Brain, Behavior, and Immunity 73 (2018) 192–204

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

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

White matter microstructure alterations correlate with terminally differentiated CD8+ effector T cell depletion in the peripheral blood in mania: Combined DTI and immunological investigation in the different phases of bipolar disorder

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ARTICLE INFO

Keywords:
Bipolar disorder
Mania
White matter abnormalities
TBSS
Immunological alterations
T cells

ABSTRACT

Background: White matter (WM) microstructural abnormalities and, independently, signs of immunological activation were consistently demonstrated in bipolar disorder (BD). However, the relationship between WM and immunological alterations as well as their occurrence in the various phases of BD remain unclear.

Method: In 60 type I BD patients – 20 in manic, 20 in depressive, 20 in euthymic phases – and 20 controls we investigated: (i) diffusion tensor imaging (DTI)-derived fractional anisotropy (FA), radial diffusivity (RD) and axial diffusivity (AD) using a tract-based spatial statistics (TBSS) approach; (ii) circulating T cell subpopulations frequencies, as well as plasma levels of different cytokines; (iii) potential relationships between WM and immunological data.

Results: We found: (i) a significant widespread combined FA-RD alteration mainly in mania, with involvement of the body of corpus callosum (BCC) and superior corona radiata (SCR); (ii) significant increase in CD4+ T cells as well as significant decrease in CD8+ T cells and their subpopulations effector memory (CD8+ CD28-CD45RA-), terminal effector memory (CD8+ CD28-CD45RA+) and CD8+ IFNγ+ in mania; (iii) a significant relationship between WM and immunological alterations in the whole cohort, and a significant correlation of FA-RD abnormalities in the BCC and SCR with reduced frequencies of CD8+ terminal effector memory and CD8+ IFNγ+ T cells in mania only.

Conclusions: Our data show a combined occurrence of WM and immunological alterations in mania. WM

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https://doi.org/10.1016/j.bbi.2018.04.017
Received 22 December 2017; Received in revised form 23 April 2018; Accepted 26 April 2018
Available online 01 May 2018

abnormalities highly correlated with reduction in circulating CD8+ T cell subpopulations that are terminally differentiated effector cells prone to tissue migration, suggesting that these T cells could play a role in WM alteration in BD.

1. Introduction

1.1. Background

Bipolar Disorder (BD) type I is a prevalent recurrent and debilitating mental disease (affecting 1–2% of the general population), characterized by the occurrence of active phases of illness – i.e., mania and depression – alternated to asymptomatic periods – i.e., euthymia (A.P.A., 2013; Kraepelin, 1902).

Several studies demonstrated white matter (WM) microstructural abnormalities as one of the most consistent neurobiological alterations in BD. In particular, a recent *meta*-analysis of whole brain magnetic resonance diffusion-tensor imaging (DTI) studies on BD patients found widespread WM alterations (Wise et al., 2015). In our previous work, we investigated WM changes across the different phases of BD. Our results showed widespread WM alterations, occurring significantly in the active phases rather than in euthymia, associated with decreased structural connectivity in midline regions in mania (Magioncalda et al., 2015; Martino et al., 2016b). However, the pathogenic mechanisms underlying this pattern of WM microstructural changes are unclear.

Independently, recent evidences suggest that BD is associated with distinct immunological abnormalities, concerning both cytokines and immune cells, in the peripheral circulation and central nervous system (Anderson and Maes, 2015; Kupka et al., 2000; Reus et al., 2015). Data on cytokines alterations in BD, although heterogeneous, mainly show increased level/activity of pro-inflammatory cytokines, e.g., interleukin (IL)-6, especially in the active phases (Anderson and Maes, 2015; Brietzke et al., 2009). Moreover, these findings are further supported by evidences of increased levels in other inflammation markers, such as C-reactive protein (CRP), in BD and in mania especially (Dickerson et al., 2007; Fernandes et al., 2016). Recently, circulating frequencies of T cells and their subpopulations, which play a key role in cell-mediated immune responses, have been found to be substantially altered in BD patients (Barbosa et al., 2014a, b; Brambilla et al., 2014; Breunis et al., 2003; Cakir et al., 2015; do Prado et al., 2013; Drexhage et al., 2011; Fries et al., 2014; Knijff et al., 2006; Kohler et al., 2016; Poletti et al., 2016; Rizzo et al., 2013; Tsai et al., 1999; Wu et al., 2017). These studies were conducted on either euthymic patients (Barbosa et al., 2014b; do Prado et al., 2013; Drexhage et al., 2011; Fries et al., 2014; Rizzo et al., 2013) or BD patients overall (regardless of the phase of illness) (Brambilla et al., 2014; Breunis et al., 2003; Cakir et al., 2015; Knijff et al., 2006; Kohler et al., 2016), while studies addressing specifically depression (Poletti et al., 2016; Wu et al., 2017) or mania (Tsai et al., 1999) are rather sparse. Thus, studies that comprehensively investigate and compare the immunological abnormalities in each different phase of BD are still lacking.

It is conceivable that WM microstructural and immunological alterations, both detected in BD, could be pathogenically related. Indeed, a previous study showed that increased levels of pro-inflammatory factors are associated with loss of WM integrity in healthy subjects (Miralbell et al., 2012). More recently, two studies demonstrated a correlation between immune alterations (concerning cytokines such as tumor necrosis factor (TNF)α, interferon (IFN)γ, IL-8, IL-10, or T cells, i.e. Th17) and WM changes in the depressive phase of BD (Benedetti et al., 2016; Poletti et al., 2016). These findings corroborate the hypothesis of a possible relationship between WM and immunological alterations in BD prompting a systematic analysis on this potential relationship across all the different phases of BD, and in mania in particular.

1.2. Aims of the study

The aims of our study were to: (i) characterize WM abnormalities in each single phase of BD (replicating our previous finding in an independent sample); (ii) characterize the immunological alterations in each single phase of BD; (iii) investigate correlations between these two potential biomarkers of the disease.

We hypothesized that: (i) WM alterations are mainly detected in the active phases of illness, and in mania especially, on the basis of our previous studies (Magioncalda et al., 2015; Martino et al., 2016b); (ii) immunological abnormalities can be detected in the manic phase, coherently with the WM changes; (iii) a correlation between WM and immunological alterations can be detected especially in the manic phase, accordingly to a potential immune-related damage of WM in BD.

Table 1
Subject Demographic and Clinical Information.

	BD				HC
	BD TOT	MANIC BD	DEPRESSED BD	EUTHYMIC BD	
Sample Size *n* (%)	60 (100%)	20 (33.3%)	20 (33.3%)	20 (33.3%)	20 (100%)
Age *mean* (SD)	47.0 (8.9)	47.2 (7.9)	45.7 (8.8)	48.2 (10.1)	41.3 (14.1)
Female *n* (%)	36 (60%)	13 (65%)	11 (55%)	12 (60%)	9 (45.0%)
Tobacco Smokers *n* (%)	39 (65%)	14 (70%)	14 (70%)	11 (55%)	8 (40%)
BMI Kg/m^2 *mean* (SD)	24.9 (4.8)	24.7 (4.7)	24.8 (5.8)	25.3 (3.9)	22.9 (2.3)
Duration of Illness *mean* (SD)	16.3 (12.7)	16.0 (12.0)	14.2 (12.0)	18.8 (14.1)	–
Number of Episodes *n* (%)	5.6 (3.7)	5.5 (4.2)	5.6 (3.8)	5.6 (3.1)	–
Inpatients *n* (%)	36 (60%)	14 (70%)	20 (100%)	2 (10%)	–
HAM-D *mean* (SD)	–	7.0 (6.0)	21.1 (4.1)	1.5 (2.6)	–
YMRS *mean* (SD)	–	19.4 (5.4)	3.4 (2.7)	2.0 (2.6)	–
Psychotic Features *n* (%)	6 (10%)	4 (20%)	2 (10%)	0 (0%)	–
Mood Stabilizers *n* (%)	47 (78.3%)	17 (85%)	15 (75%)	15 (75%)	–
Antidepressants *n* (%)	30 (50%)	4 (20%)	14 (70%)	12 (60%)	–
Antipsychotics *n* (%)	41 (68.3%)	15 (75%)	16 (80%)	10 (50%)	–
Benzodiazepines *n* (%)	41 (68.3%)	15 (75%)	18 (90%)	8 (40%)	–
Unmedicated *n* (%)	1 (1.7%)	0 (0%)	0 (0%)	1 (5%)	–

Abbreviations: BD, bipolar disorder; HC, healthy controls; BMI, body mass index; HAM-D, Hamilton Depression Scale; YMRS, Young Mania Rating Scale.

2. Methods

2.1. Subjects and clinical assessment

Subjects were recruited from the inpatients and outpatients services of the Psychiatric Clinic of Genoa (San Martino Polyclinic Hospital and Department of Neuroscience at the University of Genoa), from 2015 to 2016. The Ethical Committee of San Martino Polyclinic Hospital approved the study, and written informed consent was obtained from all the participants. The study was conducted on 60 BD type I patients – 20 in manic, 20 in depressive and 20 in euthymic phases – and 20 healthy controls (HC) (see Table 1 for a detailed description of the sample). This is an independent sample with respect to our previous work (Magioncalda et al., 2015; Martino et al., 2016b). Each participant was evaluated with standardized structured and/or semi-structured clinical instruments in order to obtain information on clinical and diagnostic features, course of illness, family history, and actual and past pharmacotherapy. The instruments were: the Mini International Neuropsychiatric Interview (MINI) (Sheehan et al., 1998); the Structured Clinical Interview for DSM Axis-I Disorders/Patient edition (SCID-I/P) (Ventura et al., 1998); the Structured Clinical Interview for DSM Axis II Personality Disorders (SCID-II) (First et al., 1994); the Structured Interview for Mood Disorder – Revised (SIMD-R) (Cassano et al., 1989); the Hamilton Depression Scale (HAM-D) with 17 items (Hamilton, 1960); the Young Mania Rating Scale (YMRS) (Young et al., 1978). Physiologic, medical and psychopathologic history was also investigated, and physical and psychiatric examination was conducted.

Inclusion criteria were as follows: diagnosis of BD type I, during manic or major depressive episodes, or euthymia, according to the DSM criteria (A.P.A., 2013) assessed by the SCID-I/P; score of 17-items HAM-D $\geq$ 18 for depressed patients; score of YMRS $\geq$ 13 for manic patients; scores of HAM-D $<$ 8 and YMRS $<$ 8 for euthymic patients; age between 18 and 60; ability to provide written informed consent. Exclusion criteria were as follows: diagnosis of schizophrenia, mental retardation, dementia, or other cognitive disorders; current somatic, immune-inflammatory or infectious illnesses, history of severe or decompensated somatic and immune-inflammatory diseases, neurological diseases (stroke, cerebral vascular malformations, or epilepsy), previous head injury with loss of consciousness (for 5 or more minutes); current alcohol and substance abuse (during the preceding 3 months), history of alcohol or substance dependence, history of abuse of synthetic and/or new drugs; pregnancy and lactation; left-handedness; the inability to undergo an magnetic resonance imaging (MRI) examination (claustrophobia, metal implants, etc); previous treatment with electroconvulsive therapy, chemotherapy or brain radiotherapy. HC did not meet the DSM criteria for psychiatric disorders, either at the time of study participation or in the past; they had a HAM-D score $<$ 8 and a YMRS score $<$ 8; they also met the same exclusion criteria indicated for patients.

For each participant, clinical assessment, blood sampling (between 8 and 10 a.m.), and then processing and analysis of blood samples, as well as MRI data acquisition were carried out during the same day. The data for all the subjects were processed following the same timeline and procedures.

2.2. DTI analyses

DTI sequences were acquired at 1.5-T with a GE scanner, and analyzed using tools from the Oxford University Centre for FMRIB software library (FSL 5.0) (Woolrich et al., 2009). After a standard preprocessing of diffusion data, fractional anisotropy (FA), radial diffusivity (RD) and axial diffusivity (AD) maps were derived. Tract-based spatial statistics (TBSS) analysis was used to perform a voxel-wise analysis of the whole-brain DTI measures (Woolrich et al., 2009).

2.3. Immunological analyses

Analysis of cell expression of membrane antigens was performed by immunofluorescence and flow cytometry. In particular, analyses were performed to achieve information on frequency in the peripheral blood of total CD4+ and CD8+ T cells as well as their subpopulations CD28 + CD45RA+ (naïve), CD28 + CD45RA- (central memory), CD28-CD45RA- (effector memory), CD28-CD45RA+ (terminal effector memory) (Fenoglio et al., 2013; Larbi and Fulop, 2014). The cytokine profile of peripheral CD4+ and CD8+ T lymphocytes, in terms of IFNγ, IL-17A, IL-4 and IL-10 production, was analyzed by intracellular staining and flow cytometry analyses, as already described (Fenoglio et al., 2011; Parodi et al., 2016; Serpero et al., 2013). Data were expressed as percentages of total CD3 + T cell population.

Plasma levels of IL-6 cytokine were analyzed by ELISA (ELISA Kit High Sensitivity, ABCAM). Plasma concentrations of IFNγ, IL-10, IL-4, IL-17A, IL-1β, TNFα, and IL-6 cytokines were measured by a bead-based immunoassay and flow cytometry (Parodi et al., 2015).

2.4. Statistical analyses

2.4.1. Comparison between groups of DTI parameters

Firstly, voxel-wise differences in FA values between manic, depressed, euthymic patients, and HC were tested using a permutation-based inference for non-parametric statistical thresholding ("randomize" program), with 5,000 permutations and age and gender as confound regressors (Nichols and Holmes, 2002). Analysis of variance (ANOVA) followed by two-sample t-tests were performed to detect differences between the various groups. A $p < 0.05$, corrected for family-wise error using the threshold-free cluster enhancement (TFCE) (Smith and Nichols, 2009), and for multiple comparisons using Bonferroni correction, was set. Subsequently, differences in RD and AD values between groups were investigated separately using the same methodology.

Secondly, the clusters showing combined alterations across different DTI parameters were identified according to the standard WM atlas, and the DTI values from these significant clusters of each altered tract were extracted and then entered into correlation analyses (see below).

2.4.2. Comparison between groups of immunological parameters

In order to detect differences between the various groups, each immunological parameter – i.e., CD4+ T cells (and their subpopulations), then CD8+ T cells (and their subpopulations), and finally the plasma levels of the various cytokines – were entered into between-groups comparisons followed by Bonferroni-corrected post-hoc tests. Age and gender were used as covariates. Correlations between cytokines level and T cells frequency were also explored.

For all the analyses, a $p < 0.05$, corrected for multiple comparisons using Bonferroni correction, was set.

2.4.3. Relationship between DTI, immunological and clinical parameters

Firstly, regression analyses were performed between whole brain voxel-wise data of DTI parameters that showed significant differences among groups and data relative to the frequency of those T cell subsets that showed significant differences among groups (with age and gender as covariates), in the whole cohort of subjects. A TFCE and Bonferroni-corrected $p < 0.05$ was set.

Secondly, the DTI values extracted from the significant clusters of each altered tract in a specific group were correlated with data relative to the frequency of those T cell subsets that showed significant alterations in the same group, with age and gender as covariates (the same analyses were also performed in the other groups that did not show such significant abnormalities, as control).

Finally, potential correlations of either DTI or immunological data with clinical parameters, namely YMRS and HAM-D total scores, were explored.

Furthermore, our findings were controlled for potential confounds, such as body mass index (BMI), tobacco smoking, illness duration and medication load.

For a detailed description of methods, see the supplemental materials, as well as Supplemental Fig. 1 and Supplemental Fig. 2.

3. Results

3.1. Clinical information

The sample was composed of patients affected by BD type I during manic episode, major depressive episode or euthymia, and HC. Demographic and clinical information for all the different groups are described in Table 1. The mean age did not show any significant difference between the various groups ($F = 1.669$; $p = 0.188$), as well as the gender distribution ($\chi^2 = 1.778$; $p = 0.620$). No significant difference between groups was found in BMI ($\chi^2 = 2.920$; $p = 0.404$). Finally, no significant difference was detected in illness duration ($F = 0.660$; $p = 0.521$) and number of episodes lifetime ($\chi^2 = 0.098$; $p = 0.956$) between patients groups, suggesting a similar course of illness.

3.2. DTI alterations in BD patients

The TBSS analysis was performed in order to detect significant changes in the DTI parameters in the different phases of BD (in an independent sample with respect to our previous work).

A widespread voxel-wise significant reduction in FA values in the manic phase and, to a lesser extent, in the depressive phase was found with respect to HC; no significant changes were detected in the euthymic phase. Furthermore, a significant RD increase was found in the manic phase only, with respect to HC, while no significant differences in the depressive and euthymic phases were detected; AD values did not show alterations in any group (Fig. 1a and Fig. 1b).

The clusters showing combined FA-RD alterations were mainly located in the body of corpus callosum (BCC), genu of corpus callosum (GCC), bilateral anterior corona radiata (ACR R and ACR L), left superior corona radiata (SCR L) and left posterior corona radiata (PCR L). Interestingly, a significant decrease in FA and increase in RD values in the BCC and SCR L were exclusively found in the manic phase, while the other tracts showed DTI changes both in manic and depressive phases (Fig. 1c, Fig. 1d, Table 2 and Supplemental Table 1).

3.3. Immunological alterations in BD patients

Phenotypic analyses were performed in order to measure the frequencies of different circulating T cell subpopulations in the various phases of BD.

The frequency of total CD4+ T cells showed a significant increase in the manic phase with respect to HC. Analysis of CD4+ T cell subpopulations revealed that such increase was dependent on the expansion of CD4+ CD28+ T cell subsets (that include both naïve and central memory CD4+ T cells) (Fig. 2a, Table 3 and Supplemental Table 2).

The frequency of total CD8+ T cells showed a significant decrease in the manic phase with respect to HC. Interestingly, this reduction was dependent on contraction of CD8+ CD28- T cell subsets, including both effector memory (CD8+ CD28-CD45RA-) T cells and terminal effector memory (CD8+ CD28-CD45RA+) T lymphocytes, and was associated with significant reduction of CD8+ IFNγ+ T cells (Fig. 2b, Table 3 and

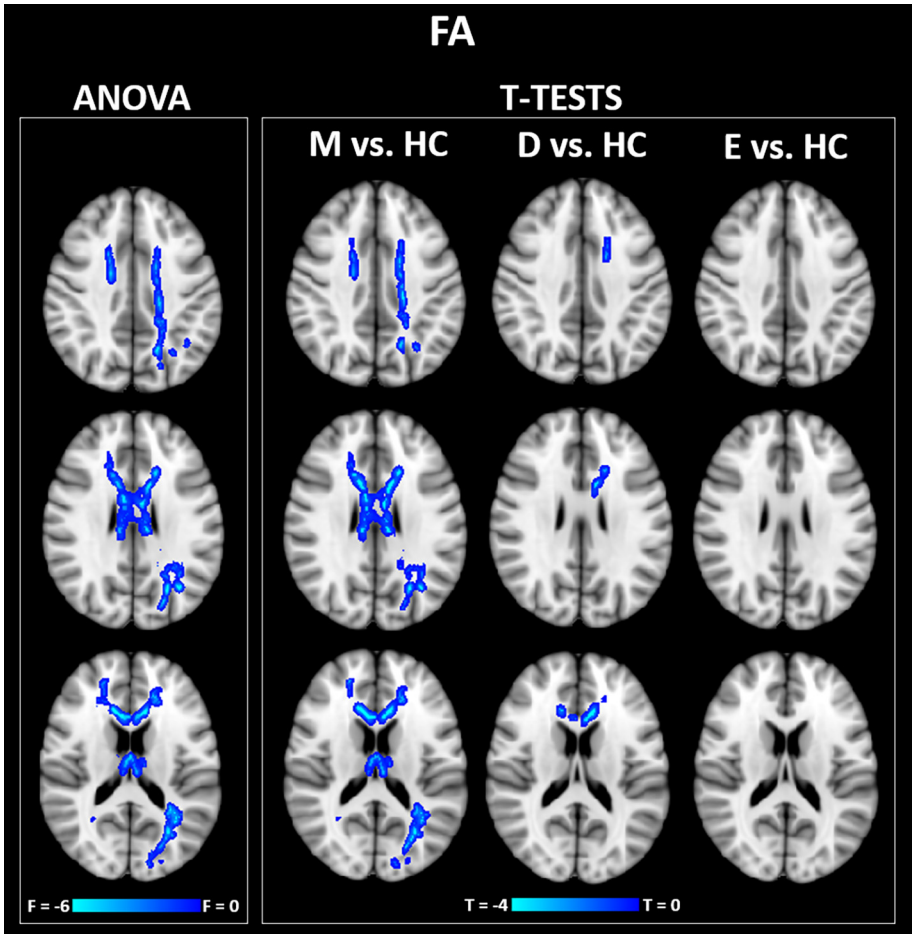

Fig. 1a. WM alterations in the various phases of BD. Results from between-group comparison (ANOVA and t-tests, with age and gender as covariates) showing clusters with significantly decreased FA values (*blue-light blue*), in BD patients in the different phases of illness (i.e. mania, depression and euthymia) compared with HC. The f-map of FA values (ANOVA) is thresholded at a TFCE corrected $p < 0.05$. The t-maps of FA values (t-tests) are thresholded at a TFCE and Bonferroni corrected $p < 0.05$, and masked with the significant clusters resulting from the correspondent ANOVA. Group differences are mapped onto standard T1 Montreal Neurological Institute (MNI) template at $z = 37$, $z = 27$ and $z = 17$. The color bar represents voxel-wise f-values and t-values. The significant clusters have been modified using the fill function of FSL software for visual purpose. Abbreviations: WM, white matter; TBSS, tract-based spatial statistics; DTI, diffusion tensor imaging; FA, fractional anisotropy; BD, bipolar disorder; M, mania; D, depression; E, euthymia; HC, healthy controls.

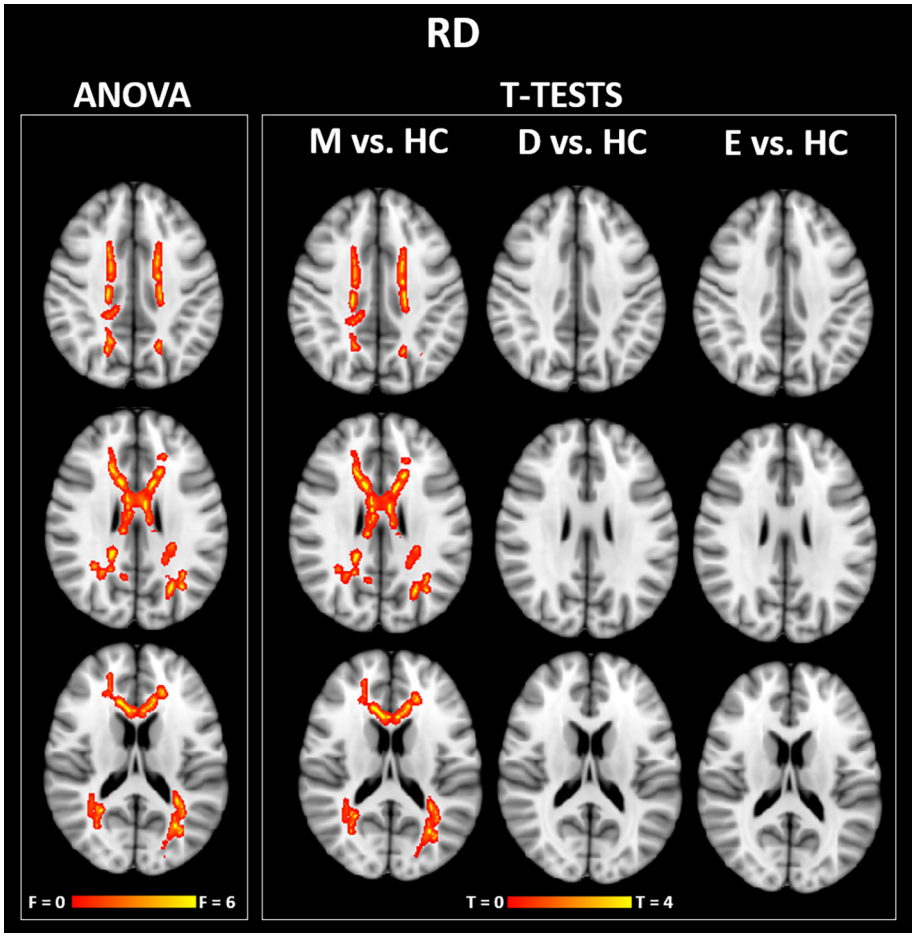

Fig. 1b. WM alterations in the various phases of BD. Results from between-group comparison (ANOVA and t-tests, with age and gender as covariates) showing clusters with significantly increased RD values (*red-yellow)*, in BD patients in the different phases of illness (i.e. mania, depression and euthymia) compared with HC. The f-map of RD values (ANOVA) is thresholded at a TFCE and Bonferroni corrected $p < 0.05$. The t-maps of RD values (t-tests) are thresholded at a TFCE and Bonferroni corrected $p < 0.05$, and masked with the significant clusters resulting from the correspondent ANOVA. Group differences are mapped onto standard T1 Montreal Neurological Institute (MNI) template at $z = 37$, $z = 27$ and $z = 17$. The color bar represents voxel-wise f-values and t-values. The significant clusters have been modified using the fill function of FSL software for visual purpose. Abbreviations: WM, white matter; TBSS, tract-based spatial statistics; DTI, diffusion tensor imaging; RD, radial diffusivity; BD, bipolar disorder; M, mania; D, depression; E, euthymia; HC, healthy controls.

Supplemental Table 2).

Finally, the levels of cytokine concentrations in the plasma of BD patients and HC showed a significant increase of IL-6 exclusively in the manic phase, while no significant changes in plasma levels of the other tested cytokines were found (Table 4 and Supplemental Table 3). Furthermore, IL-6 levels showed a significant inverse correlation with CD8+ CD28- T cells frequency (Table 5).

3.4. Correlations between DTI and immunological alterations in BD patients

In order to verify the existence of statistical associations between DTI and immunological alterations, a whole brain voxel-wise regression analysis was performed between those DTI parameters and peripheral frequencies of CD8+ T cell subpopulations that were found to be altered in the previous analyses. Taking into consideration the whole cohort of subjects, a significant direct relationship between decreased FA values (especially in the corpus callosum and corona radiata) and reduced frequencies of circulating CD8+ terminal effector memory and CD8+ IFNγ+ (but not CD8+ effector memory) T cells was observed. Coherently, an inverse relationship was found between increased RD values and reduced peripheral frequencies of both CD8+ terminal effector memory and CD8+ IFNγ+ T cell subpopulations (Fig. 3 and Table 6).

Then, the relationship between DTI and immunological changes was investigated in the manic phase in particular, since only mania was found to be characterized by combined and specific WM and immunological alterations. Consistently, a significant correlation of FA-RD abnormalities in the BCC and SCR L with reduced frequencies of circulating CD8+ terminal effector memory and CD8+ IFNγ+ T cells (but not CD8+ effector memory T cells, coherently with the whole brain regression analysis) was observed in the manic phase. These DTI-immunological relationships were specific for the manic phase because not present in depression, euthymia or HC (Table 7).

With regard to clinical correlations, YMRS total score was found to be inversely correlated with peripheral frequencies of effector memory and terminal effector memory CD8+ T cells, and directly correlated with plasma levels of IL-6 (Table 8).

4. Discussion

4.1. Main findings

The main findings of our study are the following: (i) a widespread combined FA-RD alteration was found mainly in the manic phase, with relatively specific involvement of the BCC and SCR L; (ii) peripheral immunological alterations were detected in the manic phase, mainly characterized by an increase in CD4+ T cells as well as a decrease in total CD8+ T cells and their subpopulations effector memory (CD8+ CD28-CD45RA-), terminally differentiated effector memory (CD8+ CD28-CD45RA+) and CD8+ IFNγ+; (iii) a statistical association between WM and immunological alterations was found in the whole cohort, and a correlation of FA-RD alterations in the BCC and SCR L with reduced CD8+ terminally differentiated effector memory and CD8+ IFNγ+ T cells was detected in mania (Fig. 4).

4.2. WM alterations in mania

In our study, a widespread combined FA-RD alteration was detected in BD patients, confirming previous results (Wise et al., 2015). Importantly, these WM alterations were significantly detected in the active

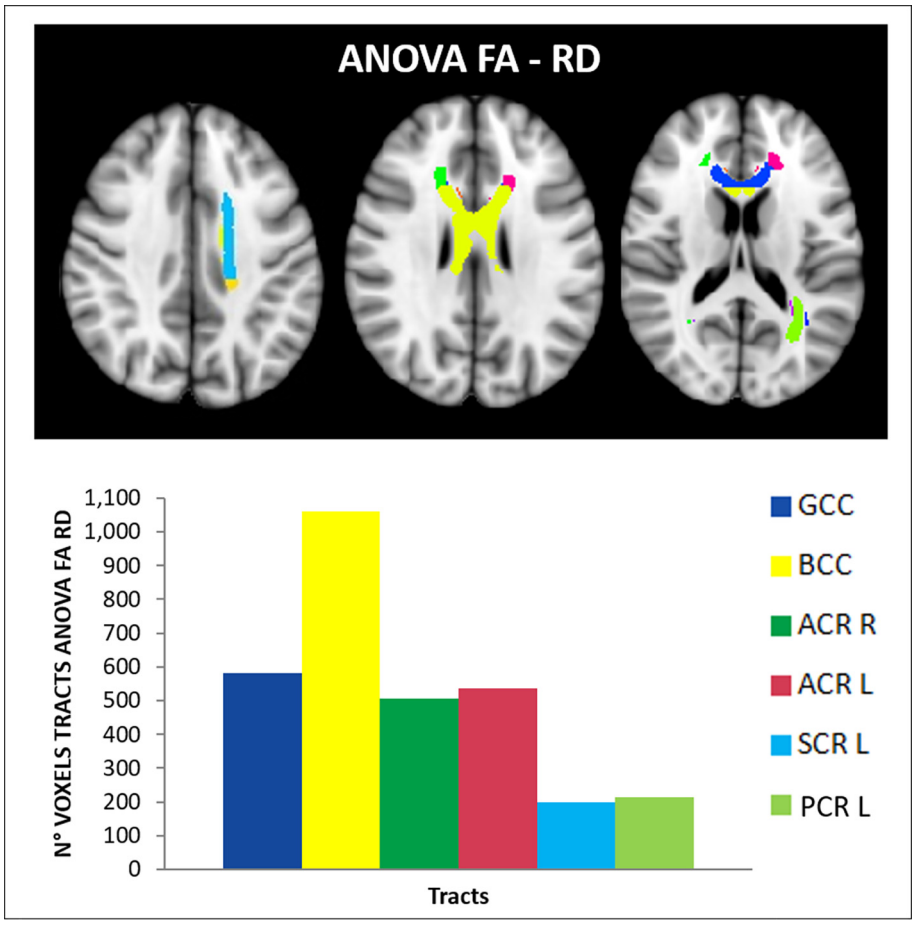

Fig. 1c. WM alterations in the various phases of BD. *Upper part*: The main altered WM tracts showing overlapping FA-RD alterations (in the ANOVAs, see Figs. 1a and 1b) in BD patients. The resulting WM alterations are differently colored according to the JHU-ICBM-DTI-81 White-Matter Labels atlas and mapped onto standard T1 Montreal Neurological Institute (MNI) template at z = 37, z = 27 and z = 17. *Lower part:* The cluster size of altered voxels is displayed for each altered tract (number of altered voxels > 200). Abbreviations: WM, white matter; FA, fractional anisotropy; RD, radial diffusivity; CGC, genu of corpus callosum; BCC, body of corpus callosum; ACR R, anterior corona radiata right; ACR L, anterior corona radiata left; SCR L, superior corona radiata left; PCR L, posterior corona radiata left.

phases of illness, confirming our previous findings on an independent BD sample (Magioncalda et al., 2015). In the present work, altered WM tracts showed a progressively decreasing trend of FA-RD changes from mania (high alterations) to depression (moderate alterations) and to euthymia (low alterations). The prominence of WM abnormalities in mania is in accordance to another study from our group which investigated the midline regions (with an *a priori* region-of-interest approach) and showed significant structural disconnectivity in mania only (Martino et al., 2016b). In the present study, by using a TBSS approach, the BCC and SCR L were found to be the most specific sites of WM alterations in mania. Interestingly, these WM tracts connect different regions of the sensorimotor network mainly (van den Heuvel et al., 2009; Wakana et al., 2004; Zakszewski et al., 2014), and, accordingly, functional alterations of this network were previously detected in mania (Martino et al., 2016a). Taken together, these data may suggest, among others, that WM abnormalities have some degree of dynamic changes across the different phases of illness, with a more consistent impairment in mania. In order to complement the present results, future longitudinal studies are needed to investigate WM alterations (and relative network dysfunctions eventually) in the same patients across the different phases of disease.

4.3. Immunological alterations in mania

Based on the hypothesis that immunological alterations may be pathogenically involved in BD, we analyzed the distribution of T cell subpopulations and cytokine concentrations in the peripheral blood of BD patients in the different phases of illness. Interestingly, the most significant immunological alterations were observed in mania, which was characterized by increased peripheral levels of CD4+ T cells and IL-6, as well as decreased frequency of CD8+ T cells, belonging specifically to the effector memory, terminal effector memory and IFNγ+ subpopulations. Trends of circulating T cell subset frequencies similar to those observed in manic phase were also detected in depressive and euthymic phases of BD, although here they did not reach statistically significant differences compared to those of controls. Although a previous work suggested an activation of cell-mediated immunity in bipolar mania (Tsai et al., 1999), studies detailing the immunological alterations are lacking so far. Our findings might identify the immunological stigmata of mania in an increase of early activated (CD4+ CD28+) T cells and a decrease of effector memory (CD8+ CD28-CD45RA-) and terminal effector memory (CD8+ CD28-CD45RA+) T cells, paralleled by reduction of CD8+ IFNγ+ T cells (corresponding to functionally activated CD8+ T cells). Interestingly, the loss of the CD28 co-receptor, which is observed in a heterogeneous CD8+ T cell population, occurs after prolonged stimulation. The expression of the CD45RA molecule within these cells characterizes the shift from effector memory to terminal effector memory cells. This represents a progression to a low proliferative/high release of cytokines profile that is associated to increasing expression of adhesion molecules and chemokine receptors, ultimately dictating their localization in peripheral tissues (Nolz, 2015). Hence, terminal effector memory CD8+ T cells are effectors cells prone to migration in the peripheral tissues (Ahlers and Belyakov, 2010). Together, our data suggest the occurrence of an acute immune response in manic patients, as further corroborated by the increase of a cytokine such as IL-6, which is related to acute inflammation. Interestingly, in our sample we could also observe a correlation between the increased IL-6 serum levels and the decrease of CD8+ CD28- T cells, hence the chronically stimulated and/or activated CD8+ T cells, suggesting that the two phenomena may be pathogenically related. Signs of immune activation in BD have been hypothetically related to autoimmune and infection processes, which

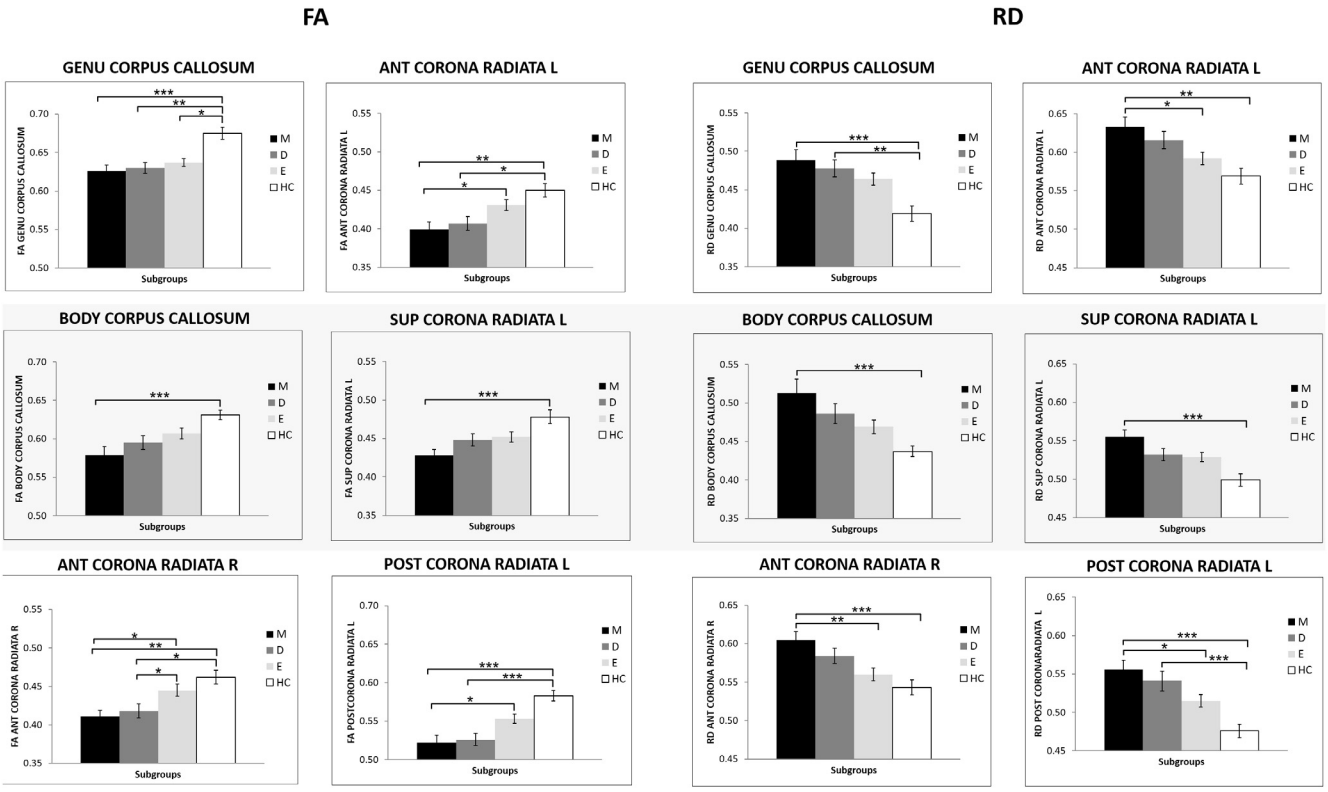

Fig. 1d. WM alterations in the various phases of BD. Between-group comparison (ANOVAs, with age and gender as covariates, followed by Bonferroni-corrected post-hoc tests) of FA and RD values extracted from each altered tract (see Fig. 1c). Corrected $p < 0.05$*; $p < 0.01$**; $p < 0.001$***. Abbreviations: WM, white matter; FA, fractional anisotropy; RD, radial diffusivity; BD, bipolar disorder; M, mania; D, depression; E, euthymia; HC, healthy controls.

Table 2
Comparisons between groups of the DTI parameters of the altered WM tracts.

WM TRACTS	ANOVA	M vs. HC	D vs. HC	E vs. HC	M vs. D	M vs. E	D vs. E
	F (p)	*(p)*	*(p)*	*(p)*	*(p)*	*(p)*	*(p)*
FA GCC	**6.530 (0.001)**	**M < HC (0.001)**	**D < HC (0.002)**	**E < HC (0.036)**			
FA BCC	**5.792 (0.001)**	**M < HC (0.001)**					
FA ACR R	**6.826 (0.000)**	**M < HC (0.004)**	**D < HC (0.011)**			**M < E (0.015)**	**D < E (0.049)**
FA ACR L	**6.108 (0.001)**	**M < HC (0.005)**	**D < HC (0.021)**			**M < E (0.027)**	
FA SCR L	**5.111 (0.003)**	**M < HC (0.002)**					
FA PCR L	**11.219 (0.000)**	**M < HC (0.000)**	**D < HC (0.000)**			**M < E (0.022)**	
RD GCC	**6.167 (0.001)**	**M > HC (0.001)**	**D > HC (0.006)**				
RD BCC	**5.755 (0.001)**	**M > HC (0.001)**					
RD ACR R	**6.765 (0.000)**	**M > HC (0.001)**				**M > E (0.005)**	
RD ACR L	**5.787 (0.001)**	**M > HC (0.003)**				**M > E (0.025)**	
RD SCR L	**6.882 (0.000)**	**M > HC (0.000)**					
RD PCR L	**9.734 (0.000)**	**M > HC (0.000)**	**D > HC (0.001)**			**M > E (0.023)**	

ANOVAs and Bonferroni-corrected post hoc tests (with age and gender as covariates) showing differences in the DTI parameters of altered WM tracts between the various groups, i.e. manic, depressed, euthymic patients, and HC. The significant results are highlighted in bold.
Abbreviations: DTI, diffusion tensor imaging; FA, fractional anisotropy; RD, radial diffusivity; WM, white matter; GCC, genu corpus callosum; BCC, body corpus callosum; ACR R, anterior corona radiata right; ACR L, anterior corona radiata left; SCR L, superior corona radiata left; PCR L, posterior corona radiata left; M, manic patients; D, depressed patients; E, euthymic patients; HC, healthy controls.

interestingly were found to be more prevalent in patients with affective disorders; these data have made attractive the autoimmune and infectious hypotheses of BD, but clear proof supporting them needs still to be provided (e.g. (Barbosa et al., 2014a; Tanaka et al., 2017; Yolken and Torrey, 1995)). Thus, the potential specific mechanisms of immune-mediated alterations in BD, and in particular in its manic phase, need to be specifically investigated in the future.

4.4. Relationship between WM and immunological alterations in mania

In order to investigate potential underpinnings for WM abnormalities in BD, we then investigated their relationship with immunological abnormalities, and a significant correlation between WM and immunological alterations was found in our sample. In this context, a previous study on the depressive phase of BD showed a significant correlation between WM alterations and Th17 cells levels, but no significant differences were detected when patients were compared to HC (coherently with our findings in depression) (Poletti et al., 2016). Our work extends previous results by showing that, notably, both the prominent WM alterations and the reduction of effectors CD8+ T cells were found to be strongly associated to the manic phase. In particular, reduced FA and increased RD in the BCC and SCR L were found to significantly correlate with a reduction of circulating CD8+ terminal effector memory T cells (which are cells prone to migration in the

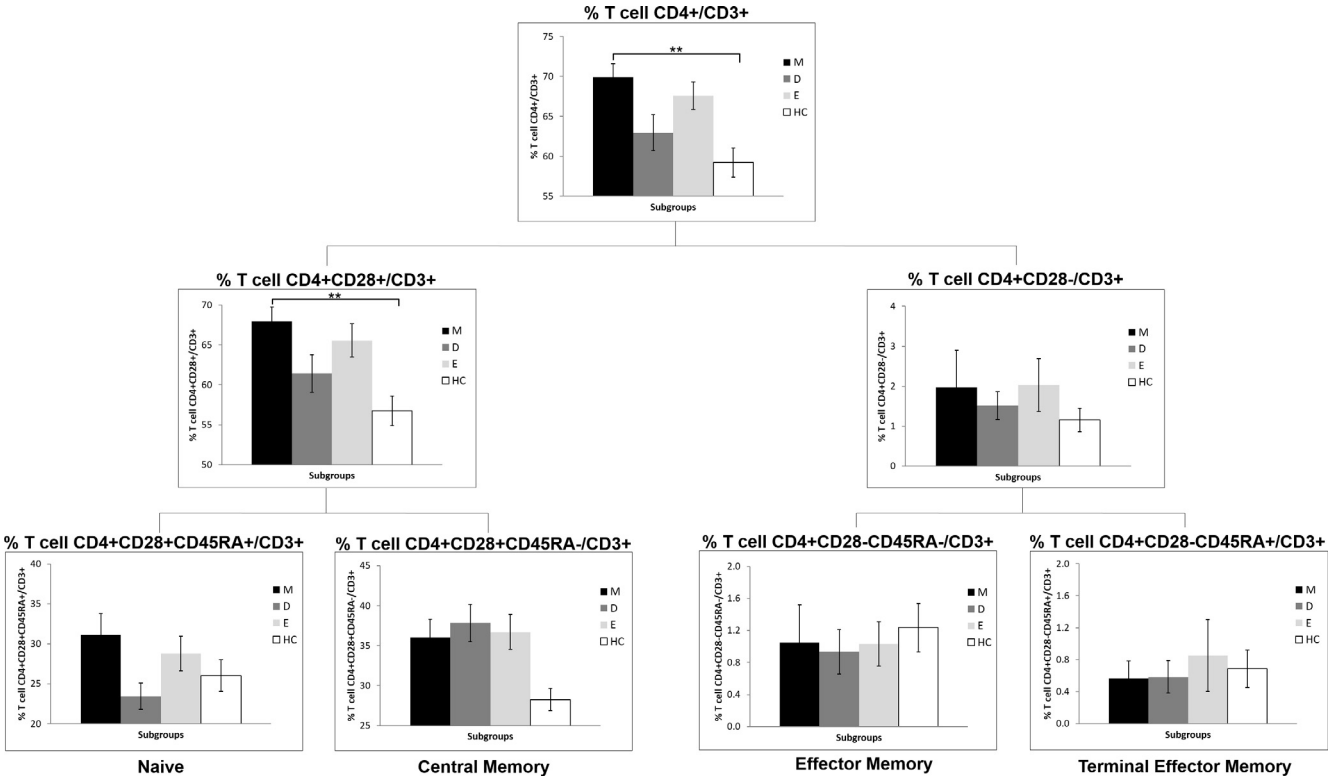

Fig. 2a. Alterations of circulating T cell subsets in the various phases of BD. Comparative analyses (ANOVAs, with age and gender as covariates, followed by Bonferroni-corrected post-hoc tests) on different CD4+ T cell subsets (total CD4+ T cells as well as naïve CD4+ CD28+CD45RA+, central memory CD4+ CD28+CD45RA-, effector memory CD4+ CD28-CD45RA- and terminal effector memory CD4+ CD28-CD45RA+ T cell subsets) among BD patients in the various phases of illness and HC. Corrected $p < 0.05$*; $p < 0.01$**; $p < 0.001$***. Abbreviations: BD, bipolar disorder; M, mania; D, depression; E, euthymia; HC, healthy controls.

Table 3
Comparisons between groups of T cells.

T CELLS	KRUSKAL WALLIS TEST	ANOVA	M vs. HC	D vs. HC	E vs. HC	M vs.D	M vs. E	D vs. E
	χ^2 (p)	F (p)	(p)	(p)	(p)	(p)	(p)	(p)
CD3+		1.587 (0.200)						
CD4+		**4.850 (0.004)**	**M > HC (0.005)**					
CD4+ CD28+		**4.629 (0.005)**	**M > HC (0.005)**					
CD4+ CD28+CD45RA+		2.115 (0.106)						
CD4+ CD28+CD45RA-		3.597 (0.017)		D > HC (0.019)				
CD4+ CD28-	2.989 (0.393)							
CD4+ CD28-CD45RA-	3.840 (0.279)							
CD4+ CD28-CD45RA+	0.908 (0.824)							
CD4+ IFNγ+		1.080 (0.363)						
CD4+ IL17+		0.305 (0.822)						
CD4+ IL10+	1.553 (0.670)							
CD4+ IL4+	3.152 (0.369)							
CD8+		**3.069 (0.033)**	**M < HC (0.026)**					
CD8+ CD28+		1.601 (0.196)						
CD8+ CD28+CD45RA+		3.370 (0.023)	M > HC (0.026)					
CD8+ CD28+CD45RA-		2.165 (0.099)						
CD8+ CD28-		**7.869 (0.000)**	**M < HC (0.000)**			**M < D (0.007)**	**M < E (0.014)**	
CD8+ CD28-CD45RA-		**9.858 (0.000)**	**M < HC (0.000)**			**M < D (0.008)**	**M < E (0.015)**	
CD8+ CD28-CD45RA+		**6.641 (0.000)**	**M < HC (0.000)**			**M < D (0.030)**	**M < E (0.027)**	
CD8+ IFNγ+		**4.702 (0.005)**	**M < HC (0.004)**					
CD8+ IL17+	1.402 (0.705)							
CD8+ IL10+	0.418 (0.937)							
CD8+ IL4+	6.296 (0.098)							

ANOVAs and Bonferroni-corrected post hoc tests (with age and gender as covariates) for normally distributed data, or Kruskal-Wallis test for non-normally distributed data, showing differences in T cells frequencies between the various groups, i.e. manic, depressed, euthymic patients, and HC. The p values of the ANOVAs are Bonferroni-corrected. The significant results are highlighted in bold.
Abbreviations: M, manic patients; D, depressed patients; E, euthymic patients; HC, healthy controls.

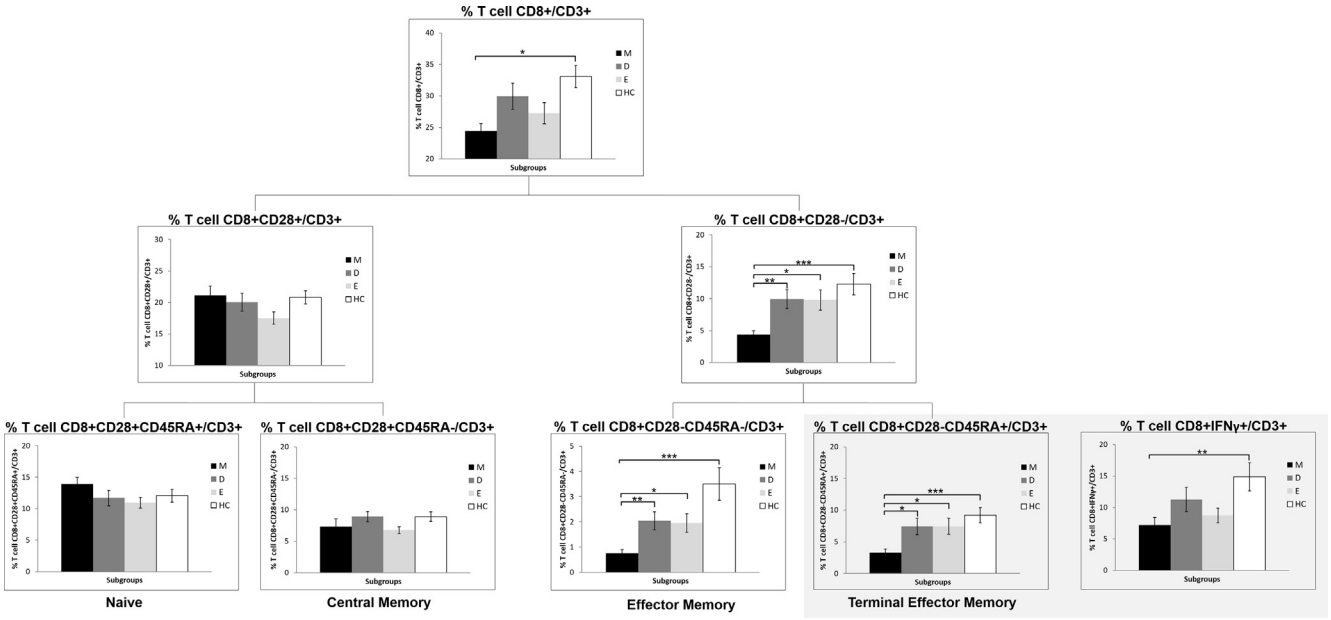

Fig. 2b. Alterations of circulating T cell subsets in the various phases of BD. Comparative analyses (ANOVAs, with age and gender as covariates, followed by Bonferroni-corrected post-hoc tests) on different CD8+ T cell subsets (total CD8+ T cells, naïve CD8+ CD28+CD45RA+, central memory CD8+ CD28+CD45RA-, effector memory CD8+ CD28-CD45RA- and terminal effector memory CD8+ CD28-CD45RA+ T cell subsets, as well as CD8+ IFNγ+ T cells) among BD patients in the various phases of illness and HC. Corrected $p < 0.05$*; $p < 0.01$**; $p < 0.001$***. Abbreviations: BD, bipolar disorder; M, mania; D, depression; E, euthymia; HC, healthy controls.

peripheral tissues) and CD8+ IFNγ+ T cells (which are the activated CD8+ T cells and include activated CD8+ terminal effector memory T lymphocytes), but not with CD8+ effector memory T cells, in the manic phase. A decrease in FA with concomitant increase in RD was suggested to reflect alterations in oligodendroglial and myelin microstructure of WM (Heng et al., 2010; Versace et al., 2014), which, in turn, have been related to T cell-mediated pathogenesis in various diseases (Willing and Friese, 2012). In the absence of an easily accessible tissue where confirmatory histological investigations could be performed, it is remarkable that our study, combining sensitive radiological investigations with immunological analyses, had the possibility to specifically constrain correlative analyses between structural and immunological alterations. The high statistical level of correlation observed in these analyses might indicate a direct relationship between the two abnormalities, leading to the hypothesis of a reciprocal interdependency.

Collectively, these findings suggest an acute immune response in mania, sustained by early generated CD4+ T cell compartment (likely with T helper function), leading to activation of CD8+ T cell subpopulations that leave the circulation to migrate in peripheral tissues such as, hypothetically, the brain areas where WM damage occurs. This phenomenon is reminiscent of what observed in chronic inflammatory neurological diseases such as multiple sclerosis. There, reduction of CD8+ T cells and increased CD4+/CD8+ ratio in peripheral circulation associated with accumulation of CD8+ T cells in acute and chronic inflammatory WM lesions are observed, suggesting that CD8+ T cells recognize components of the myelin sheath and could contribute to the WM damage as effector cells (Melzer et al., 2009; Pender, 2012). However, future studies are needed to formally demonstrate presence and localization of specific subsets of CD8+ T effector cells in post-mortem brains of BD subjects to prove their pathogenic potential in mania.

4.5. Limitations

A number of factors could affect our findings, including BMI and tobacco smoking. However, the differences between groups in all the relevant DTI and immunological data, as well as their correlations, were still significant even when further controlled for BMI or tobacco

Table 4
Comparisons between groups of plasma cytokines.

CYTOKINES	KRUSKAL WALLIS TEST	M vs. HC	D vs. HC	E vs. HC	M vs. D	M vs. E	D vs. E
	χ^2 (p)	U (p)	U (p)	U (p)	U (p)	U (p)	U (p)
ELISA							
IL-6	**17.767 (0.000)**	**M > HC 79 (0.002)**				**M > E 66 (0.000)**	
CBA							
IL-6	5.694 (0.127)	M > HC 123.5 (0.024)					
TNFα	2.651 (0.449)						
IL-1b	0.592 (0.898)						
IFNγ	2.026 (0.567)						
IL-4	0.003 (1.000)						
IL-17	6.080 (0.108)						
IL-10	2.453 (0.484)						

Kruskal-Wallis tests and Bonferroni-corrected Mann-Whitney tests showing differences in cytokines levels between the various groups, i.e. manic, depressed, euthymic patients, and HC. The significant results are highlighted in bold.
Abbreviations: IL, interleukin; TNFα, tumor necrosis factor alpha; IFNγ, interferon gamma; M, manic patients; D, depressed patients; E, euthymic patients; HC, healthy controls.

Table 5
Correlations between IL and 6 and T cells.

T CELLS	IL-6
	ρ *(p)*
CD3 +	−0.182 (0.108)
CD4 +	0.122 (0.284)
CD4 + CD28 +	0.108 (0.343)
CD4 + CD28 + CD45RA +	−0.051 (0.657)
CD4 + CD28 + CD45RA-	0.211 (0.061)
CD4 + CD28-	−0.141 (0.215)
CD4 + CD28-CD45RA-	−0.121 (0.290)
CD4 + CD28-CD45RA +	−0.127 (0.265)
CD4 + IFNγ +	−0.204 (0.073)
CD4 + IL17 +	0.001 (0.993)
CD4 + IL10 +	−0.132 (0.248)
CD4 + IL4 +	−0.001 (0.993)
CD8 +	−0.059 (0.604)
CD8 + CD28 +	**0.229 (0.043)**
CD8 + CD28 + CD45RA +	0.126 (0.270)
CD8 + CD28 + CD45RA-	0.101 (0.374)
CD8 + CD28-	**−0.279 (0.013)**
CD8 + CD28-CD45RA-	**−0.257 (0.022)**
CD8 + CD28-CD45RA +	−0.205 (0.070)
CD8 + IFNγ +	−0.182 (0.111)
CD8 + IL17 +	−0.080 (0.485)
CD8 + IL10 +	−0.120 (0.297)
CD8 + IL4 +	−0.050 (0.663)

Spearman correlation analysis (ρ) between IL-6 level and T cells frequency. The significant results are highlighted in bold.

Table 6
Correlation between DTI and immunological alterations.

WM TRACTS	CD8 + CD28-CD45RA +	CD8 + IFNγ +
	r (p)	*r (p)*
FA GCC	0.354 (0.001)	0.396 (0.000)
FA BCC	0.367 (0.001)	0.369 (0.001)
FA ACR R	0.376 (0.001)	0.299 (0.008)
FA ACR L	0.288 (0.011)	0.298 (0.009)
FA SCR L	0.359 (0.001)	0.325 (0.004)
FA PCR L	0.298 (0.008)	0.265 (0.020)
RD GCC	−0.349 (0.002)	−0.390 (0.000)
RD BCC	−0.369 (0.001)	−0.357 (0.001)
RD ACR R	−0.354 (0.001)	−0.357 (0.001)
RD ACR L	−0.303 (0.007)	−0.305 (0.002)
RD SCR L	−0.335 (0.003)	−0.360 (0.001)
RD PCR L	−0.324 (0.004)	−0.305 (0.007)

Partial correlation analysis (r), with age and gender as covariates, of DTI parameters of WM tracts with peripheral frequencies of CD8 + T cell subpopulations - i.e. CD8 + CD28-CD45RA + and CD8 + IFNγ + T cells, in the whole sample.
Abbreviations: DTI, diffusion tensor imaging; WM, white matter; FA, fractional anisotropy; RD, radial diffusivity; GCC, genu corpus callosum; BCC, body corpus callosum; ACR R, anterior corona radiata right; ACR L, anterior corona radiata left; SCR L, superior corona radiata left; PCR L, posterior corona radiata left.

smoking.

Other confounding factors could be represented by illness duration and medications. However, illness duration did not show any significant correlation with immunological data or DTI parameters in the

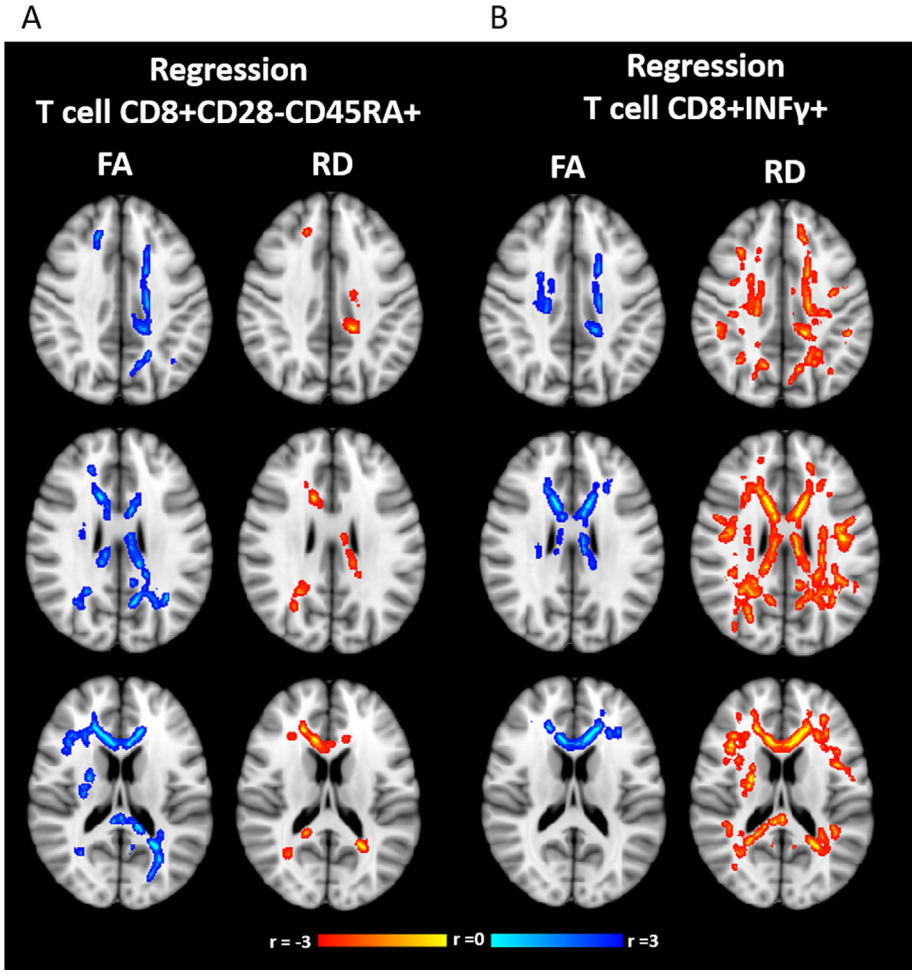

Fig. 3. Correlations between WM and T cells alterations. Results from whole brain voxel-wise regression analyses between DTI and T cells alterations (with age and gender as covariates) showing clusters with significant correlations of FA and RD with terminal effector memory CD8 + CD28-CD45RA + and CD8 + IFNγ + T cells. The regression maps of FA and RD values are thresholded at a TFCE and Bonferroni-corrected $p < 0.05$, and mapped onto standard T1 Montreal Neurological Institute (MNI) template at z = 37, z = 27 and z = 17. The color bar represents voxel-wise r-values. Direct correlations between decreased FA and reduced frequencies in the circulation of the different T cell subsets are shown in *blue-light blue*, while inverse correlations between increased RD and reduced frequencies in the circulation of the different T cell subsets are shown in *red-yellow*. The significant clusters have been modified using the fill function of the FSL software for visual purpose. Abbreviations: WM, white matter; DTI, diffusion tensor imaging; FA, fractional anisotropy; RD, radial diffusivity; BD, bipolar disorder; M, mania; D, depression; E, euthymia; HC, healthy controls.

Table 7
Correlations between WM and T cells abnormalities in the various subgroups.

	MANIA			
	FA *r (p)*		RD *r (p)*	
T CELLS	BCC	SCR L	BCC	SCR L
CD8+ CD28-	0.478 (0.045)	0.404 (0.096)	−0.504 (0.033)	−0.396 (0.103)
CD8+ CD28- CD45RA-	0.325 (0.189)	-0.227 (0.366)	−0.351 (0.153)	−0.142 (0.575)
CD8+ CD28- CD45RA+	**0.485 (0.041)**	**0.487 (0.040)**	**−0.483 (0.042)**	−0.368 (0.133)
CD8+ IFNγ+	**0.611 (0.007)**	**0.661 (0.003)**	**−0.582 (0.011)**	**−0.576 (0.012)**
	DEPRESSION			
	FA *r (p)*		RD *r (p)*	
T CELLS	BCC	SCR L	BCC	SCR L
CD8+ CD28-	0.298 (0.230)	0.067 (0.792)	−0.345 (0.161)	−0.211 (0.400)
CD8+ CD28- CD45RA-	0.191 (0.447)	0.146 (0.562)	−0.247 (0.323)	−0.293 (0.239)
CD8+ CD28- CD45RA+	0.302 (0.223)	0.063 (0.804)	−0.340 (0.168)	−0.176 (0.484)
CD8+ IFNγ+	0.126 (0.619)	0.017 (0.946)	−0.127 (0.614)	−0.086 (0.733)
	EUTHYMIA			
	FA *r (p)*		RD *r (p)*	
T CELLS	BCC	SCR L	BCC	SCR L
CD8+ CD28-	0.085 (0.739)	0.312 (0.207)	−0.093 (0.713)	-0.042 (0.868)
CD8+ CD28- CD45RA-	0.086 (0.733)	0.120 (0.635)	−0.115 (0.648)	0.126 (0.619)
CD8+ CD28- CD45RA+	0.205 (0.415)	0.375 (0.125)	−0.207 (0.409)	−0.152 (0.546)
CD8+ IFNγ+	0.007 (0.978)	0.351 (0.167)	0.017 (0.947)	−0.267 (0.301)
	HEALTHY CONTROLS			
	FA *r (p)*		RD *r (p)*	
T CELLS	BCC	SCR L	BCC	SCR L
CD8+ CD28-	−0.102 (0.688)	0.067 (0.793)	0.100 (0.692)	0.001 (0.998)
CD8+ CD28- CD45RA-	−0.108 (0.670)	−0.033 (0.897)	0.048 (0.849)	−0.029 (0.910)
CD8+ CD28- CD45RA+	−0.031 (0.902)	0.337 (0.171)	0.075 (0.769)	−0.144 (0.569)
CD8+ IFNγ+	0.382 (0.118)	0.184 (0.464)	−0.365 (0.136)	−0.171 (0.499)

Partial correlation analysis (r), with age and gender as covariates, of FA and RD values in the BCC and SCR L with peripheral frequencies of CD8+ T cell subpopulations - i.e. CD8+ CD28-CD45RA+ and CD8+ IFNγ+ T cells (as well as CD8+ CD28-CD45RA- T cells, as control) - in the manic group (as well as in the other groups, i.e. depressed and euthymic patients and HC, as control). The significant results are highlighted in bold.
Abbreviations: WM, white matter; FA, fractional anisotropy; RD, radial diffusivity; BCC, body corpus callosum; SCR L, superior corona radiata left.

WM altered tracts (with the only exception of GCC). The medication load correlated with DTI values in different WM tracts (with the relevant exception of BCC) when the whole cohort of BD patients was considered, but it was not associated with immunological data. However, when manic patients were considered separately, no significant correlation between medication load and both DTI and immunological parameters was detected. Interestingly, the significant correlations found between DTI and immunological alterations in mania held, or even increased, when corrected for medication load.

Table 8
Correlations of clinical parameters with WM and immunological alterations in BD.

WM TRACTS	YMRS	HAM-D
	ρ *(p)*	ρ *(p)*
FA GCC	−0.061 (0.642)	−0.046 (0.727)
FA BCC	−0.131 (0.318)	−0.025 (0.852)
FA ACR R	−0.204 (0.118)	−0.241 (0.063)
FA ACR L	−0.165 (0.208)	−0.199 (0.126)
FA SCR L	−0.050 (0.706)	0.008 (0.952)
FA PCR L	−0.220 (0.090)	−0.251 (0.053)
RD GCC	0.052 (0.695)	0.018 (0.892)
RD BCC	0.094 (0.476)	0.031 (0.815)
RD ACR R	0.278 (0.031)	0.164 (0.211)
RD ACR L	0.208 (0.111)	0.138 (0.295)
RD SCR L	0.156 (0.234)	−0.113 (0.390)
RD PCR L	0.253 (0.051)	0.158 (0.228)
IMMUNOLOGICAL PARAMETERS		
CD8+ CD28-	**−0.284 (0.028)**	0.064 (0.628)
CD8+ CD28-CD45RA-	**−0.326 (0.011)**	0.142 (0.279)
CD8+ CD28-CD45RA+	**−0.290 (0.025)**	0.020 (0.877)
CD8+ IFNγ+	−0.220 (0.094)	0.018 (0.891)
IL-6 (ELISA)	**0.420 (0.001)**	0.216 (0.101)

Spearman correlation analysis (ρ) of clinical parameters - i.e. YMRS and HAM-D - with altered DTI (FA and RD values of the altered WM tracts) and immunological (CD8+ CD28-CD45RA+, CD8+ IFNγ+ and CD8+ CD28-CD45RA- T cells, and plasmatic IL-6) parameters, in the BD sample. The significant results are highlighted in bold.
Abbreviations: DTI, diffusion tensor imaging; WM, white matter; FA, fractional anisotropy; RD, radial diffusivity; GCC, genu corpus callosum; BCC, body corpus callosum; ACR R, anterior corona radiata right; ACR L, anterior corona radiata left; SCR L, superior corona radiata left; PCR L, posterior corona radiata left; BD, bipolar disorder; YMRS, Young mania rating scale; HAM-D, Hamilton depression scale.

Another potential limitation is the relatively small sample size of individual subgroups. However, the DTI results of this study confirmed our previous findings on an independent BD sample. Our immunological findings are well in accordance with previous data on immunological activation in BD. And our DTI-immunological correlation findings were detected in the whole sample of 80 subjects, and then confirmed and specified in the manic phase especially. Finally, our study suffers from the typical limits of cross-sectional studies.

For a detailed description of limitations, see the supplemental materials, as well as Table 1, Supplemental Table 4a, Supplemental Table 4b, Supplemental Table 4c, Supplemental Table 5a, Supplemental Table 5b, Supplemental Table 5c, Supplemental Table 6, Supplemental Table 7a, Supplemental Table 7b and Supplemental Fig. 3.

4.6. Conclusions

In summary, both the prominent WM and immunological alterations were found to be strongly associated to mania. This finding seems to be in accordance with the “primacy of mania” hypothesis, where mania, broadly considered as a wide range of excitatory processes which clinically occur before depression, is described as the *fire* of BD and seen as the core of the pathophysiology of the illness (Kotzalidis et al., 2017; Koukopoulos and Ghaemi, 2009). Moreover, our data highlight immunological markers of mania highly correlating with structural alterations in WM tracts. They also support an innovative pathogenic mechanism that recognizes in a subpopulation of CD8+ effector T cells a major candidate to be responsible for the structural WM lesions underlying mania. If confirmed, this view could pave the way to a better understanding of the pathophysiology of BD and, in turn, to the development of novel and more effective therapies (e.g.,

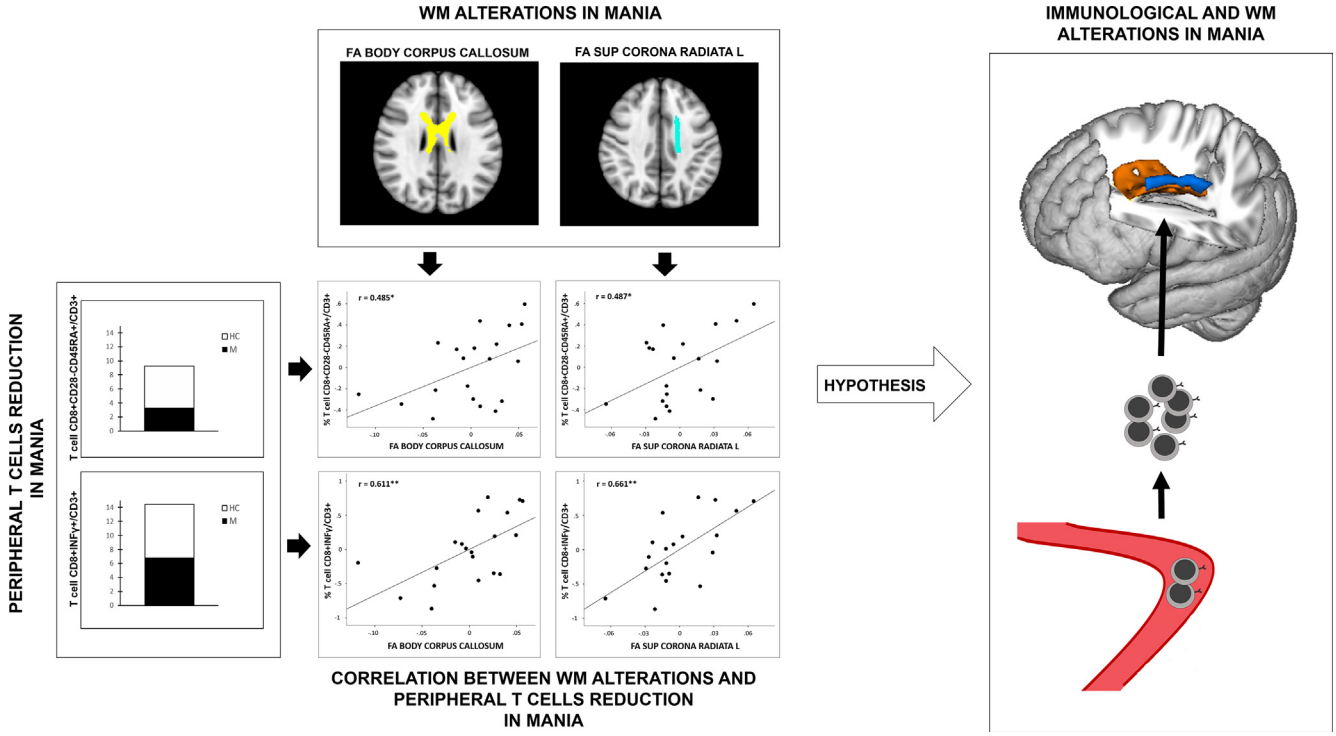

Fig. 4. Schema of results and hypothesis on the relationship between WM and immunological alterations in mania. The most remarkable DTI and immunological alterations in mania consist in: (i) relatively specific WM alterations mainly located in the body of corpus callosum and left superior corona radiata (*upper panel)*; (ii) reduction in the frequency of circulating terminal effector memory CD8+ CD28-CD45RA+ and CD8+ IFNγ+ T cell subpopulations (*left panel*); (iii) significant partial correlation (with age and gender as covariates) between the cited WM and immunological alterations (*middle panel*). Our hypothesis is that in mania CD8+ CD28-CD45RA+ T cells (which are cells prone to migration in the peripheral tissues) and CD8+ IFNγ+ T cells (which are the activated CD8+ T cells and include activated CD8+ CD28-CD45RA+ T lymphocytes) leave the blood stream to migrate into the brain where they induce an immune-related WM damage *(right panel)*. Abbreviations: WM, white matter, DTI, diffusion tensor imaging, FA, fractional anisotropy, M, mania, HC, healthy controls.

immunotherapies).

Acknowledgements

The authors thank Prof. Gianluigi Mancardi for the access to the MRI Unit (Magnetic Resonance Research Center, Department of Neuroscience, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health, University of Genoa and IRCCS Ospedale Policlinico San Martino, Genoa, Italy).

5. Declaration of interest

The authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.bbi.2018.04.017.

References

A.P.A., 2013. Diagnostic and Statistical Manual for Mental Disorders. 5th ed. (DSM-5). Washington: American Psychiatrich Association.
Ahlers, J.D., Belyakov, I.M., 2010. Memories that last forever: strategies for optimizing vaccine T-cell memory. Blood 115, 1678–1689.
Anderson, G., Maes, M., 2015. Bipolar disorder: role of immune-inflammatory cytokines, oxidative and nitrosative stress and tryptophan catabolites. Curr. Psychiat. Rep. 17, 8.
Barbosa, I.G., Machado-Vieira, R., Soares, J.C., Teixeira, A.L., 2014a. The immunology of bipolar disorder. Neuroimmunomodulation 21, 117–122.
Barbosa, I.G., Rocha, N.P., Assis, F., Vieira, E.L., Soares, J.C., Bauer, M.E., Teixeira, A.L., 2014b. Monocyte and lymphocyte activation in bipolar disorder: a new piece in the puzzle of immune dysfunction in mood disorders. Int. J. Neuropsychopharmacol. 18.
Benedetti, F., Poletti, S., Hoogenboezem, T.A., Mazza, E., Ambree, O., de Wit, H., Wijkhuijs, A.J., Locatelli, C., Bollettini, I., Colombo, C., Arolt, V., Drexhage, H.A., 2016. Inflammatory cytokines influence measures of white matter integrity in Bipolar Disorder. J. Affect. Disord. 202, 1–9.
Brambilla, P., Bellani, M., Isola, M., Bergami, A., Marinelli, V., Dusi, N., Rambaldelli, G., Tansella, M., Finardi, A.M., Martino, G., Perlini, C., Furlan, R., 2014. Increased M1/decreased M2 signature and signs of Th1/Th2 shift in chronic patients with bipolar disorder, but not in those with schizophrenia. Transl. Psychiat. 4, e406.
Breunis, M.N., Kupka, R.W., Nolen, W.A., Suppes, T., Denicoff, K.D., Leverich, G.S., Post, R.M., Drexhage, H.A., 2003. High numbers of circulating activated T cells and raised levels of serum IL-2 receptor in bipolar disorder. Biol. Psychiat. 53, 157–165.
Brietzke, E., Stertz, L., Fernandes, B.S., Kauer-Sant'anna, M., Mascarenhas, M., Escosteguy Vargas, A., Chies, J.A., Kapczinski, F., 2009. Comparison of cytokine levels in depressed, manic and euthymic patients with bipolar disorder. J. Affect. Disord. 116, 214–217.
Cakir, U., Tuman, T.C., Yildirim, O., 2015. Increased neutrophil/lymphoctye ratio in patients with bipolar disorder: a preliminary study. Psychiatr. Danub. 27, 180–184.
Cassano, G.B., Akiskal, H.S., Musetti, L., Perugi, G., Soriani, A., Mignani, V., 1989. Psychopathology, temperament, and past course in primary major depressions. 2. Toward a redefinition of bipolarity with a new semistructured interview for depression. Psychopathology 22, 278–288.
Dickerson, F., Stallings, C., Origoni, A., Boronow, J., Yolken, R., 2007. Elevated serum levels of C-reactive protein are associated with mania symptoms in outpatients with bipolar disorder. Progr. Neuro-Psychopharmacol. Biol. Psychiat. 31, 952–955.
do Prado, C.H., Rizzo, L.B., Wieck, A., Lopes, R.P., Teixeira, A.L., Grassi-Oliveira, R., Bauer, M.E., 2013. Reduced regulatory T cells are associated with higher levels of Th1/TH17 cytokines and activated MAPK in type 1 bipolar disorder. Psychoneuroendocrinology 38, 667–676.
Drexhage, R.C., Hoogenboezem, T.H., Versnel, M.A., Berghout, A., Nolen, W.A., Drexhage, H.A., 2011. The activation of monocyte and T cell networks in patients with bipolar disorder. Brain Behav. Immun. 25, 1206–1213.
Fenoglio, D., Battaglia, F., Parodi, A., Stringara, S., Negrini, S., Panico, N., Rizzi, M., Kalli, F., Conteduca, G., Ghio, M., De Palma, R., Indiveri, F., Filaci, G., 2011. Alteration of Th17 and Treg cell subpopulations co-exist in patients affected with systemic sclerosis. Clinical immunology 139, 249–257.
Fenoglio, D., Traverso, P., Parodi, A., Tomasello, L., Negrini, S., Kalli, F., Battaglia, F., Ferrera, F., Sciallero, S., Murdaca, G., Setti, M., Sobrero, A., Boccardo, F., Cittadini, G., Puppo, F., Criscuolo, D., Carmignani, G., Indiveri, F., Filaci, G., 2013. A multipeptide, dual-adjuvant telomerase vaccine (GX301) is highly immunogenic in patients with prostate and renal cancer. Cancer Immunol. Immunother. CII 62, 1041–1052.
Fernandes, B.S., Steiner, J., Molendijk, M.L., Dodd, S., Nardin, P., Goncalves, C.A., Jacka, F., Kohler, C.A., Karmakar, C., Carvalho, A.F., Berk, M., 2016. C-reactive protein

concentrations across the mood spectrum in bipolar disorder: a systematic review and meta-analysis. Lancet Psychiat. 3, 1147–1156.
First, M.B., Spitzer, R.L., Gibbon, M., et al., 1994. Structured Clinical Interview for DSM-IV Axis II Personality Disorders (SCID-II). Version 2.0. New York: Biometrics Research Deprtment. New York State Psychiatric Institute.
Fries, G.R., Vasconcelos-Moreno, M.P., Gubert, C., Santos, B.T., da Rosa, A.L., Eisele, B., Sartori, J., Pfaffenseller, B., Kapczinski, F., Kauer-Sant'anna, M., 2014. Early apoptosis in peripheral blood mononuclear cells from patients with bipolar disorder. J. Affect. Disord. 152–154, 474–477.
Hamilton, M., 1960. A rating scale for depression. J. Neurol. Neurosurg. Psychiat. 23, 56–62.
Heng, S., Song, A.W., Sim, K., 2010. White matter abnormalities in bipolar disorder: insights from diffusion tensor imaging studies. J. Neural. Transm. 117, 639–654.
Knijff, E.M., Breunis, M.N., van Geest, M.C., Kupka, R.W., Ruwhof, C., de Wit, H.J., Nolen, W.A., Drexhage, H.A., 2006. A relative resistance of T cells to dexamethasone in bipolar disorder. Bipolar Disord. 8, 740–750.
Kohler, O., Sylvia, L.G., Bowden, C.L., Calabrese, J.R., Thase, M., Shelton, R.C., McInnis, M., Tohen, M., Kocsis, J.H., Ketter, T.A., Friedman, E.S., Deckersbach, T., Ostacher, M.J., Iosifescu, D.V., McElroy, S., Nierenberg, A.A., 2016. White blood cell count correlates with mood symptom severity and specific mood symptoms in bipolar disorder. Aust. N Z J Psychiat.
Kotzalidis, G.D., Rapinesi, C., Savoja, V., Cuomo, I., Simonetti, A., Ambrosi, E., Panaccione, I., Gubbini, S., De Rossi, P., De Chiara, L., Janiri, D., Sani, G., Koukopoulos, A.E., Manfredi, G., Napoletano, F., Caloro, M., Pancheri, L., Puzella, A., Callovini, G., Angeletti, G., Del Casale, A., 2017. Neurobiological evidence for the primacy of mania hypothesis. Curr. Neuropharmacol. 15, 339–352.
Koukopoulos, A., Ghaemi, S.N., 2009. The primacy of mania: a reconsideration of mood disorders. Eur. Psychiat. 24, 125–134.
Kraepelin, E., 1902. Clinical Psychiatry. Macmillan.
Kupka, R.W., Hillegers, M.H., Nolen, W.A., Breunis, N., Drexhage, H.A., 2000. Immunological aspects of bipolar disorder. Acta Neuropsychiatr. 12, 86–90.
Larbi, A., Fulop, T., 2014. From “truly naive” to “exhausted senescent” T cells: when markers predict functionality. Cytometry A 85, 25–35.
Magioncalda, P., Martino, M., Conio, B., Piaggio, N., Teodorescu, R., Escelsior, A., Marozzi, V., Rocchi, G., Roccatagliata, L., Northoff, G., Inglese, M., Amore, M., 2015. Patterns of microstructural white matter abnormalities and their impact on cognitive dysfunction in the various phases of type I bipolar disorder. J. Affect. Disord. 193, 39–50.
Martino, M., Magioncalda, P., Huang, Z., Conio, B., Piaggio, N., Duncan, N.W., Rocchi, G., Escelsior, A., Marozzi, V., Wolff, A., Inglese, M., Amore, M., Northoff, G., 2016a. Contrasting variability patterns in the default mode and sensorimotor networks balance in bipolar depression and mania. Proceed. Natl. Acad. Sci. U.S.A. 113, 4824–4829.
Martino, M., Magioncalda, P., Saiote, C., Conio, B., Escelsior, A., Rocchi, G., Piaggio, N., Marozzi, V., Huang, Z., Ferri, F., Amore, M., Inglese, M., Northoff, G., 2016b. Abnormal functional-structural cingulum connectivity in mania: combined functional magnetic resonance imaging-diffusion tensor imaging investigation in different phases of bipolar disorder. Acta psychiatr. Scand. 134, 339–349.
Melzer, N., Meuth, S.G., Wiendl, H., 2009. CD8+ T cells and neuronal damage: direct and collateral mechanisms of cytotoxicity and impaired electrical excitability. FASEB J. Off. Pub. Feder. Am. Soc. Exp. Biol. 23, 3659–3673.
Miralbell, J., Soriano, J.J., Spulber, G., Lopez-Cancio, E., Arenillas, J.F., Bargallo, N., Galan, A., Barrios, M.T., Caceres, C., Alzamora, M.T., Pera, G., Kivipelto, M., Wahlund, L.O., Davalos, A., Mataro, M., 2012. Structural brain changes and cognition in relation to markers of vascular dysfunction. Neurobiol. Aging 33 (1003), e1009–1017.
Nichols, T.E., Holmes, A.P., 2002. Nonparametric permutation tests for functional neuroimaging: a primer with examples. Hum. Brain Mapp. 15, 1–25.
Nolz, J.C., 2015. Molecular mechanisms of CD8(+) T cell trafficking and localization. Cell. Mol. Life Sci. CMLS 72, 2461–2473.
Parodi, A., Kalli, F., Svahn, J., Stroppiana, G., De Rocco, D., Terranova, P., Dufour, C., Fenoglio, D., Cappelli, E., 2015. Impaired immune response to Candida albicans in cells from Fanconi anemia patients. Cytokine 73, 203–207.
Parodi, A., Traverso, P., Kalli, F., Conteduca, G., Tardito, S., Curto, M., Grillo, F., Mastracci, L., Bernardi, C., Nasi, G., Minaglia, F., Simonato, A., Carmignani, G., Ferrera, F., Fenoglio, D., Filaci, G., 2016. Residual tumor micro-foci and overwhelming regulatory T lymphocyte infiltration are the causes of bladder cancer recurrence. Oncotarget 7, 6424–6435.
Pender, M.P., 2012. CD8+ T-cell deficiency, epstein-barr virus infection, vitamin d deficiency, and steps to autoimmunity: a unifying hypothesis. Autoimm. Dis. 2012, 189096.
Poletti, S., de Wit, H., Mazza, E., Wijkhuijs, A.J., Locatelli, C., Aggio, V., Colombo, C., Benedetti, F., Drexhage, H.A., 2016. Th17 cells correlate positively to the structural and functional integrity of the brain in bipolar depression and healthy controls. Brain Behav. Immun.
Reus, G.Z., Fries, G.R., Stertz, L., Badawy, M., Passos, I.C., Barichello, T., Kapczinski, F., Quevedo, J., 2015. The role of inflammation and microglial activation in the pathophysiology of psychiatric disorders. Neuroscience.
Rizzo, L.B., Do Prado, C.H., Grassi-Oliveira, R., Wieck, A., Correa, B.L., Teixeira, A.L., Bauer, M.E., 2013. Immunosenescence is associated with human cytomegalovirus and shortened telomeres in type I bipolar disorder. Bipolar Disord. 15, 832–838.
Serpero, L.D., Filaci, G., Parodi, A., Battaglia, F., Kalli, F., Brogi, D., Mancardi, G.L., Uccelli, A., Fenoglio, D., 2013. Fingolimod modulates peripheral effector and regulatory T cells in MS patients. J. Neuroimm. Pharmacol. Off. J. Soc. NeuroImmune Pharmacol. 8, 1106–1113.
Sheehan, D.V., Lecrubier, Y., Sheehan, K.H., Amorim, P., Janavs, J., Weiller, E., Hergueta, T., Baker, R., Dunbar, G.C., 1998. The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. J. Clin. Psychiat. 59 (Suppl 20), 22–33 quiz 34-57.
Smith, S.M., Nichols, T.E., 2009. Threshold-free cluster enhancement: addressing problems of smoothing, threshold dependence and localisation in cluster inference. Neuroimage 44, 83–98.
Tanaka, T., Matsuda, T., Hayes, L.N., Yang, S., Rodriguez, K., Severance, E.G., Yolken, R.H., Sawa, A., Eaton, W.W., 2017. Infection and inflammation in schizophrenia and bipolar disorder. Neurosci. Res. 115, 59–63.
Tsai, S.Y., Chen, K.P., Yang, Y.Y., Chen, C.C., Lee, J.C., Singh, V.K., Leu, S.J., 1999. Activation of indices of cell-mediated immunity in bipolar mania. Biol. Psychiat. 45, 989–994.
van den Heuvel, M.P., Mandl, R.C., Kahn, R.S., Hulshoff Pol, H.E., 2009. Functionally linked resting-state networks reflect the underlying structural connectivity architecture of the human brain. Hum. Brain Mapp. 30, 3127–3141.
Ventura, J., Liberman, R.P., Green, M.F., Shaner, A., Mintz, J., 1998. Training and quality assurance with the structured clinical interview for DSM-IV (SCID-I/P). Psychiat. Res. 79, 163–173.
Versace, A., Andreazza, A.C., Young, L.T., Fournier, J.C., Almeida, J.R., Stiffler, R.S., Lockovich, J.C., Aslam, H.A., Pollock, M.H., Park, H., Nimgaonkar, V.L., Kupfer, D.J., Phillips, M.L., 2014. Elevated serum measures of lipid peroxidation and abnormal prefrontal white matter in euthymic bipolar adults: toward peripheral biomarkers of bipolar disorder. Mol. Psychiat. 19, 200–208.
Wakana, S., Jiang, H., Nagae-Poetscher, L.M., van Zijl, P.C., Mori, S., 2004. Fiber tract-based atlas of human white matter anatomy. Radiology 230, 77–87.
Willing, A., Friese, M.A., 2012. CD8-mediated inflammatory central nervous system disorders. Curr. Opin. Neurol. 25, 316–321.
Wise, T., Radua, J., Nortje, G., Cleare, A.J., Young, A.H., Arnone, D., 2015. Voxel-based meta-analytical evidence of structural disconnectivity in major depression and bipolar disorder. Biol. Psychiat.
Woolrich, M.W., Jbabdi, S., Patenaude, B., Chappell, M., Makni, S., Behrens, T., Beckmann, C., Jenkinson, M., Smith, S.M., 2009. Bayesian analysis of neuroimaging data in FSL. Neuroimage 45, S173–186.
Wu, W., Zheng, Y.L., Tian, L.P., Lai, J.B., Hu, C.C., Zhang, P., Chen, J.K., Hu, J.B., Huang, M.L., Wei, N., Xu, W.J., Zhou, W.H., Lu, S.J., Lu, J., Qi, H.L., Wang, D.D., Zhou, X.Y., Duan, J.F., Xu, Y., Hu, S.H., 2017. Circulating T lymphocyte subsets, cytokines, and immune checkpoint inhibitors in patients with bipolar II or major depression: a preliminary study. Sci. Rep. 7, 40530.
Yolken, R.H., Torrey, E.F., 1995. Viruses, schizophrenia, and bipolar disorder. Clin. Microbiol. Rev. 8, 131–145.
Young, R.C., Biggs, J.T., Ziegler, V.E., Meyer, D.A., 1978. A rating scale for mania: reliability, validity and sensitivity. Br. J. Psychiat. 133, 429–435.
Zakszewski, E., Adluru, N., Tromp do, P.M., Kalin, N., Alexander, A.L., 2014. A diffusion-tensor-based white matter atlas for rhesus macaques. PLoS One 9, e107398.