Role of dopamine in the physiology of T-cells and dendritic cells

Rodrigo Pachecoa,b,c,⁎, Carolina E. Pradoa,b, Magaly J. Barrientos a,b, Sebastián Bernales a,b,c

a Fundación Ciencia para la Vida, Santiago, Chile
b Instituto Milenio de Biología Fundamental y Aplicada, Avenida Zañartu #1482, Santiago, Chile
c Universidad San Sebastián, Chile

Abstract

Dendritic cells (DCs) are responsible for priming T-cells and for promoting their differentiation from naïve T-cells into appropriate effector cells. Because of their fundamental roles in controlling immunity, DCs and T-cells require tight regulatory mechanisms. Several studies have shown that dopamine, not only mediate interactions into the nervous system, but can also contribute to the modulation of immunity. Here, we review the emerging role of this neurotransmitter as a regulator of DC and T-cell physiology and, in turn, immune response. Moreover, we discuss how alterations in the dopamine-mediated immune regulatory mechanisms could contribute to the onset of immune-related disorders.

© 2009 Elsevier B.V. All rights reserved.

1. Introduction

1.1. The key role of T-cells and dendritic cells in the adaptive immune response

Adaptive immune responses against foreign antigens (Ags) are orchestrated by Ag-specific T-cells. Two main T-cell populations have been described, phenotypically differentiated by expression of CD4 or CD8 on the cell surface. These molecules function as co-receptors for
the MHC molecule. CD8+ and CD4+ T-cells recognize Ags as peptides bound to class I and class II MHC molecules, respectively (Nouri-Shirazi et al., 2000). Effector CD8+ T-cells may directly recognize tumor cells or infected cells expressing foreign Ags as surface peptide–MHC complexes (pMHCs). After recognition they mediate killing of those cells by secreting cytotoxic granules (Schoenborn and Wilson, 2007). In addition, by secreting IFN-γ, cytotoxic CD8+ T-cells may also potentiate function of other immune cells, including macrophages and NK cells (Schoenborn and Wilson, 2007). Thus CD8+ T-cells are key players during adaptive immune response against intracellular pathogens and tumors (Nouri-Shirazi et al., 2000).

Effector CD4+ T-cells not only contribute to efficient activation of CD8+ T-cells (Bennett et al., 1998; Ridge et al., 1998; Schoenberger et al., 1998) and B-cells (Smith et al., 2000), but they also regulate function of several cells of the innate arm of immune system, orchestrating the immune response. Depending on the signals that dendritic cells (DCs) provide to T-cells in addition to antigenic pMHC, they can promote the differentiation of CD4+ T-cell toward distinct subsets, including T helper (Th) 1 (Th1), Th2 and Th17 (Banchereau and Steinman, 1998; Lanzavecchia and Sallustio, 2001; McGeachy and Cua, 2008). The Th1 phenotype is favoured by the secretion of IL-12 by DCs. In contrast, Th2 phenotype is induced in the absence of IL-12 secretion during Ag recognition (Watford et al., 2003). Th1 cells predominantly secrete IFN-γ which promotes cellular immunity against intracellular pathogens and tumor cells. In contrast, Th2 cells primarily secrete IL-4 which facilitates responses that can be efficient at eliminating extracellular pathogens, such as helminths and extracellular bacteria (Del Prete, 1998). The Th17 phenotype, which primarily secrete IL-17, has been more recently described. Differentiation into Th17 is promoted by TGF-β and IL-6, which can be secreted by DCs during Ag-presentation. It is thought that Th17 cells protect against extracellular bacteria, particularly in the gut (Schoenborn and Wilson, 2007), however they have also been extensively associated with autoimmune diseases (McGeachy and Cua, 2008). Another type of CD4+ T-cells consists on T regulatory cells (Tregs), a phenotype favoured by the presence of TGF-β (DiPaolo et al., 2007) which mainly secrete IL-10 and TGF-β and suppress several functions of effector Ag-specific T-cells (Cosentino et al., 2007; Nouri-Shirazi et al., 2000). 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 functional phenotype of CD4+ T-cells could results in cancer or the onset of autoimmunity.

DCs are the most potent Ag-presenting cells (APCs) specialized in initiation of adaptive immune responses by directing the activation and differentiation of naïve T lymphocytes (Banchereau and Steinman, 1998; Lanzavecchia and Sallustio, 2001). These cells can capture both self and foreign Ags in diverse tissues and migrate to secondary lymphoid organs to present captured and processed Ags on MHC molecules to T-cells (Nouri-Shirazi et al., 2000). T-cell–DC synapses can comprise a diverse range of contact modes and distinct molecular arrangements that ultimately determine the nature of the T-cell response (Davis and Dustin, 2004; Friedl et al., 2005). Thus, T-cell–DC interaction can control and regulate T-cell activation, effector function and induction of tolerance (Friedl et al., 2005). Important functional components of the immunological synapse are activating/inhibitory receptor pairs expressed either on the DC or the T-cell surface (Herrada et al., 2007; Iruaratgoyena et al., 2006; Pacheco et al., 2005). In addition, the synapse recruits several modulating receptors that, upon engagement, contribute to polarization of effector T-cell responses. The main interactions in the T-cell–DC interface are TCR/pMHC, costimulatory interactions mediated by CD80/CD86–CD28 and cytokines released from DCs toward T-cells. All of these signals are integrated together to modulate the differentiation of naïve T-cell toward the appropriate effector/regulator phenotype necessary to eliminate the invading pathogen or tumor.

1.2. Emerging role of neurotransmitters on the regulation of T-cells and DCs physiology

Traditionally, it has been thought that the functions of immune cells such as T-cells and DCs are regulated by soluble protein mediators known as cytokines. However, a number of studies have more recently shown that immune system cells can be also regulated by neurotransmitters (Franco et al., 2007). Accordingly, it has been described that several receptors for neurotransmitters classically expressed in the nervous system, such as glutamate (Glu)-receptors (Glurs), acetylcholine (ACh)-receptors (AChRs), serotonin (5-HT)-receptors (5-HTRs) and DA-receptors (DARs) are also expressed on the surface of immune system cells. For instance, T-cells express Glurs (Pacheco et al., 2004, 2007), DARs (Besser et al., 2005; Saha et al., 2001b; Sarkar et al., 2006; Watanabe et al., 2006), 5-HTRs (Leon-Ponte et al., 2007), AChRs (Kawashima and Fujii, 2003), noradrenaline/adrenaline (NA/A) receptors (Elvenk et al., 2000) and γ-aminobutric acid receptors (Tian et al., 2004). On the other hand, DCs express Glurs (Pacheco et al., 2007), DARs (Nakano et al., 2008), 5-HTRs (Katoh et al., 2006), AChRs (Kawashima et al., 2007), and NA/A receptors (Maestroni and Mazzola, 2003). The identification of these receptors on these and other immune system cells suggests that neurotransmitters play a physiological role in the regulation of the immune response and that deregulation of the activation of these receptors could contribute to the development of autoimmunity or malignancies. Furthermore, this evidence implies that different physiological or pathophysiological states of the nervous system could be involved in the regulation of immunity.

Importantly, a number of recent studies have revealed that some cells involved in adaptive and innate immune responses such as DCs, T-cells and others, are capable of synthesizing and/or capturing classical neurotransmitters. Under specific conditions, these cells may release neurotransmitters from intracellular storages thus involving autocrine, paracrine, yuxtacrine and eventually endocrine communications between different leukocytes (Franco et al., 2007). For instance, T-cells may release 5-HT (O’Connell et al., 2006), NA/A (Nishibori et al., 2003), DA (Beck et al., 2004; Cosentino et al., 2007) and ACh (Kawashima and Fujii, 2003) while DCs may release Glu (Pacheco et al., 2006), DA (Nakano et al., 2009a) and 5-HT (O’Connell et al., 2006).

Important studies in this area have shown relevant regulatory function of some neurotransmitters in the function and differentiation of T-cells and DCs. Regarding the role of Glu, it has been demonstrated that during Ag-presentation, DCs release Glu, which subsequently act over T-cells (Pacheco et al., 2007, 2006). This mediator initially stimulates metabotropic-GluR5 (mGluR5), which by coupling to adenylyl cyclase, triggers inhibitory signals to impair T-cell activation. However, when the interaction TCR-pMHC is productive, T-cell activation overcomes the inhibitory mGlu5R-induced effect and they begin to express mGluR1. Further stimulation of mGlu1R by DCs-derived Glu potentiates T-cell activation and induces increased secretion of Th1- and pro-inflammatory cytokines, thus contributing to the polarization of the functional phenotype of T-cells (Pacheco et al., 2004, 2007, 2006). Addressing the role of ACh in the regulation of adaptive immune response, interesting studies have revealed the presence of the cholinergic system in T-cells. These studies have shown that T-cells not only express nicotinic and muscarinic AChRs (Kawashima and Fujii, 2003), but they also express ACh-transporter and they are able to store ACh in intracellular vesicles (Kawashima et al., 1998; Rinner and Schauenstein, 1993). Upon TCR-stimulation, T-cells release ACh, which in an autocrine way stimulates AChRs, potentiating T-cell activation and favouring differentiation toward Th1 phenotype (Fujii and Kawashima, 2000; Fujino et al., 1997; Hallquist et al., 2000). Another example is the serotoninergic system that regulates the T-cell response. 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 on T-cells, thus stimulating 5-HTRs expressed on these cells (O’Connell et al., 2006). Actually, it has been demonstrated that, indeed, 5-HT is required for proper T-cell activation early by stimulating 5-HTR1 (Leon-Ponte et al., 2007) and 5-HTR2 (Khan and Hichami, 1999; Khan and Poisson, 1999) expressed on naive T-cells, and later by stimulating 5-HTR1B and 5-HTR2C expressed on activating T-cells (Yin et al., 2006).

These and other examples not only suggest that neurotransmitters could mediate communication between immune cells, but also that these molecules may be involved in bidirectional cross-talk between immune and nervous systems. Importantly, DA not only has a highly relevant role in the nervous system, but it has also been shown to be involved in immune-related diseases (Giorelli et al., 2005; Kavtaradze and Mosidze, 2007; Lechin et al., 1990; Saha et al., 2001a,b; Zaffaroni et al., 2008). Due to the pivotal roles of T-cells and DCs during adaptive immune responses, we have analysed and discussed recent studies relative to the contribution of DA as mediator in the function of these cells.

1.3. The dopaminergic system

DA plays an essential role as neurotransmitter and neuromodulator in the nervous system. Neurotransmission associated to DA in the brain has been related with diverse functions including movement (Cenci, 2007), drug addiction (Dayan, 2009), pain perception (Potvin et al., 2009), hormone secretion (Ben-Jonathan and Hnasko, 2001), motivation and pleasure (Wise, 2008). DA synthesis and storage involves a number of cofactors and proteins, including enzymes and transporters. For instance, the rate-limiting enzyme in DA synthesis is tyrosine hydroxylase (TH), which converts tyrosine to L-DOPA. This product subsequently is metabolized by aromatic amino acid decarboxylase (AADC) to produce DA (Weihle et al., 2006). In addition to TH, DA-transporters are also relevant markers for dopaminergic systems. In the nervous system, the plasma membrane transporter for DA (DAT) removes this neurotransmitter from the extracellular space thereby controlling half-life of DA action (Mignini et al., 2009). On the other hand, vesicular monoamine transporters (VMAT) type-1 and type-2, mediate mobilization of cytosolic DA (de novo synthesised or captured) toward vesicular storages (Mignini et al., 2006).

DA exerts its effects in susceptible cells by stimulating DARS expressed on the cell surface. So far, five DARs have been described (D1–D5), which are hepta-spanning membrane receptors and belong to the superfamily of G protein-coupled receptors (Strange, 1993). Whereas type I DARs (D1 and D5) are generally coupled to Goα and subsequent stimulation of cAMP production, type II DARs (D2, D3 and D4) are often coupled to Goi promoting inhibition of cAMP synthesis (Sibley et al., 1993). However, it has been shown that type I and type II DARs also can be coupled to other Go proteins, thus triggering signal pathways different to the increase or decrease of cAMP production respectively (Neve et al., 2004; Sidhu, 1998). This differential coupling of DARs allows that DA might promote distinct cellular effects in two different kinds of cells expressing the same DAR. Furthermore, differential expression of DARs on different cells also contributes to DA exerts distinct effects in those cells. According to this idea and to the fact that there is differential expression and differential coupling of DARs in distinct neurons, DA may play very different roles on the distinct zones of the nervous system (Sidhu, 1998). Due to the extensive and important role that DA plays in the nervous system, the imbalance on the capture/release of DA or DARs expression have been related with a number of neurological or psychiatric disorders such as Parkinson’s disease, Huntington’s disease and schizophrenia (Hoenicka et al., 2007; Strange, 1993).

Dopaminergic components have not only described in the nervous system, but they have also been found in other organs and tissues including various vascular beds, the heart, the gastrointestinal tract, and the kidney. Therefore DA may exert regulation in the functioning of those tissues and organs. For instance, whereas DARs expressed in kidney contribute to the control of renal electrolyte balance (Gildea, 2009), there is evidence involving DA as an endogenous gastroprotective element (Glavin, 1992). Importantly, during the last decade, numerous evidences showing dopaminergic components in the immune system and involving to DA as a key modulator of the immune response, have emerged. Due to the central role that DCs and T-cells play in the development of adaptive immune responses, in this review we are focused to integrate and discuss the role that DA plays in the regulation of the functioning of these cells.

2. Modulation on the function of T-cells, DCs and other immune cells by dopamine

2.1. Sources of dopamine for immune cells

By stimulating different DARs expressed on T-cells or DCs, DA from diverse sources may strongly regulate the initiation and development of immune responses. The primary source of DA for patrolling resting naïve or memory T-cells and peripheral migrating effector T-cells into the blood vessels is plasma DA, which in normal individuals reaches levels of 10 pg/ml (Saha et al., 2001a,b). Furthermore, dopaminergic innervation of primary and secondary lymphoid organs through sympathetic nerves has been described (Mignini et al., 2003), which suggests the existence of direct DA-mediated regulation by nervous system on activating T-cells, resting T-cells and DCs in lymphoid organs (Fig. 1A). Accordingly, it has recently been described that early sympathetic innervation of animals ameliorates the development of autoimmunity by a mechanism involving stimulation of Tregs (Harle et al., 2008). This data therefore demonstrates a pro-inflammatory role of sympathetic-derived catecholamines on the early onset of autoimmunity.

Another source of DA for activating T-cells could be autocrine and paracrine secretion of DA by immune cells in secondary lymphoid organs. Accordingly, it has recently been described that human Tregs constitutively express TH and contain substantial amounts of DA and other catecholamines, while effector T-cells only contain trace amounts (Cosentino et al., 2000; Cosentino et al., 2007). Moreover, Tregs as well as effector T-cells express VMAT-1 and -2 which allow to these cells to accumulate catecholamines into specific vesicular storages (Cosentino et al., 2007). However, only Tregs store and release physiologically relevant amounts of DA from vesicular storages (Cosentino et al., 2007) (Fig. 1A). Interestingly, an autocrine/paracrine release of this neurotransmitter by DCs during Ag-presentation to naive CD4+ T-cells has been recently described (Nakano et al., 2009a,b) (Fig. 1A). Production and storage of DA in DCs is discussed in Section 2.3.

Depending on the context of the immune response and on the location of inflammation, another important source of DA could be the central nervous system (CNS). Due to the presence of the blood–brain-barrier (BBB) and the lack of lymphatic vessels, the entrance of immune cells into the CNS is normally avoided. However, during inflammatory processes in the CNS such as those which occur in multiple sclerosis (MS), an autoimmune disorder directed against CNS constituents, circulating immunocompetent cells readily enter the CNS (Engelhardt, 2006). In this regard, it has been shown that effector T-cells are able to cross the BBB where they may be exposed to a variety of neurotransmitters (Owens et al., 1998). Effector T-cells can cross the BBB and enter the CNS due to their expression of particular cytokines, chemokine receptors and adhesion molecules. These molecules interact in a multistep process with surface molecules expressed on the BBB, allowing entrance of T-cells (Fig. 1B). Initially, a low-affinity interaction between α4-integrins on activated T-cells and VCAM-1 on endothelial surface cells (Vajkoczy et al., 2001) and the
recently described interaction between the endothelial molecule ALCAM and the costimulatory surface molecule CD6 on T-cells (Cayrol et al., 2008), mediate the first step of T-cell/BBB interaction. Following this step, key chemokines such as CXCL9, CXCL10 or CXCL11 (Kivisakk et al., 2002), CCL19, CCL21 (Engelhardt, 2006) and MCP-1 (exclusive for Th2 cells) stimulate their respective receptors. These interactions play a crucial role in the successful recruitment of T-cells through BBB (Biernacki et al., 2001). After activation of chemokine receptors, cell surface integrins on leukocytes undergo conformational changes and engage their interactions with their receptors on the BBB surface, thus becoming high-affinity interactions and promoting strong adhesion of leukocytes to the endothelium. Subsequent to these strong interactions involving adhesion molecules such as LFA-1/ICAM-1 (Biernacki et al., 2001) takes place the extravasation of leukocytes through the BBB. Another mechanism to cross the BBB involves specific interactions between surface molecules of T-cells with surface molecules expressed on the BBB including specific cytokines, chemokine receptors and adhesion molecules. Solid lines represent cellular differentiation/migration while dotted lines represent DA secretion.

2.2. Modulation of T-cell signaling and function by DARs stimulation

The TCR-pMHC interaction determines the specificity of T-cell activation and the nature of T-cell response (Carreno et al., 2006). Recognition of pMHC by the TCR is required to trigger all downstream events which occur during T-cell function. Parallel to TCR-pMHC signaling, interaction between CD86/CD80 on DCs and CD28 on the T-cell surface triggers costimulatory signals necessary for efficient T-cell activation. The signaling pathways which contribute to efficient activation of T-cells after specific Ag recognition include the PKC/Ca²⁺.
pathway and the mitogen-activated-protein-kinases (MAPK) ERKs, JNKs and p38. Together these signaling molecules promote the activation of transcriptional factors NF-κB, NF-AT and AP-1 complexes (Fig. 2A). The simultaneous efficient stimulation of all of these pathways leads to T-cell activation with expansion of Ag-specific T-cell clones and differentiation into effector and memory cells. Type I and Type II DARs are often coupled to stimulation and inhibition of intracellular CAMP production respectively (Sibley et al., 1993). Regarding regulation of TCR-triggered signaling by cAMP in T-cells, PKA (a protein kinase activated by cAMP) as well as cAMP induce inhibition of ERKs phosphorylation (Ramstad et al., 2000) and of JNK activation (Harada et al., 1999), activate C-terminal Src kinase (Vang et al., 2001) and block NF-κB activation (Hershfield, 2005; Jimenez et al., 2001). All of these intracellular biochemical events induce a marked impairment on T-cell activation with inhibition of T-cell proliferation and of cytokine production (Aandahl et al., 2002). Thus, by stimulating DARs, DA could regulate T-cell function either negatively or positively (Fig. 2A).

Studies carried out on human and murine T-cells have demonstrated that these cells express all five DARs (D1–D5), each of which has diverse modulatory effects on the T-cell physiology (Fig. 2B and C). These studies have shown that stimulation of the D1/D5 receptor impairs T-cell function by causing the rise of intracellular cAMP. Further evidence indicates that stimulation of type I DARs not only inhibits cytokotoxic function of CD8+ T-cells (Saha et al., 2001b) but also impairs function and differentiation of Tregs (Cosentino et al., 2007; Kipnis et al., 2004). Moreover, stimulation of D1/D5 receptors has also been involved in the polarization of naïve CD4+ T-cells toward Th1 cells (Nakano et al., 2008; Nakano et al., 2008b). Because Th1 and Treg cells are involved in autoimmunity as auto-aggressive and beneficial cells respectively, it is likely that type I DARs expressed on T-cells are involved in the interface between autoimmunity and health. Interestingly, a decreased expression of D5 in peripheral-blood-mononuclear-cells has been found in patients suffering the autoimmune disease MS (Giorelli et al., 2005). Type II DARs are also involved in modulation of T-cell physiology. Addressing the role of D2 receptor on the T-cell function, it has been demonstrated that stimulation of this receptor promotes enhanced production of IL-10, a cytokine that negatively regulates the function of effector T-cells (Besser et al., 2005). This inhibition could be involved in the polarization toward Tregs. Regarding D4 receptor stimulation, evidence indicates that this receptor triggers T-cell quiescence by up-regulating Krüppel-like factor-2 (KLF-2) expression (Buckley et al., 2001; Sarkar et al., 2006) (Fig. 2C). On the other hand, whereas D3-stimulation facilitates differentiation of naïve CD8+ T-cells into CTLs (Besser et al., 2005), it also contributes to polarization of naïve CD4+ T-cells toward Th1 effector phenotype (Ilani et al., 2004) (Fig. 2B and C). Furthermore, stimulation via D3 receptor is thought to be involved in migration and adhesion of T-cells, thus modulating the homing of these cells (Ilani et al., 2004; Kivisakk et al., 2002; Watanabe et al., 2006). An integrative scheme summarizing the involvement of DARs stimulation in the function and polarization of effector phenotype of T-cells is shown in Fig. 2B and C.

2.3. Involvement of dopamine in the function and signaling of DCs

The key role that DCs play in T-cell differentiation and development of immune responses is carried out via secretion of some regulatory cytokines and expression of specialized surface molecules during Ag-presentation. So far, among the most relevant surface molecules and regulatory cytokines in the function of DCs are costimulatory membrane-bound CD80 and CD86 molecules and soluble cytokines IL-12, IL-10 and IL-6. CD80 and CD86 (Zheng et al., 2004) are two costimulatory molecules that contribute to the immunogenicity of DCs. At the T-cell–DC synapse, CD80/CD86 can bind to CD28 on T-cells and provide activating signaling for T-cells. The presence or absence of costimulatory surface molecules allows DCs to modulate the nature of T-cell responses, promoting either immunity or tolerance, respectively (Iruetagoyena et al., 2006). Regulatory cytokines produced by DCs are IL-12, IL-10 and IL-6, among others. IL-12 favours differentiation toward Th1 and impairs Th2 and Th17 differentiation (McGeachy and Cua, 2008; Watford et al., 2003). Whilst IL-6 together with TGF-β facilitates Th17 differentiation (McGeachy and Cua, 2008), the cooperative action of IL-6 and IL-4 allow development of Th2-mediated responses (Diehl and Rincon, 2002). Because TGF-β alone promotes differentiation of naïve T-cells into Tregs, but in the presence of IL-6 induces differentiation to Th17, IL-6 plays a pivotal role in the decision of differentiation between Th17/Tregs (Dardalhon et al., 2008; McGeachy and Cua, 2008; Oukka, 2008). Interestingly, both of these phenotypes of T-cells are involved in autoimmunity, Th17 being the phenotype of pathogenic cells while Tregs perform the function of beneficial cells (Dardalhon et al., 2008; Oukka, 2008). On the other hand, by secreting the suppressive cytokine IL-10, tolerogenic DCs provide a suitable environment to stimulate function of Tregs (Gaudreau et al., 2007; Mahnke et al., 2007).

Inflammatory signals triggered in DCs by stimulation of Toll-like receptors (TLRs) or by pro-inflammatory cytokine receptors generally involve activation of several signaling pathways including NF-κB, MAPKs, PI-3K, IFN-regulatory factors (IRFs) and STATs (Makel et al., 2009). When turned on, these signaling pathways stimulate DCs maturation with consequent strong expression of costimulatory molecules CD80/CD86 and production of pro-inflammatory cytokine (Fig. 3A). Depending on the precise combination of signaling pathways triggered in the stimulation of DCs, these cells may be induced to produce and release different regulatory cytokine and to express surface molecules (Ardesha et al., 2001; Arrighi et al., 2001; Iijima et al., 2003; Loscher et al., 2005; Makel et al., 2009; Mann et al., 2002; Nakahara et al., 2004; Usluoglu et al., 2007). Thus, the combination of signaling pathways triggered in DCs is pivotal for T-cell fate and the development of proper immune response (Fig. 3A).

With regard to the expression of DARs on DCs, type I as well as type II DARs have recently been described on these cells (Nakano et al., 2008), which are generally coupled to stimulation and inhibition of adenyate cyclase respectively (Sibley et al., 1993). Importantly, it has recently been described that adenyate cyclase stimulation potentiates LPS-induced ERKs and p38 phosphorylation. However adenyate
A

Efficient T-cell activation

B

C

Retention into the CNS
cyclosome stimulation can also inhibit LPS-promoted IRFs expression (Hickey et al., 2008) (Fig. 3A). In addition, there is evidence that augmented cAMP evokes inhibition of some Src-kinases with subsequent decrease on IRF-1 and c-Jun expression in LPS-treated DCs (Galgani et al., 2004). Together, these cAMP-induced effects in LPS-treated DCs stimulate strong IL-10 secretion coupled with attenuated IL-12 production by these cells (Galgani et al., 2004; Hickey et al., 2008). Nevertheless, whether activation of NF-κB is affected by elevated levels of cAMP in DCs remains controversial (Galgani et al., 2004; Jing et al., 2004; Zhou et al., 2007). Another signaling pathway modulated by increased cAMP in DCs involves PI-3K activation (Jing et al., 2004). These authors have demonstrated that by inhibiting PI-3K activity and consequently the participation of PKB and GSK3, cAMP exerts a dramatic decrease in the production of pro-inflammatory chemokines CCL3 and CCL4 (Fig. 3).

Addressing the physiological relevance of stimulation of DARs expressed on DCs in a pathological scenario, it has been demonstrated that DCs pre-treated with type I DARs-antagonists are enabled to promote amelioration of an autoimmune disease in mice (Nakano et al., 2008). This effect was possibly due to the fact that treatment with type I DARs-antagonists promotes DC-dependent inhibition of the pathogenic Th1-effector phenotype and favours Th1 differentiation in stimulated T-cells (Nakano et al., 2008) (Fig. 3B). In contrast, antagonism of type II DARs in DCs increase their capacity to promote the differentiation toward Th17 cells, whereas reducing Th1 polarization (Nakano et al., 2008). In order to elucidate the mechanism involved in DA-mediated regulation of the capability of DCs to evoke Th1 versus Th17 polarization, the same authors have described that under stimulation of type I DARs or antagonism of type II DARs, the increased cAMP induces enhanced DA production and storage by DCs (Nakano et al., 2009a,b). This effect was due to that cAMP stimulates activity of TH and thus DA synthesis (Nakano et al., 2009a). Subsequently, during Ag-presentation to specific CD4+ T-cells, DCs release the DA secretory vesicles next to naïve CD4+ T-cells stimulating type I DARs and thus promoting polarization of these cells to the Th2/Th17 effector phenotype (Nakano et al., 2008, 2009a,b). Nonetheless, further efforts are necessary to identify specific DARs and more detailed mechanisms involved in DA-mediated regulation of DCs function. Similarly, more detailed studies on the mechanisms involved in DA-mediated polarization of effector phenotype of CD4+ T-cells should be considered as well. An integrative scheme for the role of DA and DARs in the physiology of DCs and on the interplay of these cells with T-cells is represented in the Fig. 3.

2.4. Modulation of other immune cells by dopamine

Components of the dopaminergic system have been identified not only in T-cells and DCs, but also in other immune cells such as B-cells or monocytes/macrophages, which — like DCs — constitute APCs for T-cells. Interestingly, evidences have shown that the involvement of DA in the functioning of these immune cells is not limited to DARs expression, but they also are capable of synthesizing and eventually release DA. Thereby, the release of DA during Ag-presentation seems to be a general feature for APCs.

Due to that reserpine induces intracellular DA depletion with concomitant increase of extracellular DA levels, it is thought that B-cells store DA in cytoplasmic vesicles (Cosentino et al., 2000). These vesicular storages of DA contains almost the same intracellular DA levels that Tregs (Cosentino et al., 2000, 2007). Although until now the physiologic stimuli triggering DA release from B-cells has not been determined, it is likely that DA release could be triggered by Ag-presentation. Thus, DA derived from B-cells could exert modulation on T-cells and perhaps on B-cell physiology. In this regard, B-cells show the greatest expression of D2, D3 and D5 receptors among leukocytes (McKenna et al., 2002; Meredith et al., 2006). However, the role of DARs stimulation on the B-cell function remains controversial. For instance, Morkawa et al., have shown that treatment of B-cells with bromocriptine, a D2 receptor agonist, inhibits proliferation and immunoglobulin production in vitro (Morkawa et al., 1993). Nonetheless, Tsao et al., have concluded that the treatment of these cells with D1 or D2 receptor agonists enhances B-cell proliferation in vivo and in vitro (Tsao et al., 1997).

Similarly to B-cells and DCs, it has been described that monocytes/macrophages also contain intracellular DA storages, probably in cytoplasmic vesicles (Cosentino et al., 2000). On the other hand, unlike other APCs, the presence of DARs on monocytes/macrophages has not clearly been demonstrated (Beck et al., 2004; McKenna et al., 2002). Interestingly, it has been described that DA or DARs agonists significantly attenuate production of pro-inflammatory cytokines secreted by LPS-stimulated macrophages. However, because the action of DA was partially prevented by propanolol and not influenced by DARs-antagonists, this effect is mainly mediated via β-adrenoreceptors (Hasko et al., 2002). This evidence suggests that DA also may modulate physiology of immune cells by DARs-independent mechanisms.

The presence of intracellular DA storages as a common feature for different kind of APCs suggests an important role of DA during Ag-presentation. However, further efforts are necessary to identify both, physiologic mechanisms triggering DA release from APCs and the relevance of these DA-mediated stimuli in the auto-regulation of APCs function and on the development of immune responses in vivo.

3. Alterations in DA-mediated T-cell regulation and involvement on the development of diseases

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 involved in 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 malignancies but decreased in autoimmunity (Table 1). 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 (Ghosh et al., 2003; Saha et al., 2001a). It is likely that this

---

**Fig. 3.** DARs-dependent modulation of DC signaling and function. A. Putative modulation of inflammatory signaling in DCs by DARs. Pro-inflammatory agents such as pathogen-associated molecular patterns or inflammatory cytokines, by stimulating TLRs or cytokine receptors respectively, trigger activation of several signaling pathways in DCs. The production of cytokines which are key for DC function such as IL-10, IL-6 and IL-12 as well as the up-regulation of costimulatory CD80 and CD86 surface molecules on the DC surface are triggered by activation of different combinations of signaling pathways in DCs, as indicated. The capability of DCs to induce differentiation toward a specific effector phenotype in a naïve T-cell is determined by the production of regulatory cytokines and expression of costimulatory molecules, as indicated by thick lines. Stimulation of Type I or Type II DARs, by activation or inhibition of adenylylate cyclase (AC) activity, may modulate cell signaling triggered by inflammatory agents in DCs. This modulation controls the capability of these cells to produce different cytokines and surface molecules and thereby alters the ability to induce a particular effector phenotype on T-cells. B. Role of DARs on the modulation of DC function. DCs express Type I and Type II DARs which are coupled to stimulation and inhibition of adenylylate cyclase (AC) respectively (i). Thus, type I DARs stimulation, by cAMP accumulation, could inhibit CCL3 and CCL4 production (ii) and promote increased IL-10 and IL-6 secretion (iii) with attenuated IL-12 production (iv). cAMP also can promote DA synthesis by stimulating Tyrosine-Hydroxylase (TH) (v). During Ag-presentation by DCs to naïve T-cells in secondary lymphoid tissues, DCs release DA (vi) which subsequently stimulates different DARs expressed on T-cells, modulating T-cell differentiation. Whereas null arrows represent signaling pathways turned on after receptor stimulation in (A), they symbolize reactions or flux in (B). Dotted lines represent cAMP-mediated effects in (A), while secretion, production or effects in (B). Arrowheads symbolize activation/stimulation/up-regulation/secretion whereas flat heads represent inhibition/down-regulation.
DA-mediated inhibition of T-cell function contributes to the depression of anti-neoplastic immune response in cancer-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 taken in account 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 state of stress promotes increased levels of plasma DA (Table 1), which results in the inhibition of T-cell function and therefore immunosuppression (Jagmurov and Ogurtsov, 1996; La Via et al., 1996). Indeed, it is likely that elevation of plasma DA levels found in malignancy could be due to the stress suffered by patients with this illness. These findings collectively suggest that selective stimulation of specific DARs in 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 plasma levels of DA can alter T-cell function, but also the deregulation of DARs expressed on T-cells can cause similar effects. Accordingly, abnormal expression of DARs on T-cells has been described in some neurological and immune-related disorders (Table 2). For example, decreased expression of the D5 receptor, which performs an inhibitory role in T-cell function, has been described in T-cells from patients with MS, an autoimmune disorder (Giorelli et al., 2005). Furthermore, it has recently been shown that amelioration of MS by treatment with IFN-β is accompanied by restoration of levels of D5 receptor expressed on T-cells (Zaffaroni et al., 2008), DARs deregulation on T-cells has been also described in several neurological disorders (Table 2), which could have serious consequences on the function of the immune system. Indeed, 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 2). 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. According to this idea, it has been demonstrated that administration of an D2-agonist to mice during EAE, produces beneficial outcomes in the clinical course of development of this disease (Dijkstra et al., 1994). Another work supporting this notion has recently been performed by Nakano et al. (Nakano et al., 2008). These authors described that DCs pre-treated with type I DARs-antagonists are able to promote amelioration of EAE by impairing differentiation of naïve T-cells toward Th17 cells, the pathogenic effector phenotype in this pathology (Nakano et al., 2008). In fact, recently DA has been shown to be highly involved in the regulation of the phenotype of the effector T-cell response (Fig. 2B and C). Indeed, it has been demonstrated that the ratio IFN-γ/IL-4 in plasma is significantly higher in schizophrenic patients with early treatment or without medication than in healthy controls. The increased levels of IFN-γ/IL-4 were attenuated by effective neuroleptic treatment (Augustin et al., 2005; Kim et al., 2004), indicating how dopaminergic regulation may influence Th1/Th2 polarization in vivo. 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.

4. Concluding remarks and future perspectives

The evidence presented suggests that DA and DARs play an important role in the regulation of physiology of T-cells and DCs. We have proposed that by modulating signaling pathways, DARs stimulation on DCs could modify the pattern of cytokine secretion promoted by inflammatory stimuli, thus skewing polarization of T-cell differentiation toward a particular phenotype. In addition, by modulating intra-cellular signals in T-cells, DARs stimulation on these cells may induce further regulation of adaptive immune responses. Thereby DA has important roles in the development and progression of immune- or neuroimmune-related diseases.

Identification of sources of DA for modulation of T-cells constitutes an important and active research field. Neurotransmitters have not only been involved in nervous-driven regulation of immunity, but also in leukocyte-driven regulation of immunity. In this regard, DCs as well as T-cells and other immune cells have been described as sources of neurotransmitters, released under particular conditions. However, target cells and physiological roles for these leukocyte-derived neurotransmitters remains, in several cases, not well defined. Further studies are still necessary to determine physiological relevance of immune-driven release of DA, its roles, both individual and synergistic with other mediators, and their mechanism of action. In this direction, the role of leukocyte-derived DA on the physiology of nervous system should be explored as well. These studies would allow elucidation of bidirectional mechanisms of DA-mediated neuro-immune communication, which could give a better understanding of physiological mechanisms or those operating in pathologies involving leukocyte infiltration into the brain such as occurs in MS.

Further insight into DA-mediated regulation of immune function is critical to understanding its role in imbalanced immune responses, including autoimmunity, immunodeficiency and tumor growth. The relevant involvement of DA-mediated regulation in the immune response is evidenced by deregulation of DARs expressed on T-cells and alteration of plasma DA levels, both conditions found as part of the

---

**Table 1**

<table>
<thead>
<tr>
<th>Neurologic disorders</th>
<th>Pathology</th>
<th>Increase (in-fold)²</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parkinson’s disease</td>
<td>D3</td>
<td>1.00</td>
<td>(Tomei et al., 2007)</td>
</tr>
<tr>
<td>Schizophrenia</td>
<td>D3</td>
<td>0.50</td>
<td>(Tomei et al., 2007)</td>
</tr>
<tr>
<td>Schizophrenia</td>
<td>D5</td>
<td>0.50</td>
<td>(Nagai et al., 1996)</td>
</tr>
<tr>
<td>Schizophrenia</td>
<td>D5</td>
<td>0.50</td>
<td>(Boneberg et al., 2006)</td>
</tr>
<tr>
<td>Multiple sclerosis</td>
<td>D3</td>
<td>0.25</td>
<td>(Boneberg et al., 2006)</td>
</tr>
</tbody>
</table>

²Increase in plasma DA levels is expressed as the ratio of average of plasma DA concentration from patients versus average plasma DA concentration from healthy donors.

---

**Table 2**

<table>
<thead>
<tr>
<th>Pathology</th>
<th>Receptors³</th>
<th>Cells*</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alzheimer’s disease</td>
<td>D1, D5</td>
<td>PBMCs</td>
<td>(Barbanti et al., 2000a)</td>
</tr>
<tr>
<td>Migraine</td>
<td>D1, D4, D5</td>
<td>PBMCs</td>
<td>(Barbanti et al., 1996; Barbanti et al., 2000b)</td>
</tr>
<tr>
<td>Parkinson’s disease</td>
<td>D3</td>
<td>PBMCs</td>
<td>(Nagai et al., 1996)</td>
</tr>
<tr>
<td>Schizophrenia</td>
<td>D3</td>
<td>T-cells</td>
<td>(Boneberg et al., 2006; Ilani et al., 2001; Kwak et al., 2001)</td>
</tr>
<tr>
<td>Schizophrenia</td>
<td>D4</td>
<td>CD4 + T-cells</td>
<td>(Boneberg et al., 2006)</td>
</tr>
<tr>
<td>Multiple sclerosis</td>
<td>D5</td>
<td>PBMCs</td>
<td>(Giorelli et al., 2005)</td>
</tr>
</tbody>
</table>

³Arrows indicate increased or decreased expression of receptors compared with control conditions from healthy individuals. ⁴PBMCs, peripheral-blood mononuclear-cells.
pathophysiological scenario in some immune-related and neurolog-
dical disorders. In this regard, deregulation of DARs expression and plasma DA levels follow a trend geared toward exacerbate the im-
balance of immune response. For instance, plasma DA levels, which in general inhibit T-cell function, are increased in malignancies, but decreased on autoimmune disorders. The precise knowledge of the deregulation of plasma DA concentration and DARs expression on T-cells under different pathophysiological conditions, together with an understanding of the precise role of stimulation of each DARs subtype on T-cell physiology could facilitate to the design of therapies for the treatment of autoimmunity, immunodeficiency and cancer.

Acknowledgements

We would like to thank Brita Hult for critically reading this manuscript. This work was supported by grants FONDECYT 1095114 and CONICYT PFB-16. CEP is a CONICYT fellow.

References


Bordetella pertussis infection associated with haemophagus in children through recruitment of dendritic cells and subsequent development of Th1 and Th2 immunity by inhibiting SLE-like cells through protein kinase A-mediated signalling. J. Biol. Chem. 279, 32507–32514.


