SERUM ALBUMIN BIOSYNTHESIS AND SECRETION BY RESTING AND LECTIN STIMULATED HUMAN LYMPHOCYTES

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SUMMARY: Normal human peripheral lymphocytes, cultured in serum-deprived medium, synthetized and released serum albumin and some glycoproteins into the culture supernatant. With the use of [^{3}H]leucine, it was shown that this biosynthetic activity was increased about 2-3 times when the mitogenic lectin from Robinia pseudo acacia was added to the lymphocyte culture medium.

Some lectins are known to transform the small lymphocytes into blast cells. Such lectins are called mitogenic and are not generally blood group specific (1). The lectin from Robinia pseudo acacia is one of them and many of its biological effects were previously demonstrated on various types of cells (2).

This morphologic transformation is accompanied by some biochemical modifications: for instance, it was shown that mitogenic lectins increased the ribosomal protein biosynthesis (3-5) and the release of protein factors in the culture medium (6). However almost nothing is known about the biochemical features of these proteins. So it was interesting to investigate the secreted proteins by Robinia lectin-stimulated lymphocytes in comparison with control resting cells.

In this paper are shown the de novo synthesis and secretion of serum albumin and some glycoproteins by resting and stimulated human lymphocytes cultured in presence of [^{3}H]leucine. Furthermore a lymphocyte culture method in absence of autologous serum was specially designed to avoid contamination of the newly synthetized proteins.

MATERIALS AND METHODS

Human peripheral blood lymphocytes were prepared by the Ficoll-Hypaque method (7) and cultured in the presence of 3 ml of 199 medium with antibiotics only, in Falcon dishes at the concentration of 6 x 10^6 cells/ml. 6 µCi of [^{3}H] leucine (specific activity: 30 Ci/mM) were added to each dish at the beginning

Abbreviations: PAS, periodic acid-Schiff reagent staining; PAGE, poly-acrylamide gel electrophoresis; SDS, sodium dodecyl sulfate; HSA, human serum albumin; BSA, bovine serum albumin; CLS, control lymphocyte supernatant; RLS, Robinia lectin-stimulated lymphocyte supernatant; TCA, trichloracetic acid.

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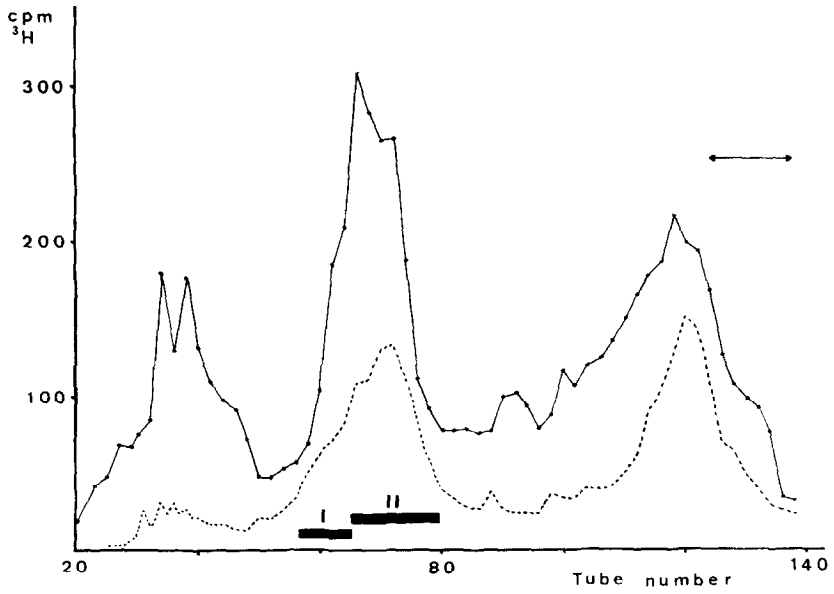

Figure 1. Sephadex G-200 elution patterns of control resting lymphocyte supernatant (CLS) and Robinia lectin stimulated lymphocyte supernatant (RLS). Column volume: 105 x 3 cm. Elution buffer: Tris-HCl 0.1 M, pH: 8. ----- : CLS radioactivity; ——— : RLS radioactivity; ←——→ : Phenol red containing fractions; ▬▬ : Studied fractions.

of the culture. An amount of 15 µg of Robinia pseudo acacia lectin per dish was added in the half of the dishes.

Seventy two hours latter, the cells were harvested by centrifugation and discarded. The supernatants were collected, re-spun at high speed and exhaustively dialyzed to remove the [^{3}H]leucine excess.

The supernatants were concentrated by dialysis against poly-ethyleneglycol and put separately on a column of Sephadex G-200 (Pharmacia)(105 x 3cm) in a Tris-HCl buffer 0.1 M, pH: 8. Phenol red was added to the samples. Fractions of 6 ml were collected and an aliquot of 0.5 ml was counted for tritium in a spectrometer (Intertechnique, France) with PCS (Amersham-Searle, USA) as scintillation liquid.

Fractions corresponding to the radioactivity peaks were gathered, dialyzed and lyophilized for further investigations.

Electrophoresis was carried out in several ways:

1) Zone electrophoresis on cellulose acetate strips (Cellogel. Chemetron, Italy) in Veronal buffer 0.05 M, pH: 8.6, 200 V, 75 minutes. Proteins were stained by Amido-Schwarz and carbohydrates by PAS.

2) PAGE in SDS according to Davis (8). Gels were stained by Coomassie blue and PAS (9). Apparent molecular weights were calculated by the method of Shapiro et al. (10). Another set of gels was cut in 1.5 mm slices, dissolved overnight in H_2O_2 at 37° C in counting vials and counted for tritium as before.

3) Immuno-electrophoresis according to Hirschfeld (11). The used antisera were:

-Horse anti human protein serum (Institut Pasteur, France).
-Rabbit anti human albumin serum (Nederland Red Cross, Amsterdam).
-Rabbit anti immunoglobulin G and M sera (Centre de transfusion, Bois-Guillaume, France).

Proteins were assayed according to Lowry et al. (12) with BSA as standard.

TABLE I

	Fraction I	Fraction II
CLS	170 µg 8,900 dpm	223 µg 29,800 dpm
RLS	525 µg (+ 3.1) 21,400 dpm (+ 2.4)	675 µg (+ 3.02) 60,200 dpm (+ 2.02)

Protein and radioactivity content of Fraction I and II from control lymphocyte supernatant (CLS) and Robinia lectin stimulated lymphocyte supernatant (RLS) for 5.4 x 10^7 cells. Values in parentheses are the respective increases of protein amount and radioactivity in RLS versus CLS.

RESULTS

1) Fractionation of the proteins released in the culture medium.

The Sephadex G-200 elution patterns of the labelled proteins synthetized either by resting or by lectin-stimulated lymphocytes were shown on the figure I. The supernatants from the resting lymphocytes (CLS) and from lectin-stimulated lymphocytes (RLS) were issued from the same number of cells.

Three radioactive peaks were delineated on the two patterns.

The first peak was excluded and heterogeneous. It was no longer studied.

It was likely that the third peak was made of the free remaining [^{3}H] leucine because this radioactivity was eluted just before the Phenol red.

The second peak only was further studied. It exhibited, in both cases, a front shoulder and two fractions were delineated and called respectively fractions I and II. Table I gives the total amount of secreted proteins and radioactivity found in the fractions I and II. A 2-3 fold increase of the RLS content in comparison with the CLS one was clearly demonstrated.

Though the amounts of proteins in CLS and RLS were different, the elution profiles were quite similar, suggesting that the difference between CLS and RLS was only quantitative and not qualitative. The further electrophoresis analysis confirmed this hypothesis.

2) Fractions I and II analysis by electrophoresis.

Fraction I: On cellulose acetate, fraction I gave only one band stained by Amido-Schwarz, PAS negative and migrating to the same distance as standard HSA.

In PAGE with SDS, fraction I exhibited one stained band with corresponding radioactivity. The apparent molecular weight of this band was about 65,000 daltons.

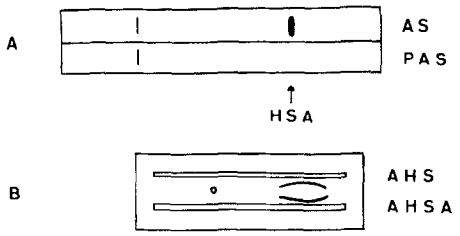

Figure 2. Electrophoresis analysis of Fraction I.
A) Cellulose acetate electrophoresis. Veronal buffer 0.05 M, pH: 8.6, 200 V, 75 minutes.
AS: Amido-Schwarz staining; PAS: Schiff reagent staining after periodic oxidation; HSA: migration area of human serum albumin.
B) Immunoelectrophoresis.
AHS: Anti human protein serum; AHSA: Specific anti human serum albumin.

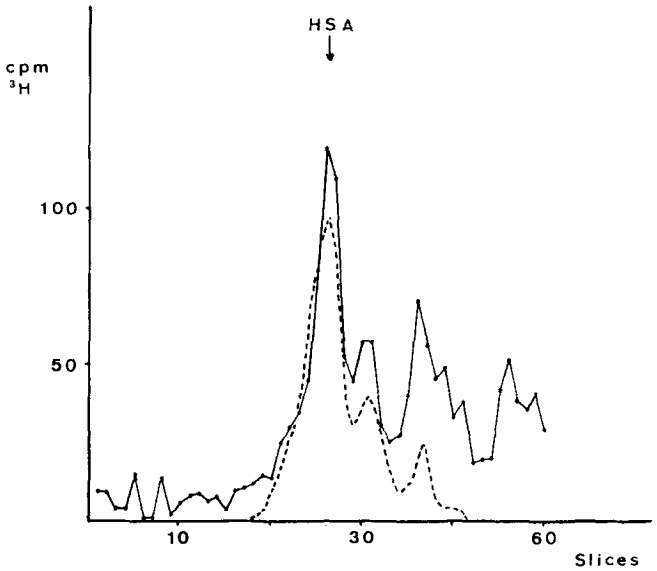

Figure 3. SDS-poly-acrylamide gel electrophoresis of [^{3}H]leucine-labelled fraction II. Proteins were detected by scanning a Coomassie blue stained gel at 600 nm. The presence of [^{3}H]leucine in proteins was detected by gel slicing and scintillation counting. The mobility of the standard human serum albumin is indicated as HSA. ——— : [^{3}H]leucine; ------: E_{600}.

A single precipitation arc was shown on immuno-electrophoresis slab when tested with respectively horse anti human protein serum and specific anti HSA serum (Fig. 2).

Furthermore radioactivity was found in the TCA precipitate of fraction I, proving the incorporation of [^{3}H]leucine into proteins which were mainly represented by serum albumin.

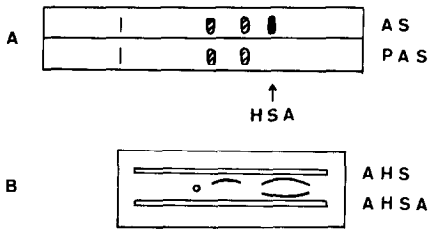

Figure 4. Electrophoresis analysis of Fraction II. Same legend as Figure 2.

Fraction II: Three Amido-Schwarz positive bands were seen on cellulose acetate strip with fraction II. The strongest band was PAS negative and migrated to the same distance as standard HSA. In addition the two other lighter bands were PAS positive and migrated to the α_2 and β regions respectively.

In PAGE with SDS three Coomassie blue positive bands were observed with corresponding radioactivity (Fig. 3). The apparent molecular weights of these proteins were respectively 65,000, 54,000 and 45,000 daltons. The two last bands were PAS positive.

Only two precipitation lines were observed in immuno-electrophoresis with the use of anti human protein serum. The mainest line represented serum albumin since it was also revealed when specific anti HSA serum was used (Fig. 4).

It can be noticed that immunoglobulins G and M were never characterized in any Sephadex G-200 fractions when tested with specific anti IgG and IgM sera.

COMMENTS

From the given data, it can be assumed that human peripheral lymphocytes are able to synthetize de novo and to secrete in the culture medium serum albumin and some minor glycoproteins. Evidences are given about the strong similarities between "lymphocyte albumin" and HSA: electric mobility, apparent molecular weight, non staining by PAS and immunological features.

The biosynthesis and secretion of albumin by lymphocytes are proved by several reasons:

- the incorporation of [^{3}H]leucine into albumin as shown by TCA precipitation and PAGE with SDS.

- the 2-3 fold increase of the amount of secreted albumin and [^{3}H]leucine incorporation with the use of Robinia pseudo acacia lectin. If albumin originated from the blood donor's serum, the amount of albumin and radioactivity found in the culture medium would be the same with resting and stimulated cells.

This increase of biosynthesis and secretion of albumin by a mitogenic

lectin is in agreement with the stimulation by lectins of the ribosomal protein biosynthesis (13).

This paper shows that this secretion seems to be selective since only few proteins and mainly albumin were released into the culture medium. The lack of immunoglobulins, in the used experimental conditions, confirms the only stimulation of T-lymphocytes by mitogenic lectins (14).

Furthermore it is obvious that human peripheral lymphocytes can be easily cultured for three days in a serum-deprived medium while maintening a metabolic activity like protein biosynthesis and a normal morphology as shown by Acridine orange staining of the cells.

Finally this albumin secretion increase could be used to follow and measure, by immunological assays for instance, the effects of mitogenic lectins on cultured T-lymphocytes.

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