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Full-length Article

Reduced number of peripheral natural killer cells in schizophrenia but not in bipolar disorder

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ABSTRACT

Overwhelming evidence indicates that subthreshold inflammatory state might be implicated in the pathophysiology of schizophrenia (SCZ) and bipolar disorder (BPD). It has been reported that both groups of patients might be characterized by abnormal lymphocyte counts. However, little is known about alterations in lymphocyte proportions that may differentiate SCZ and BPD patients. Therefore, in this study we investigated blood cell proportions quantified by means of microarray expression deconvolution using publicly available data from SCZ and BPD patients. We found significantly lower counts of natural killer (NK) cells in drug-naïve and medicated SCZ patients compared to healthy controls across all datasets. In one dataset from SCZ patients, there were no significant differences in the number of NK cells between acutely relapsed and remitted SCZ patients. No significant difference in the number of NK cells between BPD patients and healthy controls was observed in all datasets. Our results indicate that SCZ patients, but not BPD patients, might be characterized by reduced counts of NK cells. Future studies looking at lymphocyte counts in SCZ should combine the analysis of data obtained using computational deconvolution and flow cytometry techniques.

1. Introduction

Although the immune-inflammatory concept of schizophrenia (SCZ) was proposed more than a century ago, it still serves as a rapidly evolving field of research (Khandaker et al., 2015). Epidemiological studies have revealed that prevalence of several autoimmune disorders, with exception of rheumatoid arthritis, is significantly higher in SCZ patients compared to the general population (Severance et al., 2014). Furthermore, it has been reported that prenatal infections with influenza virus, herpes simplex virus type 2, cytomegalovirus and *Toxoplasma gondii* increase the risk of SCZ in the offspring (Fineberg and Ellman, 2013). These findings support an epidemiological trend that SCZ patients tend to be born in winter/spring months (Davies et al., 2003). Similarly, bipolar disorder (BPD) is increasingly being recognized as a multisystem immune-inflammatory disease (Leboyer et al., 2012).

To date, alterations in a number of peripheral immune-inflammatory markers have been reported in BPD and SCZ including elevated levels of pro-inflammatory cytokines (Miller et al., 2011a; Munkholm et al., 2013), overproduction of acute phase proteins (Maes et al., 1997; Yang et al., 2006) and increased levels of various autoantibodies (Margari et al., 2015; Pollak et al., 2015). It has long been argued that SCZ patients are characterized by a relative predominance of Th1 lymphocytes over Th2 lymphocytes (Schwarz et al., 2001). A recent meta-analysis has confirmed these findings (Guo et al., 2015). In turn, the meta-analysis performed by Miller et al. (2013) provided a broader insight into abnormal counts of blood lymphocytes observed in peripheral blood of SCZ patients. The authors revealed a significant increase in the percentage of Th lymphocytes (CD4+) and natural killer (NK) cells (CD56+) in acutely relapsed inpatients. Absolute levels of total lymphocytes, T lymphocytes (CD3+), Th lymphocytes (CD4+) and CD4+/CD8+ lymphocyte ratio were also significantly increased, and the percentage of CD3 lymphocytes was significantly decreased in drug-naïve FEP patients. The CD4/CD8 ratio appeared to be a state marker as it decreased following antipsychotic treatment in acutely relapsed patients. On the contrary, absolute levels of NK cells were found to increase during antipsychotic treatment. More recent studies have also investigated total counts of lymphocytes and monocytes in schizophrenia patients. In one study, lower total counts of peripheral blood lymphocytes have been reported (Semiz et al., 2014), while another study revealed no significant differences in the total count of lymphocytes and monocytes between patients with non-affective psychosis and healthy

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controls (Miller et al., 2015). Various alterations in proportions of blood leukocytes have been also reported in BPD, including increased proportions of monocytes, reduced proportions of T cells (CD3+) and cytotoxic T cells (CD3+CD8+), higher percentage of activated T cells (CD4+CD25+), the Th1/Th2 shift as well as lower percentage of Treg cells (Barbosa et al., 2015; Brambilla et al., 2014; do Prado et al., 2013; Drexhage et al., 2011).

The majority of previous studies related to immune system alterations in SCZ have focused on single or few blood cell subtypes that likely limits a detection of meaningful changes occurring simultaneously in multiple cell subsets. Recent computational deconvolution approaches such as linear least-squares regression, digital sorting algorithm or linear support vector regression have provided the ability to estimate individual signal components from their mixtures (Newman et al., 2015; Shen-Orr and Gaujoux, 2013). Consequently, deconvolution methods enable to retrieve a wide range of information specific for distinct cell subsets (such as relative proportions and/or cell-specific expression profiles) directly from heterogeneous samples such as whole blood gene expression profile (Abbas et al., 2009; Newman et al., 2015; Zhong et al., 2013). Importantly, deconvolution algorithms allow to differentiate and count multiple blood cell subsets concurrently in one sample. Given that immune-inflammatory alterations may be potentially connected to shifts in blood cell subset composition, we aimed to investigate blood cell proportions quantified by means of microarray expression deconvolution using publicly available datasets from SCZ and BPD patients.

2. Material and methods

2.1. Human expression data and preprocessing

All expression datasets were downloaded from the Gene Expression Omnibus (GEO) (Barrett et al., 2013). Detailed information on dataset accession number, platform, disease, mRNA source and application is provided in Supplementary Table 1. In general, our datasets included 199 SCZ patients, 49 BPD patients and 218 healthy controls. In addition, we used other 73 samples to validate the preprocessing and deconvolution steps. All Affymetrix arrays were obtained as CEL files and assessed for RNA quality using the "AffyRNADegradation" package (Fasold and Binder, 2013). All samples with $d^k < 0.45$ were removed from further analysis (see (Fasold and Binder, 2012) for the details). Remaining samples were normalized with MAS5 using the "affy" package, mapped to the NCBI Entrez gene identifiers using a custom chip definition file (Brainarray, Version 19) and converted to HUGO gene symbols as suggested previously (Dai et al., 2005; Gautier et al., 2004; Newman et al., 2015). We used the ComBat algorithm implemented in the "swamp" package to correct the data for a batch effect (scan date) (Johnson et al., 2007; Lauss et al., 2013). The ComBat algorithm could be applied to GSE20300, GSE27383 and GSE46449 datasets, in which samples representing single batches were removed. For remaining Affymetrix datasets batch correction could not be used because study groups were not evenly distributed across batches or most of the batches included single samples. Due to a lack of publicly available raw data, Illumina datasets (GSE23848, GSE48072, GSE38481 and GSE38484) were downloaded as normalized matrices from GEO. Subsequently, the "WGCNA" package was used to collapse probes into a single gene measurement by selecting probes with highest mean expression across all samples (Miller et al., 2011b). Since GSE38481 and GSE38484 datasets included a unique subgroup of patients without exposition to antipsychotic treatment (drug-naïve patients), we combined antipsychotic-naive subjects and controls from both datasets using the ComBat algorithm to remove batch effects (control samples from GSE38484 were matched for age, gender and size with controls from GSE38481). Medicated schizophrenia patients exclusively included in GSE38484 were treated as a separate dataset. Due to large technical differences, each dataset has been preprocessed and normalized separately.

2.2. Deconvolution of gene expression profiles

After data normalization, we utilized the CIBERSORT algorithm (1000 iterations) and the LM22 gene signature to predict relative proportions of 21 human hematopoietic cell phenotypes (mast cells were excluded from signature) (Newman et al., 2015). Subsequently, we aggregated 21 cell subsets into 10 major cell types (B cells, plasma B cells, T CD4+, T CD8+, NK cells, gamma delta T cells, monocytes together with macrophages, dendritic cells, eosinophils and neutrophils) (Newman et al., 2015). Cell proportions were predicted in each dataset separately.

2.3. Statistics

Estimated cell proportions were assessed for distribution (normal, non-normal) using the Anderson–Darling test. Homogeneity of variance was tested using the Levene's test. Subsequently, cell proportions were compared between groups (affected individuals *versus* healthy controls) using the *t*-test (in case of normal distribution) or the Kruskal–Wallis test (in case of non-normal distribution). Bonferroni correction, taking into account the number of tested datasets, was applied to the level of significance in order to control for multiple testing. Analysis of covariance (ANCOVA) was used to test the effects of group (affected individuals vs. healthy controls) on estimated numbers of NK cells (dependent variable) after co-varying for age and sex Analysis of variance (ANOVA) was performed for one dataset (GSE48072) since all available data were categorical variables (group and sex). In case of three datasets (GSE38481, GSE38484 and GSE46449), estimated proportions of NK cells were transformed using arcsine transformation to obtain normal distribution and homogeneity of variance. All testes were two-tailed with 0.05 level of significance. STATISTICA 10 software was used to perform ANCOVA, while the rest of statistical analyses were performed using the "nortest" and "compareGroups" packages in R statistical software (Subirana et al., 2014).

3. Results

3.1. Deconvolution evaluation

Although CIBERSORT has been extensively evaluated and outperformed other deconvolution methods as reported by Newman et al. (2015), we decided to conduct additional analyses to assess its performance in our study-specific setting (see Supplementary data for details). In brief, we applied CIBERSORT to GSE20300 dataset which includes 19 whole blood cell samples from a cohort of kidney transplant patients for which proportions of blood cells were provided (Shen-Orr et al., 2010). The scatter plot (Supplementary Fig. 1) shows that estimated proportions of lymphocytes ($R^2 = 0.77$) and neutrophils ($R^2 = 0.74$) were relatively well-correlated with corresponding raw data. These results were within the range of correlations reported by other authors using other deconvolution algorithms (Abbas et al., 2009; Gaujoux and Seoighe, 2013). Given that preprocessing steps may affect the relative cell proportion estimations, we assessed the reproducibility of estimated proportions in the dataset that includes 35 biological replicates (blood samples were split into two portions prior the RNA isolation step, GSE46449) (Clelland et al., 2013). After the