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ORIGINAL ARTICLE

Increased M1/decreased M2 signature and signs of Th1/Th2 shift in chronic patients with bipolar disorder, but not in those with schizophrenia

P Brambilla[1,2], M Bellani[3], M Isola[4], A Bergami[5], V Marinelli[3], N Dusi[3], G Rambaldelli[3], M Tansella[3], A Maria Finardi[5], G Martino[5], C Perlini[3] and R Furlan[5]

We here present data on immune gene expression of *chemokines, chemokine receptors, cytokines and regulatory T-cell (T-reg) markers* in chronic patients suffering from either schizophrenia (SCZ, $N = 20$) or bipolar disorder (BD = 20) compared with healthy controls (HCs, $N = 20$). We extracted RNA from peripheral blood mononuclear cells and performed real-time (RT)-PCR to measure mRNA levels of chemokines, chemokine receptors, cytokines and T-reg markers. All the analyses were Bonferroni-corrected. The classical monocyte activation (M1) markers *il6, ccl3* were significantly increased in BD as compared with both HC and SCZ patients ($P = 0.03$ and $P = 0.002$; $P = 0.024$ and $P = 0.021$, respectively), whereas markers of alternative (M2) monocyte activation *ccl1*, *ccl22* and *il10* were coherently decreased (controls: $P = 0.01$, $P = 0.001$ and $P = 0.09$; SCZ subjects: $P = 0.02$, $P = 0.05$ and $P = 0.011$, respectively). Concerning T-cell markers, BD patients had compared with HC downregulated *ccr5* ($P = 0.02$) and upregulated *il4* ($P = 0.04$) and compared with both healthy and SCZ individuals downregulated *ccl2* ($P = 0.006$ and $P = 0.003$) and *tgf*β ($P = 0.004$ and $P = 0.007$, respectively). No significant associations were found between any immune gene expression and clinical variables (prior hospitalizations, Brief Psychiatric Rating Scale, medications' dosages and lifetime administration). Although some markers are expressed by different immune cell types, these findings suggest a coherent increased M1/decrease M2 signature in the peripheral blood of BD patients with potential Th1/Th2 shift. In contrast, all the explored immune marker levels were preserved in SCZ. Further larger studies are needed to investigate the relevance of inflammatory response in BD, trying to correlate it to psychopathology, treatment and outcome measures and, possibly, to brain connectivity.

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INTRODUCTION

The immune system is considered a key factor in brain homeostasis and plasticity.[1] Immune response can be achieved by the *innate* or the *adaptive* immune response, both of which include the activation of leukocytes. Cytokines represent the signal for communication between leukocytes. Briefly, the *innate* immune system induces a fast and general immune response and includes cells (that is, monocytes and macrophages) and soluble mediators, whereas the *adaptive* system is slower but more specific and the main component of it are B and T lymphocytes (the complement system and cytokines; for a detailed description see Sperner-Unterweger[2]). Cytokines are therefore the key signaling molecules of the immune system and regulators of inflammation.

Inflammation and immunity have been recently proposed as potential components of the etiology of schizophrenia (SCZ) and bipolar disorder (BD).[3–5] Hope *et al.*[6] found no major differences in BD and SCZ patients compared with healthy controls (HCs) except for an increase in the plasma level of soluble TNF-receptor 1 and von Willebrand factor in both types of patients. In contrast, other authors[7,8] found only interleukin 1β (IL1β) to be increased in BD, as compared with HC, whereas IL1β, IL-6, tumor necrosis factor α (TNFα) and CCL2 were all increased, as compared with HC, in SCZ patients. Some recent lines of evidence demonstrated the presence of both pro-inflammatory activation of the innate immune system and of the T cells of the adaptive immune system in SCZ and BD,[9,10] showing that levels of soluble TNF-receptor 1, IL-1Ra, osteoprogesterin and IL-6 are state-related for BD but not in patients with SCZ. This finding would suggest a different immunological pattern in the two diseases.

Concerning SCZ, a putative role in its etiopathogenesis was already proposed 40 years ago[11] and led to the hypothesis of a possible cytokine imbalance. Indeed, some investigations demonstrated alterations in circulating inflammatory cytokine levels in patients with SCZ[12,13] and their relatives,[14–16] but other studies had controversial results.[17–21] Recently, a population-based study found IL-1 receptor antagonist to be marker of metabolic comorbidity, rather than of an inflammatory etiology in SCZ.[22]

Few studies have been conducted in BD that found a lack of T-regulatory (T-reg) cells and an overall inflammatory gene expression 'signature' in the circulating monocytes[8,9,23] including PDE4B, IL1B, IL6, TNF, TNFAIP3, PTGS2, PTX3, CCL2, CCL7, CCL20, CXCL2, CCR2 and CDC42. Moreover, acute symptomatology has been associated with higher levels of pro-inflammatory cytokines (IL-1Ra, IL-8, IL-4, C reactive protein, TNF-α and IL-6)[13,24–27] but not in all studies.[28,29] There is however a lack of studies exploring BD,

[1]DISM, Inter-University Center for Behavioural Neurosciences (ICBN), University of Udine, Udine, Italy; [2]IRCCS "E. Medea" Scientific Institute, Udine, Italy; [3]Section of Psychiatry and Section of Clinical Psychology, Department of Public Health and Community Medicine, ICBN, University of Verona, Verona, Italy; [4]Department of Medical and Biological Sciences, University of Udine, Udine, Italy and [5]Department of Neuroscience, Institute of Experimental Neurology, San Raffaele Scientific Institute, Milan, Italy. Correspondence: Dr P Brambilla, DISM, Inter-University Center for Behavioural Neurosciences (ICBN), University of Udine, P.le Kolbe no. 3, Udine 33100, Italy.
E-mail: paolo.brambilla@uniud.it
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Table 1. Description of populations' features

	Controls (N = *20*)	*Schizoprenia* (N = *20*)	*Bipolar disorder* (N = *20*)	P
Years	36.6 ± 7.69	39.75 ± 7.86	42.45 ± 6.58	0.051
Females/males	11/9	6/14	13/7	0.074
Ethnic group	Caucasian	Caucasian	Caucasian	
Smoking (n/y)	17/3	11/9	8/11	0.018
BMI	23.66 ± 3.13	26.85 ± 6.20	26.85 ± 4.15	0.054
Age of onset	—	25.45 ± 6.57	27.44 ± 6.13	0.318
Length of illness (years)	—	14.7 ± 9.4	14.56 ± 6.43	0.714
Hospitalizations (*n*)	—	3.75 ± 5.56	5.32 ± 7.07	0.557
Chlorpromazine equivalent	—	237.04	50.5	0.002
Antipsychotic lifetime (years)	—	10.42 ± 8.36	5.91 ± 7.03	0.061
Mood stabilizer lifetime (years)	—	—	8.54 ± 5.21	
GAF	82.25 ± 4.85	45 ± 11.49	58.79 ± 13.67	0.0001
BPRS				
Total	—	45.83 ± 12.03	32.22 ± 5.83	0.006
Anxiety and depression	—	11.93 ± 5.12	11 ± 4.00	0.813
Negative symptoms	—	12.7 ± 4.82	7.44 ± 0.73	0.0005
Positive symptoms	—	12.75 ± 6.07	8.4 ± 6.35	0.026
Mania	—	12.75 ± 3.99	10.22 ± 1.99	0.077
BRMRS	—	—	2.95 ± 5.2	
HDRS (21 items)	—	—	14.21 ± 11.78	

Abbreviations: BMI, body mass index; BPRS, Brief Psychiatric Rating Scale; BRMRS, Bech–Rafaelsen Mania Rating Scale; GAF, global assessment of functioning; HDRS, Hamilton Depression Rating Scale; n/y, no/yes.

and, due to methodology variability and small sample sizes, findings are still controversial. However, recent meta-analyses have confirmed the importance of cytochine alterations in BD.[30] In this regards, it should be noted that cytokines and chemokines control both the traffic of immune cells into the central nervous system and the formation of perivenular inflammatory infiltrates, which may lead to demyelination and axonal loss.[31,32] Moreover, it has been shown that pro-inflammatory cytokines are associated with cognitive disturbance in humans[33] and that, in rodents, T cells are needed for normal cognitive functioning.[34] Therefore, an altered immune system may potentially affect brain connectivity, and cognition, having a major role in the pathophysiology of BD.

In order to test the hypothesis of an immunological activation in BD, the objective of the current study was to investigate whether the levels of inflammatory parameters in a sample of chronic BD are different compared with chronic SCZ and HC.

MATERIALS AND METHODS

Participants

Three groups of participants were enrolled in the study. The first group included 20 outpatients with clinical diagnosis of SCZ (14 males, 6 females; mean age = 39.75 ± 7.86). The second group was composed of 20 outpatients with BD (7 males, 13 females; mean age = 42.45 ± 6.58).

The two groups of patients were selected from the South-Verona Psychiatric Case Register,[35] a community-based mental health register that refers to the four Psychiatric Services of Verona. Diagnoses of SCZ and BD were established according to the DSM-IV criteria, using the Structural Clinical Interview for DSM-IV, SCID-I, Italian version[36] and subsequently confirmed with the clinical consensus of two staff psychiatrists. Clinical consensus of two staff psychiatrists was also regularly taken into account in order to double-check all the diagnoses, according to the DSM-IV criteria. Patients with other Axis I disorders, alcohol or substance abuse, history of traumatic head injury, neurological or medical diseases and mental retardation were excluded from the study.

Additional exclusion criteria for the patient group were: electroconvulsive therapy during 6 months before the recruitment and treatment with immunomodulatory drugs in the prior 6 months, pregnancy, head injury with loss of consciousness, family history of hereditary neurologic disorder or floating metallic objects in the body. Symptoms at the moment of the assessment (in the same day or within 1 week) were evaluated by administering the Brief Psychiatric Rating Scale[37] for SCZ and BD patients, whereas only for the BD group manic or depressive symptoms were characterized by using the Bech–Rafaelsen Mania Rating Scale (BRMRS) and the Hamilton Depression Rating Scale (HAM-D), respectively.[38]

The third group included 20 HCs (9 males, 11 females; mean age = 36.6 ± 7.69) with no DSM-IV Axis I and Axis-II disorders, no history of psychiatric disorders among their first-degree relatives, no history of alcohol or substance abuse, no history of head injury and no current neurological or medical illness, including hypertension and diabetes. The absence of psychiatric disturbances was determined by a brief interview modified from the SCID-IV non-patient version.[39] Sociodemographic and clinical data of the three groups are outlined in Table 1. Except for three patients on treatment with clopixol, all patients with SCZ were receiving atypical antipsychotic medications: four olanzapine, four clozapine, four risperidone, three quetiapine, two aripiprazole. Three of them were receiving also haloperidol in combination with the atypical. All BD participants but three were receiving medications at the time of assessment. Specifically, four patients were on atypical neuroleptics (olanzapine), five were on typical neuroleptics (two haloperidol and three clotiapine), four patients were on antidepressant medications (one citalopram, one fluoxetine, one imipramine and one escitalopram); 11 of BD patients were taking one mood stabilizer (one oxcarbamazepine, four lithium, four valproic acid and two lamotrigine).

As for BD, based on the HAM-D, eight had mild depression (HAM-D score between 8 and 17), four had moderate depression (HAM-D score between 18 and 24) and one had severe depression (HAM-D score $\geqslant 25$). On the basis of the BRMRS, three BD patients had a hypomania state (BRMRS score between 8 and 24). On the basis of both HAM-D and BRMRS, five patients were euthymic (HAM-D score $\leqslant 7$; BRMRS score $\leqslant 7$) and two were in a mixed state (HAM-D score >7; BRMRS score >7).

All control participants were recruited in the Hospital/University and in the community areas by word of mouth and through advertisements. Informed consent was obtained from all participants after they had understood the aims and the procedures of the study and the issues involved in study participation. The study was approved by the Ethical Committee of the Azienda Ospedaliera Universitaria Integrata of Verona.

RNA extraction and complementary DNA (cDNA) synthesis

Blood has been collected in PAXgene Blood RNA Tubes (Qiagen, Milano, Italy). RNA has been extracted using PAXgene Blood RNA Kit (Qiagen), according to the manufacturer's protocol. cDNA has been synthesized from 3–5 μg of RNA using a random hexamer-primed kit (Ready-to-go, Amersham Bioscience, Milan, Italy). Blood samples were collected in the morning, and the subjects were asked not to drink coffee or smoke cigarettes before the blood draw.

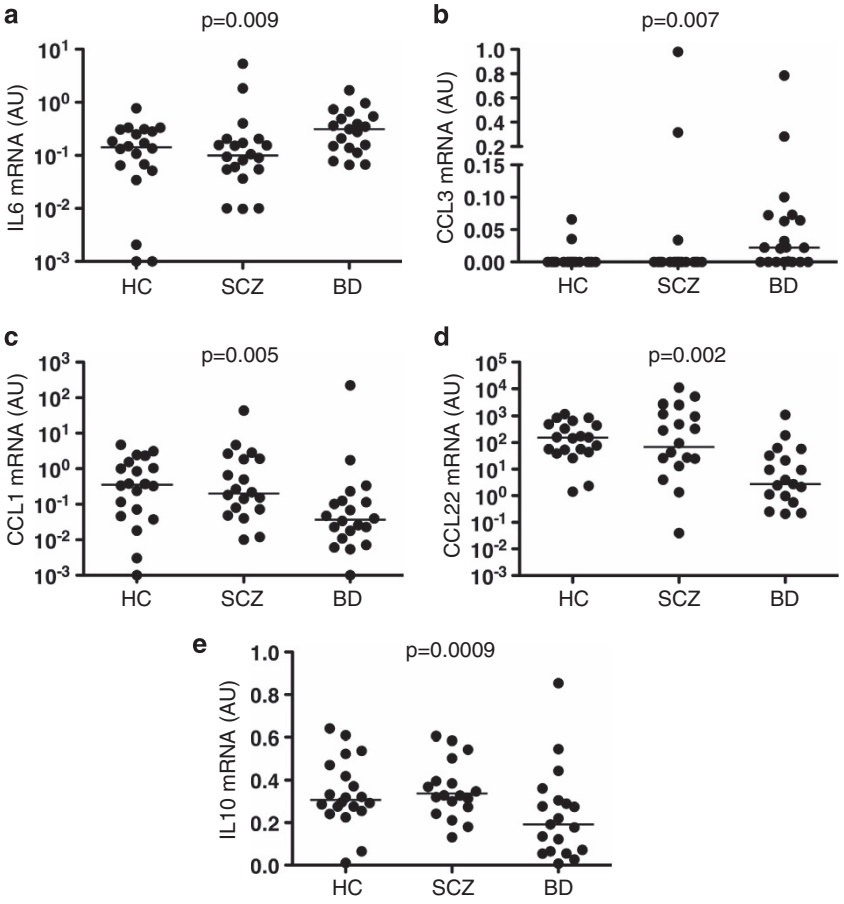

Figure 1. The M1 markers *il6* (**a**) and *ccl3* (**b**) differed among the three groups ($P=0.009$ and $P=0.007$, respectively; Kruskal–Wallis test, Bonferroni-corrected) and were upregulated in bipolar disorder patients (BD) compared with both healthy controls (HCs; $P=0.03$ and $P=0.002$, respectively) and schizophrenia patients (SCZ; $P=0.024$ and $P=0.021$, respectively; Mann–Whitney *U*-test, Bonferroni-corrected). The M2 markers *ccl1* (**c**), *ccl22* (**d**) and *il10* (**e**) significantly differed among the groups ($P=0.005$, $P=0.002$ and $P=0.009$, respectively; Kruskal–Wallis test, Bonferroni-corrected) and were downregulated in individuals with BD compared with both HC ($P=0.01$, $P=0.001$ and $P=0.09$, respectively) and SCZ subjects ($P=0.02$, $P=0.05$ and $P=0.011$, respectively; Mann–Whitney *U*-test, Bonferroni-corrected).

Real-time PCR

The cDNA from ~100 ng of starting RNA was used for real-time (RT) PCR using Pre-developed Taqman Assay Reagents (Applied Biosystems, Monza, Italy) on an ABI Prism 7700 thermal cycler (Applied Biosystems) according to the manufacturer's protocol. Reactions were performed in 25 µl volume and each reaction contained the FAM-labeled probe and primers for the given target. Human *GAPDH* was used as the housekeeping gene. Threshold parameters were maintained constant for GAPDH and for each target throughout the study. Relative quantification was obtained using the same reference sample, cDNA from peripheral blood mononuclear cells from a healthy blood donor, in which all targets were amplifiable, throughout the study, and results were expressed as arbitrary units (a.u.), according to the manufacturer's instructions (User Bulletin no. 2, Applied Biosystems). The following mRNAs have been quantified: chemokine (*ccl1, ccl2, ccl3, ccl4, ccl5, ccl20, ccl22* and *cxcl10*), chemokine receptors (*ccr3, ccr4, ccr5, ccr6, ccr7* and *cxcr5*), cytokines (*il1*α, *il1*β, *il4, il6, il10, il17, ifn*γ, *tgf*β and *tnf*α) and T-reg cell markers (*CD25/il2ra* and *foxp3*).

Auto-antibodies

On paired serum samples obtained from patients and controls, the clinical laboratory of the San Raffaele Scientific Institute, Laboraf, measured antinuclear antibodies (using indirect immunofluorescence): anti-cardiolipin (IgG and IgM), anti-transglutaminase (IgA and IgG), anti-B2microglubulin, anti-tireoperoxidase and anti-tireoglobulin antibodies (using standard enzyme-linked immunosorbent assay).

Statistics

All statistical analyses were performed using the STATA 13 (College Station, TX, USA). Continuous variables were described as the mean and s.d. or median and range, based on the results of the Shapiro–Wilk test. Categorical variables were described as frequencies and percentages and X^2 or Fisher exact test were used to compare distributions. To compare age and body mass index among the three diagnostic groups, analysis of variance was used after verifying the assumptions. To compare GAF score and to explore differences in marker levels, Kruskal–Wallis test was used, as there was no homogeneity of variances. Two group comparisons were made using the Mann–Whitney test. Spearman's correlation analyses were used to explore possible associations between quantitative variables. Bonferroni's correction for multiple comparisons was applied to all the analyses.

RESULTS

We measured peripheral blood mononuclear cell mRNA levels of ccl1, ccl2, ccl3, ccl4, ccl5, ccl20, ccl22, cxcl10, ccr3, ccr4, ccr5, ccr6, ccr7, cxcr5, il1α, il1β, il4, il6, il10, il17, ifnγ, tgfβ tnfα, CD25/il2ra and foxp3 in HCs, patients affected by SCZ and in patients affected by BD, trying to group them according to their most likely cell of origin.

M1 and M2 monocyte activation markers

We considered *il1*α, *il1*β, *il6, ccl3, ccl1, ccl22* and *il10* as innate immune cell markers.

The M1 markers *il6* and *ccl3,* but not *il1*α, and *il1*β, significantly differed among the three groups ($P=0.009$ and $P=0.007$, respectively, Kruskal–Wallis test, Bonferroni-corrected), being higher in BD patients compared with both HC ($P=0.03$ and $P=0.002$, respectively) and SCZ patients ($P=0.024$ and $P=0.021$, respectively; Mann–Whitney *U*-test, Bonferroni-corrected; Figures 1a and b).

As per the M2 markers, *ccl1, ccl22* and *il10* significantly differed across the three groups ($P=0.005$, $P=0.002$ and $P=0.009$, respectively; Kruskal–Wallis test, Bonferroni-corrected), being significantly lower in individuals with BD compared with both control ($P=0.01$, $P=0.001$ and $P=0.09$, respectively) and SCZ subjects ($P=0.02$, $P=0.05$ and $P=0.011$, respectively; Mann–-Whitney *U*-test, Bonferroni-corrected) (Figures 1c–e). In contrast, no abnormal values were found in subjects with SCZ for any M1 or M2 markers ($P>0.05$; Mann–Whitney *U*-test, Bonferroni-corrected).

In Table 2, a tentative classification of the analyzed immune genes according to the M1/M2 paradigm is reported. However, it should be kept in mind that genes that have been linked to monocyte/macrophage polarization can have important roles in other cell types such as T-helper cells.

Th1, Th2 and T-reg cell markers

The Th1 chemokine receptor *ccr5* significantly differed across the three groups ($P=0.015$; Kruskal–Wallis test, Bonferroni-corrected), being lower in BD patients compared with HC ($P=0.02$) but not with SCZ patients ($P=0.26$; Mann–Whitney *U*-test, Bonferroni-corrected; Figure 2a). No differences were found for the other putative Th1 markers *ccl3* (which is also a M1 marker, as mentioned above), *ccl5*, *ccl20*, *ifnγ*, cxcl*10, tnf*α across the three groups ($P>0.05$; Kruskal–Wallis test, Bonferroni-corrected).

Moreover, the prototypical Th2 cytokine *ccl2* and *il4* mRNAs were significantly different among the three groups ($P=0.036$ and $P=0.002$, respectively; Figures 2b and c), whereas no differences were found for the other Th2 marker mRNA levels including *il10* (which is also a M2 marker, as mentioned above), *ccl4*, *ccl11*, *ccr3*, *ccr4* ($P>0.05$; Kruskal–Wallis test, Bonferroni-corrected). In particular, BD patients had significantly lower *ccl2* compared with control and SCZ subjects ($P=0.006$ and $P=0.003$, respectively) and higher *il4* significantly compared with HC ($P=0.04$) but not to SCZ patients ($P=0.145$; Mann–Whitney *U*-test, Bonferroni-corrected).

Finally, the T-reg cell marker *tgf*β significantly differed across groups ($P=0.001$; Kruskal–Wallis test, Bonferroni-corrected) being significantly lower in BD patients compared with both HC and SCZ individuals ($P=0.004$ and $P=0.007$, respectively; Mann–Whitney *U*-test, Bonferroni-corrected; Figure 2d). No significant differences of any other T-reg cells markers, including *foxp3*, *il2ra* (*CD25*), nor of *ccr7* mRNAs, across the three groups were found ($P>0.05$; Kruskal–Wallis test, Bonferroni-corrected).

No significant differences were found between subjects with SCZ and HC for any T-cell markers ($P>0.05$; Mann–Whitney *U*-test, Bonferroni-corrected).

Marker correlations

Spearman correlation analyses (Bonferroni-corrected) were conducted among the immunological markers in each group. *CD25*/*il2ra* and *foxp3* significantly directly correlated in each group (controls: Spearman's rho = 0.739, $P<0.001$; bipolar patients: Spearman's rho = 0.854, $P<0.0001$; SCZ patients: Spearman's rho = 0.681, $P<0.0001$). Moreover, *il1a* significantly directly associated with cxcl10 in subjects with BD (Spearman's rho = 0.651, $P=0.025$) and with *il1b* in those with SCZ (Spearman's rho = 0.611, $P=0.043$).

Table 2. Classification of the analyzed immune genes according to the M1/M2 paradigm (see references 62–66)

Gene	*Phenotype*
ccl1	M2b
ccl2	Both M1 and M2
ccl3	M1
ccl4	M1
ccl5	M1
ccl11	M2
ccl20	No confirmed association
ccl22	M2
cxcl10	M1
cc3	No confirmed association
ccr4	No confirmed association
ccr5	M1
ccr6	No confirmed association
ccr7	M1
cxcr5	No confirmed association
*il1*α	M1
*il1*β	M1
il4	M2
il6	M1
il10	M2
il17	No confirmed association
ifnγ	M1
*tgf*β	No confirmed association
*tnf*α	M1
CD25/il2ra	No confirmed association
foxp3	No confirmed association

It should be noted that not all the analyzed genes have been clearly linked to one of the two activation phenotypes. In addition, genes that have been linked to monocyte/macrophage polarization can have important roles in other cell types such as T-helper cells.

Clinical variable impact

Immunological variables did not show any significant association with GAF score and body mass index in the three groups or with number of prior hospitalizations, Brief Psychiatric Rating Scale subscores (anxiety and depression, negative symptoms, positive symptoms and mania) and medications (both dosages and lifetime administration of antipsychotics and mood stabilizers) in BD and SCZ patients (Spearman's correlation analyses, $P>0.05$, Bonferroni-corrected). In addition, no significant differences were found between smokers and non-smokers for the markers that had different results across the three groups ($P>0.05$; Mann–-Whitney *U*-test, Bonferroni-corrected).

Anti-cardiolipin IgGs are more frequent among bipolar patients

In light to detect signs of a dis-immune status in BD and SCZ, we measured a small panel of auto-antibodies including antinuclear antibodies, IgG and IgM anti-cardiolipin antibodies, anti-β2 microglobulin antibodies, IgG and IgA anti-transglutaminase antibodies, anti-thyreoglobulin and anti-thyreoperoxidase antibodies. We found no differences among groups for all these autoreactivities but for anti-cardiolipin IgGs, where the only four positive samples were clustered in the group of BD patients ($P=0.030$; Fisher exact test). Further, all samples have been used to stain mouse cerebellum, a typical procedure used in diagnostic neuroimmunology to detect antineural antigen autoreactivity. No reactivity, however, was detected, neither in HCs nor in patients affected by either BD or SCZ (not shown).

DISCUSSION

We interrogated blood samples from a relatively small but very selected and homogenous cohort of patients affected by major

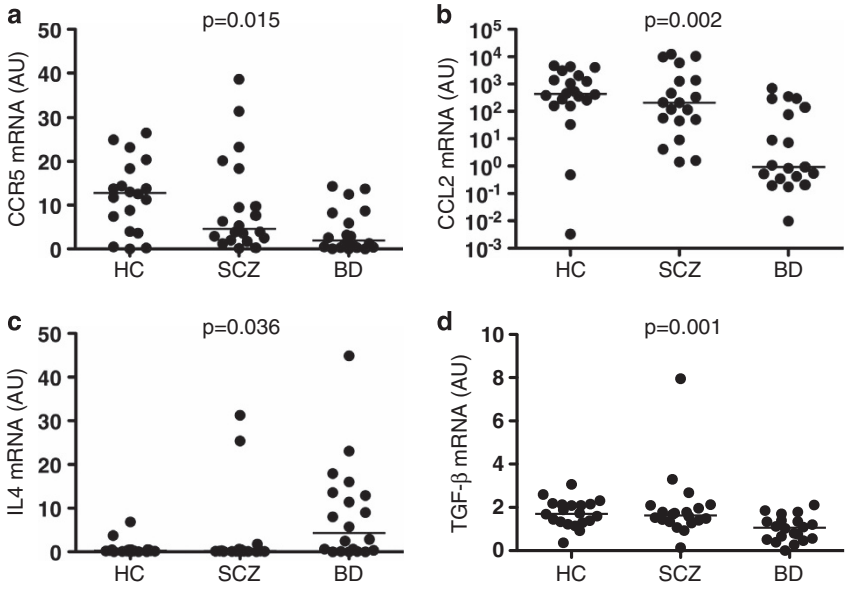

Figure 2. The prototypical Th1 cytokine *ccr5* (**a**) significantly differed across the three groups ($P = 0.015$; Kruskal–Wallis test, Bonferroni-corrected) and was downregulated in BD patients compared with HC ($P = 0.02$) but not to SCZ patients ($P = 0.26$; Mann–Whitney *U*-test, Bonferroni-corrected). The prototypical Th2 cytokine *ccl2* (**b**) and *il4* mRNA (**c**) and the T-regulatory cell marker *tgf*β (**d**) were significantly different among the three groups ($P = 0.036$, $P = 0.002$ and $P = 0.001$, respectively). In particular, BD patients had downregulated *ccl2* and *tgf*β compared with controls ($P = 0.006$ and $P = 0.004$, respectively) and individuals with SCZ ($P = 0.003$ and $P = 0.007$, respectively) and upregulated *il4* compared with HC ($P = 0.04$) but not to SCZ patients ($P = 0.145$; Mann–Whitney *U*-test, Bonferroni-corrected).

psychoses, either BD or SCZ, to identify an immune-signature in comparison with age and gender-matched healthy individuals. We found a number of myeloid cell markers to be modulated in patients affected by BD as compared with SCZ patients and HCs. Myeloid cells have been recently in the spotlight of innate immunity studies because of their newly discovered ability to have different functional phenotypes. In macrophages, this plasticity has been simplified in two main polarization types: M1 macrophages, regarded as pro-inflammatory, phagocytic, potentially tissue damaging; and M2 macrophages, considered immunomodulatory, tissue remodeling and pro-reparative.[40–43] Macrophages derive from circulating monocytes, and it is not completely clear whether circulating macrophages can be polarized. Nevertheless, we, as many authors in other diseases, found a coherent M1 signature, implying also the decrease of M2 markers, in the peripheral blood of bipolar patients. This is not new: myeloid cell alteration has been already reported in bipolar and psychotic patients. It is interesting that also the first reports on monocyte modulation in depressed patients describe the decrease in beta-2 adrenoceptors,[44] and increased phagocytosis,[45] phenomena associated today to M1 skewing.[40,46] No doubt, however, that the field has been pioneered by Drexhage *et al.*[23] describing a raised number of pro-inflammatory (M1) monocytes in bipolar patients, by several technical means.[47–49] Thus, our study is the first independent confirmation of an M1 signature in monocytes from peripheral blood mononuclear cells of bipolar patients without medical comorbidities and strengthens the case for the identification of a subgroup of BD patients who may benefit by anti-inflammatory therapies. Altered monocyte cytokine profile can be the result of an immune or inflammatory process occurring in the central nervous system. For example, substance P release has been linked to major depression[50] and is also well known to activate monocytes.[51] On the other hand, a large literature has shown how peripheral pro-inflammatory cytokines induce depression behavior on both experimental conditions and human patients.[52] Available data do not indicate whether the M1 shift in monocytes from bipolar patients is cause or consequence of the disease. In the latter case, anti-inflammatory therapeutic strategies would be less relevant. If, however, this monocyte modulation precedes disease onset, it is most likely connected to its etiology, and its therapeutic targeting may be extremely efficacious.

Considering the T-helper markers, the Th1 chemokine receptor *ccr5* was downregulated in BD, whereas the prototypical Th2 marker *il4* was upregulated in BD, suggesting a Th1/Th2 shift in BD. However, the downregulaton of *ccl2,* considered a Th2 marker, limits the possibility to fairly discuss this shift. Nonetheless, *ccl2* is mostly released by monocytes and might very well be considered in the context of the M1 shift described above, thus leading to a more coherent picture. Future larger studies in BD should further investigate the potential Th1/Th2 shift in BD. Interestingly, as already been described, we also detected a downregulation of *tgf*β in BD,[53,54] which is considered a potent anti-inflammatory mediator.[55] Therefore, reduced *tgf*β expression may expose the brain of subjects with BD to an increased susceptibility to neurotoxicity. In contrast, we were not able to confirm alterations in the expression of the major T-regulatory markers (that is, *CD25* and *foxp3*) previously reported.[56] However, in this perspective, downregulated *tgf*β may cause a functional impairment of these cells, being released by a variety of leukocytes including T-reg cells.

Finally, anti-cardiolipin auto-antibodies were detected only among BD patients. However, relatively low frequency and sample size do not allow definitive conclusions. Nonetheless, it has been reported that anti-phospholipid auto-antibodies, of which anti-cardiolipin auto-antibodies are a subset, are associated with BD.[57,58] Therefore, this suggestive finding clearly needs to be confirmed in a larger series of samples.

Some limitations of our study must be taken into account in the interpretation of these results: first, the mean duration of illness, as our patient groups were chronically ill, the cross-sectional design of the study and the relatively small sample size of our population. Second, elevated cytokine levels might also reflect general medical comorbidity and lifestyle-related factors of psychiatric disorders such as smoking, alcohol use and poor physical condition. Smoking was differently present in the three groups, nevertheless all our patients had no medical comorbidities. Third,

our study was naturalistic and most of patients were receiving psychotropic drugs, the majority was on atypical antipsychotics. These medications are thought not to induce but rather correct the abnormal inflammatory set-point of patient monocytes.[59,60] Regarding BD, half of them were treated also with mood stabilizers, four of them were on lithium, considered in general to be anti-inflammatory.[61] Thus, we cannot rule out that some of the findings may have been influenced by medications.

Future larger studies should further investigate the relevance of M1/M2 and potential Th1/Th2 signature in BD, trying to correlate it to episode type, treatment and outcome measures and, possibly, to brain connectivity.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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