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An Altered Response to Cyclic AMP Stimulating Hormones in Intact Human Leukemic Lymphocytes (39701)[1]

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Introduction. Adenylate cyclase, in particulate preparations from lymphocytes of patients with chronic lymphatic leukemia (CLL), has previously been shown by us to possess a lowered basal activity and a diminished response to prostaglandin (PG) E_1, E_2, $F_{2\alpha}$ (1). Reports of cyclic AMP and PG participation in cell division and maturation (2–4) and modulation of certain functions in lymphocytes (5) suggested that the observed reduction in adenylate cyclase activity and response to stimulation may prove important in the abnormal behavior of leukemic lymphocytes. In this report, we demonstrate observations with intact human lymphocytes, normal and malignant, which indicate further that the response of adenylate cyclase to extracellular effectors has been altered in leukemic lymphocytes.

Methods and materials. Isolation of lymphocytes. Normal human lymphocytes were isolated from the plasma layer of freshly drawn heparinized venous blood (1). The blood was allowed to settle in 15-cc screw-capped conical tubes at 37°. The lymphocytes were isolated by careful removal of the upper plasma fraction, which contained high concentrations of lymphocytes. Only cell mixtures containing a minimum of 95% lymphocytes were accepted for study. Leukemic cells were obtained similarly from previously untreated patients with leukemia. The lymphocytes were freed of platelets by repeated washing and centrifugation at 100*g*.

Lymphocyte incubation. The intact cells (normal or neoplastic lymphocytes) were preincubated for 30 min at 37° in medium "199," containing 20% calf serum. Unless otherwise stated, 6×10^6 cells were used per incubation. Incubation was initiated with the addition to each tube of 0.05 ml of a solution containing the given stimulant or equivalent solution not containing the stimulant. Prostaglandins were placed into aqueous solution with the aid of $NaHCO_3$. The incubation was terminated with 70% $HClO_4$ (containing [^{3}H]cAMP, 0.1 pmole). The final concentration of the perchlorate was 3.5%. The samples were centrifuged and the supernatant containing cAMP was neutralized with 3 *N* KOH. The insoluble salt precipitate was removed by centrifugation and the supernatant was passed through Dowex 50 W-X2 (H^+), lyophilized, resuspended, and assayed with a radioimmunoassay (1). The cAMP content of the medium was minimal, 0.5 cc containing approximately 0.5 pmole. No difference in medium cAMP content was observed before or after incubation. The prostaglandins were kindly supplied by Dr. Pike of the Upjohn Co.

Results. Under the incubation conditions described, the CLL cells contained about twice the cAMP per cell of normal lymphocytes (Table I). The cAMP content of the leukemic cells showed a much wider range of sample variability.

Table I demonstrates the cAMP levels of normal and CLL lymphocytes in response to treatment with isoproterenol, epinephrine, and glucagon. The relative response (stimulated/basal) was also determined. Both of the catecholamines showed stimulation relative to the basal solution in normal cells; glucagon had no appreciable effect. The relative response to epinephrine was significantly reduced in the malignant cells. Glucagon, again, had no appreciable effect on cAMP levels. This is best demonstrated in the stimulated/basal ratio, which illustrates that the CLL response to epinephrine was reduced by a factor of at least 2. The response to isoproterenol also appears reduced. However, with isoproterenol, enough variations existed from sample to

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[2] Deceased.

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TABLE I. cAMP LEVELS IN NORMAL AND LEUKEMIC LYMPHOCYTES IN RESPONSE TO VARIOUS AGONISTS.[a]

Additions	Normal lymphocytes		Leukemic lymphocytes		
	(pmole of cAMP/10^7 cells) Mean	Stimulated/ basal	(pmol cAMP/10^7 cells) Mean	Stimulated/ basal	*P*
None	15.7 ± 1.3		28.5 ± 5.5		0.05
Isoproterenol (5×10^{-5} *M*)	26.3 ± 4.3	1.7	28.2 ± 16.8	N.S.	
Epinephrine (2×10^{-5} *M*)	34.8 ± 11.7	2.2	31.2 ± 4.8	1.1	0.05
Glucagon (1.4×10^{-7} *M*)	17.6 ± 1.4	1.1	27.2 ± 7.8	<1	N.S.
Prostaglandin					
E_1 (5×10^{-5} *M*)	121.0 ± 39.0	7.7	54.0 ± 13.0	1.9	0.01
E_2 (5×10^{-5} *M*)	127.0 ± 41.0	8.1	44.4 ± 20.6	1.6	0.01
$F_{2\alpha}$ (5×10^{-5} *M*)	85.2 ± 44.8	5.1	42.0 ± 10.0	1.5	0.1

[a] After a 30-min preincubation at 37°, 6×10^6 lymphocytes in 0.45 ml of medium "199" containing 20% calf serum were exposed to 0.05 ml of a solution containing the given hormone. The cultures were then incubated, fixed with PCA, and assayed for cAMP as described in the text. The hormone concentrations indicated are the final concentrations. These concentrations were determined to produce maximal stimulation in both the normal and leukemic cells. Equivalent solutions not containing the given hormone were added to the control cultures; this addition had no effect on the cAMP content of these untreated cultures. N.S. not significant. Values represent 17 leukemic and 12 normal samples. Each sample was determined in triplicate. *P* Values were calculated for normal basal vs leukemic basal, and normal (stimulated/basal) vs leukemic (stimulated/basal) for each hormone.

sample to make this observation statistically questionable.

In the case of prostaglandins, the altered response in the CLL leukocytes is dramatic. In normal lymphocytes, prostaglandins E_1 and E_2 showed approximately an 8-fold stimulation over basal. Prostaglandin $F_{2\alpha}$ was less active, and showed about a 5-fold stimulation over basal. The leukemic cells displayed a decreased response to all three prostaglandins tested to about 1/3 to 1/4 of that found in normal lymphocytes.

The prostaglandin stimulation of lymphocytes was not β-adrenergic. Propranolol (5×10^{-6} *M*), a β-adrenergic blocker, inhibited isoproterenol and epinephrine stimulation by 80 and 95%, respectively, but did not affect cAMP levels consequent to PG stimulation. These results indicate that the adenylate cyclase response of the CLL lymphocytes to effectors is very poor.

Discussion. The breakage of intact cells to produce particulate preparations often causes artifactual results in basal and signal-responding activity of adenylate cyclase. For this reason, to substantiate our previous results obtained with particulate preparations, we proceeded to determine the effect of prostaglandins and other extracellular effectors on cellular cAMP levels in intact, viable lymphocytes from normal and leukemic subjects. Our present results again show a reduced stimulation of cAMP levels in leukemic cells by PGE and $PGF_{2\alpha}$. These results may prove meaningful, in view of the reported regulatory roles of the PGs and cyclic nucleotides in the immune response and cell proliferation of lymphocytes (5–7).

Since the reduction in the cAMP response in CLL cells is not limited to the PGs but also occurs with other effectors, our results further suggest that a more generalized refraction to extracellular signals may be occurring in the CLL lymphocytes. Much of the function of the lymphocyte occurs in response to extracellular proding (8).

The increased basal cAMP levels which we found in intact leukemic cells, as opposed to the decreased basal adenylate cyclase content in particulate preparations from leukemic cells, which we reported formerly, represent a contradiction which we cannot explain with the available data. It is possible that the latter was a result of a more pronounced destruction of adenylate cyclase in leukemic cells during cell breakage. Another point to be considered with these data is that human chronic lymphatic leukemia has been described as a B-cell disease (9, 10). More recently, this conclusion has been

modified somewhat to include a possible T-cell etiology, in some CLL cases (11). The normal lymphocyte population studied in this report contained a mixture of B and T cells. Data comparing the cAMP systems of B and T human lymphocytes and their response to extracellular effectors will have to be obtained in future experiments.

Summary. Lymphocytes from normal subjects and patients with chronic lymphatic leukemia were assayed for cyclic AMP content and their response to effector stimulation, in terms of increased cellular cyclic AMP levels. Concentrations of isoproterenol, epinephrine, and prostaglandins E_1, E_2, and $F_{2\alpha}$ which produced potent responses in normal lymphocytes were much less effective in increasing cyclic AMP levels of leukemic lymphocytes. The response to PGE_2 was depressed from an 8-fold stimulation over basal, in the normal lymphocyte, to a 1.6-fold response in the leukemic lymphocyte. These data, along with those obtained previously with particulate preparations, indicate that a dysfunction in receptor–adenylate cyclase interaction may exist within the membrane of leukemic cells.

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