

Modulation of T-cell Mediated Immunity by Dopamine Receptor D5

Carolina Prado^{1,2}, Sebastián Bernales¹ and Rodrigo Pacheco^{1,3,*}

¹Fundación Ciencia & Vida, ²Universidad Andrés Bello, and ³Universidad San Sebastián, Santiago, Chile

Abstract: CD4⁺ T-cells are central players orchestrating antigen-specific immunity and tolerance. Importantly, dendritic cells (DCs) are responsible for priming T-cells and for promoting their differentiation from naïve T-cells into appropriate functional cells. Because of their fundamental roles in controlling immunity, activation and differentiation of DCs and CD4⁺ T-cells require tight regulatory mechanisms. Several studies have shown that dopamine, not only mediates interactions into the nervous system, but it can also contribute to the modulation of immunity. Here, we review the emerging role of this neurotransmitter as a regulator of DCs and CD4⁺ T-cells physiology and its consequent involvement, in the regulation of immune response. We specially focus the analysis in the role of dopamine receptor D5 expressed on DCs and CD4⁺ T-cells in the modulation of immunity. We also discuss how alterations in the dopamine-mediated regulation of immunity could contribute to the onset and development of immune-related disorders.

Keywords: CD4⁺ helper T-cells, dendritic cells, dopamine, regulatory T-cells, T-cell mediated immunity.

THE NEUROTRANSMITTER-MEDIATED REGULATION OF T-CELL MEDIATED IMMUNITY

Dendritic cells (DCs) are the most potent antigen-presenting cells (APCs) specialized in initiation of adaptive immune responses by directing the activation and differentiation of naïve T-cells [1, 2]. These cells can capture both self and foreign antigens (Ags) in diverse tissues and migrate to secondary lymphoid organs to present captured and processed Ags on Major Histocompatibility Complex molecules to T-cells [3]. T-cell-DC synapses involve both soluble and contact dependent interactions that determine the nature of the T-cell response, by regulating T-cell activation and the acquisition of functional capability by CD4⁺ T-cells to promote immunity (effector T-cells, Teffs) or tolerance (regulatory T-cells, Tregs) [4]. Thus, depending on the signals that DCs provide to T-cells, they can promote the differentiation of naïve CD4⁺ T-cell toward distinct Teffs subsets, including T helper (Th) 1 (Th1), Th2 and Th17 [2, 5]. The Th1 phenotype is stimulated by the secretion of IL-12 by DCs. In contrast, a Th2 phenotype is favoured in the absence of IL-12 and in the presence of IL-4 during Ag presentation [6]. Th1 cells predominantly secrete IFN- γ to promote cellular immunity against intracellular pathogens and tumor cells. On the other hand, Th2 cells primarily secrete IL-4 which facilitates responses that efficiently eliminate extracellular pathogens, such as helminths and extracellular bacteria [7]. The Th17 phenotype, which primarily secretes IL-17, is promoted by TGF- β and IL-6 and expanded by IL-23, which can be secreted by DCs during Ag presentation [5, 8]. It is thought that Th17 cells protect against extracellular bacteria, particularly in the gut, however they have also been extensively associated with autoimmune

diseases [5]. Another functional type of CD4⁺ T-cells are Tregs, a phenotype induced in the presence of TGF- β [9] which mainly secrete IL-10 and TGF- β and suppress several functions of Teffs, thus promoting Ag-specific tolerance [10]. The success of a particular immune response depends on the polarization of specific naïve CD4⁺ T-cells toward appropriate functional phenotype; thereby this process is tightly regulated. In this regard, deregulation in the differentiation of the functional phenotype of CD4⁺ T-cells could result in cancer, exacerbated susceptibility to infections or the development of autoimmunity.

Traditionally, it has been described that the function of immune cells, such as T-cells and DCs, is regulated by soluble protein mediators known as cytokines. However, an emerging number of studies have shown that immune system cells can be also regulated by neurotransmitters [11]. In this regard, it has been described that several receptors for neurotransmitters classically expressed in the nervous system, are also expressed on the surface of immune system cells. For instance, T-cells and DCs express some subtypes of glutamate receptors (GluRs), acetylcholine receptors (AChRs), serotonin receptors (5-HTRs), dopamine receptors (DARs), adrenergic receptors, and others (see some examples in Table 1). The identification of these receptors on immune system cells suggests that neurotransmitters play a physiological role in the regulation of the immune response and that deregulation in the activation or in the expression of these receptors could contribute to the development of autoimmunity or malignancies. Recent studies have revealed that some immune cells not only expressed neurotransmitters receptors, but they are also capable of synthesizing and/or capturing classical neurotransmitters and store them in intracellular vesicles (Table 1). Under specific conditions, these cells may release neurotransmitters from intracellular storages thus involving autocrine, paracrine, juxtacrine and eventually endocrine communications between different leukocytes [10, 11, 12-26].

*Address correspondence to this author at the Fundación Ciencia y Vida. Avenida Zañartu #1482, Santiago, Chile; Tel: 56-2-3672046; Fax: 56-2-2372259; E-mail: rpacheco@cienciavida.cl; rpachecr@gmail.com

In this regard, some studies have revealed relevant neurotransmitters-mediated regulatory mechanisms in the function and differentiation of T-cells and DCs. For instance, it has been demonstrated that during Ag-presentation, DCs release glutamate and this neurotransmitter acts subsequently on T-cells [13, 27]. This neurotransmitter initially stimulates metabotropic GluR5 (mGluR5), triggering inhibitory signals mediated by a rise of cAMP, which impairs T-cell activation. However, when the interaction of T-cell receptor (TCR) with the peptide presented in the Major Histocompatibility Complex is productive, T-cells overcome the mGluR5-dependent inhibition and begin to express mGluR1. Further stimulation of mGluR1 by DCs-derived glutamate potentiates T-cell activation and induces increased secretion of Th1- and pro-inflammatory cytokines, thus contributing to the acquisition of a functional phenotype of T-cells [12, 13, 27]. Other example of DC-derived neurotransmitter that regulates the T-cell response is Serotonin (5-HT). Under maturation stimuli, DCs begin to express the 5-HT-transporter, which mediates uptake of 5-HT from the extracellular compartment to be stored into intracellular vesicles. Subsequently, when mature DCs present Ags to T-cells in lymph nodes, DCs release these 5-HT-containing

vesicles over T-cells, thus stimulating 5-HTRs expressed on these cells [18]. It has been demonstrated that, indeed, 5-HT is required for proper T-cell activation early by stimulating 5-HT⁷ [17] and 5-HT³ [28, 29] expressed on naïve T-cells, and later by stimulating 5-HT^{1B} and 5-HT^{2C} expressed on activating T-cells [30]. Another interesting group of studies have revealed the presence of the cholinergic system in T-cells [31]. These studies shown that CD4⁺ T-cells not only express nicotinic and muscarinic AChRs [19], but they also express ACh-transporter and they are able to store ACh in intracellular vesicles [32, 33]. Upon TCR-stimulation, CD4⁺ T-cells release ACh that stimulates AChRs in an autocrine manner, potentiating T-cell activation and favouring differentiation toward Th1 phenotype [34-36].

These and other examples support the notion that neurotransmitters can mediate communication between immune cells. Among neurotransmitters, dopamine (DA) seems to play a very relevant role in the regulation of immunity, as alteration of dopaminergic components has been associated with several immune-related diseases (Tables 2 and 3). Due to the pivotal roles of CD4⁺ T-cells and DCs during adaptive immune responses, we have

Table 1. Neurotransmitter receptors expressed on and capability to store/release neurotransmitters by DCs and T-cells.

Cells	Neurotransmitter	Capability to Store/Release (a)	Receptors	References
T-cells	Glutamate	ND	metabotropic and ionotropic GluRs	[12, 13]
	Dopamine	+	DARs	[10, 14-16]
	Serotonin	+	5-HTR	[17, 18]
	Acetylcholine	+	nicotinic and muscarinic AChR	[19]
	Noradrenaline/Adrenaline	+	NAR/AR	[20]
	γ -amino butiric acid (GABA)	ND	GABARs	[21]
DCs	Glutamate	+	metabotropic GluR	[13, 27]
	Dopamine	+	DARs	[22, 23]
	Serotonin	+	5-HTR	[24]
	Acetylcholine	ND	nicotinic AChR	[25]
	Noradrenaline/Adrenaline	ND	NAR/AR	[26]

(a) + Symbol indicates the capability to synthesize or capture the corresponding neurotransmitter from the extracellular compartment and store it intracellularly. ND, not determined.

Table 2. Imbalance of plasma DA levels described in some pathologies.

Disease Classification	Pathology	Increase (a)	References
Neurological	Stress	1.85	[37]
Autoimmunity	Rheumatic diseases	0.62	[38]
Malignancies	Lung cancer	4.76	[39]
	Advanced cancer (several types)	5.59	[40]

(a) Increase in plasma DA levels is expressed as the ratio of average of plasma DA concentration from patients versus average of plasma DA concentration from healthy donors.

Table 3. Altered expression of DARs in human T-cells in some pathologies.

Disease Classification	Pathology	Receptor (a)	Cells (b)	References
Neurological	Alzheimer's disease	↓ Type II DARS	PBMC	[41]
	Parkinson's disease	↓D3	PBMC	[42]
	Schizophrenia	↑D3	T-cells	[43-45]
		↓D4	CD4 ⁺ T-cells	[43]
Autoimmunity	Multiple Sclerosis	↓D5	PBMC	[46]
		↑D5	CD4 ⁺ CD25 ^{high} Tregs	[47]
		↓D5	CD4 ⁺ CD25 ⁻ Teff	[47]

(a) Arrows indicate increased or decreased expression of DARs respect to the control conditions from healthy individuals.

(b) PBMC, peripheral blood mononuclear cells.

analysed and discussed recent studies relative to the contribution of DA as mediator in the function and differentiation of these cells [37-47].

DOPAMINE AS A MODULATOR OF ADAPTIVE IMMUNE RESPONSE

DA from diverse sources can stimulate DARs expressed on CD4⁺ T-cells and DCs, and thus, this neurotransmitter may strongly regulate the initiation and development of the immune response. The primary source of DA for immune cells circulating into the blood vessels is plasma DA (see Table 2). Additionally, dopaminergic innervation of primary and secondary lymphoid organs through sympathetic nerves has been described [48], which suggests the existence of direct DA-mediated regulation from nervous system over T-cells and DCs in lymphoid organs. Another source of DA available for immune cells corresponds to the autocrine and paracrine secretion of DA by some kinds of immune cells in secondary lymphoid organs. Accordingly, it has been described that human Tregs constitutively express tyrosine hydroxylase, the rate-limiting enzyme involved in DA synthesis, and they contain substantial amounts of DA and other catecholamines, while Teffs only contain trace amounts [10]. Tregs also express vesicular monoamine transporters. VMAT-1 and -2, which allow them to accumulate catecholamines in vesicular stores [10]. Interestingly, physiologically relevant amounts of DA are released by Tregs when stimulated by reserpine [10], nonetheless, physiological stimuli inducing DA release from Tregs are still pending to be determined. DCs constitute another kind of immune cells capable to synthesize and to store DA in intracellular compartments [23, 49]. In this regards, we have recently shown that murine DCs express the machinery necessary to produce and to store DA but not to take up DA from extracellular compartments [23]. Importantly, upon exposition to maturation stimuli, release of DA-containing intracellular vesicles is induced in DCs. In addition, Nakano *et al.*, have proposed that human DCs contain intracellular DA, which is released upon Ag-presentation to T-cells [49].

Another source of DA and others neurotransmitters is the central nervous system (CNS). For a long time the CNS was

considered to be an immune privileged site due to the presence of the blood-brain-barrier (BBB) and to the lack of lymphatics. Nevertheless, it is currently known that, during inflammatory processes, circulating immunocompetent cells are able to access to the CNS parenchyma [50]. In this regard, it has been shown that Teffs as well as Tregs and DCs are able to cross the BBB [51-56] where they may be exposed to a variety of neurotransmitters. The presence of infiltrating immune cells into the CNS parenchyma has been detected in most of the neurodegenerative diseases studied [57]. In a pathological scenario involving the CNS, such as neurodegeneration or imbalance of glial homeostasis, initial neuroinflammatory processes induce brain endothelial cells to express a specialized pattern of adhesion molecules. These adhesion molecules induced by inflammatory processes subsequently allow activated T-cells to adhere to the vessels walls and to be recruited into the CNS parenchyma. T-cells that infiltrate the CNS are previously activated in the periphery, presumably in cervical lymph nodes, in which a sampling of CNS-associated Ags is constantly arriving through the cerebrospinal fluid that drains these lymph nodes. Interestingly, the expression of the chemokine receptor CCR6 by Th17 T-cells and the expression of its corresponding ligand, the chemokine CCL20, by epithelial cells of the choroid plexus have been shown to play an important role in facilitating T-cell entry into the CNS during the development of Experimental Autoimmune Encephalomyelitis (EAE) [58], the murine model of the autoimmune disease Multiple Sclerosis (MS). Once T-cells enter into the CNS, they are re-stimulated by resident APCs, such as astrocytes, microglia, or by infiltrated APCs such as DCs and macrophages [59]. Cytokine production by infiltrating T-cells and activated APCs during inflammatory process can contribute significantly to the recruitment of innate immune cells and further circulating T-cells, since parenchymal BBB is disrupted following exposure to pro-inflammatory cytokines [55, 60, 61].

DA may modulate the initiation and development of the immune response by stimulating different DARs expressed on immune cells. Five DARs have been identified to date: D1R, D2R, D3R, D4R and D5R. All of these receptors are hepta-spanning membrane proteins that belong to the superfamily of G protein-coupled receptors [62]. Based on their sequence homology, signal transduction machinery and

pharmacological properties, DARs have been classified into two subgroups. Generally, type I DARs (D1R and D5R) are coupled to G α s and subsequent stimulation of cAMP production, whereas type II DARs (D2R, D3R and D4R) are coupled to G α i promoting inhibition of cAMP synthesis [63]. Despite Type I and Type II DARs are often coupled to stimulation and inhibition of intracellular cAMP production respectively [63], they also have been found coupled to regulation of phospholipase C and ion channel activity [64-66]. On the other hand, due to the fact that different DARs present different affinity for DA, differential stimulation of DARs is induced depending on DA levels. In this regard, D3R has the major affinity for DA ($K_i \approx 27$ nM), followed by D5R ($K_i \approx 228$ nM) and then D4R, D2R and D1R ($K_i \approx 450$, 1705 and 2340 nM, respectively) [67-70]. Thus, depending on the concentration of dopamine, the differential coupling to cell-signaling, the specific DARs expressed and the kind of immune cell bearing DARs in the place where dopamine is available, this neurotransmitter may induce different effects in the immune response.

Emerging studies carried out on T-cells and DCs have demonstrated that these cells express mainly type I DARs, although they also express type II DARs. By using pharmacological approaches, a number of studies have described diverse functional effects for DARs expressed on T-cells and DCs [71]. The role of type I DARs in the physiology of these cells will be analyzed and discussed in next sections. Regarding the role of D2R on T-cell function, it has been demonstrated that stimulation of this receptor promotes enhanced IL-10 production, a cytokine that negatively regulates the function of T eff s [14]. Addressing D4R stimulation, evidence indicates that this receptor triggers T-cell quiescence by up-regulating the Krüppel-like factor-2 (KLF-2) expression [72]. On the other hand, stimulation of D3R on CD4 $^{+}$ T-cells favours production of IFN- γ [73]. Furthermore, stimulation *via* D3R is thought to be involved in migration and adhesion of T-cells, thus modulating the homing of these cells [16, 73, 74]. With regard to type II DARs expressed on DCs, pharmacological evidence has suggested the involvement of these receptors in the attenuation of DA synthesis by regulating tyrosine hydroxylase phosphorylation [49], which results in the subsequent attenuation of naïve T-cells differentiation toward Th17 phenotype [22]. However, the precise mechanism involved in this inhibition of Th17 response by type II DARs expressed on DCs remains to be elucidated.

REGULATION OF T-CELL MEDIATED IMMUNITY THROUGH D5R EXPRESSED ON CD4 $^{+}$ T-CELLS

Emerging evidence has shown a relevant role of D5R expressed on CD4 $^{+}$ T-cells in the function of these cells. Moreover, alterations in the expression of this receptor on CD4 $^{+}$ T-cells have been associated to the progression of immune related disease. As mentioned previously, all five DARs have been identified in CD4 $^{+}$ T-cells, nevertheless, there is a preferential expression of D1R and D5R in Tregs [75]. Unlike T eff s, Tregs express tyrosine hydroxylase and contain high amounts of intracellular DA stored in specialized vesicles expressing VMAT2 [10]. The capability of synthesizing and store DA and the expression of DARs

have suggested an autocrine mechanism mediated by DA in Tregs. According to this notion, Cosentino *et al.*, [10] have shown that the treatment of Tregs with reserpine, a drug that reverses the functioning of VMAT, promotes release of intracellular DA, which in turn exerts dramatic effects on the function of Tregs. However, physiological stimuli inducing DA release from Tregs are still pending to be determined. Addressing the role of DA on the Tregs physiology, two independent groups have shown pharmacological evidence indicating that, by stimulating D1R/D5R, DA reduces the suppressive function of Tregs [10, 75]. This DA-mediated inhibitory mechanism involves a reduction in IL-10 and TGF- β production and diminished CTLA-4 expression, which participate in the cytokine-mediated and contact-mediated suppression by Tregs, respectively. Thus, D1R/D5R-stimulation results in an impaired ability of Tregs to suppress T eff s proliferation [10, 75] (Fig. 1). These findings support a pro-inflammatory role for DA-D1R/D5R-Tregs axis in which, by reducing Tregs suppressive activity, it can lead to an increased ability of T eff s to develop inflammatory responses. According to these findings, a recent study has analyzed suppressive function and expression of dopaminergic machinery in Tregs obtained from patients undergoing MS or from healthy controls [47]. This study shows that D5R as well as tyrosine hydroxylase were up-regulated in Tregs from untreated MS patients when compared with those from healthy controls, however both, tyrosine hydroxylase and D5R, were down-regulated when Tregs were obtained from IFN- β -treated MS patients. Importantly, IFN- β treatment of MS patients improved their disease manifestation [76]. In addition, suppressive function was partial and completely inhibited by dopamine when Tregs were obtained from healthy controls and untreated MS patients respectively, however dopamine-mediated inhibition of Treg function was abolished when Tregs were obtained from IFN- β -treated MS patients [47]. Together, these findings support a key role for D5R in the regulation of Tregs activity and its association with autoimmunity. Thus, this data encourage the study and development of novel therapeutic strategies targeting D5R expressed on Tregs to fight against immune-related disorders such as autoimmunity or cancer.

MODULATION OF T-CELL RESPONSES BY D5R EXPRESSED ON DCs

A number of studies published during last five years have shown pharmacological evidence pointing toward an important role of type I DARs expressed on DCs as a relevant modulator of T-cell mediated immune responses, especially in autoimmune disorders. The first work addressing the role of DARs in DCs physiology, showed that antagonizing D1R/D5R expressed on human DCs altered acquisition of functional phenotype by naïve CD4 $^{+}$ T-cells increasing the Th1/Th17 ratio in DCs-T-cells co-cultures *in vitro* [22]. Moreover, the same authors reported later that human DCs contain intracellular DA storages, which are released during Ag-presentation to naïve CD4 $^{+}$ T-cells *in vitro* [49]. The *in vivo* relevance of these observations were evaluated by using a pharmacological approach in several animal models of autoimmune diseases such as EAE [22], spontaneous diabetes mellitus in NOD mice [77],

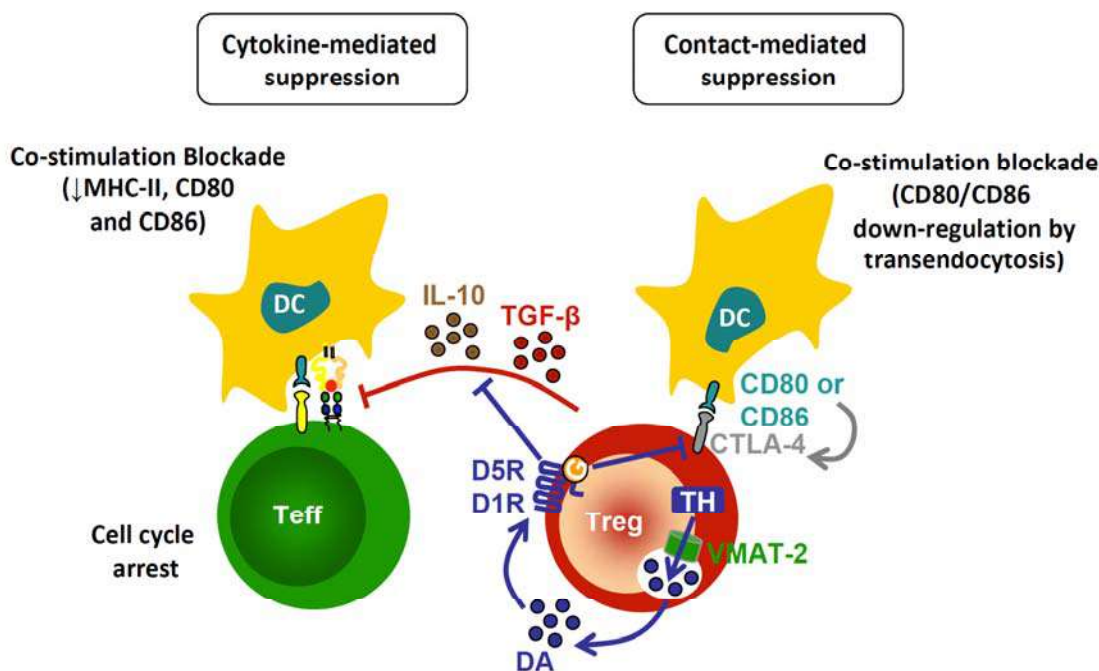


Fig. (1). Stimulation of D5R/D1R expressed on Tregs inhibits cytokine-mediated and contact-mediated suppressive mechanisms. Tregs express tyrosine hydroxylase, the rate-limiting enzyme in the synthesis of catecholamines, and store dopamine in specialized VMAT2-expressing vesicles. By still unknown stimuli, Tregs release DA, which acts in an autocrine manner stimulating D1R/D5R. Signaling triggered by stimulation of these receptors inhibits the suppressive function of Tregs by a reduction in IL-10 and TGF- β production and by down-regulation of surface expression of CTLA-4. The inhibition of both mechanisms results in an impairment of the ability of Tregs to suppress Teffs activation/proliferation. DA, dopamine; TH, tyrosine hydroxylase; Teff, effector T-cell; VMAT2, vesicular monoamine transporter 2.

Nephrotoxic serum nephritis [78] and rheumatoid arthritis in a SCID chimera mice [79]. In all these models, the treatment of mice with the systemic administration of a type I DARs antagonist, SCH23390, reduced disease severity by impairing the Th17 response. However, this pharmacologic approach does not allow the discrimination between the effects of D5R or D1R since the drug inhibits both type I DARs. In fact, SCH23390 not only displays similar affinities for D1R and D5R ($K_i=0.2$ and 0.3 nM, respectively) [80], but also displays comparable affinities for serotonin receptors 5-HT^{1C} and 5-HT^{2C} ($K_i=6.3$ and 9.3 nM, respectively) [81, 82]. Furthermore, these studies could not confine the cell-type responsible for the amelioration effect. Importantly, EAE and the other autoimmunity models used in those studies are mediated mainly by T-cells but B-cells also contribute to the initiation and development of the disease [55, 83-87]. Type I DARs as well as 5-HTRs have been found to be expressed on many types of immune cells including DCs [88], T-cells [10, 14, 16] and B-cells [89, 90]. Therefore, systemic treatment with SCH23390 could affect several types of immune cells. To restrict the contribution of DCs signaling to the amelioration of EAE, authors treated DCs with SCH23390 *ex vivo* and transferred treated cells into WT recipient mice [22]. A slight decrease in EAE severity was seen in mice transferred with SCH23390-treated DCs, however, such differences were not statistically significant [22]. Thus, this group of studies did not clarify the precise mechanism by which the type I DARs antagonist inhibits Th17 participation in autoimmune responses *in vivo*.

Recently, we have contributed to elucidate this mechanism using a genetic approach that allows us to determine the contribution of DCs and specific DARs to CD4⁺ T-cell differentiation and the consequences in EAE development. In agreement with previous studies [22], we demonstrate that D5R-deficient mice exhibited delayed EAE progression with reduced severity compared with normal mice. By comparing WT and D5R-deficient DCs *in vitro* we determined that D5R signalling selectively affects IL-23 and IL-12 production by DCs and also contributes to CD4⁺ T-cell activation and proliferation [23]. Since IL-23 and IL-12 share the p40 subunit, it is probably that D5R expressed on DCs could regulate activation of a transcription factor involved in the production of this common subunit, such as STAT3 [91]. Nevertheless, this possibility is pending to be demonstrated. The *in vivo* relevance of the impairment in cytokine secretion by D5R-deficient DCs was demonstrated in EAE experiments with prophylactic transfer of DCs. These experiments show that mice transferred with D5R-deficient DCs displayed significant attenuation of severity in EAE manifestation and had fewer Th17 CD4⁺ T-cells infiltrating into the CNS at the peak of disease severity [23]. Initially, it was described that polarization to Th17 phenotype requires solely IL-6 plus TGF- β , and IL-23 was just considered as a survival factor for differentiated Th17 cells *in vivo* [8]. In this regard, it has been demonstrated that signaling triggered by stimulation of IL-23 receptor in T-cells is essential for the Th17 program in two important

processes *in vivo*: First, promoting expansion and terminal differentiation toward Th17 cells in lymph nodes (between days 4-6 after naïve T-cell priming) and; Second during Th17 effector phase in target tissues by enhancing inflammatory activity and production of pro-inflammatory factors, such as IL-22 [92, 93] and GM-CSF [94]. These findings support the critical function observed for IL-23 in inflammation and autoimmunity [94, 95]. According to the first key role of IL-23 in Th17 responses, our recent findings [23] have associated D5R deficiency in DCs with a strongly impaired IL-23 production, which consequently results in a decreased CD4⁺ T-cell proliferation in Ag-specific co-cultures *in vitro*. This observation could explain the reduced frequency of Th17 cells infiltrated into the CNS of mice undergoing EAE [23]. Another possibility explaining the reduced Th17

frequency into the CNS of mice bearing D5R-deficient DCs during EAE could be an impaired Th17-migration into the CNS. In this regard, a defect in cell migration due to decreased IL-23 production can be ruled out since IL-23 does not affect the expression of Th17 migratory factors such as chemokine receptor CCR6 and the sphingosine 1-phosphate receptor [8]. However, an impaired migration of Th17 cells due to another factor involving D5R-deficiency in DCs, different of IL-23, can not be ruled out. Regarding the second key role of IL-23 in Th17-mediated immunity in the context of D5R expressed on DCs, it remains pending to be determined whether the reduced EAE severity in mice bearing D5R-deficient DCs is related with a lower production of IL-22 and GM-CSF by Th17 cells infiltrated into the CNS during the inflammatory process (Fig. 2).

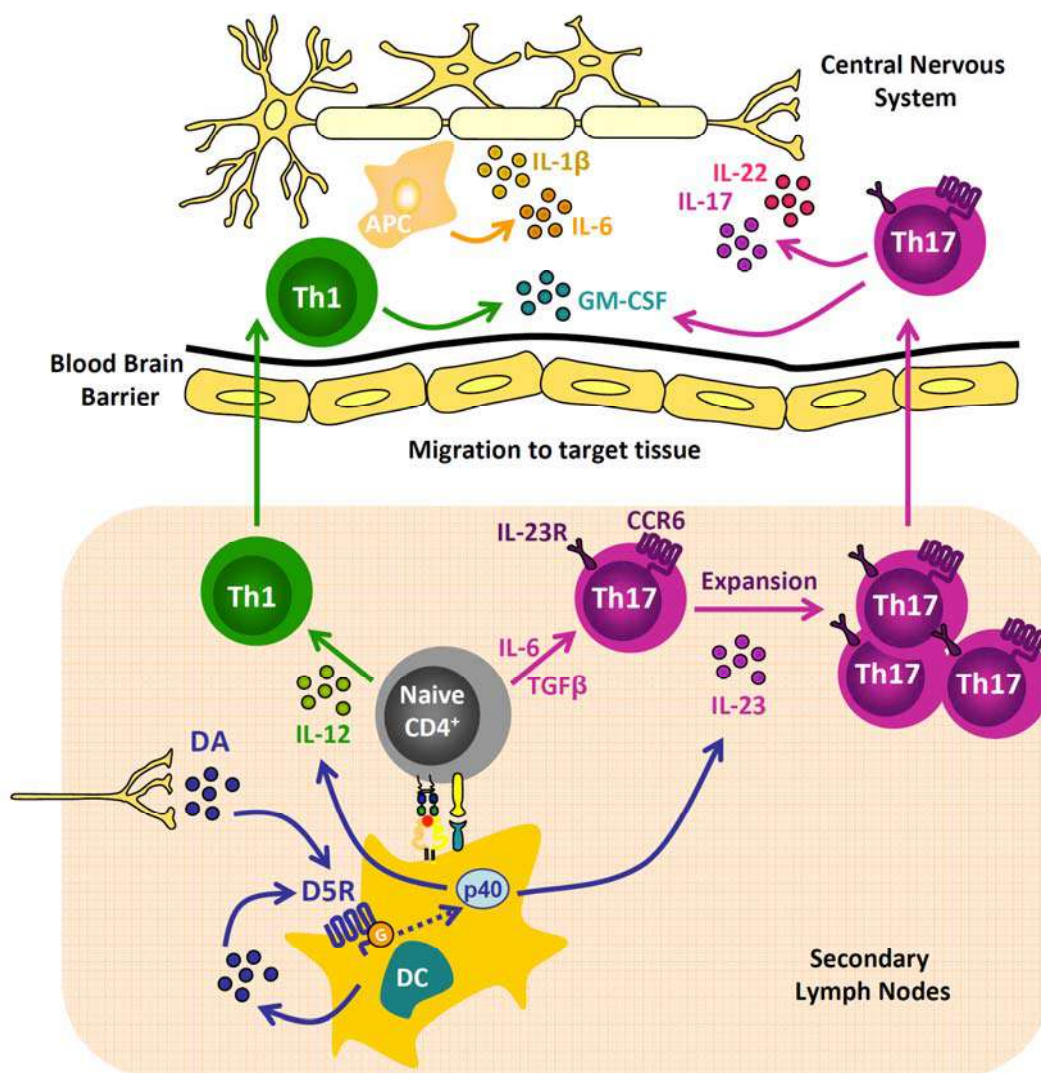


Fig. (2). Stimulation of D5R expressed on DCs potentiates Th17-mediated immunity in EAE. During Ag-presentation in secondary lymphoid tissues, D5R expressed on DCs is stimulated by DA released from sympathetic nerves or from themselves. Signaling triggered by D5R-stimulation, favours selective production of IL-12 and IL-23, cytokines necessary for Th1 differentiation and Th17 expansion, respectively. Early after EAE-induction, Th17 cells infiltrate into the CNS by a CCR6-dependent mechanism. Infiltrated T-cells are re-stimulated by local APCs, leading to local cytokine production. Pro-inflammatory cytokines secreted by infiltrating T-cells and activated APCs promote vasculature activation, which in turn, allows infiltration of further peripheral circulating T-cells into the brain parenchyma. T-cell derived cytokines, especially GM-CSF, promote subsequent myelin destruction and loss of motor function in mice. APC, Ag-presenting cell, DA, dopamine; IL-23R, IL-23 receptor; CCR6, C-C chemokine receptor 6; p40, p40 sub-unit shared by IL-12 and IL-23.

ALTERATIONS ON T-CELL FUNCTION DUE TO DEREGLATION ON DOPAMINE-MEDIATED NEUROIMMUNE COMMUNICATION AND THE DEVELOPMENT OF DISEASE

The presence of DA-mediated regulatory mechanisms involved in the modulation of the immune response suggests that deregulation of components of these mechanisms could be associated to the triggering, development or progression of immune- or neuroimmune-related diseases. Accordingly, an imbalance of plasma DA levels and deregulation of the expression of DARs on T-cells have been correlated with some disorders.

Regarding the deregulation of plasma DA levels in immune-related disorders, it seems that there is a trend in which plasma DA is increased in cancer [39, 40] but decreased in autoimmunity [38]. It has been described that *in vitro* exposure of T-cells to DA concentrations similar to those found in the plasma of cancer patients results in a general inhibition of T-cell proliferation and cytokine secretion [39, 96]. It is likely that this DA-mediated inhibition of T-cell function contributes to impair the anti-neoplastic immunity in cancer patients. Due to that stress is another condition in which plasma DA is significantly increased (Table 2), it can not be ruled out that increased DA levels in cancer is due to the stress of patients. On the other hand, low levels of plasma DA found on autoimmunity could have a role in these pathologies by allowing an exacerbated response of autoreactive T-cells. Thereby, the trend of high DA levels in malignancies and low DA levels in autoimmunity could constitute an important factor in the pathophysiology of these diseases and consequently should be considered for treatment and/or diagnosis. Given the imbalanced levels of plasma DA seen in diverse neurologic disorders, it may be too complex a process to establish the cause of the imbalance. Nevertheless, the effect of this imbalance on the T-cell function may sometimes results quite predictable. For instance, the pathophysiological condition of stress promotes increased levels of plasma DA (Table 2), which results in the inhibition of T-cell function and therefore increased susceptibility to infections and cancer [97, 98]. It is important to consider that, due to the fact that DARs display different affinity constants, differential expression in different immune cells, and differential coupling to cell signaling, altered DA levels could promote diverse effects in the immune response. These findings collectively suggest that selective stimulation of specific DARs on T-cells, which may be carried out by administration of selective agonist/antagonists, could improve T-cell response in some neurological and immune-related disorders.

Not only an imbalance of DA levels can alter T-cell function, but also the deregulation of DARs expressed on T-cells could result in similar effects. Accordingly, abnormal expression of DARs on immune cells has been described in some neurological and immune-related disorders (Table 3). For example, it has been detected an increased expression of the D5R on CD4⁺CD25^{high} Tregs [47] and a decreased D5R expression on CD4⁺CD25⁻ Teffs in MS patients when compared to healthy individuals. In both subsets of T-cells, D5R stimulation promotes impaired T-cell function [10, 15,

75]. Thereby, alterations in D5R expression occurring in T-cells during MS would result in attenuated Tregs function and exacerbated Teffs activity, thus favouring the inflammatory process. Furthermore, it has recently been shown that amelioration of MS by treatment with IFN- β is accompanied by restoration of levels of D5R expressed on T-cells subsets [47, 99]. DARs deregulation on T-cells has been also described in several neurological disorders. Some studies have suggested that the RNA levels of specific DARs subtypes expressed on T-cells could be used as peripheral markers for clinical diagnosis of these neurological and immune-related diseases (Table 3). More importantly, all of these studies suggest that the functional dialogue between DA and T-cells might be either up- or down-regulated in these diseases, playing a key element in the pathological scenario. Therefore, imbalanced neurotransmitter receptors expression on T-cells seems to be also an additional important factor that can be considered for the design of therapies for immune- or neuroimmune-related disorders.

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

Emerging evidence has indicated an important role of type I DARs, specially D5R, in the regulation of T-cell mediated immunity. These studies have shown pharmacological and genetic evidence suggesting a relevant role of D5R in the function of both, DCs and CD4⁺ T-cells. Whereas D5R expressed on DCs favours Th17-mediated immune responses, D5R expressed in CD4⁺ T-cells contributes to Tregs-mediated suppressive activity. Furthermore, a number of studies have shown alteration of plasma DA levels and expression of DARs on immune cells in the context of some immune-related diseases and some neurological disorders, suggesting an important role of DA-mediated regulation of immune cells in diverse pathologies. Of note, clinical evidence has shown altered expression of D5R in immune cells obtained from MS patients, which is reestablished upon treatment with IFN- β , a treatment that improve the course of disease manifestation. Taken together this data indicates that D5R, and probably other DARs, expressed in DCs and CD4⁺ T-cells constitutes a key regulator of T-cell mediated immune responses. Thereby it should be considered as target molecule for future immunotherapies for the treatment of immune-related-disorders such as cancer and autoimmunity. Currently, some efforts in this field are focused in pharmacologically targeting this kind of receptors as therapeutic approaches *in vivo* in animal models of immune-related diseases. However, for many cases, such as the case of type I DARs (D1R and D5R), no specific drugs are available. Thereby, future efforts should be focused into evaluate the targeting of these receptors in an receptor-specific way: for example developing new drugs (agonists/antagonists) specific for a particular receptor or, alternatively, targeting specific receptor expression (*i.e.* by ectopic up- or down-regulation with viral vectors). Moreover, not only receptor-specific therapeutic approaches are necessary, but the targeting (stimulation/antagonism or expression/repression) of these receptors in a cell-specific way is also a future pending challenge. Furthermore, not only isolated targeting of neurotransmitter receptors should be assessed as therapeutic approaches in

immune-related diseases, but also targeting different receptors in combination should be addressed. Finally, because during last decade immune-response has been strongly involved in the physiopathology of neurodegenerative diseases such as Parkinson's disease, Amyotrophic Lateral Sclerosis or Alzheimer disease, targeting neurotransmitter receptors in immune cells should be also considered as therapies in these disorders.

CONFLICT OF INTEREST

The authors declare no conflict of interest. This work was supported by grants 1095114 from FONDECYT (to RP), PFB-16 from CONICYT (to RP and SB) and 2011-0001-R from Universidad San Sebastián (to RP). CP holds CONICYT graduated fellowship.

ACKNOWLEDGEMENTS

Designed the study: CP and RP; Performed the study: CP and RP; Collected data: CP and RP; Analyzed data: CP and RP; Wrote the paper: CP, SB and RP.

ABBREVIATIONS

APC	=	Antigen-presenting cell
AChR	=	Acetylcholine receptor
Ag	=	Antigen
CNS	=	Central Nervous System
DCs	=	Dendritic cells
DA	=	Dopamine
DARs	=	DA receptors
Teffs	=	Effector T-cells
EAE	=	Experimental Autoimmune Encephalomyelitis
GluR	=	Glutamate receptor
MS	=	Multiple Sclerosis
5-HT	=	Serotonin
5-HTR	=	5-HT receptor
TCR	=	T-cell Receptor
Tregs	=	regulatory T-cells

REFERENCES

- [1] Banchereau, J. and Steinman, R.M. (1998) Dendritic cells and the control of immunity. *Nature*, **392**, 245-252.
- [2] Lanzavecchia, A. and Sallusto, F. (2001) Regulation of T cell immunity by dendritic cells. *Cell*, **106**, 263-266.
- [3] Nouri-Shirazi, M.; Banchereau, J.; Fay, J. and Palucka, K. (2000) Dendritic cell based tumor vaccines. *Immunol. Lett.*, **74**, 5-10.
- [4] Friedl, P.; den Boer, A.T. and Gunzer, M. (2005) Tuning immune responses: diversity and adaptation of the immunological synapse. *Nat. Rev. Immunol.*, **5**, 532-545.
- [5] McGeachy, M.J. and Cua, D.J. (2008) Th17 cell differentiation: the long and winding road. *Immunity*, **28**, 445-453.
- [6] Watford, W.T.; Moriguchi, M.; Morinobu, A. and O'Shea, J.J. (2003) The biology of IL-12: coordinating innate and adaptive immune responses. *Cytokine Growth Factor Rev.*, **14**, 361-368.
- [7] Del Prete, G. (1998) The concept of type-1 and type-2 helper T cells and their cytokines in humans. *Int. Rev. Immunol.*, **16**, 427-455.
- [8] McGeachy, M.J.; Chen, Y.; Tato, C.M.; Laurence, A.; Joyce-Shaikh, B.; Blumenschein, W.M.; McClanahan, T.K.; O'Shea, J.J. and Cua, D.J. (2009) The interleukin 23 receptor is essential for the terminal differentiation of interleukin 17-producing effector T helper cells *in vivo*. *Nat. Immunol.*, **10**, 314-324.
- [9] DiPaolo, R.J.; Brinster, C.; Davidson, T.S.; Andersson, J.; Glass, D. and Shevach, E.M. (2007) Autoantigen-specific TGFbeta-induced Foxp3+ regulatory T cells prevent autoimmunity by inhibiting dendritic cells from activating autoreactive T cells. *J. Immunol.*, **179**, 4685-4693.
- [10] Cosentino, M.; Fietta, A.M.; Ferrari, M.; Rasini, E.; Bombelli, R.; Carcano, E.; Saporiti, F.; Meloni, F.; Marino, F. and Lecchini, S. (2007) Human CD4+CD25+ regulatory T cells selectively express tyrosine hydroxylase and contain endogenous catecholamines subserving an autocrine/paracrine inhibitory functional loop. *Blood*, **109**, 632-642.
- [11] Franco, R.; Pacheco, R.; Lluís, C.; Ahern, G.P. and O'Connell, P.J. (2007) The emergence of neurotransmitters as immune modulators. *Trends Immunol.*, **28**, 400-407.
- [12] Pacheco, R.; Ciruela, F.; Casado, V.; Mallol, J.; Gallart, T.; Lluís, C. and Franco, R. (2004) Group I metabotropic glutamate receptors mediate a dual role of glutamate in T cell activation. *J. Biol. Chem.*, **279**, 33352-33358.
- [13] Pacheco, R.; Gallart, T.; Lluís, C. and Franco, R. (2007) Role of glutamate on T-cell mediated immunity. *J. Neuroimmunol.*, **185**, 9-19.
- [14] Besser, M.J.; Ganor, Y. and Levite, M. (2005) Dopamine by itself activates either D2, D3 or D1/D5 dopaminergic receptors in normal human T-cells and triggers the selective secretion of either IL-10, TNFalpha or both. *J. Neuroimmunol.*, **169**, 161-171.
- [15] Saha, B.; Mondal, A.C.; Majumder, J.; Basu, S. and Dasgupta, P.S. (2001) Physiological concentrations of dopamine inhibit the proliferation and cytotoxicity of human CD4+ and CD8+ T cells *in vitro*: a receptor-mediated mechanism. *Neuroimmunomodulation*, **9**, 23-33.
- [16] Watanabe, Y.; Nakayama, T.; Nagakubo, D.; Hieshima, K.; Jin, Z.; Katou, F.; Hashimoto, K. and Yoshie, O. (2006) Dopamine selectively induces migration and homing of naive CD8+ T cells via dopamine receptor D3. *J. Immunol.*, **176**, 848-856.
- [17] Leon-Ponte, M.; Ahern, G.P. and O'Connell, P.J. (2007) Serotonin provides an accessory signal to enhance T-cell activation by signaling through the 5-HT7 receptor. *Blood*, **109**, 3139-3146.
- [18] O'Connell, P.J.; Wang, X.; Leon-Ponte, M.; Griffiths, C.; Pingle, S.C. and Ahern, G.P. (2006) A novel form of immune signaling revealed by transmission of the inflammatory mediator serotonin between dendritic cells and T cells. *Blood*, **107**, 1010-1017.
- [19] Kawashima, K. and Fujii, T. (2003) The lymphocytic cholinergic system and its contribution to the regulation of immune activity. *Life Sci*, **74**, 675-696.
- [20] Elenkov, I.J.; Wilder, R.L.; Chrousos, G.P. and Vizi, E.S. (2000) The sympathetic nerve--an integrative interface between two supersystems: the brain and the immune system. *Pharmacol. Rev.*, **52**, 595-638.
- [21] Tian, J.; Lu, Y.; Zhang, H.; Chau, C.H.; Dang, H.N. and Kaufman, D.L. (2004) Gamma-aminobutyric acid inhibits T cell autoimmunity and the development of inflammatory responses in a mouse type 1 diabetes model. *J. Immunol.*, **173**, 5298-5304.
- [22] Nakano, K.; Higashi, T.; Hashimoto, K.; Takagi, R.; Tanaka, Y. and Matsushita, S. (2008) Antagonizing dopamine D1-like receptor inhibits Th17 cell differentiation: preventive and therapeutic effects on experimental autoimmune encephalomyelitis. *Biochem. Biophys. Res. Commun.*, **373**, 286-291.
- [23] Prado, C.; Contreras, F.; Gonzalez, H.; Diaz, P.; Elgueta, D.; Barrientos, M.; Herrada, A.A.; Lladser, A.; Bernal, S. and Pacheco, R. (2012) Stimulation of dopamine receptor d5 expressed on dendritic cells potentiates th17-mediated immunity. *J. Immunol.*, **188**, 3062-3070.
- [24] Katoh, N.; Soga, F.; Nara, T.; Tamagawa-Mineoka, R.; Nin, M.; Kotani, H.; Masuda, K. and Kishimoto, S. (2006) Effect of

- serotonin on the differentiation of human monocytes into dendritic cells. *Clin. Exp. Immunol.*, **146**, 354-361.
- [25] Kawashima, K.; Yoshikawa, K.; Fujii, Y.X.; Moriwaki, Y. and Misawa, H. (2007) Expression and function of genes encoding cholinergic components in murine immune cells. *Life Sci.*, **80**, 2314-2319.
- [26] Maestroni, G.J. and Mazzola, P. (2003) Langerhans cells beta 2-adrenoceptors: role in migration, cytokine production, Th priming and contact hypersensitivity. *J. Neuroimmunol.*, **144**, 91-99.
- [27] Pacheco, R.; Oliva, H.; Martinez-Navio, J.M.; Climent, N.; Ciruela, F.; Gatell, J.M.; Gallart, T.; Mallol, J.; Lluis, C. and Franco, R. (2006) Glutamate released by dendritic cells as a novel modulator of T cell activation. *J. Immunol.*, **177**, 6695-6704.
- [28] Khan, N.A. and Hichami, A. (1999) Ionotropic 5-hydroxytryptamine type 3 receptor activates the protein kinase C-dependent phospholipase D pathway in human T-cells. *Biochem. J.*, **344**, 199-204.
- [29] Khan, N.A. and Poisson, J.P. (1999) 5-HT₃ receptor-channels coupled with Na⁺ influx in human T cells: role in T cell activation. *J. Neuroimmunol.*, **99**, 53-60.
- [30] Yin, J.; Albert, R.H.; Tretiakova, A.P. and Jameson, B.A. (2006) 5-HT_{1B} receptors play a prominent role in the proliferation of T-lymphocytes. *J. Neuroimmunol.*, **181**, 68-81.
- [31] Rosas-Ballina, M.; Olofsson, P.S.; Ochani, M.; Valdes-Ferrer, S.I.; Levine, Y.A.; Reardon, C.; Tusche, M.W.; Pavlov, V.A.; Andersson, U.; Chavan, S.; Mak, T.W. and Tracey, K.J. (2011) Acetylcholine-synthesizing T cells relay neural signals in a vagus nerve circuit. *Science*, **334**, 98-101.
- [32] Kawashima, K.; Fujii, T.; Watanabe, Y. and Misawa, H. (1998) Acetylcholine synthesis and muscarinic receptor subtype mRNA expression in T-lymphocytes. *Life Sci.*, **62**, 1701-1705.
- [33] Rinner, I. and Schauenstein, K. (1993) Detection of choline-acetyltransferase activity in lymphocytes. *J. Neurosci. Res.*, **35**, 188-191.
- [34] Fujii, T. and Kawashima, K. (2000) Calcium signaling and c-Fos gene expression via M3 muscarinic acetylcholine receptors in human T- and B-cells. *Jpn J. Pharmacol.*, **84**, 124-132.
- [35] Fujino, H.; Kitamura, Y.; Yada, T.; Uehara, T. and Nomura, Y. (1997) Stimulatory roles of muscarinic acetylcholine receptors on T cell antigen receptor/CD3 complex-mediated interleukin-2 production in human peripheral blood lymphocytes. *Mol. Pharmacol.*, **51**, 1007-1014.
- [36] Hallquist, N.; Hakki, A.; Wecker, L.; Friedman, H. and Pross, S. (2000) Differential effects of nicotine and aging on splenocyte proliferation and the production of Th1- versus Th2-type cytokines. *Proc. Soc. Exp. Biol. Med.*, **224**, 141-146.
- [37] LeBlanc, J. and Ducharme, M.B. (2007) Plasma dopamine and noradrenaline variations in response to stress. *Physiol. Behav.*, **91**, 208-211.
- [38] Kavtaradze, S. and Mosidze, T. (2007) Neuro-endocrinal regulation and disorders of intracranial hemocirculation in rheumatic diseases in children. *Georgian. Med. News*, 32-35.
- [39] Saha, B.; Mondal, A.C.; Basu, S. and Dasgupta, P.S. (2001) Circulating dopamine level, in lung carcinoma patients, inhibits proliferation and cytotoxicity of CD4⁺ and CD8⁺ T cells by D1 dopamine receptors: an *in vitro* analysis. *Int. Immunopharmacol.*, **1**, 1363-1374.
- [40] Lechin, F.; van der Dijs, B.; Vitelli-Florez, G.; Lechin-Baez, S.; Azocar, J.; Cabrera, A.; Lechin, A.; Jara, H.; Lechin, M.; Gomez, F. and *et al.* (1990) Psychoneuroendocrinological and immunological parameters in cancer patients: involvement of stress and depression. *Psychoneuroendocrinol.*, **15**, 435-451.
- [41] Barbanti, P.; Fabbri, G.; Ricci, A.; Bruno, G.; Cerbo, R.; Bronzetti, E.; Amenta, F. and Luigi Lenzi, G. (2000) Reduced density of dopamine D2-like receptors on peripheral blood lymphocytes in Alzheimer's disease. *Mech. Ageing Dev.*, **120**, 65-75.
- [42] Nagai, Y.; Ueno, S.; Saeki, Y.; Soga, F.; Hirano, M. and Yanagihara, T. (1996) Decrease of the D3 dopamine receptor mRNA expression in lymphocytes from patients with Parkinson's disease. *Neurology*, **46**, 791-795.
- [43] Boneberg, E.M.; von Seydlitz, E.; Propster, K.; Watzl, H.; Rockstroh, B. and Illges, H. (2006) D3 dopamine receptor mRNA is elevated in T cells of schizophrenic patients whereas D4 dopamine receptor mRNA is reduced in CD4⁺ T cells. *J. Neuroimmunol.*, **173**, 180-187.
- [44] Ilani, T.; Ben-Shachar, D.; Strous, R.D.; Mazor, M.; Sheinkman, A.; Kotler, M. and Fuchs, S. (2001) A peripheral marker for schizophrenia: Increased levels of D3 dopamine receptor mRNA in blood lymphocytes. *Proc. Natl. Acad. Sci. USA*, **98**, 625-628.
- [45] Kwak, Y.T.; Koo, M.S.; Choi, C.H. and Sunwoo, I. (2001) Change of dopamine receptor mRNA expression in lymphocyte of schizophrenic patients. *BMC Med. Genet.*, **2**, 3.
- [46] Giorelli, M.; Livrea, P. and Trojano, M. (2005) Dopamine fails to regulate activation of peripheral blood lymphocytes from multiple sclerosis patients: effects of IFN-beta. *J. Interferon. Cytokine Res.*, **25**, 395-406.
- [47] Cosentino, M.; Zaffaroni, M.; Trojano, M.; Giorelli, M.; Pica, C.; Rasini, E.; Bombelli, R.; Ferrari, M.; Ghezzi, A.; Comi, G.; Livrea, P.; Lecchini, S. and Marino, F. (2012) Dopaminergic Modulation of CD4⁺CD25⁺ Regulatory T Lymphocytes in Multiple Sclerosis Patients during Interferon-beta Therapy. *Neuroimmunomodulation*, **19**, 283-292.
- [48] Mignini, F.; Streccioni, V. and Amenta, F. (2003) Autonomic innervation of immune organs and neuroimmune modulation. *Auton. Autacoid. Pharmacol.*, **23**, 1-25.
- [49] Nakano, K.; Higashi, T.; Takagi, R.; Hashimoto, K.; Tanaka, Y. and Matsushita, S. (2009) Dopamine released by dendritic cells polarizes Th2 differentiation. *Int. Immunol.*, **21**, 645-654.
- [50] Engelhardt, B. (2006) Molecular mechanisms involved in T cell migration across the blood-brain barrier. *J. Neural. Transm.*, **113**, 477-485.
- [51] Brochard, V.; Combadiere, B.; Prigent, A.; Laouar, Y.; Perrin, A.; Beray-Berthet, V.; Bonduelle, O.; Alvarez-Fischer, D.; Callebert, J.; Launay, J.M.; Duyckaerts, C.; Flavell, R.A.; Hirsch, E.C. and Hunot, S. (2009) Infiltration of CD4⁺ lymphocytes into the brain contributes to neurodegeneration in a mouse model of Parkinson disease. *J. Clin. Invest.*, **119**, 182-192.
- [52] Reynolds, A.D.; Stone, D.K.; Hutter, J.A.; Benner, E.J.; Mosley, R.L. and Gendelman, H.E. (2010) Regulatory T cells attenuate th17 cell-mediated nigrostriatal dopaminergic neurodegeneration in a model of Parkinson's disease. *J. Immunol.*, **184**, 2261-2271.
- [53] Codarri, L.; Gyulveszi, G.; Tosevski, V.; Hesse, L.; Fontana, A.; Magrenat, L.; Suter, T. and Becher, B. (2011) RORgamma⁺ drives production of the cytokine GM-CSF in helper T cells, which is essential for the effector phase of autoimmune neuroinflammation. *Nat. Immunol.*, **12**, 560-567.
- [54] Petermann, F.; Rothhammer, V.; Claussen, M.C.; Haas, J.D.; Blanco, L.R.; Heink, S.; Prinz, I.; Hemmer, B.; Kuchroo, V.K.; Oukka, M. and Korn, T. (2010) gammadelta T cells enhance autoimmunity by restraining regulatory T cell responses via an interleukin-23-dependent mechanism. *Immunity*, **33**, 351-363.
- [55] Goverman, J. (2009) Autoimmune T cell responses in the central nervous system. *Nat. Rev. Immunol.*, **9**, 393-407.
- [56] Anandasabapathy, N.; Vitorica, G.D.; Meredith, M.; Feder, R.; Dong, B.; Kluger, C.; Yao, K.; Dustin, M.L.; Nussenzweig, M.C.; Steinman, R.M. and Liu, K. (2011) FcγR3 controls the development of radiosensitive dendritic cells in the meninges and choroid plexus of the steady-state mouse brain. *J. Exp. Med.*, **208**, 1695-1705.
- [57] Lucin, K.M. and Wyss-Coray, T. (2009) Immune activation in brain aging and neurodegeneration: too much or too little? *Neuron*, **64**, 110-122.
- [58] Reboldi, A.; Coisne, C.; Baumjohann, D.; Benvenuto, F.; Bottinelli, D.; Lira, S.; Uccelli, A.; Lanzavecchia, A.; Engelhardt, B. and Sallusto, F. (2009) C-C chemokine receptor 6-regulated entry of TH-17 cells into the CNS through the choroid plexus is required for the initiation of EAE. *Nat. Immunol.*, **10**, 514-523.
- [59] Carson, M.J.; Doose, J.M.; Melchior, B.; Schmid, C.D. and Ploix, C.C. (2006) CNS immune privilege: hiding in plain sight. *Immunol. Rev.*, **213**, 48-65.

- [60] Kebir, H.; Kreymborg, K.; Ifergan, I.; Dodelet-Devillers, A.; Cayrol, R.; Bernard, M.; Giuliani, F.; Arbour, N.; Becher, B. and Prat, A. (2007) Human TH17 lymphocytes promote blood-brain barrier disruption and central nervous system inflammation. *Nat. Med.*, **13**, 1173-1175.
- [61] Ransohoff, R.M. and Engelhardt, B. (2012) The anatomical and cellular basis of immune surveillance in the central nervous system. *Nat. Rev. Immunol.*, **12**, 623-635.
- [62] Strange, P.G. (1993) New insights into dopamine receptors in the central nervous system. *Neurochem. Int.*, **22**, 223-236.
- [63] Sibley, D.R.; Monsma, F.J. Jr. and Shen, Y. (1993) Molecular neurobiology of dopaminergic receptors. *Int. Rev. Neurobiol.*, **35**, 391-415.
- [64] Undie, A.S.; Weinstock, J.; Sarau, H.M. and Friedman, E. (1994) Evidence for a distinct D1-like dopamine receptor that couples to activation of phosphoinositide metabolism in brain. *J. Neurochem.*, **62**, 2045-2048.
- [65] Salter, M.W. (2003) D1 and NMDA receptors hook up: expanding on an emerging theme. *Trends Neurosci.*, **26**, 235-237.
- [66] Beaulieu, J.M.; Gainetdinov, R.R. and Caron, M.G. (2007) The Akt-GSK-3 signaling cascade in the actions of dopamine. *Trends Pharmacol. Sci.*, **28**, 166-172.
- [67] Wu, W.L.; Burnett, D.A.; Spring, R.; Greenlee, W.J.; Smith, M.; Favreau, L.; Fawzi, A.; Zhang, H. and Lachowicz, J.E. (2005) Dopamine D1/D5 receptor antagonists with improved pharmacokinetics: design, synthesis, and biological evaluation of phenol bioisosteric analogues of benzazepine D1/D5 antagonists. *J. Med. Chem.*, **48**, 680-693.
- [68] Strange, P.G. (2001) Antipsychotic drugs: importance of dopamine receptors for mechanisms of therapeutic actions and side effects. *Pharmacol. Rev.*, **53**, 119-133.
- [69] Sunahara, R.K.; Guan, H.C.; O'Dowd, B.F.; Seeman, P.; Laurier, L.G.; Ng, G.; George, S.R.; Torchia, J.; Van Tol, H.H. and Niznik, H.B. (1991) Cloning of the gene for a human dopamine D5 receptor with higher affinity for dopamine than D1. *Nature*, **350**, 614-619.
- [70] Malmberg, A.; Jackson, D.M.; Eriksson, A. and Mohell, N. (1993) Unique binding characteristics of antipsychotic agents interacting with human dopamine D2A, D2B, and D3 receptors. *Mol. Pharmacol.*, **43**, 749-754.
- [71] Pacheco, R.; Prado, C.E.; Barrientos, M.J. and Bernal, S. (2009) Role of dopamine in the physiology of T-cells and dendritic cells. *J. Neuroimmunol.*, **216**, 8-19.
- [72] Sarkar, C.; Das, S.; Chakraborty, D.; Chowdhury, U.R.; Basu, B.; Dasgupta, P.S. and Basu, S. (2006) Cutting Edge: Stimulation of dopamine D4 receptors induce T cell quiescence by up-regulating Kruppel-like factor-2 expression through inhibition of ERK1/ERK2 phosphorylation. *J. Immunol.*, **177**, 7525-7529.
- [73] Ilani, T.; Strous, R.D. and Fuchs, S. (2004) Dopaminergic regulation of immune cells via D3 dopamine receptor: a pathway mediated by activated T cells. *FASEB J.*, **18**, 1600-1602.
- [74] Kivisakk, P.; Trebst, C.; Liu, Z.; Tucky, B.H.; Sorensen, T.L.; Rudick, R.A.; Mack, M. and Ransohoff, R.M. (2002) T-cells in the cerebrospinal fluid express a similar repertoire of inflammatory chemokine receptors in the absence or presence of CNS inflammation: implications for CNS trafficking. *Clin. Exp. Immunol.*, **129**, 510-518.
- [75] Kipnis, J.; Cardon, M.; Avidan, H.; Lewitus, G.M.; Mordechai, S.; Rolls, A.; Shani, Y. and Schwartz, M. (2004) Dopamine, through the extracellular signal-regulated kinase pathway, downregulates CD4+CD25+ regulatory T-cell activity: implications for neurodegeneration. *J. Neurosci.*, **24**, 6133-6143.
- [76] Rudick, R.A. and Goelz, S.E. (2011) Beta-interferon for multiple sclerosis. *Exp. Cell Res.*, **317**, 1301-1311.
- [77] Hashimoto, K.; Inoue, T.; Higashi, T.; Takei, S.; Awata, T.; Katayama, S.; Takagi, R.; Okada, H. and Matsushita, S. (2009) Dopamine D1-like receptor antagonist, SCH23390, exhibits a preventive effect on diabetes mellitus that occurs naturally in NOD mice. *Biochem. Biophys. Res. Commun.*, **383**, 460-463.
- [78] Okada, H.; Inoue, T.; Hashimoto, K.; Suzuki, H. and Matsushita, S. (2009) D1-like receptor antagonist inhibits IL-17 expression and attenuates crescent formation in nephrotoxic serum nephritis. *Am. J. Nephrol.*, **30**, 274-279.
- [79] Nakano, K.; Yamaoka, K.; Hanami, K.; Saito, K.; Sasaguri, Y.; Yanagihara, N.; Tanaka, S.; Katsuki, I.; Matsushita, S. and Tanaka, Y. (2011) Dopamine induces IL-6-dependent IL-17 production via D1-like receptor on CD4 naive T cells and D1-like receptor antagonist SCH-23390 inhibits cartilage destruction in a human rheumatoid arthritis/SCID mouse chimera model. *J. Immunol.*, **186**, 3745-3752.
- [80] Bourne, J.A. (2001) SCH 23390: the first selective dopamine D1-like receptor antagonist. *CNS Drug Rev.*, **7**, 399-414.
- [81] Briggs, C.A.; Pollock, N.J.; Frail, D.E.; Paxson, C.L.; Rakowski, R.F.; Kang, C.H. and Keabian, J.W. (1991) Activation of the 5-HT1C receptor expressed in *Xenopus* oocytes by the benzazepines SCH 23390 and SKF 38393. *Br. J. Pharmacol.*, **104**, 1038-1044.
- [82] Millan, M.J.; Newman-Tancredi, A.; Quentric, Y. and Cussac, D. (2001) The "selective" dopamine D1 receptor antagonist, SCH23390, is a potent and high efficacy agonist at cloned human serotonin2C receptors. *Psychopharmacol. (Berl)*, **156**, 58-62.
- [83] Murphy, A.C.; Lalor, S.J.; Lynch, M.A. and Mills, K.H. (2010) Infiltration of Th1 and Th17 cells and activation of microglia in the CNS during the course of experimental autoimmune encephalomyelitis. *Brain Behav. Immun.*, **24**, 641-651.
- [84] Matsushita, T.; Yanaba, K.; Bouaziz, J.D.; Fujimoto, M. and Tedder, T.F. (2008) Regulatory B cells inhibit EAE initiation in mice while other B cells promote disease progression. *J. Clin. Invest.*, **118**, 3420-3430.
- [85] Gregersen, J.W. and Jayne, D.R. (2012) B-cell depletion in the treatment of lupus nephritis. *Nat. Rev. Nephrol.*, **8**, 505-514.
- [86] Konigsberger, S.; Prodohl, J.; Stegner, D.; Weis, V.; Andreas, M.; Stehling, M.; Schumacher, T.; Bohmer, R.; Thielmann, I.; van Eeuwijk, J.M.; Nieswandt, B. and Kiefer, F. (2012) Altered BCR signalling quality predisposes to autoimmune disease and a pre-diabetic state. *Embo J.*, **31**, 3363-3374.
- [87] Liubchenko, G.A.; Appleberry, H.C.; Striebich, C.C.; Franklin, K.E.; Derber, L.A.; Holers, V.M. and Lyubchenko, T. (2012) Rheumatoid arthritis is associated with signaling alterations in naturally occurring autoreactive B-lymphocytes. *J. Autoimmun.*, **40**, 111-121.
- [88] Pacheco, R.; Riquelme, E. and Kalergis, A.M. (2010) Emerging evidence for the role of neurotransmitters in the modulation of T cell responses to cognate ligands. *Cent. Nerv. Syst. Agents Med. Chem.*, **10**, 65-83.
- [89] McKenna, F.; McLaughlin, P.J.; Lewis, B.J.; Sibbring, G.C.; Cummmerson, J.A.; Bowen-Jones, D. and Moots, R.J. (2002) Dopamine receptor expression on human T- and B-lymphocytes, monocytes, neutrophils, eosinophils and NK cells: a flow cytometric study. *J. Neuroimmunol.*, **132**, 34-40.
- [90] Meredith, E.J.; Holder, M.J.; Rosen, A.; Lee, A.D.; Dyer, M.J.; Barnes, N.M. and Gordon, J. (2006) Dopamine targets cycling B cells independent of receptors/transporter for oxidative attack: Implications for non-Hodgkin's lymphoma. *Proc. Natl. Acad. Sci. USA*, **103**, 13485-13490.
- [91] Hoentjen, F.; Sartor, R.B.; Ozaki, M. and Jobin, C. (2005) STAT3 regulates NF-kappaB recruitment to the IL-12p40 promoter in dendritic cells. *Blood*, **105**, 689-696.
- [92] Liang, S.C.; Tan, X.Y.; Luxenberg, D.P.; Karim, R.; Dunussi-Joannopoulos, K.; Collins, M. and Fouser, L.A. (2006) Interleukin (IL)-22 and IL-17 are coexpressed by Th17 cells and cooperatively enhance expression of antimicrobial peptides. *J. Exp. Med.*, **203**, 2271-2279.
- [93] Zheng, Y.; Danilenko, D.M.; Valdez, P.; Kasman, I.; Eastham-Anderson, J.; Wu, J. and Ouyang, W. (2007) Interleukin-22, a T(H)17 cytokine, mediates IL-23-induced dermal inflammation and acanthosis. *Nature*, **445**, 648-651.
- [94] El-Behi, M.; Ciric, B.; Dai, H.; Yan, Y.; Cullimore, M.; Safavi, F.; Zhang, G.X.; Dittel, B.N. and Rostami, A. (2011) The encephalitogenicity of T(H)17 cells is dependent on IL-1- and IL-23-induced production of the cytokine GM-CSF. *Nat. Immunol.*, **12**, 568-575.
- [95] Langrish, C.L.; Chen, Y.; Blumenschein, W.M.; Mattson, J.; Basham, B.; Sedgwick, J.D.; McClanahan, T.; Kastelein, R.A. and

- Cua, D.J. (2005) IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. *J. Exp. Med.*, **201**, 233-240.
 - [96] Ghosh, M.C.; Mondal, A.C.; Basu, S.; Banerjee, S.; Majumder, J.; Bhattacharya, D. and Dasgupta, P.S. (2003) Dopamine inhibits cytokine release and expression of tyrosine kinases, Lck and Fyn in activated T cells. *Int. Immunopharmacol.*, **3**, 1019-1026.
 - [97] Iagmurov, O.D. and Ogurtsov, R.P. (1996) [Functional activity of spleen and peripheral blood lymphocytes during stress-induced immunodepression]. *Biull Eksp. Biol. Med.*, **122**, 64-68.
 - [98] La Via, M.F.; Munno, I.; Lydiard, R.B.; Workman, E.W.; Hubbard, J.R.; Michel, Y. and Paulling, E. (1996) The influence of stress intrusion on immunodepression in generalized anxiety disorder patients and controls. *Psychosom. Med.*, **58**, 138-142.
 - [99] Zaffaroni, M.; Marino, F.; Bombelli, R.; Rasini, E.; Monti, M.; Ferrari, M.; Ghezzi, A.; Comi, G.; Lecchini, S. and Cosentino, M. (2008) Therapy with interferon-beta modulates endogenous catecholamines in lymphocytes of patients with multiple sclerosis. *Exp. Neurol.*, **214**, 315-321.
-