Protein Synthesis by Ribosomes from Blood Lymphocytes of Normals and Patients with Chronic Lymphocytic Leukemia (CLL) (36885)

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Human blood lymphocytes incubated under standard cell culture conditions synthesize only small amounts of RNA and protein and remain in a resting, non-dividing state. Under the influence of several mitogens such as phytohemagglutinin (PHA), lymphocytes increase their synthesis of RNA and protein, replicate DNA, and divide. Several investigators have demonstrated that the blood lymphocytes of chronic lymphocytic leukemia (CLL) respond poorly to *in vitro* stimulation by PHA or specific antigens, showing a delay in the appearance of increased macromolecular synthesis and a less than maximal response compared to lymphocytes from normal controls (1, 2). The decreased capacity of CLL lymphocytes to undergo transformation may be responsible in part for the immunologic deficits noted clinically in this disease.

One feature of lymphocyte transformation that has not been previously examined is the intrinsic ability of ribosomes isolated from lymphocytes to catalyze protein synthesis. We have developed a cell-free protein synthesizing system capable of incorporating significant amounts of ^{14}C-amino acids into protein and have used this system to study ribosomal activity in normal and CLL lymphocytes both before and after PHA stimulation.

Venous blood was drawn into a heparinized syringe and allowed to sediment. Separation of lymphocytes from other blood elements was achieved by passage of white cell-rich plasma diluted with an equal volume of Eagle's minimal essential medium through a column of washed nylon fiber (3). The usual yield was 6–8 $\times$ 10^8 cells ($>$ 98% lymphocytes) from 500 ml of normal blood. The patients with CLL were untreated individuals with white blood cell counts between 15,000 to 120,000/mm^3. Ribosomes were immediately isolated from a portion of the cells. The remainder of the cells were cultured with PHA for 72 hr (4) after which the ribosomes were isolated. The lymphocytes were pelleted and disrupted in a motor-driven, tight-fitting tissue grinder. The homogenate was spun at 13,000*g* $\times$ 10 min. The supernatant was spun at 104,000*g* $\times$ 2 hr. The ribosomes from both normal and CLL lymphocytes had an absorbancy ratio (260:280 nm) of 1.7 to 1.8. The procedure yields particles of reasonable purity and with characteristics similar to those of ribosomes from other mammalian tissues (5). Soluble RNA from *Escherichia coli* was charged with ^{14}C-phenylalanine, ^{14}C-leucine, ^{14}C-lysine, ^{14}C-arginine, ^{14}C-valine, and sixteen additional ^{12}C-amino acids (6). The supernatant enzymes were prepared from the soluble fraction of rat liver (7). The exact conditions of the assays are summarized in the legend of Table I. The requirements for incorporation were similar to those found in other mammalian systems, showing an absolute dependence on supernatant enzymes, an energy source, and Mg^{2+}. All activity could be abolished by ribonuclease. The number of aminoacyl transfers from ^{14}C-aminoacyl tRNA into protein was proportional to the concentration of ribosomes as shown in Fig. 1. Incorporation could be increased only by adding more ribosomes or more labeled amino-acid precursors and we concluded that different activities in the cell-free system represented different catalytic abilities of the added ribosomes in

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TABLE I. Characteristics and Requirements for Incorporation into Protein of ^{14}C-amino Acids by Ribosomes from Blood Lymphocytes.[a]

	Incorporation (cpm)	
	CLL	Normal
Complete	1404	8232
— Supernatant protein	63	104
— ATP, PEP, pyruvate kinase	182	169
— GTP	948	4651
— $MgCl_2$	20	12
— 2-Mercaptoethanol	1450	8211
+ 100 μg ribonuclease	12	0

[a] The protein synthesis reaction mixture contained 30 μg rRNA; 100 μg ^{14}C-aminoacyl tRNA; 5.2 mg supernatant protein; 1 μmole ATP; 0.4 μmoles GTP; 10 μmoles phosphoenolpyruvate; 0.1 mg pyruvate kinase; 70 μmoles KCl; 10 μmoles $MgCl_2$; 50 μmoles Tris–HCl, pH 7.8; 10 μmoles 2-mercaptoethanol. The amount of poly U, when present, was 100 μg. When poly U was added, the only labeled precursor present was ^{14}C-phenylalanyl tRNA. The reaction was carried out at 37° for 10 min in a final volume of 1 ml.

both normal and CLL.

As seen in Table II, ribosomes isolated from resting normal lymphocytes stimulated 10 times as much incorporation as did ribosomes from resting CLL cells. The addition of poly U increased incorporation in the resting normal cell ribosomes by 45-fold. The addition of poly U increased incorporation in the resting CLL cell ribosomes by more than 100-fold. Even in the presence of poly U, however, the normal derived ribosomes were still more active than the CLL ribosomes, the ratio between normal and CLL being about 4.

Ribosomes isolated from lymphocytes 72 hr after PHA stimulation showed increased activity in all experimental conditions compared to the resting cell ribosomes. Ribosomes from normal lymphocytes were more active than ribosomes from CLL lymphocytes both with and without the addition of poly U. PHA stimulation produced nearly identical proportional increases in the activity of the ribosomes from both normal and CLL lymphocytes when poly U was added.

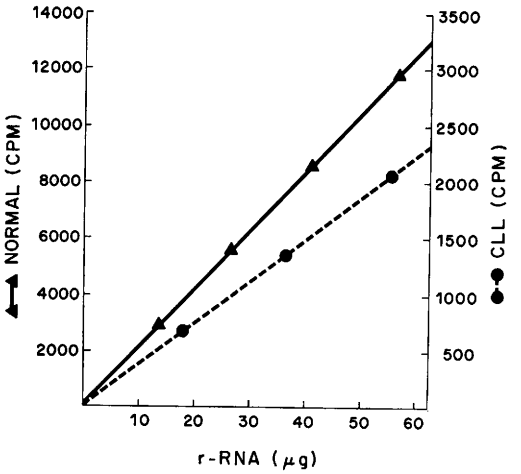

FIG. 1. Incorporation into protein of ^{14}C-amino acids as a function of ribosomal concentration in one normal and one patient with CLL. The assay was carried out as described in the legend of Table I.

Our experiments have shown that ribosomes from CLL lymphocytes are less active in catalyzing protein synthesis than ribosomes from normal lymphocytes both before and after PHA stimulation. In the experiments without added poly U, different activities may be due to increased natural messenger RNA associated with the normal

TABLE II. Incorporation of ^{14}C-amino Acids (10^{-2} $\mu\mu$moles) into Protein by Ribosomes from Lymphocytes of Normal Donors and Patients with Chronic Lymphocytic Leukemia.

	Resting		72 Hr after PHA	
	Without poly U	With poly U	Without poly U	With poly U
Normals (7)	3.20	147	9.90	827
CLL's (5)	0.35	36.9	2.90	162
p	<.05	<.05	<.05	<.05

ribosomes. The finding that the addition of poly U produced a greater increase in activity in the CLL resting cell ribosomes than in the normal resting cell ribosomes is consistent with this idea. However, normal ribosomes were still more active than CLL ribosomes following the addition of saturating amounts of poly U. This means that the differences in the amount of natural m-RNA do not entirely account for the differences in activity between normals and CLL's.

Normal blood lymphocytes are a collection of bone marrow-derived (B) and thymus-derived (T) cells. There is increasing evidence that CLL lymphocytes are predominantly or entirely B cells (8, 9). The decreased ability of CLL lymphocyte ribosomes to catalyze protein synthesis compared to normal lymphocyte ribosomes may be due to normal differences between T and B cell ribosomes and/or a defect in the protein synthesizing apparatus of CLL lymphocytes. It is of interest that PHA produced nearly identical proportional increases in the activity of the ribosomes from both normal and CLL lymphocytes when poly U was added. PHA appears to induce changes in the protein-synthesizing apparatus of both normal (T + B) lymphocytes and of CLL (B) lymphocytes to a similar degree.

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