Acta Physiologica Sinica, August 25, 2006, 58 (4): 309-317
http://www.actaps.com.cn

Brief Review

Immunoregulatory role of endogenous catecholamines synthesized by immune cells

JIANG Jian-Lan[1,2], QIU Yi-Hua[2], PENG Yu-Ping[2,*], WANG Jian-Jun[1]

[1]Department of Biological Science and Technology, School of Life Sciences, Nanjing University, Nanjing 210093, China; [2]Department of Physiology, School of Basic Medical Sciences, Nantong University, Nantong 226001, China

Abstract: It has been well known that catecholamines (CAs) in the body, including norepinephrine (NE), epinephrine (E) and dopamine (DA), are synthesized and secreted by neurons and endocrine cells and mainly modulate visceral activities such as cardiovascular, respiratory and digestive functions. The studies over the past nearly 30 years have shown that CAs can also regulate immune function. The immunomodulation of CAs is generally considered as a role mediating the regulation of nervous and endocrine systems. However, recent studies reveal that immune cells can also synthesize CAs, which is an update of traditional concept. A classical metabolic pathway of CAs shared by the nervous and endocrine systems is present in the immune cells, i.e., the immunocytes have the enzymes for synthesis of CAs [e.g. tyrosine hydroxylase (TH)] and the enzymes for degradation of CAs [e.g. monoamine oxidase (MAO) and catechol-O-methyl transferase (COMT)]. The endogenous CAs synthesized by immune cells can regulate many immune functions, including cellular proliferation, differentiation, apoptosis and cytokine production. These roles of the endogenous CAs may be mediated by an autocrine/paracrine pathway via relevant receptors on the immunocytes and intracellular cAMP. Intracellular oxidative mechanism may also be involved in immunoregulation of endogenous CAs in immune cells. In addition, some metabolic abnormalities of CAs in the immune cells probably induce some autoimmune diseases, such as multiple sclerosis (MS) and rheumatoid arthritis. These findings not only provide evidence for the new concept that the immune system is possible to become the third CA system other than the nervous and endocrine systems, but also extend our comprehension on functional significance of the endogenous CAs synthesized by immune cells.

Key words: catecholamines; lymphocytes; tyrosine hydroxylase; adrenergic receptor; cyclic AMP

免疫细胞内源性儿茶酚胺的免疫调节作用

姜建兰[1,2]，邱一华[2]，彭聿平[2,*]，王建军[1]

[1]南京大学生命科学院生物科学与技术系，南京 210093；[2]南通大学基础医学院生理学教研室，南通 226001

摘　要：机体内儿茶酚胺(catecholamines, CAs)包括去甲肾上腺素(norepinephrine, NE)、肾上腺素(epinephrine, E)和多巴胺(dopamine, DA)。CAs 由神经元和内分泌细胞合成和分泌，其主要功能是调节心血管、呼吸和消化等内脏活动。近三十年来的研究说明，CAs 也参与调控机体的免疫功能，但 CAs 的这种免疫调节作用一般视为神经和内分泌系统调节的介导作用。然而，近年来的研究发现，免疫细胞也能合成 CAs，这是对传统观念的一种补充和提高。免疫细胞内存在经典的 CAs 代谢途径，既有合成 CAs 的酪氨酸羟化酶(tyrosine hydroxylase, TH)又有降解 CAs 的单胺氧化酶(monoamine oxidase, MAO)和儿茶酚氧位甲基移位酶(catechol-O-methyl transferase, COMT)。免疫细胞合成的内源性 CAs 可以调控细胞的增殖、分化、凋亡和细胞因子生成等多种免疫功能。CAs 的这些作用可能主要通过自分泌或旁分泌途径作用于免疫细胞上相应受体和细胞内环磷酸腺苷(cyclic AMP, cAMP)实现。细胞内氧化应激机制可能也参与免疫细胞内源性 CAs 的免疫调节作用。此外，一些自身免疫性疾病如多发性硬化、风湿性关节炎可能也与免疫细胞内 CAs 的代谢异常有关。上述发现不仅为免疫系统有可能成为除神经

Received 2006-05-08　Accepted 2006-06-18

This work was supported by the Natural Science Foundation of Jiangsu Province (No. 04KJA180110), Social Development Foundation of Nantong (No. S40057, S5040), the National Natural Science Foundation of China (No. 30370462), the NSFC-RGC Joint Research Scheme of the National Natural Science Foundation of China (No. 30318004), and RFDP of the State Educational Ministry of China (No. 20050284025).

*Corresponding author. Tel: +86-513-85051714; Fax: +86-513-85051543; E-mail: yppeng@ntu.edu.cn

和内分泌系统以外的第三个CA能系统提供了证据，而且为免疫系统内源性CAs的功能意义拓展了认识。

关键词：儿茶酚胺；淋巴细胞；酪氨酸羟化酶；肾上腺素受体；环磷酸腺苷
中图分类号：R392.1

Over the past 20 to 30 years, an interesting development in the studies of neuro-endocrine-immune interaction is that some cells in the neuroendocrine and immune systems can synthesize neuropeptides/neurotransmitters, hormones and cytokines, as well as their relative receptors. These mediators coexisting in the neuroendocrine and immune systems can be used as common mediators or common language in the neuro-endocrine-immune modulating network[1]. Through these common mediators, the nervous, endocrine and immune systems are able to regulate their activities reciprocally and make the body respond properly according to various changes of internal and external environments. Thus, functional homeostasis of the body can be maintained and diseases can be prevented.

Catecholamines (CAs) in the body, including dopamine (DA), norepinephrine (NE) and epinephrine (E), have been well known to be modulators from nervous and endocrine systems and to regulate various functional activities of the body. The modulation of cardiac muscles, smooth muscles and glands by CAs allows the functional homeostasis of various systems in the body, such as cardiovascular, respiratory, digestive and renal systems. However, recent studies have found that CAs are not only important modulators regulating various functional activities, but also common language in the neuro-endocrine-immune interactive network. The sympathetic nerve fibers directly innervate lymphoid organs[2-4]. CAs can modulate immunocyte proliferation, differentiation, apoptosis and cytokine production through the receptors on the immunocytes, including β_2-, α_1-, α_2-adrenoreceptors and D_1, D_2-like DA receptors[5-13]. Our previous studies also have shown that NE can suppress concanavalin A (Con A)-induced lymphocyte proliferation[12] and interleukin-2 (IL-2) production, and also attenuate cytotoxicity of natural killer (NK) cells[13]. For many years, we have believed that only neurons and endocrine cells can synthesize CAs. Therefore, the immunomodulation of CAs is considered as a role of nervous and endocrine systems. Recent studies, however, have revealed that besides neuronal and endocrine cells, many kinds of immune cells can synthesize and secrete CAs. The endogenous CAs synthesized by immunocytes may regulate various immune functions through paracrine/autocrine or other pathways. These new findings not only pose a challenge to the traditional views on origins and roles of CAs, but also extend our understanding of neuroimmunomodulation of CAs, and meanwhile provide more research space for the common mediators in the neuro-endocrine-immune modulating network.

1 Evidence for synthesis of CAs by immune cells

In 1994, Bergquist *et al*. firstly determined CAs in lymphocytes of human cerebrospinal fluid (CSF) by means of capillary electrophoresis with electrochemical detection and found that intracellular CAs per lymphocyte are about 2×10^{-18} mol[14]. Three years later, they reported that intracellular DA in human peripheral blood mononuclear cells (PBMCs) is 1.6×10^{-18} mol and NE 1.0×10^{-18} mol per cell[15]. Further, they utilized the technology of electrospray ionization mass spectrometry to show structural characteristics of these compounds in immune cells and proved that they are CAs containing a specific structure called catechol[16]. Moreover, Knudsen *et al*.[17] and Cosentino *et al*.[18] also confirmed the existence of CAs in PBMCs by the aid of radioenzymatic assay and high-performance liquid chromatography, respectively. In human neutrophils[19] and macrophages[20], CAs are found as well. Besides human, mice are also detected for CAs in their splenocytes and peritoneal macrophages[21] as well as mast cells[22].

However, it is possible that the intracellular CAs detected in the immune cells are not from an active synthesis of the cells, but from a passive uptake from exterior of the cells. Further investigations have clarified the issues. Cosentino *et al*.[18] and Josefsson *et al*.[21] respectively examined human hematopoietic cell lines (NALM-6 and U937) and T and B cell hybridomas (HCQ6 and 6B9E4) and found CAs in these cells, although CAs in these clones are less than those mentioned above in immunocytes freshly isolated from human and animal body. Since these clones have been cultured for long time *in vitro*, the CAs detected in the cells are impossible to originate from neuronal and endocrine cells. Thus, these results on the one hand demonstrate that immunocytes are able to synthesize CAs and on the other hand suggest that CAs in the immunocytes may partly be due to the uptake of the cells from the exterior.

Recently, some evidence from our laboratory further reveals the ability of lymphocytes to synthesize CAs. We found that there was the expression of tyrosine hydroxylase (TH), an initial rate-limiting enzyme in the process of CA synthesis, in the mesenteric lymph nodes, spleen and thymus of rats, and the distributive density of TH-positive cells was highest in the lymph nodes, lowest in the thymus and middle in the spleen[23]. Resting lymphocytes can express TH mRNA and Con A-activated lymphocytes up-regulate the expression of TH mRNA[24]. More directly, we detected the three kinds of CAs, DA, NE and E, in the cultured lymphocytes, and found that all the three kinds of CAs were more in the Con A-activated lymphocytes than in the resting lymphocytes[24]. These results from our laboratory not only further demonstrate the ability of lymphocytes to synthesize CAs but also suggest a change of CA synthetic ability depending on various lymphoid organs and different functional states of the lymphocytes.

2 Metabolic pathways of CAs in immune cells

Some evidence proposes that metabolic pathways of CAs in immune cells are similar to the classical metabolic routes of CAs in nervous and endocrine systems, including their synthesis, storage, release, reuptake and degradation.

2.1 Synthesis of CAs in immune cells

As mentioned above, lymphocytes have TH mRNA expression and TH protein, and the TH mRNA expression is up-regulated and meanwhile CA synthesis increases when the lymphocytes are activated by Con A or phytohemagglutinin (PHA)[23-26]. These facts show that synthesis of CAs in lymphocytes relies on TH.

Report from laboratory of Musso *et al*. indicates synthetic characteristics of CAs in immune cells. They added *L*-tyrosine and *L*-dopa to lymphocyte cultures and found that CAs increased in the lymphocytes in a dose-dependent way, but *D*-dopa did not influence the CA synthesis of the lymphocytes[27]. Further, they added [^{3}H]-*L*-dopa to the lymphocyte cultures and one hour later [^{3}H]-NE and [^{3}H]-DA were detected in the lymphocytes[27]. The data suggest that lymphocytes are able to take the precursors of CAs from extracellular fluid and synthesize CAs. Moreover, they found that CAs in lymphocytes decreased in a dose-dependent manner after the lymphocytes were treated with either α-methyl-*p*-tyrosine (α-MT), an inhibitor of TH activity, or benserazide, an inhibitor of dopa decarboxylase[27]. Recent studies of our laboratory support the report of Musso *et al*. We observed that α-MT decreased content of intracellular DA, NE and E in Con A-activated lymphocytes and pargyline, an inhibitor of monoamine oxydase, increased the content of intracellular DA, NE and E in the Con A-activated lymphocytes[24]. Besides, NE content decreased but DA increased in lymphocytes after the lymphocytes were treated with disulfiram and fusaric acid, inhibitors of DA-β-hydroxylase (DβH)[27]. Taken together, these findings suggest that as nervous and endocrine cells do, immunocytes can utilize tyrosine to produce *L*-dopa under catalysis of TH; subsequently the *L*-dopa is converted to DA via action of dopa decarboxylase; and lastly, DA is transformed to NE by the enzyme DβH.

2.2 Storage and release of CAs in immune cells

Reserpine has been known to inhibit the uptake and storage of CAs into vesicles[28]. After immune cells are incubated with reserpine for one hour, intracellular CAs including DA, NE and E are remarkably reduced, but CAs in the culture supernatants of the cells are significantly increased[18,19]. The information suggests that CAs in immunocytes may be stored in vesicle-like structures, similar to that in neurons.

Although the mechanisms, through which immunocytes release CAs out of the cells, are still less clear, some authors present some differences between lymphocytes in human peripheral blood and chromaffin cells in adrenal medulla in the characteristics of CA release. NE secretion in both the lymphocytes and the chromaffin cells seems to be acetylcholine (ACh)- and calcium-dependent, since ACh can facilitate NE release of the two kinds of cells and ionomycine, KCl and veratridine can also promote NE release of these cells via stimulation of calcium inflow[29,30]. However, as far as the lymphocytes are concerned, tetraethylammonium (TEA), a blocker of nicotinic receptors, only partly (50%) blocked the ACh-induced NE release; and D600, a blocker of Ca^{2+} channel, only attenuated the ACh-induced NE release by 30%[29,30]. Unlike the phenomena of the lymphocytes, the ACh-induced NE release of the chromaffin cells can be completely blocked by TEA and D600. The differences of lymphocytes from chromaffin cells in the NE release suggest that some distinct ion channels or other mechanisms may be involved in CA release of lymphocytes.

2.3 Reuptake and degradation of CAs by immune cells

2.3.1 Reuptake of CAs by immune cells

Faraj *et al*. are among the first to indicate a DA uptake system in lymphocytes. They found DA specific binding sites on human lymphocytes by using radioligand-binding

assay[31,32]. [^{3}H]-labeled DA was added to cultures of human lymphocyte, and ten minutes later, the [^{3}H]-DA was detected in the lymphocytes[31,32]. Two kinds of selective inhibitors of monoamine transporters, cocaine and GBR 12909, suppressed both the DA binding to the specific sites and the uptake of [^{3}H]-DA[31,32]. On the basis of the facts, Faraj *et al*. presume that a DA transporter (DAT) exists on human lymphocytes, which is similar to that on neurons[32]. Similarly, *in vivo*, significant uptake of labeled DA into lymphoid tissues was observed [33]. However, Krieger *et al*. present a different view on the supposition of Faraj *et al*. They consider that the phenomena of the active uptake of [^{3}H]-DA by lymphocytes are probably due to contamination of the lymphocytes by platelets in the process of isolation of the lymphocytes[34]. Recently, some studies from other laboratories prove the existence of DAT on lymphocytes. Amenta *et al*. observed that there were DAT-immunoreactivity and vesicular monoamine transporter (VMAT)-immunoreactivity on cellular membrane and vesicle-like structures of lymphocytes in peripheral blood[35]. Human lymphocytes have DAT mRNA expression[36]. Incubation of human PBMCs with both desipramine, an inhibitor of NE uptake, and GBR 12909, a blocker of DAT, induced an increase of DA and NE in the culture medium[37], suggesting that besides DAT, NE transporters (NET) may also be present on immune cells and they both participate in the active uptake of CAs. However, further evidence for NET on immunocytes still needs to be provided[38,39].

2.3.2 Degradation of CAs by enzymes in immune cells

The physiological effects of the released CAs are primarily terminated through reuptake mechanism, but their final inactivation still relies on two enzymes in the cells, monoamine oxidase (MAO) and catechol-O-methyl transferase (COMT). As early as in 1983, Bidart *et al*. discovered that human T and B lymphocytes had COMT immunoreactivity[40]. Later, Balsa *et al*. reported that in lymphocytes and granulocytes of human blood existed monoamine oxidase activity[41]. Treatment of lymphocytes with pargyline resulted in an increase of intracellular CAs and a decrease of intracellular CA metabolites in the lymphocytes[24,37]. Metabolites of all the three kinds of CAs (DA, NE and E) can be detected in immune cells[18,19]. These results imply that immunocytes not only synthesize CAs but also degrade and inactivate CAs via MAO and COMT in the cells.

3 Roles and mechanisms of endogenous CAs synthesized by immune cells in immunomodulation

3.1 Roles of endogenous CAs in immunocytes in immunomodulation

Large quantities of studies have showed that CAs, as mediators of nervous and endocrine systems, can adjust immune functions. Recently, we investigated effect of the endogenous CAs of lymphocytes on function of lymphocytes themselves. We found that Con A-activated lymphocytes up-regulated TH mRNA expression, increased TH-immunoreactive protein and enhanced intracellular content of DA, NE and E compared with resting lymphocytes[23,24], suggesting that CA synthesis in lymphocytes is related to functional state of the lymphocytes. In addition, treatment of lymphocytes with α-MT, which decreased all the three kinds of CAs, both intracellular and supernatant of the cultured lymphocytes, led to enhancement of both Con A-induced lymphocyte proliferation and IL-2 production; while treatment of lymphocytes with pargyline, which increased all the three kinds of CAs, both intracellular and supernatant of the cultured lymphocytes, resulted in attenuation of the Con A-induced lymphocyte proliferation[23,24]. Our findings, from positive and negative profiles, strongly show that the endogenous CAs derived from lymphocytes can modulate function of lymphocytes themselves. Our findings are similar to some other relevant reports. Activation of RAW 264.7 macrophage cell line with LPS caused increase of extracellular NE and intracellular DA of the cultured cells[20]. The activated lymphocytes may up-regulate expression of adrenoreceptors[42,43]. Treatment of α-MT and MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), another inhibitor of TH, and haloperidol, an antagonist of DA receptors, depressed growth of T cell hybridoma (10I) in a dose-dependent manner[25]. Interestingly, there was no influence of α-MT on spontaneous apoptosis of resting PBMCs, but there was notable suppression of α-MT on the activation-induced apoptosis of PBMCs[44]. We reveal that CAs in resting lymphocytes are lower, but are dramatically increased in Con A-activated lymphocytes[23], implying that the increase of CAs in the activated lymphocytes may be necessary to their immunoregulatory role.

3.2 Immunoregulatory mechanisms of endogenous CAs in immune cells

There are two mechanisms that may be involved in immunoregulation of endogenous CAs in immune cells: one is autocrine/paracrine mechanism, through which CAs are secreted out of immunocytes and act on the cells; and the other is intracellular mechanism, through which CAs directly modulate function of the immunocytes themselves without being secreted out of the cells (Fig.1).

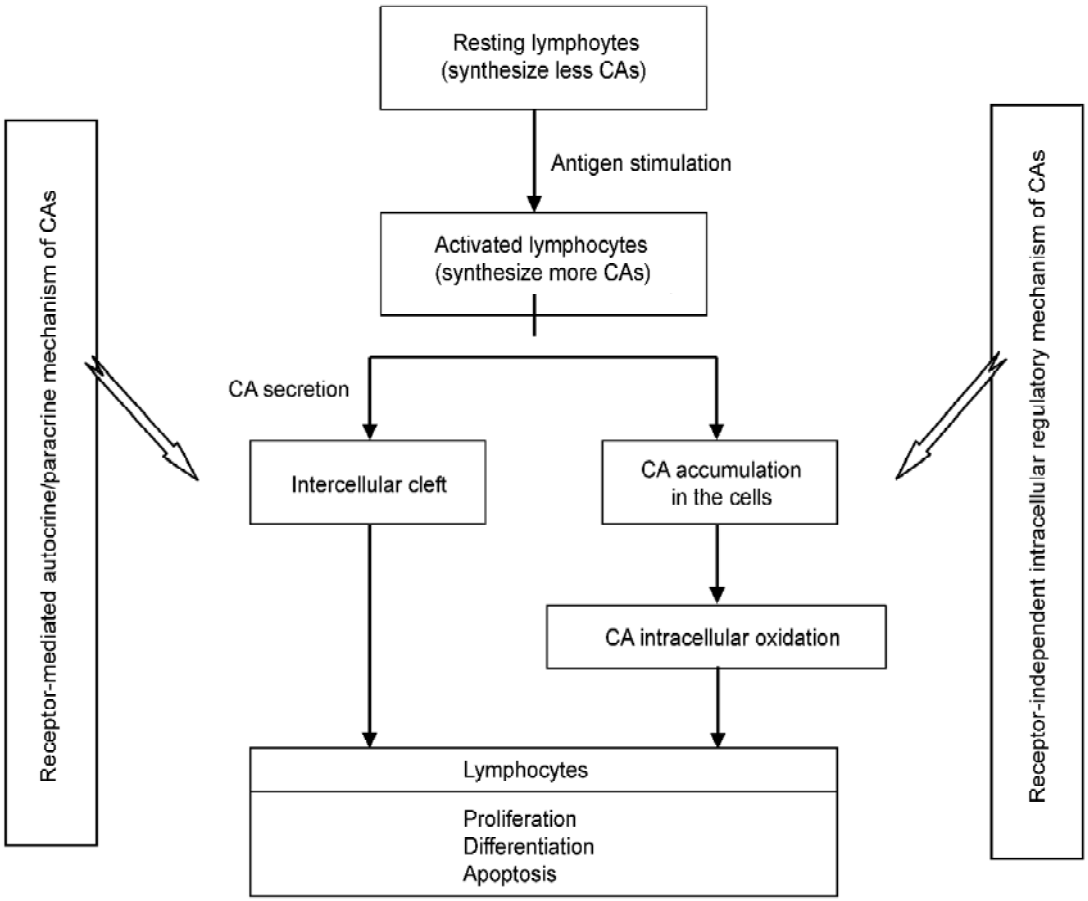

Fig. 1. Immunoregulatory mechanisms in endogenous CAs in immune cells.

3.2.1 Receptor-mediated autocrine/paracrine mechanism

It has been well established that β_2-, α_1-, α_2-adrenoreceptors[45-49] and D_1, D_2-like DA receptors[50-52] exist on immune cells by using radioenzymatic assay, RT-PCR and Western blot. These findings available provide a structural prerequisite for the paracrine/autocrine regulatory mechanism of CAs. Recently, our studies showed that α-MT induced a notable reduction of intracellular and supernatant DA, NE and E of the cultured lymphocytes, while pargyline caused a marked augment of the intracellular and supernatant DA, NE and E in the cultured lymphocytes, suggesting that intracellular CAs can be secreted out of lymphocytes[24]. Moreover, we found that treatment of lymphocytes with pargyline plus phentolamine (α-adrenoreceptor antagonist) partly reversed the suppressive effect of pargyline on Con A-induced lymphocyte proliferation, while treatment of lymphocytes with pargyline plus propranolol (β-adrenoreceptor antagonist) completely blocked the inhibition of pargyline on the lymphocyte proliferation[24]. These results propose that both α- and β-adrenoreceptors take part in mediating modulation of the endogenous CAs on lymphocyte function, but probably β-adrenoreceptors are dominant in mediating the immunomodulation of CAs. In addition, we observed that cAMP content in the lymphocytes treated with pargyline remarkably increased and propranolol completely blocked the cAMP increase induced by pargyline[24]. Reports from other laboratories that murine macrophage-derived CAs modulated LPS-induced tumor necrosis factor and interleukin-1_β production through adrenoreceptor-mediated autocrine/paracrine mechanism are in line with our findings[53,54]. Thus, we can propose that an autocrine/paracrine pathway conducts the immunoregulation of endogenous CAs in immunocytes, i.e., CAs are secreted out of immunocytes, subsequently act on β-adrenoreceptors on the immunocytes, then increase intracellular cAMP in the cells, and regulate functions of the immunocytes themselves.

3.2.2 Receptor-independent intracellular regulatory mechanism

When intracellular CAs are oxidated by MAO on mito-

chondrial membrane, they produce a large quantity of oxygen species and oxidative metabolites, which have evident cytotoxic effect at high concentrations and induce apoptosis of the cells[55-61]. This kind of reaction also occurs in immune cells. The intracellular CAs newly synthesized by immune cells may not be released immediately, but accumulate in the cells, which results in receptor-independent and oxidative stress-induced apoptosis of the cells[38]. An anti-oxidant, ascorbic acid, can completely or partially prevent CAs from their suppression of proliferation of mastocytes and macrophages, and block the CA-induced enhancement of cellular apoptosis[20]. Contrarily, preincubation of lymphocytes with *L*-buthionine-[*S*,*R*]-sulfoximine, an inhibitor of glutathione synthesis, increased sensitivity of the lymphocytes to CA-stimulated apoptosis. These data propose that CAs in immune cells may employ intracellular oxidative mechanism to exert their immunoregulatory function. A specific transporter for CAs also exists on cellular nuclear membrane of lymphocytes, via which CAs in cytoplasm can be transported into nuclei of the cells [62]. More directly, Bergquist *et al*. determined levels of CAs in nuclei of lymphocytes by using capillary electrophoresis with electrochemical detection and found that an even level in nucleus per cell was $(5.3\pm2.6)\times10^{-21}$ mol for DA and $(2.1\pm0.9)\times10^{-21}$ mol for NE, accounting for 0.1%~0.2% of total amount of CAs in the cells[12]. In addition, they observed that CAs in the nuclei interacted with nuclear receptors (e.g. steroid receptors) and regulated lymphocyte function[12]. CAs in the nuclei also influenced some transcription processes of immunocytes, such as expression of nuclear transcription factor *κB*, and then induced apoptosis of the cells[63]. CAs may facilitate the expression of proto-oncogene *Bax*, while attenuate *Bcl-2* expression[15,22]. Since both MAO and COMT, the two major catabolic enzymes for CA degradation, are on the face of mitochondrial membrane or in the cytoplasm, CAs in the nuclei are generally not degraded.

3.3 Correlation between endogenous CAs in immunocytes and autoimmune diseases

Recently, some studies pointed out that the endogenous CAs in immunocytes may also be related to pathogenesis and progression of some inflammatory autoimmune diseases. Multiple sclerosis (MS) is an autoimmune disease characterized by demyelination of the central nervous system. Although the pathogenesis of MS still has not been well known, some evidence has indicated that CAs in immune system may participate in MS pathogenesis[64,65]. The key event in the pathogenesis of MS is represented by the autoimmune recognition of myelin sheath antigens by T lymphocytes[66], which may be favored by a failure of the activation-induced apoptotic mechanisms leading to survival of autoreactive cells[66-69]. A recent clinical study from Cosentino *et al*. revealed that incubation of PHA-stimulated PBMCs with α-MT led to reduction of the activation-induced apoptosis[44]. NE level in PBMCs from MS patients is increased[70]. PBMCs from MS patients in the active period synthesize less DA than those from both healthy controls and MS patients in the inactive period after the PBMCs are activated by PHA. It seems that less CAs in immunocytes may aggravate MS due to the attenuation of apoptotic mechanism. Moreover, recent studies show cytokine interferon-β (IFN-β) facilitates synthesis and release of CAs by PBMCs, while IFN-γ inhibits the synthesis of CAs and expression of TH mRNA[71]. Importantly, IFN-β and IFN-γ are found to be implicated in MS. Clinical attacks of MS are preceded by the increased IFN-γ in cerebrospinal fluid and peripheral blood[72-74]; administration of recombinant IFN-γ to MS patients leads to a worsening of disease course[75]; but IFN-β is an effective immunomodulatory drug for the treatment of MS[76]. Since IFN-β and IFN-γ can influence CA synthesis in immune cells, the effects of IFN-β and IFN-γ on MS may be related to the endogenous CAs. Besides MS, other autoimmune diseases such as Parkinson's disease[77,78] and rheumatoid arthritis[79] are also affected by the endogenous CAs in immunocytes. At present, the knowledge about the correlation between endogenous CAs in immunocytes and autoimmune diseases is still less known. Thus, exploring and clarifying these issues will extend our comprehension of the pathogenesis of these diseases and develop our strategy for cure of these diseases[43,80-82].

4 Concluding remarks

The discoveries that immune cells are able to synthesize CAs and the endogenous CAs in immunocytes are involved in the regulation of immune functions lead to such a concept that the immune system is likely to become the third CA system other than the nervous and endocrine systems. Although this aspect of research is still superficial and needs to be explored further, the confirmation of the third new CA system and its role in neuroimmunomodulation will bring great advancement in comprehension of some immunoregulatory issues and in prevention and therapy of some autoimmune diseases.

REFERENCES

1 Blalock JE. The syntax of immune-neuroendocrine

communication. Immunol Today 1994; 15(11): 504-511.
2 Dahlstroem AB，Zetterstroem BE. Noradrenaline stores in nerve terminals of the spleen: changes during hemorrhagic shock. Science 1965; 147: 1583-1585.
3 Meltzer JC, Grimm PC, Greenberg AH, Nance DM. Enhanced immunohistochemical detection of autonomic nerve fibers, cytokines and inducible nitric oxide synthase by light and fluorescent microscopy in rat spleen. J Histochem Cytochem 1997; 45(4): 599-610.
4 Panuncio AL, De La Pena S, Gualco G, Reissenweber N. Adrenergic innervation in reactive human lymph nodes. J Anat 1999; 194: 143-146.
5 Swanson MA, Lee WT, Sanders VM. IFN-gamma production by Th1 cells generated from naive $CD4^+$ T cells exposed to norepinephrine. J Immunol 2001; 166(1): 232-240.
6 Sanders VM, Baker RA, Ramer-Quinn DS, Kasprowicz DJ, Fuchs BA, Street NE. Differential expression of the beta2-adrenergic receptor by Th1 and Th2 clones: implications for cytokine production and B cell help. J Immunol 1997; 158(9): 4200-4210.
7 Kohm AP, Mozaffarian A, Sanders VM. B cell receptor- and beta 2-adrenergic receptor-induced regulation of B7-2 (CD86) expression in B cells. J Immunol 2002; 168(12): 6314-6322.
8 Ghosh MC, Mondal AC, Basu S, Banerjee S, Majumderc J, Bhattacharya D, Dasgupta PS. Dopamine inhibits cytokine release and expression of tyrosine kinases, Lck and Fyn in activated T cells. Int Immunopharmacol 2003; 3(7): 1019-1026.
9 Dong J, Mrabet O, Moze E, Li K, Neveu PJ. Lateralization and catecholaminergic neuroimmunomodulation: prazosin, an alpha 1/alpha 2-adrenergic receptor antagonist, suppresses interleukin-1 and increases interleukin-10 production induced by lipopolysaccharides. Neuroimmunomodulation 2003; 10(3): 163-168.
10 Torres KC, Antonelli LR, Souza AL, Teixeira MM, Dutra WO, Gollob KJ. Norepinephrine, dopamine and dexamethasone modulate discrete leukocyte subpopulations and cytokine profiles from human PBMC. J Neuroimmunol 2005; 166(1-2): 144-157.
11 Offen D, Ziv I, Gorodins S, Barzilai A, Malik Z, Melamed E. Dopamine-induced programmed cell death in mouse thymocytes. Biochim Biophys Acta 1995; 1268(2): 171-177.
12 Peng YP (彭聿平), Qiu YH, Zhang QQ, Wang JH. Effect of noradrenaline on T lymphocyte proliferation. Chin J Appl Physiol (中国应用生理学杂志) 1995; 11: 75-78 (Chinese, English abstract).
13 Peng YP, Qiu YH, Jiang JL, Wang JJ. Effect of catecholamines on IL-2 production and NK cytotoxicity of rats *in vitro*. Acta Pharmacol Sin 2004; 25(10): 1354-1360.
14 Bergquist J, Tarkowski A, Ekman R, Ewing A. Discovery of endogenous catecholamines in lymphocyte evidence for catecholamine regulation of lymphocyte function via an autocrine loop. Proc Natl Acad Sci USA 1994; 91(26): 12912-12916.
15 Bergquist J, Josefsson E, Tarkowski A, Ekman R, Ewing A. Measurements of catecholamine-mediated apoptosis of immunocompetent cells by capillary electrophoresis. Electrophoresis 1997; 18(10): 1760-1766.
16 Bergquist J, Sliberring J. Identification of catecholamines in the immune system by electrosprayionization mass. Rapid Commun Mass Spectrom 1998; 12(11): 683-688.
17 Knudsen JH, Christensen NJ, Bratholm P. Lymphocyte norepinephrine and epinephrine, but not plasma catecholamines predict lymphocyte cAMP production. Life Sci 1996; 59(8): 639-647.
18 Cosentino M, Bombelli R, Ferrari M, Marino F, Rasini M, Maestroni GJ, Conti A, Boveri M, Lecchini S, Frigo G. HPLC-ED measurement of endogenous catecholamines in human immune cells and hematopoietic cell lines. Life Sci 2000; 68(3): 283-295.
19 Cosentino M, Marino F, Bombelli R, Ferrari M, Lecchini S, Frigo G. Endogenous catecholamine synthesis, metabolism, storage and uptake in human neutrophils. Life Sci 1999; 64(11): 975-981.
20 Brown SW, Meyers RT, Brennan KM, Rumble JM, Narasimhachari N, Perozzi EF, Ryan JJ, Stewart JK, Fischer-Stenger K. Catecholamines in a macrophage cell line. J Neuroimmunol 2003; 135(1-2): 47-55.
21 Josefsson E, Bergquist J, Ekman R, Tarkowski A. Catecholamines are synthesized by mouse lymphocytes and regulate function of these cells by induction of apoptosis. Immunology 1996; 88(1): 140-146.
22 Freeman JG, Ryan JJ, Shelburne CP, Bailey DP, Bouton, LA, Narasimhachari N, Domen J, Simeon N, Couderc F, Stewart JK. Catecholamines in murine bone marrow derived mast cells. J Neuroimmunol 2001; 119: 231-238.
23 Qiu YH, Peng YP, Jiang JM, Wang JJ. Expression of tyrosine hydroxylase in lymphocytes and effect of endogenous catecholamines on lymphocyte function. Neuroimmunomodulation 2004; 11(2): 75-83.
24 Qiu YH, Cheng C, Dai L, Peng YP. Effect of endogenous catecholamines in lymphocytes on lymphocyte function. J Neuroimmunol 2005; 167(1-2): 45-52.
25 Tsao CW, Lin YS, Cheng JT. Inhibition of immune cell proliferation with haloperidol and relationship of tyrosine hydroxylase expression to immune cell growth. Life Sci 1998; 62(21): 335-344.
26 Cosentino M, Marino F, Bombelli R, Ferrari M, Rasini E, Lecchini S, Frigo G. Stimulation with phytohaemagglutinin induces the synthesis of catecholamines in human peripheral blood mononuclear cells: role of protein kinase C and contribution of intracellular calcium. J Neuroimmunol 2002; 125(1-2): 125-133.
27 Musso NR, Brenci S, Settim M, Indiveri F, Lotti G. Catecholamine content and *in vitro* catecholamine synthesis in peripheral human lymphocytes. J Clin Endocrinol Metab 1996; 81(10): 3553-3557.
28 Shore PA. The mechanism of norepinephrine depletion by

reserpine, metaraminol and related agents. The role of monoamine oxidase. Pharmacol Rev 1966; 18(1): 561-568.

29 Musso NR, Brenci S, Indiveri F, Lotti G. Acetylcholine-induced, calcium-dependent norepinephrine outflow from peripheral human lymphocytes. J Neuroimmunol 1998; 87(1-2): 82-87.

30 Kilpatrick DL, Slepetis RJ, Corcoran JJ, Kirshner N. Calcium uptake and catecholamine secretion by cultured bovine adrenal medulla cells. J Neurochem 1982; 38(2): 427-435.

31 Faraj BA, Olkowski ZL and Jackson RT. Binding of [^{3}H]-dopamine to human lymphocytes: possible relationship to neurotransmitter uptake sites. Pharmacology 1991; 42(3): 135-141.

32 Faraj BA, Olkowski ZL, Jackson RT. A cocaine-sensitive active dopamine transport in human lymphocytes. Biochem Pharmacol 1995; 50(7): 1007-1014.

33 Basu S, Dasgupta PS, Lahiri T, Roychowdhury J. Uptake and biodistribution of dopamine in bone marrow, spleen and lymph nodes of normal and tumor bearing mice. Life Sci 1993; 53(5): 415-424.

34 Krieger K, Klimke A, Henning U. Active [^{3}H]-dopamine uptake displayed by native lymphocyte suspensions is mainly due to contaminating platelets. Pharmacopsychiatry 1998; 31(5): 193-198.

35 Amenta F, Bronzetti E, Cantalamessa F, El-Assouad D, Felici L, Ricci A, Tayebati SK. Identification of dopamine plasma membrane and vesicular transporters in human peripheral blood lymphocyte. J Neuroimmunol 2001; 117(1-2): 133-142.

36 Mill J, Asherson P, Browes C, D' Souza U, Craig I. Expression of the dopamine transporter gene is regulated by the 3' UTR VNTR: Evidence from brain and lymphocytes using quantitative RT-PCR. Am J Med Genet 2002; 114(8): 975-979.

37 Marino F, Cosentino M, Bombelli R, Ferrari M, Lecchini S, Frigo GM. Endogenous catecholamine synthesis, metabolism, storage, and uptake in human peripheral blood mononuclear cells. Exp Hematol 1999; 27(3): 489-495.

38 Cosentino M, Marino F, Bombelli R, Ferrari M, Lecchini S, Frigo G. Unravelling dopamine (and catecholamine) physiopharmacology in lymphocytes: open questions. Trends Immunol 2003; 24(11): 581-582.

39 Gordon J, Barnes NM. Lymphocytes transport serotonin and dopamine: agony or ecstasy? Trends Immunol 2003; 24(8): 438-443.

40 Bidart JM, Motte PH, Assicot M, Bohuon C, Bellet D. Catechol-O-methyltransferase activity and aminergic binding sites distribution in human peripheral blood lymphocyte subpopulations. Clin Immunol Immunopathol 1983; 26(1): 1-9.

41 Balsa MD, Gomez N, Unzeta M. Characterization of monoamine oxidase activity present in human granulocytes and lymphocytes. Biochim Biophys Acta 1989; 992(2): 140-144.

42 Rouppe van der Voort C, Kavelaars A, van de Pol M, Heijnen CJ. Noradrenaline induces the phosphorylation of ERK-2 in human peripheral blood mononuclear cells after induction of α_1-adrenergic receptors. J Neuroimmunol 2000; 108(1-2): 82-91.

43 Zoukos Y, Kidd D, Woodroofe MN, Kendall BE, Thompson AJ, Cuzner ML. Increased expression of high affinity IL-2 receptors and β-adrenoceptors on peripheral blood mononuclear cells is associated with clinical and MRI activity in multiple sclerosis. Brain 1994; 117: 307-315.

44 Cosentino M, Zaffaronid M, Marino F, Bombelli R, Ferrari M, Rasini E, Lecchini S, Ghezzi A, Frigo G. Catecholamine production and tyrosine hydroxylase expression in peripheral blood mononuclear cells from multiple sclerosis patients: effect of cell stimulation and possible relevance for activation-induced apoptosis. J Neuroimmunol 2002; 133(1-2): 233-240.

45 Pochet R, Delespesse G, Gausset PW, Collet H. Distribution of β-adrenergic receptors on human lymphocyte subpopulations. Clin Exp Immunol 1979; 38(3): 578-584.

46 Brodde OE, Engel G, Hoyer D, Bock KD, Weber F. The beta-adrenergic receptor in human lymphocytes: subclassification by the use of a new radio-ligand, (±)-^{125} iodocyanopindolol. Life Sci 1981; 29(21): 2189-2198.

47 Miles K, Atweh S, Otten G, Arnason BG, Chelmicka-Schorr E. Beta-adrenergic receptors on splenic lymphocytes from axotomized mice. Int J Immunopharmacol 1984; 6(3): 171-177.

48 Jetschmann JU, Benschop RJ, Jacobs R, Kemper A, Oberbeck R, Schmidt RE, Schedlowski M. Expression and *in-vivo* modulation of alpha- and beta-adrenoceptors on human natural killer (CD16+) cells. J Neuroimmunol 1997; 74(1-2): 159-164.

49 Kavelaars A. Regulated expression of alpha-1 adrenergic receptors in the immune system. Brain Behav Immun 2002; 16(6): 799-807.

50 McKenna F, McLaughlin PJ, Lewis BJ, Sibbring GC, Cummerson JA, Bowen-Jones D. Dopamine receptor expression on human T- and B-lymphocytes, monocytes, neutrophils, eosinophils and NK cells: a flow cytometric study. J Neuroimmunol 2002; 132 (1-2): 34-40.

51 Ricci A, Veglio F, Amenta F. Radioligand binding characterization of putative dopamine D_3 receptor in human peripheral blood lymphocytes with [^{3}H]7-OH-DPAT. J Neuroimmunol 1995; 58 (2): 139-144.

52 Kwak YT, Koo MS, Choi CH, Sunwoo I. Change of dopamine receptor mRNA expression in lymphocyte of schizophrenic patients. BMC Med Genet 2001; 2: 3-11.

53 Spengler RN, Chensue SW, Giacherio DA, Blenk N, Kunkel SL. Endogenous norepinephrine regulates tumor necrosis factor-α production from macrophages *in vitro*. J Immunol 1994; 152(6): 3024-3031.

54 Engler KL, Rudd ML, Ryan JJ, Stewarta JK, Fischer-Stenger K. Autocrine actions of macrophage-derived catecholamines on interleukin-1β. J Neuroimmunol 2005; 160(1-2): 87-91.

55 Velez-Pardo C, Del Rio MJ, Ebinger G, Vauquelin G. Monoamine and iron-related toxicity: from "erotonin-binding proteins" to lipid peroxidation and apoptosis in PC12 cells. Gen Pharmacol

1998; 31(1): 19-24.
56 Offen D, Ziv I, Gorodin S, Barzilai A, Malik Z, Melamed E. Dopamine-induced programmed cell death in mouse thymocytes. Biochim Biophys Acta 1995; 1268(2): 171-177.
57 Del Rio MJ, Velez-Pardo C. Monoamine neurotoxins-induced apoptosis in lymphocytes by a common oxidative stress mechanism: involvement of hydrogen peroxide (H_2O_2), caspase-3, and nuclear factor kappa-B (NF-kB), p53, c-Jun transcription factors. Biochem Pharmacol 2002; 63(4): 677-688.
58 Jones DC, Gunasekar PG, Borowitz JL, Isom GE. Dopamine induced apoptosis is mediated by oxidative stress and is enhanced by cyanide in differentiated PC12 cells. J Neurochem 2000; 74(6): 2296-2304.
59 Cosentino M, Rasini E, Colombo C, Marino F, Blandini F, Ferrari M, Samuele A, Lecchini S, Nappi G, Frigo G. Dopaminergic modulation of oxidative stress and apoptosis in human peripheral blood lymphocytes: evidence for a D_1-like receptor-dependent protective effect. Free Radic Biol Med 2004; 36(10): 1233-1240.
60 Graham DG, Tiffany SM, Bell WR, Gutknecht WF. Autoxidation *versus* covalent binding of quinones as the mechanism of toxicity of dopamine, 6-hydroxydopamine, and related compounds toward C1300 neuroblastoma cells *in vitro*. Mol Pharmacol 1978; 14(4): 644-653.
61 Burke WJ, Kristal BS, Yu BP, Li SW, Lin TS. Norepinephrine transmitter metabolite generates free radicals and activates mitochondrial permeability transition: a mechanism for DOPEGAL-induced apoptosis. Brain Res 1998; 787(2): 328-332.
62 Buu NT. Uptake of 1-methyl-4-phenylpyridinium and dopamine in the mouse brain cell nuclei. J Neurochem 1993; 61(4): 1557-1560.
63 Bergquist J, Ohlsson B, Tarkowski A. Nuclear factor-kappa B is involved in the catecholaminergic suppression of immunocompetent cells. Ann NY Acad Sci 2000; 917: 281-289.
64 Noseworthy JH, Lucchinetti C, Rodriguez M, Weisenhenker BG. Multiple sclerosis. N Engl J Med 2000; 343(13): 938-952.
65 Lassmann H, Bruck W, Lucchinetti C. Heterogeneity of multiple sclerosis pathogenesis: implications for diagnosis and therapy. Trends Mol Med 2001; 7(3): 115-121.
66 Martin R, McFarland HF. Immunological aspects of experimental allergic encephalomyelitis and multiple sclerosis. Crit Rev Clin Lab Sci 1995; 32(2): 121-182.
67 Pender MP. Genetically determined failure of activation-induced apoptosis of autoreactive T cells as a cause of multiple sclerosis. Lancet 1998; 351(9017): 978-981.
68 Comi C, Leone M, Bonissoni S, DeFranco S, Bottarel F, Mezzatesta C, Chiocchetti A, Perla F, Monaco F, Dianzani U. Defective T cell Fas function in patients with multiple sclerosis. Neurology 2000; 55(7): 921-927.
69 Sharief MK, Douglas M, Noori M, Semra YK. The expression of pro- and anti-apoptosis Bcl-2 family proteins in lymphocytes from patients with multiple sclerosis. J Neuroimmunol 2002; 125(1-2): 155-162.
70 Rajda C, Bencsik K, Vecsei LL, Bergquist J. Catecholamine levels in peripheral blood lymphocytes from multiple sclerosis patients. J Neuroimmunol 2002; 124(1-2): 93-100.
71 Cosentino M, Marco Z, Zaffaroni M, Marino F, Bombelli R, Rasini E, Frigo G, Ghezzi A, Comi G, Lecchini S. Interferon-γ and interferon-β affect endogenous catecholamines in human peripheral blood mononuclear cells: Implications for multiple sclerosis. J Neuroimmunol 2005; 162(1-2): 112-121.
72 Olsson T, Zhi WW, Hojeberg B, Kostulas V, Jiang YP, Anderson G, Ekre HP, Link H. Autoreactive T lymphocytes in multiple sclerosis determined by antigen-induced secretion of interferon-gamma. J Clin Invest 1990; 86(3): 981-985.
73 Rieckmann P, Albrecht M, Kitze B, Weber T, Tumani H, Broocks A, Luer W, Poser S. Cytokine mRNA levels in mononuclear blood cells from patients with multiple sclerosis. Neurology 1994; 44(8): 1523-1526.
74 Hohnoki K, Inoue A, Koh CS. Elevated serum levels of IFN-γ IL-4 and TNF-α/unelevated serum levels of IL-10 in patients with demyelinating diseases during the acute stage. J Neuroimmunol 1998; 87(1-2): 27-32.
75 Panitch HS, Hirsch RL, Haley AS, Johnson KP. Exacerbations of multiple sclerosis in patients treated with gamma interferon. Lancet 1987; 1(8538): 893-895.
76 Revel M. Interferon-beta in the treatment of relapsing-remitting multiple sclerosis. Pharmacol Ther 2003; 100(1): 49-62.
77 Caronti B, Tanda G, Colosimo C, Ruggieri S, Calderaro C, Palladini G, Pontieri FE, Di Chiara G. Reduced dopamine in peripheral blood lymphocytes in Parkinson's disease. Neuroreport 1999; 10(14): 2907-2910.
78 Rajda C, Dibo G, Vecsei L, Bergquist J. Increased dopamine content in lymphocytes from high-dose *L*-Dopa-treated Parkinson's disease patients. Neuroimmunomodulation 2005; 12 (2): 81-84.
79 Roupe van der Voort C, Heijnen CJ, Wulffraat N, Kuis W, Kavelaars A. Stress induces increases in IL-6 production by leucocytes of patients with the chronic inflammatory disease juvenile rheumatoid arthritis: a putative role for α_1-adrenergic receptors. J Neuroimmunol 2000; 110(1-2): 223-229.
80 Chelmicka-Schorr E, Arnason BG. Nervous system-immune system interactions and their role in multiple sclerosis. Ann Neurol 1994; 36: S29-S32.
81 Baerwald CG, Laufenberg M, Specht T, von Wichert P, Burmester GR, Krause A. Impaired sympathetic influence on the immune response in patients with rheumatoid arthritis due to lymphocyte subsetspecific modulation of beta 2-adrenergic receptors. Br J Rheumatol 1997; 36(12): 1262-1269.
82 Miller LE, Justen HP, Scholmerich J, Straub RH. The loss of sympathetic nerve fibers in the synovial tissue of patients with rheumatoid arthritis is accompanied by increased norepinephrine release from synovial macrophages. FASEB J 2000; 14(13): 2097-2107.