Advances in Neuroimmune Biology 3 (2012) 111–155
DOI 10.3233/NIB-2012-012044
IOS Press

Role of Peripheral and Brain-Derived Dopamine (DA) in Immune Regulation

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Abstract. There is a well-defined influence of dopamine (DA) within the immune system. It can be synthesized not only in neurons, but also in immune cells, especially in T cells. In addition, these cell are bearing an active uptake mechanism, which could serve another source of DA. Therefore, it is highly likely that a functional DA-erg autocrine/paracrine regulatory loop exists, where lymphocytes-derived DA acting through its own receptors, also expressed on the same cells, can have an influence on its own function. However, the possibility that immune cell derived DA may act in accordance with DA secreted by other sources, i.e. from sympathetic terminals, cannot be ruled out. In harmony with these observations have provided evidences for the existence of a functional DA-erg system in the thymus, indicating that DA may have also a role in the maturation and selection of a certain subpopulation of lymphocytes as well. Based upon all of this information and evidences, for being able to summarize this topic, a much broader survey, including all direct and indirect immune-modulatory role of DA is required. Therefore, in this review we are going to discuss the most relevant aspects of this regulatory function. Facts and theories based upon experimental (pre-clinical) data are extended with the evidences accumulated by clinical observations.

Keywords: Dopamine, neurotransmitter, immune system, autocrine/paracrine regulation, lymphocyte, lymphokine, clinical trial

INTRODUCTION

Dopamine (DA) as a neurotransmitter

It was first recognized more than 40 years ago by demonstrating that it was a genuine neurotransmitter and it was not just a precursor of norepinephrine (NE) and epinephrine (E) [23]. DA is synthesized mainly by the nervous system (central and peripheral) and the medulla of the adrenal glands. The first and rate limiting step of its biosynthesis is the hydroxylation of the L-tyrosine to L-DOPA via the enzyme tyrosine hydroxylase (TH). This first step is followed by the decarboxylation of L-DOPA by aromatic L-amino acid decarboxylase (AADC). In authentic DA-erg neurons it is packaged into the secretory vesicles, which are then released into the synapse. There are also neurons at both the central and peripheral nervous system, where DA is further processed into NE by DA-beta-hydroxylase (DBH). In the adrenal medulla as well as in certain nuclei of the brain, there is one more enzyme i.e. the phenylethanolamine-N-methyltransferase (PNMT), which makes E from NE. DA is basically released from DA-erg nerve terminals then it can bind to its appropriate receptors located at the post-synaptic membrane followed by the initiation of the biological response. Once DA is released, it can bind to and activate five distinct but closely related G protein-coupled receptors (GPCRs).

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ISSN 1878-948X/12/$27.50

They are divided into two major groups: D1 and D2 classes of DA receptors [20, 210]. While D1 class includes D1 and D5 subtypes has a stimulatory effect on intracellular signaling pathways, D2 class includes D2, D3 and D4 subtypes has an inhibitory influence on cAMP and Akt resulting in a decrease in PKA and GSKβ activity, respectively [20]. The excess amount of DA released into the synaptic cleft is inactivated by reuptake via the DA transporter (DAT) located at the presynaptic membrane. NE transporter (NET) can also uptake DA especially in areas where the concentration of DAT is low [153, 220]. The uptake is followed by enzymatic breakdown by two consecutive enzymatic steps, monoamine oxidase (MAOA or MAOB) and aldehyde dehydrogenase (ALDH), into 3,4-dihydroxyphenylacetic acid (DOPAC).

DA has many important, although diverse functions in the brain

These functions include roles in behavior, voluntary movement, motivation, attention, learning, mood, reward and tonic inhibition of prolactin secretion from the anterior lobe of the pituitary gland. DA-erg neurons, whose primary neurotransmitter is DA, are located in the substantia nigra (SN) and the ventral tegmental area (VTA) of the midbrain, in the zona incerta (ZI), the arcuate nucleus (ARC) and the rostral and caudal periventricular regions of the hypothalamus. In addition to the central nervous system (CNS), DA can be produced at several peripheral sites, i.e. neuronal fibers of sympathetic neurons, adrenal medulla and at some peripheral organs like kidney [212], islands of pancreas ([183] or retinal cells [125].

In addition, several studies have demonstrated that DA can be synthesized by different immune cells that has significance from our point of view [29, 154]. Expression of TH could be detected in lymphocytes by both immunohistochemistry and western-blot technique [171]. Furthermore, it has been demonstrated that lymphocytes can not only synthesize DA, but the rate of synthesis can increase when they are activated. Experimental data strongly support that DA, once produced in lymphocytes, can act in an autocrine/paracrine mode in order to modulate lymphocyte proliferation and differentiation, by acting through DA receptors that are also expressed in the same cells [22, 59, 114, 141, 147, 158, 189].

Therefore, an obvious conclusion is that DA is one of the key neurotransmitter molecules that maintains multiple connections between the nervous and the immune systems.

Multiple aspects of the regulatory role of DA

The regulatory role of DA on the immune system has been investigated and evaluated during the last two decades, and new and essential knowledge have been discovered:

- DA can modify the function of specific regulatory T cells (*Treg*) as well as the function of other immune cells, including B cells, macrophages and monocytic-dendritic cells [59, 106, 158].
- DA seems to have a dual role, it activates resting T cells, but inhibits activated T cells [29, 30, 32, 189].
- Changes in the expression of DA receptors and their signaling pathways, especially on T cells, are associated with altered immune functions in disorders like schizophrenia and Parkinson's disease [108, 110, 189, 213]. These data are supported by evidence based on clinical findings, like: (i) elevation of D3, but not of D4 DA receptor mRNA of lymphocytes in schizophrenic patients [108], or (ii) decrease of D3 receptor mRNA, and reduction of D3 receptor binding sites but no change of D5 receptor mRNA expression in Parkinson's disease patients [155].
- Moreover, abnormal plasma level of DA may also be associated with the altered functions of T cells, which can also be responsible for the changes of immune functions detected in patients in schizophrenic as well as in Parkinsonian patients.

In summary, there is a well defined influence of DA within the immune system [110]. We are going to summarize that DA can be synthesized not only in neurons, but also in immune cells, especially in T cells. In addition, these cells possess an active uptake mechanism, which could serve another source of DA. Therefore, it is highly likely that a functional DA-erg autocrine/paracrine regulatory loop exists, where lymphocytes-derived DA acting through its own receptors, also expressed on the same cells, can have an influence on its own function [15, 189]. However, the possibility that immune cell derived DA may act in accordance of DA secreted by other sources, i.e. from sympathetic terminals, cannot be ruled out [29, 59]. In harmony with these observations, a recent report has provided evidences for the existence of a functional DA-erg system in the thymus of rats [148], indicating that DA may have also a role in the maturation and selection of certain subpopulation of lymphocytes as well. Clearly a lot of information is available,

and in order to give a fair evaluation of this topic, a much broader survey, including all direct and indirect immune-modulatory roles of DA is required.

In this review we are going to discuss the most relevant aspects of DA regulatory function. Facts and theories based upon experimental (preclinical) data are discussed in relation to the evidence accumulated in clinical observations. The following aspects will be taken into consideration:

1. There are multiple data indicating that DA itself (in concert with other catecholamines) serves as one of the most effective mediators of the neuroimmune modulation. Therefore, the **structural basis of direct influence of DA on immune cells,** including (i) specific subtypes of **DA receptors**, or (ii) reuptake carriers, **like DA transporters (DAT)**, which can internalize extracellular DA, and **vesicular monoamine transporters (VMAT)**, that can transport newly synthesized DA into the secretory vesicles, need to be discussed. It must be emphasized that all of these are **present in different immune cells**.
2. Recent findings support the view that an **effective concentrations of DA can be reached locally**, **surrounding the immune cells at the periphery**. Moreover, in certain diseases, out-of-normal level of peripheral DA co-exists with altered immune functions, which also underlines the suspected role of central or peripheral DA in immune regulation.
3. A functional "**endogenous catecholaminergic/ DA-ergic system**" exists in lymphocytes. Further, the **DA-specific autocrine/paracrine** regulation of the immune system may serve as an essential element in harmonization of the immune response with the modulating effect of the hypothalamo-hypophyseal-adrenal (HPA) hormones.
4. DA regulates **prolactin (PRL). PRL** has multiple immunoregulatory roles; therefore, this way DA can indirectly affect immune function. Related questions: (i) Is this relationship functional? (ii) To what extent of the effects mentioned above can be considered as secondary (therefore, indirect) effects mediated through the hypothalamo-pituitary peripheral axis? These questions are yet to be answered.
5. **Brain-derived DA** may also play a role in immune function. Restricted areas of the brain are involved. Only those lymphocytes may be affected that can pass the blood brain barrier (BBB) and may localize in restricted areas of the brain. These "wandering immune cells" can be "educated" by locally secreted neurotransmitters, including DA, while they are inside of the BBB". Following that, they can transmit **brain-driven messages** to other cells of the immune system via direct or indirect pathways.

DA, ITS RECEPTORS AND MECHANISM OF ACTION

DA in the peripheral blood and tissues

Synthesis, forms and elimination of plasma DA

DA arises from the amino acid L-tyrosine, via the enzyme TH that forms an immediate precursor of DA, namely L-DOPA. This is the rate limiting step for DA production. L-DOPA is converted by another enzyme, AADC to generate DA [15]. Following intravenous administration of DA, it is widely distributed in the body but does not cross the blood-brain barrier (BBB). The plasma half-life of DA is about 2 minutes, the onset of action occurs within 5 minutes. In humans, plasma level of L-DOPA exceeds NE by about 10-fold, due to a much more rapid clearance of NE compared to L-DOPA. Until recently it has been thought that all of the L-DOPA synthesized in sympathetic nerve endings is rapidly converted to DA. Release of L-DOPA from sympathetic nerve endings into the bloodstream has not been expected, in spite of the fact that there are always increments of plasma L-DOPA levels between the arterial inflow and venous outflow in the limbs, heart, head, leg, adrenal gland, and gut. At the same time, some experimental and clinical findings indicate the existence of important additional, non-neuronal sources of L-DOPA in arterial plasma as well [95, 98]. The APUD cells constitute an additional source of DA in peripheral tissues; can be found in kidney [212] both exocrine [145] and endocrine [183] pancreas, retinal cells [125].

At the periphery, DA is metabolized in the liver, kidneys, and plasma by monoamine oxidase (MAO) to form dihydroxyphenylacetic acid (DOPAC) and catechol-0-methyltransferase (COMT) to form the inactive compounds homovanillic acid (HVA). DOPAC level in plasma is about 50 times higher then DA, due to the much slower clearance of DA from the circulation. The origin of plasma DOPAC, at least some of it, is the sympathetic nerve terminals [95]. Plasma HVA is derived mainly from DOPAC that is O-methylated by COMT. This explains why COMT inhibition increases plasma DOPAC levels as HVA levels fall. The liver and kidneys possess high levels

of COMT activity; however, in humans, a substantial proportion of HVA production takes place in the wall of mesenteric organs [76].

Besides the above mentioned metabolites, DA sulfate (DA-S) is the major form of circulating DA [95, 97]. Sulfoconjugation is carried out in the gastrointestinal (GI) tract by sulfotransferase 1A3 (SULT1A3) [77, 95]. Basal level of plasma DA-S exceeds by 5 folds the combined levels of free DA, NE or E. In the circulation, the biologically inactive DA-S has a half-life of 3–4 hr, compared to few minutes for unmodified DA [78]. Unlike inactivation of DA by deamination, O-methylation or glucuronidation, sulfoconjugation is reversible. DA-S can be converted back to bioactive DA by arylsulfatase A (ARSA), a releasable lysosomal enzyme [91, 199]. It has been recently shown that adipocytes express DA receptors and possess an active ARSA, indicating a regulatory role of circulating peripheral DA converted back from DA-S in adipose tissues [36]. Therefore, it can be reasoned that if target cells, like lymphocytes may also produce ARSA, circulating DA-S may serve as a regulator of immune functions as well.

Sources, precursors and metabolites of DA in lymphoid organs

DA can be released from the sympathetic nerve terminals at the sites of peripheral tissues including lymphoid organs. However, it is primarily considered to be the precursor of NE and E synthesis, but it can function as a neurohormone by its own right. In the spleen for example, the NE-erg sympathetic fibers can reuptake, thus accumulate circulating DA, particularly during stress, which can then be released during sympathetic activation at close proximity of neighboring splenocytes. Apart from neuronal sources such as sympathetic nerve terminals, adrenal medulla, the peripheral DA can originate also from immune cells. Therefore, the major source of DA for lymphocytes might be within the immune system itself. It has been shown that DA is present in lymphocytes, macrophages and neutrophils and, at least in case of the first two of them; therefore they are capable of actively synthesizing DA from amino acid precursor tyrosine through the intermediate, L-DOPA. Lymphocytes can also uptake DA through active transport via DAT [17, 22, 29, 99].

The lymphoid organs are massively innervated by nerves, which can release DA and other neurotransmitters and neuropeptides within these organs. The secondary lymphoid tissues are highly innervated by sympathetic nerve fibers. Thus, numerous direct contacts exist between nerve terminals and immune cells at the periphery [217]. Since blood vessels are also intensely innervated by nerves releasing neurotransmitters, thus providing conditions also for direct contacts between neurotransmitters and blood-borne T-cells in both health and disease [130].

Physiologically, production of catecholamines is required for normal functions of the immune system. For example, DA plays an important role in modulation of T cell-mediated immunity [1]. Since both the primary and secondary lymphoid organs are highly innervated by the sympathetic nerves that can store and release a large amount of DA [105, 146, 171, 217], it can be a major source.

In conclusion, besides the fact that DA can be synthesized in, and released from the immune cells [29, 59], the sympathetic innervations of lymphoid tissues can also be DA-erg, which can increase or decrease DA levels related to physiological and pathological (like stress situations) circumstances [147]. Once DA is released from presynaptic terminals, for example in the spleen, bone marrow or even in the blood circulation, it can affect immune cells [17]. The presence of D2 receptors in rat thymus and spleen suggests a role of this receptor subtype in the communications between peripheral sympathetic nervous and immune systems [148].

Control and release of DA in immune cells

Neurotransmitters or neurohormones, such as NE, E and DA can affect but also be produced by various cells of the immune system. Lymphocytes are "indeed a privileged target of catecholamines" and vice versa, DA has been recently identified as a direct modulator of the immune system. Cell specific functions have been described and proved to be regulated by DA originating from either external or intracellular sources [17, 29, 58, 59, 87, 114, 136, 154, 208].

Cosentino et al., [61] have demonstrated that mitogen stimulation of human peripheral blood mononuclear cells have got a fairly similar path of subsequent activation of T and B cells that has been detected by the effect of DA [61]. The induction of catecholamine synthesis through a protein kinase C-dependent mechanism triggers de novo expression of the mRNA of TH resulting in a substantial accumulation of catecholamines, including DA [86] in these activated lymphocytes. It can likely provide an autocrine/paracrine supply of these mediators for lymphocytes themselves and/or for neighboring cells, since most of them express a variety of DA receptors [60].

Treatment with reserpine that inhibits the uptake of catecholamines into secretory granules induces a huge release of DA and E and a decrease of intracellular levels without affecting their metabolites. Depletion of intracellular stores consequently triggers the whole synthetic pathway of catecholamines, which is indicated by the overall increase of DA and E content [59].

In a specific subpopulation of T cells (CD4+CD25+ regulatory T lymphocytes, *Tregs*) Cosentino et al., [59] have described an autocrine regulatory loop that has significance in immune homeostasis. Since *Tregs* express TH it contains releasable amount of DA as well as NE and E. The consequences of reserpine treatment on *Treg* functions has also provided experimental evidence for the existence and the physiological significance of an autocrine/paracrine loop for *Tregs* that is mediated by the endogenous DA acting on the specific DA receptors expressed on the same cells.

These catecholamines when released through autocrine and/or paracrine paths can reduce production of immunosuppressive cytokines, IL-10 and TGF-β, and may cause a consequent down regulation of other *Treg*-dependent mechanisms, such as suppression of effector T cells (*Teffs)*. Inhibition of D1 DA receptors selectively reversed down-regulation of *Tregs*, proving evidence for the regulatory role of DA [59, 61, 141, 181].

TNF-α production is also induced by DA in T lymphocytes, but reserpine does not affect TNF-α (and IFN-γ) production in *Teff/Treg* co-cultures, where it is known that DA is released from *Tregs* themselves. A possible explanation can be that *Treg*-derived DA is released in amounts sufficient to act in an autocrine manner on *Tregs* themselves but it does not have paracrine influence on neighboring *Teffs*, and/or TNF-α production of *Teffs* is not modulated by DA-erg receptors [59].

The paracrine/autocrine immune-regulatory role of DA has also been demonstrated [158] between immune competent human monocytic-dendritic cells (Mo-DCs) and T cells [158]. DA stored in and released from DCs stimulates D1 receptors present on the surface of T cells that increases cAMP and results in a differentiation to the "Th2 lineage". In the absence of DA this differentiation goes to the direction towards Th1 lineage. Since DA acts through the D1 receptors, cAMP subsequently can further increase DA concentration in dendritic cells, thus the released DA potentially able to enhance and auto-regulate via autocrine manner its synthesis acting through the D1 receptors [158].

Similarly, Hasko et al., [104] and Kohut et al., [122] have also concluded that DA affects macrophage functions mediated through an autocrine/paracrine loops [104, 122], since it has been demonstrated that macrophages accumulate catecholamines into functional pools [154], which serves as an indirect evidence [104, 122].

Concentration of DA in plasma and in situ

In general, DA primarily serves as a precursor for the synthesis of NE and E in both the sympathetic neurons and the adrenal medulla. Therefore, the endogenous DA levels in the peripheral blood and tissues, with the exception of the enteric nervous system, have been considered to be low compared to other catecholamines [82]. Although, plasma concentration of DA is similar to those of E, but until recently, because of its much lower potency, circulating DA has not been considered to act as a neurohormone [95]. DA levels in the circulation have been shown to be in the picomolar to femtomolar range. The half life of it is very short, since its concentration is not only determined by the rate of synthesis and entry into the plasma but also by the clearance (metabolism by different pathways) from there.

Moreover, it is clear that this level of DA is several orders of magnitude lower than concentration which has been used in most of the *in vitro* studies. Data obtained using human lymphocytes have suggested a predominant immunosuppressive activity of DA, however the concentration of DA used in those *in vitro* experiments might have been too high compared to physiological concentration of DA in the immune system. The forms detectable are sulfoconjugate or glycoconjugate, which may represent biologically altered status of DA, which are not proved to be directly related to the major sources of catecholamines. Plasma level of the sulfate-conjugated metabolite, i.e. DA-S is generally high because a lot slower clearance rate of it [64, 95, 215].

Eldrup et al., [80] have measured basal levels of free and conjugated DA in the plasma of men. The level of free DA has been found to be between 0–0.72 nmol/l and concentration of the conjugated one is between 6.34–45.03 nmol/l (the average is 15.16 nmol/l). The level of DA-S (but not the free one) has been markedly increased after meals (up to the average value of 33.01 nmol/l) [80].

The concentration of DA, comparable to the level found in the plasma of such individuals who have had cancer related stress, has been used *in vitro* to evaluate its effect on proliferation and cytotoxicity of CD4+

and CD8+ T cells [186]. Using this elevated level of DA, it can inhibit the proliferation and cytotoxicity of CD4+ and CD8+ T cells, which has been more prominent on CD8+ T cells compared to CD4+ T cells. It has been also shown that the mechanism of these DA related inhibition is mediated through the D1 type of DA receptor and stimulation of intracellular cAMP [187].

The supposedly higher level of DA in the lymphoid microenvironment, as discussed above can likely come from two sources: (i) from the immune cells themselves, and/or (ii) from the sympathetic nerve terminals, which are in close opposition to T lymphocytes and other immune cells in the lymphoid organs [148]. Local concentration of DA in these tissues might be much higher than in peripheral blood, since DA can be elevated following the inhibition of dopamine β-hydroxilase (DBH) [17, 187].

As far as the secondary lymphoid tissues are concerned, they are also innervated by the sympathetic nerves that can potentially store a large amount of DA. Nerve fibers are particularly concentrated around vascular endothelial cells [217]. The estimated concentration of DA in these synapses is between 100–300 μM. At the same time, lymphocytes themselves can also produce DA at the concentration between $1.6–8.6 \times 10^{-18}$ mol/cell [29]. Thus, lymphocytes may be exposed to a relatively high concentration of DA within the microenvironment of the secondary lymphoid tissues, especially in close vicinity of blood vessels, including the high endothelial venules (HEV), which serves as the gateway for lymphocyte homing as well [10, 42, 196, 214]. This suggests that DA may play an important regulatory role in the homing of naïve CD8+ T cells into secondary lymphoid tissues [215].

DA receptors on immune cells

As it has been already mentioned, DA-erg agents can profoundly influence immune response acting at certain levels and/or on different subtypes of cells of the immune system [17]. However, the overall outcome of immune response to physiological or pathological changes, like stress situations, is likely depend upon the integration of the effects exerted by various components of the immune system, such as lymphokines on one hand, or other active immunomodulatory agents, on the other hand. Additionally, the function of DA-erg receptors depend upon the resting or activated state of lymphocytes. This has already been shown on T lymphocytes, but similar results have recently been collected on other immune cells as well. Therefore, it must be emphasized that the functional status of lymphocytes and other immune competent cells may substantially affect their response to DA resulting in diverse and sometimes conflicting results of experimental data [59, 60, 86, 92, 110, 131, 186, 206]. All of those may lead to conclusions in a specific DA-ergic-immune environment but not being relevant for more general aspects in drug development and for clinical practices.

Cells of the immune system, including T-, and B-lymphocytes, monocytes, neutrophils, eosinophils, macrophages as well as natural killer (NK) cells express specific DA receptors on their surface [17, 141, 180], but there are substantial differences between them. For example, T lymphocytes, monocytes have low, neutrophils, eosinophils have moderate and B lymphocytes, NK cells have high and more consistent expression of DA receptors [141]. As it has been shown in the central nervous system the expression of DA receptors on immune cells is dynamic. It means that they can change in response to different signaling-, and modulating molecules, like cytokines or neuropeptides and neurotransmitters, for example, they can potentially be up-regulated or down-regulated by different physiological stimuli or pathophysiological situations [130].

DA receptor classes and their subtypes

DA acts through its two main, D1 and D2 classes of DA receptors. This classification was originally based upon their ability to affect cAMP production and adenylyl cyclase (AC) activity as summarized in recent comprehensive review of Beaulieu and Gainetdinov [20]. D1 type of DA receptors (D1 and D5 subtypes) increases the intracellular cAMP and AC activity following receptor activation. In contrast, D2 type of DA receptors (D2, D3 and D4 subtypes) inhibits intracellular cAMP and AC activity upon stimulation [17, 189]. Moreover, D2 type of receptor exists in two alternatively spliced isoforms, termed D2-short and D2-long receptors, which differ from each other in the insertion of 20 amino acids of the third cytoplasmic loop. Meanwhile, all subtypes of these two classes have already been classified and characterized on the basis of their structural, pharmacological, and biochemical properties [20].

Expression of DA receptors on immune cells

Besides their broad expression patterns in the brain and certain peripheral sites, all subtypes of DA receptors (D1, D2, D3, D4 and D5) are expressed in normal

leukocytes [5, 32, 81, 86, 121, 131, 141, 157, 158, 179, 215]. Expression of the two isoforms (long and short) of D2 receptors has not been investigated yet on immune cells. In most cases the expression of DA receptors on lymphocytes has been demonstrated by RT-PCR showing the specific mRNA expression [14, 177, 178, 180, 201], as well as by immunocytochemistry using receptor subtype-specific antibodies (Table 1) [6, 59, 141, 215].

Nowadays, there is a general agreement that D3 subtype is the predominant DA receptor in lymphoid tissues, and it is mainly expressed in naïve or resting T (CD8+) cells in both humans and mice, which indicates that its expression is highly conserved across the species. In contrast, expression of other subtypes of DA receptors appears to have a more restricted distribution [215].

Both normal and malignant B cells can also express subtypes of DA receptor (with an exception of the D4 type). The expression levels of these receptors are different between normal and neoplastic B cell. For example, transcripts of D1 and D2 receptors have frequently been found, whereas D3 and D5 revealed to be restricted only to specific subpopulations [141, 144]. In human, dendritic cells also express D1 DA receptor [157]. The presence of both D1 and D2 classes of DA receptors have been also shown in microglial cells (the immune-competent elements of the brain) by Chang and Liu [55], and this finding has been recently confirmed by Färber et al., [83]. Similar to other immune cells, the presence of specific DA receptors has also been shown on the surface of macrophage [17, 18, 29]. Consonant with this finding, recent observation has suggested that functions of macrophage are regulated by the endogenous DA-erg tone [52].

Mechanisms of DA receptor mediated actions on immune cells

There are unique features of DA mediated response of immune cells: It is dependent on the functional status of the cells. Activated cells convey stronger response via D3 receptor and react differently with the D2, D4, and D5 subtypes compared to resting (inactive) T cells [59]. Resting and activated T cells also differ in mRNA levels of D3 receptors. Moreover, the same receptor can use different signal transduction pathways. D3 receptors on T cells can be coupled to both inhibitory and stimulatory G proteins (Gi and Gs) resulting in respective changes (decrease and increase) in cAMP accumulation [109, 161]. Both Gi and Gs coupling can be abolished by addition of D3 receptor antagonist. Changes in cAMP accumulation following DA-erg activation using either the stimulatory or inhibitory pathways are greater in mature than in blast T cells [110].

DA induces a huge and sustained increase of intracellular cAMP in *Treg* cells. This cAMP elevation occurs almost immediately and it can still be detected after hours. In contrast to *Tregs*, no similar change observed in *Teff* cells. This DA-induced cAMP rise in *Treg* cells is specific to D1 receptors, since it can be dose-dependently blocked by D1-specific receptor antagonists [59].

Catecholamines have the ability to block nitric oxide production by microglia, which could partially explain the impaired immune protection against viral infection in the central nervous system in stressed animals. In microglial cells both the D1 and D2 classes of DA receptors are present. Brain derived DA act via D1 receptors to regulate the synthesis of microglial nitric oxide. During stress situation, catecholamine secretion is enhanced and immune responses are diminished. Additionally, it has been also shown that DA regulates the migration of immune cells *in vitro*, since tonic DA receptor stimulation enhanced migratory activity [55, 83].

Expression and function of DA transporters on immune cells

Plasma as well as vesicular membrane transporters (DAT and VMAT, respectively) are probably the most specific markers of DA-erg neurons [137]. DAT and the type 1 and 2 VMAT (VMAT1 and VMAT2) reuptake and remove DA from the extracellular space or transport DA into the secretory vesicles for controlled release, respectively [143, 144].

Vesicular monoamine transporters (VMAT)

VMAT-2 is the predominant VMAT in the CNS but it is also present in adrenal medulla and peripheral blood cells (see also Table 1). Western blot and RT-PCR analysis have confirmed the previous results and further characterized these systems in peripheral tissue preparations, including in peripheral blood lymphocytes, thymus and spleen [148, 149]. The function of VMAT-2 in peripheral blood lymphocytes is similar to the neuronal counterpart, i.e. it is responsible for transporting cytoplasmic DA into vesicles, which provide storage and depot for release [4, 137, 148]. Similar to DA-erg terminals, following the transport through VMAT-2, the intra-vesicle DA cannot be oxidized, consequently DA-related reactive free radicals, reactive oxygen species (ROS) cannot be produced. Therefore,

Table 1
Dopamine Receptors/Transporters on Immune Competent Cells

Cell type	Receptor class	Reference	Observation/summary/comment
B lymphocytes, human	D5, D2, D3, D4	McKenna 2002	B cells have higher and consistent expression. DA receptors D3R and D5R have been found in most individuals whereas D2 and D4 have more variable expression. D1R was never found
B lymphocytes neoplastic, normal	D1, D5, D2, D3	Meredith 2006	Transcripts for D1R and D2R were frequently found, whereas D3R and D5R revealed restricted expression, D4R was not detected
Dendritic cell, T lymphocytes CD4+ CD45RA+ naïve	D2-like	Nakano 2008 Nakano 2009	D2-like-receptor antagonists, L750667 induced dendritic cell DC-mediated Th17 differentiation; sulpiride and nemonapride induced a DC-mediated T(h)2 differentiation. DC is a source of DA, which functions as a T(h)2-polarizing factor in DC-naive T-cell interface
Dendritic cells	D1/D5, D2	Nakano 2008 Nakano 2009	D2-like receptor is functionally dominant over D1-like receptor in Mo-DCs. Antagonizing D2-like receptors increases dopamine synthesis and storage by sustaining the cAMP elevation. Antagonizing D1-like-receptors inhibits Th17 differentiation and IL17 production
Eosinophils	D2, D3, D4, D5	McKenna 2002	Eosinophils have moderate expression D3R and D5R receptors whereas D2R and D4R have not. D1R could not be found at all
Immune cell derived lines	DAT	Meredith 2006	DAT transcripts have been found in most immune cell derived lines
Microglial cells	D1, D2, D3, D4	Chang 2000 Faber 2005	DA by acting via its receptors have the ability to block nitric oxide production by microglia, which could partially explain the impaired immune protection against viral infection in stressed animals. Chronic dopamine receptor stimulation enhanced migratory activity
Monocyte	D2, D3, D4, D5	McKenna 2002	Monocytes have low expression of D2R, D3R, D4R and D5R
Neutrophils	D2, D3, D4, D5	McKenna 2002	Eosinophils have moderate expression D3R and D5R receptors whereas D2R and D4R have not. D1R could not be found at all
NK cells	D2, D3, D4, D5	McKenna 2002	NK cells have high and consistent expression of D2R, D3R, D4R and D5R
PBL	D2, D3	Levite 2001	D2R and D3R were both shown
PBL, human	D3, D4, D5	Amenta 1999 Amendta 2002	D3 and D4 receptor subtypes on human peripheral blood lymphocytes, from D2-like receptors, but no D2 receptor immunostaining
PBL, human	DAT, VMAT	Amenta 2002	PBL express plasma membrane and vesicular dopamine transporters
PBL, human	D3, D4, D5	Ricci 1995 Ricci 1998	D3R, D4R receptor subtypes identified by radioligand binding assay technique and antibodies against dopamine D2-like receptor subtypes
PBL, human	D5	Ricci 1999	D5R was found to be the only D1-like receptor in human peripheral blood lymphocytes (no D1 mRNA) expressed on human leukocytes. Confrimed also by radioligand binding assay and immunocytochemistry
PBL, human	D5	Takahashi 1992	RT-PCR products contained three types of sequences of D5 dopamine receptor gene and the two related pseudogenes
PBL, normal human leukocytes	D1-like, D2-like	Ferrari 2004	DA-ergic D1-like receptor agonist inhibited TPA-induced TH mRNA expression, and consequently promotes cell survival. D2-like antagonist domperidone did not interfere
PBL, normal human leukocytes	D1, D5, D2, D3, D4	McKenna 2002, Kirillova 2008	DA receptors D3R and D5R were found in most individuals, D2 and D4R had more variable expression. D1R was never found, confirmed by radioligand binding and quantitative RT-PCR assays
Splenocytes, macrophages in mice	DAT	Kavelaars 2005	The activity of the immune system in DAT(-/-) mice have been challenged and concluded that interference with the DAerg system has major consequences for both the acquired and the innate immune response. Splenocytes had reduced NK cell activity and reduced mitogen-induced cytokine responses. Macrophages had enhanced LPS-induced cytokine production

Table 1
(*Continued*)

Cell type	Receptor class	Reference	Observation/summary/comment
T and B lymphocytes, human	D2, D3, D4	Santambrogio 1993	Pharmacological characterization of the binding site suggests similarities mainly with the D2R and D4R rather than D3R subtype of DA receptors
T lymphocytes	D2, D3, D4, D5	McKenna 2002	T-lymphocytes and monocytes have low expression of D2R, D3R, D4R and D5R. No D1R was shown
T lymphocytes Treg /Teff CD4+	D1, D5, D2, D3, D4	Kipnis 2004, Cosentino 2007	Tregs and Teffs express on the cell membrane both D1-like and D2-like dopaminergic receptors. Treg expressed significantly more D1R and D5R transcripts then Teff. No significant difference in D2 type receptors family (D2R, D3R, and D4R) in Treg and Teff
T lymphocytes, human, normal	D1/D5, D2, D3	Besser 2005	DA triggers secretion of IL-10 and TNF-α by T-cells, without affecting IFNgamma and IL-4. Secretion of TNFα selective to D3 or IL-10 via D2; for TNF-α+IL-10 via D1/D5R
T lymphocytes, human, normal	D3, D2	Levite 2001	DA activates naïve/resting human peripheral T-cells, by stimulating D2R and D3R. Induction of integrin-mediated adhesion to fibronectin/activation of β1 integrins of human T-cells
T lymphocytes, naive CD4+	D1/D5	Nakano 2009	DA dose dependently increased cAMP levels via D1-like receptors and shifts T-cell differentiation to T(h)2, in response to anti-CD3 plus anti-CD28 mAb
T lymphocytes, human, normal	D4	Sarkar 2006	D4Rs in T cells isolated from normal volunteers, demonstrated by RT-PCR and Western blot analysis
T lymphocytes, naive CD8+ human, mice	D3	Watanabe 2006	D3R is the predominant subtype of DA receptor in secondary lymphoid tissues, selectively expressed by naive CD8+ T cells. Induces chemotactic responses both in humans and mice
Thymus and spleen lymphocytes, rat	D1, D2, D3, D4, D5	Mignini 2009	D1R, D2R, D3R, D4R and D5R receptor immunoreactivity primarily found in the thymic cortical-medulla transitional zone and to a lesser extent in the medulla. In spleen immunoreactivity was located in the white pulp border and to a lesser extent in the white pulp
Thymus and spleen lymphocytes, rat	VMAT-1; VMAT-2	Mignini 2009	Thymus and spleen express elements of the DAerg system: presence of DA, all types of receptors and both froms of VMAT
Treg CD4+CD25+	D3*	Cosentino 2007	The mRNA of DAerg receptor subtypes have been detected *(D2 and D4 were not investigated)
Treg CD4+CD25+	D1/D5	Cosentino 2007	D5Rs is the main receptor, functionally coupled to intracellular production of cAMP which is responsible for DA-erg inhibition of Treg function, via autocrine/paracrine loop to downregulate Treg function

Notes: ConA: concanavalin A; cAMP: cyclic-AMP; DA: dopamine; DAT: dopamine transporter; DC: Dendritic cell; IFN-γ: interferon-γ; IL: Interleukin; LPS: lipopolysaccharide; NK: natural killer cells; PBMC: peripheral blood mononuclear cells; PBL: peripheral blood lymphocytes, Th1/Th2: T helper type 1/type 2 cells.

one of the possible function of VMAT is to prevent lymphocytes from apoptosis (vide infra) [148, 163].

Studies using immunohistochemistry have provided evidences that the pattern of DA immunoreactivity in immune organs is similar to that of VMAT-1 and VMAT-2 and mostly co-expressed with DAT in the thymic cortical-medulla transitional zone and to a lesser extent in the medulla, but in the cortex not at all. In the spleen, VMAT-1 and VMAT-2 immunoreactivity are primarily located at the border of white pulp (co-localized with DA receptors), and to a lesser extent inside the white pulp.

However, it should be mentioned that the precise location of DA stores in immune organs and whether they express the same or different catecholamine transporters are not yet fully described [148].

DA transporter (DAT)

This member of the Na^+/Cl^--dependent transporter superfamily displays the characteristic twelve transmembrane domains and actively transports DA. Hence, not only DAT can remove DA from the synaptic cleft but under certain conditions can also pump DA out of cells [15], which is generally referred to as the "reverse transport".

A growing number of data in the literature highlights the potential for most of the immune cells to express DAT or at least a complement of it (see also

Table 1). DAT transcripts have been shown in most immune cell derived lines [143, 144]. The functional characteristics of DAT detected on human peripheral blood lymphocytes or in primary lymph organs are fairly similar to that observed in the brain [4, 99, 118, 148, 149]. Highly selective uptake inhibitors, like GBR 12909 have been used to show the presence of DAT in peripheral lymphocytes [29].

The expression of the mRNA of DAT in both the thymus and the spleen has been shown by Mignini et al., [149]. However, as far as the thymus is concerned, DAT has not appeared to be co-localized with thymocytes [148]. Catecholamines are located in both, neural and non-neuronal compartments of the thymus. Neural compartment contains sympathetic fibers, where DA is co-localized with NE. While in the non-neural compartment catecholamines (mainly DA and NE) are supposed to be predominantly present at the medullary side of the corticomedullary junction and also in the subcapsular cortex. It should be mentioned that due to the relatively low concentration of catecholamines in the thymus, it is difficult to demonstrate the exact localization of DA itself [170]. In rat thymus, immunohistochemistry revealed DAT immune reaction in the wall of arteries located in the septa. On the other hand, in the spleen, DAT immunoreactivity was located primarily in the wall of white pulp arteries and in the marginal zone between the red-, and white pulp within small cells, likely corresponding to lymphocytes. As an indication to its possible role, inhibition of the function of DAT resulted in an altered natural killer (NK) cell activity and insufficient mitogen-induced cytokine responses [118, 149].

DAT is a common target of several drugs used in both the therapeutic field of psychiatry (psychostimulants, antidepressants), and drug abuse (like cocaine or amphetamine). Manipulations with the function of DAT and its potential influence on DA levels in and surrounding immune cells (that is supposed to have regulatory potential) might disturb immune functions as well. In theory, this may open a new avenue for drug development [43, 135, 205].

A possible role of VMAT and DAT in the maturation and activation of immune cells

The maturation and activation of immune cells involve complex processes including steps of proliferation and differentiation. Contribution of DA in immunomodulation is based on the hypothesis that DA is released into the lymphoid microenvironment and consequently able to achieve modulation of immune cells. In other words, it is present at the right place and at the proper stage of, for example, the early phases of thymocytes development in the thymus. The observation that DA and VMATs are located at the border of the thymic medulla, where most of single-positive (CD4+ and CD8+) lymphocytes reside suggests that DA may play a role in the modulation of T cell maturation process. It may be slowed down or accelerated. In a recent work of Mignini et al., [148] it has been demonstrated that D1-, and D2-like DA receptors are also expressed in the same areas where DA is being stored and its transporter is mostly located in the thymus [148]. It is in concert of the hypothesis of Swarzenski et al., [200] that D2-like activity can stimulate mitogenesis and it may also induce differentiation of immune cells [148, 200].

Based on the microanatomical localization of DA-erg markers in the spleen underlines the potential role of DA and its receptors. DA must have been involved in the maturation and selection of lymphocytes, and even more, in the induction of the activation cascade of immune responses [148, 149].

Malignant B cells also express DAT [144]. The presence and the localization of DAT in spleen has provided additional evidences that DA release from either lymphoid or neural elements in the lymphoid microenvironment may have its distinct role in immune modulation. However, the importance of any other sites, such as direct or indirect DA-erg induction or activation of lymphocytes in primary and secondary immune organs should not be diminished [149].

DAT knockout (KO) mice have also been used to investigate the possible role of DA uptake system on both the acquired and the innate immune responses. DAT KO mice are generally characterized by being hyper-DA-erg phenotype, due to the lack of DA uptake and metabolism. Deletion of DAT results in a reduced NK cell activities and delayed type hypersensitivity responses. In contrast, cytokine production of macrophages induced by mitogen (LPS) is increased in the same animals [118].

Actions independent from dopaminerg receptors

Actions of DA via reactive intermediers

In sets of experiment, human B cell lines and mitogen induced proliferating normal lymphocytes had been exposed to DA, L-DOPA and apomorphine. All of them have delivered significant cytostasis to a spectrum of B cell malignancies representing stages of maturation arrest from pre-B cells through to plasma cells, and similarly to proliferating nonmalignant B cells. The oxidative stress might be constituted as the

primary mechanism of cytostasis. The action of DA being mimicked by hydrogen peroxide and reversed by exogenous catalase can also serve as an evidence for the contribution of intracellular redox protein thioredoxin. Since pharmacological analysis using specific receptor blockers disclosed that DA targeted cycling B cells using the classical "DA-erg components" (like DA receptors or DAT), thus a completely independent and still unknown event, called "receptor and transporter independent" mechanism may be applicable to understand the interactions in neurotransmitter-immune communication. This mechanism seems to be applicable for normal B cells and their lymphoma counterparts as well [144].

Experimental findings have indicated an increased sensitivity of proliferating B cells to DA's actions. A few hours of treatment with increasing DA concentrations resulted in steadily enhanced apoptosis, while viability of resting peripheral blood mononuclear cells (PBMC) or tonsil B cells were relatively uncompromised even after 24 h of exposure to DA [144]. This pro-apoptotic effect of DA may induce: (1) loss of mitochondrial potential; (2) relocation of Bax to the mitochondria; (3) cytochrome c release; (4) caspase-3 activation, and (5) nuclear fragmentation, resulting in apoptosis [113].

Interestingly, the rapidly dividing B cells are targeted by DA for cell-cycle arrest, which may proceed to apoptosis, where Bcl-2 levels are low. Although Bcl-2, Bax, or Trx levels could not individually explain all variations across the populations, it is suggested that a composite of their actions may have influence the outcome: Bcl-2 and Trx contributing resistance and Bax conferring sensitivity [144].

References in relation with the above discussed DAT or D1/D2 receptors suggested an indirect role within the CNS that results in DA-related toxicity due to increased intracellular concentrations of DA or DA-like molecules independent from DA receptors. Oxidative stress generated by DA oxidation triggers apoptosis via a signaling pathway that is initiated by the generation of ROS [115]. The DA toxicity mechanism generally involves both auto-oxidation and monoamine oxidase-mediated oxidation of catecholamine to form ROS and quinones, which ultimately contribute to inducing oxidative stress in cells [16]. DA exerts its effect on normal cells via a D2-independent mechanism. This similar effect in lymphocytes involves DA-derived ROS formation as well as oxidative stress. Apoptosis and consequent cell-death of thymocytes have been reported to be mediated through a mechanism similar to that observed in the central nervous system [163].

Microglia cells in CNS, produce a variety of toxic substances to eliminate infectious agents. Releasing these toxic substances like ROS, reactive nitrogen species, proinflammatory cytokines, and prostaglandins can accelerate neuronal injury and even lead to cell death. Since the substantia nigra contains the highest concentration of microglia in the brain, this region especially susceptible to altered microglial activation responses. Increased levels of pro-inflammatory cytokines such as TNF-α and IL-1β found in post-mortem PD tissues, similarly elevated IL-6 appears to increase the risk for developing PD [203].

In a GH3 pituitary cell line DA rapidly increased the formation of intracellular ROS. The antioxidant N-acetyl-L-cysteine applied in the same culture effectively blocked DA-induced ROS formation and prevented the consequent apoptosis, therefore that DA triggers apoptosis via a mechanism involving DAT and oxidative stress [113].

In summary, DA delivered significant cytostasis at low micromolar concentrations, resulted in as antiproliferative effect for normal and malignant B cells as well as for stimulated PBMC. The growth arrest for normal and malignant B cells was concluded to be independent from dopaminergic receptors and transporter components; performed via reactive oxidation products that can significantly contribute to the mechanism of cell cytostasis. Other agents, similarly involved in redox-cycle that generates superoxide or reactive intermediates, are also effective inducing cytostasis. Interestingly, some other antioxidants (N-acetyl cysteine, ascorbic acid, reduced glutathione, trolox, and cell-permeable antioxidants) were reported without effect in similar lymphocyte cultures [144]. On the other hand, the *in situ* DA concentration in lymphocyte cultures and also in the microenvironment *in vivo* is being mediated also by dopamine transporters and *vice versa*. DAT induces the increase of intracellular concentrations of the DA-derived products; while in the VMAT embedded DA seems to be hidden and consequently prevents the induction of apoptotic cascade.

Direct actions of DA thru beta-adrenerg receptors

Similar to the direct suppressor effects of other catecholamines (NE, E) on immune cells demonstrated by IL-12 regulation, there is a distinct and direct action of DA on beta-adrenerg receptors that may contribute as an alternate path to regulate the function of specific immune cells [106]. It has been

demonstrated on macrophages that there is a specific beta-adrenerg receptor action of DA causing pretranslational suppression in mRNA accumulation and this effect in modulation of immune response is specific to cell type. However, due to the limitations in this review we aimed to be focused on interactions of DA receptors and transporters, thus not discussed these beta-adrenoceptor-dependent pathways.

SUMMARY OF IMMUNE SYSTEM SPECIFIC ACTIONS OF DA

Direct DA-erg control of immune cells

Theory and the aspects of direct DA-erg control

It has been already mentioned that elements of both peripheral and central nervous systems are supposed to modulate immune functions, in a different manner, by releasing soluble factors such as neurohormones and neurotransmitters. Moreover, lymphocytes themselves are also capable of producing DA [29, 171, 215]. On the other hand, the existence of DA receptors on lymphocytes has been demonstrated by specific binding of DA-erg ligands as well as by RT-PCR for specific mRNA expression [34, 92, 155, 156, 201].

Lymphocytes are always the mixture of functional subsets and indeed, different lymphocyte classes and clones may express different DA receptor subtypes [215]. Due to these facts, some of the experimental results are still contradictory or may be difficult to harmonize with the clinical observations. The overall effects of neurotransmitters on T-cells are still diverse *in vivo* and are "rarely exclusive" for stimulatory or inhibitory influence. In a recent review Levite [130] summarized these effects to four pragmatic categories of "crucial factors" that will determine the outcome of the effect of a neurotransmitter [130]:

- Activation state of T-cells
- T-cell subpopulation subjected to stimuli
- The subtype of receptors expressed on T-cells
- Concentration of neurotransmitter

Catecholamine markers and immune cells. Both the primary and secondary lymphoid organs are highly innervated by the sympathetic nerves that store a large amount of DA [104, 217]. Catecholamines are located both in the neural (sympathetic nerve endings) and also in the non-neuronal (medulla or cortico-medullary junction with T lymphocytes or thymic epithelial cells compartments of the thymus [170], or primarily in the white pulp border and to a lesser extent in the red pulp in the spleen [147, 148]. Similarly, DA receptors are found on most of the immune cells in primary and secondary lymph organs and also on peripheral blood lymphocytes [148]. The effects where DA can elicit its biological actions are its own receptors [148, 162] or via reactive intermediers [144].

The most important evidences for the direct control of immune system by DA are based on the fact that variety of DA receptors as well as DAT and VMAT are present in lymphocytes [148, 149]. DAT and VMAT-2 transporters are considered probably the most specific markers for localization of DA-erg neurons [137]. DAT of immune cells (also on human peripheral blood lymphocytes) displays similar characteristics to those expressed in the brain and other peripheral tissues [4, 99]. However, the location of DA stores in other immune organs and the consequences of different micro-anatomical circumstances are still under discussion [148].

The neuroimmune communication via neurotransmitters. The effects of DA on the function of immune cells and its role as neuroimmune mediator recently became into the focus of basic research (Table 2). A theory of "interactive neuroimmune communication" focuses on substances, which are proved to be present within the nervous as well in the immune systems, such as neurotransmitters. Catecholamines have a key role in neurotransmission and indeed these molecules are "crucially involved in several physiological and pathological conditions" of the immune system. The sympathetic control of the innate immune response is "believed to be immunosuppressive", because the beta-adrenerg receptors coupled Gs pathway, however the involvement of other G-protein dependent mechanisms (Gs/Gi switch) in sympathetic modulation of immune response [132] may have some other and more like a cell-specific importance. It has been known for several years now that DA receptors are expressed on immune cells [6, 17, 59, 130, 141, 180, 215] and these are coupled to both inhibitory and stimulatory G proteins (Gi and Gs) [109, 110, 161]. Their functional activity and their direct coupling to signal transduction pathways are depend on the activation state and the relative expression of specific receptors of these cells [110].

As discussed above, the action of DA on beta-adrenerg receptors may contribute as an alternate path to modulate the function of specific immune cells [106].

Cell-to-cell autocrine/paracrine communication. Until recently, DA has been considered mainly as a

conventional neurotransmitter and a neuroendocrine regulator. Due to the mass of experimental evidences concerned about the existence and function of DA receptors on T cells, the immunomodulatory role of DA has became the focus of experimental research as well as a potential aspect for drug development. Since subsets of DA receptors (D1–D5) have been identified, and DA has been proved to be synthesized, stored and released from immune competent cells, an autocrine/paracrine regulatory loop of DA has been hypothesized.

The other theory of the "immune-synapse" communication is based on the observation that T-cell activation requires a zone of adhesive and direct contact with antigen presenting cells (APC). This, in many aspects, carries similarities with classical neuronal synapses, including a cell-to-cell adhesion and close membrane apposition giving the possibility for the autocrine/paracrine interactions [174]. Several pairs of adhesion molecules are involved in these interactions such as leukocyte function-associated antigen (LFA)-3–CD2, ICAM-1,-3–LFA-1 or DC-SIGN–ICAM-3. Because naïve T cells highly express ICAM-3, which can provide co-stimulatory signals to T cells and which has been localized at the APC-T cell interface DC-SIGN–ICAM-3 is thought to be the most important adhesive interaction. Within that triggering of cell surface receptors leads to intracellular signal transduction in both structures. Recent studies have revealed that some neurotransmitters secreted by DCs, have been released for naïve T cells during DC–T-cell interaction, leading to an activation of T cells in mice and humans. The Mo-DCs in periphery are one of the major sources of DA, which can be released upon interaction with naïve $CD4^+$ T cells, thereby regulating the cytokine profile and T_h1/T_h2 differentiation [158].

Experimental evidences obtained from in vitro and in vivo studies. Using high doses of DA *in vitro* has been shown to inhibit the mitogen-induced proliferation and it can also promote apoptosis. Several studies looked at the effects of DA on T cells and demonstrated a down-regulation of cytokine expression [92]. In most of these studies, peripheral mechanisms have been taken into consideration for this neurotransmitter effect on T cells. Subtype of lymphocytes or the status of activation of lymphocyte population have to be specified to predict and moreover to draw a conclusion from these studies [50, 110, 118, 148].

Data from *in vivo* studies have also provided conflicting results. For example, the effect of DA can be either stimulatory or inhibitory on lymphocyte functions [57, 207] or DA can only affect a certain subpopulation of lymphocytes, like blast but not the resting T cells [110]. Another explanation can be that the experimental design has allowed investigating only a specific function of lymphocytes [8, 131, 215].

On the other hand, direct and indirect modulation by DA may also cause a diversity of its actions on the immune system. Besides the direct influence of DA on cells like T and B lymphocytes, the amount of released DA can also change the profile of cytokine secretion, consequently functions of specific cells in the immune system [17].

However, we should note that the experimental design, dosage applied and the relative sensitivity of immune cells to other *in vitro* or *in vivo* environmental *in situ* factors may not have been taken into (full) consideration. Therefore, the results and the conclusions made by different authors still may have contradictory statements or data with relative conflicts.

Besides massive experimental evidences, increasing number of clinical observations has been published over the last decade, regarding the ability of DA to affect the function of most subtypes of lymphocytes, including *Treg* cells (see also Table 2) [8, 32, 59, 92, 110, 119, 131, 186, 215]. The most prominent aspects of the immune-modulatory or immune-regulatory role of DA clearly indicate that these results can have an impact on developing a novel therapeutic approach, will be outlined in the last chapter of this review.

Endogenous DA-erg system in lymphocytes

Over the last few years several observations indicated the ability of DA to affect various functions of lymphocytes, including proliferation and differentiation. These results support the hypothesis that in lymphoid tissues and cells an "endogenous DA-erg system" exists, because:

- they can produce DA via *de novo* synthesis,
- or progress with functional uptake of DA from other sources,
- suggesting the presence of an active DA-erg control via autocrine/paracrine loop,
- through which lymphocytes can down-regulate their own activity.

Pharmacological inhibition of TH reduces catecholamine levels of lymphocytes suggesting an active catecholamine synthesis. On the other hand, the intracellular DA levels are shown to be increased by extracellular DA, underlining an active cellular-uptake mechanism. The effect of addition of L-DOPA or DA from external sources resulted in a dose dependent

inhibition of proliferation and differentiation of lymphocytes indicate that catecholamines produced by lymphocytes may act in an autocrine or a paracrine way [29].

We should note that the effect of DA-erg compounds as well as the function of the DA receptors may depend upon the status of T lymphocytes. For example, D3-type of DA receptor induces a stronger response in activated T lymphocytes compared with resting T cells.

One of the recently characterized example of DA dependent activation of immune cells are the *Tregs*, which can produce a substantial amounts of DA, then upon release it can act on DA receptors and consequently regulate the subsequent responses of the same or other immune competent cells. These effects can potentially manifest in an autocrine/paracrine manner. This hypothesis has been supported by the result that DA content is significantly higher (>100×) in *Tregs* than in naïve *Teff* lymphocytes, indicating also the presence of a DA dependent immune competent inhibitory loop sustained by endogenous catecholamines regulating *Treg* modulatory function. In this respect, DA, like some other regulatory molecules, is important, since via these mechanisms specific subpopulation of immune cells can become potentially effective to modulate the cascades of immune response [59, 61, 158].

DA-erg control of T lymphocytes

Activation as well as suppression of T cells induced by neurotransmitters including DA has been reported. This modulatory influence of DA on T cell function has been shown in both normal or stress conditions [17, 110, 130, 147, 190, 215].

Activation: migration, adhesion and cytotoxicity. Expression of receptors mediating cell activation and trafficking (integrins, inflammatory chemokine receptors and others) is a critical if not a fundamental aspect of the immune cell response.

- DA induces chemotactic responses selectively in CD8+ T cells. Levite et al., [131] has first reported a dose range of DA that activates the naïve /resting human peripheral T-cells (CD8+) through the D2/D3 DA receptors. This D3 receptor-mediated activation promotes the adhesion of human T cells to fibronectin and ICAM-1 by triggering activation of the β1 integrins (VLA-4 and VLA-5). This T cell function is closely associated with trafficking and tissue localization of the cells in the microenvironment, during extravasation across the blood vessels or other tissue barriers [131, 215].
- Activated T blast cells have a unique response to DA, which is distinct to resting T cell response, since in the latter one DA does not have significant effect on the expression of the surface marker molecules (VLA-4 CD25, CXCR3). Activation of D3 DA receptor on T blast cells by quinpirole led to the activation and increase in CD25 expression, a decrease in CXCR3, and no change in VLA-4 levels. This is similar to the situation of cytokine as well as surface markers expression: only activated blasts and not resting T cells responded to DA-erg activation. These results demonstrate another aspect of D3-mediated alteration in the immune response of T blast cells [110].
- DA is synergistic with chemokines in attraction of naïve CD8+ T cells. DA induced chemotactic response via D3 receptors coupled with the Gαi class of G proteins has been demonstrated in naïve CD8+ T cells both in human and mouse. D3 is the predominant receptor type in secondary lymphoid tissues and it is also expressed quite selectively in naïve CD8+ T cells. Intraperitoneal injection of DA to mice rapidly attracts naïve CD8+ T cells into the peritoneal cavity via D3 mediated action. When D3 antagonist was applied it selectively reduced the homing of naïve CD8+ T cells into lymph nodes [215].
- In multiple aspects that has been already stated that neurotransmitters, inlcuding DA influence the function of CD8+ T lymphocytes, can act either stimulatory or suppressive. The outcome depends mainly on the activation status of the T cells but clearly can not induce any new or diverse function of these T cells. These agents act more likely as a modulator to enhance or fine-tune the specific responses or functions, such as migration, adhesion and cytotoxicity otherwise coded or induced within the cascade mechanisms of immune response in CD8+ T lymphocytes [198].

Cytokine and surface marker expression profile. Besides the direct actions of DA on immune cells like it has discussed on T lymphocytes, DA can also change the profile of cytokine secretion or DA induced cytokine secretion from resting T cells [32]. Consequently to the changes in cytokine profile, DA can indirectly regulate the functions of other specific cells in the immune system.

- DA acting on specific sets of receptors on T cells can selectively trigger changes in cytokine secretion and surface marker expression profile. That includes the production and regulation of the mRNA levels of the anti-inflammatory IL-10 and the pro-inflammatory tumor necrosis factor-α (TNF-α) and also IL-4, and INF-gamma.
- Stimulation of D3 and D1/D5 receptors increase the secretion of TNF-α, but stimulation of D2 receptors induces IL-10 secretion without affecting the secretion of IFN-γ and IL-4 in resting human T-cells. When D2/D3 agonist quinpirole applied, IFN-γ levels increased suggesting that this effect is mediated through D3 receptor [32].
- The activation status of T cell can remarkably affect its response to DA via D3 receptor activation, however, CD4+ and CD8+ derived subsets of them are affected differtially [110].
- In CD4+ T blasts DA induces a marked shift in cytokine profile and change in surface marker expression. The Th2 to Th1 switch in cytokine profile decreases IL-4 and IL-10 to negligible levels but pronounced IFN-γ is induced.
- CD8+ T blasts, on the other hand, have essentially undetectable mRNA levels of all three cytokines before treatment, but in response to D2/D3 agonist, quinpirole, an increased level of IFN-γ have been detected.
- In contrast, mRNA levels of all three cytokines have been found to be undetectable in resting CD4+ and CD8+ T cells even after quinpirole treatment.
- There is a similar blasts specific effect of D3 receptor, for the three surface markers: namely VLA-4, CD25 and CXCR3, which are involved in trafficking of T cells due to their increased activation state. DA-erg activation of resting T cells had no significant effect on the expression of these three molecules [110].

In conclusion, cytokine expression as well as surface markers respond to DA-erg stimulation on activated blasts only and not on resting T cells. This activation path markedly differs from the classical TCR-induced non-selective cytokine-secretion. Thus, it may have significance in treatments of various immunological and neurological pathologies that are either mediated or can be treated with IL-10 or TNF-α [32, 110].

A Th2 shift in cytokine profile during the peripheral activation of CD4+ T cells have reported by Nakano et al., [158], in response to anti-CD3 plus anti-CD28 mAb. The human monocyte-derived immune competent dendritic cells (Mo-DCs) can release DA that may act on D1 receptors, which are present on naïve CD4+ T cells. The Mo-DCs reside in an immature state in many non-lymphoid tissues playing a critical role in the induction as well as in the regulation of Th1 and Th2 immune responses. The D1-like DA receptors are functional and dominant in CD4+ CD45RA+ T cells. As a result of the antigen-specific interaction with naïve CD4+ T cells induced release of DA, which dose dependently increases cAMP levels via D1-like receptors naïve CD4+ T cells resulting in a shift of T-cell differentiation to Th2, in response to anti-CD3 plus anti-CD28 mAb. This finding also support the hypothesis that these subsets of powerful immune competent cells (Mo-DCs) which play a critical role in the induction of primary immune responses and immunological tolerance, is a source of DA. Moreover, they carry the potential for being Th2-polarizing factor due to D1-induced cAMP-elevation, which predisposes DC-naïve CD4+ T cells to differentiate toward the Th2 lineage [158].

Suppression of T cell functions

- Based on the results of Koussai (1987) certain subsets of T lymphocytes are depressed by DA, like generation of cytotoxic T cells or the number of spleen T cell populations indicating that suppression of selective T cells are mediated by a direct peripheral action of DA [124]. A specific receptor related mechanism has already been demonstrated by several research groups. In contrast to D1 class of receptors whose function is associated with an increase of intracellular cAMP, D2 class of receptors, upon stimulation, inhibits intracellular cAMP [150].
- High concentration of DA increases intracellular cAMP via activation of D1 receptor, consequently significantly inhibits proliferation and cytotoxicity of stimulated CD4+ and CD8+ T cells obtained from normal human subjects [186, 187].
- Similar inhibition of cell proliferation and secretion of IL-2, IFN-c and IL-4 of activated T cells, by down-regulating the expressions of non receptor tyrosine kinase (non RTK) *lck* and *fyn*, has also been demonstrated. This is mediated through stimulation of D2 and D3 receptors [92].
- Stimulation of D4 receptors of activated T cells (i) inhibits the induction of T cell activation via T cell receptor (TCR), while IL-2 concentration remains similar to the unstimulated cells; (ii)

inhibits the ERK1/ERK2 phosphorylation, consequently changes of KLF2 expression in the presence of anti-CD3/CD28. KLF2 is a dominant factor in regulating the critical quiescence state of T cells. A correlation between D4 DA receptor activation and the expression of KLF2 in human T cells may be one of the mechanisms of DA-mediated regulation of T cell homeostasis [190].

- The existence of a DA-erg inhibitory loop sustained by endogenous catecholamines regulating *Treg* function. DA-erg inhibition of IL-10 and TGF-β production and impairment of the ability of *Tregs* to suppress *Teff* proliferation has been demonstrated. The effect seems to be specific to *Tregs*, since reserpine did not change the production of IL-10 or TGF-β, or proliferation rate of *Teff* in the absence of *Tregs*. Since *Teffs* contain only very small amounts of DA, therefore an autocrine/paracrine action is unlikely [59].

Effects of DA on normal and malignant B cells

The experimental data obtained about the effect of DA on B cells are still controversial. Most of the influences of DA are associated with inhibitory effect of cell proliferation, which results in apoptosis. The role of classical β-adrenergic receptor/adenylyl cyclase/cAMP-mediated inhibitory path may also be relevant in case of B lymphocytes, since β-adrenergic receptors are also present in these cells. However, similar to T-cells, both D1 and D2 class of DA receptor subtypes exists in B lymphocytes that can carry the specific ligand related stimulatory or inhibitory actions, respectively, as discussed above [141, 144].

- DA promotes apoptosis on active/cycling B cells (but not in resting lymphocytes) through mechanisms of oxidative stress. Incubation with DA could completely abolish the production of antibodies by B cells or in a dose-dependent manner the inhibition of lymphocyte proliferation and differentiation, as observed by Bergquist et al., [29, 28].
- The effects of DA, noradrenaline and other adrenergic agonists on lymphocyte activation were studied by Cook-Mills [57] to test function of the classical β-adrenergic receptor/adenylyl cyclase/cAMP-mediated inhibitory pathway in B and T cells. DA and also noradrenaline, adrenaline, isoproterenol all inhibited the specific mitogen (LPS; ConA) activation of splenocytes. The inhibition of lymphocyte activation could not be reversed by neither DA-erg receptor antagonist (haloperidol) nor β-adrenergic receptor antagonist, i.e. ruling out the classical β-adrenergic cAMP-mediated inhibitory pathway [57].

According to the data published by Meredith et al., [144] DA inhibits proliferation of B cells. Upon exposure to DA in a low micromolar concentration range proliferation of both the mitogen stimulated normal (nonmalignant) B lymphocytes and polyclonally stimulated peripheral blood mononuclear cells were inhibited. Malignant clones of B cells were also arrested by DA using the same range of dose. Similar results have been observed with L-DOPA or apomorphine [144].

A 6-hour-long *in vitro* treatment with DA leads to apoptosis, where Bcl-2 levels are low. Bcl-2 is considered critical in DA-induced cytostasis when accompanied by apoptosis. However, this action of DA is not found in resting lymphocytes. Resting cell viability (PBMC or tonsil B cells) has been relatively uncompromised even after 24 h of exposure. The impact of DA-related selective modulatory effects on lymphocytes that are found in active cycle indicates a potential axis for therapeutic intervention not only in case of B cell neoplasia, but also in lymphoproliferative disturbances [144].

The presence of DA is required to maintain the proliferation of splenocytes in response to mitogens like lipopolysaccharide (LPS) and concanavalin A (Con A). Artificially lowered endogenous DA by intraperitoneal administration of alpha-methyl-p-tyrosine (aMpT) or 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) resulted in suppression in response to mitogens. Application of specific receptor agonists *in vivo* and similarly, in cell cultures *in vitro*, D1 or D2 agonists or DA directly promoted cell proliferation in response to mitogens. These results indicate that DA enhances mitogen induced proliferation of B- and T-cell both *in vivo* and *in vitro* [207].

It has been shown that treatment of murine splenocytes with DA or L-DOPA stimulates proliferation by acting through D2 receptors [51].

Macrophages, NK cells, dendritic and other immune competent cells

DA can cause reduced NK cell activity and ovalbumin (OVA) induced delayed type hypersensitivity responses as reported in animals with the deletion of dopamine transporter (DAT) genes in consequence of the hyperdopaminergic phenotype of DAT(-/-) animals. Interestingly the cytokine production in LPS

Table 2
Immunomodulatory Effects Mediated via Cellular DA Elements

Cell type	Immunomodulatory effects mediated or triggered by DA or receptor agonist/antagonist	Receptors involved	Reference/year
B and T lymphocytes, from BALB/c mice	DA inhibits cell proliferation and the activation of spleen and thymus B and T cell by LPS and Con A, respectively. (D2 antagonist failed to reverse)	D1	Cook-Mills 1995
PBMC, human	Coincubation of human PBMC with DA inhibited the PKC activator induced TH mRNA expression. The effect of DA was concentration-dependent and D1-like receptor mediated	D1	Ferrari 2004
T cells, CD4+/CD8+, human	IL-2 induced activated CD4+ and CD8+ T cells significantly inhibited the proliferation and cytotoxicity, via increase in the intracellular cAMP levels, in lung cancer patients and also in normal volounteers. Effects reversed by D1-like receptor antagonist	D1	Saha 2001(a) Saha 2001(b)
Microglial cells, mouse and rat	DA enhances the migration of these cells *in vitro* by acting through DA receptors; attenuates the lipopolysaccharide-induced nitric oxide (NO) release	D1-D2-like receptor subtypes	Färber, 2005 Chang 2000 Sarkar 2010
Thymocytes, rat	DA may have a role in the maturation and selection of lymphocytes, within the immune organs. Intraparenchymal branching of the phrenic nerve and sympathetic-parasympathetic fibers into thymus parenchyma along vessels was observed	D1-D2-like receptor DAT, VMAT	Mignini 2009 Mignini 2010
Microglial cells	DA by acting via its D1/D2 receptors have the ability to block NO production by microglia, which could partially explain the impaired immune protection against viral infection in stressed animals. Chronic dopamine receptor stimulation enhanced migratory activity	D1/D2	Chang 2000 Färber 2005
T lymphocytes	D1-like receptors are functionally dominant in CD4+ CD45RA+ T cells. D1R antagonists inhibit IL-17-producing Th cells and induce IFN-γ -producing Th1 cells	D1/D5	Nakano 2008, Nakashioya 2011
T lymphocytes, CD4+ naïve	DA dose dependently increased cAMP levels via D1-like receptors and shifts T-cell differentiation to Th2 in naive CD4(+) T cells, in response to anti-CD3 plus anti-CD28 mAb	D1/D5	Nakano 2009
Treg, CD4+CD25+	DA is the only neurotransmitter that reduces the activity of Tregs, affected both the suppressive and the trafficking activities. D1-type agonist increased spontaneous neuroprotection. Depletion of Treg increases the ability to withstand the nural injury	D1/D5	Kipnis 2002, Kipnis 2004, Cosentino 2007
Treg, CD4+CD25+	Systemic DA, or *in situ* released DA (via autocrine/paracrine loop) induces cAMP rise via D1/D5 inhibits the immunosuppressive cytokines (TGF-β and IL-10) produced by Tregs. That can be reversed by blockade of D1/D5 receptors, but not via D2-type receptors	D1/D5	Kipnis 2004, Cosentino 2007
Treg, CD4+CD25+ Teff, CD4+CD25-	D1R-dependent signaling uses the ERK (MAP/ERK kinase) pathway, phospho-ERK1/2 was found to be downregulated in the presence of DA. Reduction of suppressive effect of Tregs subsequently releases inhibition on Teff cells	D1/D5	Kipnis 2004
PBL, normal human leukocytes	DA-ergic D1-like receptor agonist inhibited TPA-induced TH mRNA expression, and consequently promotes cell survival. D2-like antagonist domperidone did not interfere	D1-like-receptors	Ferrari 2004
Murine splenic lymphocytes	Chronic treatment with DA and L-DOPA enhances the proliferation of murine splenocytes. Production of IFN-γ, but not IL-4-was significantly inhibited by DA/L-DOPA	D2	Carr 2003
T lymphocytes, human, resting	Production of IL-10 mRNA and secretion of IL-10 (primarily via D2). Both TNFα and IL-10 mRNA, by D1/D5R	D2, D1/D5	Besser 2005
T cells, human	DA can induce of integrin-mediated adhesion to fibronectin/activation of β1 integrins of human T-cells	D2, D3	Levite 2001
Activated T cells, human	DA suppress non-receptor tyrosine kinases, Lck and Fyn expression, resulting in inhibition of clonal cell proliferation of T cells. DA inhibited anti-CD3 mAb-induced release of both Th1 and Th2 cytokines, IL2, IFN-gamma and IL-4 from T cells	D2, D3, non-RTK	Ghosh 2003

Table 2
(*Continued*)

Cell type	Immunomodulatory effects mediated or triggered by DA or receptor agonist/antagonist	Receptors involved	Reference/year
T and B lymphocytes, human	DA treatment was able to reduce the intracellular cAMP levels of lymphocytes stimulated by forskolin	D2, D3, D4	Santambrogio 1993
Dendritic cell (DC); T lymphocytes CD4+ naïve	D2-like-receptor antagonists, L750667 induced dendritic cell DC-mediated Th17 differentiation; (sulpiride and nemonapride), induced a DC-mediated Th2 differentiation. DC is a source of DA, which functions as a T(h)2-polarizing factor in DC-naive T-cell interface	D2-like-receptors	Nakano 2008, Nakano 2009
T cell, human and mouse	DA induce chemotactic responses, enhance induction of integrin-mediated adhesion of these T-cells to fibronectin and ICAM-1	D3	Watanabe 2006
T cell, naive CD8+, human and mouse	DA plays a significant role in migration and homing of naive CD8+ T cells via D3R	D3	Watanabe 2006
T lymphocytes human peripheral blood, activated (blast)	Dopaminergic activation affected differentially the CD4 and CD8-derived blasts: induces Th1 bias in the cytokine profile, induces changes in the surface markers expression CD4+ cells, and CD8+ cells are triggered to produce IFN-γ	D3	Ilani 2004
T lymphocytes human, rat peripheral blood, activated (blast)	Only activated blasts but not the resting T cells responded to dopaminergic D3R activation with cytokine expression, and change in surface markers: increase in CD25 (IL-2R α chain), decrease in CXCR3, a principal inflammatory chemokine receptor, involved in trafficking of T cells to the CNS	D3	Ilani 2004
T lymphocytes, human, resting	Induce cytokine secretion/production of TNFα mRNA and secretion of TNFα (primarily via D3R), without affecting IFN-γ and IL-4	D3, D1/D5	Besser 2005
T lymphocytes, human	Stimulation of D4R induces quiescence by up-regulating lung Krüppel-like factor-2 expression through the inhibition of ERK1/ERK2 phosphorylation	D4	Sarkar 2006
T lymphocytes, human	D4 receptor agonist inhibits the cell activation via a TCR; Stimulation of D4R inhibits IL-2 secretion from anti-CD3/CD28-stimulated T cells *in vitro*	D4	Sarkar 2006
NK cells and macrophages, mouse	Hyperdopaminergic status resulted in reduced NK cell activities and delayed type hypersensitivity responses, but macrophages had enhanced cytokin production by LPS	DAT (-/-)	Kavelaars 2005
Speloncytes, mouse	Plasma levels of catecholamines did not differ significantly in DAT(-/-) animals. Splenocytes displayed reduced NK cell activity and reduced mitogen-induced cytokine responses	DAT (-/-)	Kavelaars 2005
Normal and malignant cycling B cells	DA promotes apoptosis in cycling B cells through oxidative stress. Inhibition of proliferation in both resting and malignant B lymphocytes, independent of both DAT and DA receptors	N/A	Meredith 2006
B cells, T cells, in mouse	DA enhanced mitogen induced B- and T-cell proliferation in mice, *in vivo* and *in vitro*	N/D	Tsao 1997
PBL, T and B lymphocytes, human	DA or L-DOPA dose-dependently inhibits lymphocyte proliferation and differentiation, abolishes the IFN-γ, IL-4 and antibody production	N/D	Bergquist 1994, Bergquist 1998, Bergquist 1997
PBMC, human	DA increase lymphocyte activation and Th1 or Th2 type cytokine production	N/D	Torres 2005
PBL, T and B lymphocytes, human	Extracellular DA or L-DOPA elevated the intracellular DA levels suggesting a cellular-uptake mechanism	N/D (DAT)	Bergquist 1994

Notes: ND indicates not defined; NA indicates not applicable; ConA: concanavalin A; cAMP: cyclic AMP; DA: dopamine; DAT: Dopamine transporter; IFN-γ: interferon-γ; IL: Interleukin; LPS: lipopolysaccharide; NK: natural killer cells; PBMC: peripheral blood mononuclear cells; PBL: peripheral blood lymphocytes, Th1/Th2: T helper type 1/type 2 cells.

induced macrophages increased, as well as the OVA-induced humoral response was increased. In DAT(-/-) mice the *in vivo* consequences of the defected DA-erg system affected both the acquired and the innate immune responses [118].

The presence of both D1 and D2 classes of DA receptors in the microglial cells have been demonstrated in recent studies by Chang and Liu, [55] and by Färber et al., (2005). Accordingly, when DA by acting via its D1 receptors attenuates the synthesis of microglial

nitric oxide and enhances the migration of these cells *in vitro* by acting through its receptors present in these cells [55, 83].

Indirect effects of DA on immune function

Suppressive control by regulatory T cells (Treg)

As discussed above specific attention has been given to a subpopulation of T cells (CD4+ CD25+ regulatory T lymphocytes, *Tregs*). These cells exert their suppressive activity on *Teff* ("autoimmune" T-cells), but there is also an autocrine effect of DA after being released in the microenvironment. DA acts on the D1 receptors present in these *Treg* cells resulting in down-regulation of *Treg* function and suppresses IL-10 and TGF-β synthesis [59]. DA is considered as one of the physiological compounds that controls *Treg* activity on a daily basis probably by a suppression of *Treg* activity via the ERK pathway; similarly modulates the adhesive and migratory abilities of *Treg* cells. *Treg* might exert their suppressive activity on *Teff* at the site of the neural tissue degeneration or in lymphoid organs. These regulatory cells are widely recognized to be capable of controlling innate immune reactivity and suppressing both CD4+ and CD8+ effectors T cell responses [119]. As recently reported that DA reduces the suppressive and trafficking activities of CD4+CD25+ *Treg* cells in mouse through a family of D1 type of DA receptors via the extracellular signal-regulated kinase (ERK) signaling pathway. Injection of activated *Treg* cells immediately after CNS injury of mice (BALB/c) significantly inhibits the spontaneous neuroprotective response. As the consequences of the insult less neurons can survive. On the other hand depletion of *Treg* increases the ability to withstand it [120]. The physiological and pharmacological effects of DA are able to downregulate the *Treg* cell activity that would improve the recovery after a mechanical CNS injury. Mediation of the suppressive activity of *Treg* has been attributed partially to IL-10 and CTLA-4, and TGF-β production and decreased ability of *Tregs* to suppress *Teff* proliferation. Whereas the suppression of specific chemokines receptors, like CD44 and CCR-4, may participate in the function of migration and adhesion, respectively, catecholamine release of these *Tregs* cells results in a similar effect, which occurs without influencing the production of TNF-α or interferon-β [59, 119].

Both *Tregs* and *Teffs* express D1 and D5 DA-erg receptors on the cell membrane, but Kipnis et al., (2004) found significantly more D1 than D5 receptors expressed by *Treg* compared to *Teff* [119]. Catecholamine-dependent down-regulation of *Tregs* is, however, selectively reversed by pharmacological blockade of D1-like receptors. The effects seem to be selectively exerted on *Tregs*: demonstrated in *Tregs* only (and not in *Teffs*) and expressed at the level of mRNA that functionally coupled to intracellular production of cAMP [59]. According to the described D1/D5 receptor mediated feedback mechanisms specific to *Treg* cells, DA is one of the potential candidates to act as an immunomodulatory agent in autoimmune disorders [119].

DA induces a huge and sustained increase of intracellular cAMP in regulatory *Tregs* cells. This is mainly related to the high number of D1 receptors present in *Tregs.* This cAMP elevation occurs almost immediately as the DA receptor stimuli and it can still be detected after hours (no similar effects in *Teff* cells were seen). This DA-induced cAMP rise in *Tregs* is specific to DA receptors, since it can be dose-dependently inhibited by DA receptor antagonists [59].

Shift in cytokine secretion profile

Besides the direct influence of DA on immune cells like the one observed on T and B lymphocytes, the released DA can also change the profile of cytokine secretion, as discussed in details in chapters above. Consequent to change in cytokine profile indirectly effect the functions of other specific cells in the immune system [17].

In addition, triggering of cell surface receptors by DA leads to intracellular signal transduction in both structures. In both *in vitro* and *in vivo* studies, DA has been shown to either inhibit the production of cytokines and proliferation or enhance specific functions. Sets of experimental results suggested that high levels of DA within the specific relation either induces cAMP production via dominant D1 receptors mediated pathways in naïve during activation of T-cell or by D2/D3 receptor pathway in activated blasts T cells, thereby facilitating the to Th2/Th1 differentiation. For cytokine expression as well as for surface markers, only activated blasts and not the resting T cells respond to DA-ergic activation. Moreover, the activation affected differently the CD4- and CD8-derived blasts [110, 158].

In theory, which was partially confirmed by *in vitro* experiments by Ilani et al., [110], the lymphokines secreted by these blasts may indicate that dopaminergically activated blasts could trigger other T cells to mimic their properties by means of soluble factors. These direct changes on specific T cells provide indirect but specific DA-erg changes to their activation status.

Immune functions maintained by pituitary hormones

The other aspects of immunological effects related to changes of DA in dopaminergic pathways in the brain is manifested via alterations in hypothalamic-releasing factors and consequent changes in hormones circulating at the periphery. Indeed, central catecholamine reduction does transiently affect the secretion of prolactin (PRL), growth hormone (GH) and luteinizing hormone (LH) [85, 87, 219]. Since these pituitary hormones may be able to alter lymphocyte activity in periphery, the model of neuroimmunomodulation should be expanded to the network of neuro-endocrine-immune interactions and include the (indirect) regulatory and inhibitory effects via pituitary hormones related to levels of DA [62, 111].

PRL and GH are quoted as lactogenic/somatomammotrop hormones, apart from their primary functions are playing roles for the development and maintaining the normal and stress-related function of the immune cells. It is well supported by experimental evidences targeting specific population of the immune cells, that the immune system is being influenced by hormones (and/or receptor antagonists), neurotransmitters and neuropeptides, mostly by the modulation of signal delivery pathways. Cells from the immune system may respond to either pituitary PRL or PRL-like molecules (such as GH) [89].

A broad literature exists describing the physiology of PRL: additional to the classical roles in lactation, reproduction, growth and development, water and electrolyte balance, it is also active in immunoregulation. The PRL receptor has been shown to be a member of the superfamily of cytokine receptors involved in the growth and differentiation of lympho-hematopoietic lineages [19]. PRL acts through its receptors (PRL-R), which have been shown to be present in immunocompetent cells [88]. Evidences indicate the participation of PRL in the regulation of humoral and cell-mediated immune responses as well as enhancing macrophage function:

- it has been shown to *stimulate* not only T and B cells, but also the natural killer cells, macrophages, neutrophils, CD34+ hematopoietic cells and antigen-presenting dendritic cells as well [21, 31, 72, 123, 128, 139, 185, 195]. PRL acts through its receptors (PRL-R), which have been shown to be present in immunocompetent cells [33, 38, 63, 165].
- moreover, PRL (and GH) are involved in promotion of the immune cells for proliferation but also in a *process of differentiation* and some other, more specific functions.

Further down of this path, certain pituitary hormones might also be regarded as cytokines. The activation process of lymphocyte proliferation is controlled by the harmony of secreted cytokines, finally promoting cells to differentiation and maturation. Since the HPA-axis derived hormones generally may regulate the levels of immune cell's activity including the response to immune activation, inflammatory stimuli, signal transduction, gene activation, the production and activity of cytokines and other immune system-specific functions, "therefore these hormones and growth factors, which playing roles like interleukins" may all function as cytokines [24, 25]. In this regard, direct evidence of PRL-like message and production of structural and bioactive PRL-like molecules by certain cells of the immune system in normal and mitogen-induced conditions has been conclusively provided in rodents and human as well [53, 123, 127, 152]. These locally released agents are in a close proximity and ready for an autocrine/paracrine action with PRL receptors that present on immune cells, therefore they act as cytokine.

In concert with the conclusion that Berczi et al., suggested [25], those hormones that under the hypothalamic DA control should similarly and specifically regulate the immune cell's activity. This theory has been extended with multiple direct and indirect evidences during the last decade. Taken also into the account that PRL, GH are considered as non-obligate immunoregulators, but more like a "stress-modulating" hormone in cells of the immune system, a constant interaction of neuroendocrine agents and internal immunoregulatory mechanisms are in harmony in controlling and fine-tuning the function of the cells of the immune system [17, 25, 53, 73].

In vitro studies demonstrated the dual role of PRL, acting as a cytokine in addition to "its endocrine control on the immune system". The theoretical and experimental conclusions are in accordance with the clinical observation: altered prolactin levels would affect the normal immune response. PRL is necessary *in situ,* and via autocrine/paracrine route potentially capable of modulating the immune response, thus is suspected to be involved also in autoimmunity [53, 138].

- Sets of clinical data, specifically in SLE implicate the involvement of prolactin in autoimmunity. Since PRL has a general effect as immunostimulatory agent, it enhances the production of immunoglobulins and potentially also autoantibodies, therefore in some disorders it

is suspected to promote autoimmunity. PRL affects the selection of autoreactive B lymphocytes occurring during immune cell maturation process. Negative selection of autorecative (follicular) B cells are susceptible pathway for the immunostimulatory effects of PRL, since these are T cell dependent, CD4+ T cells which are required for the prolactin-mediated breakdown of B cell tolerance [169].

– Clinical manifestations of hyperprolactinaemia usually described as a multi-organ specific autoimmune disease included; systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), Sjogren's syndrome (SS), Hashimoto's thyroiditis (HT) and multiple sclerosis (MS). Interestingly, there was no consistent correlation between the circulating PRL and disease activity, however increased serum PRL levels have been reported in SLE patients of both genders and that have been associated with accelerated disease expression. Studies in SLE patients suggest some potential role of DA agonists in therapy. The results, however must be interpreted with cautious, since some of the DA agonists (like bromocriptine) does effect only pituitary-derived PRA but would not affect other sources such as lymphocyte-derived PRL secretion. The role of DA agonists in treatment of autoimmune diseases is yet to be determined [111, 142, 165] and DA system-based therapies [119, 157, 159] will be discussed further (vide infra).
– The D2 agonist bromocriptine *in vitro* has no effect on lymphokine production (IFN-γ and IL-2), or expression of IL-2 receptor or lymphocyte cytotoxic function. In summary, Neidhart [160] demonstrated that the major immunosuppressive activity of bromocriptine is probably dependent on its effect in downregulation of PRL secretion. The D2 agonist bromocriptine seems to have only little direct effect on murine lymphocytes: the immunomodulatory role is mainly based on indirect effects mediated by the suppression of PRL secretion [160].

Harmony in actions during immune challenges: Would direct or rather indirect actions of DA regulate the immune system?

Understanding the role of DA in a direct control of pituitary PRL secretion would raise the dispute whether change in DA-driven immunoregulation is due to the indirect control of immune function via PRL, or it is rather the DA itself, due to the changes in production or in sensitivity of immune cells for peripheral DA? Additionally to the results of *in vitro* experimental data, there are still concerns in clinical practice related to treatment and influence of DA agonists on autoimmune diseases: is the clinical effect rather indirect and only a consequence of the reduced pituitary PRL secretion or more like a direct effect of the DA agonist acting on the DA receptors of immune cells?

In theory, the hypothalamic DA can inhibit the PRL released from the AL, thereby blocking the above mentioned enhancing effects of PRL on T cell function [123], including protection against infections. The question has been addressed by Alaniz et al., (1999) in mice lacking DA β-hydroxylase (dbh-/-): these animals characterized by essentially no NE and EP in circulation, but a 10-fold-increase in DA levels. The serum PRL concentrations, however, were similar to dbh+/- heterozygous controls both in pathogen free environment and after infection. The fact that these (dbh-/-) mice with normal PRL levels resulted in impaired T cell function and were more susceptible for infections, arguing against that DA-mediated inhibition of PRL secretion being responsible for an impaired cellular immunity [1, 123]. The HPA axis and the corticosterone production in response to infections or stress inducing elevated level of glucocorticoid inhibits certain T cell functions. The immune system in *dbh-/-* mice seems to be normal in the absence of immune stimuli, but shifted to abnormal and markedly impaired during repeated cases of infections or other means of immunological challenges. It can be concluded that in dbh-/- mice the corticosterone production, but neither the elevated DA nor the (otherwise normal) PRL levels are causing the immune malfunction during immunological challenges [1].

In clinical studies, on the other hand, administration of DA antagonists restored the depressed cell-mediated immune functions. This "beneficial" immunomodulatory action is rather a secondary effect of DA, due to PRL upregulation. That is also supported by the results that a positive correlation of serum PRL levels with the intensity of oxidative burst of peritoneal macrophages observed, suggesting an indirect participation of hyperprolactinaemia, induced by D2 antagonist treatment in macrophage activity [52].

The development and function of the immune system is regulated by neuroendocrine factors. Any sudden rise of cytokines in the circulation, such as IL-1, IL-6, and TNF-alpha are considered as an acute phase response [26]. As the cytokines act on the brain, on the active regulatory elements of the neuroimmune and

also neuroendocrine system, may lead to changes in secretion profile of neurotransmitters or subsequently hormones. The involvement of the hypothalamus in this loop and also HPA axis serve as the potential indirect regulator of immune function. Direct and indirect control by DA may have an additional regulatory or modulatory role supporting and fine-tuning in concert of immune actions.

Combined direct and indirect DA actions regulating the immune function. Understanding the manner and complexity of immune responses balancing between effective actions mediated by subgroups of immune cells to uncontrolled autoimmune outcome as results from broken control mechanisms, in these interactions DA may have coupled with the neural or neuroendocrine mechanisms. In discussion of experimental data or mechanisms of action, reader must exercise caution to distinguish between normal "i.e. physiological" action and environment from "diseases with abnormal DA levels" or "artificial" concentrations in experimental conditions. We should, however note that direct and indirect control mechanisms and factors may work in concert in steps of physiological immune modulation, resulting in the right balance and the right mode of actions as such:

- PRL and DA receptors, both might be involved in the modulation of macrophage activity, providing means of communication between the nervous and immune systems.
- DA receptor antagonists increase serum levels of PRL. Positive correlation was reported between serum levels of PRL and the intensity of oxidative burst i.e. the rate of phagocytosis: the macrophage function has been regulated by the endogenous DA in rats treated with antagonist of dopamine D2 receptors, domperidone. The dopamine receptor antagonist on macrophage activity might be due to the blockade of DA receptors on the macrophage surface, to the effects of the drug on the synthesis and release of PRL or to the combination of both effects [52].
- Based on these experimental results it is suggested that both PRL and DA may exert their action directly on the immune cells, therefore immune functions are regulated by an endogenous DA-erg tone, via direct DA, or indirectly via DA-erg control of PRL release [53].
- DA however may have a biphasic direct action on immune cells: DA but also norepinephrine and adrenaline stimulated macrophage phagocytosis at lower concentrations (10–11 to 10–15 M), whereas they suppressed phagocytic activity at higher concentrations (10–7 to 10–5 M), suggesting the existence of a concentration-related differential effect of catecholamines on macrophage function [182].
- Taken the experimental evidences into account DA or the blockade of dopamine receptors can modulate the immune cell activity: it is possible to suggest that both PRL and DA can modulate immune cell activity which may be due to both its (i) direct effects via DA-ergic tone on immune competent cells, (ii) indirect regulatory effects on synthesis and release of pituitary PRL, which proved to modulate certain functions in cells of the immune system.

The approach to analyze the role on direct or indirect (via pituitary hormones) control of DA in regulation of immune function, rather use a broad scope to include the potential effects of multiple factors of normal and also in altered immune milieu. In experimental approach we may also consider the developmental aspects of immune competent cells from even from intra-uterine environment, throughout lactation to childhood.

Brain-derived DA: Impact and actions on immune cells and direction of communication pathways

The brain and spinal cord are noted for their lack of immune system. Nevertheless, the primary function of the immune response is the protection of the host against infection by pathogens, including viruses. Since viruses can penetrate to any tissue of the body, including the CNS, it is obvious that cells of the immune system should have access to brain tissues. Furthermore, as immune responses do occur in the CNS, which are also confirmed in certain neurologic disorders; frequently associated with deleterious effects such as inflammatory and/or demyelinating pathology [166]. On the other hand, certain cytokines or cell-surface molecules were identified to play the dual role of mediating either cytotoxicity or cell survival.

An increased number of immune cell subsets have been implicated as mediators of immune regulation with different mechanisms of action. Results that specific subpopulations of immune cells can transfer disease resistance indicate that an active immune-regulatory mechanism may contribute in the disconnection between the presence of autoreactive T cells and the development of autoimmune disease

[7]. More recently, a functional derangement in a subset of suppressor CD4+CD25+ regulatory T cells as acting via cell–cell-dependent mechanisms, was found to be involved in multiple sclerosis and correlate with the disease activity [211].

Taken together, in the theory of a bi-directional communication link between the CNS and the (peripheral) immune cells, and vice versa, the immune system sends specific signals or activated cells can invade the restricted parts of the CNS, and provoke changes on the profile of neurosecretion. The role of DA is still subject of experimental and clinical investigations to evaluate its involvement in a modulatory function for both directions (Fig. 1). Furthermore, to identify the therapeutic consequences and possible additional clarifications in cases when patients characterized by a treatment–driven altered or a CNS-restricted elevated DA levels, in Parkinson's disease or schizophrenia, respectively.

Can brain-derived DA affect the function of peripheral T cells?

The brain, where neurotransmitters are synthesized and secreted, is considered an "immune-privileged organ" where the blood brain barrier (BBB) tightly prevents peripheral cells to entry. However, activated T blasts, termed blasts, can cross the BBB and these cells can enter the brain regardless of their antigen specificity, due to high expression of adhesion molecules. Ilani et al., [110] suggested the existence of a pathway by which the brain affects immune cells [110]. The pathway as described is mediated by DA receptors expressed on activated T cells. Since blasts cross the BBB can therefore potentially encounter neurotransmitters in the brain. The novel theory here is that these blasts have a unique response that carried on to DA-erg activation (but in resting T cells) as a Th1 bias in their cytokine profile and causes changes in surface marker expression. The conveyed "message of DA-erg activation" can be transmitted to the periphery either by those cells leaving the CNS or via messenger-soluble factors. The theory has been validated *in vivo* both in rats and human, thus providing a possible explanation on the communication between the CNS and the periphery using the function of neurotransmitter receptors and activation of T cells [110, 167, 209].

In experimental conditions when rats were injected with L-dopa/carbidopa (a treatment known to elevate DA levels exclusively in the CNS), had similar features to those of DA activated human blasts: higher mRNA expression of INF-γ and D3R, and also higher levels of VLA-4 and CD25, as compared with control groups. The observed changes in peripheral immune cells most probably influenced by changes in DA levels in the brain [110]. The described DA-erg regulation of T cells is not restricted only to the brain: it may also involve some similar peripheral mechanism. Once these blasts exposed to DA-erg ligands, undergo immunological changes driven by DA, which may be transmitted to the periphery either by these cells leaving the CNS or via messengers of soluble cytokines that secreted by the DA induced blasts. These secreted cytokines carried by the cerebrospinal fluid [3], which drains to the venous system of the circulation and consequently transmitting the same message as the DA activated blasts do within the BBB. This theory can serve as a conclusion on the dispute on how the CNS-born DA in normal conditions can affect the immune function at the periphery via circulating DA levels: even though is were shown to be in the order of picomolar to femtomolar and ∼95% is in sulfoconjugate or glycoconjugate forms, it is not necessary that brain-derived DA has to leave the BBB. Very much like the hormones, in this case the cytokines of activated lymphocytes staying within the BBB can do the same job as they would at the periphery: secreting and transmitting lymphokines [3, 110].

The presence of catecholamines in lymphocytes and the effect of L-dopa and DA on lymphocyte proliferation and differentiation, indicates that DA synthesized and accumulated by lymphocytes seems to be important regulatory molecule, thus potentially important during an ongoing immune response. It has been discovered that cloned CSF lymphocytes contain catecholamines. This is consistent with findings that immune system cells carry adrenergic and DA-erg receptors, which is a prerequisite for subsequent interaction with catecholamines [56]. The discovery of catecholamines in single CSF lymphocyte emphasizes a functional role for catecholamines involving the control of T and B cells. Regulation of lymphocyte function by catecholamines proves to be an important part of immune cell activation within the CNS and consequently provides the missing connection between brain-derived DA and the immune system at the periphery.

Influences of cytokines on DA secretion in the brain: Reverse function loop

The effects of the immune system on the function of the brain among the clinically well characterized behavioral responses, such as depression, anxiety, fatigue, psychomotor depression, etc. with the consequences of neurotransmitter metabolism and

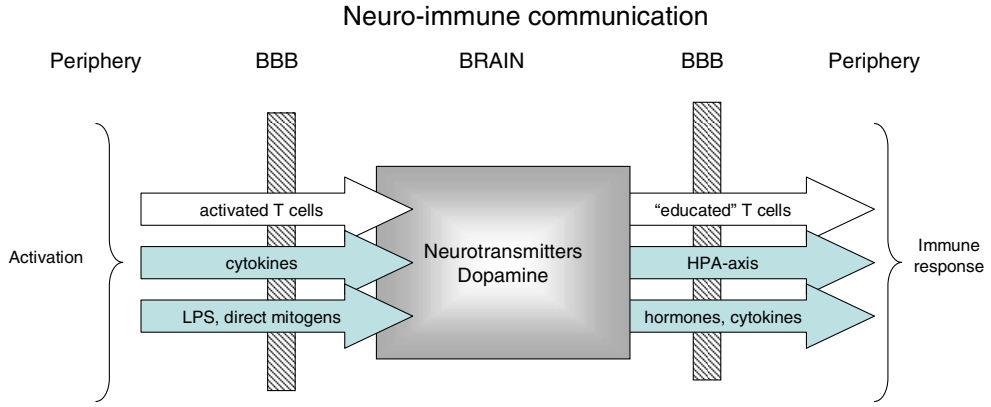

Fig. 1. Schematic representation showing the theory of a bi-directional communication link between CNS and the peripheral immune system. Immune system sends specific signals or activated cells invade the restricted parts of the CNS. Changes within the brain in profile of neurotransmitters (including DA) have a unique response that controls the hypothalamo-pituitary-adrenal gland (HPA) axis, or carried on T blast cells followed by DAerg activation as suggested by Ilani et al., [110]. That "message of DA-erg activation" can be transmitted to the periphery either by those cells leaving the CNS or via messenger-soluble factors.

neuroendocrine functions are also well known. In the context of inflammation, pro-inflammatory cytokines can access the CNS and interact with a cytokine network within the BBB to influence virtually every aspect of brain function. These pathways and mechanisms by which the immune system can influence the brain have been explored recently more extensively [44].

Peripheral cytokines influence the release and metabolism of several CNS-derived neurotransmitters, including DA [112], NE [116]. Several mechanisms proposed for cytokines produced in the periphery to access to the CNS included: the circumventricular organs; through the blood-brain barrier by selective saturable transport systems; actions on brain perivascular cells; or via neurally mediated mechanisms involving visceral sensory nerves [11, 66, 84, 133, 192]. Furthermore, the elevation of pro-inflammatory cytokines, TNF-α and IL-1β are followed the similar manner of elevation in the CNS in experimental conditions in rats. The findings from this study suggest those interactions of cytokines and the RAAS with central processes, such as activation of the HPA axis, intercommunication that can influence downstream functions such as the release of corticosterone or catecholamines [101].

It has been shown that prenatal exposure to infection mediated proinflammatory cytokines released by the maternal immune system may disrupt the development of the fetal brain and that may associated with increased liability to schizophrenia due to an altered DA levels during adulthood [221]. This suggests that cytokines can elicit direct effects on neurons of the brain altering neurotransmission, which may consequently underline the neuroendocrine and behavioral effects. Peripheral and central administration of IL-1 induces NE and in a smaller extend DA release in the brain, the former most markedly in the hypothalamus. The changes of DA in brain are occasionally observed, and these effects were not regionally selective. IL-2 has modest effects on DA, NE and 5-HT. On the other hand, IL-6 activates the HPA axis, although it is much less potent in these respects than IL-1 [74].

Since LPS increase the DA concentrations in hypothalamus, and PRL is mainly under tonic inhibitory control of DA that will have an indirect effect on plasma PRL levels. Similar to LPS, the pro-inflammatory cytokine TNF-alpha also increases the DA turnover in the hypothalamic-pituitary axis, TNF-α may act directly or indirectly to affect neurotransmitters [75]. TNF-alpha significantly increased also the basal and K(+)-evoked DA release posterior pituitary [67]. The increase in DA-ergic activity could indirectly mediate the inhibitory effect of LPS and TNF-alpha on PRL release, and thus subsequently via PRL modulate the peripheral immune function.

CLINICAL IMPLICATIONS AND PROSPECTIVE FOR FUTURE THERAPIES

Preclinical results as discussed above indicated that DA and the other monoamines are directly or indirectly involved in processes that regulating both the neuronal and non-neuronal cells and consequently the function

of the immune system. Elements of the DA system offer potential targets to treat various neurological or psychiatric conditions (e.g. Parkinson's disease, schizophrenia) and also provide the neurochemical basis of reward, including forms of drug abuse or side effects of psychostimulant-drugs [17]. Understanding the mechanisms of lymphocyte activation and consequent up/down-regulation in immune disorders that associated with abnormal DA-ergic activities provides a solid approach to elucidate the immunomodulatory role of DA. Additionally, that also helps to understand the adverse drug reactions of potential DA-ergic agents (analogues/inhibitors/receptor blockers/partial or specific antagonists) and provides an insight to potential side effects of applied therapies.

After two decades of research on DA-erg system, now it is commonly accepted that lymphoid tissues can be DA-ergic in nature. As a summary, the experimental data proved at least four different interfaces available for DA to regulate the immune function:

1. Co-localization: Both the primary and the secondary lymphoid organs are innervated with nerve fibers and even under non-pathological conditions the co-localization of DA-erg fibers and immune cells supports the interactions. The innervations of lymphoid tissues can be DA-ergic in nature during stress, thus providing a source of catecholamines for immune cells, since DA is actively synthesized in these cells from tyrosine via the intermediary L-DOPA.
2. De novo synthesized DA via autocrine/paracrine regulation: DA that produced by the immune cells can be utilized in regulation via autocrine/paracrine manner since immune cells are actively expressing and utilizing various types of DA receptors
3. Via cytokine secretion profile, since DA can trigger a change in cytokine secretion and surface marker profile of lymphocyte and a consequent Th1/Th2 switch.
4. Remotely from CNS-born DA via activated lymphocytes utilizing the communication between elements of CNS and peripheral immune cells, through a direct transfer with activated T lymphocytes or soluble lymphokines secreted by these activated cells.

Known functions and interactions of DA-ergic agents (more generally the endogenous catecholamines) on human lymphocytes still requires clarification before it can provide clear targets for therapeutic interventions. Several pharmacological strategies are potentially available utilizing DA-ergic agents (based on a summary by Cosentino) [59]:

(a) regulate intracellular storage and release (or packaging);
(b) utilize selective agonists/antagonists for activation/block specific DA receptors and activate cells or modulate cytokine profile;
(c) inhibit or regulate the DA/catecholamine synthesis (e.g., using specific or selective inhibitors);
(d) enhance or restore DA synthesis (e.g., using direct DA precursors, LDOPA).

The vision of this novel approach in drug development, which is based on clinical observations and a creative combination of pharmacology, pathophysiology and physiology of neurotransmitters, to convert experimental approaches to a potential therapy for immune disorders, led us to introduce the term of "*neuro-immuno-pharmacology*". Increasing amount of evidences [15, 99, 130, 143] support the novel theory and considering the DA-regulated systems as one of the targets for new pharmaceutical agents. This approach potentially offers treatment options for human immune and autoimmune disorders, neurodegenerative diseases, as well for lymphoid malignancies. The following chapter first reviews the major clinical disorders associated with abnormalities in DA system, highlighting the similarities found in preclinical studies, then summarizes the current and potential future treatment options with an insight to drug development avenues.

Clinical observations of altered DA levels and receptor function

Altered DA levels associated with CNS disorders, coupled with immune-system abnormalities of expression or function of DA receptors in T-cells reported in cases of *schizophrenia*, *Parkinson's disease* (PD) [35, 108, 110, 155, 213]; *Alzheimer's disease* (AD) [12], migraine, and *Multiple Sclerosis* (MS) [13, 93]. These potentially exciting findings, however should be taken with caution as summarized in the review by Levite [130] "some of these studies studied only the presence of abnormal receptor mRNA levels rather than the functional receptor itself" [130]. There are still a gap between clinical observations and the aimed pharmacological actions, thus more specific clinical studies needed before risk/benefits conclusions or safety of

these pharmacological agents can be fully evaluated. DA elements can be stimulatory to lymphocytes or regulatory, however DA can also act as a specific suppressor in some of the T-cell functions. Proliferation and cytotoxicity are primarily affected when DA interacts with activated T-cells (in contrast to that of naïve /resting T-cells). The major clinical disorders associated with the most prominent abnormalities found in the DA system are summarized in Table 3.

Diseases associated with altered levels of DA

Non-transient dysfunction of the central DA-ergic system in human is known to be associated with a combination of neurologic and psychiatric disorders. Clinical data accumulated from patients suffering from *schizophrenia* (with an increased DA-erg activity in specific brain areas) or *Parkinson's diseases* (diminished function of the central DA-erg system in the brain), are both formally discussed as a brain-restricted abnormal DA-erg transmission. These are, however connected to consequent changes in expression of DA receptors or in cytokine profile. Altered immune functions with abnormal DA-erg system have been also observed in patients suffering from other psychiatric diseases, such as *depression, addictions* or drug abuse.

In this review we discuss only those clinical observations that had been associated with altered DA-erg system and related immune malfunctions. Most of the clinical observations have been already connected to preclinical models, and at least partially, the potential mechanisms have been discussed or summarized earlier by experimental or *in vitro* studies.

In a close relation to DA, also cytokines are likely involved in the pathophysiology of psychiatric disorders, such as depression, schizophrenia or neurodegenerative diseases like PD or Alzheimer's (AD). Additional to the above mentioned clinical classes of central DA abnormalities, similar, however more periphery-related observations of DA-erg alterations are being observed in certain *cancer* types or *stress related disorders*. Our focus is to highlight the clinical findings that can be associated with the central or peripheral abnormalities of the DA-erg system in relation to the altered immune function or regulation via cytokines.

Schizophrenia

As a brief overview, the clinical manifestations of schizophrenia affects 1% of the population and ranks among the top 10 causes of disability worldwide. It is considered as a neurodevelopmental psychiatric disorder in which multiple neurotransmitter systems have been implicated. The pathogenesis yet to be fully characterized, at least various environmental factors, genetic predispositions, inflammatory immune processes such as cytokine hypothesis have been discussed in relation to the etiology and pathology of schizophrenia [216]. As a concept of the last two-decades, schizophrenia can serve as a natural human model for CNS-restricted alterations in DA, because the prevailing theory of schizophrenia suggests an excessive activity at DA-erg synapses in specific brain regions [47, 49, 108]. Increased and intermittently decreased transmitter substances like GABA, glutamate, serotonin and DA transmission in the subcortical meso-limbic and meso-cortical systems can cause imbalance that might be closely linked to the "positive" and "negative" symptoms of schizophrenia, respectively [45]. The key elements of the modified DA-erg involvement are summarized as:

- The "DA hypothesis" of schizophrenia specifically relates to the D2 subclass of receptors. An increased level of DA in the brain coupled with an overexpression of D2-like receptors in lymphocytes: significantly higher expression of D3R mRNA in T cells, but reduced D4R mRNA was detected in schizophrenic patients [35, 108].
- Administration of a selective D1-like receptor antagonist has been reported to result in the worsening of symptoms [35].
- Lymphocytes from schizophrenia patients' exhibit elevated CD25 expression and increase in serum IL-2 and IFN-γ levels [54, 108]. Most drugs that actively possess clinical efficacy in treatment of schizophrenia exhibit D2 type of receptor antagonistic activity. On the other hand, therapies with D2-like antagonists may also modulate the cytokine balances via D2/D3R on immune cells, thus the increased levels of IFN-γ–IL-4 are also attenuated by the effective neuroleptic treatment [108].
- The mRNA levels of D3 receptors in lymphocytes of schizophrenic patients are higher than those of healthy controls. Although some typical or atypical treatments applied, the approx of 3–4-fold increase in D3R RNA levels is consistent among schizophrenic patients. Interestingly, it can be noted that all patients exhibited a similar range of increase indicating, that was not a result of specific dopamine-receptor subtype blockade or reactive up-regulation [108].

Table 3
Clinical Disorders with Altered DA Elements or Immune Abnormalities

Clinical Disorders	Abnormalities in elements of DA-erg system	Immune and/or cytokine abnormalities	Reference
Schizophrenia	Excessive activity at DA-ergic synapses and DA levels in specific brain regions may have impact on interactions between different neurotransmitters in complex neurocircuits		Carlsson 1997
Schizophrenia	The mRNA expression of D2 class of receptors increased in lymphocytes, the increase is not affected by different antipsychotic drug treatments (typical or atypical)	Treatment with D2-like antagonists modulates cytokine balances	Ilani 2001
Schizophrenia	Increased (2–7 fold) expression of D3R mRNA in lymphocytes, but not in D4R mRNA detected		Ilani 2001, Boneberg 2006
Schizophrenia	quinpirole-induced increase of cAMP accumulation in T lymphocytes are higher compared to healthy controls		Ilani 2001, Boneberg 2006
Schizophrenia	IL-1beta, TNF-alpha ICAM-1 and IL-2 specific receptors were found significantly higher. Increased number of activated CD4+ and CD16+ NK cells		Theodoropoulou 2001
Schizophrenia	Elevated CD25 expression	Significantly higher IL-2 and IFN-γ levels in serum	Cazzullo 2001, Ilani 2001, Boneberg 2006
Parkinson's disease (PD)	Decreased mRNA expression of D3 receptors but no change of D5R. Similar decrease seen in both medicated or nonmedicated patients. Decrease in D3R expression correlated with the degree of clinical severity in PD patients	Decreased IFN-γ synthesis by peripheral lymphocytes	Nagai 1996, Ilani 2001
Parkinson's disease (PD)	Decreased central DA production, low circulating levels of DA within the CNS or at the periphery	Decreased productions of IL-2 and IFN-γ without treatment. IL-2 returned to normal when treated with amantadine	Wandinger 1999
Parkinson's disease (PD)	Central dopaminergic hypoactivity reduces DA-ergic control on PRL and corticosterone secretion	Immune disturbances, can be direct effect of low DA or maybe indirect effect e.g. via pituitary hormones. Central DA depletion induced transient changes in blood leukocyte distribution and cytokine production	Engler 2009
Alzheimer's disease (AD)	Reduced density of dopamine D2-like receptors on peripheral blood lymphocytes. No change in density of D1-like receptors		Barbanti 2000(a)
Alzheimer's disease (AD)	Clinical symptoms in correlation with specific DA-ergic elements in CNS: higher D2 receptor availability found in most behaviorally disturbed patients		Reeves 2009
Alzheimer's disease (AD)	Cholinergic loss associated with progression of AD is mainly due to a relative striatal hyper-dopaminergia		Reeves 2009
Migraine	Increased density of dopamine D3R and D4R on PBL. PD patients reported improvement in or remission of migraine after PD onset and treatment		Barbanti 2000(b)
Migraine		Cytokines involved in as a cause of migraine pain due to activation of trigeminal nerves	Bruno 2007
Migraine	DAergic nigrostriatal denervation prevents the neuronal activation and nitroglycerin induced hyperalgesia		Greco 2008
Multiple sclerosis (MS)	Diminished mRNA and protein levels of D5R, normal D3R in PBMCs of untreated MS patients	No inhibition of DA on T cell proliferation, or downregulation on MMP-9 mRNA or on IFN-γ expression are observed in MS	Giorelli 2005

Table 3
(*Continued*)

Clinical Disorders	Abnormalities in elements of DA-erg system	Immune and/or cytokine abnormalities	Reference
Multiple sclerosis (MS)		Reduced thymic output and number of naïve CD4+ T-cells resulted in peripheral homeostatic alterations of immune function, including autoimmune responses in RRMS and PPMS	Haegert 2011
Malignancy associated stress	High DA concentration in plasma	Altered cytokine secretion. DA significantly inhibited the IL-2 induced CD4+ and CD8+ T cell activation	Saha 2001(a), Saha 2001(b)
Malignancy	Neuroblastoma cells contain TH that elevates DA and L-DOPA levels		Eldrup 2001
Restraint stress	Increased plasma concentrations of DA in stress	Decrease the size of certain T and B lymphocyte subsets	Kanemi 2005
Stress		Stress induces a redistribution of lymphocytes, increase in NK cells in acute stressing of humans	Dhabhar 1996

Notes: ND indicates not defined; NA indicates not applicable; ConA: concanavalin A; cAMP: cyclic-AMP; DA: dopamine; DAT: Dopamine transporter; IFN-γ: interferon-γ; IL: Interleukin; LPS: lipopolysaccharide; NK: natural killer cells; PBMC: peripheral blood mononuclear cells; PBL: peripheral blood lymphocytes, Th1/Th2: T helper type 1/type 2 cells.

- The clinical manifestation and the immune symptoms are closely correlated with the altered expression of D3R. The consequent changes on signaling pathways in T cells and the increased IFN-γ synthesis in untreated schizophrenic patients has been demonstrated [108]. Similar shift from Th2 to Th1 of cytokine secretion profile has been recorded both in CD4+ and CD8+ blast cells [110].
- The applied therapy does not really change the high levels of IL-1β and TNF-α in schizophrenic patients. In the mean time, increased percentage of activated CD4+ and CD16+ natural killer cells, high level of ICAM-1 adhesion molecules were also reported. Interestingly higher percentage of cells expressing IL-2 specific receptors were detected in patients under chronic treatment compared with drug naïve subjects [202].
- The novel theory on brain derived DA controlling the peripheral lymphocyte activation has been demonstrated by an elegant design of experiments on immune cells obtained from human donors [110]. The change of D3R mRNA levels in lymphocytes of schizophrenic patients compared to those of healthy controls is similar to the increase observed in human activated blasts vs. resting T cells. Specific D2/D3 agonist applied in T cells can produce ∼8-fold increase in cAMP accumulation in schizophrenia patients vs. healthy individuals, which is similar to the increase in activity that has been observed in blasts vs. resting T cells from normal donors. This substantial increase of D2/D3-induced cAMP accumulation in schizophrenia patients may reflect the higher proportion of activated peripheral T cells associated with the disease [110]. These results can further support the theory that CNS-restricted change in DA can induce alterations on immune cells at the periphery.

On the standpoint of pharmacology, increasing knowledge about the interactions between different neurotransmitters in complexity opens up possibilities to develop more efficient treatment for symptoms. The original "DA hypothesis" of schizophrenia has nowadays been changed to a multifactorial view with a focus on neurotransmitter interactions in complex neurocircuits. The traditional DA theory is still in place, although at a "higher level of sophistication" utilizing the new concept of receptor specific antagonist stabilizing imbalanced neurocircuits, whereas theories on other neurotransmitters, such as serotonin and glutamate just recently added [48].

Treatments with classical D2R blocking agents are based on the conventional view of DA theory of schizophrenia. The new classes of partial DA antagonists or so called "DA stabilizers". These receptors show low affinity for dopamine D2R, and proposed recently to target extrasynaptic receptors while leaving the basic DA functions intact. This latter approach is based on the "dopaminergic deficit hypothesis" where the receptor heterogeneity is reflected by synaptic versus "extrasynaptic" (presynaptic) D2 receptors [46]. These extrasynaptic receptors are known to be

much more responsive than postsynaptic receptors. Dopaminergic deficit can cause a consequent receptor upregulation in schizophrenia, which is resulting in the well-characterized increase in DA-ergic tone in certain area of the brain [46]. The clinical deployment of compounds based on hypothesis of DA-ergic receptor heterogeneity is still remain experimental and in development phases I-III, currently over 50 studies with aripiprazole listed in indication of schizophrenia.

Parkinson's disease

Parkinson's disease (PD) is a complex multifactor disease based on extensive neuropathology of DA system in the midbrain. There is a considerable amount of evidences supporting that a combination of genetic susceptibilities and environmental factors play a critical role in the pathogenesis of this disease. PD is the second most common neurodegenerative disorder that affects millions of elderly population worldwide. The etiology of PD is still unknown, although an increasing number of evidences suggested that PD starts by the intake of a toxin, bacteria or virus. The first morphological abnormalities may (i.e. Lewy bodies, Lewy neurites and alpha-synuclein deposition) occur in the olfactory bulb and the vagal and glossopharyngeal nuclei rather than in the substantia nigra [37, 176] The very first Lewy bodies or alpha-synuclein inclusions are found in the enteric nervous system i.e. the gastric, myenteric and submucosal plexuses, idenifying the so called stage 1 and stage 2 of preclinical phases of PD. Similar pathology has been found in the submandibular glands of patients who died in Parkinson's disease [68].

Besides the "intake theory of a toxin", bacteria, virus or the consequences of oxidative stress and mitochondrial dysfunction, the innate and adaptive immune system also play crucial roles in the pathogenesis of PD. Cellular and regulatory humoral elements of the immune system within the brain, such as microglia, complement system, cytokines are known to participate in the neuroinflammatory processes resulting in manifestation of PD [197].

As the clinical signs are not restricted to CNS, patients often suffer from symptoms seem to be unrelated to PD. The current therapies are palliative, neither can control the symptoms which appear at later-stages, nor can address the ongoing degeneration of the DA-erg (and the non-DA-erg) systems [203]. The selective and prominent loss of DA neurons, followed by diminished central DA production by the nigrostriatal system are associated with significant changes in immune response, also confirmed by clinical observations. The altered DA-erg system is considered the main cause of disturbances in the immune function, as summarized below:

- In contrast to schizophrenia, PD patients characterized by decreased D3R mRNA expression and a reduced IL-2, IFN-γ synthesis in peripheral lymphocytes [155, 213]. This significant and consistent decrease of D3R mRNA expression found to be correlated with the clinical progression of the disease [155].
- The immune abnormalities seemed to be associated not only with the loss of DA in the CNS or in the peripheral circulation, but more importantly, with the changes in decreased expression of D3Rs and the consequent downregulation of signaling pathways in T cells of PD patients [110].
- Cellular toxicity induced by DA through a generation of reactive free radicals is well documented in cell line models of PD. This oxidative stress may play a fundamental role in the mechanism in the pathogenesis of PD since DA-ergic neurons are specifically vulnerable to ROS induced toxicity. Based on the clinical efforts to identify early signs for diagnosis the clinical interest explored the possibility to measure the oxidative damage. The level of reduced glutathione in DA-erg neurons occurs much earlier than actual neuronal loss and more importantly, it may be one of the important triggering event in development of PD [203]. Interestingly, concentrations of DA required to affect neuronal cells appeared to be substantially higher than levels against the immune cells [144].
- Central DA depletion in the rat model of experimental PD induces a four-week-long transient change in blood leukocyte distribution and in cytokine production, before it is normalized. The reduced DA production within the nigrostriatal system is associated with an altered pro-inflammatory response to bacterial LPS. This finding also provides clear evidence that the nigrostriatal DA-erg system is actively involved in peripheral immune regulation and in response to mitogen induced immune response. The central DA-erg activity controls the excessive inflammation during infection or tissue injury, and it can be speculated whether an impaired DA-ergic control of the inflammatory response contributes to increased susceptibility to infections and mortality observed in PD patients. During the early phase of central DA depletion the increased sensitivity to the inflammatory stimulus seems to be either

(i) as a result of an impaired DA-erg (i.e. indirect) control through pituitary hormones (PRL and corticosterone), or (ii) with a direct effect on immune cell receptors (via D1 or D2 receptor subtypes) and a consequent changes of cytokine secretion profile. It is plausible that treatments (with L-DOPA) can normalize the levels of DA in the brain and most likely the immune response at the periphery resulting in reduced risk of inflammation [82].

Current therapy of PD is largely based on a dopamine replacement strategy, primarily using the dopamine precursor levodopa. Medical treatment is palliative at best by providing effective control of symptoms, particularly in the early stages of the disease with a lack significant disease-modifying effect. Symptomatic therapies cannot completely ameliorate later-stage symptoms and neither can address the ongoing degeneration in the dopaminergic and non-dopaminergic systems [164, 203].

The most effective medical therapy continues to be levodopa mixed with a peripheral decarboxylase inhibitor. DA agonists such as ropinerole or pramipexole may delay motor complications of PD, but less effective than levodopa in symptomatic relief [191]. The most recent available symptomatic medical therapies focused to prolong the effect of levodopa through the use of COMT inhibition and by changing the availability and formulation of older medications such as selegiline and apomorphine.

Identification of monogenetic forms of PD has uncovered a role for proteasomal and mitochondrial dysfunction, oxidative stress, protein misfolding, and aberrant phosphorylation in the pathophysiology of PD [191]. A great deal of preclinical research has focused on finding the cause of dopaminergic cell loss and on exploring protective, restorative agents or a replacement therapies [203]. Experimental models of PD aimed to identify effective strategies targeting oxidative stress, mitochondrial dysfunction, or the exploration of neuroimmune pathways to achieve neuroprotection.

Alzheimer disease

The pathology of Alzheimer's disease (AD) is characterized by intracellular neuro-fibrillary tangles formed by the hyperphosphorylated microtubule-binding protein and the extracellular senile plaques composed by beta-amyloid protein. Genetic studies suggest that the presynaptic dysfunction might be a converging early pathogenic event before neurodegeneration in AD that is similar to pathogenesis of PD [193].

The dysfunction within the cortico-striatal DA-erg neurocircuitry has been discussed and implicated as a neuronal base of the characterized neuropsychiatric symptoms associated with AD as outlined below:

- Increased availability of striatal dopamine (D2/D3) receptors in AD patients and that is correlated with the clinical symptoms of delusions to a similar extent that observed in drug-naïve patients with schizophrenia.
- Perturbation of cholinerg–DA-erg balance within cortico-striatal neurocircuitry may play a crucial role in the development of psychosis or apathy as well. Since the relative excess in striatal DA activity can be viewed as the "final common pathway" in the development of psychotic symptoms, thus the cholinergic loss associated with progression of AD is mainly due to a relative striatal hyper-DA-ergia. The consequences of the imbalanced cholinerg–DA-erg activity may increase the propensity of patients with AD to develop psychosis, even with a functionally intact DA-erg system [173].
- Evaluation of clinical symptoms resulted in correlation with specific DA-ergic elements: higher D2R availability found in most behaviorally disturbed patients and controversially clinical signs of apathy associated with higher DAT availability in striatum. Increased DA (D2/D3) receptor availability, and possibly other indices of striatal DA-erg function, might act as markers of psychosis proneness in AD. The psychosis however, required to be characterized further in terms of pre-synaptic and post-synaptic DA-erg indices, to identify those most vulnerable to its development [173].
- On the other hand some earlier observations revealed lower density of dopamine D2-like receptors on PBL than controls at the periphery AD patients, which was reported to be consistent with the observation of changes in the expression of D2-like receptors in DA-erg brain areas in AD [12].
- The other aspect in pathogenesis of AD is based on inflammatory response in the brain which is tightly regulated at multiple levels. Microglial cells are capable of producing neuronal damage through the production of bioactive molecules such as cytokines, as well as ROS, and nitric oxide (NO). Just recently reported

that in experimental conditions the exogenous transmembrane chemokine CX3CL1, produced also by neurons in high level within the CNS, in young but not in elderly patients, suppresses the activation of microglia and consequently neurodegeneration [168].

The current pharmacotherapy of AD is based on a combination of symptomatic and disease-modifying agents. For future therapeutic targets in many inflammation based neurodegenerative diseases, like AD or PD, can be the pharmacological or maybe the immunological regulation of immune-competent elements of the brain such as microglia cells controlling the activation and aim a balance the production of microglia-born bioactive molecules, with a consequent effect of neuroprotection.

Multiple sclerosis

Multiple sclerosis (MS) is also a chronic inflammatory disease of the CNS, associated with intermittent progress of demyelination and variable degree of axonal and neuronal degeneration. Experimental data suggest that energy failure is a major factor driving tissue injury, which is mainly due to mitochondrial malfunction triggered by ROS and nitric oxide species (NOS). The mechanisms of neural tissue injury are still poorly understood, however the active multiple sclerosis lesions show profound alterations of mitochondrial respiratory chain proteins [103].

In MS, similarly to PD, also various immune mechanisms may actively contribute to the pathogenesis of the different MS subtypes.

- Recent review by Haegert [102] provided direct evidences that peripheral T-cell alteration in relapsing-remitting MS (RRMS) or in primary progressive MS (PPMS) are secondary to an immune system abnormality which overall may contribute to the pathogenesis of these subtypes [102]. Proliferation of CD3-CD4-CD8- thymocytes decreases progressively with age, which explains the normal process of age-associated decrease of thymic output [2]. Detailed analysis of naïve T cell homeostasis showed that patients who had an early-onset of thymic involution and a consequent reduced thymic output, resulted in peripheral homeostatic alterations of immune function mainly due to the reduced number of naïve CD4+ T-cells. Homeostatic T-cell receptor (TCR) signaling and proliferation of naïve T cells are favoring the development of autoimmunity, which is a subject of "considerable interest". Both RRMS and PPMS patients have increased naïve CD4 T-cell expression of Bcl-2, anti-apoptotic molecule. Increased expression of this survival signal is probably a mechanism to compensate the otherwise reduced thymic output in RRMS and PPMS by helping to maintain the size of the naïve T cell pool [102].
- One other possibility focusing on the disturbed development and function of *Treg* cells in PPMS causing continuous injury of CNS. Since the early-onset of thymus involution consequently changes in T-cell homeostasis which alters quantitatively or qualitatively the natural *Treg* cells. The subpopulation of naïve CD4 T cells has a central role initiating the immune responses, including autoimmune responses in RRMS. Remissions that are seen in RRMS could be driven by regulatory *Treg* cells and similarly the absence of remissions in PPMS that may indicate the critical or rate limiting function of this T cell subset [140].
- Untreated MS patients have diminished mRNA and protein levels of D5R, but not of D3R, in peripheral PBMCs. DA counteracts T cell functions through its specific receptor subtype D5 and D3. Specific treatment of MS patients with IFN-β, resulted in reduced the level of D3 and interestingly restored the DA-related regulatory functions on cell proliferation and adhesion in PBMCs [93].
- The involvement of B cells in the patho-etiology of MS has been recently also supported by the results of potent immunomodulatory therapy applied in MS. Rituximab is an anti-CD20 monoclonal antibody that specifically depletes B cells and does not affect the size of plasma cell population or the antibody levels in serum or in CSF. There are a few of important co-stimulatory molecules in B-T cell interactions including cell or function specific cytokines, or the DA-ergic agents which may play a role in regulation of B cell functions. Activated B cells are effective in activating T cells to proliferate and differentiate as demonstrated by an antigen-specific presenting B cell (APC) function, during the induction of T cell tolerance or expansion of regulatory T cells in mouse and in human [175]. Since T cells are considered as mediators of the pathology of MS, based on the interactions between B cells and T cells, the current emerging lines of immunotherapies may impact these cell interactions in treatment of MS.

Migraine

Migraine is a neurovascular disorder that induces debilitating headaches associated with multiple symptoms including facial allodynia and characterized by an hypersensitivity mainly through the trigeminal system otherwise to normally innocuous mechanical stimuli. The pathology of migraine can be associated with an up-regulation of receptors within the immune system, since the activation of immune-derived inflammatory mediators enhances pain [218]. Cytokines are important mediators of the immune and inflammatory pathways and their receptors are widely expressed in cells of CNS, including neurons. Accordingly, a role for cytokines, as a pain mediator in neurovascular inflammation has been suggested.

- Cytokines may be a cause of the migraine pain due to activation of trigeminal nerves, release of vasoactive peptides or other biochemical mediators (like NO), and cause acute inflammation. Since the results of the studies focused on peripheral or central cytokines are highly controversial, the exact pathogenesis of migraine is still unclear and there is not a conclusive evidence of the role played by cytokines in the pathology of migraine pain [41].
- D2 subclass of DA receptors is involved in the determination of the so-called migraine trait. Peripheral blood lymphocytes (PBL) may represent an alteration of receptors on DA system associated with the disease, which may give a cue for diagnoses or treatment. Increased density of both D3R and D4R on PBL observed in patients suffered from migraine, compared with healthy controls. This up-regulation of receptors might be a reflection due to hypofunction of the DA-ergic system. On the other hand in experimental conditions nigrostriatal denervation prevented the neuronal activation and inhibited hyperalgesia [100]. This finding is in harmony with the clinical observations, that most of PD patients reported improvement or remission of migraine after onset of PD [13].

Malignancies associated with elevated DA/L-DOPA levels

Plasma L-DOPA levels found elevated indicating the catecholamine synthesis in a variety of disorders, including tumors. Clinical reports indicated significant elevation of plasma DA levels in malignancies due to stress of the disease process, and the altered DA levels are associated with the depressed T cell functions.

- Patients with lung carcinoma showed significant elevation of plasma DA (48.6 ± 5.1 pg/ml) vs. normal controls (10.2 ± 0.9 pg/ml) which significantly inhibited the proliferation and cytotoxicity of T cells. This D1 receptor mediated action resulted in elevation of intracellular cAMP in these cell populations *in vivo*, similarly that observed *in vitro* [186].
- Neuroblastoma constitutes one of the most common solid tumors of children. These tumor cells derive from the neural crest in embryo and they contain TH that elevates DA and L-DOPA levels [79, 95].
- Malignant pheochromocytoma contains catecholamine-synthesizing cells, also have elevated plasma L-DOPA levels and lead to consequent effects of DA-ergic activities. Malignant pheochromocytoma cells appear to be so undifferentiated that although they can hydroxylate tyrosine to form L-DOPA, but they do not decarboxylate L-DOPA efficiently to form DA or hydroxylate DA to form norepinephrine [96].
- High plasma L-DOPA levels occur in malignant melanoma. These tumor cells do not contain tyrosine hydroxylase, but they do contain high levels of tyrosinase, and L-DOPA is produced in phase I melanogenesis, either from direct oxidation of tyrosine or from dopaquinone [129].

Acute and chronic stress

The effects of the stressors (various physical, chemical environmental, anthropogenic factors or other stressors of emotional origins) on the body constitute the "stress response" which also causes a "response" from the immune system. DA also has known as one of the catecholamines and considered that during stress influences the immune cells included lymphocytes and macrophages [90, 101, 107, 122]. Stress induces a redistribution of lymphocytes in parallel to elevation of plasma concentrations of DA, epinephrine and norepinephrine. It has been proposed, that skin is the main tissue that during the onset of stress infiltrated by lymphocytes. The activation of the physiologic stress-response systems of the body can enhance the cell-mediated immune response and activation of specific immune functions in general. *In vivo*, it is observed either as a long lasting increase in allergic contact sensitivity, or a delayed-type hypersensitivity [70]. There is however, a difference during acute stress in redistribution of blood lymphocytes: the number of blood lymphocytes, especially NK cells increase immediately after psychological or physical stress in

humans. These results discussed as a bidirectional relationship between acute/chronic stress and the immune function: acute stress enhances, while chronic stress suppresses the adaptive immune responses *in vivo* [40, 69, 94, 126]. The innate immune response is amplified by chronic stress and by acute phase response [27]. The role of DA seems to be only secondary modulating those immune reactions observed during acute or chronic stress, however the reactive elevations of DA and the triggering elements still need to be elucidated.

- Pharmacological doses of DA have been shown to modulate T cell functions. The IL-2 induced activated CD4+ and CD8+ T cells obtained from healthy human subjects are significantly inhibited by high concentrations of DA *in vitro*. In vivo, a 3–5 folds increase of DA concentration (compared to normal) observed during chronic uncoping stress in human malignancies [186, 187]. Similar decrease in the absolute number of peripheral lymphocytes, as well as in CD4+ and CD8+ T-cells and B-cells in acute and repeatedly amphetamine-treated rats exposed to stress were reported in rats [9]. These results provide evidences that DA can play a general role within the immune regulatory function during stress, since indirectly but consequently connecting the status of chronic or acute stress and elevated DA levels in periphery. The mechanism has been described *in vitro* and attributed to the D1 type of receptors induced increase in the intracellular cAMP [186, 187]. Thus, the attenuated T cell proliferation and altered cytokine secretion are expected to be the key immune modulatory actions of DA *in vivo*.
- Increased plasma concentrations of DA in acute stress have been reported by Kanemi et al., [117]. DA in parallel with E and NE are elevated shortly after acute restraint stress, which immediately followed by the decrease of certain T and B lymphocyte subsets in lung and blood, followed by a substantial recovery within a few hours after release from stress [117]. Similar results observed in healthy volunteers (in good physical conditions) when exposed to repeated stress that caused significant increase of plasma DA levels [204].
- Environmental factors (overexposure to light, noise, urban stress, etc.) or diet (like high-fat) may contribute to acute stress that induces tyrosine hydroxylase enzyme and increases plasma DA levels. Chronic stress can lead to hyperplasia and hypertrophy of the adrenal glands and has detrimental secondary effects on the DA-ergic system. Interestingly enough, social interactions can also activate DA-ergic regions that alters brain DA signaling [184]. Other common life stressors in patients can be pathologic in response coupled with a hyperresponsive DA-ergic system, e.g. DA may play role as a potential factor triggering and relapse of psychosis. There is no direct evidence to confirm the role of DA in psychosis, but sensitized DA-ergic response to stress in a psychiatric condition may have important theoretical as well as clinical implication [151].

5.3. Potential therapeutic value of DA

Pharmacological actions of DA with a potential therapeutic value

The class of immunomodulatory agents is considered as another area of therapeutic drug development that has been just opened within the recent years: utilizing preclinical concepts of neuroimmunomodulation and transforming the results to therapies. DA as a conventional neurotransmitter and/or neuroendocrine mediator has received much higher attention because of the presence of specific subsets of DA receptors on most of the immune cell, included normal and malignant B and T cells. Direct or indirect/inhibitory pathways mediated by these receptors are capable to modulate/regulate/suppress specific functions of immune-competent cells by:

(1) Action of DA-agonists/antagonists
(2) DA itself, mainly via autocrine/paracrine manner
(3) Alternatively, via modified cytokine production

As discussed in relation with DA, cytokines are also involved in the pathophysiology of psychiatric disorders. This link between the immune system and the pathogenesis of CNS disorders leads to the concept that immunomodulatory agents may possess efficacy alone or in combination with other drugs in therapy. Both *in vitro* and *in vivo* studies as described above demonstrated that DA has the capacity to regulate immune cell function via specific receptors: either activates resting cells or inhibits the cell proliferation and lead to apoptosis. In parallel, it may change the production profile of cytokines in peripheral lymphocytes, thus initiating a general immunoregulatory activity. Moreover as the Treg cells and polymorphonuclear cells, such as macrophages and neutrophils, are also capable of *de novo* production of DA or other catecholamines, the autocrine/paracrine mechanisms may enhance the desired effect.

These preclinical evidences contribute to understand the cross-talk between the immune and nervous systems and support drug development approach that can provide effective future therapies. The recognized, immune cell and function specific pharmacological actions of DA, or the DA-ergic agents are in consideration of scientific approach to extend the therapeutic regimes either of these directions:

(i) provide mediation or control of lymphoproliferative disturbances;
(ii) apply DA (or analogues/receptor agonists/antagonists/blockers) to promote specific immune cell proliferation or production of favorable cytokine profile;
(iii) utilize the mechanisms of DA-erg action to support/potentiate/enhance basic therapies of other agents;
(iv) regulate the balance of Th1/Th2 and Th17/Treg immune function on autoimmune/allergy/cell protection;
(v) apply immune cell modulation to mitigate the occurrence of medication related side effects or disease specific symptoms.

Some of the agents possess DA-ergic actions are currently in clinical use for various (or maybe other then immune related) indications and with a usually favorable therapeutic index. Characterization in-depth of these extended therapeutic mechanisms for the purpose of functional modulation of human immune cells could provide the rationale for further investigations. Examining in a first instance the therapeutic potential of the available and registered drugs to extend they therapeutic indications maybe to allergy, autoimmune disorders, other immunological or major disease conditions [59], would be continued with a specific design of DA-ergic elements, as discussed later on. Some of the recent examples of preclinical results reported as:

Counteraction with cell cycle to treat B cell malignancies

- The antiproliferative actions of DA on normal and malignant B cells have no interference with DAT or the DA receptors when receptor antagonists applied concomitantly. It is discussed in details above, that steps of oxidative stress caused by DA involved in the process of cell cytostasis: scavenger of H_2O_2, superoxide and hydroxyl radicals have impact on DA's antiproliferative activity. DA promoting or triggering only the cycling cells to apoptosis, but resting B cells are not affected [144].
- Proliferating normal lymphocytes and dividing malignant clones of B cells are clearly arrested upon exposure to DA in the low micromolar concentration range. The impact of DA-related selective modulatory effects on cell cycle indicates a potential axis for new therapeutic intervention not only in case of B cell neoplasia, but also in other lymphoproliferative disturbances [144].

Treatments utilizing cytokines in immunological and neurological pathologies

- The selective cytokine secretion profile of T-cells is mediated by stimulation of D1- and D2-like receptors. Application of DA on its own or specific receptor agonists induces a five-fold elevation of the TNF-α and/or IL-10 secretion by resting normal-human T-cells. DA has a unique ability to trigger a selective secretion of either TNFα only (via D3R), or IL-10 only (via D2R) or both (via D1/D5R). That differs from the general and non-selective cytokine-secretion model which is induced by 'classical' TCR-activation in immune cells. These DA-induced modulations of T-cell cytokine secretion profile may have therapeutic implications in fighting pathologies that are either mediated or can be cured by IL-10 or TNFα [32, 130].

Control of T cell proliferation remotely

- Converting the results that D4 receptors stimulation in human T cells during TCR activation is resulted in quiescence via inhibition of ERK1/ERK2, into the concept indicating that D4 receptor agonists may have a therapeutic value in diseases of uncontrolled T cell proliferation [190].
- Remote control of peripheral immune system from brain-derived DA has been demonstrated in animal studies. Existence of such a pathway by which the brain *in situ* can regulate immune cells via DA signals opens a new direction in neuroimmune interactions and may be also explore mechanism for future therapies [17, 110].

Regulate the balance of Th1/Th2 and Th17/Treg immune function. Balance of Th1 (producing IFN-γ) and Th2 (characterized by IL-4 secretion) cell activation in concert with the other Th cell subsets, i.e. IL-17-producing by Th17 cells and Treg cells have

been considered to be one of the most important factors for homeostatic maintenance of the immune system.

- Imbalances may result in the development of autoimmune diseases (e.g. accumulation of Th1 cells) or induction of allergic diseases (characterized by an accumulation of Th2 cells) [158],
- Antagonizing D1-like receptors inhibit IL-17-producing Th cells and induce IFN-γ -producing Th1 cells [157, 159], moreover, DA induced down-regulation of Treg cells is selectively reversed by D1-like receptor antagonists [59],
- Antagonists applied for D1-like or D2-like receptors polarize the cells to Th1 and Th2 differentiation, respectively [158].

Potential therapeutic conclusions on applications of D1-receptor antagonists are:

– DA-erg elements regulating the balance of Th1/Th2/Th17/Treg cell activation have a recognized role in several autoimmune or allergic disorders [158];
– application of D1-antagonists or D1-agonists may be a useful treatment where DA and/or DA receptors on immune cells are considered as therapeutic targets [119, 158].

Prospective for new "NeuroImmuno-Pharmacology" therapies

In pipelines of drug development avenues there is a potential role for DA, or DA-analogues/precursors to target specific DA-erg components of immune cells upon dysfunction. These approaches, in order achieve clinical efficacy but maintain the normal immune function attention should be taken to:

– focus on a proper delivery systems capable targeting right at the sites of pathologies;
– discriminate the normal cells from the malfunctioning immune cells.

Regulatory function through specific Th/Treg cells. One of the recent alternative approaches in NeuroImmunoPharmacology might focus on the regulatory function of CD4+CD25+ cells. These *Treg* cells are able to regulate immune responses of other T cell subtypes, including *Teff* or Th17 cells.

Stimulation of D1 receptors (by DA present at the same loci, autocrine/paracrine manner) suppresses the cytokine secretion, or down-regulates *Treg*-dependent inhibition on effector *Teff* [8, 59]. Applying D1-antagonists and inhibiting the D1-receptor driven downregulation of *Tregs*, should consequently reverse that loop and downregulates the autoreactive stimuli on *Teff*s, while restore the *Treg*-specific inhibition on *Teff* cells, resulting in suppression of autoimmunity.

Due to the fact that depletion of *Tregs* cells disrupts the regulation on autoreactive clones therefore changes the normal protection/autoimmunity balance: (i) increase the development of "spontaneous autoimmunity", thus predisposes animals to autoimmune diseases; on the other hand, (ii) it promotes survival of neurons after CNS insults, as well as (iii) boosts the antitumor autoimmunity [119, 120].

Tregs are known to exhibit regulatory activities by suppression of immune responses via secretion of antiinflammatory cytokines. The proper numbers of active Tregs in order to suppress proinflammatory T cells might also provide an environment for increased neuroprotection. Thus, the size and activity of naturally occurring *Treg* clones intended to maintain a balance between the ability to manifest the beneficial (auto)immune response required to maintain normal immunity (i.e. for neuroprotection and repair) and in the same time these cells are need to avoid development of autoimmunity [120, 194].

Pharmaceutically designed compounds capable specifically regulate the suppressing activity of Tregs or reducing the trafficking ability (adhesion and migration) of *Treg*, might be promising candidates to boost anticancer therapies or mitigate neurodegeneration. On the other side of the balance, compounds capable for upregulation of inhibitory or trafficking activity of *Treg*, or both, might be potential candidates for therapy against autoimmune diseases. From the most available candidates, DA-ergic elements affect both the suppressive and the trafficking activities of *Treg*. The concept from both directions has been proved: when D1-agonists applied in experimental models of neurodegenerative conditions of CNS resulting in neuroprotection after mechanical or chemical injuries [119]; or in experimental autoimmune models when treatment with D1-antagonists suppressed the severity of rheumatoid arthritis [159].

As a conclusion of *Th/Treg* function, the immunomodulation-based strategies with a specific attention to DA level-driven measure of efficacy should have a distinct rationale based on the demonstration that T cells themselves are orchestrating also in the progress of (cyto)toxic events [197] or protective autoimmune actions. A greater understanding of T cell interactions as a regulatory *Treg* cell function in the brain both in normal and disease conditions could lead to an identification of methods generating

Treg cells for therapeutic modalities would be a great interest in future therapies.

Selection and maturation of T cells. Drug development approach specific to immunomodulatory or immunotherapy in treatment of PD much likely focusing on specific subpopulations of cells; such as the CD4+ T cells that are responsible for cytotoxicity and selecting those which may enhance neuroprotection. Brochard and colleagues [39] suggested that CD4+ T cells mediate cytotoxicity in the mouse model of PD, thus the Th1 and Th17 cells become potential targets in efforts to minimize the hostile neuronal microenvironment. Based on this experimental finding the suppression of these differentiating signals as well as Th1 and/or Th17 cells themselves has now become a potentially meaningful target for immunotherapy [8, 39].

Experimental evidences supported by recent *in vivo* data have been similarly concluded the selective impact of dopamine on lymphocyte function. This may serve a novel approach for adjuvant therapy utilizing DA not only in B-cell neoplasia but the similar way that can be extended that also in other lymphoproliferative disturbances in general [15].

DA may have a role also in the maturation and (de)selection of lymphocytes. Cellular mechanisms by which DA activates resting T cells may have a role in the maturation and selection of lymphocytes within the microenvironment of the Thymus or other primary lymph organs. To model the same cellular path that DA inhibits stimulated T cell subsets will be essential to design new and effective therapies in future to modulate the functions of T cells both in health and in diseases [148, 189].

Natural or semi synthetic biological agents specific to induce molecular switches that regulate lymphocyte quiescence and keep the cell cycle in control of T lymphocytes could have a great scientific or therapeutic interest. In addition, strategies for inducing quiescence by using specific D4 receptor agonists might be useful in the treatment of diseases in which uncontrolled T cell proliferation plays an important pathogenic role [190].

On the other hand, studies of genetics lead us to focus for neuroprotective and restorative therapies that in the future may also consider the aspects of NeuroImmunoPharmacology. Recent studies have explored adaptive immune systems in pathogenesis of PD. They consist of highly specialized cells with specific immunologic effectors, the ones with regulatory, and memory capabilities (T and B lymphocytes) that specifically eliminate or prevent pathogenic insults; but is activated by the nonspecific innate immune system [197].

Design for specific drug delivery systems. A variety of experimental delivery strategies including liposomes, solid lipid nanoparticles and biocompatible microparticles have been under the interest of pharmaceutical investigations for PD but being nowadays extended to immune disorders. The design of these new drug delivery systems which would utilize the DA-ergic components similarly to those currently under development for PD could provide potential vehicle to carry the desired elements e.g. to promote oxidative stress acting at pathological sites. Preclinical studies investigating lymphocytes and other immune cells with the presence of multiple regulatory components lead to these novel therapeutic opportunities for NHL or other nonmalignant lymphoproliferative disorders [71, 144].

- The liposomal packaging of L-DOPA is utilized for treatment in PD. In order to minimize unfavorable side effects, L-DOPA is encapsulated in unilamellar liposomes with higher lipophilicity or biodegradable polymeric microspheres as a depot system. The success of this formulation demonstrated with remarkably elevated levels of L-DOPA and DA in striatum after i.p./s.c. administration of the new formulations. The success of this formulation demonstrated by 2.5-fold increase in the basal levels of DA in striatum. The polymeric microsphere matrix protects the prodrug from chemical and enzymatic degradation and additionally provides sustained levels of DA [65, 71].
- Based on similar principle the combination of liposomal packaging and carrier system designed for L-DOPA in PD with the anti-CD19/CD20-targeted "immunoliposomes" for B lymphoma is another relevant drug development approach for NHL or other lymphoproliferative disorders [144, 188].
- Development of new drugs and delivery systems are targeting the DA-ergic components in treatment of Parkinson's disease. The clinical observation that lymphocytes of those PD patients who are receiving regular high-dose L-DOPA treatment have an increased DA content [172] opened another theory whether the forced release of this DA would generate a sufficient local source of extracellular H_2O_2 to regulate the malignant

cell proliferation and consequently lead them to apoptosis [144].

Receptor driven immunomodulation. The use of "reinvented" pharmaceutical agents originally developed for CNS indications to manipulate central monoamine function could have the availability to offer suitable candidates to test the therapeutic potential in NeuroImmunoPharmacology. Recent evidences have been implicated that the D3 receptors functioning in immunomodulation as "the neurotransmitter-mediated regulation of peripheral T lymphocytes." The theory briefly discussed above in this review: activated T-blasts cross the blood brain barrier and exposed to DA and activated within the CNS, return to the circulation and transmit "dopamine-dependent" information by specific cytokine release to modulate other T cells remaining in the periphery [110]. This novel paradigm could extend to interactions of neurotransmitters and a spectrum of sensitive and mobile immune cells (e.g. T or B cell clones) to communicate between the brain and peripheral immune system [15]. It is still theoretical and rather "exciting proposition" that will need to be proved with solid evidences, considering proper control and risk/benefit evaluations.

Abnormal level of receptor expression in T-cells has been observed and associated with human diseases discussed above. Since T-cells produce, store and secrete DA and other neurotransmitters thus, they may be affected by an autocrine/paracrine manner as well. The expression of the neurotransmitter receptors in T-cells can be changed significantly even:

- during the TCR-activation,
- activation by cytokines,
- by the presence neurotransmitter itself,
- by the interactions with other co-released neurotransmitters or additional factors.

These interesting findings would be extended in future studies to prove that abnormal interactions between neurotransmitters and T-cells, for example due to: (i) too high or low level of neurotransmitter receptors in T-cells; or (ii) malfunction of the brain because of chronic stress or injury may play an active role in the pathogenesis of the respective diseases or counteract the efforts of T-cells' in fighting against pathologies during the normal immune function [130].

As it is today, DA analogues, also specific receptor blockers, agonists or antagonists are all available and more of them are in a daily use by clinicians during treatments of other conditions, which might have direct or indirect side effects manifested. As a recommendation (rather then a conclusion), well designed, controlled clinical trials would be necessary to evaluate the therapeutic use of the available (and as most of them also inexpensive) drugs with immunomodulatory function for treatments of immune disorders or mitigation of side effects [162, 189].

However, these drugs should be used with caution in patients in certain clinical conditions (e.g. specific psychiatric conditions, microbial sepsis, autoimmune manifestations, etc...) as it may suppress or demodulate the immune functions in these patients, which may raise safety concerns. The proper risk/benefits evaluation over efficacy is critically important for future treatment combinations using these potential immunomodulatory agents.

DA transporter systems. Another new approach for future development is based on the role of DAT, since that is one of the common targets of several drugs used in the therapeutic field of psychiatry, such as psychostimulants, or antidepressants. Cells expressing an active transporter for (DAT) similarly that the DA receptors were varied among T cells, or in normal/neoplastic B cell populations. The role and function of DAT in lymphocytes have been characterized recently [4, 134, 143, 144, 148]. Monoamines have been hypothesized to be critically involved in the pathophysiology of a number of brain disorders, including PD, schizophrenia and drug addiction. The availability of DA in the synaptic cleft is strictly limited by selective, active re-uptake mechanisms performed by specific proteins, the DA transporters. DAT play the major role of terminating the activity of the neurotransmitter, once it is released in the synaptic cleft [135].

Preclinical results of lymphocytes that express and actively utilize DAT in their normal function provide a theoretical approach for immunomodulation. Regulation of DAT function and its potential to influence the DA levels within the cells and also for the surrounding immune cells convey a microenvironmental direct regulatory potential. In theory, it may also open a potential new approach for drug development, however the literature on this topic is still quite controversial and different studies are not easily comparable. Data in human are quite few: the available findings, as expected and also based on the interactions between the lymbic system and the immune systems would suggest that drugs interacting with lymphocyte DAT transporters might be beneficial particularly in severe immune disturbances, or autoimmune disorders [15, 135]. Prospective, controlled, double blind clinical studies are strongly recommended to elucidate

clinical efficacy. However, relevant clinical data also required to clarify the immunological interactions with antidepressants or psychostimulants or other pharmacological targets of DAT.

CONCLUDING REMARKS

It is well documented that DA can be synthesized, vesicular storage mechanism is present, and active uptake carrier for DA are expressed in immune cells. The expression of DA receptor subtypes on immune cells is dynamic. It is related with the maturation or with the phase of immune response, it is function and lineage specific and characteristic to certain immune disorders. It should be emphasized that DA seems to have a dual role on immune cell regulation: it activates resting cells, but inhibits activated cells or malignant cell clones. Based upon clinical observations, DA, at low micromolar concentrations can deliver significant cytostasis. Therefore, it is likely that an autocrine/paracrine regulatory loop exists in lymphocytes, where DA produced and released by the cells then acts through its own receptors, and can have an influence on its own function.

At the same time, elements of DA signaling and metabolites can serve as a communication interface between central nervous system and immune system that can work to both directions. Therefore, brain-derived DA may also be in context with immune functions. Permanent dysfunctions of either the central or the peripheral DA-ergic system are frequently associated with immune malfunctions. Current DA replacement or receptor blocking therapies are based upon the supposed action of these drugs at the target site, and several occasions it achieves mainly and only symptomatic efficacy. These may need to be revisited or at least topped up with the concepts of neuroimmunomodulatory influence of those treatment, and focus on the events of cross-talk between the immune and nervous systems. The characterized and specific pharmacological actions of DA or the DA-ergic agonists or antagonist on DA-ergic systems should be considered to extend for therapies of lymphoproliferative disorders; for enhancement of classical therapies in neuroprotection; and controlling autoimmune/allergy immune status or modulation of the immune response.

Finally, the pharmacological design of targeted drug delivery systems could provide potential vehicle to carry a desired compound right to the sites of cellular pathologies. Well designed clinical trials are needed for a critical evaluation of this new theory in therapy; either by the use of the available drugs with an extended immunomodulatory functions, or newly designed compounds or the combination of both. Evaluation of clinical efficacy and data on safety of patients should provide an answer for these questions.

DATA REVIEW METHODOLOGY

Inclusion of data to this review was guided by the following principles. Studies and relevant clinical data conducted after 1990 were included. We were over-inclusive and did not restrict our data inclusion on any standardized methodology. The intent was to include as much research and aspects were possible. Wherever it was applicable, the strengths and the limitations of the cited research were also discussed.

ACKNOWLEDGMENTS

This work was supported by OTKA-81522 grant (for GMN) and by TÁMOP-4.2.1/B-09/1/KMR-2010-0001 (for GMN and TZ).

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