Title

Altered populations of natural killer cells, cytotoxic T lymphocytes, and regulatory T cells in major depressive disorder: Association with sleep disturbance

Authors

Suzuki, Hideo
Savitz, Jonathan
Teague, T Kent
et al.

Publication Date

2017-11-01

DOI

10.1016/j.bbi.2017.06.011

Peer reviewed

HHS Public Access

Author manuscript

Brain Behav Immun. Author manuscript; available in PMC 2018 November 01.

Published in final edited form as:

Brain Behav Immun. 2017 November ; 66: 193–200. doi:10.1016/j.bbi.2017.06.011.

Altered Populations of Natural Killer Cells, Cytotoxic T Lymphocytes, and Regulatory T Cells in Major Depressive Disorder: Association with Sleep Disturbance

Hideo Suzuki[a,b,*], **Jonathan Savitz**[a,c,*], **T. Kent Teague**[d,e,f], **Siva K. Gandhapudi**[g], **Chibing Tan**[d], **Masaya Misaki**[a], **Brett A. McKinney**[h], **Michael R. Irwin**[i], **Wayne C. Drevets**[j], and **Jerzy Bodurka**[a,k]

[a]Laureate Institute for Brain Research, Tulsa, OK, United States

[b]Department of Educational Psychology, University of Nebraska-Lincoln, Lincoln, NE, United States

[c]Oxley College of Health Sciences, The University of Tulsa, Tulsa, OK, United States

[d]Departments of Surgery and Psychiatry, University of Oklahoma School of Community Medicine, Tulsa, OK, United States

[e]Department of Pharmaceutical Sciences, University of Oklahoma College of Pharmacy, Tulsa, OK, United States

[f]Department of Biochemistry and Microbiology, Oklahoma State University Center for the Health Sciences, Tulsa, OK, United States

[g]Department of Microbiology, Immunology and Molecular Genetics, University of Kentucky School of Medicine, Lexington, KY 40536

[h]Tandy School of Computer Sciences, The University of Tulsa, Tulsa, OK, United States

[i]Cousins Center for Psychoneuroimmunology, Department of Psychiatry and Biobehavioral Sciences, David Geffen School of Medicine at UCLA, Los Angeles, CA, United States

[j]Janssen Research & Development, LLC, of Johnson & Johnson, Inc., Titusville, NJ, United States

[k]Stephenson School of Biomedical Engineering, University of Oklahoma, Norman, OK, United States

Abstract

A subset of individuals with major depressive disorder (MDD) have impaired adaptive immunity characterized by a greater vulnerability to viral infection and a deficient response to vaccination along with a decrease in the number and/or activity of T cells and natural killer cells (NKC).

Corresponding author: Jerzy Bodurka, Laureate Institute for Brain Research, 6655 S. Yale Ave., Tulsa, OK 74136-3326, United States, Telephone: +1-918-502-5110, jbodurka@laureateinstitute.org.

*Joint first authors

Nevertheless, it remains unclear which specific subsets of lymphocytes are altered in MDD, a shortcoming we address here by utilizing an advanced fluorescence-activated cell sorting (FACS) method that allows for the differentiation of important functionally-distinct lymphocyte sub-populations. Furthermore, despite evidence that sleep disturbance, which is a core symptom of MDD, is itself associated with alterations in lymphocyte distributions, there is a paucity of studies examining the contribution of sleep disturbance on lymphocyte populations in MDD populations. Here, we measured differences in the percentages of 13 different lymphocytes and 6 different leukocytes in 54 unmedicated MDD patients (partially remitted to moderate) and 56 age and sex-matched healthy controls (HC). The relationship between self-reported sleep disturbance and cell counts was evaluated in the MDD group using the Pittsburgh Sleep Quality Index (PSQI). The MDD group showed a significantly increased percentage of $CD127^{low}/CCR4^{+}$ T_{reg} cells, and memory T_{reg} cells, as well as a reduction in $CD56^{+}CD16^{-}$ (putative immunoregulatory) NKC counts, the latter, prior to correction for body mass index. There also was a trend for higher effector memory $CD8^{+}$ cell counts in the MDD group versus the HC group. Further, within the MDD group, self-reported sleep disturbance was associated with an increased percentage of effector memory $CD8^{+}$ cells but with a lower percentage of $CD56^{+}CD16^{-}$ NKC. These results provide important new insights into the immune pathways involved in MDD, and provide novel evidence that MDD and associated sleep disturbance increase effector memory $CD8^{+}$ and T_{reg} pathways. Targeting sleep disturbance may have implications as a therapeutic strategy to normalize NKC and memory $CD8^{+}$ cells in MDD.

Keywords

flow cytometry; depression; sleep; regulatory T cells; CD8+ cells; natural killer cells

1. Introduction

While most recent studies have focused on the link between inflammation and depression, major depressive disorder (MDD) also is considered to have an immunosuppressive component, especially involving the adaptive immune system. This perspective evolved from the stress literature which demonstrated that chronic psychological stressors were associated with declines in the total numbers of circulating B-cells and T-cells, reduced mitogen-induced lymphocyte proliferation and natural killer cell (NKC) activity, and the mobilization of regulatory T-cells (T_{reg}), along with greater susceptibility to viral infections, reduced immune responses to vaccines, reactivation of latent herpesviruses, and slowed wound healing (Dhabhar, 2014; Glaser and Kiecolt-Glaser, 2005; Irwin, 2008; Toben and Baune, 2015). This impairment in the adaptive immune response is hypothesized to be mediated by the suppressive effects of cortisol on the expression of anti-viral genes as well as the effects of adrenergic signaling on the expression of type I interferons (Irwin and Cole, 2011). Chronic sympathetic nervous system and hypothalamic-pituitary-adrenal axis activation additionally suppress the trafficking of immune cells from the blood to the tissue and draining lymph nodes (Dhabhar and McEwen, 1997).

A similar impairment of the adaptive immune response has been reported in depression, in particular, decreases in NKC cytotoxicity and reduced mitogen-stimulated T-lymphocyte

proliferation (Blume et al., 2011; Irwin and Miller, 2007; Miller, 2010; Toben and Baune, 2015; Zorrilla et al., 2001). Consistent with these data, depressed individuals show a decreased response to viral vaccines (Irwin et al., 1998; Irwin et al., 2013), and reportedly, a worse prognosis in cases of infectious disease (Leserman, 2008) and cancer (Sephton et al., 2009). Given the close link between stress and depression, glucocorticoid and adrenergic signaling also may underlie reduced NKC and T cell numbers and/or function in depression. However, Miller (2010) also raised the possibility that depression-associated decreases in T lymphocytes may result from apoptotic processes that are induced by inflammation-driven tryptophan depletion in the context of kynurenine pathway activation. One potential upstream cause of the depression-associated dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis, sympathetic nervous system (SNS), and the kynurenine pathway, is a disturbance in sleep.

Despite the fact that sleep disturbance is a significant risk factor for depression (Baglioni et al., 2011; Cho et al., 2008; Lee et al., 2013), the mechanisms underlying this association remain poorly understood. In addition to the direct effect of sleep on inflammatory mediators, one possibility is that sleep disturbance impairs the viral immune response by increasing adrenergic signaling of the SNS (Irwin, 2015; Irwin and Cole, 2011), thus rendering the individual more vulnerable to infection-induced inflammation (Mechawar and Savitz, 2016). Specifically, sleep disturbance appears to lead to a shift away from Type 1 T helper cell (T_h1) immunity (Axelsson et al., 2013; Lange et al., 2010; Petrovsky and Harrison, 1997; Redwine et al., 2003; Sakami et al., 2002), potentially explaining why shortened or fragmented sleep is associated with increased vulnerability to viral infection (Cohen et al., 2009), and a reduction in the efficacy of viral vaccines (Miller et al., 2004; Prather et al., 2012; Spiegel et al., 2002). Arguably, however, the most robust finding in the literature is the association between sleep disturbance and reduced NKC counts and/or activity. Partial sleep deprivation was originally shown to reduce NKC activity in healthy volunteers (Irwin et al., 1994; Irwin et al., 1996) and more recently, a night of naturally short sleep (<7 hours) was reported to be associated with reduced NKC and T-cell function compared to a normal (7–9 hours) nights sleep (Fondell et al., 2011).

Nevertheless, few studies have examined the relationship between sleep disturbance and NKC and T cell immunity within depressed populations. Cover and Irwin (1994) reported that insomnia was associated with a reduction of NKC activity in depressed patients that was independent of the severity of other depressive symptoms, raising the possibility that sleep disturbance is a critical pathway by which depression leads to a reduction in NK activity. A subsequent study of MDD patients partially replicated these results by showing that about 20% of the variance in NKC number in the MDD group was accounted for by sleep disturbance (Frank et al., 2002). Regarding T cells, in contrast, mitogen-induced lymphocyte stimulation was found to be reduced in depressed subjects compared with controls, but the differences were not attributable to sleep disturbance (Cosyns et al., 1989; Miller et al., 1999).

Here we advance the literature in several respects. Firstly, sleep disturbance, a core symptom of MDD, has itself been associated with alterations in lymphocyte distributions similar to those observed in MDD. However, there is a paucity of studies examining the association

between sleep disturbance and lymphocyte populations within non-medically ill MDD populations. This is one of the few studies to examine the link between sleep disturbance and circulating lymphocyte distributions in depressed subjects with MDD who were otherwise medically healthy and unmedicated.

Second, most studies reporting abnormalities of lymphocytes in depressed subjects were conducted when fluorescence-activated cell sorting (FACS) methods were unable to differentiate between many functionally-distinct lymphocyte sub-populations. Here, we employed a novel FACS method based on the immunophenotyping protocol developed for the Human Immunology Project (Maecker et al., 2012). This technique allowed for a more fine-grained analysis of cell populations – for instance distinguishing between putative cytotoxic and regulatory NKC, nine different populations of $CD4^+$ cells and four different populations of $CD8^+$ cells (Table 2). Additionally, we obtained measures of less commonly studied cell populations such as myeloid dendritic cells and plasmacytoid dendritic cells.

Third, there has been debate in the literature concerning the two potentially discrepant findings in the psychoneuroimmunology literature, i.e. the counterintuitive presence of both “inflammation” and “immune suppression” in MDD (Blume et al., 2011). Few publications have explicitly addressed the relationship between these two phenomena. Here, we examine the relationship between “inflammation” (indexed by CRP) and “immune suppression” (indexed by changes in lymphocyte populations).

Although we measured multiple different cell types, our primary focus in this paper is on monocytes, T-cells, and NKC, not only because of the research discussed above, but also because in a previous pilot study we reported changes in monocytes, NKC and T_{reg} cell frequencies in an independent sample of individuals with MDD (Savitz et al., 2013).

2. Method

2.1. Subjects

The current research was approved by the Western Institutional Review Board, and the study was conducted according to the principles expressed in Declaration of Helsinki. All participants gave written informed consent to participate and received financial compensation.

Volunteers between the ages of 18 and 55 years were recruited through a variety of sources including: the clinical services of the Laureate Psychiatric Clinic and Hospital (LPCH), newspaper, flyer, radio, Facebook or other media advertisements in the Tulsa metropolitan area. A total of $N = 110$ subjects (83 females) comprising 54 subjects who met DSM-IV-TR criteria for MDD (mean Montgomery-Åsberg Depression Rating Scale (MADRS) score = 22.3±7.9, partial remission ($n = 18$), mild depression ($n = 11$), and moderate depression ($n = 25$)) and 56 healthy control (HC) subjects, were included in the data analyses. The diagnosis of MDD was established using the Structural Clinical Interview for DSM-IV-TR Axis I Disorders (SCID-I/NP) (First, January, 2010) and confirmed by an unstructured interview with a psychiatrist. All MDD participants were free from psychotropic medications for at least 3 weeks prior to study entry. Exclusion criteria for both the MDD and HC samples

included major medical or neurological illness (including autoimmune and infectious diseases), psychosis, traumatic brain injury, and a history of drug/alcohol abuse within one year (for details please see Table S1). An additional exclusion criterion that applied to the control sample was a history of any major psychiatric disorder in a first-degree relative. Table 1 lists the clinical and demographic characteristics of the subjects.

2.2. Assessments

The severity of depressive symptoms was rated using the clinician-administered Montgomery-Åsberg Depression Rating Scale (MADRS) (Williams and Kobak, 2008), and self-reported sleep quality was measured using the Pittsburgh Sleep Quality Index (PSQI) (Buysse et al., 1989). The PSQI is a valid method of identifying sleep disturbance. It shows both a high sensitivity (98.7%) and specificity (84.4%) in identifying insomnia, as well as significant correlations with other sleep measures including sleep diaries and polysomnography (Backhaus et al., 2002). Further, a recent paper showed a robust association between remission of insomnia and a decrease in PSQI scores (Irwin et al., 2017). PSQI score was treated both a continuous variable and as a binary variable, defined using the standard PSQI cutoff score to denote sleep disturbance (≤5 vs. >5). The Physical Activity Questionnaire (PAQ) was used to assesses the frequency of the participants' physical exercise (ranging from light to vigorous), as well as home-related activities (e.g., cleaning, repairs, yard maintenance, child care, shopping). A higher score indicates more engagements in physical activities.

2.3. Blood processing and flow cytometry

Morning blood samples were obtained from the participants and peripheral blood mononuclear cells (PBMC) were isolated using cell preparation tubes (CPTs). The FACS analysis was based on the methods used in the Human Immunology Project (Maecker et al., 2012) and the NKC subtyping was based on the methods of Michel et al. (2016) (Table 2, Figures S1–S3). Frozen PBMC were thawed using a 37°C water bath, and the cell suspension was transferred into 15mL centrifuge tubes containing 10mL of cRPMI buffer (RPMI1640 with 10% Fetal Bovine Serum (FBS)). Cells were pelleted by centrifugation at 500g for 10 min at 4°C. The pellet was re-suspended in FACS buffer containing human immunoglobulin G (lgG) (Life Technologies, 1:20 dilution) and incubated at 4°C for 60 min to block F_C receptors. Then, at a concentration of 5×10^6 cells/mL, PBMCs were stained with fluorescent antibody conjugates at 4°C for 60 min. The following fluorochrome human monoclonal antibodies (mAbs) were used in immunophenotyping stains: CD8-PE (Biolegend), CD3 PERCP (Biolegend), CxCR3 APC (BD Bioscience), CCR6 PE-CY7 (Biolegend), CD38 APC-CY7 (Biolegend), CD4 Pacific Blue (Biolegend), CCR7 Brilliant Violet 711™ (BD Bioscience), HLA-DR PE-CY5.5 (Biolegend), CD45RA PE/Dazzle™ 594 (Biolegend), CD127 PE (BD Bioscience), CCR4 APC (Biolegend), CD25 APC-CY7 (Biolengend), CD45RO PE-CY7 (Biolegend), CD8 Brilliant Violet 711™ (Biolegend), CD56 PE (Biolegend), CD16 APC (Biolegend), CD14 APC-CY7 (Biolegend), CD123 PE-CY7 (Biolegend), CD19 Pacific Blue (Biolegend), CD20 Brilliant Violet 711™ (Biolegend), and CD11c PE/Dazzle™ 594 (Biolegend). After two washes, the cells were resuspended in FACS buffer containing 1× SYTOX® dead cell stain and incubated for 15 min at 4°C. Cell population percentages were analyzed using a BD LSRII 4-laser flow cytometer and FACS

Diva software (BD Biosciences, San Jose, CA). The stained cells were passed through a laser beam to record the fluorescence emission from their bound antibody conjugates and to calculate cell percentages within PBMCs. The cell populations comprised: cluster of differentiation $CD3^+$ T cells, $CD4^+$ T cells (including central memory cells, naive cells, effector memory cells, and effector cells), $CD8^+$ T cells (including central memory cells, naive cells, effector memory cells, and effector cells), T helper (T_h) cells (including T_h1 cells, T_h2 cells, and T_h17 cells), regulatory T (T_{reg}) cells (including $CD25^+$ T_{reg} cells, $CD127^{low}/CCR4^+$ T_{reg}, and Memory T_{reg} cells), $CD3^-/CD19^-$ cells, $CD14^-/CD20^-$ cells, human $HLA\text{-}DR^+$, (including myeloid dendritic cells and plasmacytoid dendritic cells), $CD56^+CD16^+$ natural killer (NK) cells, $CD56^+CD16^-$ NK cells, and monocytes (including non-classical monocytes and classical monocytes).

FACS was performed blind-to-diagnosis. For each subject, the cell counts were normalized to the total cell count for that subject and expressed as a percentage. In order to avoid batch effects, samples were run in groups of 12 subjects consisting of (6 MDDs and 6 HCs matched for age and sex). In addition, PBMCs from one individual (independent of the study participants) were used as controls to bridge each set of stains in order to adjust gating for laser drift or any staining inconsistencies across all of the assays. High-sensitivity C-reactive protein (hs-CRP) was measured immunoturbidimetrically with the Kamiya Biomedical K-Assay in a CLIA-certified hospital laboratory.

2.4. Statistics

T-tests or χ^2 tests were performed to test for group differences in age, sex, ethnicity, occupational status, educational status, nicotine use, body mass index (BMI), MADRS score, PSQI score, PAQ score, and hs-CRP. ANOVA and ANCOVAs (with BMI, age, sex, and batch effect as covariates) were performed to test for diagnostic group differences in the percentages of immune cells. On the basis of our previous pilot study implicating monocytes, NKC, and T_{reg} cells in depression (Savitz et al., 2013) as well as the extensive literature reporting T-cell and NKC abnormalities in depression, our primary outcome variables were monocyte, T_{reg} cell, and NKC counts, while differences in other cell populations (i.e. monocytes, myeloid dendritic cells and plasmacytoid dendritic cells) were assessed in secondary analyses. For the primary outcomes, a statistical threshold of $p<0.05$ (two-tailed) was used for determining statistical significance, whereas for the secondary outcomes we employed a Bonferroni correction for multiple testing with a statistical threshold of $p<0.004$ (two-tailed).

Pearson correlations were subsequently performed in order to evaluate the relationship between sleep disturbance (PSQI total score as a continuous variable) or CRP and the counts of cell populations that differed significantly between the MDD and HC groups. A two-tailed statistical threshold of $p<0.05$ was used for determining significance. In order to control for depression severity in the sleep analyses, the MADRS score was recalculated without the single sleep item. Partial correlations were then calculated by partialling out the revised MADRS score. In addition, differences in cell counts between MDD subjects with (PSQI score >5) and without (PSQI score ≤5) sleep disturbance were tested with Analysis of Variance.

Intraclass correlation coefficient (ICC) estimates (with their 95% confidence intervals) were calculated to test the reliability of the flow cytometry measurements across 10 batches, using R (with a multiple-rating (k=10), consistency, two-way mixed-effects model). A higher ICC indicates that the batches resembled each other for each of the 19 cell types.

3. Results

Descriptive statistics are shown in Table 1. There were no significant group differences in age, sex, ethnicity, occupational status, educational status, tobacco use, and physical activity. As expected, the MDD group had significantly higher BMI, MADRS, and PSQI scores than the HC group.

The ICC value for the FACS analyses was 1.00 ($F_{(19,171)}$ = 6987, p<0.00001), indicating excellent reliability across batches.

Consistent with our *a priori* hypothesis, the MDD group had significantly higher percentages of $CD127^{low}/CCR4^{+}$ T_{reg} cells (0.42% in MDD versus 0.35% in HC, η^2 = 0.04) after controlling for BMI, age, sex, and batch effects (Table 2). The percentage of memory T_{reg} cells also was higher in the MDD group (0.39%) than the HC group (0.33%), when the model controlled for BMI (η^2 = 0.04). Within the MDD group both $CD127^{low}/CCR4^{+}$ T_{reg} cells and memory T_{reg} cells were inversely correlated with CRP concentrations: r = −0.32, p = 0.02 and r = −0.34, p = 0.01, respectively. However, sleep disturbance was not related to T_{reg} cell counts.

Also consistent with our *a priori* hypothesis, the MDD group had a lower percentage of $CD56^{+}CD16^{-}$ NKC than the HC group (0.57% versus 0.77%, η^2 = 0.04) although this difference was not significant after age, sex, BMI, and batch were controlled. Further, $CD56^{+}CD16^{-}$ NKC counts were inversely correlated with PSQI scores both with and without controlling for MADRS scores (Pearson: r = −0.35, p = 0.018; partial: r = −0.45, p = 0.005). In addition, compared to the MDD group without self-reported sleep disturbance, the MDD participants with sleep disturbance showed a trend towards lower $CD56^{+}CD16^{-}$ NKC counts both with ($F_{1,43}$ = 3.30, p=0.076) and without ($F_{1,43}$ = 3.51, p=0.068) controlling for depression severity. The percentage of $CD56^{+}CD16^{+}$ NKC did not differ significantly between the MDD and HC groups (p's<0.1) and therefore the association between this cell type and sleep disturbance was not tested.

In contrast to the results obtained in our pilot study, there were no significant group differences in either classical or non-classical monocyte counts (all p's > 0.3; table 2).

Regarding the secondary analyses, the MDD group showed a trend towards significantly higher percentages of effector memory $CD8^{+}$ cells (5.73% in MDD versus 4.09% in HC, η^2 = 0.06) independent of BMI, age, sex, and batch effects (Table 2). Within the MDD group, PSQI score was positively associated with effector memory $CD8^{+}$ cell counts (Pearson: r = 0.30, p = 0.049; partial correlation: r = 0.29, p = 0.061). In addition, compared to the MDD group without self-reported sleep disturbance, the MDD participants with sleep disturbance showed a trend towards greater effector memory CD8+ cell counts both with ($F_{1,43}$ = 3.35, p=0.074) and without ($F_{1,43}$ = 3.43, p=0.071) controlling for depression severity.

4. Discussion

There were four main findings: (1) Compared with HCs, the MDD group had a significantly greater percentage of $CD127^{low}/CCR4^{+}$ T_{reg} cells as well as a trend towards a greater percentage of memory T_{reg} cells. (2) The MDD group showed reduction in $CD56^{+}CD16^{-}$ NKC count compared with the HC group although this group difference depended on BMI. Further, MDD subjects with greater reductions in $CD56^{+}CD16^{-}$ NKC numbers reported more sleep disturbance. (3) Higher counts of effector memory $CD8^{+}$ cells in the MDD group, as well as greater numbers of effector memory $CD8^{+}$ in MDD subjects with more self-reported sleep disturbance. (4) Within the MDD group, $CD127^{low}/CCR4^{+}$ T_{reg} cells and memory T_{reg} cells were inversely correlated with CRP concentrations.

Firstly, MDD subjects had a greater percentage of $CD127^{low}/CCR4^{+}$ T_{reg} cells as well as a trend towards a greater percentage of memory T_{reg} cells. Our finding of increased T_{reg} levels in MDD differs from a number of studies that have reported reduced T_{reg} counts in depressed populations (Grosse et al., 2016b; Li et al., 2010). In contrast, our results are consistent with data from a study of elderly subjects that reported that an elevated percentage of T_{reg} cells was associated with worse physical and mental health status, as well as higher levels of depressive symptomatology (Ronaldson et al., 2016). Our finding also is potentially consistent with several preclinical studies. For instance, stressed mice that developed UV-induced tumors had greater numbers of T_{reg} cells in both the skin and blood (Saul et al., 2005). Second, T cells reactive to brain-associated self-proteins are important for brain tissue homeostasis and the ability to cope with a stressor (odor of a predator). This anti-stress effect was blocked by $CD4^{+}CD25^{+}$ T_{reg} cells and further, depletion of the T_{reg} cells from wild-type mice improved the ability of the mice to withstand the stressor (Cohen et al., 2006). This phenomenon which extends more broadly to various types of CNS insults, was discovered by Schwartz, Kipnis, and colleagues, and is known as protective autoimmunity. Protective autoimmunity is an adaptive response in which autoreactive T cells are harnessed to mitigate neuronal damage and is correlated with resistance to the development of autoimmune disease (Kipnis et al., 2004b; Schwartz and Kipnis, 2002, 2005). Conceivably, excessive upregulation of T_{reg} cells renders individuals incapable of mounting an effective protective autoimmune response, making them vulnerable to stressors and hence the subsequent development of depression. The binding of dopamine to D_1 receptors, which are only found on naive T_{reg} cells, reduces the suppressive activity and the adhesive and migratory abilities of T_{reg} cells (Kipnis et al., 2004a). Interestingly, positron emission tomography (PET) studies are indicative of decreases in D_1 receptor binding (BP_{ND}) in depressed samples (Savitz and Drevets, 2013).

Another possibility is that the increased levels of T_{reg} cells are indicative of an accelerated aging process in MDD. During aging, thymic T-cell output decreases resulting in a diminished capacity to produce new T-cells. There is however, a compensatory age-related increase in peripheral T_{reg} cells (Vadasz et al., 2013), and there is emerging evidence that MDD is characterized by accelerated aging. For instance, several studies have reported a reduction in the length of telomeres, an index of cellular aging, in MDD cohorts (Darrow et al., 2016), a finding that is accentuated by increased severity and duration of symptoms suggesting a “dose” effect (Verhoeven et al., 2014).

Secondly, consistent with the literature, MDD subjects showed a trend towards a significant decrease in NKC compared to HC. The reduction in NKC was observed for both the $CD56^+CD16^+$ and $CD56^+CD16^-$ populations although the effect was only statistically significant for the $CD56^+CD16^-$ population and depended on BMI (see ANOVA in Table 2). Thus, we noted that findings of NKC reductions in MDD need to be interpreted with a caution. $CD56^+CD16^+$ cells are the predominant population of NKC in the blood and possess significant cytotoxic capacity (Moretta et al., 2006). In contrast, $CD56^+CD16^-$ NKC are the main NKC subset in lymphoid tissue and play an important immunoregulatory role, producing anti-inflammatory cytokines and the immunoregulatory purine molecule, adenosine, as well as playing an important role in T cell proliferation (Fu et al., 2013; Laroni et al., 2011; Morandi et al., 2015; Schepis et al., 2009). Since $CD56^+CD16^-$ cells are a major producer of IFNγ (Cooper et al., 2001), the decrease in this cellular subset could partly explain the results of previous studies which have reported that sleep disturbance and/or sleep deprivation is associated with a shift away from T_h1 immunity and an increased vulnerability to viral infection.

Thirdly, the MDD group exhibited a higher percentage of effector memory $CD8^+$ cells than HCs. To our knowledge, the percentage of *memory* $CD8^+$ cells has not previously been evaluated specifically in depression, although several studies reported higher levels of $CD8^+$ cells *per se*, in depressed populations (Grosse et al., 2016a; Pavon et al., 2006). Potentially consistent with our finding, non-responders to treatment with either venlafaxine or imipramine showed higher levels of $CD8^+$ cells compared to depressed subjects who responded to treatment (Grosse et al., 2016a). Additionally, a study of HIV positive women reported that depressive and anxiety symptoms were significantly associated with higher activated $CD8^+$ counts and higher viral load levels suggesting a mechanism by which depression may have a negative effect on HIV disease progression (Evans et al., 2002). Similarly, compared with multiple sclerosis (MS) patients without comorbid MDD, depressed MS patients had greater numbers of pro-inflammatory cytokine-producing CD8+ cells after stimulation with phytohemagglutanin (PHA) (Gold et al., 2011).

One possible explanation for the fact that the increase in $CD8^+$ cells in MDD was limited to the effector memory population is impaired viral immunity leading to reactivation of latent herpesviruses (Koch et al., 2007). Usually a small population of memory $CD8^+$ cells is preserved after viral infection, however, periodic reactivation of herpesviruses such as cytomegalovirus causes the virus-specific $CD8^+$ cell population to reach high frequencies (up to 20% of all $CD8^+$ cells) and to specifically acquire the status of effector memory $CD8^+$ cells (Kim et al., 2015; Lang et al., 2009; O'Hara et al., 2012; Snyder et al., 2008). This effect may explain why the MDD and HC groups did not differ significantly from each other in the percentage of central memory $CD8^+$ cells. Effector memory $CD8^+$ cells express chemokine receptors and adhesion molecules that give them the ability to migrate to inflamed peripheral tissues and rapidly activate to produce perforin granules and IFNγ (Sallusto et al., 2004). In contrast, the central memory $CD8^+$ cells behave more like true memory cells, expressing the receptors CCR7 and CD62L which allow them to home to T cell areas of the secondary lymphoid organs. Here they display no effector function, but readily proliferate and differentiate into effector cells in response to antigenic stimulation (Sallusto et al., 2004).

Effector memory $CD8^+$ counts were positively correlated with sleep disturbance within the MDD group. Conceivably, sleep disturbance increases vulnerability to a range of infectious agents many of which elicit the formation of effector memory $CD8^+$ cells. For instance, short (≤ 6 hours) sleepers were found to be at increased risk for developing a cold after a rhinovirus challenge (Prather et al., 2017; Prather et al., 2015).

Fourth, this is one of the few studies to address the relationship between inflammation and immune suppression in MDD, a question which has been the subject of debate (Blume et al., 2011). In elderly stressed, but not necessarily depressed caregivers receiving the influenza vaccine, decreased stimulated IL-1β release from monocytes and IL-2 release from PBMCs co-occurred with decreased antibody titers (Kiecolt-Glaser et al., 1996), suggesting that in stressed individuals a *reduced* inflammatory response is associated with immune suppression. In contrast, Segerstrom et al. (2012), who reported a negative correlation between psychological stress and influenza vaccine titers, found that the inflammatory response to the vaccine (defined by IL-6 concentration), was *independent* of the antibody response.

Here, we found that within the MDD group, but not the HC group, both $CD127^{low}/CCR4^+$ T_{reg} cells, and memory T_{reg} cells were inversely correlated with CRP concentrations. However, there was no significant association between effector memory $CD8^+$ cells and CRP, nor between NKC and CRP. Thus, our results do not support the hypothesis that depression-associated abnormalities in $CD8^+$ and NKC populations are driven by current inflammation. The relationship between T_{reg} cell numbers and CRP is deserving of further study. It is conceivable that an upregulation of T_{reg} cell numbers occurs as a compensatory response to high levels of inflammation, leading to a subsequent decrease in CRP concentrations. Irrespective of the mechanism involved, the inverse association between CRP and Treg counts is consistent with a recent study reporting an inverse correlation between IL-6 responses to acute psychophysiological stress and Treg cell counts in the Whitehall II cohort (Ronaldson et al., 2016).

There are a number of limitations of the study that deserve mention. First, we defined T_{reg} cells based on expression of the cell surface proteins, CD127 and CCR4, rather than the classical intracellular marker of T_{reg} cells, FOXP3. It is theoretically possible that a proportion of the $CD127^{low}/CCR4^+$ T_{reg} cells may be recently activated T cells. Nevertheless, this possibility is mitigated by the fact that there was no group difference in monocyte cells. Second, we performed multiple statistical tests when evaluating diagnostic group differences in cell populations, raising the possibility of false positive results. Nevertheless, this possibility is mitigated by the facts that: (a) we used an uncorrected p-value for just three classes of immune cells, i.e. $CD4^+$, $CD8^+$, and NKC; (b) two subtypes of T_{reg} cells, and two subtypes of NKC differed significantly (or trended toward significance) between groups, and false positive results would presumably be more randomly distributed; (c) we had a strong rationale for our *a priori* selection of cell populations based on the literature as well as our previous pilot study. Third, we measured the normalized percentages of immune cell populations rather than the functional activity of these cell types although in the case of NKC (Frank et al., 2002; Maes et al., 1994) there is a significant correlation between a reduction in numbers and a reduction in activity. Fourth, the assessment of sleep

disturbance relied on a self-report questionnaire. However, the PSQI shows a high sensitivity (99%) and specificity (84%) in identifying insomnia in addition to significant correlations with other sleep measures including sleep diaries and polysomnography (Backhaus et al., 2002). Fifth, it is possible that sleep disturbance could affect waking time, such that differences in cellular distribution could reflect blood sampling at different points in the circadian cycle among those with sleep disturbance rather than an abnormality in adaptive immune function *per se*. Sixth, we measured the distribution of circulating immune cell populations and these results might not reflect the cellular changes taking place in the tissues, spleen, and lymph nodes. Seventh, the depressed participants were unmedicated and in relatively good health. Further, approximately half of the sample met DSM-IV criteria for partial remission or were mildly depressed. Thus, it is possible that the results are not generalizable to a severely ill population. Eighth, the association between sleep disturbance and immune cell populations reported here is derived from cross-sectional data and we cannot draw causal inferences regarding the effect of sleep on immune function.

In sum, our results not only replicate previous studies showing that sleep disturbance in the context of depression significantly reduces NKC numbers, but also provide important new leads in our understanding of the immunological pathways that are preferentially affected in MDD. Specifically, we have shown that there is a deficit in effector memory $CD8^+$ cells in MDD and that effector memory $CD8^+$ cells may be impacted by sleep disturbance. Finally, our results highlight the potential importance of T_{reg} cell function in MDD. Further research employing experimental designs is needed to better understand the mechanisms underlying these abnormalities.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This study was supported by the National Institute of Mental Health grant R01MH098099. The authors thank all staff members at Laureate Institute for Brain Research, especially Brent Wurfel, M.D., Matthew Meyer, M.D., and William Yates, M.D. for conducting psychiatric interviews, Tim Collins for administering clinical interview and assessments, Megan Cole, Saman Aziz, and Cindy Bonebright for performing blood draws, and Julie Owen, Julie Crawford, Leslie Walker, and Tressia Lewis for working on all MRI technical issues. In addition, the authors acknowledge the contributions of Julie H. Marino, Ashlee Taylor, Brenda Davis, and Debbie Neal from the laboratory of TKT towards the transport, processing, and handling of the blood samples.

WCD is an employee of Janssen Research & Development, LLC, of Johnson & Johnson, Inc. The other authors declare no financial interests in relation to the present work.

References

Axelsson J, Rehman JU, Akerstedt T, Ekman R, Miller GE, Hoglund CO, Lekander M. Effects of sustained sleep restriction on mitogen-stimulated cytokines, chemokines and T helper 1/T helper 2 balance in humans. PLoS One. 2013; 8:e82291. [PubMed: 24349251]

Backhaus J, Junghanns K, Broocks A, Riemann D, Hohagen F. Test-retest reliability and validity of the Pittsburgh Sleep Quality Index in primary insomnia. J Psychosom Res. 2002; 53:737–740. [PubMed: 12217446]

Baglioni C, Battagliese G, Feige B, Spiegelhalder K, Nissen C, Voderholzer U, Lombardo C, Riemann D. Insomnia as a predictor of depression: a meta-analytic evaluation of longitudinal epidemiological studies. J Affect Disord. 2011; 135:10–19. [PubMed: 21300408]

Blume J, Douglas SD, Evans DL. Immune suppression and immune activation in depression. Brain, behavior, and immunity. 2011; 25:221–229.

Buysse DJ, Reynolds CF 3rd, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. Psychiatry Res. 1989; 28:193–213. [PubMed: 2748771]

Cho HJ, Lavretsky H, Olmstead R, Levin MJ, Oxman MN, Irwin MR. Sleep disturbance and depression recurrence in community-dwelling older adults: a prospective study. Am J Psychiatry. 2008; 165:1543–1550. [PubMed: 18765482]

Cohen H, Ziv Y, Cardon M, Kaplan Z, Matar MA, Gidron Y, Schwartz M, Kipnis J. Maladaptation to mental stress mitigated by the adaptive immune system via depletion of naturally occurring regulatory CD4+CD25+ cells. J Neurobiol. 2006; 66:552–563. [PubMed: 16555237]

Cohen S, Doyle WJ, Alper CM, Janicki-Deverts D, Turner RB. Sleep habits and susceptibility to the common cold. Arch Intern Med. 2009; 169:62–67. [PubMed: 19139325]

Cooper MA, Fehniger TA, Caligiuri MA. The biology of human natural killer-cell subsets. Trends Immunol. 2001; 22:633–640. [PubMed: 11698225]

Cosyns P, Maes M, Vandewoude M, Stevens WJ, De Clerck LS, Schotte C. Impaired mitogen-induced lymphocyte responses and the hypothalamic-pituitary-adrenal axis in depressive disorders. J Affect Disord. 1989; 16:41–48. [PubMed: 2521650]

Cover H, Irwin M. Immunity and depression: insomnia, retardation, and reduction of natural killer cell activity. J Behav Med. 1994; 17:217–223. [PubMed: 8035453]

Darrow SM, Verhoeven JE, Revesz D, Lindqvist D, Penninx BW, Delucchi KL, Wolkowitz OM, Mathews CA. The Association Between Psychiatric Disorders and Telomere Length: A Meta-Analysis Involving 14,827 Persons. Psychosom Med. 2016; 78:776–787. [PubMed: 27359174]

Dhabhar FS. Effects of stress on immune function: the good, the bad, and the beautiful. Immunol Res. 2014; 58:193–210. [PubMed: 24798553]

Dhabhar FS, McEwen BS. Acute stress enhances while chronic stress suppresses cell-mediated immunity in vivo: a potential role for leukocyte trafficking. Brain Behav Immun. 1997; 11:286–306. [PubMed: 9512816]

Evans DL, Ten Have TR, Douglas SD, Gettes DR, Morrison M, Chiappini MS, Brinker-Spence P, Job C, Mercer DE, Wang YL, Cruess D, Dube B, Dalen EA, Brown T, Bauer R, Petitto JM. Association of depression with viral load, CD8 T lymphocytes, and natural killer cells in women with HIV infection. Am J Psychiatry. 2002; 159:1752–1759. [PubMed: 12359683]

First, M. Biometrics Research Department. New York State Psychiatric Institute; New York, NY: Jan. 2010 Structured Clinical Interview for DSM-IV-TR Axis I Disorders -Non-Patient Edition (SCID-I/NP).

Fondell E, Axelsson J, Franck K, Ploner A, Lekander M, Balter K, Gaines H. Short natural sleep is associated with higher T cell and lower NK cell activities. Brain Behav Immun. 2011; 25:1367–1375. [PubMed: 21496482]

Frank MG, Wieseler Frank JL, Hendricks SE, Burke WJ, Johnson DR. Age at onset of major depressive disorder predicts reductions in NK cell number and activity. J Affect Disord. 2002; 71:159–167. [PubMed: 12167512]

Fu B, Li X, Sun R, Tong X, Ling B, Tian Z, Wei H. Natural killer cells promote immune tolerance by regulating inflammatory TH17 cells at the human maternal-fetal interface. Proc Natl Acad Sci U S A. 2013; 110:E231–240. [PubMed: 23271808]

Glaser R, Kiecolt-Glaser JK. Stress-induced immune dysfunction: implications for health. Nat Rev Immunol. 2005; 5:243–251. [PubMed: 15738954]

Gold SM, Kruger S, Ziegler KJ, Krieger T, Schulz KH, Otte C, Heesen C. Endocrine and immune substrates of depressive symptoms and fatigue in multiple sclerosis patients with comorbid major depression. J Neurol Neurosurg Psychiatry. 2011; 82:814–818. [PubMed: 21296901]

Grosse L, Carvalho LA, Birkenhager TK, Hoogendijk WJ, Kushner SA, Drexhage HA, Bergink V. Circulating cytotoxic T cells and natural killer cells as potential predictors for antidepressant

response in melancholic depression. Restoration of T regulatory cell populations after antidepressant therapy. Psychopharmacology (Berl). 2016a; 233:1679–1688. [PubMed: 25953327]

Grosse L, Hoogenboezem T, Ambree O, Bellingrath S, Jorgens S, de Wit HJ, Wijkhuijs AM, Arolt V, Drexhage HA. Deficiencies of the T and natural killer cell system in major depressive disorder: T regulatory cell defects are associated with inflammatory monocyte activation. Brain Behav Immun. 2016b; 54:38–44. [PubMed: 26674997]

Irwin M, Costlow C, Williams H, Artin KH, Chan CY, Stinson DL, Levin MJ, Hayward AR, Oxman MN. Cellular immunity to varicella-zoster virus in patients with major depression. The Journal of infectious diseases. 1998; 178(Suppl 1):S104–108. [PubMed: 9852986]

Irwin M, Mascovich A, Gillin JC, Willoughby R, Pike J, Smith TL. Partial sleep deprivation reduces natural killer cell activity in humans. Psychosom Med. 1994; 56:493–498. [PubMed: 7871104]

Irwin M, McClintick J, Costlow C, Fortner M, White J, Gillin JC. Partial night sleep deprivation reduces natural killer and cellular immune responses in humans. Faseb J. 1996; 10:643–653. [PubMed: 8621064]

Irwin MR. Human psychoneuroimmunology: 20 years of discovery. Brain Behav Immun. 2008; 22:129–139. [PubMed: 17911004]

Irwin MR. Why sleep is important for health: a psychoneuroimmunology perspective. Annu Rev Psychol. 2015; 66:143–172. [PubMed: 25061767]

Irwin MR, Cole SW. Reciprocal regulation of the neural and innate immune systems. Nature reviews Immunology. 2011; 11:625–632.

Irwin MR, Levin MJ, Laudenslager ML, Olmstead R, Lucko A, Lang N, Carrillo C, Stanley HA, Caulfield MJ, Weinberg A, Chan IS, Clair J, Smith JG, Marchese RD, Williams HM, Beck DJ, McCook PT, Zhang JH, Johnson G, Oxman MN. Varicella zoster virus-specific immune responses to a herpes zoster vaccine in elderly recipients with major depression and the impact of antidepressant medications. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2013; 56:1085–1093. [PubMed: 23413415]

Irwin MR, Miller AH. Depressive disorders and immunity: 20 years of progress and discovery. Brain, behavior, and immunity. 2007; 21:374–383.

Irwin MR, Olmstead R, Carrillo C, Sadeghi N, Nicassio P, Ganz PA, Bower JE. Tai Chi Chih Compared With Cognitive Behavioral Therapy for the Treatment of Insomnia in Survivors of Breast Cancer: A Randomized, Partially Blinded, Noninferiority Trial. J Clin Oncol. 2017 JCO2016710285.

Kiecolt-Glaser JK, Glaser R, Gravenstein S, Malarkey WB, Sheridan J. Chronic stress alters the immune response to influenza virus vaccine in older adults. Proceedings of the National Academy of Sciences of the United States of America. 1996; 93:3043–3047. [PubMed: 8610165]

Kim J, Kim AR, Shin EC. Cytomegalovirus Infection and Memory T Cell Inflation. Immune Netw. 2015; 15:186–190. [PubMed: 26330804]

Kipnis J, Cardon M, Avidan H, Lewitus GM, Mordechay S, Rolls A, Shani Y, Schwartz M. Dopamine, through the extracellular signal-regulated kinase pathway, downregulates CD4+CD25+ regulatory T-cell activity: implications for neurodegeneration. J Neurosci. 2004a; 24:6133–6143. [PubMed: 15240805]

Kipnis J, Cohen H, Cardon M, Ziv Y, Schwartz M. T cell deficiency leads to cognitive dysfunction: implications for therapeutic vaccination for schizophrenia and other psychiatric conditions. Proc Natl Acad Sci U S A. 2004b; 101:8180–8185. [PubMed: 15141078]

Koch S, Larbi A, Ozcelik D, Solana R, Gouttefangeas C, Attig S, Wikby A, Strindhall J, Franceschi C, Pawelec G. Cytomegalovirus infection: a driving force in human T cell immunosenescence. Ann N Y Acad Sci. 2007; 1114:23–35. [PubMed: 17986574]

Lang A, Brien JD, Nikolich-Zugich J. Inflation and long-term maintenance of CD8 T cells responding to a latent herpesvirus depend upon establishment of latency and presence of viral antigens. J Immunol. 2009; 183:8077–8087. [PubMed: 20007576]

Lange T, Dimitrov S, Born J. Effects of sleep and circadian rhythm on the human immune system. Ann N Y Acad Sci. 2010; 1193:48–59. [PubMed: 20398008]

Laroni A, Gandhi R, Beynon V, Weiner HL. IL-27 imparts immunoregulatory function to human NK cell subsets. PLoS One. 2011; 6:e26173. [PubMed: 22039443]

Lee E, Cho HJ, Olmstead R, Levin MJ, Oxman MN, Irwin MR. Persistent sleep disturbance: a risk factor for recurrent depression in community-dwelling older adults. Sleep. 2013; 36:1685–1691. [PubMed: 24179302]

Leserman J. Role of depression, stress, and trauma in HIV disease progression. Psychosom Med. 2008; 70:539–545. [PubMed: 18519880]

Li Y, Xiao B, Qiu W, Yang L, Hu B, Tian X, Yang H. Altered expression of CD4(+)CD25(+) regulatory T cells and its 5-HT(1a) receptor in patients with major depression disorder. J Affect Disord. 2010; 124:68–75. [PubMed: 19900711]

Maecker HT, McCoy JP, Nussenblatt R. Standardizing immunophenotyping for the Human Immunology Project. Nat Rev Immunol. 2012; 12:191–200. [PubMed: 22343568]

Maes M, Meltzer HY, Stevens W, Calabrese J, Cosyns P. Natural killer cell activity in major depression: relation to circulating natural killer cells, cellular indices of the immune response, and depressive phenomenology. Progress in neuropsychopharmacology & biological psychiatry. 1994; 18:717–730.

Mechawar N, Savitz J. Neuropathology of mood disorders: do we see the stigmata of inflammation? Transl Psychiatry. 2016; 6:e946. [PubMed: 27824355]

Michel T, Poli A, Cuapio A, Briquemont B, Iserentant G, Ollert M, Zimmer J. Human CD56bright NK Cells: An Update. J Immunol. 2016; 196:2923–2931. [PubMed: 26994304]

Miller AH. Depression and immunity: a role for T cells? Brain Behav Immun. 2010; 24:1–8. [PubMed: 19818725]

Miller GE, Cohen S, Herbert TB. Pathways linking major depression and immunity in ambulatory female patients. Psychosom Med. 1999; 61:850–860. [PubMed: 10593638]

Miller GE, Cohen S, Pressman S, Barkin A, Rabin BS, Treanor JJ. Psychological stress and antibody response to influenza vaccination: when is the critical period for stress, and how does it get inside the body? Psychosom Med. 2004; 66:215–223. [PubMed: 15039506]

Morandi F, Horenstein AL, Chillemi A, Quarona V, Chiesa S, Imperatori A, Zanellato S, Mortara L, Gattorno M, Pistoia V, Malavasi F. CD56brightCD16- NK Cells Produce Adenosine through a CD38-Mediated Pathway and Act as Regulatory Cells Inhibiting Autologous CD4+ T Cell Proliferation. J Immunol. 2015; 195:965–972. [PubMed: 26091716]

Moretta L, Ferlazzo G, Bottino C, Vitale M, Pende D, Mingari MC, Moretta A. Effector and regulatory events during natural killer-dendritic cell interactions. Immunol Rev. 2006; 214:219–228. [PubMed: 17100887]

O'Hara GA, Welten SP, Klenerman P, Arens R. Memory T cell inflation: understanding cause and effect. Trends Immunol. 2012; 33:84–90. [PubMed: 22222196]

Pavon L, Sandoval-Lopez G, Eugenia Hernandez M, Loria F, Estrada I, Perez M, Moreno J, Avila U, Leff P, Anton B, Heinze G. Th2 cytokine response in Major Depressive Disorder patients before treatment. J Neuroimmunol. 2006; 172:156–165. [PubMed: 16457895]

Petrovsky N, Harrison LC. Diurnal rhythmicity of human cytokine production: a dynamic disequilibrium in T helper cell type 1/T helper cell type 2 balance? J Immunol. 1997; 158:5163–5168. [PubMed: 9164932]

Prather AA, Hall M, Fury JM, Ross DC, Muldoon MF, Cohen S, Marsland AL. Sleep and antibody response to hepatitis B vaccination. Sleep. 2012; 35:1063–1069. [PubMed: 22851802]

Prather AA, Janicki-Deverts D, Adler NE, Hall M, Cohen S. Sleep Habits and Susceptibility to Upper Respiratory Illness: the Moderating Role of Subjective Socioeconomic Status. Ann Behav Med. 2017; 51:137–146. [PubMed: 27679462]

Prather AA, Janicki-Deverts D, Hall MH, Cohen S. Behaviorally Assessed Sleep and Susceptibility to the Common Cold. Sleep. 2015; 38:1353–1359. [PubMed: 26118561]

Redwine L, Dang J, Hall M, Irwin M. Disordered sleep, nocturnal cytokines, and immunity in alcoholics. Psychosom Med. 2003; 65:75–85. [PubMed: 12554818]

Ronaldson A, Gazali AM, Zalli A, Kaiser F, Thompson SJ, Henderson B, Steptoe A, Carvalho L. Increased percentages of regulatory T cells are associated with inflammatory and neuroendocrine responses to acute psychological stress and poorer health status in older men and women. Psychopharmacology (Berl). 2016; 233:1661–1668. [PubMed: 25678193]

Sakami S, Ishikawa T, Kawakami N, Haratani T, Fukui A, Kobayashi F, Fujita O, Araki S, Kawamura N. Coemergence of insomnia and a shift in the Th1/Th2 balance toward Th2 dominance. Neuroimmunomodulation. 2002; 10:337–343. [PubMed: 12907840]

Sallusto F, Geginat J, Lanzavecchia A. Central memory and effector memory T cell subsets: function, generation, and maintenance. Annual review of immunology. 2004; 22:745–763.

Saul AN, Oberyszyn TM, Daugherty C, Kusewitt D, Jones S, Jewell S, Malarkey WB, Lehman A, Lemeshow S, Dhabhar FS. Chronic stress and susceptibility to skin cancer. J Natl Cancer Inst. 2005; 97:1760–1767. [PubMed: 16333031]

Savitz J, Tan C, Taylor A, Drevets W, Teague K. Abnormalities in regulatory T cells and natural killer cells in major depressive disorder. Journal of Immunology. 2013; 190:43.25.

Savitz JB, Drevets WC. Neuroreceptor imaging in depression. Neurobiology of disease. 2013; 52:49–65. [PubMed: 22691454]

Schepis D, Gunnarsson I, Eloranta ML, Lampa J, Jacobson SH, Karre K, Berg L. Increased proportion of CD56bright natural killer cells in active and inactive systemic lupus erythematosus. Immunology. 2009; 126:140–146. [PubMed: 18564343]

Schwartz M, Kipnis J. Autoimmunity on alert: naturally occurring regulatory CD4(+)CD25(+) T cells as part of the evolutionary compromise between a ‘need’ and a ‘risk’. Trends Immunol. 2002; 23:530–534. [PubMed: 12401405]

Schwartz M, Kipnis J. Protective autoimmunity and neuroprotection in inflammatory and noninflammatory neurodegenerative diseases. J Neurol Sci. 2005; 233:163–166. [PubMed: 15949502]

Segerstrom SC, Hardy JK, Evans DR, Greenberg RN. Vulnerability, distress, and immune response to vaccination in older adults. Brain Behav Immun. 2012; 26:747–753. [PubMed: 22062498]

Sephton SE, Dhabhar FS, Keuroghlian AS, Giese-Davis J, McEwen BS, Ionan AC, Spiegel D. Depression, cortisol, and suppressed cell-mediated immunity in metastatic breast cancer. Brain Behav Immun. 2009; 23:1148–1155. [PubMed: 19643176]

Snyder CM, Cho KS, Bonnett EL, van Dommelen S, Shellam GR, Hill AB. Memory inflation during chronic viral infection is maintained by continuous production of short-lived, functional T cells. Immunity. 2008; 29:650–659. [PubMed: 18957267]

Spiegel K, Sheridan JF, Van Cauter E. Effect of sleep deprivation on response to immunization. Jama. 2002; 288:1471–1472.

Toben C, Baune BT. An Act of Balance Between Adaptive and Maladaptive Immunity in Depression: a Role for T Lymphocytes. J Neuroimmune Pharmacol. 2015; 10:595–609. [PubMed: 26133121]

Vadasz Z, Haj T, Kessel A, Toubi E. Age-related autoimmunity. BMC Med. 2013; 11:94. [PubMed: 23556986]

Verhoeven JE, Revesz D, Epel ES, Lin J, Wolkowitz OM, Penninx BW. Major depressive disorder and accelerated cellular aging: results from a large psychiatric cohort study. Mol Psychiatry. 2014; 19:895–901. [PubMed: 24217256]

Williams JB, Kobak KA. Development and reliability of a structured interview guide for the Montgomery Asberg Depression Rating Scale (SIGMA). Br J Psychiatry. 2008; 192:52–58. [PubMed: 18174510]

Zorrilla EP, Luborsky L, McKay JR, Rosenthal R, Houldin A, Tax A, McCorkle R, Seligman DA, Schmidt K. The relationship of depression and stressors to immunological assays: a meta-analytic review. Brain, behavior, and immunity. 2001; 15:199–226.

Highlights

- Effector memory $CD8^+$ cells and regulatory T cells were increased in depression.
- Increased memory $CD8^+$ cells were related to sleep disturbance in depression.
- Natural killer cells (NKC) were decreased in depression.
- Reduced natural killer cells were related to sleep disturbance in depression.

Table 1

Characteristics of the Sample

Variable		All (N = 110)	MDD (n = 54)	HC (n = 56)	t or χ^2
Age (in years)		33.2 (11.07)	34.3 (11.20)	32.1 (10.93)	1.05 (108)
Sex (Female/Male)		83/27	41/13	42/14	0.01 (108)
Ethnicity	Caucasian	85	40	45	2.66 (5)
	African American	8	4	4	
	Native American	4	3	1	
	Native Hawaiian/Pacific Islander	2	1	1	
	Asian American	1	0	1	
	Other	10	6	4	
Occupational Status	Employed full-time	45	20	25	7.36 (7)
	Employed part-time	17	11	6	
	Homemaker	5	1	4	
	Full-time student	18	7	11	
	Unemployed less than 6 months, but expects to work	4	2	2	
	Unemployed 6 months or more, but expects to work	1	1	0	
	Unemployed 6 months or more and does not expect to work	1	1	0	
	Other	1	0	1	
Educational Status	Some high school	2	1	1	5.00 (4)
	High school graduate	7	4	3	
	Some college /technical school	44	25	19	
	College graduate	32	11	21	
	Masters or above	7	2	5	
Smoking (Yes/No)		8/85	5/39	3/46	0.28 (1)
BMI		28.0 (6.99)	29.6 (7.62)	26.4 (6.00)	2.39 * (108)

Variable	All (N = 110)	MDD (n = 54)	HC (n = 56)	t or χ^2
MADRS	11.7 (11.63)	22.3 (7.90)	2.1 (2.64)	18.02** (105)
PSQI	5.2 (4.18)	8.3 (4.13)	2.7 (1.88)	8.95** (97)
PAQ	110.2 (75.5)	104.1 (89.60)	115.3 (61.59)	0.75 (101)
CRP (mg/L)	3.02 (3.91)	3.51 (3.90)	2.55 (3.91)	1.27 (105)

Note. Values enclosed in the parenthesis represent standard deviations (under "All," "MDD," and "HC") or degrees of freedom (under "t or χ^2"). HC = healthy controls; BMI = body mass index; MADRS = total score on Montgomery-Åsberg Depression Rating Scale; PSQI = total score on Pittsburgh Sleep Quality Index; PAQ = total score on Physical Activity Questionnaire. Note that smoking information was not available for all participants.

* $p < 0.05$;

** $p < 0.01$.

Table 2

Summary of Comparisons in the Percentages of Live Peripheral Blood Mononuclear Cell (PBMC) Type between the MDD and the Healthy Control Groups ($N = 110$)

			ANOVA		ANCOVA1		ANCOVA2	
	MDD (n = 54)	**HC (n = 56)**	***F* (1,108)**	***p***	***F* (1,107)**	***p***	***F* (1,104)**	***p***
T cells, T_H cells, and T_{reg} cells								
$CD3^+$	56.05	54.92	0.59	0.445	0.09	0.771	0.17	0.677
$CD4^+$	27.96	27.44	0.16	0.691	0.00	0.952	0.00	0.996
Central memory $CD4^+$	9.65	8.96	0.95	0.331	0.17	0.684	0.03	0.875
Naive $CD4^+$	9.75	10.22	0.27	0.602	0.38	0.537	0.20	0.655
Effector memory $CD4^+$	4.82	4.43	1.17	0.282	0.59	0.445	0.16	0.693
Effector $CD4^+$	3.74	3.84	0.05	0.818	0.02	0.890	0.10	0.756
T_H1	3.19	3.25	0.04	0.836	0.46	0.499	0.88	0.349
T_H2	18.48	18.55	0.00	0.957	0.01	0.935	0.00	0.964
T_H17	4.14	3.70	1.69	0.197	1.18	0.280	0.75	0.389
$CD25^+$ T_{reg}	0.82	0.73	1.39	0.242	2.15	0.145	2.01	0.159
$CD127^{low}/CCR4^+$ T_{reg}	0.42	0.35	3.17	0.078	**4.21**	**0.043**	**4.03**	**0.047**
Memory T_{reg}	0.39	0.33	2.92	0.090	**3.99**	**0.048**	3.78	0.054
$CD8^+$	21.90	19.93	2.65	0.107	1.96	0.165	2.09	0.151
Central memory $CD8^+$	2.65	2.33	1.24	0.269	0.58	0.449	0.50	0.481
Naive $CD8^+$	7.66	7.94	0.20	0.655	0.38	0.537	0.03	0.867
Effector memory $CD8^+$	5.73	4.09	**7.68**	**0.007**	**6.60**	**0.012**	**5.50**	**0.021**
Effector $CD8^+$	5.86	5.58	0.22	0.638	0.32	0.575	0.20	0.658
Leukocytes								
$CD3^-/CD19^-$	35.93	38.97	3.53	0.063	1.36	0.247	2.31	0.132
$CD14^-/CD20^-$	12.49	14.44	3.29	0.072	2.28	0.134	3.09	0.082
HLA-DR^+	2.47	2.58	0.12	0.727	0.03	0.872	0.09	0.765
Myeloid DCs	1.58	1.73	0.60	0.440	0.89	0.348	1.86	0.175
Plasmacytoid DCs	0.52	0.50	0.02	0.887	0.22	0.637	0.38	0.538

Brain Behav Immun. Author manuscript; available in PMC 2018 November 01.

			ANOVA		ANCOVA1		ANCOVA2	
	MDD (n = 54)	**HC (n = 56)**	***F* (1,108)**	***p***	***F* (1,107)**	***p***	***F* (1,104)**	***p***
HLA-DR$^-$	10.02	11.86	3.54	0.063	2.58	0.111	3.25	0.074
CD16$^+$ NK	7.63	9.20	3.30	0.072	2.35	0.128	2.86	0.094
CD16$^-$ NK	0.57	0.77	**4.45**	**0.037**	3.22	0.076	3.67	0.058
Monocytes	21.42	22.44	0.76	0.384	0.03	0.874	0.06	0.812
Non-classical monocytes	0.70	0.65	0.35	0.556	0.62	0.431	0.51	0.475
Classical monocytes	20.71	21.79	0.89	0.349	0.05	0.822	0.09	0.763

Note. Values under MDD and HC represent the percentages (%) of cell types in live PBMCs. ANCOVA1 controlled for body mass index, and ANCOVA2 controlled for age, sex, body mass index, and batch effect. All *p*-values in this table are uncorrected. Any statistical parameter in bold met a significance level of 0.05. CD = cluster of differentiation; T_H = T helper; T_{reg} = regulatory T; CCR = CC-chemokine receptor; HLA-DR = human leukocyte antigen-antigen D related; DC = dendritic cell; NK = natural killer.