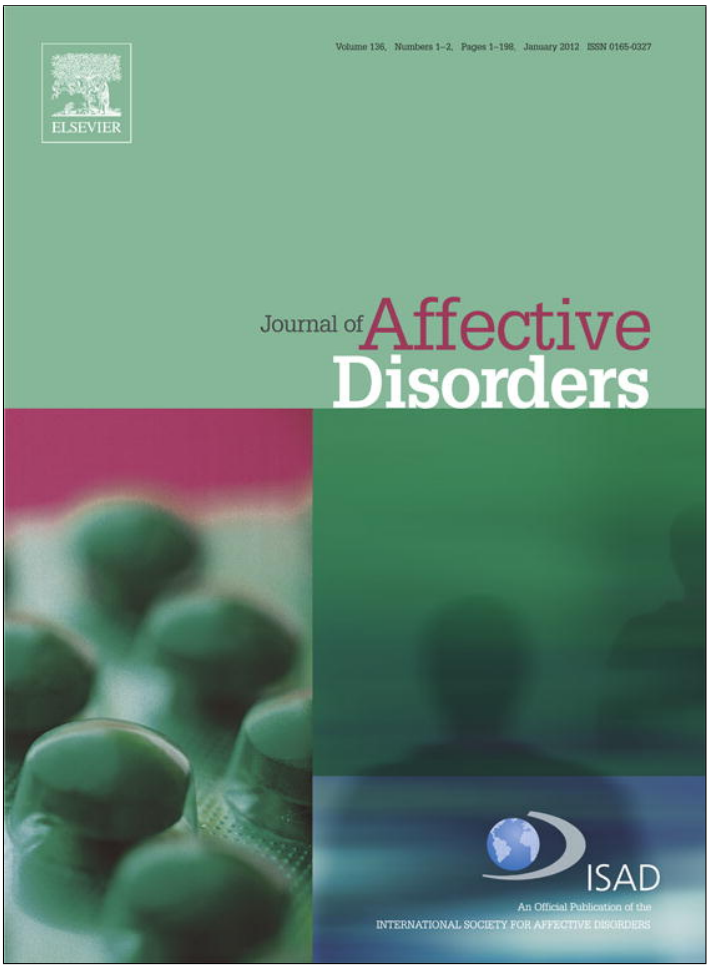

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Research report

Enhanced inflammatory and T-helper-1 type responses but suppressed lymphocyte proliferation in patients with seasonal affective disorder and treated by light therapy

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ABSTRACT

Background: Animals show seasonal changes in the endocrine and immune system in response to winter stressors. Even though increased inflammation has been implicated in the pathophysiology of depression, whether immune disorder is a key mediator in seasonal affective depression (SAD) is unknown. Here, we hypothesized that short photoperiods in winter may induce inflammatory response, which contributes to SAD, and that light treatments should normalize immune function and improve depressive symptoms.

Methods: Twenty patients with a diagnosis of SAD, and a score on the HAM-29 of 20 or higher were recruited for this study. Twenty-one healthy subjects with no personal and family history of psychiatric disorder were matched to patients according to age and sex. Patients and controls were sampled during winter between November and January, inclusive. A subset of SAD patients ($N=13$) was re-sampled after 4 weeks of light therapy. Blood samples were assayed for macrophage activity, lymphocyte proliferation and cytokine release.

Results: SAD patients showed significantly higher macrophage activity and lower lymphocyte proliferation in winter compared to healthy subjects. The concentrations of macrophage-produced proinflammatory cytokines interleukin-1β and tumour necrosis factor-α, and T-helper (Th)-1 produced cytokine, interferon-γ were all significantly increased. In contrast, no significant changes in Th2-produced cytokines were observed. Light therapy significantly improved depressive scores, which was associated with attenuation of decreased lymphocyte functions, increased macrophage activity and level of proinflammatory cytokines.

Conclusion: SAD patients have increased macrophage and Th1 type responses in winter, and light therapy normalized immune functions and depressive symptoms. These results support an inflammatory hypothesis for SAD and an immunomodulatory role of light therapy.

1. Introduction

An immune response initiated in the periphery can be transferred into the brain through cytokine transmitters and modulate brain function. Administration of proinflammatory cytokines in animals can lead to sleep interruption, lethargy, reduction of libido and exploration, poor concentration, and increased stress and anxiety-like behaviour (Dantzer, 2006; Song, 2006). Treatment with tumour necrosis factor (TNF)-α or interferon (IFN)-γ can induce depressive symptoms in healthy human volunteers or cancer patients (Meyers, 1999; Capuron et al., 2000). Increased

Abbreviations: Con, concanavalin; ELISA, a quantitative enzyme-linked immunosorbent assay; HPA, hypothalamic-pituitary-adrenal axis; IL, interleukin; IFN, interferon; MDD, major depressive disorder; PMA, phorbol 12-myristate 13-acetate; PHA, phytohemagglutinin; SAD, seasonal affective depression; SCID, Structured Clinical Interview for DSM-IV Structured Interview Guide for the Hamilton Depression Rating Scale (SIGH–SAD); Th, T-helper; TNF, tumour necrosis factor

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macrophage activity and enhanced production of proinflammatory cytokines have been consistently reported in patients with major depressive disorder (MDD) (Song et al., 1998; Maes et al., 2012; Hoyo-Becerra et al., 2014). The mechanism by which proinflammatory cytokines induced depressive symptoms and neuroendocrine dysfunction has been related to several aspects: (1) directly stimulating the hypothalamic-pituitary-adrenal axis (HPA) axis to secrete corticotrophin releasing factor and glucocorticoids (Leonard, 2014); (2) activating indoleamine 2,3-dioxygenase that may reduce the availability of serotonin (5-HT) precursor tryptophan and decrease 5-HT availability to the brain (Song et al., 1998; Maes et al., 2011) and (3) triggering glial activity in the brain, which may increase CNS inflammation and oxidative stress (Leonard and Maes, 2012; Song et al., 2013). Therefore, proinflammatory cytokines-produced changes in neuroendocrine and neurotransmission are similar to those observed in patients with MDD.

Human behaviour and mood also fluctuates with the seasons. At the extreme end of seasonality lies seasonal affective disorder (SAD, or winter depression), a subtype of major MDD characterized by recurrent episodes of depression in the winter and normal mood in the summer. Symptoms of SAD include increased sleep and sleep need, increased appetite with carbohydrate craving and weight gain, and decreased energy and feeling fatigued, which have been likened to a winter "hibernation" response. So far, the aetiology of SAD is unknown, but major hypotheses include photoperiodism, circadian phase shift and neurotransmitter dysfunction (Lam and Levitan, 2000).

In the past decade, several studies demonstrated that the changing seasons exert fundamental effects on the immune system. In animal studies, immune function is enhanced during the short photoperiods of winter as an adaptive response to energetic stress, such as low temperature, reduced food availability, etc. (Nelson et al., 2002). These seasonal changes in immune function are mediated via the duration of melatonin secretion, which acts as a signal of the changing photoperiod from long in summer to short in winter (Nelson et al., 2002; Haldar and Ahmad, 2010). Since melatonin is secreted only at night, it is increased in the winter short photoperiod and decreased in the summer long photoperiod. Melatonin receptors have been found in lymphocytes and macrophages in both humans and animals (Calvo et al., 1995; Lopez-Gonzalez et al., 1992). Melatonin can enhance macrophage activity, elevates antibody response, and increases cytokine production from macrophage and T-helper 1 (Th1) lymphocytes (Nelson et al., 2002; Shafer et al., 2001) but decreases anti-inflammatory response from Th2 lymphocytes (Kuhlwein and Irwin, 2001; Shearer et al., 2002). In human subjects, macrophages and Th1 cell-produced proinflammatory cytokines IFN-α, IFN-γ and interleukin (IL)-6 were increased in winter when compared to summer, while Th2-produced cytokine IL-10 was decreased (Maes et al., 1994; Kuhlwein and Irwin, 2001; Shearer et al., 2002). These studies indicate that immune cells, immune cell subsets, and cytokine releases exhibit significant seasonal variation, and these seasonal changes are mediated through photoperiodic changes.

Given that inflammation may contribute to the aetiology and symptoms of MDD and that seasonal variation is seen in immune function, a hypothesis could be that an increased immune-inflammatory response in winter is a mediating factor in SAD. Few studies have investigated immune changes in SAD patients, with only preliminary evidence showing higher plasma levels of IL-6 (Leu et al., 2001). However, changes in immune cellular functions and the balance between proinflammatory and anti-inflammatory cytokines and between Th1 and Th2 cell-produced cytokines in SAD are unclear. Furthermore, whether bright light therapy, an effective treatment for SAD (Lam et al., 2006), can modulate immune changes has not been studied. Thus, the present study aimed to investigate two hypotheses in SAD patients: (1) the shorter photoperiod of winter leads to the activation of macrophages, production of proinflammatory cytokines, increase in Th1 responses and shift in dynamic balance between Th1 and Th2; and (2) treatment with bright light will normalize these immune changes. To test these hypotheses, both cellular (macrophage activity and lymphocyte proliferation) and humoral immune functions (proinflammatory cytokines and Th1 and Th2 cell-produced cytokines) were measured in the blood samples from SAD patients during the four seasons of the year, and in healthy subjects and depressed SAD patients in winter, before and after light therapy.

2. Materials and methods

2.1. Subjects

This study was approved by the clinical research ethics board at the University of British Columbia and written, informed consent was obtained from all participants. Patients were recruited from the Mood Disorders Clinic at UBC Hospital in Vancouver, Canada. Inclusion criteria for SAD patients included: (1) DSM-IV criteria for MDD with a seasonal (winter pattern) as determined by the Structured Clinical Interview for DSM-IV (SCID) (Spitzer et al., 1995) modified to include criteria for seasonal pattern, (2) psychotropic drug-free for at least 2 months, (3) in winter, a score on the 29-item Structured Interview Guide for the Hamilton Depression Rating Scale (SIGH–SAD) (Williams, 1988) of 20 or higher (indicating moderately severe depression), (4) in summer, a score on the SIGH–SAD of 12 or less (indicating clinical remission). Exclusion criteria for SAD patients included: (1) other major psychiatric diagnoses, including bipolar disorder and psychotic disorders; (2) active alcohol/substance abuse or dependence within the past 12 months; (3) medical conditions or use of medications that may affect immune function; (4) retinal diseases that precluded the use of bright light therapy. Healthy control subjects were recruited by advertisement and matched to SAD patients by age. Inclusion criteria for healthy subjects included: (1) no current nor past history of mood disorder as determined by a SCID interview, (2) no family history of mood disorder, (3) score on the Seasonal Pattern Assessment Questionnaire (Rosenthal et al., 1987) of 6 or less, psychotropic drug-free for at least 2 months. The same exclusion criteria as for SAD patients were also used.

2.2. Procedure

After screening procedures for eligibility, 20 medication-free SAD patients (15 females and 5 males, age 39.0 ± 9.3 years) and 21 age-matched healthy subjects (13 females and 8 males, age 35.7 ± 12.3 years) were sampled during winter. Thirteen of the SAD patients (9 females and 4 males) had repeated blood sampling after 4 weeks of treatment with a standard regimen of light therapy, consisting of 10,000 lx fluorescent white light box fitted with a ultraviolet filter (Lam et al., 2006). Patients used the light box at home for 30–60 min in the early morning upon awakening, typically between 07:00 and 10:00 (Lam et al., 2006; Desan et al., 2007). Clinical response was determined by SIGH–SAD ratings at baseline and post-treatment. The intravenous blood samples were drawn into a heparinised syringe at 8:00–10:00 am.

2.3. Lymphocytes and monocyte/macrophages separation

Blood samples were diluted with RPMI-1640 with l-glutamine and phenol red (Sigma, Canada) at 1:1, and layered on the top of Histopaque (1.077). Then the tubes were centrifuged at 1650 rpm for 25 min at room temperature. Lymphocytes and monocytes/

macrophages at the interface of the upper layer and middle layer were collected, and washed with RPMI-1640 for twice (centrifuge at 1550 rpm for 20 min at 4 °C). To separate lymphocytes from monocytes/macrophages, the cell suspension was layered on the top of 46% Percoll, and centrifuged at 1800 rpm for 20 min at room temperature. The upper layer contains monocytes/macrophages and the bottom layer containing lymphocytes were collected separately. The monocytes/macrophages were washed with HBSS solution (Gibco, Canada) twice (centrifuged at 1600 rpm at 4 °C for 20 min), counted after Typan Blue stain and adjusted to 5×10^5 cells/ml in HBSS. The lymphocytes were washed with RPMI 1640 twice (centrifuged at 1600 rpm at 4 °C for 20 min), counted and adjusted to 2×10^6 cells/ml with the RPMI-1460 containing 1% penicillin (Sigma, Canada) and 10% heat-inactivated foetal calf serum (Sigma, Canada) (Song et al., 1996).

2.4. Measurements of macrophage activity and lymphocyte proliferation

Because monocytes, as resting macrophages, do not produce detectable chemineluminescence, the phorbol 12-myristate 13-acetate (PMA)-induced chemiluminescence of monocytes/macrophages was performed by our previously published method (Song et al., 1996). Different concentrations of PMA were tested and the optimal concentration 100 μg/ml was selected. In brief, 200 μl PMA, 200 μl cell suspension and 200 μl luminol (10^{-4} M) were mixed in a sample cuvette and then recorded by a luminometer (Turner Designs TD-20/20, USA) for 20 to 60 min, depending on the time that macrophages-released chemiluminescence to the highest peak at 37 °C. The count duration was 1 min with reading intervals of 3 min. The data were expressed as peak chemiluminescence (mV).

Mitogens have been popularly used as valuable probes for understanding the function of lymphocytes in antigen stimulation condition. Mitogen-stimulated lymphocyte proliferation may reflect lymphocyte new DNA transform and synthesis in different diseases. Both phytohemagglutinin (PHA) and concanavalin (Con) A can stimulate T-lymphocyte proliferation. In the present study, different concentrations of PHA (Sigma, Canada) and ConA (Sigma, Canada) were tested, and optimal concentrations of PHA (10 μg/ml) and Con A (5 μg/ml) were selected before carrying out the formal experiment. The cell culture procedure was the same as described before (Song et al., 1996). In brief, 200 μl of RPMI-1640 medium that contained PHA or Con A were added to a 96 well plate with lymphocytes (triplicate for each subject) at 37 °C in a 5% CO_2 atmosphere for 64 h. After adding [^{3}H]-thymidine (0.5 mCi/well, Amershall Ltd, UK) for 8 h, lymphocytes were harvested, and tritiated thymidine uptake was measured by a scintillation counter (Beckman Coulter LS 6500, Canada). Mean scintillation counts per minute (cpm) were calculated for each well.

2.5. Whole blood culture

Blood samples were diluted 1:10 with RPMI-1640 medium containing 1% of penicillin and 1% streptomycin (Sigma, Canada). 5 μg/ml PHA (Sigma, Canada) and 20 μg/ml lipopolysaccharide (LPS, stimulating macrophages) (Sigma, Canada) were used to stimulate cytokine releases (Maes et al., 1999). Unstimulated cultures reflect the spontaneous production of these cytokines, whereas stimulated cultures reflect the potential response to some pathogenic factors. For a blank, the blood from each sample was diluted with RPMI-1640. Samples (400 μl) were pipetted into 24-well plates prefilled with medium (1200 μl) and incubated for 48 h in a humidified atmosphere at 37 °C, 5% CO_2. After incubation, the plates were centrifuged at 1500 rpm for 20 min. Supernatants were taken off carefully under sterile conditions, divided into eppendorf tubes, and frozen immediately at −70 °C until thawed for assay cytokines.

2.6. ELISA for the measurement of cytokine concentrations

The release of cytokines is measured by a quantitative enzyme-linked immunosorbent assay (ELISA) (Biosource International, California, USA). Monoclonal antibodies specific for each component are pre-coated onto 96-well microtiter plates. Standards and samples (50 μl) are pipetted into the wells and then incubated at 37 °C. The cytokines will be bound by the immobilized antibody and incubated at 37 °C again. After washing any unbound ligand, an enzyme linked polyclonal antibody specific for each of these components is added to the wells and incubated at 37 °C. Unbound antibody-enzyme reagent is removed by washing and a substrate solution is added to the wells for 10 min. The colour intensity developed by the addition of sulphinuric acid is proportional to the amount of receptor or protein bound (Song et al., 1998). Cytokine concentrations are expressed as pg/ml.

2.7. Statistics

Each immune parameter was analyzed by one-way ANOVA between healthy control subjects and SAD patients before treatment, and by repeated measures ANOVA in SAD patients pre- and post-treatment. The significant changes between groups were further confirmed by Scheffe's or Newman-Keuls post-hoc test. The statistic package was from GB-STAT, Dynamic Microsystems, Inc. USA. Significance was set at $p < 0.05$.

3. Results

3.1. Clinical outcome

The 20 SAD patients experienced a mean of 8.7 ± 3.9 (range 3–17) previous winter depressive episodes, with a current episode duration of 11.3 ± 5.0 (range 4–20) weeks at time of baseline testing. The mean baseline SIGH–SAD score for the 20 SAD patients in winter was 29.7 ± 4.6, indicating a moderate severity of depression. The 21 healthy control subjects had minimal symptoms, with a mean SIGH–SAD score of 1.1 ± 1.2. For the 13 SAD patients who were treated with light therapy, 11 (85%) had a clinical response, defined as $\geq$50% improvement in SIGH–SAD score. The repeated measures ANOVA also showed a significant improvement in depressive symptoms after 4 weeks, with SIGH–SAD scores decreasing from pre-treatment (29.3 ± 4.8) to post-treatment (9.2 ± 6.4) ($F1,12 = 90.23$, $p < 0.0001$).

3.2. Comparison of immune changes between SAD patients and healthy control subjects in winter and effects of light treatment

The ANOVA analysis indicated that macrophage activity was significantly increased in the 20 SAD patients compared to the 21 healthy control subjects ($F1,38 = 52.47$, $p < 0.0001$). The repeated measures ANOVA revealed that the enhanced macrophage activity was significantly reduced in the 13 patients after light therapy when compared to their pre-treatment baseline ($F1,31 = 42.81$, $p < 0.0001$) (Fig. 1).

For the lymphocyte proliferation, the ANOVA analysis showed that both PHA and Con A stimulated lymphocyte proliferations were significantly lower in the pre-treated SAD patients than healthy controls (PHA: $F1,38 = 21.63$, $p < 0.001$; Con A: $F1,38 = 17.73$, $p < 0.001$). In the SAD patients after treatment with light therapy, the repeated measures ANOVA found that both mitogens-stimulated lymphocyte proliferations were increased to the

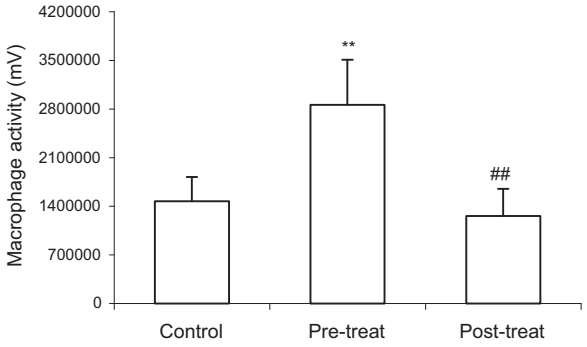

Fig. 1. Macrophage phagocytosis in healthy controls and SAD patients before and after light treatment. Results are expressed as mean+SD. $^{**}p < 0.01$ versus healthy controls; ## $p < 0.01$ versus SAD patients before light treatment.

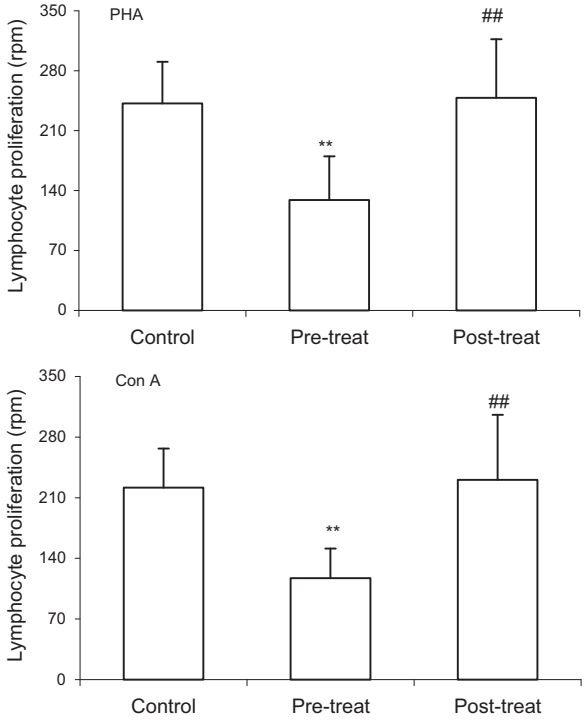

Fig. 2. Mitogens-stimulated lymphocyte proliferation in healthy controls and SAD patients before and after light treatment. Results are expressed as mean+SD. $^{**}p < 0.01$ versus healthy controls; ## $p < 0.01$ versus SAD patients before light treatment.

levels of healthy controls (PHA: F1,30=25.03, $p < 0.001$; ConA: =21.54, $p < 0.001$) (Fig. 2).

The ANOVA analysis indicated that the concentration of IL-1β was higher in SAD patients than in the healthy controls, with and without mitogen stimulations (baseline: F1,36=21.23, $p < 0.0001$; stimulated: F1,36=6.71, $p < 0.01$) (Fig. 3). Compared to the pre-treatment levels, IL-1β releases (with and without mitogen stimulation) were significantly lower in SAD patients post-light treatment (baseline: F1,30=17.84, $p < 0.001$; stimulated: F1,30=6.69, $p < 0.01$) (Fig. 3). In SAD patients compared to healthy control subjects, the release of the other proinflammatory cytokine TNF-α was lower under baseline condition (F1,36=4.24, $p < 0.05$), but mitogen-stimulated TNF-α release was higher in SAD patients than in healthy controls (F1,36=6.5, $p < 0.01$) (Fig. 4). After the light therapy, the concentrations of TNF-α was not significantly changed under baseline condition, but was significantly reduced in mitogen-stimulated condition (F1,30=6.45, $p < 0.01$).

SAD patients also produced more IFN-γ, a Th1 cells-produced

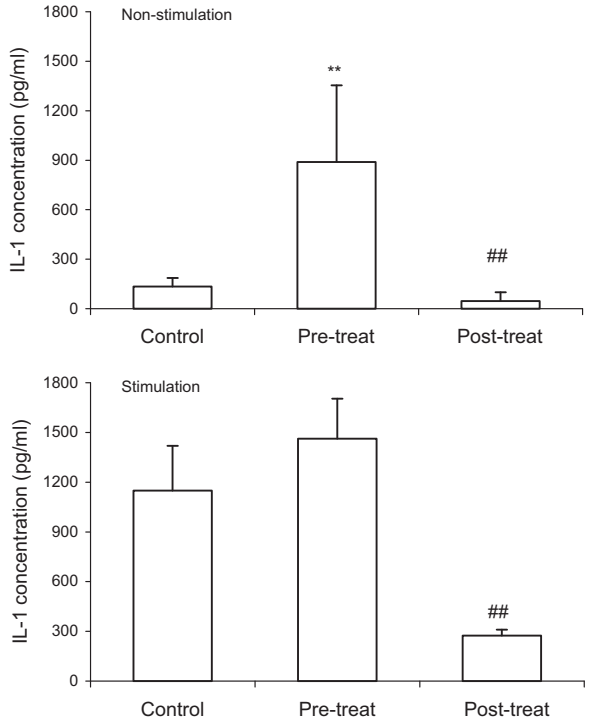

Fig. 3. IL-1β release from whole blood culture with and without mitogen stimulation. Results are expressed as mean+SD. $^{**}p < 0.01$ versus healthy controls; ## $p < 0.01$ versus SAD patients before light treatment.

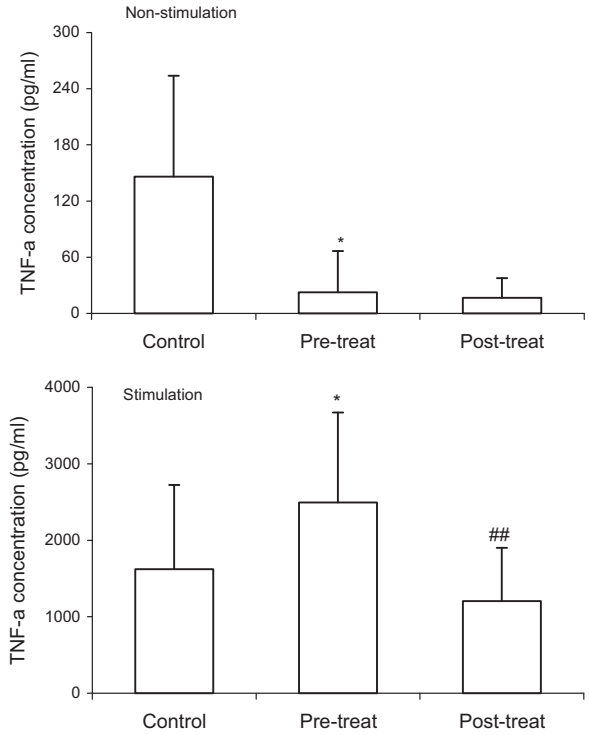

Fig. 4. TNF-α from whole blood culture with and without mitogen stimulation. Results are expressed as mean+SD. $^{**}p < 0.01$ versus healthy controls; ## $p < 0.01$ versus SAD patients before light treatment.

proinflammatory cytokine, than control subjects after culture with or without mitogens (baseline: F1,36=4.11, $p < 0.05$; stimulated: F1,36=8.24, $p < 0.001$) (Table 1). Light treatment markedly suppressed IFN-γ production from the blood, with and without mitogen stimulation (F1,30=6.73, $p < 0.01$; F1,30=4.34, $p < 0.05$,

Table 1
The concentration of Th1 and Th2 cells-produced cytokines with and without mitogen stimulation pre- and post light treatments

	Th1 cytokines				Th2 cytokines			
Cytokines	IFN-γ		IL-2		IL-10		IL-4	
Mitogen	N-ST	ST	N-ST	ST	N-ST	ST	N-ST	ST
Controls	10.65 ± 10.72	504.45 ± 862.78	6.73 ± 1.93	91.53 ± 21.35	29.58 ± 13.51	157.29 ± 72.75	19.34 ± 5.47	34.85 ± 8.66
Pre-treat	48.3 ± 37.03*	1547.73 ± 1086.73**	7.11 ± 1.88	62.47 ± 14.4*	27.38 ± 21.6	180.73 ± 74.42	16.87 ± 5.31	30.69 ± 6.45
Post-treat	12.23 ± 4.6#	366.65 ± 532.78##	6.59 ± 4.04	87.38 ± 17.24#	16.31 ± 11.34#	288.72 ± 173.06#	12.35 ± 4.1**#	35.45 ± 9.61

The results are expressed as Mean ± SD (pg/ml). *$p < 0.05$; **$p < 0.01$ versus controls, #$p < 0.05$; ##$p < 0.01$ versus pre-treatment group (n=18-20). N-ST: without mitogen stimulation; ST: with mitogen stimulation.

respectively) (Table 1).

The other Th1 cell-produced cytokine, IL-2 (a lymphocyte growth factor), was not significantly changed at baseline condition but decreased after mitogen stimulation ($F1,36 = 3.67$, $p < 0.05$) (Table 1), which was also reversed by light treatment ($F1,30 = 4.14$, $p < 0.05$).

The concentration of Th2 type of cytokine IL-4 with and without mitogen stimulation was slightly decreased in SAD patients compared to control subjects, but did not reach statistical significance (Table 1). Anti-inflammatory cytokine IL-10, another Th2 cell-produced cytokine, was also slightly decreased compared to controls under both conditions, which also did not reach statistical significance. In the stimulated blood, the repeated measures ANOVA showed a higher IL-10 release in the SAD patients after light therapy ($F1,30 = 3.24$, $p = 0.055$) (Table 1).

4. Discussion

The present study, for the first time, reported seasonal effects on cellular and humoral immunities in SAD patients. The results demonstrated that most inflammatory responses in the winter season were significantly enhanced in SAD patients. According to the inflammation theory of depression, these results are consistent with an inflammation-related mechanism in SAD.

SAD patients showed similar cytokine changes in winter season to previous findings observed in patients with major depression and animal models of depression. For example, increased IL-1β and microglial activity have been reported in suicidal depressed patients (Steiner et al., 2008) and increased IL-1β concentrations were found in patients with MDD, late life depression and dysthymia (Maes et al., 1993; Thomas et al., 2005; Anisman et al., 1999). In rodents, IL-1β administration can induce anxiety and stress-like behaviour, increased glucocorticoid secretion and dysfunction of neurotransmission (Song, 2006). However, no change in IL-1β has also been reported in patients with MDD and subtypes of depression (Huang and Lee, 2007; Kaestner et al., 2005). The present study not only showed an increase in IL-1 release in SAD patients, but also demonstrated its producer macrophage activity was activated in the same patients. Thus, this result further suggests that macrophage-triggered inflammatory response occurs in SAD patients.

In the present study, SAD patients also showed higher Th1 cells-produced proinflammatory cytokine IFN-γ in both baseline and mitogen-stimulated conditions. In contrast, Th2 cell-produced cytokines IL-4 and IL-10 were unchanged in SAD patients compared to healthy controls. Previously, elevated levels of IL-6, a Th1 cell- and macrophage-produced cytokine, was reported in SAD patients (Leu et al., 2001). These results suggest that the immune response in SAD patients is shifted to Th1 dominant type, which also indicates an enhanced inflammatory response in SAD. Increased IFN-γ concentrations have been also reported in patients with MDD (Lichtblau et al., 2013). IFN-γ can activate the enzyme indoleamine 2,3-dioxygenase (IDO) in monocyte-derived macrophages, dendritic, and other cells, which, in turn, decreases serum levels of tryptophan and reduces synthesis of serotonin in the brain (Maes et al., 2011).

In winter, lower TNF-α in baseline condition was found in the SAD patients compared to healthy controls, which was similar to the finding by Haack et al. (1999) in drug free depressed patients. We have previously reported no change or slightly decreased TNF-α in patients with MDD (Song et al., 2009). The reason could be due to a decline in NK cell and some T-lymphocyte functions, which have been reported in depressed patients (Irwin et al., 1992), and also found in the present study. After mitogen stimulation, higher TNF-α production was observed in SAD patients compared to control subjects, which may suggest an increased inflammatory response during pathogenic conditions. As TNF-α is also involved in autoimmune diseases, the change in this cytokine in SAD may reflect a secondary immune response.

On the other hand, lymphocyte proliferation was suppressed in the SAD patients compared to controls in this study. The mechanism for this decrease is unknown. Decreased lymphocyte proliferation has been associated with increased cortisol secretion in MDD patients (Musiani et al., 1998; Gloger et al., 1997; Sullivan et al., 1997), but SAD is not associated with hypercortisolism (Gold et al., 1995). Melatonin may also affect lymphocyte proliferation, with both enhanced and suppressed lymphocyte proliferation reported (Markowska et al., 2001; Drazen et al., 2001). These conflicting results may depend on methodological differences between studies, i.e., different doses and durations of melatonin, different seasons (Michelis et al., 2013) and subjects of study. For example, in an in vivo experiment, Prendergast et al. (2001) reported that melatonin suppressed mitogen-stimulated lymphocyte proliferation in short photoperiod day but not in long photoperiod day. In several in vitro experiments, the suppressed mitogen-stimulated lymphocyte proliferation was found after co-culture with melatonin (Markowska et al., 2002; Persengiev and Kyurkchiev, 1993). Since melatonin may also suppress corticotropin releasing factor in winter (Wehr, 1992), the decreased lymphocyte proliferation observed in the present study may not result from changes in the HPA system. Consistent with lower lymphocyte proliferation in SAD patients, the present study found that lymphocyte growth factor IL-2 secretion was lower after mitogen stimulation when compared to normal controls. Other factors that suppress lymphocyte proliferation could be increased noradrenalin from the sympathetic nervous system (SNS). Demas and co-workers demonstrated that long night (16 h) and short day decreased lymphocyte proliferation, which was correlated with increased splenic noradrenalin (Demas et al., 2003). Altogether, these results suggest an imbalance between macrophage and lymphocyte functions in SAD patients.

To our knowledge, this is also the first report that shows consistent immune modulation of bright light treatment. Light

therapy normalized immune function in SAD patients in three aspects. First, light therapy significantly reduced inflammatory response by suppressing macrophage activity and reducing proinflammatory cytokines IL-1β, TNF-α and IFN-γ. Second, light treatment increased anti-inflammatory cytokine IL-10 release in mitogen-stimulated blood. This result suggests that light treatment can also restore the balance between Th1 and Th2 functions. Third, light treatment reversed the lower lymphocyte proliferation and lower IL-2 production found in SAD patients. These results suggest that light therapy can modulate lymphocyte function and balance innate (macrophage) and adaptive immune response.

With regards to biological/molecular mechanisms, bright light therapy was reported to increase 5-HT levels in the anterior cingulate and prefrontal cortex by reducing 5-HT transporter binding (Harrison et al., 2015); inhibit orexinergic signalling underlies depression-like responses induced by daytime light deficiency (Deats et al., 2014) and regulate circadian (Lewy et al., 2006).

There are some limitations in this study. Melatonin concentrations should be measured. In fact, we collected salivary samples to assay melatonin, but because of technical problems the melatonin results were unusable. In addition, the sample size is small, which may affect the statistical meaning since we made multiple tests to better understand immune mechanism in SAD. Because it was an exploratory analysis, the significant findings in this study will need to be replicated in future studies.

In conclusion, this study showed significant seasonal effects on immune function in patients with SAD, with enhanced inflammatory responses in winter during a depressive episode. Furthermore, an imbalance between macrophage activity and lymphocyte proliferation, and between inflammation/Th1 type response and Th2 function were found in SAD patients when compared to healthy control subjects. These results suggest that the short photoperiod in winter, perhaps mediated by increased melatonin secretion, may enhance inflammatory response and induce seasonal depression. Light therapy significantly normalized these immune changes to the level found in the healthy subjects.

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Conflict of interest

The authors declare no conflict of interest.

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