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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 (Th)1, 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.

ORIGINAL ARTICLE

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 (Cosentino *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. Cosentino *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 cAMP-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 n a pecific 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 polyvinylidine 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, Foxp 3, 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

Tab. 1. Sequences of PCR primers.

Gene	Sense primer	Antisense primer	Product size (bp)	Accession No.
D2R	5′-CCATTGTCTGGGTCCTGT-3′	5′-TGCCCTTGAGTGGTGTCT-3′	258	NM_010077
D3R	5′-CTACGCCCTGTCCTACTGT-3′	5′-CCACCTGTCACCTCCAAG-3′	189	NM_007877
D4R	5′-GTGTTGGACGCCTTTCTTCG-3′	5′-GGGTTGAGGGCACTGTTGA-3′	120	NM_007878
IFN-γ	5′-TCAAGTGGCATAGATGTGGAAGAA-3′	5′-TGGCTCTGCAGGATTTTCATG-3′	92	NM_008337.2
IL-2	5′-CCCAAGCAGGCCACAGAATTGAAA-3′	5′-AGTCAAATCCAGAACATGCCGCAG-3′	81	NM_008366.2
IL-4	5′-ACAGGAGAAGGGACGCCAT -3′	5′-GAAGCCCTACAGACGAGCTCA-3′	95	NM_021283.1
IL-17	5′-TGGACTCTGAGCCGCAATG-3′	5′-GGCGGACAATAGAGGAAACG-3′	142	NM_010552.3
IL-22	5′-AGCGGTGATGACCAG AACA-3′	5′-CTCAGGGACATA AAC AGCAGA-3′	220	NM_016971.2
IL-10	5′-GGACAACATACTGCTAACCGAC-3′	5′-TGGATCATTTCCGATAAGGCTTG-3′	83	NM_010548.1
TGF-β	5′-GATACGCCTGAGTGGCTGTC-3′	5′-GCTGATCCCGTTGATTTCC-3′	150	NM_011577.1
T-bet	5′-CCAGTATCCTGTTCCAGCC-3′	5′-CATAACTGTGTTCCCGAGGTG TC-3′	104	NT_165773.2
GATA3	5′-GCCTGTGCAAAAGAGATTTCAGAT-3′	5′-TGATTCACAGAGCATGTAGGCC-3′	100	NT_039202.7
ROR- γt	5′-AGTAGGCCACATTAC ACTGCT-3′	5′-GACCCACACCTCACA AATTGA-3′	137	NM_011281.2
Foxp3	5′-TACACCCAGGAAAGACAGCAACCT-3′	5′-TCTGCTTGGCAGTGCTTGAGAA-3′	138	NM_001199348.1
β-actin	5′-CTGTCCCTGTATGCCTCTG-3′	5′-ATGTCACGCACGATTTCC-3′	218	NM_007393

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).

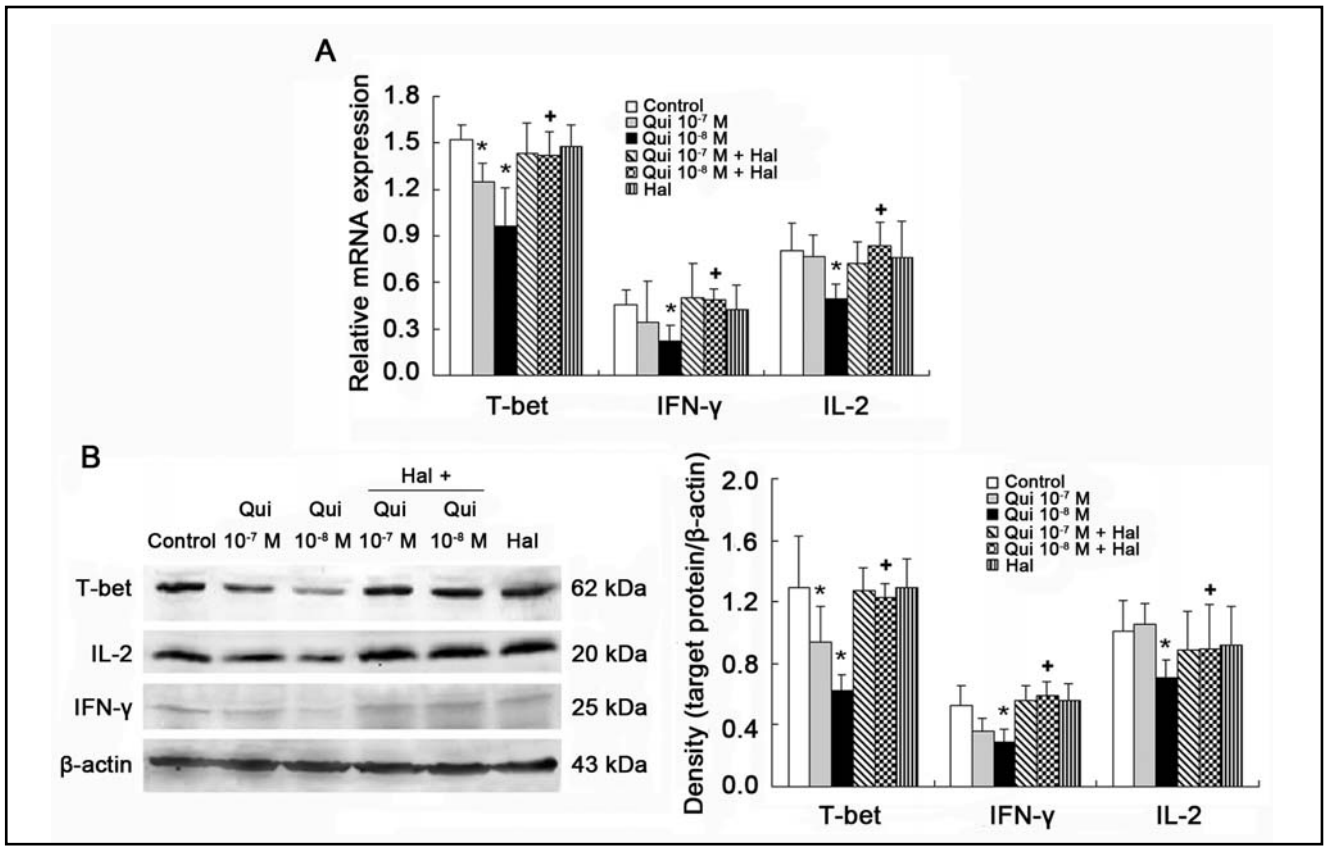

Fig. 1. Activation of D2-like receptors inhibits Th1 lymphocytes function and differentiation. The mesenteric lymph node cells were cultured with Con A for 48 h in the presence of D2-like receptor agonist quinpirole (10^{-8} or 10^{-7} M) or antagonist haloperidol (10^{-7} M). The expression of specific 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) Representive 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.

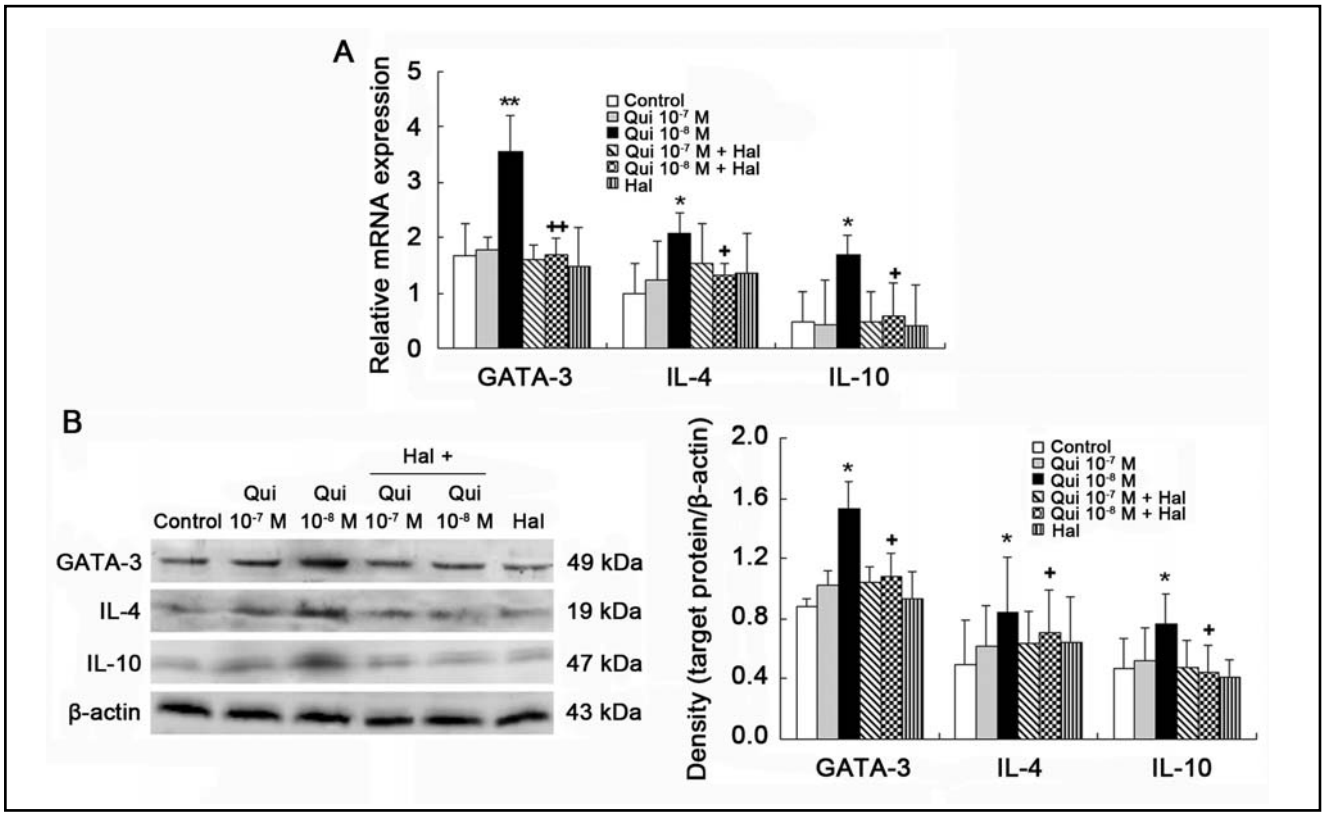

Fig. 2. Activation of D2-like receptors elevates Th2 lymphocytes function and differentiation. The mesenteric lymph node cells were cultured with Con A for 48 h in the presence of D2-like receptor agonist quinpirole (10^{-8} or 10^{-7} M) or antagonist haloperidol (10^{-7} M). The expression of specific transcription factor of Th2 cells, GATA-3 and Th2 cell-produced anti-inflammatory cytokines, IL-4 and IL-10 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 five repeated experiments. (B) Representative electrophoretic bands and statistic chart of three independent experiments for GATA-3, IL-4 and IL-10 expression in T lymphocytes. *p<0.05, **p<0.01, vs. control; +p<0.05, ++p<0.01, vs. Qui (10^{-7} M). Qui: quinpirole, Hal: haloperidol.

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 Foxp 3, 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

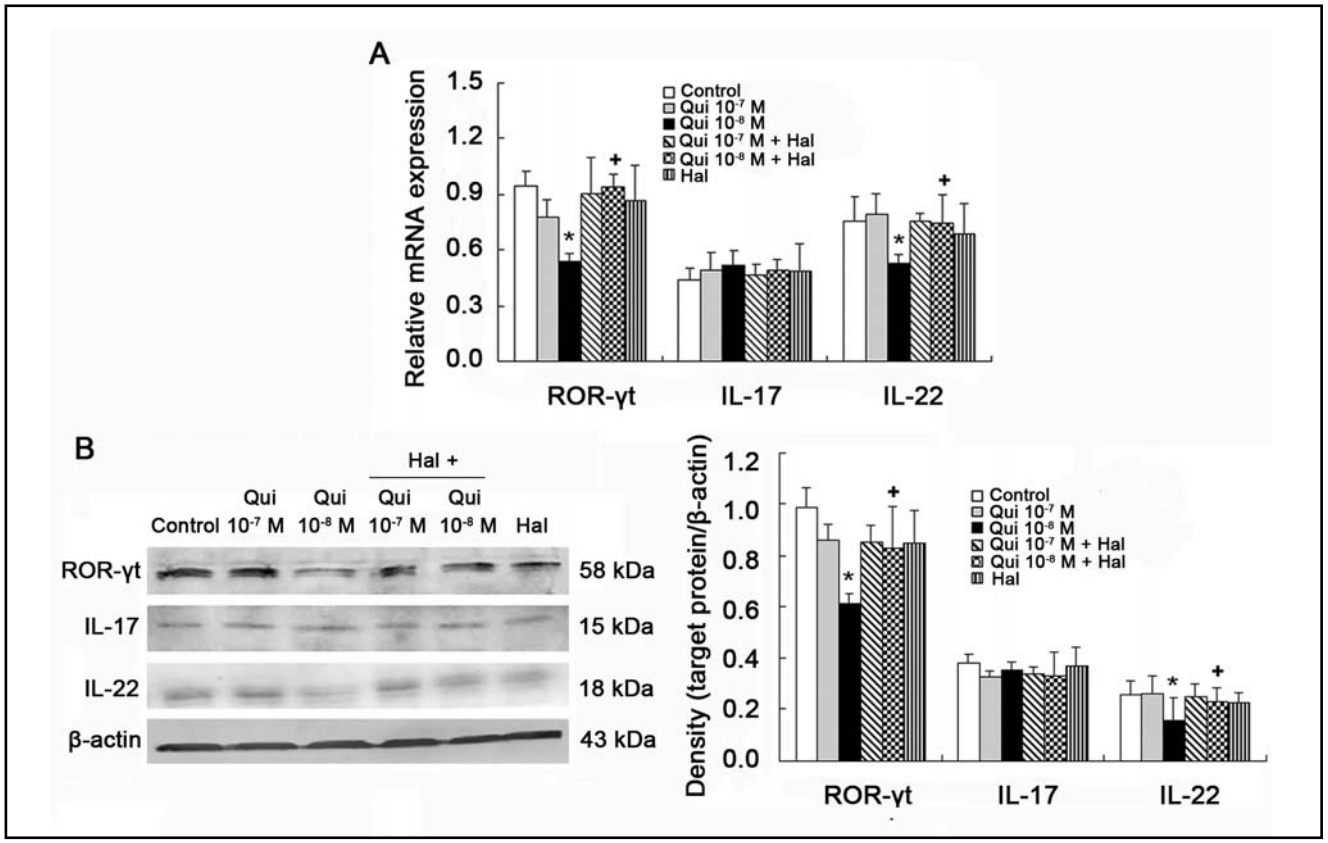

Fig. 3. Activation of D2-like receptors inhibits Th17 lymphocytes function and differentiation. The mesenteric lymph node cells were cultured with Con A for 48 h in the presence of D2-like receptor agonist quinpirole (10^{-8} or 10^{-7} M) or antagonist haloperidol (10^{-7} M). The expression of specific 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) Representive 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.* 1997; Basu *et al.* 2000; Ghosh *et al.* 2003) or on its selective stimulation of different dopamine receptor subtypes (Sealfon & Olanow 2000). We have previously demonstrated that five DA receptor subtypes of the two families, D1-like and D2-like receptors, exist on T lymphocytes of mice. Of the two families, D2-like receptors are more important in mediating modulation of T cell function than D1-like receptors (Huang *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 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 to 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.*

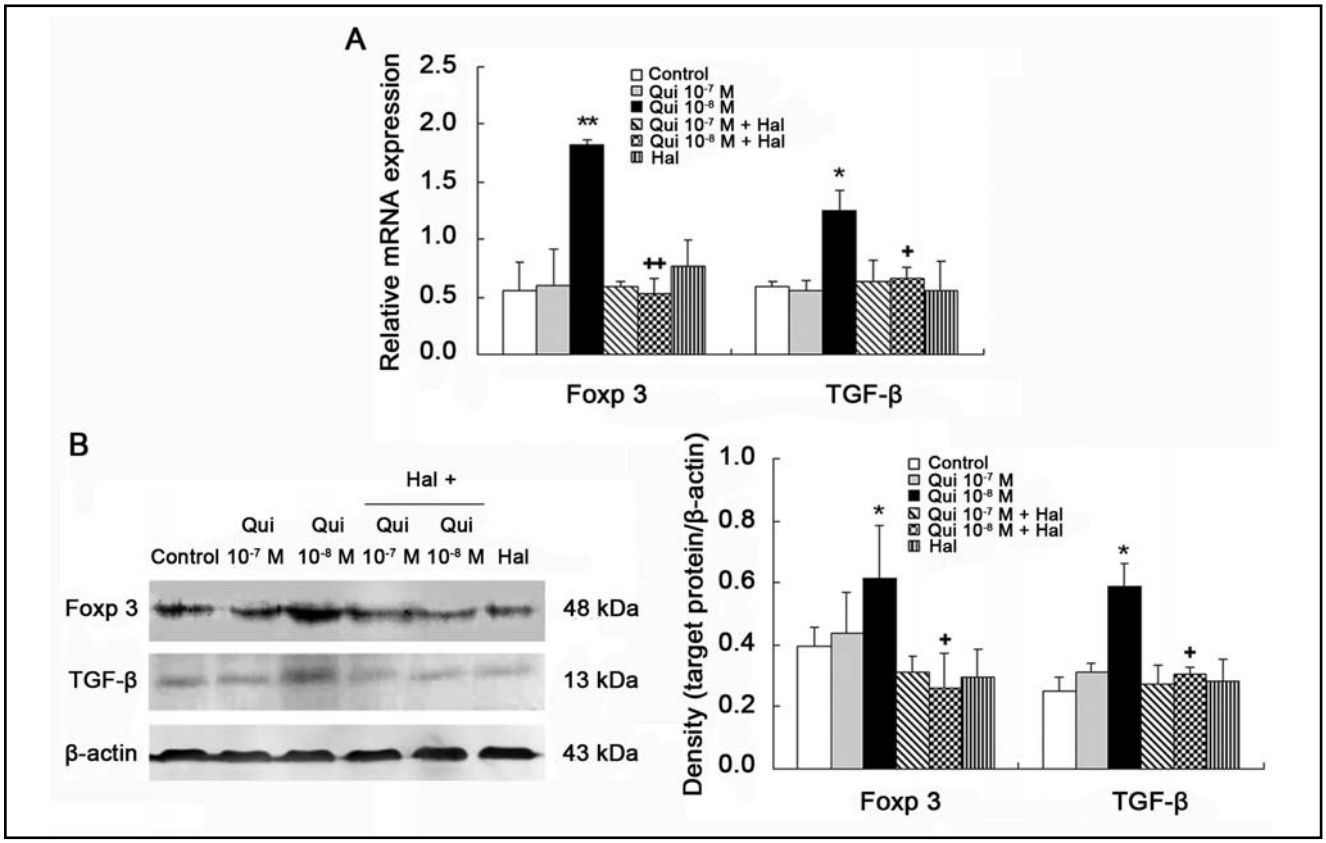

Fig. 4. Activation of D2-like receptors elevates Treg lymphocytes function and differentiation. The mesenteric lymph node cells were cultured with Con A for 48 h in the presence of D2-like receptor agonist quinpirole (10^{-8} or 10^{-7} M) or antagonist haloperidol (10^{-7} M). The expression of specific transcription factor of Treg cells, Foxp 3 and Treg cell-produced anti-inflammatory cytokine, TGF-β 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) Representive electrophoretic bands and statistic chart of three independent experiments for Foxp 3 and TGF-β expression in T lymphocytes. *p<0.05, **p<0.01, vs. control; +p<0.05, vs. Qui (10^{-7} M). Qui: quinpirole, Hal: haloperidol.

2001; Mullen *et al.* 2001). 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,

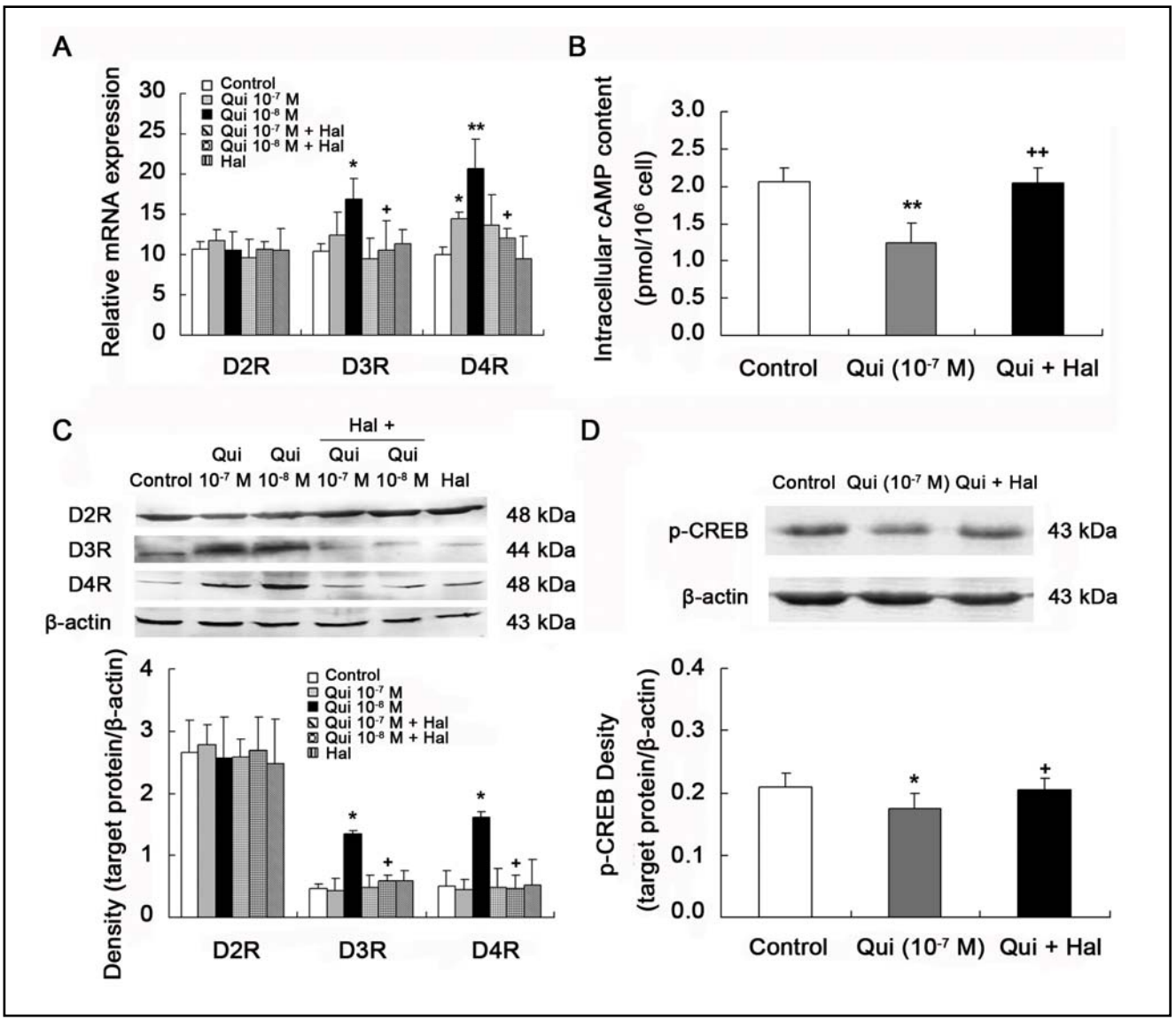

Fig. 5. Activation of D2-like receptors elevate D3R and D4R expression, reduce cAMP content and phosphorylated CREB level in T lymphocytes. T cells were exposed to D2-like receptor agonist quinpirole (10^{-8} or 10^{-7} M) or co-exposed to antagonist haloperidol (10^{-7} M) and the agonist quinpirole for 4 h, and then measured by real-time PCR, Western blot and ELISA, respectively. (A) The data was normalized to β-actin and represent M ± SD of three repeated experiments. (B) Representive electrophoretic bands and the statistic chart for three independent experiments. (C) Compilation of data for mean and standard deviation of six respective experiments. (D) Representive electrophoretic bands and compilation of data obtained from five separate experiments. *p<0.05,**p<0.01, vs. control; +p<0.05, ++p<0.01, vs. Qui (10^{-7} M). Qui: quinpirole, Hal: haloperidol.

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 tempting 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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REFERENCES

1 Agnello D, Lankford CS, Bream J, Morinobu A, Gadina M, O'Shea JJ, *et al.* (2003). Cytokines and transcription factors that regulate T helper cell differentiation: new players and new insights. J Clin Immunol. **23**: 147–161.
2 Basu S, Dasgupta PS (2000). Dopamine, a neurotransmitter, influences the immune system. J Neuroimmunol. **102**: 113–124.
3 Beaulieu JM, Gainetdinov RR (2011). The physiology, signalling, and pharmacology of dopamine receptors. Pharmacol Rev. **63**: 182–217.
4 Bergquist J, Josefsson E, Tarkowski A, Ekman R, Ewing A (1997). Measurements of catecholamine-mediated apoptosis of immunocompetent cells by capillary electrophoresis. Electrophoresis. **18**: 1760–1766.
5 Bergquist J, Tarkowski A, Ekman R, Ewing A (1994). Discovery of endogenous catecholamines in lymphocytes and evidence for catecholamine regulation of lymphocyte function via an autocrine loop. Proc. Natl. Acad. Sci. **91**: 12912–12916.
6 Besser MJ, Ganor Y, 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, TNF-α or both. J Neuroimmunol. **169**: 161–171.
7 Bourreau E, Ronet C, Darcissac E, Lise MC, Sainte Marie D, *et al.* (2009). Intralesional regulatory T-cell suppressive function during human acute and chronic cutaneous leishmaniasis due to Leishmania guyanensis. Infect Immun. **77**: 1465–1474.
8 Carr L, Tucker A, Fernandez-Botran R (2003). In vivo administration of L-dopa or dopamine decreases the number of splenic IFN-γ-producing cells. J Neuroimmunol. **137**: 87–93.
9 Cederbom L, Hall H, Ivars F (2000). CD4+CD25+ regulatory T cells down-regulate co-stimulatory molecules on antigen-presenting cells. Eur J Immunol. **30**: 1538–1543.
10 Cosentino M, Bombelli R, Ferrari M, Marino F, Rasini E, Maestroni GJ, *et al.* (2000). HPLC-ED measurement of endogenous catecholamines in human immune cells and hematopoietic cell lines. Life Sci. **68**: 283–295.
11 Cosentino M, Fietta AM, Ferrari M, Rasini E, Bombelli R, Carcano E, *et al.* (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.
12 Coombes JL, Robinson NJ, Maloy KJ, Uhlig HH, Powrie F (2005). Regulatory T cells and intestinal homeostasis. Immunol Rev. **204**: 184–194.
13 Cosentino M, Marino F, Bombelli R, Ferrari M, Rasini E, Lecchini S *et al.* (2002a) Stimulation with phytohaemagglutinin induces the synthesis of catecholamines in human peripheral blood mononuclear cells: role of protein kinase C and contribution of intracellular calcium. J Neuroimmunol. **125**: 125–133.
14 Cosentino M, Zaffaroni M, Ferrari M, Marino F, Bombelli R, Rasini E, *et al.* (2005) Interferon-gamma and interferon-beta affect endogenous catecholamines in human peripheral blood mononuclear cells: implications for multiple sclerosis. J Neuroimmunol. **162**: 112–121.
15 Cosentino M, Zaffaroni M, Marino F, Bombelli R, Ferrari M, Rasini E, *et al.* (2002b). Catecholamine production and tyrosine hydroxylase expression in peripheral blood mononuclear cells from multiple sclerosis patients: effect of cell stimulation and possible relevance for activation-induced apoptosis. J Neuroimmunol. **133**: 233–240.
16 D'Ambrosio D, Iellem A, Colantonio L, Clissi B, Pardi R, Sinigaglia F (2000). Localization of Th cell subsets in inflammation: differential thresholds for extravasation of Th1 and Th2 cells. Immunol Today. **21**: 183–186.
17 Das J, Chen CH, Yang L, Cohn L, Ray P, Ray A (2001). A critical role for NF-kappa B in GATA-3 expression and Th2 differentiation in allergic airway inflammation. Nat Immunol. **2**: 45–50.
18 Fiorentino DF, Bond MW, Mosmann TR (1989). Two types of mouse T helper cell: IV. Th2 clones secrete a factor that inhibits cytokine production by Th1 clones. J Exp Med. **170**: 2081–95.
19 Fontenot JD, Gavin MA, Rudensky AY (2003). Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. Nat Immunol. **4**: 330–336.
20 Ghosh MC, Mondal AC, Basu S, Banerjee S, Majumder J, Bhattacharya D, *et al.* (2003). Dopamine inhibits cytokine release and expression of tyrosine kinases, Lck and Fyn in activated T cells. Int Immunopharmacol. **3**: 1019–1026.
21 Harris TJ, Grosso JF, Yen HR, Xin H, Kortylewski M, Albesiano E, *et al.* (2007). An in vivo requirement for STAT3 signaling in Th17 development and Th17-dependent autoimmunity. J Immunol. **179**: 4313–4317.
22 Hori S, Nomura T, Sakaguchi S (2003). Control of regulatory T cell development by the transcription factor Foxp3. Science. **299**: 1057–1061.
23 Huang Y, Qiu AW, Peng YP, Liu Y, Huang HW, Qiu YH (2010). Roles of Dopamine Receptor Subtypes in Mediating Modulation of T Lymphocyte Function. Neuroendocrino Lett. **31**: 101–110.
24 Ivanov II, McKenzie BS, Zhou L, Tadokoro CE, Lepelley A, Lafaille JJ, *et al.* (2006). The orphan nuclear receptor ROR-γt directs the differentiation program of proinflammatory IL-17+ T helper cells. Cell. **126**: 1121–1133.
25 Kim JM, Rasmussen JP, Rudensky AY (2007). Regulatory T cells prevent catastrophic autoimmunity throughout the lifespan of mice. Nat Immunol. **8**: 191–197.

26 Kipnis J, Mizrahi T, Hauben E, Shaked I, Shevach E, Schwartz M (2002). Neuroprotective autoimmunity: Naturally occurring CD4+CD25+ regulatory T cells suppress the ability to withstand injury to the central nervous system. Proc Natl Acad Sci. **99**: 15620–15625.

27 Lahl K, Loddenkemper C, Drouin C, Freyer J, Arnason J, Eberl G, *et al.* (2007). Selective depletion of Foxp 3+ regulatory T cells induces a scurfy-like disease. J Exp Med. **204**: 57–63.

28 3. Levite M (2012). Dopamine in the immune system: dopamine receptors in immune cells, potent effects, endogenous production and involvement in immune and neuropsychiatric diseases. In: Levite M, editors. Nerve-driven-immunity-neurotransmitters and neuropeptides in the immune system. Springer, Wien, p. 1–45.

29 Missale C, Nash SR, Robinson SW, Jaber M, Caron MG (1998). Dopamine receptors: from structure to function. Physiol Rev. **78**: 189–225.

30 Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL (1986). Two types of murine helper T cell clone: I. Definition according to profiles of lymphokine activities and secreted proteins. J Immunol. **136**: 2348–2357.

31 Mullen AC, High FA, Hutchins AS, Lee HW, Villarino AV, Livingston DM, *et al.* (2001). Role of T-bet in commitment of Th1 cells before IL-12-dependent selection. Science. **292**: 1907–1910.

32 Nakano K, Higashi T, Hashimoto K, Takagi R, Tanaka Y, 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.

33 Nakano K, Higashi T, Takagi R, Hashimoto K, Tanaka Y, Matsushita S (2009). Dopamine released by dendritic cells polarizes Th2 differentiation. Int. Immunol. **21**: 645–654.

34 Nakagome K, Imamura M, Okada H, Kawahata K, Inoue T, Hashimoto K, *et al.* (2011) Dopamine D1-like receptor antagonist attenuates Th17-mediated immune response and ovalbumin antigen-induced neutrophilic airway inflammation. J Immunol. **186**: 5975–5982.

35 Nakano K, Yamaoka K, Hanami K, Saito K, Sasaguri Y, Yanagihara N, *et al.* (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. **86**: 3745–3752.

36 Ouyang W, Ranganath SH, Weindel K, Bhattacharya D, Murphy TL, Sha WC, *et al.* (1998). Inhibition of Th1 development mediated by GATA-3 through an IL-4-independent mechanism. Immunity. **9**: 745– 755.

37 Papp G, Barath S, Szegedi A, Szodoray P, Zeher M (2012a). The effects of extracorporeal photochemotherapy on T cell activation and regulatory mechanisms in patients with systemic sclerosis. Clin Rheumatol. **31**: 1293–1299.

38 Papp G, Horvath IF, Barath S, Gyimesi E, Sipka S, Szodoray P, *et al.* (2011). Altered T-cell and regulatory cell repertoire in patients with diffuse cutaneous systemic sclerosis. Scand J Rheumatol. **40**: 205–210.

39 Papp G, Horvath IF, Barath S, Gyimesi E, Vegh J, Szodoray P, *et al.* (2012b). Immunomodulatory effects of extracorporeal photochemotherapy in systemic sclerosis. Clin Immunol. **142**: 150–159.

40 Rangel-Barajas C (2015). Coronel I, Floran B: Dopamine Receptors and Neurodegeneration. Aging Dis. **6**: 349–68.

41 Rao A, Avni O (2000). Molecular aspects of T-cell differentiation: Br Med Bull. **56**: 969–984.

42 Saha B, Mondal AC, Basu S, Dasgupta PS (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.

43 Sakaguchi S (2004). Naturally arising CD4+ regulatory T cells for immunologic self-tolerance and negative control of immune responses. Ann Rev Immunol. **22**: 531–562.

44 Sarkar C, Basu B, Chakroborty D, Dasgupta PS, Basu S (2010). The immunoregulatory role of dopamine: an update. Brain Behav Immun. **24**: 525–528.

45 Sarkar C, Das S, Chakroborty D, Chowdhury UR, Basu B, Dasgupta PS, *et al.* (2006). 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.

46 Sealfon SC, Olanow CW (2000). Dopamine receptors: from structure to behavior. Trends Neurosci. **23**: S34–40.

47 Sibley DR, Monsma FJ Jr, Shen Y (1993). Molecular neurobiology of dopaminergic receptors. Int Rev Neurobiol. 35: 391–415.

48 Strange PG (1993). New insights into dopamine receptors in the central nervous system. Neurochem Int. **22**: 223–236.

49 Swain SL, Weinberg AD, English M, Huston G (1990). IL-4 directs the development of Th2-like helper effectors. J Immunol. **145**: 3796–3806.

50 Szabo SJ, Kim ST, Costa GL, Zhang X, Fathman CG, Glimcher LH (2000). A novel transcription factor. Cell. **100**: 655–69.

51 Tang C, Inman MD, van Rooijen N, Yang P, Shen H, Matsumoto K, *et al.* (2001). Th type 1-stimulating activity of lung macrophages inhibits Th2-mediated allergic airway inflammation by an IFN-γ-dependent mechanism. J Immunol. **166**: 1471–1481.

52 Vallone D, Picetti R, Borrelli E (2000). Structure and function of dopamine receptors. Neurosci Biobehav Rev. **24**: 125–132.

53 Volpe E, Servant N, Zollinger R, Bogiatzi SI, Hupe P, Barillot E, *et al.* (2008). A critical function for transforming growth factor-beta, interleukin 23 and proinflammatory cytokines in driving and modulating human T(H)-17 responses. Nat Immunol. **9**: 650–657.

54 Waite JC, Skokos D (2012). Th17 response and inflammatory autoimmune diseases. Int J Inflam. **2012**: 819467.

55 Weihe E, Nohr D, Michel S, Muller S, Zentel HJ, Fink T, *et al.* (1991). Molecular anatomy of the neuro-immune connection. Int J Neurosci. **59**: 1–23.

56 Yang L, Anderson DE, Baecher-Allan C, Hastings WD, Bettelli E, Oukka M *et al.* (2008). IL-21 and TGF-β are required for differentiation of human T(H)17 cells. Nature. **454**: 350–352.

57 Yang XO, Panopoulos AD, Nurieva R, Chang SH, Wang D, Watowich SS, *et al.* (2007). STAT3 regulates cytokine-mediated generation of inflammatory helper T cells. J Biol Chem. **282**: 9358–9363.

58 Yao WD, Spealman RD, Zhang J (2008). Dopaminergic Signaling in Dendritic Spines. Biochem Pharmacol. **75**: 2055–2069.

59 Zhao W, Huang Y, Liu Z, Cao BB, Peng YP, Qiu YH (2013). Dopamine receptors modulate cytotoxicity of natural killer cells via cAMP-PKA-CREB signaling pathway. PLoS One. **8**: e65860.

60 Zheng W, Flavell RA (1997). The transcription factor GATA-3 is necessary and sufficient for Th2 cytokine gene expression in CD4 T cells. Cell. **89**: 587–596.

61 Zhou L, Lopes JE, Chong MM, Ivanov II, Min R, Victora GD, Shen Y, *et al.* (2008). TGF-β-induced Foxp 3 inhibits Th17 cell differentiation by antagonizing ROR-γt function. Nature. **453**: 236–240.