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The circulating levels of CD4+ t helper cells are higher in bipolar disorder as compared to major depressive disorder

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ABSTRACT

Introduction: Clinical differentiation between bipolar disorder (BD) and major depressive disorder (MDD) is difficult. Research has therefore focused on discriminatory biological markers. Previous studies in MDD reported T cell deficits, while the limited studies in BD reported T cell activation. Studies directly comparing circulating numbers of T cells and T cell subsets between BD and MDD are lacking. The studies in the MOODINFLAME consortium make such a comparison possible.

Methods: The number of circulating leukocyte populations (lymphocytes, monocytes, NK cells, B cells, T cells, CD3+CD8+ T cytotoxic cells, CD3+CD4+ T helper cells, Th1, Th2, Th17 and T regulatory cells) was determined using FACS technology in a cohort of 83 euthymic BD patients, 8 BD patients with a current mood episode and 165 healthy controls (HC). Data were compared to those of 34 moderately and 56 severely depressed MDD patients.

Results: Compared to MDD patients, BD patients showed significantly increased levels of Th17, Th2, Th1 and T regulatory cells (all $p < .02$). In BD patients, levels of Th17 and T regulatory cells were *increased* compared to HC ($p = .03$, $p = .02$, respectively), while MDD patients showed *decreased* levels of Th17 and Th2 compared to HC ($p = .03$, $p = .01$, respectively). Of the various medications only SSRI/SNRI usage could explain part of the Th2 decrease in MDD.

Conclusion: This study shows CD4+ T helper cell deficits in MDD patients, while normal or even raised levels of these cells were found in BD patients. The differences in CD4+ T helper cell differentiation was most outspoken for Th17 cells.

1. Introduction

Over the years, there has been accumulating evidence that dysregulation of the immune system plays a role in the pathogenesis of major depressive disorder (MDD) (Maes, 2011) and bipolar disorder (BD) (Barbosa et al., 2014). However, there are few studies that directly compare the two disorders with regard to their underlying immune alterations. We believe this to be is of high interest, since in clinical practice it is difficult to differentiate between depressive episodes in the course of BD or MDD (Benazzi et al., 2002) on the basis of symptoms alone. Misdiagnosis as MDD and delayed recognition of BD is associated with increased risk for long-term morbidity, disability and suicide (Baldessarini et al., 2012; Nasrallah, 2015). Moreover, the pharmacological treatment of both disorders is different, with more emphasis on the use of mood stabilizers (lithium, anti-epileptics and antipsychotics) for patients with BD (Yatham et al., 2013) and more emphasis on the use of antidepressants for patients with MDD. Antidepressants have an uncertain efficacy in BD (Pacchiarotti et al., 2013). Therefore, investigating immune differences between the two disorders may give us more insight into differences in pathophysiology and may have the potential to aid accurate diagnosing.

In both disorders, cells of the myeloid lineage of the immune system

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have been examined, such as circulating monocytes and microglia. Apart from an activation of microglia (Haarman et al., 2014), an upregulation of inflammation-associated genes was found in circulating monocytes of MDD patients (Grosse et al., 2014) as well as BD patients (Padmos et al., 2008). However, although both mood disorders show similar upregulated immune gene profiles, this appeared to be conditional. In BD, upregulation of genes in circulating monocytes was high only in patients experiencing a mood episode (Becking et al., 2015), while in MDD patients, monocyte immune activation was particularly high in relatively older (Grosse et al., 2014) and in antidepressant-free melancholic patients (Carvalho et al., 2014), suggesting that immune dysregulation may differ across subgroups of the mood disorder spectrum and varying with mood states.

Other important regulators of the immune response are cells of the lymphoid lineage. This lineage consists predominantly of natural killer (NK) cells, T cells and B cells. The main function of B cells is to develop into plasma cells, which produce the antibodies important in combatting foreign intruders, mainly extra-cellular bacteria (Parham, 2009). NK-cells are not-antigen specific lymphoid cells classically known as capable of killing tumor cells and virus infected cells (Herberman and Ortaldo, 1981), yet recent research suggests that these cells also play a role in regulating immune responses, among which microglial activity (Shi et al., 2011). T cells are antigen specific, generated in the thymus and capable of differentiating into T cytotoxic or T helper cells. T cells can be identified on the basis of "cluster of differentiation" (CD) proteins expressed on their surface (Parham, 2009) and/or on the cytokines they produce. All T cells (CD3+) express the typical T cell receptor (the CD3 molecule), playing a role in antigen recognition. Cytotoxic T cells (CD3 + CD8+) additionally express CD8; their main function is to induce cell apoptosis in infected or dysfunctional cells (Parham, 2009). T helper cells (CD3+CD4+) additionally express CD4 and these cells interact with other cells of the immune system to stimulate or inhibit various components of the immune reaction. T helper cells differentiate during an active immune response into several functional subgroups: T helper(h)1, Th2, and Th17 from naïve T helper cells. Th1 cells (CD3+CD4+IFN-γ+) are capable of producing IFN-γ, while Th17 cells (CD3+CD4+IL17A+) produce interleukin (IL)-17. Both Th1 and Th17 cells are capable of activating macrophages and other myeloid cells, such as microglia. Th2 cells (CD3+CD4+IL4+) secrete IL-4, which is a cytokine capable on the one hand of stimulating B-cells to become plasma cells, and on the other hand counteracts the development of Th1 cells. The subpopulation of T regulatory cells ($CD4+CD25^{high}Foxp3+$) is partly spontaneously generated in the thymus (natural T regulatory cells), while the other part is produced from naïve T cells in an immune reaction, if down regulation for such immune reaction is required (Wing and Sakaguchi, 2009). T regulatory cells are capable of dampening the activity of Th1, Th2, Th17 cells and inflammatory monocytes/macrophages.

Studies that have examined the various cells of the lymphoid lineage in MDD and BD have found disturbances in the circulating numbers and function of these cells. In MDD, there is evidence that an impaired T cell function may directly contribute to the development of depression, since T cells may play an important role in neuroprotective and anti-inflammatory functions during stress and inflammation (Miller, 2010). Furthermore, a recent systematic review suggests that T cells in MDD patients display maladaptive characteristics in terms of increased circulating numbers of CD4+ T helper cells with decreased T regulatory cell numbers (Toben and Baune, 2015). Also deficiencies of NK, Th2, Th17 cells (Grosse et al., 2016b) and T regulatory cells (Grosse et al., 2016a) were found in MDD patients compared to healthy controls.

In BD, cells of the lymphoid lineage have not been examined as extensively as in MDD, however previous research from our team found higher levels of activated CD3+ T cells in both symptomatic and euthymic BD patients compared to healthy controls (Breunis et al., 2003), and significantly higher T regulatory cells in BD patients under 40 years compared to healthy controls (Drexhage et al., 2011). However, another study found lower levels of T regulatory cells in BD patients compared to HCs (do Prado et al., 2013). Furthermore, another study examining these cells found reduced levels of T-cells, cytotoxic T cells and a lower percentage of IL-10 expressing T regulatory cells, in combination with a higher percentage of activated CD4 + CD25+ T cells (Barbosa and Rocha, 2014). Finally, a recent study in an older euthymic BD patient group showed higher levels of Th2 and Th17 cells (Vogels et al., 2017). Taken together, these findings suggest a deficiency of T cells in MDD, while a normal to overactive T cell system might characterize BD.

Therefore, the aim of the present study was to compare percentages of circulating leukocytes between BD and MDD patients tested in the same series of experiments ("MOODINFLAME website", 2014) and using the same techniques; the studies of the EU- MOODINFLAME consortium enabled such studies. We therefore investigated the number of circulating leukocyte populations in a large cohort of bipolar patients (N = 91) of whom 8 were in a current mood episode, while 83 were euthymic at the time of blood collection. We compared data to combined data of two MOODINFLAME MDD cohorts (N = 90): 40 non-medicated melancholic depressed patients and 50 naturalistically treated depressed patients. Outcomes of the MDD cohorts have been published earlier (Grosse et al., 2016a, 2016b), but not in the context of a comparison with BD. Furthermore, we combined these two MDD groups and divided them into moderately and severely depressed patients. Of all these patients, as well as of a large cohort of healthy controls (N = 165), we determined percentages of circulating total lymphocytes, monocytes, NK cells, B cells, T cells, CD3+CD8+ T cytotoxic cells, CD3+CD4+ T helper cells, and Th1, Th2, Th17, and T regulatory cells using the same standardized techniques.

Our hypothesis was that we would find in particular differences in circulating levels of T helper cell populations between the two patient groups, based on previous analyses in the MDD cohorts and in BD patients. Since our group previously showed that particularly these populations were abnormal and that these populations regulate the inflammatory state of circulating monocytes (Grosse et al., 2016b; Snijders et al., 2016), the main focus of the paper is on the CD4+ T helper cell populations.

2. Methods

2.1. Participants

The reported studies are part of the EU-funded MOODINFLAME project ("MOODINFLAME website", 2014) and were approved by the Medical Ethical Committees of Erasmus MC Rotterdam, University Medical Center Groningen, University Hospital Leuven and University Hospital Münster. All participants provided written informed consent. In the MOODINFLAME study, adult male and female participants were included who were free of inflammation-related symptoms including fever and current or recent infectious or inflammatory disease. Exclusion-criteria were: uncontrolled systemic disease, uncontrolled metabolic disease, other significant uncontrolled somatic disorders known to affect mood, use of somatic medication known to affect mood or the immune system (corticosteroids, non-steroid anti-inflammatory drugs, and statins), present or recent pregnancy. Blood was analyzed of 165 healthy controls (HC), 83 naturalistically treated euthymic bipolar patients (BD), 34 moderately depressed patients, and 56 severely depressed patients. Furthermore, as an additional study, blood from a small group of 8 BD patients with a current mood episode (seven depressed, one manic) was analyzed. HC were recruited from Groningen (The Netherlands), Rotterdam (The Netherlands), Leuven (Belgium) and Münster (Germany). Inclusion criteria for HC were the absence of schizophrenia, psychotic disorders, mood disorders, anxiety disorders, or substance-related disorders according to Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) (American Psychiatric