Dopamine receptors modulate T lymphocytes via inhibition of cAMP-CREB signaling pathway

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Abstract

OBJECTIVES: We have previously reported that dopamine D2-like receptors including D2, D3 and D4 receptors are more important in mediating modulation of T cells than dopamine D1-like receptors (D1 and D5 receptors). Here we aimed to clarify the role of D2-like receptors in regulation of differentiation and function of T lymphocyte subsets, including helper T (Th1), Th2, Th17 and regulatory T (Treg) cells.

METHODS: Lymphocytes, separated from the mesenteric lymph nodes of mice, were stimulated with concanavalin A (Con A) and treated with the D2-like receptor agonist quinpirole or the antagonist haloperidol. Expression of lymphocyte cytokines and transcription factors and dopamine D2, D3 and D4 receptors were measured by real-time quantitative polymerase chain reaction and Western blot assay. Meanwhile, cAMP and phosphorylated cAMP-response element-binding (CREB) levels in the lymphocytes were examined by enzyme-linked immunosorbent assay and Western blot assay, respectively.

RESULTS: Activation of D2-like receptors with the agonist quinpirole upregulated the expression of Th2- and Treg-specific transcription factors and cytokines in Con A-activated lymphocytes, but downregulated the expression of Th1- and Th17-specific transcription factors and cytokines. Simultaneously, quinpirole increased dopamine D3 and D4 receptor expression, but did not alter D2 receptor expression. However, quinpirole reduced both cAMP and phosphorylated CREB levels in Con A-activated lymphocytes. All these quinpirole effects were blocked by haloperidol, an antagonist of D2-like receptors.

CONCLUSIONS: D2-like receptors, principally dopamine D3 and D4 receptors, promote differentiation and function of T lymphocytes towards anti-inflammatory T cell subsets by a negative link to cAMP-CREB pathway.
INTRODUCTION
Dopamine (DA) is a key neurotransmitter in the brain, where it is also increasingly regarded as a crucial transmitter in the neuroimmune network, contributing to the CNS-immune system interplay as well as in the communication among immune cells (Basu & Dasgupta 2000; Sarkar et al. 2010; Levite 2012). Immune cells themselves produce DA, which may act as autocrine/paracrine mediator on immune cells as well as on neighbouring cells (Costentino et al. 2000, 2002a, 2002b, 2005).

CD4+ T cells may be divided into four subpopulations, termed as helper T (Th)1, Th2, Th17 and Treg cells, according to differences in their cytokine expression profiles (Mosmann et al. 1986) T-box expressed in T cells (T-bet), a Th1-specific transcription factor, is thought to initiate Th1 development, whereas GATA binding protein 3 (GATA-3), a Th2-specific transcription factor, plays a pivotal role in development of Th2 cells. Th1 cells are mainly involved in cellular immune responses by secretion of cytokines, such as interleukin (IL)-2, interferon (IFN)-γ and tumor necrosis factor α (TNF-α). Th2 cells mainly mediate humoral immune responses and have anti-inflammatory effects via secretion of IL-4 and IL-10 (D’Ambrosio et al. 2000).

Th17 cells are a recently identified subset, which express high levels of IL-17 under the transcriptional regulation of the orphan retinoic acid nuclear receptor (ROR) family transcription factor ROR-γt. Th17 cells participate in the progression of many autoimmune diseases through the secretion of multiple inflammatory cytokines including IL-17, IL-21, and IL-22. Th17 cells can mediate inflammatory responses and functionally oppose regulatory T (Treg) cells (Volpe et al. 2008; Yang et al. 2008). Treg cells are known to maintain self-tolerance, prevent autoimmunity, and regulate immune homeostasis by attenuating excessive inflammation caused by pathogens or injury (Cederbom et al. 2000; Kipnis et al. 2002; Hori et al. 2003; Sakaguchi et al. 2004; Coombes et al. 2005; Kim et al. 2007; Bourreau et al. 2009). Treg cells are identified by expression of CD4 and CD25 cell-surface markers and transcription factor forkhead box P3 (Foxp 3), as well as secretion of cytokines, such as transforming growth factor-β (TGF-β) in mice (Fontenot et al. 2003; Hori et al. 2003).

We have previously shown that T lymphocytes are modulated by DA via its receptors (Huang et al. 2010). Other reports have also presented an extensive regulation of T cells by DA via its receptors. For example, Nakano et al. (2009, 2011) have suggested that stimulation of D1-like receptor expressed on human naive CD4+ T cells would contribute to the production of Th2 and Th17 cytokines. Moreover, Besser et al. (2005) have shown evidence suggesting that stimulation of D1-like receptor in human T-cells obtained from healthy donors would regulate production of IL-10 and TNF-α. Costentino et al. (2007) have suggested that stimulation of D1-like receptor on Treg cells would decrease production of TGF-β. Furthermore, there is a group of in vivo studies indicating that systemic administration of a D1-like receptor antagonist attenuates Th17 differentiation in mice (Nakano et al. 2008; Nakagome et al. 2011; Nakano et al. 2011). However, whether the D2-like receptor is also implicated in regulation of T lymphocyte subsets still remains to be clarified.

DA exerts its effects by stimulating five different DA receptors expressed on the cell surface. Five DA receptors have been identified to date: D1R, D2R, D3R, D4R and D5R (Sibley et al. 1993; Strange 1993) All of these receptors are hepta-spanning membrane proteins that belong to the superfamily of G protein-coupled receptors. Based on their sequence homology, signal transduction machinery and pharmacological properties, DA receptors have been classified into two subgroups. D1R and D5R belong to D1-like receptor which are often coupled with stimulatory G protein (Gs) and increase intracellular cAMP, and in turn cAMP induces phosphokinase A (PKA) activation, while D2R, D3R, and D4R constitute D2-like receptor, which generally couple to inhibitory G protein (Gi) (Beaulieu & Gainetdinov 2011; Rangel-Barajas et al. 2015) and decrease intracellular cAMP (Missale et al. 1998). We have previously found that via reduction of cAMP and cAMP-response element binding (CREB), a transcriptional factor that mediates AMP-induced gene expression via binding to cAMP-response element in gene promoter region, D2-like receptors exert a regulatory effect on T lymphocytes and NK cells, suggesting that cAMP-CREB signaling pathway is involved in modulation of T lymphocytes and NK cells by DA (Huang et al. 2010; Zhao et al. 2013).

Due to the key role of DA receptors in the adaptive immune response and to the lack of evidence demonstrating signalling pathways coupled to D2-like receptor in T lymphocytes and their functional relevance in Th1, Th2, Treg and Th17 cells, in this study we aimed to determine signalling pathways coupled to D2-like receptor and their involvement in T lymphocyte subsets function and differentiation.

MATERIALS AND METHODS

Mice
ICR mice (8–10 weeks old, 20–25 g) were obtained from Center of Experimental Animals, Nantong University, China. Animals were kept and bred in a specific pathogen-free environment with standard temperature and a 12-hour light/dark cycle. Mice were housed standard cages with 4 to 5 animals per cage, and food and water were provided ad libitum. All animal procedures were in accordance with National Institutes of Health (USA) guidelines and were approved by the Institutional Animal Care and Use Committee of Nantong University.
Cell culture and drug treatment

The mesenteric lymph nodes of the mice were harvested by celiotomy and lymphocytes were obtained by gently squeezing the lymph nodes. The cells were then washed twice and resuspended in RPMI 1640 medium supplemented with 10% heat-inactivated calf serum, 2.5×10^{-2} M HEPES, 1×10^{-3} M sodium pyruvate, 5×10^{-5} M mercaptoethanol and antibiotics (100 U/ml penicillin, 100 U/ml streptomycin), at the final concentration of 1×10^6 cells/ml. Different concentrations of D2-like receptor agonist quinpirole or antagonist haloperidol (both from Sigma) were added to the lymphocyte suspensions according to different experimental aims, and concanavalin A (Con A) was then added to the suspensions, which were cultured for 48 h. The control groups were conducted simultaneously with the experimental groups but treated without quinpirole or haloperidol.

Real-time Quantitative Polymerase Chain Reaction (PCR)

Total RNA was extracted from T cells using Trizol (Invitrogen) and cDNA was synthesized from 2 μg of total RNA using cDNA reverse transcription kit (Invitrogen, USA) in 20 μl reaction volume as described by the manufacturer. Real-time PCR was performed with Universal SYBR Green Master Mix (Roche, Germany), 0.4 μmol/L primer and 2 μl cDNA. Amplification was run in a Rotor-Gene 3000 Real-time Cycler at 95 °C for 10 min followed by 35 cycles of 94 °C for 15 s and 60 °C for 60 s. Following amplification, a melting curve analysis was performed by heating the reactions from 72 °C to 95 °C at 1 °C intervals. The analysis confirmed a single PCR product at the predicted melting temperature. β-actin was used for sample normalization.

The cycle at which each sample crossed a fluorescence threshold was determined. Relative gene expression levels were calculated by comparative cycle threshold method. The murine-specific primers of DA subtype receptors (D2R, D3R and D5R), the cytokines and transcription factors were summarized in Table 1.

Western Blot Analysis

Protein extracts (40 μg/lane) of T cells isolated from the mesenteric lymph nodes were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to a polyvinylidene difluoride membrane (Pall, USA) using a semi-dry transfer apparatus. After blocking nonspecific bindings with 5% (w/v) nonfat dry milk, the membranes were probed with antibodies specific for IL-2, IFN-γ, IL-4, IL-10, IL-17, IL-22, TGF-β, T-bet, GATA-3, ROR-γt, Foxp3, D2R, D3R, D4R (all from Santa Cruz, USA) or for phosphorylated CREB and total CREB (both from Cell Signaling Technology, USA) at room temperature for 2 h or at 4 °C overnight. They were incubated with appropriate IRDye 800-conjugated secondary antibodies (Rockland Immunochemical, USA) for 1 h at room temperature and visualized by Odyssey laser scanning system (LI-COR Inc, USA). The molecular weight and relative quantity of the protein bands were determined by an image analysis system (Odyssey 2.1, LI-COR Inc, USA).

Intracellular cAMP measurement

The concentration of cAMP in all samples was quantified using an ELISA (R&D Systems). Briefly, T cells were washed three times with PBS, incubated with HCl (100 μL, 1 mol/L) for 30 min at room temperature. They were then centrifuged at 600 × g for 15 min and

<table>
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<tr>
<th>Gene</th>
<th>Sense primer</th>
<th>Antisense primer</th>
<th>Product size (bp)</th>
<th>Accession No.</th>
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<td>D2R</td>
<td>5'-CCATTGCCTGGGTCCTG-3'</td>
<td>5'-TGGCCCTTGAGTGGTGCT-3'</td>
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<td>D3R</td>
<td>5'-CTACGCCTGGTCTCTG-3'</td>
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<td>D4R</td>
<td>5'-GTGTTGGACGCCTTTCTTG-3'</td>
<td>5'-GGGTTAGGGCAGCCTGGA-3'</td>
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<td>NM_007878</td>
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<td>IFN-γ</td>
<td>5'-TCAAGTGGGAGGATGGAAGA-3'</td>
<td>5'-TGGCTCTGGGCAAGGTACGAT-3'</td>
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<td>NM_008337.2</td>
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<td>5'-AGTCAAACTGCAACAGCCGAG-3'</td>
<td>81</td>
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<td>5'-CAGGAGAAGGGAGGGCCAC-3'</td>
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<td>IL-22</td>
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<td>IL-10</td>
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the same amount of NaOH as the HCl was added to the supernatants. Supernatants were immediately used for acetylated cAMP assays as recommended by the manufacturer. The assay is a colorimetric method that generates a yellow color read on a multi-mode microplate reader (Bio Tek, USA) at 405 nm. Obtained optical densities were used to calculate the cAMP concentrations of samples, using a standard curve.

**Statistical analysis**

Data were expressed as means ± standard deviation (M ± SD). Statistical analyses were performed with the Statistics Package for Social Science (SPSS, 12.0). The data were subjected to the one-way analysis of variance (ANOVA), followed by Student-Newman-Keul’s test to compare the data of all groups relative to each other. Differences were considered statistically significant at $p<0.05$.

**RESULTS**

**D2-like receptor agonist quinpirole inhibits Th1 lymphocytes function and differentiation**

Treatment of T cells with D2-like receptor agonist quinpirole (10$^{-8}$ or 10$^{-7}$ M) reduced the expression of T-bet, a specific transcription factor of Th1 cells and Th1 cell-produced pro-inflammatory cytokines, IFN-γ and IL-2. The co-treatment with D2-like receptor antagonist haloperidol (10$^{-7}$ M) and quinpirole (10$^{-8}$ or 10$^{-7}$ M) significantly caused the reduced T-bet, IFN-γ and IL-2 levels by the quinpirole to return to the control levels, indicating a blocking effect of haloperidol on the quinpirole-induced cytokine changes (Figure 1).

**D2-like receptor agonist quinpirole elevates Th2 lymphocytes function and differentiation**

Next we examined the effect of quinpirole on Th2-mediated immune response in T lymphocytes. We determined that quinpirole (10$^{-8}$ or 10$^{-7}$ M) elevated the expression of GATA-3, a specific transcription factor of Th2 cells and Th2 cell-produced anti-inflammatory cytokine, IL-4 and IL-10. The co-treatment with D2-like receptor antagonist haloperidol (10$^{-7}$ M) and quinpirole (10$^{-8}$ or 10$^{-7}$ M) also significantly caused the reduced GATA-3, IL-4 and IL-10 levels by the quinpirole to return to the control levels. This illustrated that haloperidol blocked the quinpirole-induced cytokine changes (Figure 2).

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**Fig. 1.** Activation of D2-like receptors inhibits Th1 lymphocytes function and differentiation. The mesenteric lymph node cells were cultured with Con A for 48 h in the presence of D2-like receptor agonist quinpirole (10$^{-8}$ or 10$^{-7}$ M) or antagonist haloperidol (10$^{-7}$ M). The expression of specific transcription factor of Th1 cells, T-bet and Th1 cell-produced pro-inflammatory cytokines, IFN-γ and IL-2 at mRNA and protein levels were detected by using real-time PCR and Western blot, respectively. (A) The data was normalized to β actin and represent M ± SD of four repeated experiments. (B) Representative electrophoretic bands and statistic chart of three independent experiments for T-bet, IFN-γ and IL-2 expression in T lymphocytes. *$p<0.05$, vs. control; +$p<0.05$, vs. Qui (10$^{-7}$ M). Qui: quinpirole, Hal: haloperidol.
Dopamine receptors and T lymphocytes

D2-like receptor agonist quinpirole inhibits Th17 lymphocytes function and differentiation

We also examined the effect of quinpirole on Th17-mediated immune response in T lymphocytes. We found that quinpirole (10⁻⁸ or 10⁻⁷ M) decreased the expression of ROR-γt, a specific transcription factor of Th17 cells and Th17 cell-produced pro-inflammatory cytokine, IL-17 and IL-22. The co-treatment with D2-like receptor antagonist haloperidol (10⁻⁷ M) and quinpirole (10⁻⁸ or 10⁻⁷ M) significantly caused the decreased ROR-γt, IL-17 and IL-22 levels by the quinpirole to return to the control levels, indicating a blocking effect of haloperidol on the quinpirole-induced cytokine changes (Figure 3).

D2-like receptor agonist quinpirole elevates Treg cells function and differentiation

Similarly, treatment of T cells with D2-like receptor agonist quinpirole (10⁻⁸ or 10⁻⁷ M) elevated the expression of Foxp3, a specific transcription factor of Treg cells and Treg cell-produced anti-inflammatory cytokine TGF-β. The co-treatment with D2-like receptor antagonist haloperidol (10⁻⁷ M) and quinpirole (10⁻⁸ or 10⁻⁷ M) significantly caused the reduced TGF-β levels by the quinpirole to return to the control levels, indicating that haloperidol could block the effect of quinpirole-induced cytokine changes (Figure 4).

Quinpirole elevates D3R and D4R expression, reduces cAMP content and CREB phosphorylation in T lymphocytes, and these effects are blocked by haloperidol

D2-like receptor agonist quinpirole (10⁻⁷ M) markedly up-regulated expression of D3R and D4R proteins but did not significantly increase D2R expression in T cells. Compared with the quinpirole exposure alone, the combined exposure to D2-like receptor antagonist haloperidol (10⁻⁷ M) and agonist quinpirole (10⁻⁷ M) down-regulated the expression of D3R and D4R proteins but did not significantly alter D2R expression, indicating a blockage of quinpirole-induced up-regulation of D3R and D4R expression by haloperidol. The treatment with haloperidol alone did not significantly affect the expression of the three subtypes of DA receptors. Quinpirole (10⁻⁷ M) decreased cAMP content in T cells, and this effect was abolished by haloperidol (10⁻⁶ M). Phosphorylated CREB level was significantly lower in quinpirole-treated T cells than in control cells. The co-treatment with haloperidol (10⁻⁶ M) and
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Fig. 3. Activation of D2-like receptors inhibits Th17 lymphocytes function and differentiation. The mesenteric lymph node cells were cultured with Con A for 48 h in the presence of D2-like receptor agonist quinpirole (10^{-8} or 10^{-7} M) or antagonist haloperidol (10^{-7} M). The expression of specific transcription factor of Th17 cells, ROR-γt and Th17 cell-produced pro-inflammatory cytokines, IL-17 and IL-22 at mRNA and protein levels were detected by using real-time PCR and Western blot, respectively. (A) The data was normalized to β-actin and represent M ± SD of four repeated experiments. (B) Representative electrophoretic bands and statistic chart of three independent experiments for ROR-γt, IL-17 and IL-22 expression in T lymphocytes. *p<0.05, vs. control; +p<0.05, vs. Qui (10^{-7} M). Qui: quinpirole, Hal: haloperidol.

quinpirole (10^{-7} M) caused the quinpirole-dependent decrease in CREB phosphorylation to return to control level (Figure 5).

DISCUSSION

Dopamine has been shown to act on receptors present on immune cells, with all subtypes of dopamine receptors found on leukocytes (Saha et al. 2001; Besser et al. 2005; Sarkar et al. 2006). The secondary lymphoid tissues are highly innervated by sympathetic nerve fibers that store high levels of dopamine (Weihe et al. 1991), and lymphocytes also produce dopamine (Bergquist et al. 1994; Cosentino et al. 2007). Previous in vitro studies demonstrated that dopamine could be either immunostimulatory or immunosuppressive depending on the experimental conditions used (Bergquist et al. 1994; Cosentino et al. 2007). Previous in vitro studies demonstrated that dopamine could be either immunostimulatory or immunosuppressive depending on the experimental conditions used (Bergquist et al. 1994; Cosentino et al. 2007). Previous in vitro studies demonstrated that dopamine could be either immunostimulatory or immunosuppressive depending on the experimental conditions used (Bergquist et al. 1994; Cosentino et al. 2007). Previous in vitro studies demonstrated that dopamine could be either immunostimulatory or immunosuppressive depending on the experimental conditions used (Bergquist et al. 1994; Cosentino et al. 2007). Previous in vitro studies demonstrated that dopamine could be either immunostimulatory or immunosuppressive depending on the experimental conditions used (Bergquist et al. 1994; Cosentino et al. 2007). Previous in vitro studies demonstrated that dopamine could be either immunostimulatory or immunosuppressive depending on the experimental conditions used (Bergquist et al. 1994; Cosentino et al. 2007). Previous in vitro studies demonstrated that dopamine could be either immunostimulatory or immunosuppressive depending on the experimental conditions used (Bergquist et al. 1994; Cosentino et al. 2007). Previous in vitro studies demonstrated that dopamine could be either immunostimulatory or immunosuppressive depending on the experimental conditions used (Bergquist et al. 1994; Cosentino et al. 2007). Previous in vitro studies demonstrated that dopamine could be either immunostimulatory or immunosuppressive depending on the experimental conditions used (Bergquist et al. 1994; Cosentino et al. 2007). Previous in vitro studies demonstrated that dopamine could be either immunostimulatory or immunosuppressive depending on the experimental conditions used (Bergquist et al. 1994; Cosentino et al. 2007). Previous in vitro studies demonstrated that dopamine could be either immunostimulatory or immunosuppressive depending on the experimental conditions used (Bergquist et al. 1994; Cosentino et al. 2007).

Several studies suggested dopamine would suppress the production of IFN-γ (Cosentino et al. 2007). For example, Bergquist et al. (1994) reported that dopamine suppressed IFN-γ production as described earlier. Carr et al. (2003) reported that dopamine suppressed the number of IFN-γ producing cells. Besser et al. (2005) reported that selective stimulation of D2R in human T-cells led to an augmented IL-10 secretion. IFN-γ, secreted from Th1 cells, is known to induce differentiation of naive CD4+ T cells into Th1 cells and to inhibit the proliferation of Th2 cells (Szabo et al. 2000; Tang et al. 2001). On the other hand, IL-4 and IL-10, secreted from Th2 cells, are known to induce the differentiation of naive CD4+ T cells into Th2 cells and to inhibit the function of Th1 cells (Fiorentino et al. 1989; Swain et al. 1990). These distinct subsets of helper T cells are responsible for specific immune functions. Th1 cells contribute to cell-mediated inflammatory immunity, while Th2 cells are responsible for humoral responses (Das et al. 2010). Therefore, in this study we have further explored roles of dopamine D2-like receptor in modulating differentiation and function of four subtypes of T lymphocytes, including Th1, Th17, Th2 and Treg cells.
Several transcription factors have been identified that control the differentiation of Th1/Th2 cells. T-bet, a specific transcription factor of Th1 cells, initiates Th1 lineage development from CD4+ T cell precursors while GATA-3, a specific transcription factor of Th2 cells, is critical for the development of Th2 cells (Agnello et al. 2003). Especially, GATA-3 is selectively induced early during Th2 development (Zheng & Flavell 1997), induced by IL-4 and inhibited by IFN-γ (Ouyang et al. 1998). In this study, we used quinpirole, a selective dopamine D2-like receptor agonist, to stimulate D2-like receptor on T lymphocytes. Our results showed that the expression of T-bet, and the Th1 cell-produced cytokines, IFN-γ and IL-2 were reduced. But the expression of GATA-3, and the Th2 cell-produced cytokines, IL-4 and IL-10 augmented. Both the effects of quinpirole were abolished by the D2-like receptor antagonist haloperidol. These results demonstrated that dopamine D2-like receptors might induced the improvement of helper T cell responses, from a Th1-dominant to a Th2-dominant pattern by increasing expression of IL-4 and GATA-3 while decreasing expression of IFN-γ and T-bet. The imbalance of Th1/Th2 type responses plays an important role in the development and perpetuation of a number of immune disorders such as allergies, systemic lupus erythematosus (SLE) and rheumatoid arthritis (Rao & Avni 2000). It has been demonstrated that the Th1/Th2 cell response was shifted to a predominantly Th1 cell response in autoimmune diseases, and thus, IFN-γ production from Th1 cells was increased in those diseases (Rao & Avni 2000). Therefore, our findings suggest that activating dopamine D2-like receptor have a tendency to regulate the balance of Th1/Th2 cell responses and to provide the basis for the treatment of immune diseases.

In addition, we found that after activating D2-like receptor by quinpirole, the expression of ROR-γt, and the Th17 cell-produced cytokines, IL-17 and IL-22 were reduced. But the expression of Foxp 3, and the Treg cell-produced cytokines, TGF-β, augmented. Both the effects of quinpirole were abolished by the D2-like receptor antagonist haloperidol. Increased Th17/Treg ratio and the altered regulatory function of Treg cells play an important role in the development and progression of some autoimmune diseases, such as sjogren syndrome (Papp et al. 2011, 2012a, 2012b). Th17 cells represent a subset of T helper cells that secrete mainly IL-17 as well as other proinflammatory cytokines.
and they have been related to many autoimmune and chronic inflammatory diseases (Waite & Skokos 2012). There is a balance between Th17 and Treg cells that depends on the activation of the transcription factor ROR-γt or Foxp 3, respectively, which regulate the immune response through the secretion of pro- and anti-inflammatory cytokines (Ivanov et al. 2006; Harris et al. 2007; Yang et al. 2007; Zhou et al. 2008). On the other hand, the importance of Treg cells to the maintenance of peripheral tolerance under noninflammatory conditions throughout life has also been confirmed. In fact, mice lacking Treg cells presented a fatal inflammatory response (Kim et al. 2007; Lahl et al. 2007).

Considering these previous studies and our animal experimental data, we are tempted to hypothesize that activating D2-like receptors could be new therapeutic strategies to some autoimmune and inflammatory diseases through inhibiting the Th17 cell response and enhancing Treg cell response.

In general, D2-like receptors are negatively coupled to cAMP/PKA-dependent signaling (Vallone et al. 2000; Yao et al. 2008). Stimulation of D2-like receptors leads to decreased adenyl cyclase activity via G protein (Gi), reducing PKA activation, which in turn decreases cAMP activity (Vallone et al. 2000; Yao et al. 2008). In the current study, activation of D2-like receptors...
on T lymphocytes by the agonist quinpirole led to an up-regulation of D3R and D4R expression, a decrease in cAMP content, and a reduction of phosphorylated CREB level. And the receptor antagonist haloperidol blocked these effects of quinpirole. The evidence demonstrates that activation of T lymphocyte D2-like receptors suppresses the downstream signal pathway, cAMP/PKA-CREB pathway, and therefore testifies a negative association of D2-like receptors with cAMP/PKA-CREB signaling in T lymphocytes. Thus, these changes in function and differentiation of T lymphocytes in this study can be considered as an outcome of cAMP/PKA-CREB signal-transduction inhibition induced by dopamine D2-like receptor activation in the T lymphocytes.

In this work, we present data that illustrate the involvement of dopamine D2-like receptor in modulation of T lymphocytes function via cAMP/PKA-CREB signaling pathway. Our data suggest that activation of D2-like receptors by the agonist quinpirole significantly regulated the balance of Th1/Th2 cells and Th17/Treg cells through inhibiting Th1 and Th17 cell responses and enhancing Th2 and Treg cell responses. Moreover, these changes can be considered as an outcome of cAMP/PKA-CREB signal-transduction inhibition induced by dopamine D2-like receptor activation in the lymphocytes. These may provide new insights into the knowledge of T cells physiology and suggest relevant molecular targets for some autoimmune and inflammatory diseases.

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