Journal of Neuroimmunology 238 (2011) 58–66

Contents lists available at ScienceDirect

Journal of Neuroimmunology

journal homepage: www.elsevier.com/locate/jneuroim

Dopamine up-regulates Th17 phenotype from individuals with generalized anxiety disorder☆,☆☆

Thais B. Ferreira [a], Taissa M. Kasahara [a], Priscila O. Barros [a], Morgana M.M. Vieira [a], Vera Carolina B. Bittencourt [a], Joana Hygino [a], Regis M. Andrade [a], Ulisses C. Linhares [a], Arnaldo F. Andrade [b], Cleonice A. Bento [a,*]

[a] Department of Microbiology and Parasitology, Federal University of the State of Rio de Janeiro, Rio de Janeiro, Brazil
[b] Department of Microbiology, State University of Rio de Janeiro, Rio de Janeiro, Brazil

ARTICLE INFO

Article history:
Received 22 March 2011
Received in revised form 11 June 2011
Accepted 17 June 2011

Keywords:
Generalized anxiety disorder
Th17/Th1
IL-10/TGF-β
Dopamine
Glucocorticoid

ABSTRACT

Our objective was to evaluate the effect of stress-related dose of dopamine (DA) on the *in vitro* proliferation and cytokine production in polyclonally-activated T cells from healthy individuals or individuals with generalized anxiety disorder (GAD). Our results demonstrated that cell cultures from GAD group proliferated less following T cell activation, as compared with control group. The addition of DA reduced the proliferative response in cell cultures from healthy but not from GAD individuals. The cytokine profile in GAD individuals revealed Th1 and Th2 deficiencies associated with a dominant Th17 phenotype, which was enhanced by DA. A similar DA-induced immunomodulation was also observed in PPD-activated cell cultures from GAD individuals. Unlike the control, DA-enhanced Th17 cytokine production in GAD individuals was not affected by glucocorticoid. In conclusion, our results show that the T cell functional dysregulation in GAD individuals is significantly amplified by DA. These immune abnormalities can have impact in increasing the susceptibility of individuals with anxiety disorders to infectious diseases and inflammatory/autoimmune disorders.

1. Introduction

It is now known that the immune system is regulated by central as well as peripheral sympathetic nervous system. This is primarily achieved by cathecolamines, such as dopamine (DA), which interact with different effector immune cells and thereby ultimately regulate the homeostatic response of an individual to environmental stresses (Sarkar et al., 2010).

In the central nervous system, DA plays an essential role as neurotransmitter by playing 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). At the peripheral level, sympathetic innervations of organs and tissues, such as lymph nodes and spleen, can be controlled by dopaminergic signaling, particularly during stress (Bencsics et al., 1997).

DA exerts its effects in susceptible cells by stimulating DA receptors (DARs) expressed on the cell surface of neurons. Five DARs have been described so far (D1–D5); DARs are hepta-spanning membrane receptors and belong to the superfamily of the G protein-coupled receptors (Strange, 1993). Whereas type I DARs (D1 and D5) are generally coupled to Gαs and stimulate cAMP production, type II DARs (D2, D3 and D4) are often coupled to Gαi promoting inhibition of cAMP synthesis (Sibley et al., 1993). This differential coupling of DARs allows DA to promote distinct effects in the same cell expressing the two different kinds of DARs.

Besides its conventional role as neurotransmitter, DA is now considered to play a pivotal role in neuroimmune communications. The first clue to this possibility was the observation that DARs are expressed in normal human leukocytes (McKenna et al., 2002; Ferrari et al., 2004; Kirillova et al., 2008; Nakano et al., 2008, 2009). Interestingly, in schizophrenic patients, who have a hiperdopaminergic activity (Birtwistle and Baldwin, 1998), severe abnormalities of immune functions have been demonstrated, such as alterations in T-cell subsets, production of cytokines, and effector functions (Muller et al., 2000; Strous and Shoenfeld, 2006; Riedel et al., 2007). Furthermore, it is known that psychological stress causes an increase in peripheral release of DA (Sinha, 2008), and individuals suffering from chronic stress, like those with generalized anxiety disorder (GAD), are more susceptible to infectious diseases by damaging Th1-mediated immune response (Boscarino, 2004; Koh and Lee, 2004; Sareen et al., 2005; Schneiderman

☆ This work was supported by Fundação de Amparo à Pesquisa Carlos Chagas Filho (FAPERJ).
☆☆ Conflict of interest statementAll authors declare that there are no conflicts of interest.
* Corresponding author at: Department of Microbiology and Parasitology, Federal University of the State of Rio de Janeiro, Frei Caneca 94, 20.261-040, Rio de Janeiro, RJ, Brazil. Tel./fax: +55 21 2531 7906.
E-mail address: cbento@unirio.br (C.A. Bento).

0165-5728/
doi:10.1016/j.jneuroim.2011.06.009

et al., 2005; Zhou et al., 2005; Godbout and Glaser, 2006; Arranz et al., 2007). On the other hand, anxiety disorders are also known to contribute to the pathogenesis of many inflammatory/autoimmune diseases by elevating systemic production of IL-1, IL-6 and TNF-α cytokines (Affleck et al., 1997; Wessa and Rohleder, 2007; Lemieux et al., 2008; Gill et al., 2009).

Generalized anxiety disorder (GAD) is a common chronic disorder characterized by long-lasting anxiety that is not focused on any object or situation. Patients suffering from generalized anxiety experience persistent non-specific fear and worry, and become over-concerned with everyday matters (Khouzam, 2009). They are prone to flare up in moments of high stress and frequently present psychosomatic symptoms such as headache, sweating, muscle spasms, palpitations, and hypertension, which, in some cases, severely compromise their quality of life. The prevalence of GAD in the USA (Kessler et al., 2005) and in Brazil (Andrade et al., 2002) was estimated in 1.3–2.8% adults, and, until now, no information is available on the effect of DA on T-cell functions in anxious subjects. Therefore, the objective of this work was to explore the impact stress-related doses of DA on T cell profile following *in vitro* non-specific activation in a group of patients with GAD.

2. Methods

2.1. Participants

Twenty individuals with generalized anxiety disorder (8 male, 12 female; mean age = 29.4 years, SD = 12.7, range 17–42) and 20 healthy subjects without any detectable psychiatric disorder matched by age (mean age = 29.7 years, SD = 10.3, range 19–40), gender, racial background and annual income were recruited to participate this study (Table 1). We used the Spielberger State (STAIS) and Trait (STAIT) Anxiety Inventory (Kohn et al., 2008), Hamilton Depression Rating scale (HAMD) and Hamilton Anxiety Rating scale (HAMA) to measure mood states in all subjects (Hamilton, 1959). Individuals with a present, past and family history of mental disorders were assessed through the Semi structured Interview for the DSMIII-R (SCID) (Spitzer et al., 1992). According to the HAMA, we included only the patients with moderate–severe symptoms of anxiety.

Subjects were excluded if they reported intake of any medication with immune-modulating effects, such as glucocorticoids, had acute or chronic organic illnesses, or met criteria for additional mental disorders other than anxiety or stress-related affective disorders. Anxiety disorders often co-exist with other mental disorders, particularly clinical depression, which may occur in as many as 60% of people with anxiety. In our study, 05/20 among anxiety patients met the DSM-IV criteria for current major depressive episode, however, they were not excluded because no significant difference was observed between them and the other non-depressive anxious individuals concerning the results from immune function analysis. Although 14/20 anxious patients reported past intake of psychotropic medication (12 anxiolytics and 2 antidepressants), all participants were completely medication-free for at least 6 months prior to blood sampling. Of note, none of the women was pregnant or lactating. Finally, the Survey of Immunological and General Health (SIGH) was used as a self-report measure of physical health (Kang et al., 1991). The SIGH also includes questions on age, weight, and demographic background and has been successfully used in a wide variety of studies (Kang et al., 1991; Strauman et al., 1993; Lemieux and Coe, 1995). Body Mass Index (BMI), that might affect immune function, was calculated as weight in kilograms divided by the square of height in meters. For our study, all subjects had BMIs ranging from 18 to 30 (Table 1). To study the impact of dopamine (DA) on the *in vitro* immunological events induced by recall antigen, we selected individuals from both groups who had a positive purified protein derivative (PPD) skin test (induration >4–10 mm). Of note, none of the PPD-positive subjects had active pulmonary tuberculosis (excluded by normal chest x-ray).

After a complete description of the study to the participants, written informed consent was obtained for each individual. The study was approved by the Ethical Committee for Research on Human Subjects of the Federal University of the State of Rio de Janeiro (UNIRIO). Finally, all immunological evaluations shown here were performed twice in each individual from blood samples collected in different times.

2.2. Isolation of plasma and PBMC

The peripheral blood (20 mL) from healthy (control, n = 20) and anxious patients (GAD, n = 20) were drawn between 10 and 11 a.m. into tubes containing heparine (BD Vacutainer, Franklin Lakes, NY). Immediately after blood collection, an aliquot (2 mL) was separated for obtaining the total plasma for systemic cytokine quantification by ELISA. The remaining blood was used to obtain the peripheral blood mononuclear cells (PBMC) by centrifugation on Ficoll–Hypaque density gradients. The PBMC were collected and washed three times in HANK's solution. The total cells were then suspended in 1 mL of RPMI-1640 and the number of viable cells was measured by trypan blue exclusion using a hemocytometer.

2.3. Immunofluorescence labeling and flow cytometry

In order to determine the percentage of $CD4^+$ T cells, $CD8^+$ T cells, B cells and monocytes among whole PBMC, 50 μL of PBMC were incubated for 20 min with antibodies (Abs) anti-CD3-FITC, CD4-PE, CD8-APC, anti-CD19-FITC, and anti-CD14-PE. All the Abs were obtained from BD PharMingen. Labeled cells were analyzed by using FACSCalibur flow cytometer and CellQuest software (BD Biosciences, Mountain View, CA). With each sample, a negative control with isotype-matched control Abs was used to determine the positive and negative cell populations.

2.4. Cell cultures and stimulus

For functional immune experiments, viable PBMC (1×10^6/mL) were cultured either in a 96-well flat-bottomed microplates with 0.2 mL of complete RPMI 1640 medium or in a 24-well flat-bottomed microplates with 1 mL of complete RPMI 1640 medium. The RPMI 1640 was considered complete when it was added with 2 mM of L-glutamine (GIBCO, Carlsbad, CA, USA), 10% of FCS, 20 U/mL of penicillin, 20 μg/mL of streptomycin and 20 mM of HEPES buffer. In order to induce T cell polyclonal activation, whole PBMC cultures (1×10^6/mL) were maintained for 3 days with phytohemaglutinin (PHA, 1 μg/mL), which correspond the time of maximal T cell activation by this mitogen. To

Table 1
Characteristics of the subjects.

	Control[a] (n = 20)	GAD[b] (n = 20)
Mean age in years (sd)	29.4 (12.7)	20.7 (10.3)
Male (%)	50	40
Mean BMI (sd)[c]	25.4 ± 4.7	23.2 ± 5.8
BCG vaccine (%)	100	100
PPD skin test positive (%)[d]	85	75
CD4 T cell (%)[e]	49.3 ± 5.1	50.1 ± 4.4
CD8 T cell (%)	24.6 ± 10.3	26.1 ± 7.6
B cell (%)	18.2 ± 3.8	18.7 ± 5.1
Monocytes (%)	8.3 ± 2.2	9.6 ± 2.6

[a] Healthy individuals.
[b] Generalized anxiety disorder individuals.
[c] Body mass index.
[d] Individuals that reacted to standard dose of 5 Tuberculin units (0.1 mL) injected intradermally. It is considered a skin positive reaction when the diameter of induration is >4 mm (read 48 to 72 h later). None of subjects had active pulmonary TB.
[e] Percentage of PBMC subsets.

evaluate the immune response to recall antigen, PBMC from PPD skin test positive individuals were maintained for 5 days in the presence of PPD (15 μg/mL) (RT48, Statens Serum Institut, Copenhagen, Denmark). The endotoxin content of PPD solution was <0.02 ng/mg, as assessed by a chromogenic *Limulus* lysate assay (BioWhittaker). The effect of dopamine (DA) on either PHA- or PPD-induced immune events was evaluated following addition of DA in stress-related doses (1×10^{-7} and 1×10^{-6} M) (Maestroni and Mazzola, 2003; Cosentino et al., 2004). To evaluate the effect of glucocorticoid (GC), 1×10^{-6} M or 1×10^{-5} M of dexamethasone (DEX) (Sigma Chemicals, St Loius, MD) was added to some wells at the beginning of cell cultures. We chose these GC concentrations because they have been related to stress-induced endogenous cortisol release (Agarwal and Marshall, 1998). Importantly, both DA and DEX concentrations used here did not induce cell death (data not shown). All cells were cultured at 37 °C in a humidified 5% CO_2 incubator. Of note, the results shown in this study are representative of two experiments done in different times for each individual.

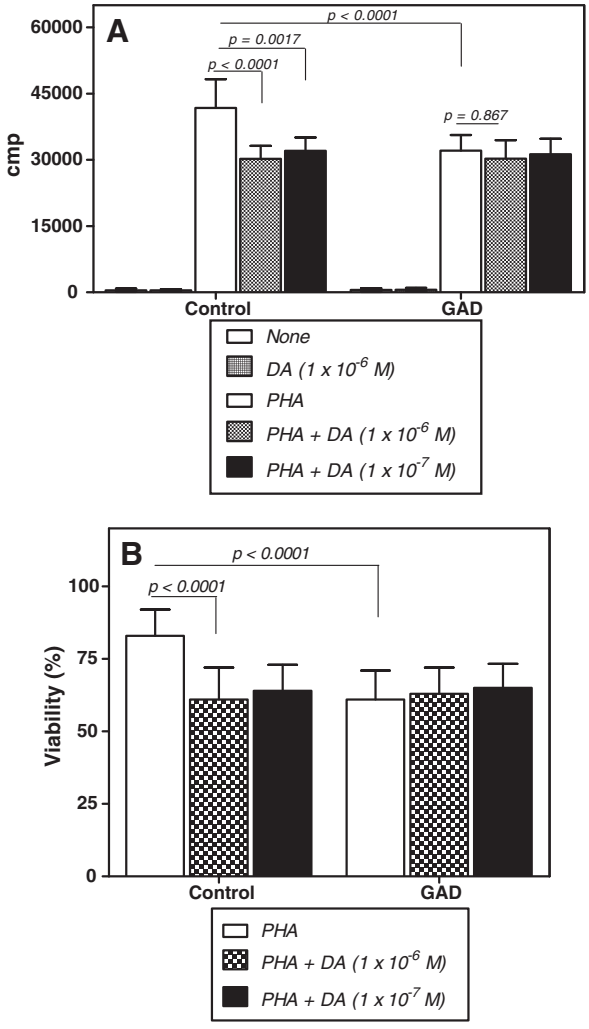

Fig. 1. The effect of dopamine (DA) on *in vitro* proliferative response and cell viability in anxious and non-anxious subjects in response to T cell polyclonal activator. PBMC (1×10^6/mL) purified from anxious (GAD, n = 20) and non-anxious (control, n = 20) individuals were kept in culture with only medium (none) or with PHA (1 μg/mL) for 3 days. The effect of stress-related dose of dopamine (DA) was evaluated by addition of 1×10^{-6} M or 1×10^{-7} M of this neurotransmitter at beginning of cultures. In (A) the proliferation was determined by the level of [^{3}H] thymidin incorporation (cpm), while in (B), the cell viability was evaluated by cell count in trypan blue. The p values are indicated in the figure.

2.5. Proliferation assay

Cultures containing approximately 1×10^6/mL of PBMC were maintained for 3 days in the presence of PHA (1 μg/mL) or for 5 days with PPD (15 μg/mL). In some wells, stress-related doses of both DA and DEX were added at beginning of the culture time. The cellular proliferation was measured by [3H] thymidine incorporation, added to cultures at 0.5 μCi/well 8 h before the end of the incubation time. The cells were harvested in glass fiber filters in an automatic cell harvester and radioactive incorporation was measured using a liquid-scintillation counter. The results were shown as mean ± sd of counts per minute (cpm).

2.6. Viability test

In order to perform the viability test, PMBC (1×10^6/mL) were seeded in a 24-well flat-bottomed microplates and stimulated or with PHA (1 μg/mL) for 3 days. In some wells, stress-related doses of both DA and DEX were added at beginning of the time cultures. At the end of culture time, the percentage of viable/unviable PBMC cultures was evaluated by trypan blue exclusion test. Briefly, 72 h after culturing, 50 μL of cell suspension was added to 50 μL of trypan blue (0.4%). The mix was left approximately 3 min at room temperature. The cells were then counted within 3 to 5 min of mixing with trypan blue using a hemocytometer. Viable (unstained) and nonviable (stained) cells and total (both) were counted. To verify the percentage (%) of viable cells, we applied the following equation: viable cells (%) = total number of viable cells per mL/total number of cells per mL × 100.

2.7. Cytokine evaluation

In order to dose the *in vivo* and *in vitro* cytokine contents, the plasma samples and the supernatants, collected from either PHA- or PPD-stimulated PBMC cultures with or without DA, were submitted to cytokine measurement by OptEIA ELISA kits (BD, Pharmigen, San Diego), according to manufacturer's protocol. Furthermore, we assayed the cytokine contents from these cell cultures following addition of stress-related dose of DEX. Briefly, each ELISA was performed using pairs of mAbs directed to human IL-2, IL-1β, IL-6,

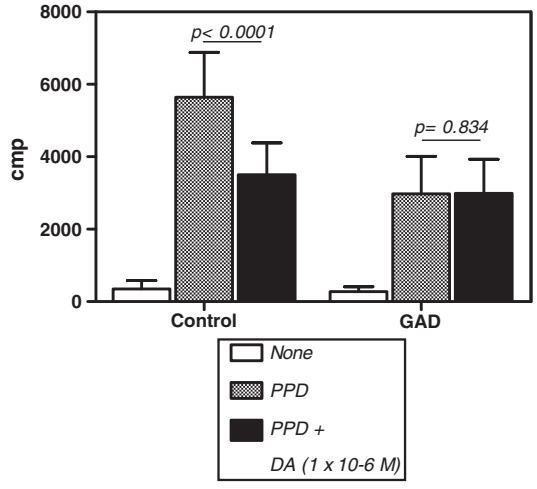

Fig. 2. The effect of dopamine (DA) on *in vitro* PPD-induced proliferative response in anxious and non-anxious subjects. PBMC (1×10^6/mL) purified from anxious (GAD, n = 12) and non-anxious (control, n = 15) individuals were kept in culture with only medium (none) or with PPD (15 μg/mL) for 5 days. The effect of dopamine (DA) was evaluated by addition of 1×10^{-6} M of this neurotransmitter at beginning of cultures. The p values are indicated in the figure.

IL-10, IL-4, IL-21, TNF-α, IFN-γ, TGF-β, and IL-17. The reaction was revealed with streptavidin-horseradish peroxidase, using 3,3′,5,5′-tetramethylbenzidine (TMB) as substrate. Recombinant human cytokine ranging from 10 to 500 pg/mL was used to construct standard curves.

2.8. Statistical analysis

The statistical test for comparison between groups was the nonparametric Kruskal–Wallis test with multiple comparisons (i. e. immunological events induced in the presence of PHA, PHA/DA and PHA/DA/DEX from healthy controls and GAD individuals). Comparison between two groups was performed using the nonparametric Mann–Whitney *U* test. The Wilcoxon signed rank test was used to assess changes within the group. The significance in all experiments was defined as $p<0.05$.

3. Results

3.1. Impact of dopamine (DA) on proliferation of peripheral blood mononuclear cells stimulated with either T cell polyclonal activator or recall antigen

To investigate the impact of chronic stress on some immune events, samples of peripheral blood were drawn from healthy (control) and anxious individuals (GAD). As shown in the table, any statistical difference was observed concerning the parameters analyzed between control and GAD individuals, even the percentage of $CD4^+$ and $CD8^+$ T cells, B cells ($CD19^+$) and monocytes ($CD14^+$). Furthermore, no participant showed any detectable sign of current infection unless 1 month before blood sampling.

The first immune event analyzed in our study was the PHA-induced T cell proliferation. As shown in Fig. 1A, the extent of T cell proliferation was statistically lower in cultures from anxious

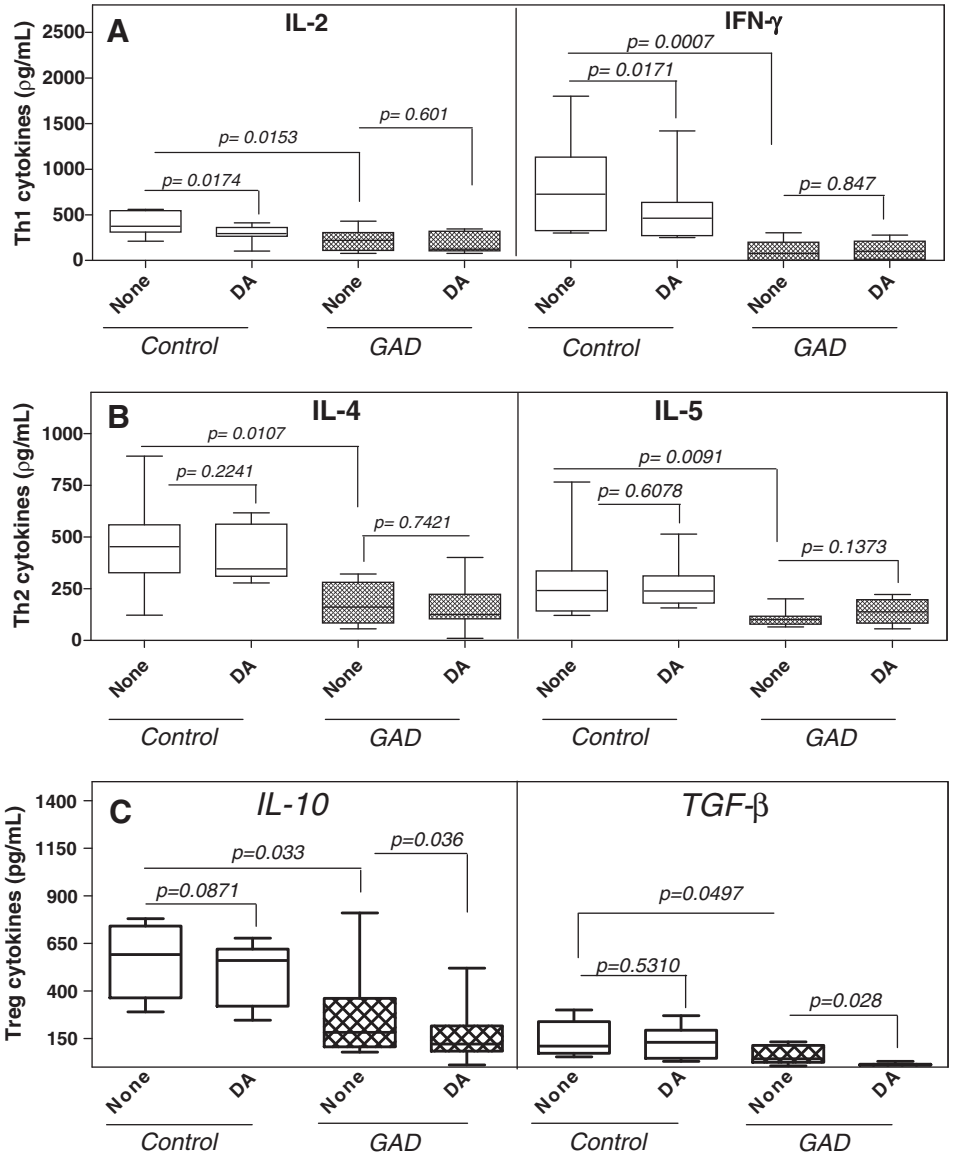

Fig. 3. Th1/Th2 and Treg cytokines in anxious and control individuals in the presence or absence of dopamine (DA). The polyclonal T cell activator (PHA, 1 μg/mL), alone or with DA (1×10^{-6} M), was added to PBMC cultures (1×10^6/mL) derived from anxious (GAD, n = 20) and non-anxious (control, n = 20) individuals and their supernatants were collected after 3 days. In the figure, [A] indicates Th1 (IL-2 and IFN-γ), [B] shows Th2 (IL-4 and IL-5), and [C] Treg (IL-10 and TGF-β) cytokines evaluated by ELISA. In the figure, the horizontal bars within boxes correspond to the median; box limits correspond to 25th and 75th percentiles and vertical lines indicate the range. The mean values of control and anxious groups were compared and the p values are indicated in the figure.

individuals, as compared with the control group. In both groups, DA alone neither induced any detectable cell proliferation nor caused cell death (data not shown). On the other hand, although DA had been able to diminish the proliferation in control cell cultures, this neurotransmitter did not change it in the GAD group (Fig. 1A). The lower cell proliferation in the anxious-derived PBMC cultures might be related to higher cell death, since the percentage of viable cells was significantly lower in the anxious group, as compared with control cultures (Fig. 1B). Nevertheless, while the addition of DA significantly reduced the cell viability in PHA-activated cell cultures from non-anxious individuals (control), this neurotransmitter did not change the viable cell yield in anxious-derived PHA-activated PBMC (Fig. 1B). Of note, the similar pattern concerning cell proliferation and viability was observed even after addition of DA concentration 10 lower (Fig. 1).

Concerning the response to recall antigen PPD, the level of thymidine uptake was also significantly lower in GAD-derived cell cultures ($p = 0.017$) (Fig. 2). As observed by PHA-activated cell cultures, the extent of PPD-induced lymphoproliferation was reduced by DA only in the control group (Fig. 2).

3.2. Impact of dopamine (DA) on cytokine profile in cell cultures from control and anxious individuals

The type of acquired immune response is mainly determined by the cytokine network produced by activated T cells. Therefore, we evaluated the cytokine profile of non-activated or activated cell cultures from the two experimental groups. Of note, no detectable spontaneous release of any cytokine was observed in both experimental groups without PHA or DA (data not shown). Following DA addition, very low, but detected, levels of TNF-α (41 ± 22 pg/mL) and IL-6 (37 ± 21 pg/mL) were observed only in anxious-derived non-activated cell cultures.

Following PHA stimulus, however, the production of the classical Th1 cytokines, IL-2 and IFN-γ, was significantly higher in cell cultures from control individuals as compared with GAD ones (Fig. 3A). DA, on the other hand, reduced significantly the level of IL-2 and IFN-γ only in cell cultures from the control group. Concerning IL-4 and IL-5, their levels were also lower in PHA-activated cell cultures from anxious individuals (Fig. 3B) and DA addition did not modify them neither in control nor in anxious-derived cell cultures. Among the anti-inflammatory cytokines, although both IL-10 and TGF-β production had been lower in anxious-derived PHA-activated cell cultures, the addition of DA was able to reduce the production of these anti-inflammatory cytokines in the anxious group (Fig. 3C). Finally, we performed the quantification of cytokines related to Th17 phenotype, particularly IL-17. As demonstrated in Fig. 4, the production of not only IL-17 but also IL-21 and TNF-α were significantly higher in the anxious group. With regard to DA, this neurotransmitter significantly up-regulated the production of TNF-α and IL-6 from both control and GAD groups (Fig. 4). Interestingly, DA enhanced IL-17 and IL-21 production only in PHA-activated cell cultures from anxious subjects.

When we analyzed the cytokine profile in PPD-activated PBMC cultures, the IFN-γ levels were significantly lower in the GAD group and it was not changed by DA (Fig. 5). On the other hand, IL-17 and TNF-α release in response to PPD was significantly up-regulated by DA addition.

Finally, when we analyzed the systemic cytokine profile, IL-1β (41 ± 18 pg/mL × 8 ± 10 pg/mL, $p < 0.0001$) and IL-6 (76 ± 31 pg/mL × 7 ± 12 pg/mL, $p < 0.0001$) were significantly higher in the anxious group. Concerning the other cytokines, their levels were undetectable or extremely low and the differences did not reach any statistical difference.

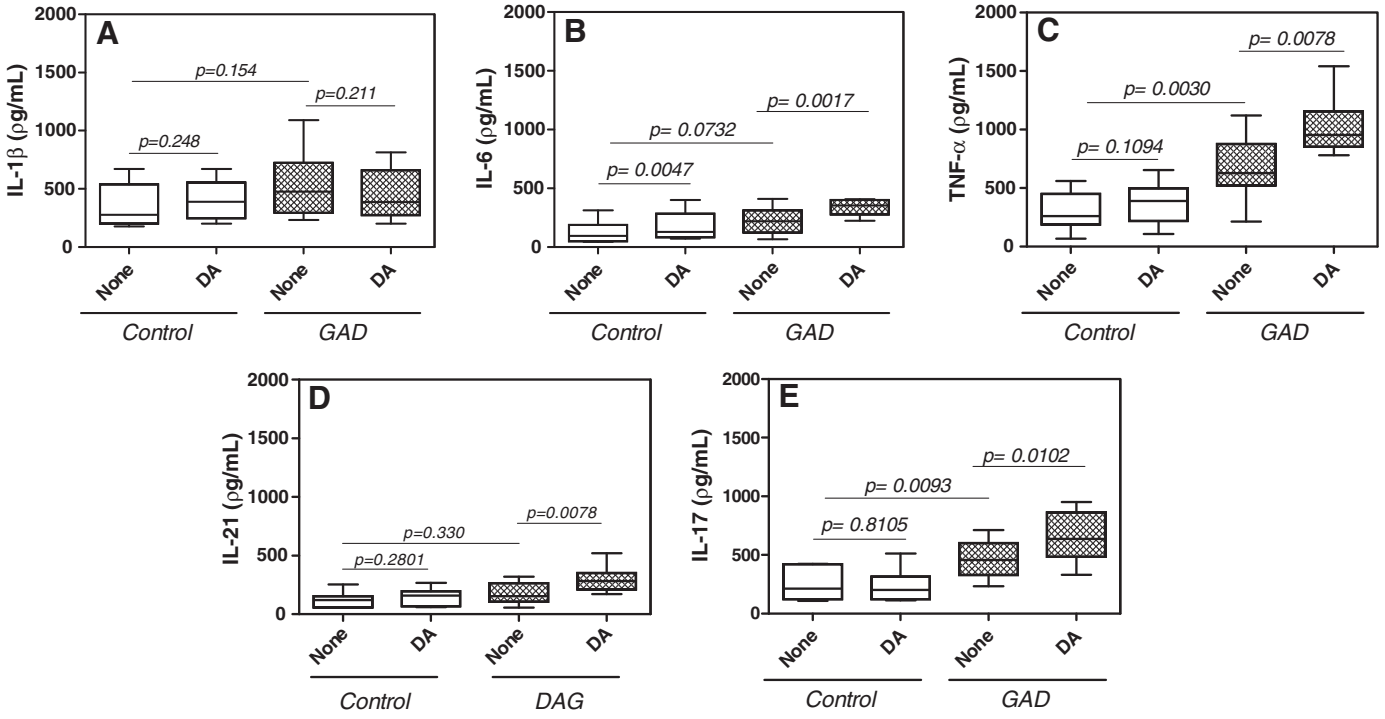

Fig. 4. The impact of dopamine (DA) on Th17 cytokines in anxious and control individuals. PBMC (1×10^6/mL) obtained from non-anxious (A, n = 20) and anxious (B, n = 20) individuals were maintained in cultures for 3 days in the presence of polyclonal T cell activator (PHA, 1 μg/mL) alone or with stress-related dose of DA (1×10^{-6} M). At the end of 3 days, the supernatants were collected and IL-1β (A), IL-6 (B), TNF-α (C), IL-21 (D), and (E) IL-17 were evaluated by ELISA. In the figure, the horizontal bars within boxes correspond to the median; box limits correspond to 25th and 75th percentiles and vertical lines indicate the range. The mean values of control and anxious groups were compared and the p values are indicated in the figure.

3.3. Impact of glucocorticoid on the production of IL-21, TNF-α and IL-17 in cultures from control and GAD individuals

Finally, our last objective was to evaluate the effect of a stress-related dose of glucocorticoid (GC) on IL-21, TNF-α and IL-17 production by PHA-activated cell cultures in the presence of DA. As shown in Fig. 5, while DEX (1×10^{-6} M) reduced IL-21, TNF-α and IL-17 levels in control cell cultures, this glucocorticoid was not able to down-regulate these pro-inflammatory cytokine production in the anxious group. This GC-insensitivity was also observed in PHA-activated cell cultures even after addition of DEX concentration 10 higher (1×10^{-5} M) (Fig. 6A). The presence of 1×10^{-7} M of DA did not change this unresponsiveness to DEX (data not shown). Finally, the lower sensitivity to DEX was also observed in the GAD-derived PPD-activated cell cultures (Fig. 6B).

4. Discussion

Chronic stress damages the immune function and, consequently, elevates the risk of infectious diseases (Boscarino, 2004; Koh and Lee, 2004; Sareen et al., 2005; Schneiderman et al., 2005; Zhou et al., 2005; Godbout and Glaser, 2006; Arranz et al., 2007). As stressful life events enhance the peripheral concentration of dopamine (DA) (Sinha, 2008), the objective of the present study was to evaluate the impact of a stress-related dose of DA on the functional profile of T cells from patients with generalized anxiety disorder (GAD). To our knowledge, this is the first report that investigates the effect of DA on individuals with GAD.

Some studies have demonstrated that DA inhibits polyclonally-activated T cell proliferation in cell cultures from healthy individuals (Saha et al., 2001a,b). In our system, the *in vitro* proliferative response induced by either T cell polyclonal activator or PPD was higher in non-anxious individuals, but, differently from GAD cell cultures, it was reduced following DA addition. The reason by which DA attenuated proliferative response in PHA-activated cell cultures from control individuals could be related to its ability to reduce both cell viability and IL-2 release. Study by Ghosh et al. (2003) demonstrated that DA, through type I DARs, reduces the expression of tyrosine kinases Lck and Fyn in polyclonally-activated T cells. Both enzymes are TCR-dependent signaling molecules involved in IL-2 production (Mills et al., 1993). Furthermore, Cosentino et al. (2004) also demonstrated that type I DARs stimulation by stress-related doses of DA induced apoptotic T cell death through oxidative stress in cell cultures from healthy individuals. Although we have decided to show only the data of 3 days of cell cultures (the peak of T cell response to mitogen), a time-course evaluation also demonstrated lower cell viability 24 and 48 h after DA addition in non-anxious polyclonally-activated cell cultures (data not shown). To date, we do not know why these phenomena were not observed in GAD patients, but this could be related to the lower type I DARs expression in anxious-derived T cells. At the moment we are dedicating our efforts to investigate, by cytometry, the expression pattern of DARs in the PBMC from anxious patients.

It is known that an efficient host defense against invading pathogenic microorganisms is coordinated by the action of the highly heterogeneous effector T cells. Th1-mediated response is characterized by the production of IL-2 and IFN-γ, and is involved mainly in cellular immunity against intracellular microorganisms (Gutcher and Becher, 2007). On the other hand, human Th2 cells produce IL-4, IL-5, and IL-13 and are required to control helminthes by stimulating the growth and activation of mast cells and eosinophils, as well as differentiating B cells into IgE secreting cells (Ekkens et al., 2003). Study by Ghosh et al. (2003) demonstrated that DA inhibited the anti-CD3-induced release of both Th1 and Th2 cytokines from healthy individuals. In our study, however, we observed that in control cell cultures DA addition only down-regulated Th1 cytokines. A possible explanation for the absence of DA effect on Th2 phenotype in the control group could be the exclusion in our study of all individuals with high tendency to develop Th2 pattern, such as subjects with chronic atopic disorders, like asthma and atopic dermatitis. Concerning the anxious individuals, the production of Th1 cytokines by polyclonally-activated T cells was significantly lower than control cultures, and it was not altered by DA addition. The Th1 phenotype deficiency in GAD individuals, which was already recently published by our group (Vieira et al., 2010), may clearly provide a reason by which anxious individuals have greater susceptibility to infectious diseases and malignancies (Cohen et al., 1993; Takkouche et al., 2001; Ben-Eliyahu, 2003; Aviles et al., 2004). It is known that protection against pulmonary TB is related to the ability of humans to develop a

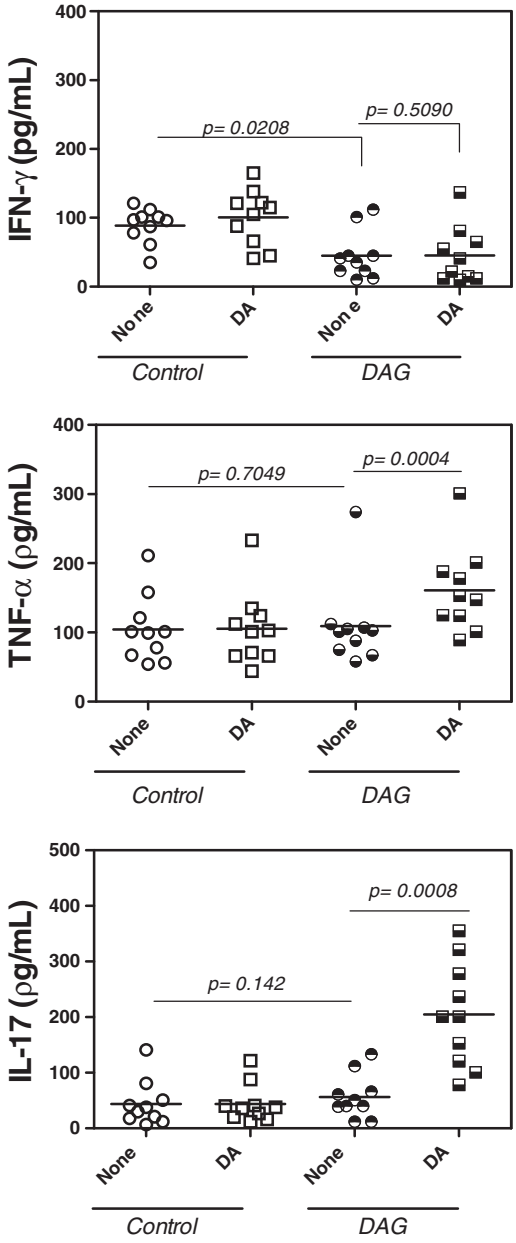

Fig. 5. The impact of dopamine (DA) on PPD-induced cytokine production in anxious and control individuals. PBMC (1×10^6/mL) obtained from non-anxious (control, n = 10) and anxious (DAG, n = 10) individuals were maintained in cultures for 5 days in the presence of PPD (15 μg/mL) alone or with stress-related dose of DA (1×10^{-6} M). At the end of 5 days, the supernatants were collected and IL-10, IFN-γ, and IL-17 were evaluated by ELISA. In the figure, the horizontal bars correspond to the median. The mean values of both groups were compared and the p values are indicated in the figure.

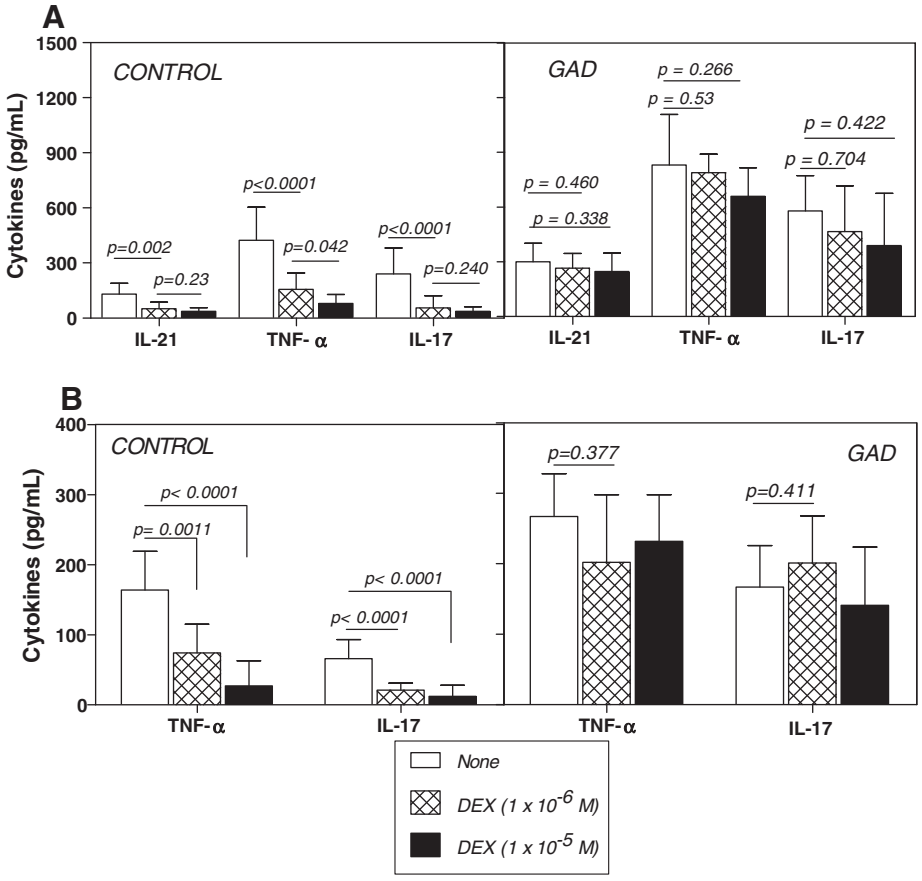

Fig. 6. The effect of glucocorticoid on DA-induced cytokine profile from anxious and control individuals. DA (1×10^{-6} M) was added to the PBMC (1×10^{6}/mL) cultures, obtained from anxious (GAD) and non-anxious (control) individuals, and maintained in (A) for 3 days in the presence of polyclonal T cell activator (PHA, 1 μg/mL) or in (B) for 5 days in the presence of PPD (15 μg/mL). To evaluate the effect of glucocorticoid on the DA-induced cytokines in PHA-activated cell cultures, we added stress-related doses of dexamethasone (DEX, at 1×10^{-6} M or 1×10^{-5} M) at the beginning of the cultures. After 3 days (PHA-activated) or 5 days (PPD-stimulated), the supernatants were collected and the cytokines were evaluated by ELISA. The mean values without and with DEX were compared and the p values are indicated in the figure.

Th1 immune response (Marchant et al., 1999; Djuardi et al., 2010). In fact, in our present study, we observed a lower IFN-γ production by PPD-activated cell cultures from anxious individuals as compared with control ones.

In our study, the most interesting result was the effect of DA on Th17-related cytokines. Th17 cells produce not only IL-17 (also referred to as IL-17A) but also IL-21, IL-1β, IL-6 and TNF-α, and play, with the help of Th1 cells, important roles in the clearance of certain microbes, particularly extracellular bacteria and fungi (Jovanovic et al., 1998; Matsuzaki and Umemura, 2007) mainly by inducing chemotaxis of neutrophils to sites of infection (Gutcher and Becher, 2007). Nevertheless, a dominate Th17 phenotype has also been related to development of inflammatory disorders (Miossec, 2009). Our results demonstrated that activated cell cultures from anxious individuals produced higher Th17 related cytokines, particularly IL-17, in response to T cell polyclonal activator. Interestingly, the *in vitro* DA addition amplified Th17-related cytokine production from both PHA- and PPD-stimulated GAD cell cultures. This same general pattern in GAD group was also observed in earlier culture times (24 and 48 h) (data not shown). In schizophrenic patients, where hyperdopaminergic activity has been emphasized (Birtwistle and Baldwin, 1998), a significantly higher expression of DA D3 receptors has been described in T cells (Ilani et al., 2001; Boneberg et al., 2006), and it appears to correlate with increased plasma levels of IL-6, IL-1β and TNF-α (Maes et al., 1995). In our study, the dosage of the cytokines in the plasma showed low but significantly higher IL-1β and IL-6 production in GAD patients, as compared with control ones. In agreement with this last result, it is well established that anxiety is related to increased circulating levels of IL-6, TNF-α, IL-1β and IL-8 (Affleck et al., 1997; Wilson et al., 1999; Muller and Schwarz, 2002; Sutherland et al., 2003; von Känel et al., 2007; Wessa and Rohleder, 2007; Gill et al., 2008; Lemieux et al., 2008; Gill et al., 2009). As revised by Khairova et al. (2009), pro-inflammatory cytokines, such as TNF-α, are associated with increased risk of anxiety and depression. Although dopamine has been directly implicated in the mood disorders (Durant et al., 2010), this neurotransmitters could indirectly elevate the risk of anxiety by up-regulating the production of these cytokines in anxious subjects.

In contrast to control group, the DA-enhanced Th17 cytokine production in either PHA- or PPD-activated anxious-derived cell cultures was more resistant to stress-related doses of glucocorticoid, known to inhibit Th1- and Th17-mediated immune response normal individuals (Ashwell et al., 2000; Vazquez-Tello et al., 2010).

Glucocorticoid (GC) hormones, particularly cortisol, can suppress inflammatory cytokine production by downregulating the activity of the transcription factor nuclear factor kappa B (NFκB) (De Bosscher et al., 2000). Although we did not measure the plasma or saliva cortisol, in chronically stressed patients the hypothalamic–pituitary–adrenal (HPA) axis is often hyperactivated, resulting in the persistent release of high levels of cortisol (Arborelius et al., 1999; Holsboer, 2000). It is believed that, after long periods of exposure to stress, glucocorticoid resistance arises, primarily through a reduction of both sensitivity and expression of GC receptors. This GC resistance compromises the

physiological regulation of inflammatory responses by HPA axis, leading to a high basal immune activation state (Gotovac et al., 2003; De Kloet et al., 2007).

Finally, besides GC resistance demonstrated here, the excessive Th17 response in anxious individuals might also be a consequence of an insufficient immunoregulation by regulatory T cells, whose cytokines were down-regulated by DA addition. Tight regulation of Th17 cells is required for effective control and avoidance of immunopathological phenomena (Costantino et al., 2008). Several lines of evidence have demonstrated that fine immunoregulation is largely performed by a pool of regulatory T cells, including those producing IL-10 and TGF-β cytokines (Vignali et al., 2008). In our system, GAD individuals showed lower IL-10 and TGF-β levels in activated cell cultures, as compared with control cultures, and DA addition reduced significantly the production of them only in cell cultures from anxious individuals. Recent evidence indicates that stimulation of type I DARs also favors the polarization of naïve CD4+ T-cells toward Th17 cells (Birtwistle and Baldwin, 1998; Nakano et al., 2009). Because Th17 and Treg cells are involved in autoimmunity acting as auto-aggressive and protective cells respectively, it is likely that type I DARs expressed on T-cells are involved in the interface between autoimmunity and health. Therefore, our next step is to identify, through selective DARs agonists and antagonists, the involvement of different kinds of DARs on these immune disturbances enhanced by stress-related doses of DA in anxious individuals.

Taken together, all results indicate that cells derived from anxious individuals show Th1, Th2 and Treg responses damage associated with elevated Th17 response that was amplified by stress-related dose of dopamine (DA). The ability of DA to favor Th17-related cytokines may be a reason for the higher susceptibility of anxious individuals to autoimmune diseases.

Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.jneuroim.2011.06.009.

References

Affleck, G., Urrows, S., Tennen, H., Higgins, P., Pav, D., Aloisi, R., 1997. A dual pathway model of daily stressor effects on rheumatoid arthritis. Ann. Behav. Med. 19, 161–170.

Agarwal, S.K., Marshall, G.D., 1998. Glucocorticoid-induced type 1/type 2 cytokine alterations in humans: a model for stress-related immune dysfunction. J. Interferon Cytokine Res. 18, 1059–1068.

Andrade, L., Walters, E.E., Gentil, V., Laurenti, R., 2002. Prevalence of ICD-10 mental disorders in a catchment area in the city of São Paulo. Brazil. Soc. Psychiatry Psychiatr. Epidemiol. 37, 316–325.

Arborelius, L., Owens, M.J., Plotsky, P.M., Nemeroff, C.B., 1999. The role of corticotropin-releasing factor in depression and anxiety disorders. J. Endocrinol. 160, 1–12.

Arranz, L., Guayerbas, N., De la Fuente, M., 2007. Impairment of several immune functions in anxious women. J. Psychosom. Res. 62 (1), 1–8.

Ashwell, J.D., Lu, F.W., Vacchio, M.S., 2000. Glucocorticoids in T cell development and function. Annu. Rev. Immunol. 18, 309.

Aviles, H., Johnson, M.T., Monroy, F.P., 2004. Effects of cold stress on spleen cell proliferation and cytokine production during chronic *Toxoplasma gondii* infection. Neuroimmunomodulation 11 (2), 93–102.

Bencsics, A., Sershen, H., Baranyi, M., Hashim, A., Lajtha, A., Vizi, E.S., 1997. Dopamine, as well as norepinephrine, is a link between noradrenergic nerve terminals and splenocytes. Brain Res. 761, 236–243.

Ben-Eliyahu, S., 2003. The promotion of tumor metastasis by surgery and stress: immunological basis and implications for psychoneuroimmunology. Brain Behav. Immun. 17 (Suppl. 1), S27–S36.

Ben-Jonathan, N., Hnasko, R., 2001. Dopamine as a prolactin (PRL) inhibitor. Endocr. Rev. 22, 724–763.

Birtwistle, J., Baldwin, D., 1998. Role of dopamine in schizophrenia and Parkinson's disease. Br. J. Nurs. 7, 832–834.

Boneberg, E.M., von Seydlitz, E., Propster, K., Watzl, H., Rockstroh, B., Illges, H., 2006. D3 dopamine receptor mRNA is elevated in T cells of schizophrenic patients whereas D4 dopamine receptor mRNA is reduced in CD4+-T cells. J. Neuroimmunol. 173, 180–187.

Boscarino, J.A., 2004. Posttraumatic stress disorder and physical illness: results from clinical and epidemiologic studies. Ann. N. Y. Acad. Sci. 1032, 141–153.

Cenci, M.A., 2007. Dopamine dysregulation of movement control in L-DOPA-induced dyskinesia. Trends Neurosci. 30, 236–243.

Cohen, F., Kemeny, M.E., Kearney, K.A., Zegans, L.S., Neuhaus, J.M., Conant, M.A., 1993. Persistent stress as a predictor of genital herpes recurrence. Arch. Intern. Med. 159, 2430–2436.

Cosentino, M., Rasini, E., Colombo, C., Marino, F., Blandini, F., Ferrari, M., Samuele, A., Lecchini, S., Nappi, G., Frigo, G., 2004. Dopaminergic modulation of oxidative stress and apoptosis in human peripheral blood lymphocytes: evidence for a D1-like receptor-dependent protective effect. Free Radic. Biol. Med. 36 (10), 1233–1240.

Costantino, C.M., Baecher-Allan, C.M., Hafler, D.A., 2008. Human regulatory T cells and autoimmunity. Eur. J. Immunol. 38, 921–924.

Dayan, P., 2009. Dopamine, reinforcement learning, and addiction. Pharmacopsychiatry 42 (1), S56–S65.

De Bosscher, K., Vanden Berghe, W., Vermeulen, L., Plaisance, S., Boone, E., Haegeman, G., 2000. Glucocorticoids repress NF-kappa B driven genes by disturbing the interaction of p65 with the basal transcription machinery, irrespective of co-activator levels in the cell. Proc. Natl. Acad. Sci. U.S.A. 97, 3919–3924.

De Kloet, C.S., Vermetten, E., Bikker, A., Meulman, E., Geuze, E., Kavelaars, A., Westenberg, H.G., Heijnen, C.J., 2007. Leukocyte glucocorticoid receptor expression and immuneregulation in veterans with and without post-traumatic stress disorder. Mol. Psychiatry 12, 443–453.

Djuardi, Y., Sartono, E., Wibowo, H., Supali, T., Yazdanbakhsh, M., 2010. A longitudinal study of BCG vaccination in early childhood: the development of innate and adaptive immune responses. PLoS One 5 (11), 14066 19.

Durant, C., Christmas, D., Nutt, D., 2010. The pharmacology of anxiety. Curr. Top. Behav. Neurosci. 2, 303–330.

Ekkens, M.J., Liu, Z., Liu, Q., Whitmire, J., Xiao, S., Foster, A., Pesce, J., VanNoy, J., Sharpe, A.H., Urban, J.F., Gause, W.C., 2003. The role of OX40 ligand interactions in the development of the Th2 response to the gastrointestinal nematode parasite *Heligmosomoides polygyrus*. J. Immunol. 170 (1), 384–393.

Ferrari, M., Cosentino, M., Marino, F., Bombelli, R., Rasini, E., Lecchini, S., Frigo, G., 2004. Dopaminergic D1-like receptor-dependent inhibition of tyrosine hydroxylase mRNA expression and catecholamine production in human lymphocytes. Biochem. Pharmacol. 67, 865–873.

Ghosh, M.C., Mondal, A.C., Basu, S., Banerjee, S., Majumder, J., Bhattacharya, D., Dasgupta, P.S., 2003. Dopamine inhibits cytokine release and expression of tyrosine kinases, Lck and Fyn in activated T cells. Int. Immunopharmacol. 3, 1019–1026.

Gill, J.M., Vythilingam, M., Page, G., 2008. Low cortisol, high DHEA, and high levels of TNF-a and IL-6 in women with PTSD. J. Trauma. Stress 21 (6), 530–539.

Gill, J.M., Saligan, L., Woods, S., Page, G., 2009. PTSD is associated with an excess of inflammatory immune activities. Perspect. Psychiatr. Care 45 (4), 262–277.

Godbout, J.P., Glaser, R., 2006. Stress-induced immune dysregulation: implications for wound healing, infectious disease and cancer. J. Neuroimmune Pharmacol. 1 (421–427), 2006.

Gotovac, K., Sabioncello, A., Rabatic, S., Berki, T., Dekaris, D., 2003. Flow cytometric determination of glucocorticoid receptor (GCR) expression in lymphocyte subpopulations: lower quantity of GCR in individuals with post-traumatic stress disorder (PTSD). Clin. Exp. Immunol. 131, 335–339.

Gutcher, I., Becher, B., 2007. APC-derived cytokines and T cell polarization in autoimmune inflammation. J. Clin. Invest. 117, 1119–1125.

Hamilton, M., 1959. The assessment of anxiety states by rating. Br. J. Med. Psychol. 32, 50–55.

Holsboer, F., 2000. The corticosteroid receptor hypothesis of depression. Neuropsychopharmacology 23, 477–501.

Ilani, T., Ben-Shachar, D., Strous, R.D., Mazor, M., Sheinkman, A., Kotler, M., Fuchs, S., 2001. A peripheral marker for schizophrenia: Increased levels of D3 dopamine receptor mRNA in blood lymphocytes. Proc. Natl. Acad. Sci. U.S.A. 98, 625–628.

Jovanovic, D.V., Di Battista, J.A., Martel-Pelletier, J., Jolicoeur, F.C., He, Y., Zhang, M., Mineau, F., Pelletier, J.P., 1998. IL-17 stimulates the production and expression of pro-inflammatory cytokines, IL-beta and TNF-alpha, by human macrophages. J. Immunol. 160, 3513–3521.

Kang, D., Davidson, R.J., Coe, C.L., Wheeler, R.E., Tomarken, A.J., Erschler, W.B., 1991. Frontal brain asymmetry and immune function. Behav. Neurosci. 105, 860–869.

Kessler, R.C., Chiu, W.T., Demler, O., Merikangas, K.R., Walters, E.E., 2005. Prevalence, severity, and comorbidity of 12-month DSM-IV disorders in the National Comorbidity Survey Replication. Arch. Gen. Psychiatry 62 (6), 617–627.

Khairova, R.A., Machado-Vieira, R., Du, J., Manji, H.K., 2009. A potential role for pro-inflammatory cytokines in regulating synaptic plasticity in major depressive disorder. Int. J. Neuropsychopharmacol. 12, 561–578.

Khouzam, H.R., 2009. Anxiety disorders: guidelines for effective primary care. Part 1: diagnosis. Consultant 49 (3).

Kirillova, G.P., Hrutkay, R.J., Shurin, M.R., Shurin, G.V., Tourkova, I.L., Vanyukov, M.M., 2008. Dopamine receptors in human lymphocytes: radioligand binding and quantitative RT PCR assays. J. Neurosci. Methods 174, 272–280.

Koh, K.B., Lee, Y., 2004. Reduced anxiety level by therapeutic interventions and cell-mediated immunity in panic disorder patients. Psychother. Psychosom. 73 (5), 286–292.

Kohn, P.M., Kantor, L., DeCicco, T.L., Beck, A.T., 2008. The Beck Anxiety Inventory-Trait (BAIT): a measure of dispositional anxiety not contaminated by dispositional depression. J. Pers. Assess. 90, 499–506.

Lemieux, A.M., Coe, C.L., 1995. Abuse-related post-traumatic stress disorder: evidence of chronic neuroendocrine activation in women. Psychosom. Med. 57, 105–115.

Lemieux, A., Coe, C.L., Carnes, M., 2008. Symptom severity predicts degree of T cell activation in adult women following childhood maltreatment. Brain Behav. Immun. 22 (6), 994–1003.

Maes, M., Bosmans, E., Calabrese, J., Smith, R., Meltzer, H.Y., 1995. Interleukin-2 and interleukin-6 in schizophrenia and mania: effects of neuroleptics and mood stabilizers. J. Psychiatr. Res. 29, 141–152.

Maestroni, G.J., Mazzola, P., 2003. Langerhans cells beta 2-adrenoceptors: role in migration, cytokine production, Th priming and contact hypersensitivity. J. Neuroimmunol. 144, 91–99.
Marchant, A., Goetghebuer, T., Ota, M.O., Wolfe, I., Ceesay, S.J., De Groote, D., Corrah, T., Bennett, S., Wheeler, J., Huygen, K., Aaby, P., McAdam, K.P., Newport, M.J., 1999. Newborns develop a Th1-type immune response to *Mycobacterium bovis* bacillus Calmette-Guérin vaccination. J. Immunol. 163, 2249–2255.
Matsuzaki, G., Umemura, M., 2007. IL-17 as an effetor molecule of innate and acquired immunity against infections. Microbiol. Immunonol. 51, 1139–1147.
McKenna, F., McLaughlin, P.J., Lewis, B.J., Sibbring, G.C., Cummerson, J.A., Bowen-Jones, D., Moots, R.J., 2002. Dopamine receptor expression on human T- and B lymphocytes, monocytes, neutrophils, eosinophils and NK cells: a flow cytometric study. J. Neuroimmunol. 132, 34–40.
Mills, G.B., Schmandt, R., Gibson, S., Leung, B., Hill, M., May, C., Shi, Y.F., Branch, D.R., Radvanyi, L., Truitt, K.E., et al., 1993. Transmembrane signaling by the interleukin-2 receptor: progress and conundrums. Semin. Immunol. 5, 345–364.
Miossec, P., 2009. IL-17 and Th17 cells in human inflammatory diseases. Microbiol. Infect. 11, 625–630.
Muller, N., Schwarz, M.J., 2002. Immunology in anxiety and depression. In: Kasper, S., den Boer, J.A., Sitsen, J.M.A. (Eds.), Handbook of Depression and Anxiety. Marcel Dekker, New York, pp. 267–288.
Muller, N., Riedel, M., Gruber, R., Ackenheil, M., Schwarz, M.J., 2000. The immune system and schizophrenia. An integrative view. Ann. N. Y. Acad. Sci. 917, 456–467.
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.
Nakano, K., Matsushita, S., Saito, K., Yamaoka, K., Tanaka, Y., 2009. Dopamine as an immune-modulator between dendritic cells and T cells and the role of dopamine in the pathogenesis of rheumatoid arthritis. Nihon Rinsho Meneki Gakkai Kaishi 32, 1–6.
Potvin, S., Grignon, S., Marchand, S., 2009. Human evidence of a supra-spinal modulating role of dopamine on pain perception. Synapse 63, 390–402.
Riedel, M., Spellmann, I., Schwarz, M.J., Strassnig, M., Sikorski, C., Möller, H.J., Müller, N., 2007. Decreased T cellular immune response in schizophrenic patients. J. Psychiatr. Res. 41, 3–7.
Saha, B., Mondal, A.C., Basu, S., Dasgupta, P.S., 2001a. 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.
Saha, B., Mondal, A.C., Majumder, J., Basu, S., Dasgupta, P.S., 2001b. Physiological concentrations of dopamine inhibit the proliferation and cytotoxicity of human CD4+CD8+ T cells in vitro: a receptor-mediated mechanism. Neuroimmunomodulation 9, 23–33.
Sareen, J., Cox, B.J., Clara, I., Asmundson, G.J., 2005. The relationship between anxiety disorders and physical disorders in the U.S. National Comorbidity Survey. Depress. Anxiety 21, 193–202.
Sarkar, C., Basu, B., Chakroborty, D., Dasgupta, P.S., Basu, S., 2010. The immunoregulatory role of dopamine: an update. Brain Behav. Immun. 24, 525–528.
Schneiderman, N., Ironson, G., Siegel, S.D., 2005. Stress and health: psychological, behavioral, and biological determinants. Annu. Rev. Clin. Psychol. 1, 607–628.
Sibley, D.R., Monsma Jr., F.J., Shen, Y., 1993. Molecular neurobiology of dopaminergic receptors. Int. Rev. Neurobiol. 35, 391–415.
Sinha, R., 2008. Chronic stress, drug use, and vulnerability to addiction. Ann. N. Y. Acad. Sci. 1141, 105–130.
Spitzer, R.L., Williams, J.B., Gibbon, M., First, M.B., 1992. The Structured Clinical Interview for DSM-III-R (SCID). I: history, rationale, and description. Arch. Gen. Psychiatry 49 (8), 624–629.
Strange, P.G., 1993. New insights into dopamine receptors in the central nervous system. Neurochem. Int. 22, 223–236.
Strauman, T.J., Woods, T.E., Schneider, K.L., Kwapil, L., Coe, C.L., 1993. Self-regulatory cognition and immune reactivity: idiographic success and failure feedback effects on the natural killer cell. Brain, Beh., Imm. 18, 544–554.
Strous, R.D., Shoenfeld, Y., 2006. Schizophrenia. Autoimmunity and immune system dysregulation: a comprehensive model updated and revisited. J. Autoimmun. 27, 71–80.
Sutherland, A.G., Alexander, D.A., Hutchison, J.D., 2003. Disturbance of pro-inflammatory cytokines in post-traumatic psychopathology. Cytokine 24, 219–225.
Takkouche, B., Regueira, C., Gestal-Otero, J.J., 2001. A cohort study of stress and the common cold. Epidemiology 12, 345–349.
Vazquez-Tello, A., Semlali, J., Chakir, J.G., Martin, D.Y., Leung, D.H., Eidelman, Q. Hamid, 2010. Induction of glucocorticoid receptor-beta expression in epithelial cells of asthmatic airways by T-helper type 17 cytokines. Clin. Exp. Allergy 40, 1312–1322.
Vieira, M.M., Ferreira, T.B., Pacheco, P.A., Barros, P.O., Almeida, C.R., Araújo-Lima, C.F., Silva-Filho, R.G., Hygino, J., Andrade, R.M., Linhares, U.C., Andrade, A.F., Bento, C.A., 2010. Enhanced Th17 phenotype in individuals with generalized anxiety disorder. J. Neuroimmunol. 229 (1–2), 212–218.
Vignali, D.A., Collison, L.W., Workman, C.J., 2008. How regulatory T cells work. Nat. Rev. Immunol. 8, 523–532.
von Känel, R., Hepp, U., Kraemer, B., Traber, R., Keel, M., Mica, L., Schnyder, U., 2007. Evidence for low-grade systemic proinflammatory activity in individuals with posttraumatic stress disorder. J. Psych. Res. 41, 744–752.
Wessa, M., Rohleder, N., 2007. Endocrine and inflammatory alterations in posttraumatic stress disorder. Expert Rev. Endocrinol. Metab. 2, 91–122.
Wilson, S., van der Kolk, B., Burbridge, J., Fisler, R., Kradin, R., 1999. Phenotype of blood lymphocytes in PTSD suggests chronic immune activation. Psychosomatics 40, 222–225.
Wise, R.A., 2008. Dopamine and reward: the anhedonia hypothesis 30 years on. Neurotox. Res. 14, 169–183.
Zhou, F.L., Zhang, W.G., Wei, Y.C., Xu, K.L., Hui, L.Y., Wang, X.S., Li, M.Z., 2005. Impact of comorbid anxiety and depression on quality of life and cellular immunity changes in patients with digestive tract cancers. World J. Gastroenterol. 11 (15), 2313–2318.