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Age-associated plasticity of α1-adrenoceptor-mediated tuning of T-cell development

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ABSTRACT

Alpha$_1$-adrenoceptors (α_1-ARs) are involved in neuro-thymic and thymic intercellular communications, and consequently modulation of T-cell development. Ageing is associated with a number of changes in noradrenergic neuro-effector transmission, and possibly intercellular noradrenaline (NA)-mediated communication resulting in altered responses of target cells to NA. Thus, in old animals an altered NA modulation of thymopoiesis via α_1-ARs may be expected. To test this hypothesis, in old and young adult Wistar rats we examined: 1) thymic NA levels, density of noradrenergic innervation and NA synthesizing cells, as well as α_1-AR expression, and 2) then the effects of 14-day-long treatment with the α_1-AR blocker, urapidil, on thymocyte development. Overall, the first part of study suggested augmented NA signalling to thymic cells via α_1-ARs due to increased NA availability and α_1-AR thymocyte surface density in old rats. The second part of study supported this assumption. Namely, although in rats of both ages urapidil affected the same thymocyte developmental steps ultimately leading to changes in the relative number of the most mature single positive TCR$\alpha\beta^{high}$ thymocytes, its effects were generally more prominent in old animals. Following urapidil treatment, the percentages of CD4+CD8− cells, including those showing a regulatory CD4+CD25+RT6.1− phenotype, were increased, while CD4−CD8+ cells decreased. In old rats, an augmented thymic escape of immature CD4+CD8+ cells was also registered. In rats of both ages the thymic changes were accompanied by alterations in the proportions of major cell populations in the T-lymphocyte compartment of both peripheral blood and spleen, leading to an increase in the CD4+/CD8+ T-cell ratio. These alterations were also more pronounced in old rats. Moreover, in old rats following urapidil treatment the proportion of TCR$\alpha\beta$+ cells in the periphery was slightly greater reflecting, most likely, partly enhanced thymic production of regulatory CD161+TCR$\alpha\beta$+ cells. Thus, the study indirectly suggests an age-associated increase in the basal α_1-AR-mediated inhibitory influence of NA on thymopoiesis.

1. Introduction

Generally, ageing is associated with a number of changes in noradrenergic neuro-effector transmission, resulting in an altered response to the neurotransmitter. Neurotransmission is likely to be affected by changes in nerve activity, density of innervation and neurotransmitter disposition mechanisms, as well as by alterations in target organ responsiveness, which may involve alterations at the receptor and post-receptor level or even alterations in tissue structure (Docherty, 2002).

The thymus is richly innervated by postganglionic sympathetic noradrenergic fibers. Noradrenergic nerve profiles have been observed not only within the thymic capsular and trabecular system, but also within thymic parenchyma ending in close proximity to thymocytes and non-lymphoid cells driving T-cell development (reviewed in Elenkov et al., 2000; Leposavić et al., 2008). In addition, noradrenaline (NA) was recently reported to be produced by thymic lymphoid and non-lymphoid cells (Pilipović et al., 2008). Moreover, it was suggested that, apart from NA of neural origin, thymic cellular NA, acting in both autocrine and paracrine ways, is involved in modulation of T-cell development (Pilipović et al., 2008). This role of NA is corroborated by the detection of β- and α_1-adrenoceptors (ARs) on thymic lymphoid and non-lymphoid cells (Loveland et al., 1981; Marchetti et al., 1994; Kavelaars, 2002; Pešić et al., 2009). Furthermore, pharmacological studies implicated both subtypes of ARs in NA-mediated tonic inhibition of T-cell development (Plećaš-Solarović et al., 2004, 2005; Leposavić et al., 2006a; Pešić et al., 2009).

In rodent thymi, ageing has an impact on the density of noradrenergic nerve fibers and NA concentration (Bellinger et al., 1988; Madden et al., 1997; Cavallotti et al., 1999), but the influence of ageing on thymic cellular NA production has not been investigated yet. Furthermore, age-associated alterations in the efficiency of β-AR-

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mediated modulation of T-cell development have been reported (Madden and Felten, 2001; Pešić et al., 2007). However, although many types of cells have been demonstrated to exhibit age-related changes in sensitivity to α_1-AR signalling (Yokoyama et al., 1984; Basso and Piantanelli, 2002), there are no data related to the influence of ageing on α_1-AR-mediated intrathymic communication, and consequently modulation of thymopoiesis.

Uncovering age-associated changes in NA-mediated intrathymic communications may be important for better understanding of complex multifactorially driven alterations in thymopoiesis in old age (Kelley et al., 1986; Li et al., 1992; Hirokawa et al., 1994; Fabris et al., 1997; Lau and Spain, 2000; Hsu et al., 2005; Leposavić and Perišić, 2008). Since T cells are central to adaptive immunity, knowledge about the mechanisms underlying detrimental changes in T-cell development with increasing age is crucial for understanding age-related decay in immune functions (Berzins et al., 2002; Globerson, 2002; Gruver et al., 2007). The changes in T-cell compartment with age encompass narrowing diversity of the T-cell repertoire and disruption of the major T-cell population balance within the peripheral lymphoid compartment (De Paoli et al., 1988; Gilman-Sachs et al., 1991; Pahlavani and Richardson, 1994; Aspinall and Andrew, 2000; Globerson, 2002; Clambey et al., 2007; Czesnikiewicz-Guzik et al., 2008), mainly due to the thymus-dependent decline in CD4+ lymphocyte abundance in combination with oligoclonal expansion in the CD8 T-cell subset (De Paoli et al., 1988; Gilman-Sachs et al., 1991; Pahlavani and Richardson, 1994; Clambey et al., 2007; Czesnikiewicz-Guzik et al., 2008). Clinical consequences are poor responses to new antigens or vaccinations, raised infection rates with higher morbidity and mortality, and increasing incidence of autoimmune diseases with advancing age (Aspinall and Andrew, 2000; Pawelec et al., 2005; Aw et al., 2007).

Having all the previously stated in mind, we aimed to explore the putative influence of ageing on neuro-thymic and thymic intercellular α_1-AR-mediated NA communications. To this end, in thymi from young adult and old Wistar rats we examined: i) the density of noradrenergic nerve fibers and NA synthesizing cells, thymic and thymocyte NA levels and α_1-AR expression and ii) the effects of 14-day-long treatment with urapidil on T-cell differentiation/maturation. In addition, to determine if the changes in thymopoiesis translated into the periphery, the proportions of major T-lymphocyte populations in peripheral blood and spleen, as an example of a secondary lymphoid organ, were examined.

2. Materials and methods

2.1. Animals

In this study male Wistar rats purchased from the Medical Military Academy, Belgrade were used. This substrain of Wistar rats was chosen since thymi from these aged animals exhibit morphological characteristics similar to those found in aged human thymus (Plećaš-Solarović et al., 2004).

2.2. Experimental protocols

The first of two sets of experiments was undertaken to explore age-associated changes in: i) thymic NA level; ii) thymocyte NA content and tyrosine hydroxylase (TH) expression, iii) distribution of noradrenergic nerve fibers and TH-immunoreactive cells and iv) distribution of α_1-AR expressing cells and α_1-AR thymocyte surface density. For this purpose, old (18-month-old) and young (2.5-month-old) rats were killed and their thymi were dissected out. Thymic left lobes were processed for quantification of NA by high performance liquid chromatography (HPLC) or immunostaining of cells expressing TH and α_1-ARs, while thymic right lobes were snap frozen for analysis of fluorescent noradrenergic nerve distribution or processed for flow cytometric analysis (FCA) of thymocyte TH and α_1-AR expression.

In the second set of experiments, old and young rats were randomly assigned to an experimental condition: the α_1-AR antagonist, urapidil, or saline administration. Accordingly, over 14 consecutive days the animals were injected subcutaneously with urapidil (Ebrantil, Byk Gulden, Germany) at 0.20 mg/kg BW/day or with an equivalent volume of saline. This particular dose and duration of treatment were chosen on the basis of previous experiments showing that such treatment significantly affected thymocyte development in young rats (Pešić et al., 2009). Following the treatment all animals were euthanized by exposure to increasing doses of CO_2 followed by cardiac puncture exsanguination. The spleen and thymus were then carefully dissected out. The left thymic lobes were processed for stereological and immunocytochemical analyses of apoptotic and proliferating cells, while the right lobes and spleens were used for cell phenotyping by FCA. Each group consisted of at least six animals.

Animal care and experimental procedures were carried out in accordance with the principles described in the European Community's Council Directive of 24 November 1986 (86/609/EEC).

2.3. Chemicals, antibodies and immunoconjugates

All chemicals and standards (L-noradrenaline hydrochloride, L-adrenaline hydrochloride, 3,4-dihydroxybenzylamine) for HPLC, Concanavalin A (Con A) and RPMI 1640 powdered medium were purchased from Sigma-Aldrich Chemie GmbH (Taufkirchen, Germany). RPMI 1640 powdered medium was dissolved in redistilled water according to the manufacturer's instructions. To prepare complete RPMI medium, 2 mM L-glutamine (Serva, Heidelberg, Germany), 1 mM sodium pyruvate (Serva), 100 units/ml penicillin (ICN, Costa Mesa, CA, USA), 100 μg/ml streptomycin (ICN) and 10% fetal calf serum (FCS) (Gibco, Grand Island, NY, USA) were added. Liquid DAB + substrate–chromogen system, LSAB + system, antibody diluent, Faramount aqueous mounting medium and target retrieval solution were obtained from DakoCytomation, Glostrup, Denmark.

For immunostaining the following first-step antibodies (Abs) were used: phycoerythrin (PE)-conjugated anti-CD4 (clone W3/25, Serotec, Oxford, UK), fluorescein isothiocyanate (FITC)-conjugated anti-CD8 (clone MRC OX-8, Serotec), peridinin chlorophyll protein (PerCP)-conjugated anti-TCRαβ (clone R73, BD Biosciences Pharmingen, Mountain View, CA, USA), biotin-conjugated anti-CD25 (clone MRC OX-39, Serotec), biotin-conjugated anti-CD90 (Thy-1.1) (clone OX-7, BD Biosciences Pharmingen), anti-RT6.1 (clone P4/16, Serotec), FITC-conjugated anti-CD161 (clone 10/78, Serotec), anti-Ki-67 (clone B56, BD Biosciences Pharmingen), anti-rat tyrosine hydroxylase (TH, clone TOH A1, BD Biosciences Pharmingen), and goat anti-rat α_{1B}-AR polyclonal Ab (N-20, Santa Cruz Biotechnology, CA, USA). As second-step reagents were used: streptavidin-PerCP (BD Biosciences Pharmingen), streptavidin-PE (BD Biosciences Pharmingen), F(ab′)$_2$-anti-rat IgG FITC (Serotec), FITC-conjugated rabbit anti-goat IgG (Sigma-Aldrich Chemie) and FITC-conjugated goat anti-mouse IgG (BD Biosciences Pharmingen). Appropriate IgG isotype controls were obtained from BD Biosciences Pharmingen.

2.4. HPLC with electrochemical detection

Briefly, for measurement of NA level, thymic tissue and 1×10^8 thymocyte suspension homogenized in ice-cold 0.1 M perchloric acid (Sigma-Aldrich) containing 0.15% $Na_2S_2O_5$ (Sigma-Aldrich) and 0.05% Na_2EDTA (Sigma-Aldrich) were centrifuged at 30,000 × *g* for 20 min as previously described (Pilipović et al., 2008). The supernatants were passed through 0.2-μm filters and 25 μl aliquots were injected into a GBC high-performance liquid chromatograph (LC1120 pump, GBC, Victoria, Australia) with an electrochemical detector (LC1260, GBC)