Cytoplasmic ^{14}C-labeled arginine basic proteins of Ehrlich ascites tumour cells[1]

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Summary. With the use of ^{14}C-arginine it is shown that among soluble cytoplasmic proteins of Ehrlich ascites tumour cells there appear basic proteins rich in arginine with the content of amino acid higher than 14%. The amount of these proteins is about 10%. The role of arginine-rich basic proteins in the cytoplasma is briefly discussed.

The presence of basic protein in the cytoplasmic fraction in Guérin epithelioma[2] and the rat ovary ascites tumours[3] has been described previously. The arginine-rich basic protein from the cytoplasma of Guérin epithelioma was isolated and characterized[2]. These findings induced the authors to study the basic proteins in the cytoplasmic fraction of Ehrlich ascites tumour cells using ^{14}C-arginine.

Materials and methods. Ehrlich ascites tumour cells were maintained by weekly intraperitoneal transplantation into Swiss white mice weighing 50 g. The tumour-bearing mice were fasted 18–20 hours before injections i.p. of 0.2 ml ^{14}C-arginine containing 10 µCi (sp.act. 336 mCi/mmole) (Radiochemical Centre, Amersham, England). All the mice (30) were killed 3 h after injection of ^{14}C-labeled L-arginine. The ascites fluid was centrifuged at 200 × g for 7 min at 4 °C. The cells were washed 3 times with cold 0.14 M NaCl and then homogenized in a medium containing 0.25 M saccharose, 0.03 mM Tris-HCl, 0.2 mM EDTA, pH 7.4 in the volume equivalent to 10% of homogenate. The homogenate was centrifuged (VAC 601 Janetzky ultracentrifuge) at 105,000 × g for 60 min. The supernatant has been accepted as the soluble cytoplasmic fraction.

The soluble cytoplasmic ^{14}C-labeled L-arginine proteins were separated in a CM-cellulose chromatography column (Whatman Biochemicals Ltd, England).

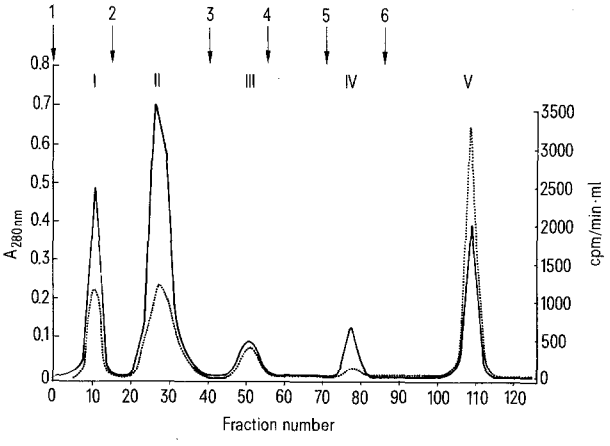
The radioactivity of all fractions was estimated in a Nuclear Chicago scintillation counter; the protein concentration was measured spectrophotometrically (VSU-2P, Carl Zeiss, Jena). Specific radioactivity of ^{14}C-arginine labeled fractions of particular peaks pooled, dialysed and concentrated by lyophilization was estimated.

The ^{14}C-labeled arginine-rich basic proteins (peak V) were analysed by electrophoresis on a polyacrylamide gel according to the method of Panyim and Chalkey[4]. The zones of proteins were stained with Coomassie brillant blue R 250.

The concentration of proteins was estimated by the procedure of Lowry et al.[5]. The content of arginine in particular protein fractions were estimated by the chemical procedure described by Sakaguchi but modified by the authors[6]. Total nitrogen was assayed by the Kjeldahl method.

Results and discussion. 5 peaks of protein were obtained from the CM-cellulose chromatography column. As can be seen from the figure, peak II contains the highest amount of protein (64.4%) and peak IV shows the lowest one (3.6%). ^{14}C-labeled arginine-rich basic proteins are strongly adsorbed in the CM-cellulose column and can be eluted only with the use of 0.02 M HCl. The highest total radioactivity was in peak V and amounted to 3270 cpm. The table summarizes the quantitative data for each of the protein peaks. Of all the proteins present in the cytosol protein mixture, peak V contains the highest amount of arginine (measured chemically) and the highest specific radioactivity – 14,384 cpm/mg.min, as well as the highest concentration of protein nitrogen (206 µg/mg).

The basic protein content of peak V amounted to about 10%. In polyacrylamide gel electrophoresis at pH 2.7 the proteins of this peak migrate to the cathode and are seen to be a mixture of 4 different proteins, possessing similar basic physicochemical properties. Their high content of nitrogen and arginine, and their high specific radioactivity in peak V, good solubility in acidic buffers, fractionation by CM-



Chromatography of cytoplasmic ^{14}C-labeled proteins of Ehrlich ascites tumour cells in a CM-cellulose column (1.5 × 30 cm). A 20 mg sample of these proteins was dialysed against 0.0175 M phosphate buffer (pH 6.3) and subsequently applied to a column equilibrated previously with phosphate buffer (pH 6.3). 1.5 ml fractions were collected at a flow rate of about 30 ml/h. Arrows indicate particular eluting buffers:
1, 0.0175 M phosphate buffer (pH 6.3); 2, 0.01 M phosphate buffer (pH 5.9); 3, 0.1 M phosphate buffer (pH 5.8); 4, 0.4 M phosphate buffer (pH 5.2); 5, 0.4 M phosphate buffer (pH 4.4) containing 2 M NaCl; 6, 0.02 M HCl. —— Absorption 28 nm; - - - - radioactivity.

Chemical characteristics of cytoplasmic proteins of Ehrlich ascites tumour cells after CM-cellulose column chromatography

Protein studied	Protein recovery (mg)	Per cent of protein*	µg nitrogen/mg of protein	µg arginine/mg of protein	Sp. act. of ^{14}C-arginine cpm/mg min
Peak I	1.70	9.9	142.8	58.9	4320
Peak II	11.02	64.4	155	113.2	5505
Peak III	1.33	7.7	163	128	7328
Peak IV	0.62	3.6	160	125	839
Peak V	2.42	14.1	206	145	14387

* Calculated from recovery of a 20 mg sample of cytoplasmic protein dialysed before placing in the chromatography column.

cellulose column chromatography, and migration to the cathode during electrophoresis at pH 2.7, indicate their basic nature.

Arginine-rich proteins have also been reported in other types of neoplastic cells[2,3,7]. Until now their role within the cells has not been precisely established. It can be postulated that the basic proteins might be modulating factors in some process of intermediary metabolism in the cells.

On the other hand, it is known that these basic proteins are easily degraded into polypeptides in vitro by intracellular proteases and endopeptidases[8]. It can be supposed that there are proper substrates for the proteolytic enzymes. Those enzymes are easily released from lysosomes into the cytosol, and into the extracellular medium, by Ehrlich ascites tumour cells[9]. The existence of a well developed lysosomal system and the association of acid hydrolases with this kind of subcellular particle in Ehrlich ascites cells was established long ago[10]. Thus, a mechanism for the specific release of lysosomal contents, similar to the process studied in macrophages under the term 'exocytosis', may be suggested[11]. Low molecular weight arginine-rich peptides have been observed previously in the cytosol of some tumour cells, in extracellular spaces, and in the blood of tumour bearing rats[12,13].

1 Supported by Polish National Cancer Programe PRC Project No. 1318/13. The authors are grateful to Mrs Anna Zbroch and Elzbieta Lipczyńska for skillful technical assistance, during the course of this work.

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Compartmentalized growth of hemopoietic stem cells within mouse Friend leukemic spleens

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Summary. A dose of 4000 rads (r) to the central portion of mouse spleens followed by Friend erythroleukemic virus infection created independent compartments where hemopoietic stem cells exhibited distinct growth kinetics. Rather than suggesting autonomous proliferation, the stem cell kinetics were indicative of the control exercised by the local microenvironment upon stem cell growth within each compartment.

There is increasing evidence that self renewal of hemopoietic stem cells (HSC) depends upon local stimulation from specialized cells of the hemopoietic microenvironment. Although the nature of these cells is uncertain, their existence is suggested by a number of experiments in vivo[2-4] and in vitro[5]. These microenvironment cells have been called 'source' cells to emphasize their function as producers of a microdiffusing stimulus which promotes HSC renewal[6]. It is of interest now to see whether HSC renewal in mouse viral leukemia is also dependent upon stimulation from the microenvironment. As suggested elsewhere[6,7], Friend virus induces a leukemic response principally by increasing stimulus production by infected sources. Consequently, the population of HSC in leukemic spleens increases greatly above normal levels. However, when the spleen microenvironment is irradiated at doses $\geq$ 950 r the source cells (as any lethally irradiated cell) become resistant to virus infection[7,8] and support only a normal population of HSC.

We tested this hypothesis by irradiating with a single 4000 r dose only the central part of DBA/2j mouse spleens before injection of a large dose of Friend erythroleukemic virus. As detailed elsewhere[2] a number of normal, adult, male DBA/2j mice were positioned in the field of a 4 MV Varian linear accelerator. 2 (2 cm thick) lead blocks separated by a 5 mm gap were positioned over the mice to protect body and tail, with the exception of a 5 mm long central spleen portion, which received the full 4000 r dose. The extreme spleen portions (each about 5 mm long) were protected by the blocks and received $\leq$ 200 r as scatter. Since the spleens (average length = 15 mm) were not surgically exposed, positioning of the 5 mm gap was done by approximating their location in situ. This yielded only 3-5% of the mice with a centrally irradiated spleen. After irradiation all mice

CFU content ($\pm$ SD) in separate portions of centrally irradiated and leukemic (CIL) spleens and in whole leukemic control (LC) spleens at various days after Friend virus injection

Day	CIL spleens			LC spleens	NC spleens
	P_1	C (4000 r)	P_2		
8	12,380 $\pm$ 3230	150 $\pm$ 60	8600 $\pm$ 2570	43,600 $\pm$ 6700	5400 $\pm$ 1580
10	15,100 $\pm$ 4060	890 $\pm$ 215	9980 $\pm$ 3200	48,320 $\pm$ 5920	5400 $\pm$ 1580
12	16,560 $\pm$ 4190	2350 $\pm$ 400	12,080 $\pm$ 2950	50,920 $\pm$ 8300	5400 $\pm$ 1580
14	17,940 $\pm$ 5280	2460 $\pm$ 520	13,830 $\pm$ 3800	60,560 $\pm$ 9870	5400 $\pm$ 1580

The P_1, C, and P_2 portions of CIL spleens are shown in the figure. NC spleens are from normal nonvirus injected control mice.