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Cyclophilin A produced by thymocytes regulates the migration of murine bone marrow cells

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Abstract

Supernatant obtained from high dose hydrocortisone resistant thymocytes can induce migration of the bone marrow cell precursors to the periphery. This biological activity depends on the presence of the 18 kDa protein, whose amino acid sequence fits with the sequence of the secretory form of murine cyclophilin A (SP-18). Cyclophilin A isolated from the supernatant of the cortisone-resistant thymoma EL-4 shows its characteristic functional features as it demonstrates isomerase activity and binds with cyclosporine A. The cyclophilin A obtained manifests chemotactic activity that regulates migration of bone marrow cell precursors of neutrophils, T-, B- and dendritic cells.

Keywords: Cyclophilin A; Thymocytes; Chemotaxis; Bone marrow precursors

1. Introduction

The thymus consists of a heterogeneous cell population in various stages of maturation and assorted localizations, cell markers, T-cell receptor expression and immunocompetent properties [1,2]. Along with these parameters, thymic cells may be differentiated by their sensitivity to corticosteroid hormones. Corticoid hormones are normally present in thymus and provide the necessary component of thymic selection [3,4]. But if the concentrations of such hormones significantly exceed physiological levels, the bulk of thymocytes expire [5]. Only a small cell population, less than 3% of the total number of thymic cells, survives [6]. Cortisone-resistant thymocytes (CRT) are the most mature thymic subpopulation and are located mainly in the medullar zone, representing about 25% of the medulla cells [7].

In previous experiments with CRT, we have demonstrated that factor(s) that enhance bone marrow cell migration *in vivo* were accumulated in the culture medium after *in vitro* incubation of these cells [8]. We observed that intravenously injected CRT supernatant induced an increase of spleen colonies in mice exposed to a sublethal dose of irradiation on the seventh day after exposure, compared to control animals injected with culture medium. Further biochemical purification and functional activity testing of different CRT supernatant fractions have demonstrated that the 18 kD protein cyclophilin A (CypA) was the substance responsible for this phenomenon. CypA is localized in cytosol and participates in the processes of intracellular protein transport acting as an ubiquitinious protein with cis–trans-isomerase activity [9]. Moreover, CypA participates in the signal transduction from T-cell receptor [10] and is a ligand for cyclosporin A, thus determining the immunosuppressing properties of the latter [11,12]. In addition to the cytosolic form, there is a secretory form

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of CypA. Activated macrophages are able to secrete this protein into the environment [13]. The secretory form of CypA has been detected in human milk [14]. Extracellular CypA acts as a chemotactic agent regulating the migration of monocytes, neutrophils, eosinophils and T-cells [15]. This protein possesses immunomodulating activity by stimulation of maturation and antigenic presentation in dendritic cells [16].

We have demonstrated that CypA can be secreted by thymic cells and that this protein acts as the chemoattractant for the precursor cells from various bone marrow growths of differentiation. Phenotypic determination of the bone marrow cells which have migrated *in vitro* under the influence of CypA revealed that the precursors which lacked the SCA-1 molecule on their surfaces were more sensitive to its action. CypA significantly enhances the migration of $CD3^+$ T-cells, precursors of B-cells ($B220^+CD19^+$), granulocytes ($GR1^+CD31^+$) and dendritic cells ($CD11b^+CD11c^+$). Thus, these findings demonstrate that CypA chemoattractive ability applies not only to the described mature cell forms [17], but to their precursors as well.

2. Materials and methods

2.1. Animals

Our experiments were carried out on specific pathogen free mice (C57BL/6 ($H\text{-}2^b$) × CBA ($H\text{-}2^k$) F1 which were obtained from the Laboratory Animal Nursery of the Russian Academy of Sciences, Institute of Bioorganic Chemistry (Puchshino Branch). Mice were kept in microisolator wards equipped with barrier filters.

2.2. Tumor cells

Cortisone-resistant murine EL-4 thymoma cells of $H\text{-}2^b$ haplotype [18] were used as a source of the CRT factor. We performed EL-4 cell cloning by the limiting dilution method using flat-bottom 96-well plates (Costar) to the final dilution of one cell per well. The phenotype of cells which were used for the experiments corresponded by 97% to $CD4^-CD8^-TCR\alpha\beta^+NK1.1^-CD2^+$ $CD48^+$.

2.3. Conditions for in vitro cell culture

EL-4 cells were routinely cultured in RPMI 1640 culture medium supplemented with 10% fetal calf serum, 5×10^{-5} M 2-mercaptoethanol, 2 mM L-glutamine, and an antibiotic. The culturing was performed at 37 °C in an atmosphere of 5% CO_2.

2.4. Obtaining of CRT and EL-4 supernatants

Mice 6 weeks of age ($m = 16$–18 g) were injected intraperitonealy with hydrocortisone (Gedeon Richter, Hungary) in a dosage of 2.5 mg per animal. Animal thymic cell isolation was performed within 48 h of injection. Cell suspension was obtained by homogenization in RPMI 1640 medium and subsequent precipitation of cells by centrifugation. For erythrocyte removal, the cells were treated with 0.85% NH_4Cl solution and then washed with Earle's solution. About 4×10^6 CRT cells were used to obtain 1 ml of supernatant. EL-4 cells were washed three times by centrifugation in a large volume of Earle's solution. About 3×10^5 of EL-4 cells were used to obtain 1 ml of supernatant. CRT and EL-4 cells were incubated for 18 h in serum-free RPMI 1640 supplemented medium. After incubation, cell viability was about 75%.

2.5. Concentration of the CRT and EL-4 cell supernatants

Concentration of the supernatants was performed by ultrafiltration on PM-10 membrane (Amicon). For aggregates removal, the samples were centrifuged on a minicentrifuge (Eppendorf) at the speed of 14,000*g*/min for 10 min. The resultant supernatant was used for further biochemical purification procedures.

2.6. CRT and EL-4 supernatant proteins gel-chromatographic purification

The separation of proteins from the supernatant concentrates was performed using gel-chromatography on "Superdex-200" column (Amersham Biosciences). A set of standards for gel-filtration (Amersham) was used for column calibration. For the protein separation, high-pressure liquid chromatography system (HPLC) (Gilson) was used; eluting buffer contained 20 mM Tris–Hcl, pH 7.2; stream velocity was 0.5 ml/min. The fractions were collected in 0.5 ml volume. Protein content in the eluate was detected by the absorption at 280 nm.

2.7. Electrophoretic analysis

Different fractions obtained after the separation of CRT and EL-4 cell supernatant proteins were concentrated in microfuge YM-10 tubes (Millipore Centrifugal Filter Devices Microcon YM-10) to the volume of 100 μl, then lyophilized and diluted in 20 μl buffer. Prepared samples were used for SDS–electrophoresis [19]. Electrophoretic separation was performed in 16.5% polyacrylamide gel in a tris–tricine system [19] with subsequent Coomassie (Bio-Rad) protein gel staining.

2.8. Evaluation of chemotactic activity in vitro

Biological activity of the supernatants and different gel-chromatographic fractions was determined *in vitro* based on the influence of the tested substances on the migration of bone marrow cells of syngenic mice (C57BL/6× CBA) F1 across a minitranswell membrane (5 micron pores) (Neuroprobe, Cabin John, MD) [20]. Tenfold NaCl solution was added to the physiological concentration to the