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REVIEW

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## Cellular and Nerve Fibre Catecholaminergic Thymic Network: Steroid Hormone Dependent Activity

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### Summary

The thymus plays a critical role in establishing and maintaining the peripheral T-cell pool. It does so by providing a microenvironment within which T-cell precursors differentiate and undergo selection processes to create a functional population of major histocompatibility complex-restricted, self-tolerant T cells. These cells are central to adaptive immunity. Thymic T-cell development is influenced by locally produced soluble factors and cell-to-cell interactions, as well as by sympathetic noradrenergic and endocrine system signalling. Thymic lymphoid and non-lymphoid cells have been shown not only to express  $\beta$ - and  $\alpha_1$ - adrenoceptors (ARs), but also to synthesize catecholamines (CAs). Thus, it is suggested that CAs influence T-cell development *via* both neurocrine/endocrine and autocrine/paracrine action, and that they serve as immunotransmitters between thymocytes and nerves. CAs acting at multiple sites along the thymocyte developmental route affect T-cell generation not only numerically, but also qualitatively. Thymic CA level and synthesis, as well as AR expression exhibit sex steroid-mediated sexual dimorphism. Moreover, the influence of CAs on T-cell development exhibits glucocorticoid-dependent plasticity. This review summarizes recent findings in this field and our current understanding of complex and multifaceted neuroendocrine-immune communications at thymic level.

### Key words

Thymus • Catecholamines • Adrenoceptors • Sexual dimorphism  
• Glucocorticoids

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### Introduction

It is well known that the brain and immune system, as major adaptive systems, communicate with each other extensively in order to maintain body homeostasis. This communication involves the autonomic nervous system, mainly its sympathetic branch, and the hypothalamo-pituitary-adrenal axis (Madden and Felten 1995, Besedovsky and Del Rey 1996, Fabris *et al.* 1997, Mocchegiani *et al.* 2006, Haddad 2008). The key node in brain-immune system crosstalk is the thymus, the primary lymphoid organ responsible for T-cell development (Dardenne and Savino 1996, Fabris *et al.* 1997, Thyagarajan and Felten 2002, Mocchegiani *et al.* 2006). The thymus receives information from both the brain and circulating lymphocytes (Madden and Felten 1995, Fabris *et al.* 1997, Mocchegiani *et al.* 2006). Lymphocytes were suggested to act as the body's sixth sense transmitting information, *via* the thymus or directly, to the brain about responses to external or internal antigenic stimuli (Blalock 1994).

There is increasing evidence that immune system cells, including thymic lymphoid and non-lymphoid cells, are capable of synthesizing and releasing not only cytokines, but also neuropeptides,

neurotransmitters and hormones, and expressing specific receptors for these agonists (Batanero *et al.* 1992, Bergquist *et al.* 1994, Dardenne and Savino 1996, Josefsson *et al.* 1996, Silva *et al.* 2006, Pilipović *et al.* 2008). Thus, coexisting in the nervous, endocrine and immune systems, these agonists provide the universal language of the neuro-endocrine-immune network. This enables the nervous, endocrine and immune systems to regulate and fine-tune their functional responses, and consequently allow the body to adapt to changes in the internal and external environments. Understanding the functional interactions among these three systems, and their relevance to various physiological processes, is needed: (i) to recognize the mechanisms underlying the onset and course of many pathologic conditions related to immune system disturbances, such as infective, autoimmune/inflammatory and neoplastic diseases, and (ii) to envisage therapeutic strategies to counteract their onset and/or progress. This review is focused on depicting the histotopography of thymic catecholamines (CAs), and elucidating the putative significance of CA-steroid hormone interactions, in particular between CA and glucocorticoids (GCs), in fine-tuning T-cell development and consequently immune responses.

## Catecholamine sources in the thymus

### *Thymic noradrenergic innervation*

The thymus receives extensive sympathetic innervation (Williams and Felten 1981, Felten *et al.* 1985, Novotny *et al.* 1990, Vizi *et al.* 1995, Cavallotti *et al.* 1999, Nance and Sanders 2007). Thus, noradrenergic nerve profiles were identified not only along the vasculature in the subcapsular and associated septal regions, but also in close apposition to thymocytes and thymic epithelial cells (TECs) (Leposavić *et al.* 1992, Vizi *et al.* 1995). In the outer cortex, TECs forming the blood-thymus barrier are also exposed to circulating noradrenaline (NA). In addition, noradrenergic nerve profiles, including their varicose terminals, were observed in close proximity to mast cells, fibroblasts and macrophages (Novotny *et al.* 1990, Vizi *et al.* 1995). However, classical synapses between these nerve profiles and thymic cells were not detected. It was assumed that NA releases nonsynaptically from free nerve endings into a large extraneuronal space with no postjunctional specializations (Novotny *et al.* 1990, Vizi *et al.* 1995). Thus, most likely, NA diffuses a considerable distance before interacting with its receptors on target cells (Vizi

and Labos 1991). It is also strongly believed that NA released from perivascular or connective tissue septa plexuses diffuses away through surrounding adventitia or collagenous fibrils, in a paracrine fashion, transmitting a signal to thymic cells (Elenkov *et al.* 2000). Moreover, nonsynaptic transmission occurs in thymic blood vessel walls (between varicose nerve terminals and smooth muscle cells), where NA is involved in the regulation of blood flow and lymphoid cell traffic (Elenkov *et al.* 2000).

Generally, NA is released in the thymus in response to axonal firing arising in the central nervous system. This release is subjected to presynaptic modulation by various endogenous ligands (e.g. NA, adrenaline, acetylcholine, adenosine, etc.) or different drugs from the circulation through various receptors ( $\alpha_2$ -ARs, N-nicotinic, P<sub>1</sub>-purinergic and prostaglandin E presynaptic receptors) (Hasko *et al.* 1995).

### *Thymic cells*

Much evidence gathered during the past 15 years indicates that, besides neuronal and endocrine cells, many types of immune cells synthesize and secrete CAs. By acting in an autocrine/paracrine manner they regulate many immune functions, including cellular proliferation, differentiation, apoptosis and cytokine production. (Bergquist *et al.* 1994, Josefsson *et al.* 1996, Cosentino *et al.* 2000, Jiang *et al.* 2006). Recently, we detected NA in rat thymocytes, whereas dopamine content was below the reliably measurable level (Leposavić *et al.* 2007, 2010, Pilipović *et al.* 2008). Given that tyrosine hydroxylase (TH), the rate limiting enzyme in CA synthesis, was found in these cells at both the protein (Leposavić *et al.* 2007, 2010, Pilipović *et al.* 2008) and mRNA levels (Fig. 1), it seems highly conceivable that thymocytes not only take up NA passively from their microenvironment, but also synthesize it. The lack of measurable dopamine in thymocytes coincides with the finding that NA represents the major portion (57-95 %) of intracellular CAs in lymphocytes (Cosentino *et al.* 2000). A possible explanation for differential amount of NA and dopamine in lymphocytes is that, quite opposite to the rate of synthesis, the secretion rate of NA in lymphocyte cultures is lower than that of dopamine (Qiu *et al.* 2005). Flow cytometry analysis revealed TH immunostaining across all thymocyte subsets delineated by CD3 surface density, but the greatest frequency of TH-immunoreactive (ir) cells was registered within the most mature CD3<sup>high</sup> thymocyte subset (Pilipović *et al.* 2008). In agreement

with this finding, TH-ir thymocytes occurred most frequently on the medullary side of the cortico-medullary junction, although they were also present in the subcapsular cortex, and rarely intracortically and intramedullary (Pilipović *et al.* 2008). Furthermore, mRNA for phenylethanolamine N-methyltransferase, which converts NA to adrenaline, was also found in rodent thymi (Andreassi *et al.* 1998, Warthan *et al.* 2002). The specific cell types expressing mRNA for this enzyme have not yet been identified. We failed to detect adrenaline in rat thymocytes (Leposavić *et al.* 2007, Pilipović *et al.* 2008).

In the rat thymus, morphologically diverse subsets of non-lymphoid cells were also shown to contain immunoreactive TH (Leposavić *et al.* 2007, Pilipović *et al.* 2008). Their distribution pattern roughly matches that of thymocytes, i.e. they densely populate the medullary side of the cortico-medullary junction, moderately the subcapsular cortex, but are extremely rare intracortically and intramedullary. Immunocytochemical staining of rat and human thymic sections and/or isolated TEC-enriched thymic stromal cell fractions showed TH expression in neural crest derived thymic nurse cells (Jones *et al.* 1998, Botham *et al.* 2001, Pilipović *et al.* 2008). These cells formed multicellular complexes with thymocytes and macrophages completely enclosed within their cytoplasm (Pezzano *et al.* 2001). They may be involved in thymocyte positive selection and early post-selection development, as well as clearance of thymocytes, which were negatively selected or dying from neglect (Pezzano *et al.* 2001). Namely, in thymic nurse cells were identified CD4+CD8+ double positive (DP) TCR $\alpha\beta$ <sup>low</sup> thymocytes. These cells derive from CD4-CD8-TCR $\alpha\beta$ -triple negative precursors following successful rearrangement of genes encoding  $\alpha$  and  $\beta$  TCR chains, and successive surface expression of TCR $\alpha\beta$  complex and CD4 and CD8 coreceptor molecules. To proceed further with development, DP TCR $\alpha\beta$ <sup>low</sup> thymocytes are obliged to interact with self-peptides associated with major histocompatibility complex (MHC) antigens expressed on the surface of thymic non-lymphoid cells, and, most likely, on the vacuole surface surrounding engulfed thymocytes in thymic nurse cells (McCormack *et al.* 1991, Pezzano *et al.* 2001). At present, the most scrutinized hypothesis proposes that thymocytes producing a TCR $\alpha\beta$  that binds either tightly or not bind to self-peptide in association with MHC antigens are selectively deleted (Marrack and Kappler 1988, McCormack *et al.* 1991). On the other hand, thymocytes

producing a TCR $\alpha\beta$  that binds self-peptide with low affinity are allowed to mature to CD4+ or CD8+ single positive (SP) cells, and are ultimately released from the thymus (Marrack and Kappler 1988, McCormack *et al.* 1991). Immunoreactive TH was also found within some other types of TECs, possibly type 1 and type 5 TECs (Kranz *et al.* 1997, Anagnostou *et al.* 2007, Pilipović *et al.* 2008). Macrophages in the subcapsular/subtrabecular cortex and at the cortico-medullary junction also showed TH immunoreactivity (Leposavić *et al.* 2008).

These findings indicate that in thymus, apart from the catecholaminergic nerve fibre network, there is cellular catecholaminergic network comprising lymphoid and non-lymphoid cells, whose functional significance still awaits full appreciation.

### Expression of adrenoceptors in the thymus

Although the expression of both  $\beta_1$ - and  $\beta_2$ -ARs has been found in rat thymus,  $\beta_2$ -ARs appears to be the major  $\beta$ -AR subtype in this tissue (Marchetti *et al.* 1990a,b, 1994). We observed  $\beta_2$ -AR-ir cells mainly in the subcapsular/subtrabecular cortex and the cortico-medullary junction, but extremely rarely in the medulla (Leposavić *et al.* 2008, 2010). Expression of  $\beta_2$ -ARs was demonstrated on both thymocytes and thymic non-lymphoid cells, and TH was found in some of them as well (Leposavić *et al.* 2008). Compared to mature T lymphocytes, thymocytes expressed fewer  $\beta_2$ -ARs per cell, with the exception of the most mature cells (Fuchs *et al.* 1988). This suggests that  $\beta_2$ -AR expression on thymocytes is developmentally regulated.

Using double immunocytochemical staining with antibodies specific for  $\beta_2$ -AR and pan-cytokeratin, we found that subsets of TECs located mainly in the cortico-medulla exhibit  $\beta_2$ -AR immunoreactivity (Leposavić *et al.* 2008). Apart from  $\beta_2$ -ARs, functional studies demonstrated  $\beta_1$ -ARs on TECs. In addition, it was shown that NA may directly inhibit TEC proliferation and cytokine secretion or modulate the efficacy of other stimuli (Kurz *et al.* 1997, von Patay *et al.* 1999). Macrophages in the subcapsular cortex and cortico-medullary junction also exhibit  $\beta_2$ -AR immunoreactivity (Leposavić *et al.* 2008). Thus, it seems plausible to conclude that CAs, *via*  $\beta$ -ARs, influence T-cell development directly and indirectly, modulating their microenvironment.

Data on the presence of  $\alpha$ -ARs on T cells are rather limited (Kavelaars 2002). Both  $\alpha_1$ -AR mRNA and

$\alpha_1$ -AR protein were demonstrated in human bone marrow cells and thymocytes and in rat thymocytes, respectively (Kavelaars 2002, Pešić *et al.* 2009, Leposavić *et al.* 2010). Expression of  $\alpha_1$ -ARs in human peripheral blood mononuclear cells was undetectable or extremely low (van der Voort *et al.* 2000a, Kavelaars 2002). Thus, one may speculate that  $\alpha_1$ -AR expression is downregulated during lymphocyte development (Kavelaars 2002). In agreement with this hypothesis our data show that a majority of  $\alpha_1$ -AR positive thymocytes are the least mature CD3-cells (Pešić *et al.* 2009). Given that bone marrow cells and CD3- thymocytes are proliferating as well as differentiating cells, whereas peripheral blood mononuclear cells are mainly in a resting stage (Kavelaars 2002), it may be further speculated that the developmental changes in  $\alpha_1$ -AR expression are associated with cellular activity. Support for this assumption comes from several line of evidence. First,  $\alpha_1$ -AR mRNA expression is higher in Con A-activated T lymphocytes than in the corresponding resting lymphocytes (van der Voort *et al.* 2000b, Bao *et al.* 2007). Second, the expression of  $\alpha_1$ -AR re-appears in secondary lymphoid organs, when lymphocytes start to proliferate and differentiate into effector cells (Kavelaars 2002). In rat thymus, non-lymphoid cells located subcapsulary/subtrabeculary, in the cortico-medullary junction and rarely intramedullary also express  $\alpha_1$ -ARs (Pešić *et al.* 2009). Further characterization of these cells demonstrated that: i) they belong either to TECs or CD68-positive macrophages and ii) some of them also express TH (Pešić *et al.* 2009). Thus, it may be assumed that  $\alpha_1$ -ARs are involved not only in intercellular thymic communication, but also in thymic non-lymphoid cell autoregulation.

### Receptor-independent intracellular catecholamine action

The agonistic actions of CAs are terminated by their inactivation *via* intracellular oxidation. During this process they are degraded into various products including large quantities of reactive oxygen species and other cytotoxic oxidative metabolites, which induce apoptosis in mouse lymphocytes, and PC12 cell lines (Josefsson *et al.* 1996, Burke *et al.* 1998). Newly synthesized CAs that are stored inside the cells could also cause oxidative stress-mediated receptor-independent apoptosis (Cosentino *et al.* 2003). Even more importantly, a CA-specific transporter was found on lymphocyte nuclear

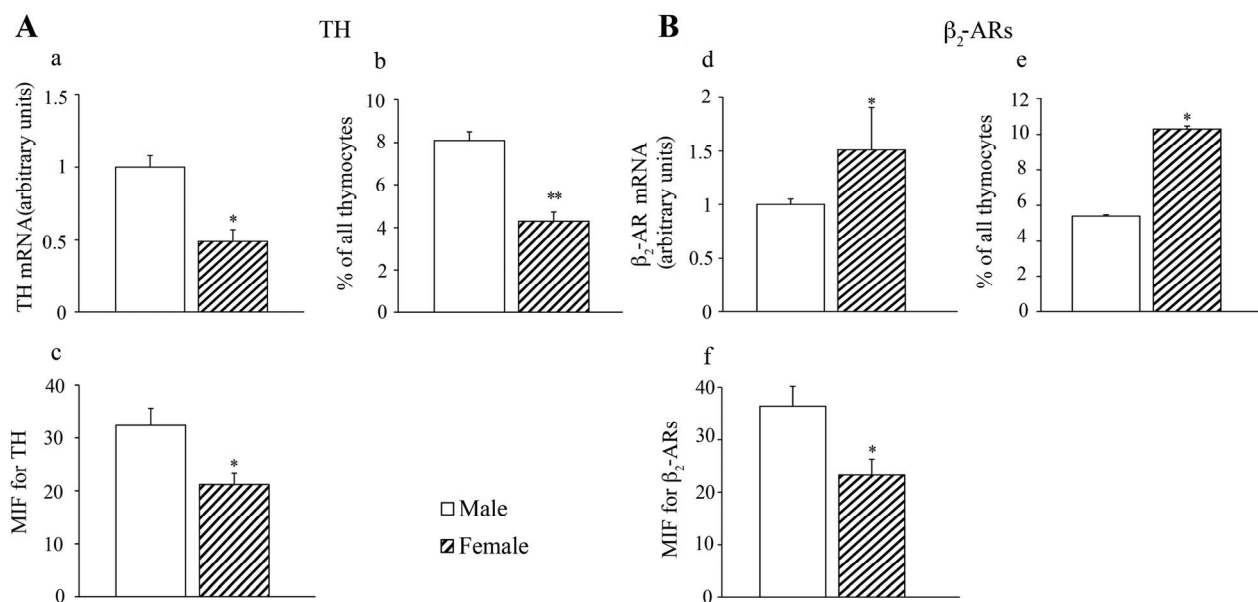
membranes (Bergquist *et al.* 1997, 2000). It actively transports CAs from the cytoplasm into the cell nucleus, where they can interact with nuclear steroid receptors and nuclear factor NF- $\kappa$ B influencing transcription processes and modulating apoptosis (Bergquist *et al.* 1997, 2000). Thus, it may be assumed that CAs may influence T-cell development also *via* non-AR mediated mechanisms.

### Gonadal steroid-dependent sexual dichotomy in thymic NA levels and AR expression

#### Noradrenaline

NA levels were higher in male than in female rat thymi (Pilipović *et al.* 2008). Considering that CAs are involved in modulating T-cell development (Rauški *et al.* 2003a,b, Leposavić *et al.* 2006, Pešić *et al.* 2009), it may be hypothesized that sexual dimorphism in thymic weight, cellularity and thymocyte subset distribution (Leposavić *et al.* 1996), at least partly, reflects gender dichotomy in thymic NA availability. Furthermore, since T-cells are central to adaptive immunity, it has been speculated that the observed sexual difference in the efficiency of the immune response establishes during T-cell development (Kovacs and Olsen 1998, Ansar-Ahmed *et al.* 1999). To elucidate mechanisms underlying sexual dimorphism in thymic NA level, we explored the density of noradrenergic nerve fibres and TH synthesizing thymic cells (Pilipović *et al.* 2008). There was no difference between male and female rats in the density of thymic noradrenergic nerve fibres (Pilipović *et al.* 2008), while the axonal NA content has not been measured. However, the densities of TH-ir lymphoid and non-lymphoid cells were greater in male than in female rats (Pilipović *et al.* 2008). In addition, TH mRNA (Fig. 1) and NA content (Pilipović *et al.* 2008) were greater in male than in female rat thymocytes. This agrees with data that in male rats nerve growth factor selectively and independently of neuronal proliferation induces synthesis of TH and dopamine  $\beta$ -hydroxylase (Thoenen and Barde 1980).

To establish any sex steroid contribution to the sexually dimorphic thymic NA level, we explored the effects of bilateral gonadectomy in male and female rats on thymic NA level (Pilipović *et al.* 2008). This was performed at the age of 30 days given that: i) sexual dimorphism in immune response efficiency and frequency of immune pathology arises soon after sexual maturation (Blazkovec and Orsini 1976) and ii) the hormonal changes occurring at the time of puberty lay the



**Fig. 1.** Gender differences in thymocyte tyrosine hydroxylase (TH) and  $\beta_2$ -adrenoceptor ( $\beta_2$ -AR) expression. A, Gender differences in (a) TH mRNA expression (as determined by real time RT-PCR), (b) frequency of TH containing thymocytes and (c) mean intensity of fluorescence (MIF) for TH, reflecting the thymocyte TH content (as measured by flow cytometry using monoclonal antibodies against rat TH and FITC-conjugated goat anti-mouse IgG antibody). B, Gender differences in (d)  $\beta_2$ -AR mRNA expression (as determined by real time RT-PCR), (e) frequency of  $\beta_2$ -AR bearing thymocytes and (f) MIF for  $\beta_2$ -AR reflecting  $\beta_2$ -AR thymocyte surface density (as measured by flow cytometry using polyclonal antibodies against rat  $\beta_2$ -AR and FITC-conjugated goat anti-rabbit IgG antibodies).

framework for biological differences that persist throughout life (Da Silva 1999). Gonadectomy diminished NA levels in thymi from adult rats of both sexes, but to a greater extent in males. Therefore, in gonadectomised rats we failed to measure any gender differences in thymic NA level (Pilipović *et al.* 2008). Irrespective of sex, the decrease in thymic NA levels in gonadectomised rats reflected the reduced density of both NA-containing fibres and cells (Pilipović *et al.* 2008). Support for these conclusions comes from data showing that: i) in male rats TH activity in the hypogastric (Melvin and Hamill 1986) and pelvic ganglion (Melvin and Hamill 1987) rises in parallel with plasma testosterone level during postnatal development (Resco *et al.* 1968), and ii) oestrous cycle-, pregnancy- and ovariectomy-related hormonal changes coincide with alterations in the TH level in the superior cervical ganglion of female rats (Anglin and Brooks 2003).

#### Adrenoceptors

We also found sexual dimorphism in  $\beta_2$ -AR thymocyte surface expression (Fig. 1). Namely, although the  $\beta_2$ -AR mRNA level was greater in thymocyte suspensions from female than male rats, the surface density of  $\beta_2$ -ARs was less on female thymocytes reflecting the greater frequency of  $\beta_2$ -AR bearing cells in their suspensions than in corresponding suspensions from

male rats (Fig. 1). These findings are corroborated by autoradiographic studies showing a sexually dimorphic pattern of postnatal changes in density of  $\beta$ -ARs in rat thymus (Marchetti *et al.* 1990b). Furthermore, it was found that alterations in gonadal steroid level influence  $\beta_2$ -AR thymocyte surface density in female (Marchetti *et al.* 1994). Moreover,  $\beta_2$ -AR stimulated activity of adenylyl cyclase was also shown to be dependent on the sex steroid hormone background (Marchetti *et al.* 1994). Thus, it may be assumed that gonadal steroids modulate the catecholaminergic influence on the thymus, affecting both availability of NA and/or density and functionality of  $\beta_2$ -ARs. On the other hand, data on the influence of gonadal steroids on  $\alpha_1$ -AR density and signalling capacity are still lacking.

### Catecholamine-mediated effects on T-cell development

#### Role of $\beta$ -adrenoceptors

There is accumulating clinical (Galbiati *et al.* 2007) and experimental evidence (Singh 1985a,b, Alaniz *et al.* 1999) suggesting a role for CAs in maintaining thymic size and T-cell output. To elucidate the mechanism underlying the influence of CAs on these parameters, the effects of long-lasting treatment with non-selective  $\beta$ -AR blockers on thymopoiesis were

investigated (Leposavić *et al.* 2000, 2006, Madden and Felten 2001, Pešić *et al.* 2007). Although  $\beta$ -AR blockade did not affect overall thymic cellularity, it significantly influenced the T-cell development (Leposavić *et al.* 2000, 2006, Madden and Felten 2001, Pešić *et al.* 2007). Thymocytes undergoing selection appeared to be particularly sensitive to  $\beta$ -AR blockade (Leposavić *et al.* 2006, Pešić *et al.* 2007). Long-lasting treatment with propranolol, a non-selective beta-blocker, diminished the frequency of DP TCR $\alpha\beta^{\text{low}}$  cells entering selection processes. This decrease, coupled with the increased frequency of DP TCR $\alpha\beta^{\text{high}}$  cells that had just passed positive selection, and their SP (CD4+CD8- and CD4-CD8+ cells) TCR $\alpha\beta^{\text{high}}$  descendants, strongly suggested enhanced positive/reduced negative selection and facilitated maturation of the selected cells. This in turn led to enhanced generation (Leposavić *et al.* 2006, Pešić *et al.* 2007), and probably subsequent egress of mature T cells into the periphery. Two lines of evidence associated alterations in thymocyte selection following propranolol treatment with the increase in Thy-1 surface density on selectable thymocytes (Leposavić *et al.* 2006, Pešić *et al.* 2007). First, it was shown that exogenous cAMP and NA can induce a decrease in steady state Thy-1 mRNA levels in T-lineage cells and murine thymocytes (Wajeman-Chao *et al.* 1998), which can be prevented by propranolol (Wajeman-Chao *et al.* 1998). Second, exaggerated negative selection and consequently markedly reduced *de novo* production of mature SP cells were found in Thy-1<sup>-/-</sup> mice (Hueber *et al.* 1997), and hence Thy-1 was implicated in the regulation of TCR-dependent thymocyte selection (Killeen 1997).

#### $\alpha_1$ -adrenoceptors

Differently from long-lasting blockade of  $\beta$ -ARs, long-term treatment with urapidil, an  $\alpha_1$ -AR antagonist, augmented thymocyte proliferation and increased thymocyte yield (Plečaš-Solarović *et al.* 2005, Pešić *et al.* 2009, Leposavić *et al.* 2010). As simultaneous administration of NA and propranolol decreased the frequency of proliferating cells in rat spleen (Stevenson *et al.* 2001), a stimulatory influence of urapidil on thymocyte proliferation was assumed (Pešić *et al.* 2009, Leposavić *et al.* 2010). In addition, urapidil affected the thymocyte phenotype profile and increased the frequency of DP TCR $\alpha\beta$ - thymocytes (Pešić *et al.* 2009, Leposavić *et al.* 2010). In the absence of changes in the frequency of precedent CD4-CD8- TCR $\alpha\beta$ - cells, this increase was related to a greater density of proliferating cells in the

outer cortex of thymi from urapidil-treated than from control rats. Prolonged cell proliferation at the DP TCR $\alpha\beta$ - developmental stage is likely to postpone TCR $\alpha$  gene rearrangement (Xi *et al.* 2006), and to impede transition to the next DP TCR $\alpha\beta^{\text{low}}$  developmental stage. Accordingly, the diminished frequency of DP TCR $\alpha\beta^{\text{low}}$  cells following urapidil treatment was attributed to decelerated differentiation of DP TCR $\alpha\beta$ - thymocytes (Pešić *et al.* 2009, Leposavić *et al.* 2010). The unaltered frequency of DP TCR $\alpha\beta^{\text{high}}$  thymocytes (being intermediary cells between the DP TCR $\alpha\beta^{\text{low}}$  and SP TCR $\alpha\beta^{\text{high}}$  stages), in conjunction with greater Thy-1 thymocyte surface density (reflecting a decline in central sympathetic flow caused by urapidil central action) in young adult rats, suggested more efficient positive/reduced negative thymocyte selection (Pešić *et al.* 2009). The greater Thy-1 thymocyte surface density in these rats, most likely, was associated with a decline in central sympathetic flow caused by urapidil central action (Pešić *et al.* 2009). The lack of urapidil influence on Thy-1 expression in old rats indicated that urapidil might also influence the thymocyte selection *via* direct thymic action (Leposavić *et al.* 2010). This is fully consistent with the observation of Bellinger *et al.* (2008) that CAs acting *via*  $\alpha_1$ -ARs on TECs involved in thymocyte selection may affect thymopoiesis. Moreover, irrespective of age, urapidil influenced thymocyte lineage commitment, causing an increase in the frequency of CD4+CD8- TCR $\alpha\beta^{\text{high}}$  and a decrease in that of CD4-CD8+ TCR $\alpha\beta^{\text{high}}$  SP cells (Pešić *et al.* 2009, Leposavić *et al.* 2010).

### Catecholamine-glucocorticoid interplay in the modulation of T-cell development

Repeated and chronic stress has an important role in the pathogenesis of almost all diseases related to immune system disturbances. Both GCs and CAs participate not only in regulating body functions during steady states, but also, often acting in concert, have a central role in maintaining the steady state of the internal milieu when it is threatened or disturbed by various internal or external challenges (Cunnick *et al.* 1990, Dobbs *et al.* 1993). Having this in mind, understanding their interplay in immunomodulation seems to be of utmost importance.

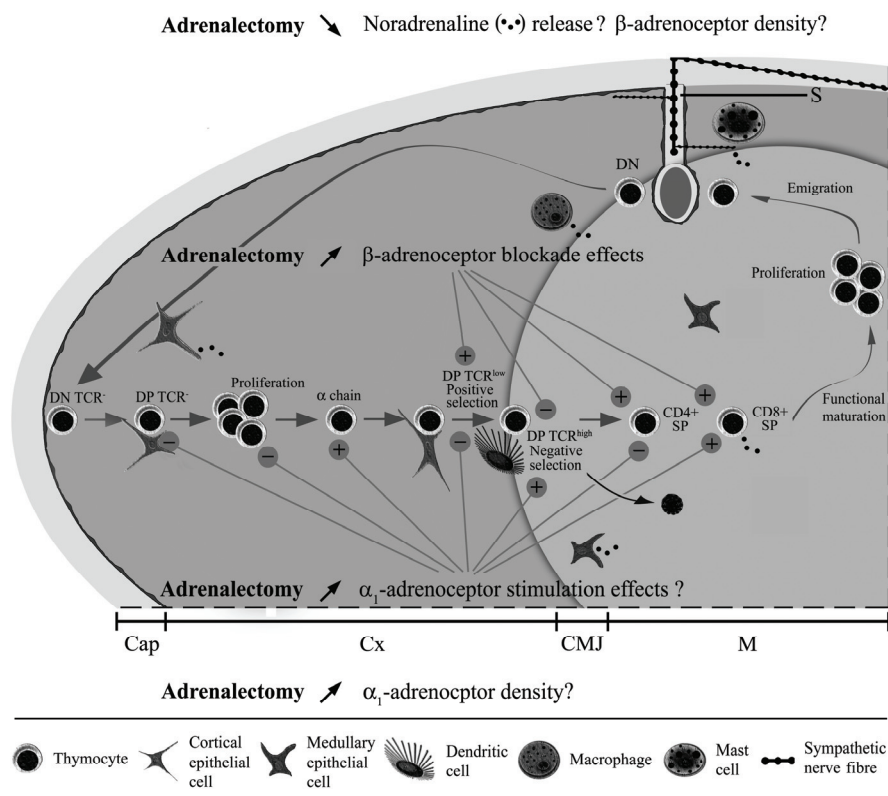
It has been shown that thymic lymphoid and non-lymphoid cells produce GCs and express glucocorticoid receptors (Lechner *et al.* 2000, Qiao *et al.* 2009). Furthermore, GCs have been implicated in the



modulation of thymocyte development (Stojić-Vukanić *et al.* 2009). Moreover, they were shown to influence CA biosynthesis and release (Hagerty *et al.* 2001) and  $\beta$ - and  $\alpha_1$ -AR gene expression (Cotecchia and De Blasi 1984). Based on these observations, we hypothesized that CA effects on thymopoiesis depend on the GC milieu (Pilipović *et al.* 2010). To test this we compared thymocyte development in rats adrenalectomized (Adx) four days before starting four-day-long propranolol treatment (0.4 mg/100g body weight/day, s.c.) with that in non-Adx propranolol-treated rats. Generally, we found that  $\beta$ -AR blockade was more efficient in Adx rats than in non-Adx rats (Fig. 2) (Pilipović *et al.* 2010). This is corroborated by data that simultaneous administration of isoproterenol and hydrocortisone produces additive effects on many thymic indices (Durant 1986). Compared with non-Adx rats, we registered a more pronounced increase in frequency of the most mature SP (CD4+CD8- and CD4-CD8+) TCR $\alpha\beta^{\text{high}}$  thymocytes in Adx rats, which, most probably reflected more efficient positive/less efficient negative selection (Fig. 2). Given that GCs augment CA synthesis and  $\beta$ -AR expression (Cotecchia and De Blasi 1984, Hagerty *et al.* 2001), it was speculated that the alterations in thymopoiesis were related to diminished thymic CA availability and  $\beta$ -AR

expression (Pilipović *et al.* 2010). However, when compared with non-Adx rats, propranolol caused unproportional increase in the frequency of the most mature SP cells in Adx rats (i.e. a more pronounced increase in the frequency of CD8+CD4- than in that of CD8-CD4+ SP cells) (Fig. 2). Thus, it was clear that the differential effect of propranolol in Adx and non-Adx rats could not be solely explained by the more efficient  $\beta$ -AR blockade in Adx rats (Fig. 2). Considering the effects of  $\alpha_1$ -AR blockade on T-cell differentiation (Pešić *et al.* 2009, Leposavić *et al.* 2010), we supposed that more efficient  $\alpha_1$ -AR-mediated mechanisms in Adx rats contributed to this phenomenon (Fig. 2). To support this assumption are data that prolonged exposure of cardiac myocytes to NA diminishes  $\alpha_1$ -AR expression (Rokosh *et al.* 1996). Thus, in propranolol-treated Adx rats an increased  $\alpha_1$ -AR density, and consequently sensitivity of  $\alpha_1$ -AR-mediated mechanisms, reflecting diminished sympathetic NA outflow induced by central propranolol action (Mora *et al.* 1983) and reduced intrathymic CA synthesis (Hagerty *et al.* 2001) could be expected (Fig. 2).

Taken together, these data indicate that catecholaminergic modulation of thymopoiesis exhibits a substantial degree of GC-dependent plasticity (Fig. 2).



**Fig. 2.** Schematic representation of putative effects of  $\beta$ -adrenoceptor blockade with propranolol (0.4 mg/100g body weight/day, s.c.) on thymocyte differentiation in adult rats adrenalectomized four days before starting propranolol administration. Adrenalectomy, most probably, diminishing availability of noradrenaline derived from noradrenergic nerve fibres and thymic cells, and density of thymic  $\beta$ -adrenoceptors, augments efficiency of  $\beta$ -adrenoceptor blockade, and thereby increases thymocyte positive/decreased negative selection, and consequently frequency of the most mature single positive (SP) TCR $\alpha\beta^{\text{high}}$  thymocytes. In addition, following propranolol treatment in adrenalectomized rats increased density of thymic  $\alpha_1$ -adrenoceptors, and consequently efficiency of  $\alpha_1$ -adrenoceptor-mediated mechanisms leading to the increase in the frequency of CD4-CD8+TCR $\alpha\beta^{\text{high}}$  and the decrease in that of CD4+CD8-TCR $\alpha\beta^{\text{high}}$  cells is likely to occur. Thus, the net effect of propranolol administration in adrenalectomized rats is favoured CD8 over CD4 cell differentiation/maturation leading to a more pronounced increase in frequency

of CD4-CD8+SP TCR $\alpha\beta^{\text{high}}$  compared with that of CD4+CD8-SP TCR $\alpha\beta^{\text{high}}$  cells. Symbols and abbreviations: ↗, increase; ↘, decrease; Cap, capsule; Cx, cortex; CMJ, cortico-medullary junction; M, medulla; S, interlobular septa; DN, double negative; DP, double positive.

## Conclusions

The findings collected to date indicate that subsets of thymic lymphoid and non-lymphoid cells also synthesize CAs, which together with CAs delivered by neural and endocrine cells, act either directly or indirectly on thymocytes at distinct stages of maturation, and consequently modulate thymic T-cell output not only numerically, but also qualitatively. Furthermore, they showed that there is sexual dimorphism in both thymic NA level and thymocyte surface density of  $\beta_2$ -ARs, which may be responsible for sexual dimorphism in many thymic indices. Moreover, it is clear that CA modulation of thymopoiesis exhibits a significant degree of GC-dependent plasticity. At the present time, it is not clear what is relationship among neural, endocrine and locally synthesized CAs in T-cell development modulation.

Furthermore, putative significance of sexual dimorphism in NA levels and AR thymic cell densities for sexual dimorphism in immune response should be established. In addition, the relationship among circulating GCs and locally synthesized GCs and CAs is not understood. Finally, further work is required to clarify the influence of naturally occurring or drug-induced variance in thymic CA action on GC-mediated modulation of T-cell development.

## Conflict of Interest

There is no conflict of interest.

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