

# The Role of the Sympathetic Nervous System in the Thymus in Health and Disease

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## Key Words

Sympathetic nervous system • ■■■ 

## Abstract

The existence of a network of immunoneuroendocrine interactions that results in the reciprocal modulation of the classical functions of each system is well established at present. Most of the evidence derives from studies on secondary lymphoid organs, such as the spleen and lymph nodes. In this article, several aspects relevant to understand the role of the sympathetic nervous system in the establishment of these interactions in the thymus are discussed. At present, the sympathetic innervation of the thymus, the expression of adrenergic receptors in thymic cells, particularly of  $\beta$ -adrenergic receptors, and the effect of sympathetic neurotransmitters, although mainly derived from in vitro or pharmacological studies, seem to be relatively well studied. However, other aspects, such as the relevance that immune-sympathetic interactions at the thymic level may have for certain diseases, specially autoimmune or other diseases that primarily involve the activation of the immune system, as well as how the integration of sympathetic and hormonal signals at local levels may affect thymic functions, certainly deserve further investigation.

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## Introduction

The thymus is a central immune organ that in mammals and other species is responsible for the maturation of T lymphocytes. After entering the thymus, progenitor T cells multiply rapidly within the cortex but proliferation is paralleled by a large rate of cell death. A small portion of mature thymocytes migrates from the thymic cortex to the medulla, where they continue to mature and finally leave the gland through postcapillary venules. During the multiple steps of maturation, thymocytes undergo processes of negative and positive selection under the guidance of thymic epithelial stromal cells (TEC). Negative selection is based on clonal deletion of cells that recognize not only major histocompatibility complex antigens but also peripheral antigens that are expressed in the thymic stromal epithelium under the control of the transcription factor AIRE (autoimmune regulator) [1]. Although not exclusively [2], the thymus assures self-tolerance. Positive selection is based on rearrangement of gene segments linked to the generation of T cell receptors, which results in the highly diversified repertoire of T cells capable of recognizing and reacting to an enormous variety of antigens. These very sophisticated processes are based on an exchange of signals between thymocytes and

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thymic stromal cells. However, there is consistent evidence that neuroendocrine signals can influence T cell maturation and differentiation in the thymus. The initial evidence of the thymus as a hormonal target was that adrenalectomy results in thymus hypertrophy, while adrenal extracts can induce thymic atrophy [3, 4]. Hormones involved in thymus development and function are discussed in other articles in this issue. There is also evidence that the thymus receives innervation and that thymocytes are exposed to signals mediated by neurotransmitters and neuropeptides. We shall refer here to the sympathetic innervation of the thymus, the expression of adrenergic receptors on thymocytes, and the relevance that the contribution of the sympathetic nervous system (SNS) may have for the regulation of thymic functions. As compared to the large number of studies reporting effects of the SNS and its main mediator noradrenaline (NA) on mature immune cells in secondary lymphoid organs, such as the spleen and lymph nodes, or in the circulation, there are less studies dealing with thymic cells. This article does not pretend to be an exhaustive review; rather it is limited to some selected aspects. Other neurotransmitters and neuropeptides that can potentially contribute to thymus physiology and pathophysiology are not addressed here, nor are SNS effects in the thymus during aging included since this is the main topic of another article in this issue.

### **The SNS in the Control of Immunity**

The existence of a network of immunoneuroendocrine interactions that results in the reciprocal modulation of the classical functions of each system is well established at present. Together with the hypothalamic-pituitary-adrenal axis, the contribution of the SNS to this network is probably the best studied. The main reason why research in this field has originally concentrated on these systems is that, under physiological conditions, they usually act in a concerted fashion, and the classical example is their nearly simultaneous response following stressful stimuli. Experimental evidence accumulated during more than 30 years has shown that the SNS can affect almost all mechanisms involved in innate and acquired immune responses [5–8]. Retrospectively, it is not surprising that the results seem to be contradictory sometimes since whether sympathetic neurotransmitters promote or inhibit immunity depends on numerous variables. Among such variables, the type of immune response, the time when the SNS is activated in relation to

the particular state of immune and accessory cell activation, and which type of adrenergic receptors is predominantly stimulated seem to be decisive for the immunological outcome, demonstrating the complexity of immune-SNS interactions. From a physiological point of view, however, this complexity is not a distinctive attribute concerning the immune system only; comparable ‘dual’ effects of sympathetic mediators are observed in most systems regulated by the SNS, such as the respiratory system, or organs, such as the heart, just to mention two examples.

A concept that seems to us worth stressing here is that, according to our actual knowledge, neither hormones nor neurotransmitters can change the specific functions for which cells, including immune cells, have been programmed, but that their main role is to modulate their activity.

### **The Sympathetic Innervation of the Thymus**

There is a large body of evidence that the thymus receives a dense sympathetic innervation. The first reference showing the presence of catecholamines in the mammalian thymus that we were able to trace is a work from 1974 [9]. Using a modification of the method developed by Falck and Hillarp in the 1960s, Sergeeva [9] localized catecholamines in the thymus of cats and mice, mainly in nerve fibers accompanying small blood vessels and directly innervating the parenchyma. He also described that Hassall’s corpuscles were closely connected with varicose adrenergic nerve fibers. Since then, the development of more refined techniques and the availability of selective and sensitive antibodies has led to a detailed description of the origin and distribution of sympathetic nerve fibers in the thymus, an aspect pioneered by Felten and Bulloch and colleagues [10–12].

The sympathetic fibers that innervate the thymus originate from postganglionic neurons in the upper paravertebral ganglia of the sympathetic chain, particularly the superior cervical and stellate ganglia [12–14]. These neurons are connected with central nervous system neuronal pathways, as shown by retrograde, transneuronal virus tracing [15]. There are connections in the spinal cord to the intermediolateral cell column at levels T<sub>1</sub>–T<sub>7</sub> that spread to the central autonomic nucleus, the intercalated cell nucleus and the lateral funiculus. Within the brain, peripheral noradrenergic ganglia are also connected to brain structures of the medulla oblongata, pons and hypothalamus. Connections with the hypothalamus

were observed within the arcuate nucleus, dorsal, lateral, and posterior hypothalamus and in all parvicellular subdivisions of the paraventricular hypothalamic nucleus [15].

Noradrenergic fibers enter the capsule and interlobular septa of the thymus as dense nerve plexuses along with large blood vessels from where they diverge into the thymic cortex and enter into the parenchyma. NA-containing fibers are predominantly found in the cortex, with a slightly higher density near the corticomedullary junction, a region where the more mature thymocytes localize. Sympathetic fibers are also found adjacent to TEC in the deep cortex and in the medulla.

The release of NA by sympathetic fibers in the thymus is modulated at prejunctional levels by  $\alpha$ -adrenergic, cholinergic, P1-purineric and prostaglandin E<sub>2</sub> receptors [8], but the main regulatory effect is mediated by the inhibitory effect of released NA acting at prejunctional  $\alpha_2$ -receptors [16, 17]. Thus, endogenous NA mediates a tonic inhibition of its own release.

In summary, there are close contacts between noradrenergic nerve fibers, thymocytes and TEC, and there is anatomical evidence that noradrenergic nerve activity in the thymus can be controlled by signals from the central nervous system due to the connectivity of the sympathetic ganglia from which they originate. Furthermore, there are intrinsic mechanisms that control NA production and release by sympathetic nerves. However, it should be kept in mind that, under physiological conditions, the effect of the SNS on thymic cell maturation and education is the outcome of multiple interactions between sympathetic and other neurotransmitters and the endocrine system, and probably also depends on the immunological status of the host.

### Adrenergic Receptors in the Thymus

Ahlquist's [18] seminal concept of the existence of two different types of adrenergic receptors was a scientific breakthrough in physiology. Although 9 subtypes of adrenergic receptors, mainly defined by their pharmacological characteristics, have been identified today, his work provided an essential advance to understand the functioning of the autonomic nervous system [19]. About 28 years later, Singh and Owen [20] postulated the presence of  $\beta$ -adrenergic receptors in mouse thymocytes based on their finding that *in vitro* treatment of fetal thymic cells with the  $\beta$ -agonist isoproterenol, but not with the  $\alpha$ -stimulant phenylephrine, induced an increase in

the proportion of Thy-1-positive cells. A few years later, they showed that developing thymocytes possess specific  $\beta_2$ -adrenergic binding sites, and that marked changes in receptor density and distribution could be detected at different stages of thymic cell maturation and differentiation [21]. Later, radioligand binding studies as well as Northern blot analysis confirmed that  $\beta_2$ -adrenergic receptors are also expressed by rat thymic cells [22]. Using autoradiography, this group also showed that the majority of the  $\beta_2$ -adrenergic receptors are localized in the medullar compartment of the thymus, an observation consistent with a maturation-dependent regulation of  $\beta$ -expression in thymocytes [22, 23]. Expression of  $\beta$ -adrenergic receptors in thymocytes has also been demonstrated in other species, including chicken [24]. In most of these studies and in others not included here, it has been shown that these receptors are functional, by evaluating, for example, classical effects of  $\beta$ -stimulation such as increases in cAMP levels following exposure of thymic cells to the appropriate agonists.

Although not so abundant as for  $\beta$ -adrenergic receptors, there is evidence that  $\alpha_1$ -adrenoceptors are present mainly on less mature CD3<sup>-</sup> and CD3<sup>low</sup> thymic cells, as shown by immunohistochemistry and flow cytometry [25]. Finally, pharmacological studies showing dual, dose-dependent effects of  $\alpha_2$ -adrenergic agonists indicate that this receptor subtype is also present on thymic cells [26, 27].

Due to the essential role of TEC in the process of positive and negative selection in the thymus, the expression of adrenergic receptors in this cell type is also of interest. mRNA for  $\beta_1$ - and  $\beta_2$ -adrenoceptors has been detected in rat cultured TEC, as well as specific, saturable binding of  $\beta$ -adrenergic agonists [28]. The functionality of these binding sites has been confirmed by increased intracellular cAMP levels following TEC exposure to specific  $\beta_1$ - and  $\beta_2$ -agonists, while no effects were elicited by a  $\beta_3$ -adrenoceptor stimulus. The other subtype of adrenoceptors reported to be present on TEC are  $\alpha_1$ -adrenoceptors [25]. Since these authors also found  $\alpha_1$ -receptors on thymocytes, they proposed that this receptor subtype may be involved in intrathymic communication.

An interesting aspect in this context is the differential expression of adrenergic receptors on thymocytes during development. Using radioligand binding studies, it has been reported that the number of  $\beta$ -adrenergic binding sites in both fetal and adult thymocytes is the same, but that the affinity is less in adult cells, the amount of bound radioligand being about 8 times greater in embryonic cells [21]. Other authors have also reported that there is a

significantly higher  $\beta$ -binding activity in the early phases of murine thymus development, with a peak at about the day of birth, followed by a sharp drop between 2 and 4 days after birth, and an even further decrease in subsequent weeks [29]. Particularly regarding  $\beta_2$ -adrenergic receptors, it has been shown that their density increases progressively between birth and 70 days of age, reaching half-maximal levels at 17 days, and a plateau at 40 days [30].

The state of maturation and differentiation also seems to be decisive for the density of  $\beta$ -adrenergic receptors present in thymocytes. While unfractionated thymocytes express low levels of high-affinity  $\beta$ -adrenergic receptors compared to mature T cells, mature medullary thymocytes had a number of receptors per cell comparable to those of splenic T cells [31]. Binding of the adrenergic radiolabeled adrenergic antagonists [ $^3\text{H}$ ]-dihydroalprenolol 1-[ring propyl- $^3\text{H}$ (N)] and 4-(3-t-butylamino-2-hydroxypropoxy)-[5,7- $^3\text{H}$ ]benzimidazol-2-one was used in these studies. Other authors have reported that a population of thymocytes, consisting mainly of immature cells, has less receptor sites per cell as evaluated by the binding of the  $\beta$ -antagonist pindolol, but that there is no difference in their affinity. Also, cortisone-sensitive, immature thymocytes bear fewer  $\beta$ -receptors than mature T cells. It has been estimated that freshly prepared thymocytes, consisting predominantly of immature T cells, express an average of approximately 500 sites per cell, with an affinity of about 39 pM [32].

### Plasticity of Adrenergic Receptor Expression in the Thymus

Besides the differences in adrenergic receptor numbers during ontogeny and at different stages of cell maturation, there is evidence that their expression in the thymus can be physiologically modulated. A few selected examples serve to illustrate that hormonal and immunological stimuli can exert such modulation.

A significantly increased number of  $\beta_2$ -binding sites is observed during the estrous cycle and pregnancy in the rat thymus [22]. Accordingly, treatment with estradiol, alone or in combination with progesterone, induces a marked increase in  $\beta$ -receptor density. Castration results in a strong reduction and in marked differences in the organization and distribution of the thymic  $\beta$ -adrenergic receptor [30].

Acute (2-hour immobilization) stress induces an increase in dihydroalprenolol- (a  $\beta$ -adrenergic blocker)

binding sites in thymocytes (but not in splenic cells), indicating an upregulation of  $\beta$ -adrenergic receptors [33].

Stimuli that result in immune activation can also affect adrenergic receptor expression in the thymus. For example, it has been reported that the number of  $\beta$ -receptors in thymocytes is decreased by in vitro culture with phorbol myristate acetate, but increased by concanavalin A [32]. Very interesting are also the studies by Morale et al. [34] showing marked changes in both number and distribution of  $\beta_2$ -adrenergic receptors in the rat thymus during the course of an in vivo immune response. A sharp decrease in receptor number paralleled by a significant loss of the autoradiographic reaction in the thymic medullary compartment was observed 3 days after the challenge with bovine serum albumin in complete Freund's adjuvant. However, at the pick of the immune response, these effects were followed by a significant increase in receptor density and number without changes in receptor affinity. These findings were corroborated by evaluation of adenylyl cyclase activity and  $\beta_2$ -adrenergic receptor mRNA in the thymus.

The inclusion of  $\beta_2$ -agonists in the diet has illegally been used to improve production performance. In this context, it is interesting to mention a publication showing that chicken raised specifically for meat production have, among other disturbances, a marked decrease in  $\beta_2$ -adrenergic receptor concentration in the thymus when they are fed for 21 days with a diet containing  $\beta_2$ -adrenergic agents [35].

Taken together, the evidence briefly described above indicates that the potential plasticity of thymic adrenergic receptor expression can contribute to immunoneuroendocrine interactions in the thymus.

### Effects of Sympathetic Mediators on Thymic Cells

As mentioned above, the postulation of the existence of adrenergic receptors on thymocytes was, in fact, based on the capacity of the  $\beta$ -agonist isoproterenol to increase the proportion of fetal thymic cells bearing Thy-1 [20]. It is interesting to mention that it has later been shown that Thy-1 (CD90), a glycoprotein that is important for cell adhesion and signal transduction in T cell differentiation and proliferation, is also present in a variety of stem cells and in the axonal processes of mature neurons. Singh [36] also showed that nonlymphoid rudiments of fetal thymus from nude mice support the development of thymocytes when transplanted into the anterior chamber of a sympathectomized eye, and that, following sympathetic

nude thymus is able to sustain lymphopoietic activity and generate lymphoid cells, characteristics present on thymocytes during *in vivo* development in normal mice [37]. This work provided the first evidence that the SNS can exert functional effects on the thymus. Since then, many reports supporting these findings have been published. It is beyond the scope of this article to review the abundant literature in detail, and the reader is referred to two excellent recent reviews for more references [8, 38].

In what follows, we provide a short overview of some selected *in vitro* and *in vivo* effects of sympathetic mediators considered relevant for thymic functions.

Most *in vitro* studies agree that the  $\beta$ -adrenergic agonist isoproterenol can induce an increase in cAMP content [39–41] and apoptosis in thymocytes [42] or in fetal organ cultures [40]. We have shown that the apoptotic effect of NA in thymocytes is mediated by  $\beta$ -receptors [43] and that this effect is Fas-independent [44].

It has also been shown that NA, adrenaline and isoproterenol can decrease concanavalin-A-induced thymocyte proliferation [45]. Since  $\alpha$ -adrenergic agonists have no effect on this parameter and the effect of NA could not be reversed by the  $\beta$ -antagonist propranolol nor by the  $\alpha$ -antagonist phentolamine, the authors proposed the existence of some alternative mechanism, such as a still unidentified adrenergic receptor or a nonreceptor-mediated effect, like binding of catecholamine oxidation products to proteins. Using fetal thymic cultures, other authors have shown that the  $\alpha_1$ -agonist phenylephrine inhibits cell proliferation [46], while others showed that  $\alpha_2$ -agonists increase apoptosis of thymic cells [27], but that the effect of these agonists on concanavalin-induced proliferation of thymocytes depends on the dose used [26].

Regarding other effects, it has been demonstrated that NA and its main metabolite 3-methoxy-4-hydroxyphenylglycol can suppress thymocyte chemotaxis, an effect that can be blocked by  $\alpha$ - and  $\beta$ -antagonists [47]. Using an electrophysiological approach, it has been shown that NA inhibits outward voltage-dependent potassium current in thymocytes, an important finding since  $K^+$  channels are believed to be involved in T cell differentiation [16].

Sympathetic agents can also affect TEC *in vitro*. Adrenaline and NA increase cAMP production by these cells, and decrease their basal and stimulated proliferation, effects that are exerted via  $\beta_1$ - and  $\beta_2$ -receptor subtypes and modulated by dexamethasone [28]. Finally, NA and isoproterenol increase IL-6 production by TEC provided that they are simultaneously costimulated with lipopolysaccharide or TNF- $\alpha$  [48, 49], an important effect

considering that IL-6 has been implicated in the modulation of thymic development. An overview of the *in vitro* effects of sympathetic agents is shown in table 1.

As already mentioned, the early *in vivo* work of Singh [15] indicated that the SNS is one important input in the control of thymocyte development. Consistent with these results are other *in vivo* studies showing that administration of the nonselective  $\beta$ -agonist isoproterenol to mice results in reduced thymus weight and thymocyte number [50] and that an increased thymocyte apoptotic index is observed in pigs after prolonged (2 months) feeding with anabolic doses of the  $\beta_2$ -agonist clenbuterol [51]. Also decreased thymus weight and cellularity are observed in the thymus of the offspring of mice treated with the  $\beta_2$ -agonist salbutamol [52]. These findings are in line with the *in vitro* immunosuppressive and proapoptotic effects of NA and  $\beta_2$ -agonists. However, *in vivo* blockade of  $\beta$ -receptors seems to exert effects comparable to those induced by their stimulation. For example, *in vivo* treatment with propranolol, a nonselective  $\beta$ -blocker, also results in increased thymic apoptosis, which prevails over its proliferative effects, and results in an increased percentage of more mature double-negative lymphocytes, and a decreased relative proportion of double-positive precursors [53].

Regarding *in vivo* effects of stimuli acting on  $\alpha$ -receptors, Stevenson et al. [54] showed that there is a decrease in all thymic populations in rats that had been treated simultaneously with NA and propranolol, thus increasing predominantly the  $\alpha$ -tone. Injection into rats of low doses of xylazine, a selective  $\alpha_2$ -agonist, stimulates *in vivo* concanavalin-induced thymocyte proliferation [26]. However, the opposite effect, namely decreased proliferation, as well as *in vivo* IL-2 production are observed when high doses of this agonist are administered. The effect of chronic  $\alpha_1$ -blockade by urapidil seems to depend on the age of the rats to which it is administered. While this treatment results in decreased thymic cellularity, mainly by decreasing the proportion of CD4<sup>+</sup>CD8<sup>-</sup> thymocytes in immature rats, it increases thymus weight and favors the maturation of this cell subtype over that of CD4<sup>+</sup>CD8<sup>+</sup> cells in adult animals [55].

The other *in vivo* approach used to study the effect of the SNS on the thymus is based on sympathetic denervation. It has been reported that adult chemical sympathectomy in mice and rats results in decreased thymic cellularity and/or weight [56–58]. Double-positive CD4<sup>+</sup>CD8<sup>+</sup> cells seem to be the most affected population [58], apoptosis is increased but there is an increased cellular proliferation in the thymus cortex [56]. On the other hand, it

**Table 1.** Effects of adrenergic mediators on thymic functions in vitro

Species	Substance	Cell type	Effect	Notes	Reference
Mouse	isoproterenol	thymocytes	↑ cAMP	cAMP (basal levels): PBL > spleen and lymph node cells > thymocytes cAMP (following β-stimulation): thymocytes >> spleen and lymph node or peripheral blood cells Corticoreistant thymocytes < whole population Corticoreistant thymocytes > peripheral T cells	39
Mouse	isoproterenol	thymocytes	↑ Thy-1 (CD90) mRNA (concentration dependent)	← propranolol	73
Mouse	isoproterenol	thymocytes	↑ cAMP	DNA fragmentation only observed if another signal activates protein kinase c	41
Mouse	NA isoproterenol	thymocytes	↑ apoptosis	β-adrenergic mediated Fas independent	43, 44
Mouse	isoproterenol	thymocytes	↑ apoptosis of CD4+CD8+ thymocytes	Tunel technique Caspase-3 activation Gα dependent; PKA independent	42
Mouse	NA, A or isoproterenol	thymocytes	↓ ConA-induced proliferation	No effect of phenylephrine, clonidine NOT ← propranolol or phentolamine	45
Mouse	isoproterenol	fetal organ cultures	↑ cAMP ↑ apoptosis		40
Mouse	phenylephrine	fetal thymic explants	↑ proliferation		46
Rat	xylazine	thymocytes	↑ ↓ ConA-induced proliferation High doses: ↓ IL-2 ↓ IL-2 receptor	Low doses: ↑ proliferation High doses: ↓ proliferation	26
	xylazine	thymocytes	↑ apoptosis		27
	NA	thymocytes	↓ ↓ chemotaxis	← propranolol	47
	MHPG			← phentolamine	
Mouse	NA or isoproterenol (costimulated)	TEC	↑ IL-6 production	Implicates modulation of thymic development No effect of the catecholamines alone, but additive with TNF-α or synergistic with LPS	48, 49
Rat	A, NA or isoprenaline	TEC	↑ cAMP ↓ basal and serum-stimulated proliferation	β <sub>1</sub> - and β <sub>2</sub> -receptor subtypes downregulated by dexamethasone or cortisol	28

A = Adrenaline; MHPG = 3-methoxy-4-hydroxyphenylglycol; PBL = peripheral blood lymphocytes; isoproterenol = β-agonist; isoprenaline = β-agonist; phenylephrine = α<sub>1</sub>-agonist; xylazine = α<sub>2</sub>-agonist; clonidine = α<sub>2</sub>-agonist; propranolol = β-antagonist; phentolamine = α-antagonist; ↑ = increase; ↓ = decrease; ↓ ↓ = inhibit; ← = reversed/blocked by.

has been reported that no changes in thymus weight are observed when chemical sympathectomy is performed in mice at birth [59].

Finally, an interesting work based on the use of mice in which dopamine β-hydroxylase, the enzyme that catalyzes the conversion of dopamine to NA, has been knocked down showed that this deficit does not affect thymic cell numbers and subpopulations nor the in vitro response of thymocytes to mitogens if mice are bred under specific pathogen-free (SPF) conditions. However, an

extreme thymic involution, mainly based on a decrease in double-positive CD4-CD8+ cells, is observed when these knockouts are kept in non-SPF conditions [60]. The results briefly discussed above are summarized in table 2.

There is some contradiction between these results since similar effects were observed independently of whether agonists or blockers were administered. Several reasons could account for this apparent discrepancy. The pharmacological in vivo administration of adrenergic agonists and blockers certainly affects many physiological

**Table 2.** Effects of adrenergic mediators on the thymus following in vivo manipulations

Species	Manipulation	Effect	Reference
Mouse	Sympathectomy + thymus and ganglia implant	↓↓ thymic lymphopoiesis	36
Mouse	Nude nonlymphoid thymic rudiments implanted into the anterior eye chambers of denervated mice	Denervated nude thymus sustains lymphopoietic activity and generates lymphoid cells	37
Mouse	Adult chemical sympathectomy (1–3 days before exp.)	↓ thymic cellularity ↓ spontaneous and in vitro ConA-induced proliferation ↓ in vivo LPS-induced proliferation	57
Mouse	Adult chemical sympathectomy	↓ thymus weight; ↓ thymic cellularity CD4+CD8- more affected than other populations ↑ apoptosis; ← desipramine pretreatment	58
Rat	Adult chemical sympathectomy	↓ thymus weight; ↓ cellularity; ↑ apoptosis ↑ numbers proliferating cells in cortex	56
Mouse	Chemical sympathectomy at birth	No changes in thymus weight ↑ number Hassall's corpuscles Huge Hassall's corpuscles engorged with cellular debris	59
Mouse	Isoproterenol	↑ cAMP, potentiated by hydrocortisone ↓ thymus weight	50
Pig	Clenbuterol ( $\beta_2$ -agonist) fed anabolic doses for 2 months	↑ apoptosis	51
Rat	Propranolol treatment 16 days	↑ ↑ apoptosis > ↑ proliferation; ↓ mature CD4+CD8- ↓ relative proportion of CD4+CD8+ precursors	53, 74
Rat	Propranolol treatment of immature rats	↓ thymus size and volume in cortex and medulla	75
Rat	Urapidil ( $\alpha_1$ -blocker) for 15 days	Age-dependent effects Immature rats: ↓ cellularity, organ size total number of thymocytes ↓ CD4+CD8-; ↑ CD4+CD8+ Adult: ↑ thymus weight and cellularity ↑ CD4+CD8-; ↓ CD4+CD8+ Favor maturation of CD4+CD8- over CD4+CD8+ thymocytes	55
Mouse	Offspring of mothers treated with salbutamol ( $\beta_2$ -agonist)	↓ thymus weight; ↓ thymus cellularity No changes in % populations	52
Rat	Xylazine ( $\alpha_2$ -agonist)	Low doses ↑ in vitro ConA-induced proliferation High doses: ↓ ConA-induced proliferation; ↓ IL-2 production in vivo; ← IL-2 administration	26
Rat	12 h s.c. NA and propranolol (↑ $\alpha$ -tone)	All populations ↓ (not due to redistribution)	54
Mouse	Dopamine $\beta$ -hydroxylase knockout	Bred in SPF conditions: normal cell number, subpopulations and in vitro response to mitogens Bred in non-SPF conditions: extreme thymic involution; ↓ CD4+CD8+	60

↑ = Increase; ↓ = decrease; ↓↓ = inhibit; ← = reversed/blocked by.

functions in the host, and blood circulation is among them. In the thymus, the major arteries and veins enter and leave via the septa, and articulate out at the cortico-medullary junction, with capillaries looping out into the cortex. Blood vessels are the main route taken by the thymic cells that reach the final steps of intrathymic maturation to leave the gland. Thus, a sympathetically induced vasoconstriction, with the consequent reduction of blood flow, could affect the outflow of single positive thymic cells. However, other mechanisms could interfere with

this effect. For example, we have shown that NA stimulates lymphoid cell mobilization from the spleen via  $\beta$ -adrenergic receptors [61], and that endogenously produced IL-1 counteracts the  $\alpha$ -adrenergic effects of sympathetic nerves on the splenic vascular structures by inhibiting the postjunctional release of NA [62]. It is therefore possible that a similar mechanism could also operate in the thymus.

Other effects should also be considered when interpreting the effects of chemical sympathectomy induced

by 6-hydroxydopamine (6-OHDA), the most commonly used neurotoxin, since it has many other effects *in vivo*. For example, it can affect behavior, adrenergic receptor expression, pre- and postsynaptic supersensitivity, and hormonal levels. It should also be taken into account that 6-OHDA results in mainly peripheral or in combined central and peripheral effects depending on whether it is injected into adult or newborn mice. Another important variable is the time after denervation when the experiments are performed, since the neurotoxin also induces an increase in corticosterone blood levels that can last for several days after 6-OHDA injection. Also, 6-OHDA does not deplete NA in the thymus to the same extent as in other tissues, such as the spleen. A detailed review of the *in vivo* effects of 6-OHDA can be found in Kostrzewa and Jacobowitz [63]. Furthermore, this drug can directly induce apoptosis of thymocytes *in vitro* [58]. However, even when all these restrictions are considered, the evidence indicates that the SNS, alone or in concerted action with other endogenous mediators, can affect thymic functions.

#### **Disturbances of Thymic Functions Related to Sympathetic Alterations?**

Anomalies of the thymus gland are rare diseases. Among them are tumors originating from TEC (thymomas), neuroendocrine neoplasms, nonneoplastic thymic hyperplasias, congenital defects including thymic aplasia, or congenital deficiency of the thymus (DiGeorge syndrome). Defects in the processes that induce immunologic central tolerance to self-antigens, thus resulting in autoimmune diseases, can, of course, also be due to alterations in thymic functions. Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy, the first systemic autoimmune disease found to be due to a defect in a single gene and characterized by a large array of clinical features, is extremely rare and is caused by a deficiency of the autoimmune regulator (AIRE) gene in the thymus. Myasthenia gravis is often associated with thymic hypertrophy. In the acquired T cell immunodeficiency syndrome (AIDS), although the major effect of the HIV virus is on mature peripheral T cells, it can also infect developing thymocytes in the thymus.

We have not been able to find published evidence strictly related to disturbances in thymic functions due to alterations in the sympathetic innervation of the gland, nor related to the opposite situation, namely that pathologies involving primarily thymic functions induce alterations in the activity of local sympathetic nerves. In what

follows, we summarize some results that might indicate the existence of such a link.

Spontaneously hypertensive rats (SHR) have increased SNS activity and moderate immune deficits that have been implicated in the onset of hypertension. Among the immune alterations, SHR rats have a reduced number of immature T lymphocytes. Purcell and Gattone [64] have reported that the SHR thymus exhibits a strain-related increase in sympathetic innervation at 2 and 12 weeks of age compared to other immunocompetent, normotensive rat strains. Morphologically, they detected that the thymus of adult SHR rats does not display the increase in medullary volume typically noted with aging [65]. Although no causal relation was established in these studies, based on reports showing that developing thymocytes possess adrenergic receptors and that catecholamines can alter the expression of T cell surface alloantigens, the authors propose that the maturation process of T cells may be altered by this increased innervation.

Arthritis induced experimentally by adjuvant or collagen injection into rats is often used as a model of rheumatoid arthritis. The fact that chemical sympathectomy exacerbates the symptoms of the disease supports a role for noradrenergic innervation in the pathology [66, 67]. However, sympathetic denervation can produce opposite effects on the outcome of the disease, depending on the route of neurotoxin administration or differences in the mechanisms by which sympathetic nerve terminals are destroyed [68] whether nerve ablation is performed before immunization or during the chronic phase of the disease [69]. Much less is known about the sympathetic nerve activity in the thymus during the disease. It has been reported that while no alterations in the sympathetic innervation of any secondary lymphoid organs from arthritic rats were observed, the density of sympathetic nerve fibers was increased in the thymus. Also, while NA concentration in the spleen and lymph nodes was decreased, it was significantly increased in the thymus of rats with arthritis [70]. However, the relevance of the alterations observed in the thymus for the development and course of arthritis is at present unknown.

The consequences of ethanol exposure at prenatal stages on the sympathetic components of the thymus are also interesting. Adult mice that had been exposed to alcohol *in utero* show a depressed ability to produce cellular immune responses, paralleled by altered noradrenergic synaptic transmission selectively in lymphoid organs, including a reduction in NA levels and  $\beta$ -adrenoceptor density in the thymus [71].

We have reported that the sympathetic innervation in the spleen of *lpr/lpr* mice, which develop an autoimmune lymphoproliferative disease, gradually disappears and that this alteration in the SNS contributes to the pathology [44]. We have also shown that NA is capable of inducing apoptosis in thymocytes of these Fas-deficient mice. However, we do not know if alterations in thymic sympathetic components have an impact on the development of the disease in this model of lupus erythematosus.

Using a parasite model, we have found that infection of mice with *Trypanosoma cruzi*, the causal agent of Chagas' disease, results in a markedly decreased NA content in the thymus [unpublished results]. However, neither in this model do we know the relevance that this sympathetic alteration in the thymus may have for the course of the infection.

Finally, there is evidence that patients with epilepsy have abnormalities in immune functions, and significant activation of both the SNS and the hypothalamic-pituitary-adrenal axis following seizure activity. Using a model of long-term kindled seizures, it has been found that kindled rats have increased thymus weight and cellularity, increased rate of apoptosis and proliferation of TEC, decreased numbers of CD3<sup>+</sup> cells, a decreased ratio of CD4<sup>+</sup>/CD8<sup>+</sup> cells and reduced proliferative response to T cell mitogens [72]. Also marked morphological changes were detected in the thymus of the rats with seizures, including disappearance of the borderline between the cortex and the medulla, large aggregates of

TEC, and pathological evidence of epithelial cell thymoma in a large proportion of kindled rats. Treatment of the animals with guanethidine, a drug that reduces the release of catecholamines, resulted in amelioration of most thymic alterations, including the level of adrenoceptors, the rate of apoptosis, and the morphological changes, as well as disappearance of thymomas in nearly all rats with kindled seizures. These results allowed the conclusion that the SNS mediates the functional and pathological changes in the thymus during kindled seizures.

## General Conclusions

We have summarized here several aspects that we consider relevant to understand the role of the SNS in the thymus. At present, the sympathetic innervation of the thymus, the expression of adrenergic receptors in thymic cells, particularly of  $\beta$ -adrenergic receptors, and the effect of sympathetic neurotransmitters, although mainly derived from in vitro or pharmacological studies, seem to be relatively well established. However, other aspects, such as the relevance that immune-sympathetic interactions at the thymic level may have for certain diseases, specially autoimmune or other diseases that primarily involve the activation of the immune system, as well as how the integration of sympathetic and hormonal signals at local levels may affect thymic functions, certainly deserve further investigation.

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