cells is known to be lower than in cultures incubated with PHA.

The experiments clearly show that redistribution of acid hydrolases among subcellular fractions (labilization of lysosomes) is not limited to PHA stimulation of lymphocytes. Indeed, the phenomenon appears in homogenates originating from lymphocytes stimulated by two unrelated, nonspecific mitogens. To prove, however, the general validity of the hypothesis that lysosomal hydrolases mediate lymphocyte transformation, further studies are necessary in which other nonspecific stimulants are employed.

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