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Somatostatin modulates T cells development in adult rat thymus

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Abstract

It is well known that somatostatin modulates thymic functions, such as binding to receptors. In order to elucidate the influence of somatostatin on the thymus architecture and the T cells maturation, young adult male rats were treated with somatostatin-28. The results showed that somatostatin-28 decreased thymus weight and cellularity, probably due to alterations in the thymic morphometric parameters. Our results also demonstrated that SRIH treatment reduces number of cells with undetectable αβTCR and cells with low expression of αβTCR, while the number of TCRαβ^{hi} cells remains approximately the same as the values obtained from the control rats. Besides, in the least mature thymocytes (DNTCR TCRαβ^{-}) and among the most mature the SPCD4 TCRαβ^{hi} subset remained unaltered, while SPCD8 TCRαβ^{hi} decreased. At last, it should be noted that SRIH treatment increases DN thymocytes subsets expressing TCRαβ$^{low/hi}$ (TCRαβ^{+}).

These results suggest that somatostatin-28 induces reshaping of T cells maturation and, at least partly, contributes to thymic weight loss, through the modulation of the complex neuroendocrine–immune network.

Keywords: Rodent; Thymus; T cells development; Somatostatin

1. Introduction

The thymus is a central lymphoid organ where bone marrow T cells progenitors migrate along morphologically similar but functionally distinct stromal regions, and mature through a series of developmental stages [1,2]. During intrathymic maturation T cells differentiate from a double negative (DN; CD4^{-}8^{-}) to the mature single positive (SP) CD4^{+}8^{-} or CD4^{-}8^{+} cells via a double positive (DP; CD4^{+}8^{+}) stage of development [1]. This chain of events includes selection at two different stages. The first is called β selection, and occurs during DN3 to DN4 transition and leads to expression of the pre-TCR. Only cells that express a functional pre-TCR undergo a marked proliferation and differentiate into DP cells [1,3]. The cells that pass this selection step undergo the selection events driven by the TCRαβ. Most DP cells are eliminated through negative selection or by neglect because their TCR has too high or too low affinity for peptide–MHC complexes. Those that mature successfully migrate to the periphery as functional SP CD4^{+}8^{-} or CD4^{-}8^{+} cells [1,4,5].

During these processes, thymocytes interact with different molecules expressed on the surface of the thymus microenvironment cells, with components of extracellular matrix [1], as well as with soluble components such as thymus hormones, cytokines, neuropeptides and other soluble factors produced by thymus epithelial cells [6]. Nevertheless, it is also very important to emphasize that a certain amount of classically produced hormones and neuropeptides enters the thymus through blood vessels, and has a great influence on differentiation and maturation processes [7].

Somatostatin (Somatotropin releasing inhibiting hormone, SRIH) is a neuropeptide, derived from the hypothalamic periventricular nucleus. There are two biologically active forms of somatostatin, composed of either 14 or 28 amino acids, SRIH-14 and SRIH-28 [8,9]. SRIH regulates neurotransmission in the brain, secretion of the anterior pituitary cells, the pancreas and gastrointestinal tract, under physiological and pathological circumstances [10]. It is well known that SRIH is

Abbreviations: DN, Double negative; DP, Double positive; SP, Single positive; TCR, T cell receptor; SRIH, Somatotropin release inhibiting hormone; TEC, Thymus epithelial cells; SSTR, Somatostatin receptor; PI, Propidium iodide.

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very potent inhibitor of basal and stimulated secretion varieties of exocrine and endocrine cells. The biological effects of SRIH are mediated via five high affinity subtypes G-protein coupled transmembrane somatostatin (SST) receptors, named SST_{1-5}, widely distributed throughout many tissues [9]. In the human and rodents the SST_2 receptor is represent by two splice variants (SST_{2A} and SST_{2B}), which diverge in their C-terminal sequence [11]. Normal human thymus expresses SST_1, SST_2 and SST_3 receptor subtypes of somatostatin receptor [12–14]. In rats, transcripts for all receptor types have been identified throughout the CNS, endocrine and exocrine gland and other peripheral organs [15]. The data, derived from adult Sprague–Dawley rats, showed expression of SST_3 in spleen and lymph nodes [15], and SST_3 and SST_4 were found in Lewis rat immune cells [16], while the rat thymocytes constitutively expressed SST_2 [17].

Growth hormone (GH) is one of the hormones which succumbs to the regulation of SRIH. GH via receptors expressed on thymocytes and thymic microenvironment cells regulates the thymus development [7]. A marked thymic hypotrophy, accompanied with cortical lymphocytes loss, is described in dwarf mice, which are deficient in growth hormone (GH), prolactine (PRL) and thyroid hormones [18]. On the other hand, the presence of nerve endings, which may hold and release SRIH [19] in the thymus, and SRIH producing cells, in the medulla and corticomedullary region [20–23], indicates a paracrine/autocrine route of SRIH activity in the thymus, through SST receptors expressed on thymocytes and thymic epithelial cells [7,13,23,24].

SRIH is involved in different functions of the thymus as: (i) secretion of cells expressing SST receptors [12]; (ii) proliferation of thymocytes and TEC [12,13,25]; (iii) apoptosis of thymocytes [14,26].

Considering the results of Starcevic et al. and Petrovic-Djergovic et al. [27–29], which pointed out that intracerebroventricular (i.c.v.) application of SRIH-28 in adult male rats changes the level of the growth hormone (GH) and prolactine (PRL) that alters T cells development in peripubertal rats. The aim of this study was to investigate whether SRIH-28, i.c.v. applied, in young adult rats contributes to thymic weight loss (size and cellularity) and alters T cell differentiation, then, if there are some changes, which part of the thymus and which thymocyte subpopulation are the most susceptible to this neuropeptide, and in addition we also examined the presence of SST receptors on thymic cells. To estimate the influence of SRIH-28 on T cell differentiation and maturation, we followed the expression of CD4, CD8 and TCRαβ cell surface molecules, as well as cell proliferative capacity by cell cycle analysis and its susceptibility to apoptosis by flow cytometry. In addition, we analyzed the expression of SSTR by flow cytometry and immunohistochemistry and thymus architecture through assessment of morphometric parameters by the stereological method.

2. Materials and methods

The study was performed on male Albino Oxford (AO) rats, 10 weeks old at the start of experiment. Animals were individually single-housed and kept in our animal colony under environmentally controlled conditions (12-h light/dark cycle, light on from 7 am until 7 pm; 22 °C ± 1 °C). Food and water were available ad libitum. Our Institutional Animal and Use Committee has approved the experimental protocol.

2.1. Animal preparation

The operative procedure of cannulation was performed as we previously described [28]. The silastic-sealed 20-gauge cannula [30] inserted into a lateral brain ventricle, 2 mm laterally to the sagittal suture, 2 mm caudally to the frontal suture and 3.0 mm ventrally to the cortical surface, was used for intracerebroventricular (i.c.v.) application of somatostatin. The cannula and small, stainless steel anchor screws placed at a remote site on the skull were cemented to the skull with dental acrylate (Simgal, Galenika, Beograd). Minimal recovery time before beginning of the treatment was 5 days as suggested [31].

2.2. Protocol

After recovery, the rats were divided into two groups ($n = 10$/group). The first group received i.c.v. three doses of 1 μg, each, of SRIH-28 (Sigma Chemical, St Louis, MO USA, S 6135) in 5-μl saline, with 48-h-intervals between administrations. The second group received 5-μl saline in the same manner as the first group. Animals were sacrificed by decapitation, 24 h after the last injection. The examination of position and permeance of the cannula was accomplished by vital dyes on the day of the sacrifice. Only animals with confirmed position in the lateral ventricle were considered for analysis.

2.3. Preparation of thymus cell suspensions

The thymuses were carefully removed. After being weighed they were used for a preparation of single-cell suspensions. Suspensions were prepared by grinding the thymus tissues between the frosted ends of microscope slides, in cold phosphate-buffered saline (PBS, pH 7.3) containing 2% heat-inactivated fetal calf serum (FCS, Gibco, Grand Island, NY, USA) and 0.01% sodium azide (NaN_3, Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany). The obtained single-cell suspensions were passed through a fine nylon mesh and washed three times in a cold buffer solution. After counting by a standard haemocytometer, cell density, 1×10^7 cells/ml buffer, was adjusted. The cells viability, determined by Trypan blue exclusion, was routinely greater than 95%.

2.4. Antibodies

For immunofluorescence staining of thymocytes, the subsequent monoclonal antibodies (mAbs) were used: fluorescein-isothiocyanate (FITC)-conjugated anti-CD4, (W3/25, Serotec, Oxford, UK), phycoerythrin (PE)-conjugated anti-CD8 (MRC OX-8, Serotec, Oxford UK), biotin-conjugated anti-TCRαβ (R73, Serotec, Oxford, UK), and polyclonal anti-SST_{2A} antibody (goat polyclonal IgG, sc-11606, Santa Cruz Biotechnology). Controls included irrelevant isotype matched antibodies tagged with FITC, PE and biotin (Sigma, St. Louis,